SECTION 2

The Patient with fluid, electrolyte, and renal tubular disorders

CHAPTER 20

An overview of tubular function

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Introduction

Of the approximately 180 L of water that is filtered by the renal glomeruli per day, only 1–2 L is excreted. Similarly, the filtered sodium (Na^+) load of approximately 25,000 mmoL/day is mostly reabsorbed with only approximately 150 mmol/day being excreted. Chloride (Cl^-), bicarbonate (HCO_3^-), calcium (Ca^{2+}), and magnesium (Mg^+) have a proportionally similar excretory profile and although secretion is important for the homeostasis of some electrolytes (particularly potassium and acid–base balance), the metabolic activity of the kidney is chiefly concerned with reabsorption. Most of the transport processes are coupled to the reabsorption of sodium, either directly through specialized transport proteins, or indirectly due to transepithelial electrochemical gradients. The 'pump-leak' model is evident in most nephron segments: the heteromeric Na^+/K^+-ATPase extrudes three sodium ions across the basolateral membrane in exchange for two potassium ions, generating a low intracellular sodium concentration and a steep electrochemical gradient for passive entry across the apical membrane via a selection of specialized transport proteins (Fig. 20.1). This process accounts for most of the energy used by the kidney, which is derived chiefly through oxidative metabolism. Renal tissue, which accounts for approximately 0.5% of total body mass, consumes approximately 7% of whole-body oxygen. Renal blood flow is about 20% of the cardiac output and the renal cortex is maintained at the high partial pressure of oxygen required for aerobic metabolism.

The proximal tubule is the powerhouse for reabsorptive processes. It reclaims all of the filtered glucose, amino acids, and small proteins, 80% of HCO_3^- and phosphate (PO_4), and 70% of Na^+, Cl^-, potassium (K^+), and water. The loop of Henle contributes a further 25% to overall reabsorption of Na^+, Cl^-, and K^+ with significant amounts of Ca^{2+} and Mg^+ also being reabsorbed, particularly in the thick ascending limb of Henle (TALH). The distal tubule and collecting duct can fine-tune overall urinary excretion in accordance with systemic balance requirements: Na^+, Cl^- Ca^{2+}, and Mg^{2+} are all reabsorbed here, whereas the net movement of K^+ is secretory. Water reabsorption in the distal nephron is variable, being influenced by vasopressin and thus hydration, and to a lesser extent, volume status. In the first part of this chapter, the major transport pathways and regulatory features for each nephron segment is described. The focus here is on the transepithelial flux of Na^+,K^+, and water. In the second part, other important aspects of renal homeostasis, including urine concentration and acid–base balance, are summarized. Throughout the chapter, the key transport proteins are given and, where different, the name of the encoding gene is also provided.

The proximal tubule

The main function of the proximal tubule is the bulk reabsorption of the glomerular filtrate. The majority of this occurs in the proximal convoluted tubule. Two structural features of the proximal tubule cells enhance the surface area for transepithelial flux. First, microvilli project into the tubule lumen from the apical membrane (the brush border membrane); and second, the basolateral membrane is extensively invaginated.

Transport in the proximal tubule is driven by the Na^+/K^+-ATPase, expressed in the basolateral membrane. Hydrolysis of ATP by this transporter underpins the high metabolic requirements of the proximal tubule, which has mitochondria densely packed to the basolateral membrane. The proximal tubule lies mainly in the cortex and relies predominantly on aerobic metabolism. This contrasts with those segments in the medulla that are in a relatively hypoxic environment and therefore have capacity for anaerobic metabolism. The proximal tubule is prone to hypoxic insult and mitochondrial dysfunction, which may reflect intrinsic differences in the mitochondrial population, compared to the distal tubule (Hall et al., 2009).

In the proximal tubule, the NHE3 isoform of Na^+/H^+ exchange is the main transporter responsible for Na^+ entry into the cells but a whole battery of specialized transporters are also expressed in this membrane, which couple sodium entry to movement of other species (Fig. 20.2A). Despite the prominence of NHE3, mutations in the encoding gene (*SLC9A3*) have not yet been associated with human renal disease. This reflects the redundancy of renal sodium transport: NHE3 knockout mice have increased distal sodium reabsorption (Bailey et al., 2004) due to activation of the renin–angiotensin–aldosterone system (RAAS); the mild hypotension and alkalosis reflect an absorptive defect in the colon.

Aquaporin-1 (AQP1) water channels are constitutively expressed in both membranes, and contribute to the high water hydraulic permeability of the proximal segment. Deletion of AQP1 in mice diminishes substantially transcellular flux and this channel is required for near isosmolar reabsorption observed in the proximal tubule (Vallon et al., 2000). However, water transport in the proximal tubule as a whole remains robust and the low-resistance paracellular shunt provides a route, perhaps the major route, of transport for water across the proximal tubule. Experiments in rodents suggest that the tubular fluid is relatively hypotonic, the transepithelial osmotic gradient of < 10 mosmol/kg being sufficient to drive large amounts of water reabsorption (Green and Giebisch, 1989). It is possible, however, that reabsorption can occur in the absence of an osmolar gradient due to 'electro-osmosis' or current-induced fluid movement (Fischbarg, 2010).

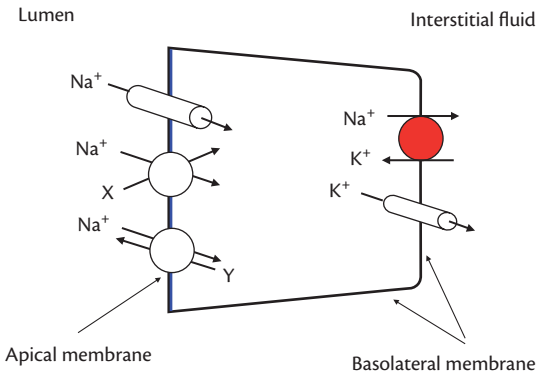

Fig. 20.1 Prototypical renal tubule cell. The Na^+/K^+-ATPase or 'sodium pump' in the basolateral membrane maintains a low intracellular Na^+ concentration, generating an electrochemical gradient for passive entry of Na^+ across the apical membrane. The transport protein facilitating movement of Na^+ across the apical membrane varies along the nephron. Cotransport (symport and antiport) systems use the kinetic energy of Na^+ entry to move another species against its electrochemical gradient. A simple system—an ion channel for Na^+—is found in the distal nephron.

The apical membrane of the proximal tubule also expresses a range of enzymes that change the composition of luminal fluid. Carbonic anhydrase type IV, for example, is vital to bicarbonate reabsorption (see later) and enzymes such as neutral endopeptidase (Walter et al., 1997) and ecto-5′-nucleotidase can influence the tubular concentration of paracrine agents (Shirley et al., 2009) and thereby influence tubular function in downstream segments.

Transport processes

The initial short segment of the proximal tubule (S1 segment) reclaims most of the filtered glucose, amino acids, and phosphate by cotransporters directly coupled to sodium entry into the cell down its electrochemical gradient (Fig. 20.2A). Glucose crosses the apical membrane via the low-affinity, high-capacity, sodium-dependent transporter SGLT2 (SLC5A2), and exits via GLUT2. The reabsorption of glucose (and amino acids) is electrogenic, generating a small, lumen negative, transepithelial potential difference (PD) (approximately −2mV) in the S1 proximal tubule. Although this should favour the paracellular reabsorption of Cl^-, the reflection coefficient of the S1 PT for Cl^- is 0.9 (a reflection coefficient of 1.0 indicates complete impermeability of the S1 epithelium to the solute in question). Cl^- reabsorption therefore lags behind that of Na^+ and water, causing the tubular fluid Cl^- concentration to rise slightly. Thus, Na^+ reabsorption in the S1 PT is preferentially 'coupled' to that of HCO_3^-. This process is indirect: sodium entry into the cell via the NHE3 exchanger causes secretion of a proton into the tubule lumen. An apical H^+-ATPase also contributes to proton secretion. In the tubule fluid, protons combine with the filtered HCO_3^- to produce carbonic acid and ultimately carbon dioxide (CO_2) and water. This is discussed in more detail in the later section on HCO_3^-.

The remainder of the proximal convoluted tubule is the S2 segment. In the cortex, the proximal straight tubule also consists of S2 cells, with S3 cells being limited to the medulla. The preferential reabsorption in the S1 segment of $NaHCO_3$ relative to NaCl creates a modest tubular-fluid to plasma concentration gradient for Cl^-. The gap junctions between S2 cells have a high permeability for Cl^-, allowing paracellular reabsorption down its concentration gradient. Since electrogenic Na^+ reabsorption in the S2 segment is now limited by the low tubular fluid concentration of glucose and amino acids, paracellular Cl^- flux causes a reversal of the transepithelial PD (now +2 mV). This lumen positivity favours paracellular reabsorption of cations (Fig. 20.2B) but the majority of sodium transport is thought to be transcellular via apical NHE3. The gradient also favours anion secretion and there is substantial plasma to tubule fluid concentration gradient for HCO_3^-. However, this gradient is not dissipated due to low paracellular permeability for HCO_3^- backflux.

Micropuncture evidence suggests that the S1 and S2 proximal tubule reabsorbs approximately 70% of the filtered Cl^-. Not all of this occurs by paracellular diffusion as some transporters, coupled ultimately to the basolateral Na^+/K^+-ATPase, help to reabsorb Cl^- by the transcellular route (Fig. 20.3). The apical entry path is either secondary or tertiary active transport involving members of Slc26 anion exchanger family (Sindic et al., 2007). These transporters are multifunctional, capable of transporting, in addition to Cl^-, anions such as sulphate, iodide, formate, oxalate and HCO_3^-. CFEX (SLC26A6) has an important role here. Expressed on the apical membrane, CFEX is capable in expression systems of chloride/formate, chloride/oxalate and chloride/bicarbonate exchange (Aronson, 2006). Studies in Slc26a6 null mice indicate that the primary mode in vivo is to mediate oxalate-dependent Cl^- reabsorption in the proximal tubule. The chloride exits via a basolateral K^+Cl^- cotransport mechanism (probably KCC1) or through Cl^- channels.

K^+ reabsorption pathways in the proximal convoluted tubule are not completely resolved. Much of the potassium that enters the cell via the Na^+/K^+-ATPase is recycled across the basolateral membrane via K^+ channels (channels of KCNK and KCNJ gene families have been identified) or K^+Cl^- cotransport. The isoforms KCC3 (SLC12A6) and KCC4 (SLC12A7) have been identified (Jentsch, 2005). These basolateral exit routes for K^+ are crucial for maintaining efficient transepithelial Na^+ flux, stabilizing intracellular K^+ concentration (and cell volume) in the face of fluctuating rates of transepithelial Na^+ transport (Hamilton and Devor, 2012).

K^+ channels have also been identified in the apical membrane but the electrochemical gradient would favour secretion of K^+ into the tubular fluid. A small secretory component has been identified but the physiological purpose of this is unknown. There is some evidence for active transport mechanisms, particularly the non-gastric isoform of H^+/K^+-ATPase (ATP12A), but the contribution that this pathway makes under normal circumstances is probably minor (Malnic et al., 2013). K^+ reabsorption lags behind that of Na^+ in the S1 segment, causing the tubular fluid concentration to rise slightly. Combined with the lumen positive PD in the S2 segment, paracellular reabsorption by simple diffusion is sufficient to account for the majority of K^+ reabsorption by the proximal tubule. A certain proportion of K^+ reabsorption is mediated by paracellular solvent drag. Since the S2 segment has a high K^+ permeability (reflection coefficient of < 0.4), significant amounts of this solute can be entrained by vectoral fluid flux.

The proximal straight tubule, or *pars recta*, has similar mechanisms of transport for the major electrolytes as the PCT and reabsorption here is also effectively isosmolar. Notably, the proximal straight tubule expresses in addition to AQP1, AQP7 (Sohara et al.,

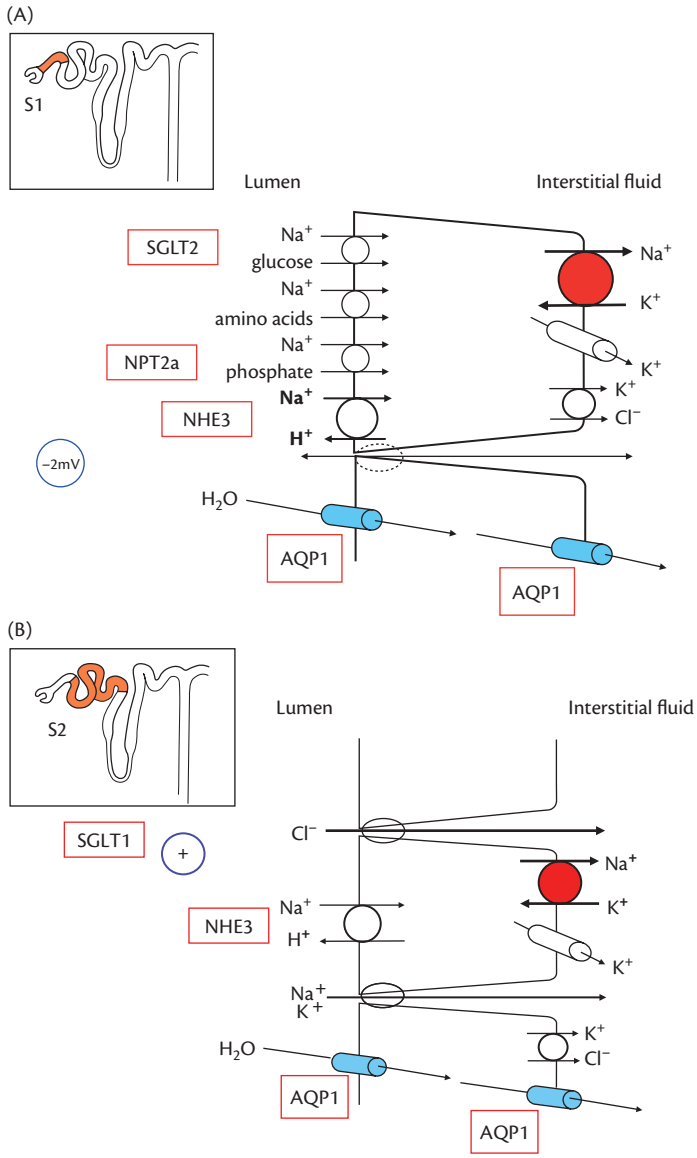

Fig. 20.2 The major Na^+ transport pathways in the proximal tubule. (A) In the S1 segment, Na^+ transport is coupled to that of glucose, amino acids, and bicarbonate, generating a small lumen negative transepithelial potential difference. (B) In the S2 segment, this potential becomes lumen positive..

2009), which contributes both to transepithelial water flux and the generation of a concentrated medulla due to its permeability to glycerol. Studies in isolated tubule segments have identified a lower Na^+/K^+-ATPase activity per unit length and the capacity for Na^+ transport is approximately 50% that of the PCT. The straight segment reclaims ('mops up') the remaining 10% of the filtered glucose load with the high-affinity, low-capacity Na^+-dependent cotransporter, SGLT1 (SLC5A1), providing entry across the apical membrane; GLUT1 facilitates basolateral exit. A major difference in the proximal straight tubule is that net K^+ flux is secretory, which may reflect the diminution (or reversal) of the concentration gradient for paracellular K^+ diffusion coupled to transcellular secretion via apical K^+ channels. The PST is also an important site for the secretion of organic acids and bases, via multiple members of the *SLC22A* gene family (Fig. 20.4). For organic acids, the first step of the secretory process is basolateral anion exchange, in which the exit of dicarboxylate (chiefly α-ketoglutarate) down a concentration gradient is coupled to entry of organic acids (Rizwan and Burckhardt, 2007). The main transporters are OAT1 and OAT3. Apical efflux utilizes a range of transporters, including OAT4 (humans only) and URAT-1, and is not rate limiting. For bases, the overall pathway is similar, with the basolateral entry step being rate limiting and mediated by the selective exchangers, OCT1 and OCT2 (Jonker and Schinkel, 2004).

Clinically important organic acids (e.g. non-steroidal anti-inflammatory drugs, diuretics, penicillin) and bases (e.g. amiloride, cimetidine and atropine) are substrates for this secretory system, which is often, therefore, defined from a pharmacological viewpoint. Indeed, the prototypic substrate for organic acid secretion is p-aminohippurate, this being the central tenet for its clearance being used as an index of effective renal plasma flow. Creatinine is secreted via the organic base pathway, which can lead

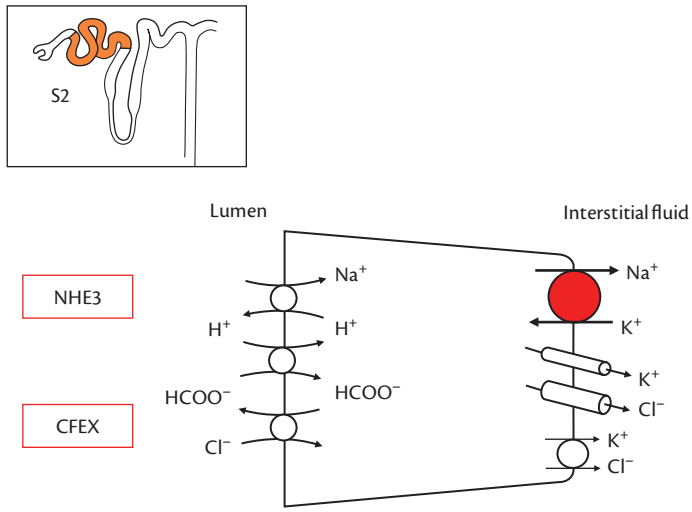

Fig. 20.3 Tertiary active transport facilitating transepithelial Cl^- reabsorption.

to an overestimation of creatinine clearance and thus glomerular filtration rate (GFR).

The physiological role of this system is less well characterized. URAT1 contributes to the urate homeostasis. Urate is the end product of purine metabolism in humans (rodents express uricase allowing metabolism of urate to allantoin) and hyperuricaemia is a risk factor for chronic kidney and cardiovascular disease (Filiopoulos et al., 2012). URAT1 reabsorbs urate in exchange for lactate or nicotinate and is therefore an attractive therapeutic target in patients with hyperuricaemia/gout. Corticosteroids have affinity for the OAT proteins and dopamine and adrenalin are transported via the organic base route. These systems may therefore contribute to intrarenal recycling of physiological active hormones. Gene deletion studies in mice also indicate that OAT3 may contribute to blood pressure control (Vallon et al., 2008), suggesting a role in Na^+ and fluid homeostasis.

Major control mechanisms

Autoregulation of blood flow is an intrinsic property of the vasculature that stabilizes renal perfusion in the face of fluctuating blood pressure. Autoregulation is so efficient that renal blood flow can be largely independent of blood pressure in the physiological range (Cupples, 2007). Whole-kidney autoregulation is governed through the combined influence of at least two mechanisms: tubuloglomerular feedback (see 'The macula densa') and the intrinsic myogenic response of the vascular smooth muscle. These regulatory systems have different, but overlapping, operational frequencies. Of the two major components, only the intrinsic myogenic response is both necessary and sufficient for full whole-kidney autoregulation (Cupples, 2007). The myogenic response operates along the preglomerular vascular tree, responding to increased transmural pressure by channel-mediated calcium influx and reflex vasoconstriction of the vascular smooth muscle. The exact signalling mechanisms are not defined, but local release of ATP is implicated, causing vasoconstriction through activation of P2X1 channels (Shirley et al., 2013).

Glomerulotubular balance (GTB) is a mechanism intrinsic to the haemodynamic and structural properties of the proximal tubule. It ensures that fluctuations in GFR, and therefore filtered solute load, are matched by near proportionate changes in proximal tubular reabsorption such that the fractional reabsorption of the proximal tubule is held almost constant. This serves to prevent loss of solute in the event of increased GFR; as GFR drops it maintains delivery of sodium to the distal nephron, permitting efficient regulation of potassium and proton secretion. Mechanistically, the balance of peritubular Starling forces is a major component of GTB. Thus, when filtration rate rises due to an increased filtration fraction, the oncotic pressure in the peritubular capillaries is elevated, stimulating reabsorption. When GFR rises without a change in filtration fraction (influenced by glomerular plasma flow), flow-dependent reabsorption may contribute to GTB. The underlying mechanism of flow-dependence couples mechanical forces exerted on the microvilli to altered intracellular calcium signalling (Weinbaum et al., 2010) and modulation of paracrine agents, such as ATP (Shirley et al., 2013), dopamine (Du et al., 2012b) or angiotensin II (Du et al., 2012a).

Pressure natriuresis is the process through which increases in arterial blood pressure lead to an increase in renal sodium excretion. This process has infinite feedback gain, that is, it continues to

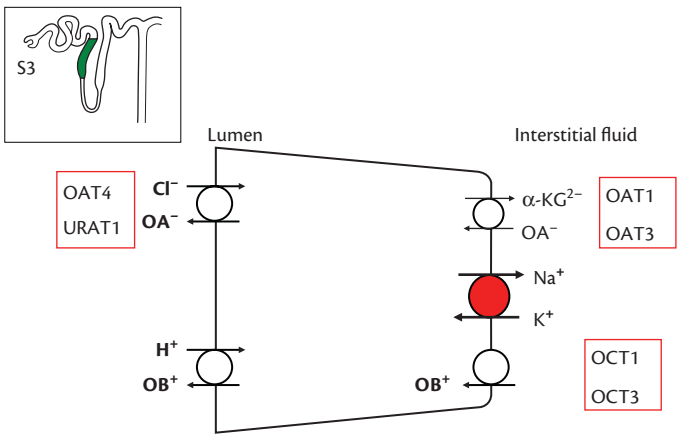

Fig. 20.4 The S3 proximal tubule is a major site for the secretion of organic acids (OA) and bases (OB), which include several classes of clinically important drugs. The physiological role of this system is less well defined.

function until blood pressure returns to set point. Pressure natriuresis is central to the integrated maintenance of sodium homeostasis and thus the long-term regulation of blood pressure (Wadei and Textor, 2012). When kidney function is normal, sustained elevation of arterial pressure of only a few mmHg can evoke large changes in sodium excretion. An impaired pressure natriuresis response is a hallmark of hypertensive states or conditions of sodium sensitivity of blood pressure (Ivy and Bailey, 2014) (Fig. 20.5). Pressure natriuresis is ascribed to a diminution of sodium reabsorption in the proximal tubule following an acute increase in blood pressure. The main mechanism is an increase in renal interstitial hydrostatic pressure, particularly in the medulla. Decapsulation of the kidney prevents the rise in interstitial pressure and blunts the natriuresis, indicating that the response is largely determined by physicochemical factors. Pressure natriuresis occurs in the absence of large changes in renal blood flow due to autoregulation. This at first seems counterintuitive, since the increase in systemic arterial pressure must be transduced to the kidney by changes in renal perfusion pressure. One possibility is that medullary vasculature autoregulates less well than the cortex and small increases in whole-kidney blood flow exert large changes in the medulla. Nevertheless, factors that directly affect tubular sodium transport, particularly those that determine nitric oxide bioavailability are important (Menzies et al., 2015).

Renal sympathetic nerve activity (RNSA) can directly stimulate proximal tubule sodium transport by increasing NHE3 and Na^+/K^+-ATPase activity. RSNA is increased following a reduction in circulating volume or a modest (< 5 mmol/L) elevation of plasma sodium, such as can occur following a meal. RNSA thus contributes to an integrated view of blood pressure control (Kopp, 2011).

Renal sympathetic overactivity has long been linked to sodium retention in experimental hypertension and recent clinical data indicate that bilateral sympathetic efferent denervation effects sustained reductions in blood pressure in selected hypertensive patients (Esler et al., 2010).

Plasma angiotensin II, within the physiological range stimulates sodium transport by activation of NHE3; pharmacological levels are inhibitory. These effects are mediated by AT1 receptors on the basolateral membrane.

Glucocorticoids, acting via the glucocorticoid receptor, stimulate the reabsorption of sodium bicarbonate due to activation of NHE3 and the $Na^+HCO_3^-$ cotransporter on the basolateral membrane. From an integrated standpoint, however, glucocorticoids promote natriuresis, kaliuresis and phosphaturia. Some of this reflects inhibition of transporters but the majority reflects the haemodynamic effects of increased GFR (Hunter et al., 2014).

Intraluminal factors produced by proximal tubule cells will regulate transport by autocrine/paracrine signalling. The concentration of angiotensin II in tubular fluid is much higher than that in plasma due to the intrarenal RAAS (Ramkumar and Kohan, 2013). Angiotensin II can either stimulate (via AT1R) or inhibit (via AT2R) proximal transport by regulating NHE3 activity. Similarly, the proximal tubule has a local dopaminergic system (Zhang et al., 2012); activation of D1 receptor contributes to the natriuretic effect of dopamine. Finally, intraluminal nucleotides inhibit NHE3 activity via P2Y1 receptors (Shirley et al., 2013).

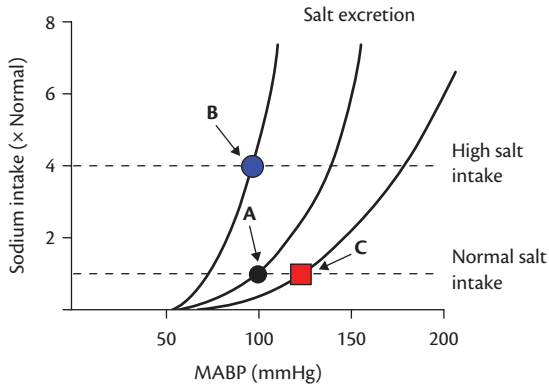

Fig. 20.5 Renal function curve showing the relationship between arterial blood pressure (MABP) and renal sodium excretion. (A) The equilibrium pressure that is maintained through adjustment in sodium balance. (B) On sustained increases in salt intake, the function curve shifts to the left to give a higher level of excretion at any given pressure. (C) If natriuretic capacity is impaired, the curve shifts to the right and is flattened, so that a higher equilibrium pressure is required to match sodium output to input.

The loop of Henle

The loop of Henle comprises the proximal straight tubule (see above), the thin descending limb, the thin ascending limb, and the cortical and medullary segments of the TALH.

The thin descending limb has a high expression of AQP1 in both apical and basolateral membranes and has, therefore, a large hydraulic permeability. The permeability to sodium varies among species and transepithelial transport processes are not defined. In contrast to the proximal tubule, the thin limb is simple epithelial of flat cells with short microvilli and few mitochondria. Consistent with this, only a low level of Na^+/K^+-ATPase activity has been detected and few other transport proteins detected: there is evidence for Cl^- and K^+ channels and K^+Cl^- cotransport and a sodium-borate (or potentially $Na^+HCO_3^-$ transporter), encoded by *SLC4A11*, has been identified (Groger et al., 2010). UT-A2 (SLC14A2) transporters have been identified in the thin descending limb, giving this segment a high permeability to urea.

The transition from thin descending limb to thin ascending limb is evident only in the deep nephron population and occurs just before the bend of the loop. The thin ascending limb is a simple epithelium, having low water permeability due to absence of any aquaporin. Although Na^+/K^+-ATPase activity is low, the epithelial permeability to sodium, potassium and chloride is high. A bumetanide-sensitive pathway, presumably a Na^+-K^+-$2Cl^-$ cotransporter has been identified in several species (Nishino et al., 2007) and mRNA for NHE1, NHE3, H^+-ATPase and H^+/K^+-ATPase is expressed (Pannabecker et al., 2002). In addition, Cl^- channels are identified in both apical and basolateral membrane (Liu et al., 2002). The thin descending limb has significant permeability to urea, allowing rapid equilibrium of the tubular fluid and medullary interstitium.

Thick ascending limb of Henle

The TALH is a major site of Na^+Cl^- reabsorption, accounting for approximately 20% of the filtered load. Cells of the TALH contain a high number of mitochondria, reflecting the significant activity of Na^+/K^+-ATPase. Sodium crosses the apical membrane of TALH

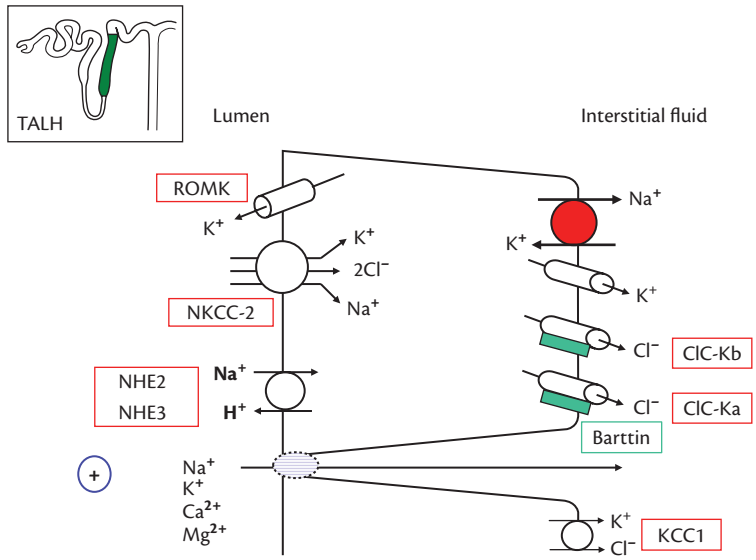

Fig. 20.6 Major transport pathways in the thick ascending limb of Henle.

cells by several routes (Fig. 20.6). The NHE3 and NHE2 exchangers but their contribution to transcellular Na^+ flux is marginal (Shirley et al., 1998). More recently mRNA for a sodium-activated sodium channel has been found in the TALH. Expression is regulated by dietary sodium content and although it is hypothesized that this may be the elusive renal sodium sensor, its physiological function is unclear (Lara et al., 2012).

The main route of entry is via NKCC2 (Fig. 20.6). This protein is inhibited by loop diuretics, which compete with Cl^- at one of its binding sites. NKCC2 is encoded by a single gene, *SLC12A1*, mutations in which cause the salt-wasting Bartter syndrome type 1 (Seyberth and Schlingmann, 2011) (Fig. 20.7). Alternative splicing of *SLC12A1* produces variants of NKCC2, which differ in their ion affinity and in their distribution along the TALH. Physiologically, the distribution is such that the affinity matches the concentration of the transported ions in the tubular fluid, thereby permitting maximum rates of Na^+Cl^- reabsorption (Ares et al., 2011). Chloride exits the basolateral membrane via K^+Cl^- cotransport or through a heteromeric chloride channel assembled from CLC-Kb and Barttin (BSDN). Mutations in these subunits cause Bartter's type 3 and 4, respectively (Fig. 20.7).

Most of the potassium that enters the cell via NKCC2 diffuses back across the apical membrane through apical K^+ channels ROMK/KCNJ1, driven by the electrochemical gradient. This recycling of K^+ has two consequences. First it ensures the continued availability of K^+ to the cotransporter. Na^+ and K^+ must bind in a 1:1 stochiometry and the K^+ concentration in the fluid delivered into the TALH is an order of magnitude lower than that of Na^+. Second, it confers electrogenicity on a transporter that is intrinsically electroneutral. The charge separation caused by movement of K^+ back into the tubule

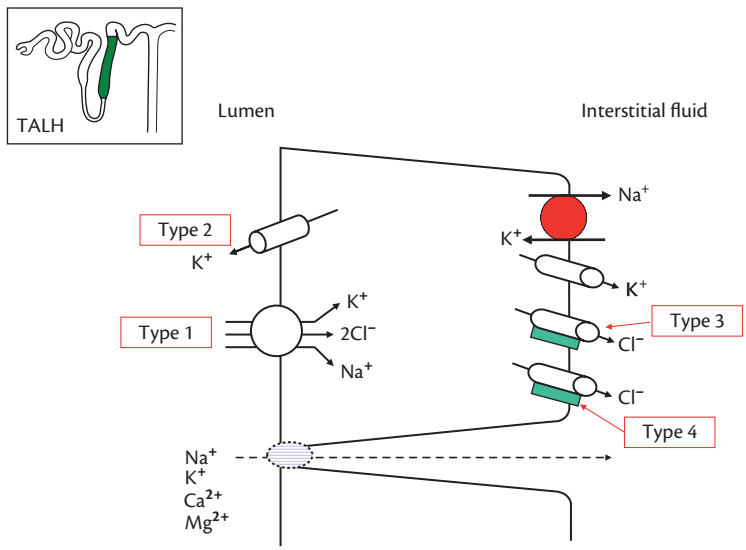

Fig. 20.7 Mutations causing Bartter syndrome impair the transcellular reabsorption of Na^+Cl^-, leading to a diminution of the gradient for paracellular reabsorption of cations.

fluid and Cl^- out across the basolateral membrane generates a transepithelial PD that is lumen positive (~ +10 mV). Theoretically, the transepithelial PD can drive paracellular reabsorption of cations or secretion of anions. The tight junctions of the TALH do have a high ionic conductance (and a very low hydraulic conductance) but are selective for cations. Approximately 50% of the Na^+ reabsorbed in the TALH is via this voltage-driven paracellular shunt. This also provides a major route for the reabsorption of Ca^{2+} and Mg^{2+}. The TALH reabsorbs approximately 25% of the filtered K^+ load. Despite a significant K^+ conductance in both apical and basolateral membrane, the majority of this reabsorption is paracellular and under conditions of high Na^+ reabsorption, the net transcellular K^+ flux can be secretory. Nevertheless the apical K^+ channels are a vital for the efficient functioning of the TALH. Patch-clamp studies have identified two K^+ channels in this membrane. The smaller conductance channel has the electrophysiological 'fingerprint' of the cloned ROMK channel, encoded by *KCNJ1*. Mutations in this gene cause Bartter syndrome type 2 and small molecule inhibitors of ROMK are in development as novel diuretics (Bhave et al., 2011). Although the molecular identity of the second channel is unknown, it is not expressed in the TALH of KCNJ1 knockout mice (Lu et al., 2004), suggesting that it is formed as a heteromeric complex in which ROMK is essential for physiological function.

The macula densa

The macula densa, a small plaque of approximately 20 cells found at most distal segment of the cTALH, is the senor unit of the juxtaglomerular apparatus and influences the secretion of renin from granular cells of the afferent arteriole (Kurtz, 2011). Macula densa cells also exert a countervailing influence on GFR in the glomerulus of origin (and perhaps also in neighbouring nephrons) via vasoactive effects on the afferent arteriole and mesangial cells. This process, called tubule-glomerular feedback, serves to stabilize the moment-to-moment delivery of NaCl into the distal nephron and thereby optimize the fine-tuning of Na^+ (and K^+/H^+) homeostasis by the kidney: aberrant tubuloglomerular feedback has been implicated in the progression of diseases such as diabetic nephropathy (Vallon and Thomson, 2012).

Macula densa cells express the high-affinity isoform of NKCC2 in the apical membrane. The exit pathways for Na^+ are unclear and intracellular Na^+ concentration is not well buffered compared to other renal epithelial cells, rising and falling along with apical entry. As Cl^- exits through channels, the membrane becomes depolarized, activating a maxi-anion channel through which ATP is released. The release of ATP, directly proportional to Na^+ delivery to the apical membrane, is the critical effector of tubule-glomerular feedback, promoting vasoconstriction of the afferent arteriole either directly or following metabolism to adenosine (Bell et al., 2009).

The TALH is also the site for the production and secretion of Tamm–Horsfall protein (aka uromodulin). This glycoprotein is the most abundant protein in normal urine, with up to 150 mg/day being excreted. The physiological roles are poorly understood. Mutations in the encoding gene, *UMOD*, cause a cluster of rare autosomal dominant kidney diseases, characterized by progressive tubule-interstitial damage, hyperuricaemia, urinary concentrating defects, cyst formation, and progressive renal failure. Genome-wide association studies have linked common UMOD variants to chronic kidney disease, hypertension, and type 2 diabetes. Studies in *umod* knockout mice provide a more mechanistic link to physiological function. Tamm–Horsfall protein may guard against infection by inhibiting adherence of bacteria to tubular cells and act as a constitutive inhibitor of calcium crystallization in tubular fluid (Rampoldi et al., 2011).

Major control mechanisms

As with the proximal tubule, the loop of Henle is well endowed with sympathetic nerve endings: the medullary thick limb is the most densely innervated of any tubule segment. Increased RNSA increases Na^+ reabsorption in the loop of Henle as a whole and β-adrenergic stimulation *in vitro* stimulates Na^+ flux in isolated TALH. *In vivo* bilateral denervation studies suggest that RNSA increases NKCC2 by a cyclic AMP-dependent mechanism (Torp et al., 2012).

Aldosterone has a stimulatory effect on Na^+ and K^+ reabsorption in superficial loops of Henle perfused, although this has not been a consistent finding. NKCC2 does not seem to be an aldosterone-induced protein, but studies report stimulation of basolateral Na^+/K^+-ATPase (Grossman and Hebert, 1988), which would increase the driving force for Na^+ transport reabsorption. The receptor through which these effects are mediated is undefined: both mineralocorticoid and glucocorticoid receptors are expressed in the thick limb but 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), the enzyme that governs ligand access, is absent (Ackermann et al., 2010).

Circulating vasopressin stimulates Na^+ reabsorption in the TALH through V2 receptor-mediated activation of adenylate cyclase and increased production of cAMP. This cascade culminates in a coordinated activation of key components in the transcellular reabsorptive pathway: apical K^+ and basolateral Cl^- channels are activated and there is increased trafficking of NKCC2 to the apical membrane Vasopressin also phosphorylates two threonine residues in the N-terminal of NKCC2, which may contribute to enhanced transporter activity (Ares et al., 2011).

Other hormones that lead to increased intracellular cAMP such as parathyroid hormone (PTH), calcitonin, and glucagon increase NKCC2 activity and stimulate reabsorption in the TALH.

Intraluminal factors inhibiting reabsorption in the loop of Henle include eicosanoids (e.g. prostaglandin E_2), produced intrarenally from arachadonic acid metabolism by cyclooxygenase, endothelin-1 (ET-1) and extracellular ATP. The TALH is second only to the collecting duct in terms of ET-1 synthesis. ET-1, via ETB receptors, inhibits sodium transport via an effect on NKCC2 (Ramseyer et al., 2011). ATP is released by TALH cells in response to increased flow and inhibits NKCC2 activity via P2 receptors in both the apical and basolateral membranes. Nitric oxide production (through NOS3) may be the final inhibitory mediator for these paracrine factors (Garvin et al., 2011).

Increased extracellular Ca^{2+} inhibits Na^+ transport in the TALH, reducing the driving force for calcium reabsorption by the paracellular cation shunt pathway. The increased interstitial Ca^{2+} is detected by the G-protein coupled Ca^{2+}-sensing receptor, localized on the basolateral membrane. Activation of the receptor increases the production of 20-HETE and thereby inhibits NKCC2, ROMK, and the basolateral Na^+/K^+-ATPase. The cognate ligand is Ca^{2+} but the receptor is activated by a number of other divalent and trivalent cations, including Mg^{2+} (Gamba and Friedman, 2009). Mutations in this receptor can cause a bartter-like syndrome, sometimes called Bartter type 5, but this nomenclature is not widely accepted.

Distal tubule and collecting duct

The distal tubule lies between the macula densa and the point of confluence with another distal tubule at which a collecting duct is formed. The distal tubule incorporates several subsections: the distal convoluted tubule (DCT), the connecting tubule (CNT), and a short section of the cortical collecting tubule, often referred to as the initial collecting tubule. The collecting duct is divided into the cortical (CCD), outer medullary (OMCD), and inner medullary collecting duct (IMCD).

In addition to a cell type characteristic of each subsegment, intercalated cells are found in much of the distal nephron. In general, adjacent cells have a much deeper contact than in the proximal tubule and the tight junctions are much less 'leaky': paracellular flux is not so common and the epithelia is able to maintain a larger transepithelial PD.

Distal convoluted tubule

The DCT makes up approximately 50% of the total distal tubule. The first part (DCT1) consists exclusively of DCT cells while DCT2 also includes a small proportion of intercalated cells. The DCT has the highest density of mitochondria along the nephron. These cluster along the basolateral membrane, which has a considerably higher surface area than that of the apical membrane. The epithelial of the DCT is very plastic and will amplify or regress depending on sodium delivery. The exact cue to this is unknown but transepithelial flux of Na^+ is likely to be a major factor.

In the apical membrane, DCT cells express a Na^+Cl^- cotransporter (TSC/NCC) sensitive to thiazide diuretics binding to the chloride-binding site. The apical membrane also contains the epithelial calcium channel (ECaC or TRPV5), a ROMK-like potassium channel, a K^+Cl^- cotransporter and NHE2 (Fig. 20.8). In keeping with a central role for the DCT in Na^+ homeostasis, basolateral Na^+/K^+-ATPase is greater than in any other nephron segment. The basolateral membrane also has a chloride exit channel (CLC-K2) and a Ca^{2+}-ATPase. An inwardly rectifying potassium channel (Kir4.1) mediates K^+ recycling across the basolateral membrane. The importance of this process is underscored by the description of the salt wasting EAST syndrome that results from mutations in encoded by *KCNJ10*, the encoding gene (Bockenhauer et al., 2009).

The major route for Na^+Cl^- transport across the apical membrane is NCC, coupled to exit across the basolateral membrane via Na^+/K^+-ATPase and ClC-K2 respectively. Loss of function mutations in the encoding gene, *SLC12A3*, cause Gitelman syndrome (Seyberth and Schlingmann, 2011), which presents as a milder form of Bartter syndrome. A point of differential diagnosis, however, is that Gitelman syndrome presents with hypocalciuria and hypomagnesaemia, while Bartter syndrome patients have normal or hypercalciuria and typically normal Mg^{2+} levels. The basis for these differences is explained in the section on Ca^{2+} transport.

Pseudohypoaldosteronism type 2 (Gordon disease or familial hypertension with hyperkalaemia (FHH)) presents as a gain of function of NCC: hypertension in these patients is very sensitive to low-dose thiazide diuretics. Human genetic screening identified mutations in two members of a novel family of regulatory kinases, the with-no-lysine (WNK) kinases isoforms 1 and 4. The expression of WNK4 is limited to the aldosterone-sensitive distal nephron. In the DCT is normally exerts an inhibitory effect on thiazide-sensitive Na^+ transport, partly by reducing expression of NCC at the apical membrane. This inhibitory effect is no longer observed in PHAII-WNK4 mutants, giving rise to dysregulated NCC activity. The physiological effects of WNK1 are not well understood. It is expressed along the nephron but there is no evidence to suggest a direct interaction with NCC and it is likely that WNK1 affects the activity of other regulatory kinases (Gamba, 2009).

The DCT is not a major site of potassium secretion: the absence of electrogenic sodium reabsorption in DCT1 results in an apical membrane potential close to the K^+ equilibrium potential and there is thus little driving force for ROMK-mediated section. DCT2 contains hybrid cells that also express the epithelial sodium channel (ENaC) in the apical membrane (Nesterov et al., 2012). ENaC-mediated Na^+ entry depolarizes the apical membrane, sustaining some K^+ secretion. Potassium secretion can also occur via K^+Cl^- cotransport (KCC1) but only if the luminal chloride concentration is unusually low. This can happen if the delivery of HCO_3^- is high (Amorim et al., 2003), a situation that also favours

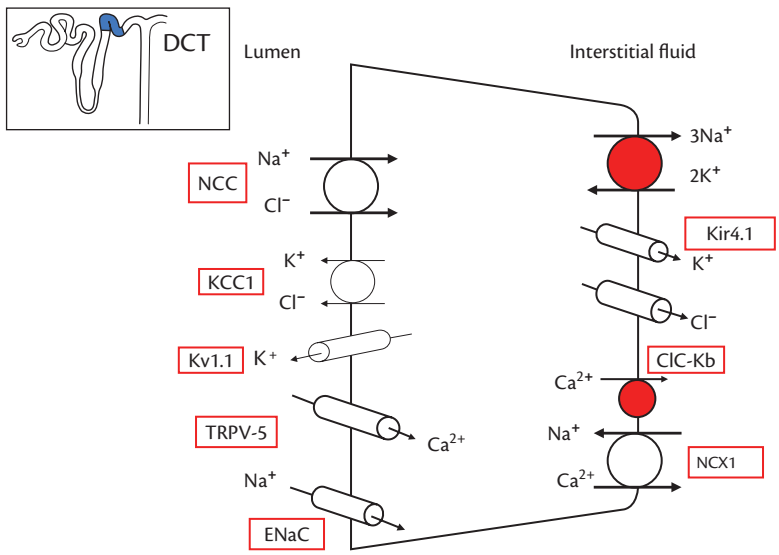

Fig. 20.8 Major transport pathways in the distal convoluted tubule.

NHE2-mediated $NaHCO_3$ reabsorption in this segment (Bailey et al., 2004).

Major control mechanisms

In order to be physiologically active, NCC must be phosphorylated on threonine and serine residues in a regulatory domain in the N-terminus (Rafiqi et al., 2010) and correctly trafficked to the apical membrane of the DCT. Recent studies indicate that angiotensin II, via activation of AT1R, can increase NCC phosphorylation (van der Lubbe et al., 2011) and stimulate thiazide-sensitive Na^+ transport (Ashek et al., 2012).

In vivo studies have shown that corticosteroids can increase NCC-mediated sodium transport in the DCT (Velazquez et al., 1996). The exact mechanisms of action are not clear and both MR and GR are expressed in the DCT, but not 11β-HSD2 (Ackermann et al., 2010).

Other hormones, such as vasopressin and insulin can phosphorylate NCC and increase apical expression. In the chronic setting, the kidney escapes from the Na^+-retaining effects of aldosterone (aldosterone escape) by downregulation of NCC (Turban et al., 2003).

The connecting tubule

The CNT consists of two cell types: CNT cells, the majority, and intercalated cells, the remainder (~ 30% of the total). Like DCT cells, CNT cells exhibit basolateral amplification and have mitochondria along this membrane. The majority of Na^+ transport in the CNT is amiloride-sensitive. These cells express NHE2, which can be inhibited by amiloride analogues, but the major route for sodium reabsorption, is via the ENaC in the apical membrane, coupled to basolateral Na^+/K^+-ATPase activity. Notably, ENaC activity in the CNT does not seem to be regulated by aldosterone (Nesterov et al., 2012).

There is functional evidence for thiazide-sensitive Na^+ transport, but NCC expression is virtually zero. This may reflect a novel, pathway for Na^+ reabsorption in the intercalated cell (see Fig. 20.14), in which Cl^- entry through pendrin is recycled on a newly identified apical transporter, NDCBE (Na^+-driven, Cl^-, bicarbonate exchanger (SLC4A8)), in exchange for Na^+ (Leviel et al.). This system is best described in the collecting duct and may explain the long-standing observation that a significant proportion of Na^+ transport in the cortical collecting duct is sensitive to thiazides. The remainder is amiloride-sensitive and in the CNT, ENaC-mediated Na^+ entry depolarizes the apical membrane with respect to the basolateral membrane, generating a large-lumen negative PD of 30–40 mV.

The apical membrane also expresses two K^+ channels in the apical membrane: the low conductance (~ 35 pS) K^+ channel, ROMK (KCNN1) and a larger (~ 100 pS), big potassium (BK) (KCNMA1) channel (Malnic et al., 2013). The high K^+ permeability of the apical membrane favours K^+ secretion and exerts a repolarizing influence on the apical membrane potential, thereby sustaining ENaC-mediated Na^+ transport.

The CNT is also an important site for Ca^{2+} reabsorption, accounting for approximately 15% of the filtered load and exerting the final regulation of urinary excretion under the control of PTH (see below). CNT is the most proximal site of vasopressin-regulated water transport, expressing both AQP2 water channels and the V2 receptor. The route of Cl^- transport is not clear. A basolateral exit pathway (ClC-K2) is available and Cl^- might enter the cell via NDCBE in the β-intercalated cell (Leviel et al.). Alternatively, chloride might be reabsorbed paracellularly.

The collecting duct

The initial collecting duct is the final segment of the distal tubule and has the same structural makeup as the CNT, comprising mainly

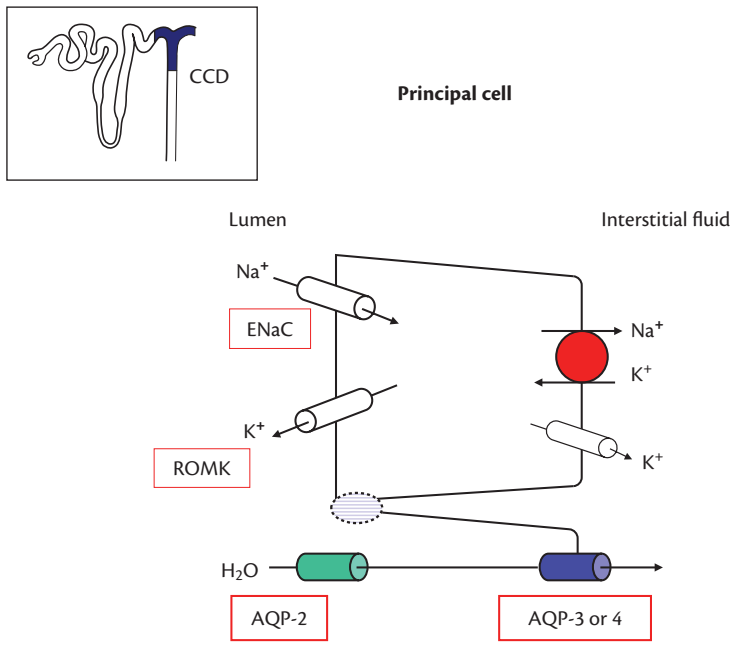

Fig. 20.9 Na^+ reabsorption in the principal cell of the connecting tubule and collecting duct.

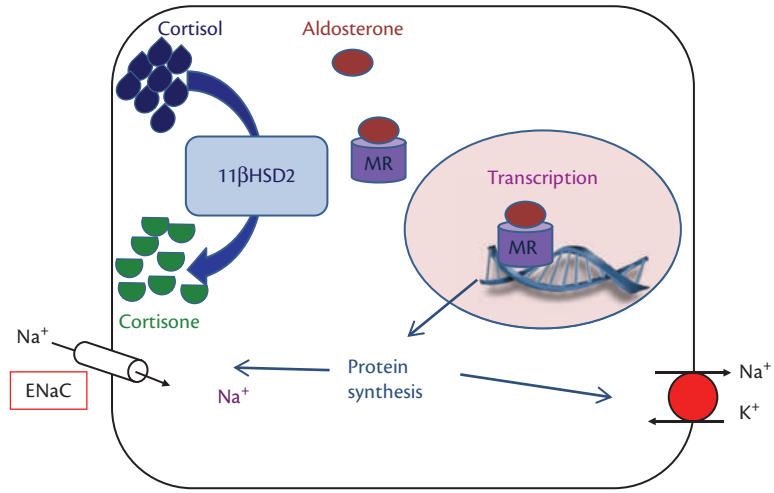

Fig. 20.10 Aldosterone specificity in the distal nephron is governed by the enzyme 11β-dehydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts glucocorticoids into metabolites that are not able to activate the mineralocorticoid receptor (MR).

of principal cells with intercalated cells making up the remainder. The OMCD is much the same as the CCD, both in appearance and cell structure. There is a diminution in the proportion of intercalated cells during the transition from the outer to the inner stripe and are absent from the terminal IMCD. In this segment, structurally distinct IMCD cells replace principal cells.

In the initial collecting tubule (ICT), CCD, and OMCD, the major entry pathway for Na^+ is apical ENaC. Expression of this channel, and of the basolateral Na^+/K^+-ATPase, declines progressively along the collecting duct and the principal cells become less densely packed with mitochondria. ENaC is not found beyond the first third of the IMCD, being replaced by an amiloride-sensitive, non-selective cation channel. Mechanistically, Na^+ reabsorption in the CCD proceeds via the same mechanism as in the CNT: entry through ENaC is electrophysiologically coupled to K^+ secretion via ROMK/BK channels. The OMCD and IMCD do not express apical K^+ channels: ENaC-mediated Na^+ reabsorption is, therefore, self-limiting as depolarization of the apical membrane attenuates the electrochemical gradient for Na^+ entry (Fig. 20.9).

Under sodium replete conditions, ENaC reabsorbs approximately 1–2% of the filtered Na^+ load (Ashek et al., 2012). Sodium transport in this segment is plastic and strongly influenced by the RAAS. In the short term (< 4 hours), aldosterone stimulates Na^+/K^+-ATPase activity and regulates ENaC trafficking, prolonging the half-life of the channel complex in the apical membrane. In the long term, MR activation increases the expression of the basolateral pump, ENaC and ROMK potassium channels. Constitutive activation of ENaC causes the hypertensive Liddle syndrome. ENaC is composed of three subunits (alpha, beta, and gamma), encoded by distinct genes (*SCNN1a, SCNN1b*, and *SCNN1c*). Genetically, Liddle syndrome arises from mutations in β or γ subunits that impair the removal of the channel complex from the apical membrane (Palmer et al., 2012). Conversely, the salt-wasting disorder of pseudohypoaldosteronism type 1 is caused by loss-of function mutations in the mineralocorticoid receptor (PHA1A) or by mutations in the ENaC genes (*PHA1B*), rendering the channel non-functional (Mullins et al., 2006).

In mice, deletion of *Scnn1a, Scnn1b*, or *Scnn1c* causes perinatal lethality. More sophisticated genetic approaches have deleted specifically the αENaC subunit from cells of collecting duct lineage (Rubera et al., 2003) or from cells of the CNT (Christensen et al., 2010). These studies strongly suggest that sodium balance, even under conditions of dietary sodium restriction, is critically dependent on ENaC in the late DCT and CNT.

Aldosterone action and 11β-hydroxysteroid dehydrogenase type 2

In vitro, MR can be activated with equal potency both by aldosterone and cortisol; in vivo, ligand access to MR is determined by co-localization with 11β-HSD2 (Fig. 20.10). By catalysing the rapid conversion of cortisol into cortisone, which does not activate MR, 11β-HSD2 confers upon MR the specificity to aldosterone that it inherently lacks (Funder et al., 1988). MR and 11β-HSD2 have overlapping distributions in the collecting duct, helping to define the 'aldosterone-sensitive distal nephron'. The DCT, however, does not express the enzyme (Ackermann et al., 2010) and the control by corticosteroids of sodium transport is not fully understood.

In rats, pharmacological inhibition of 11β-HSD2 increased sodium reabsorption by the collecting duct (Bailey et al., 2001). In humans, excess intake of liquorice, which contains the 11β-HSD2 inhibitor glycyrrhetinic acid, causes a sodium-dependent hypertension. Inactivating mutations in the encoding gene (*HSD11B2*) cause the hypertensive syndrome of apparent mineralocorticoid excess (AME): a mouse model of this disorder presents with hypertension and severely hypokalaemia and impaired renal Na^+ excretion due to activation of ENaC (Bailey et al., 2008). AME is an extreme phenotype and, like the other Mendelian blood pressure disorders, is very rare. Nevertheless, these disorders illustrate the fundamental role of renal Na^+ transport in blood pressure. Moreover, mild mutations in these same genes may be prevalent in the essential hypertensive population (Wagner, 2008), particularly in those individuals with low-renin or salt-sensitive hypertension. For example, several association studies link polymorphisms in *HSD11B2* to blood pressure and mice with 50% of the normal levels of enzyme salt-sensitive blood pressure due to an impaired ability regulate ENaC activity (Craigie et al., 2012).

Major control mechanisms

The major regulator of Na^+ reabsorption and K^+ secretion in the distal nephron is aldosterone, which is secreted from the zona

glomerulosa of the adrenal cortex in response to plasma angiotensin II (reflecting effective plasma volume) and K^+ concentration. The glucocorticoid receptor is also expressed throughout the distal nephron and provides an alternative pathway for ENaC regulation, particularly when the hypothalamic–pituitary–adrenal axis is activated (Bailey et al., 2009).

Vasopressin is emerging as a powerful regulator of Na^+ reabsorption in the collecting duct, acting synergistically with aldosterone activating ENaC via V2 receptor activation (Stockand, 2010). Another neurohypophysial hormone, oxytocin, is natriuretic due to nitric oxide-dependent inhibition of tubular reabsorption and by stimulating the release of atrial natriuretic peptide (Gimpl and Fahrenholz, 2001).

The natriuretic peptides are a complex family of peptides influencing cardiovascular and renal function. Atrial natriuretic peptide, released from atrial myocytes in response to stretch, increases sodium excretion through coordinated inhibition of the apical cation channel and basolateral Na^+/K^+-ATPase in the IMCD (Beltowski and Wojcicka, 2002). Natriuretic peptides also act as antagonists of the RAAS.

A wide-range of paracrine factors influences collecting duct function. Extracellular nucleotides regulate Na^+ and water transport through activation of P2X and P2Y receptors (Bailey and Shirley, 2009). Endothelins also influence ENaC activity (Kohan et al., 2011). These systems have complex levels of interaction and probably serve to modify the overall 'tone' set by the RAAS.

Proteolytic cleavage of the γ subunit by serine proteases (e.g. prostatin, trypsin, chymotrypsin, and elastase) will increase ENaC activity (Rossier and Stutts, 2009). ENaC cleavage is regulated *in vivo* by aldosterone and may also underpin the sodium retention observed in proteinuric states such as nephrotic syndrome.

Several of the key transporters in the distal nephron have marked circadian rhythm of expression (Stow and Gumz, 2012). ENaC, for example, is under control of the clock gene per1 and disruptions in circadian control have been linked to non-dipper patterns of blood pressure (nocturnal hypertension), a risk factor for cardiovascular and kidney disease.

Urine concentration and dilution

(See Chapter 22.)

The loop of Henle reabsorbs a considerable amount of sodium, potassium, and water. In addition to these transepithelial fluxes, a major function of the loop of Henle is the generation and maintenance of the interstitial osmotic gradient that increases from approximately 290 mOsm/kg in the renal cortex to approximately 1200 mOsm/kg the tip of the medulla. The driving force for this is the reabsorption in the TALH of solute without water, which generates a 'horizontal' osmotic gradient of approximately 200 mOsm/kg at any point between the tubule fluid and interstitium. This 'single osmotic effect' also exists at any given level between the ascending and descending limb because the latter has high osmotic permeability and is in equilibrium with the surrounding interstitium. The hairpin structure of the loop, in which flow in the ascending limb is in the opposite direction to that in the descending limb, multiplies the single effect (countercurrent multiplication), creating a much larger 'vertical' or corticomedullary gradient (Fig. 20.11). The highest degree of urine concentration is found in mammals with the longest renal papilla. This partly reflects the increased length of the loop of Henle, which in species adapted to arid climates can multiply the single effect to 11 Osmol/L/kg at the papillary tip.

The countercurrent multiplier function of the loop of Henle dilutes the tubule fluid (fluid exiting the TALH has an osmolarity of ~ 100mOsm/kg) and concentrates the medullary interstitium. This creates the potential to produce hypotonic or hypertonic urine

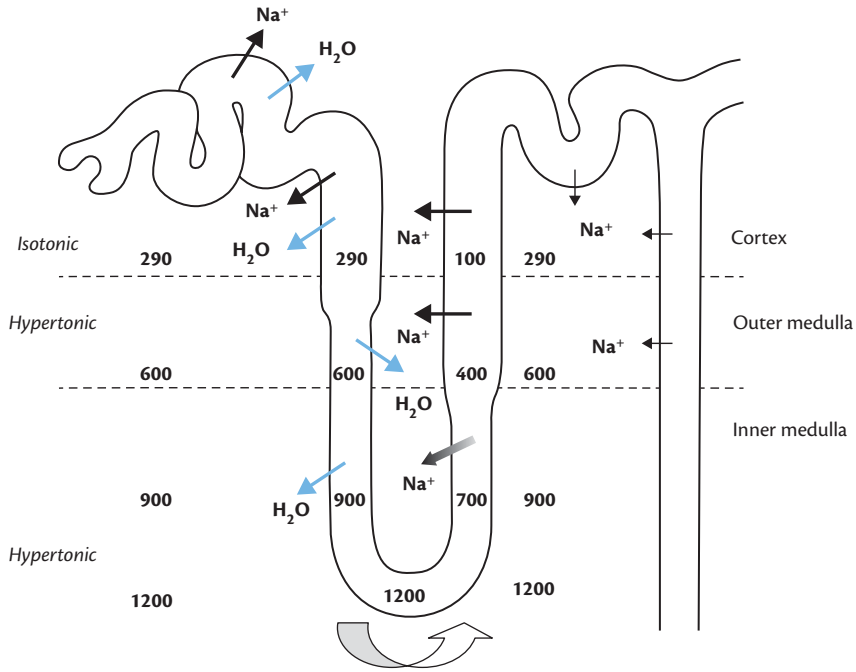

Fig. 20.11 Countercurrent multiplication of the 'single osmotic effect' in the loop of Henle.

in response to homeostatic requirements, by varying the permeability to water of the collecting duct system. The key hormone is vasopressin (antidiuretic hormone), released from the hypothalamus, via the posterior pituitary, in response to increased plasma osmolarity or decreased circulating volume. Vasopressin binds the V2 receptor, activating a signalling cascade that leads ultimately to insertion of AQP2 into the apical membrane of the principal cell. The basolateral membranes of these cells constitutively express AQP3 and AQP4: apical insertion of AQP2 is the rate-limiting step for transepithelial water reabsorption. When plasma vasopressin is high, the hypotonic fluid delivered into distal tubule becomes isotonic by the ICT and progressively hypertonic as it descends through the MCD. In conditions of hydration, when vasopressin is low, little water is extracted during passage through the distal nephron and the final urine can be further diluted by the continued reabsorption of sodium chloride.

The role of urea

The thin limbs of the loop of Henle are permeable to urea (ascending > descending), the TALH and distal nephron are impermeable up to the terminal section of the IMCD. In this part of the nephron, vasopressin-dependent water reabsorption has led to a high urea concentration within the tubule fluid. Vasopressin also determines the urea permeability of the terminal IMCD: V2 receptor activation increases the expression of urea transporters (UT-A1 and UT-A3) in the apical and basolateral membrane respectively, facilitating the passive reabsorption of urea into the inner medullary interstitium (Fenton and Knepper, 2007). Some of this urea enters the vasa recta and some the S3 segment of the proximal tubule and the descending and ascending thin limbs of the loop of Henle. This is then returned to the IMCD to be reabsorbed.

The net result of this urea 'recycling' process is to add urea to the inner medullary interstitium, thereby increasing interstitial osmolality. The high urea concentration within the MCD is rendered osmotically ineffective since it is balanced by a similarly high urea concentration in the medullary interstitium. This allows large quantities of urea to be excreted without obligate osmotic diuresis. Moreover, the concentration of urea in the medullary interstitium increases water abstraction from the thin descending limbs of deep nephrons, raising the intraluminal Na^+ concentration within these structures. Thus urea recycling also creates a concentration gradient that in theory could account for passive Na^+ reabsorption from the thin ascending limbs, which lack Na^+/K^+-ATPase. However, this concept is challenged by studies in mice with genetic deletion of UT-A1 and UT-A3, which have reduced urea concentration in the inner medullary interstitium but a normal interstitial Na^+Cl^- gradient (Fenton and Knepper, 2007). Thus, the mechanisms responsible for the inner medullary electrolyte gradient are still undefined. An interesting theory is that peristaltic contractions observed in the renal pelvis compress rhythmically the hyaluronic acid matrix in the inner medulla, generating a hydrostatic pressure gradient to create the single effect: paralysis of the papillary wall reduces the osmolarity of the inner medulla (Pruitt et al., 2006). It is worth emphasizing, however, that the ultimate driving force for countercurrent multiplication is active Na^+ reabsorption in the TALH. This is underscored by the disruption of the osmotic gradient when loop diuretics are given.

Countercurrent exchange in the vasa recta

If the capillaries supplying the renal medulla had the usual anatomical arrangement of a capillary network, then medullary blood would rapidly dissipate the medullary osmotic gradient as the hypertonic interstitium equilibrated with isotonic capillary blood. This does not happen to any appreciable extent, because the vasa recta also have a special anatomical arrangement, embracing the epithelial structures in a U-shape. The blood does indeed equilibrate with the neighbouring interstitium but solute entry and water loss in the descending vasa recta are offset by solute loss and water entry in the ascending vasa recta. Although countercurrent exchange is a passive process, contractile cells, called pericytes, control vasa recta flow. This epithelial vascular cross-talk is modulated by a variety of autocrine/paracrine agents (e.g. nitric oxide, eicosanoids, adenosine ATP), and helps to match blood flow to transport in the TALH. Countercurrent exchange applies also to oxygen, which diffuses from descending to ascending vasa recta. This 'shunting,' combined with ongoing energy-dependent salt transport in the TALH, renders medullary tissue relatively hypoxic. Nevertheless, medullary cells are adapted to this hostile environment. They have a higher capacity for glycolysis than do cells in the cortex and hypoxia and hyperosmolarity induce, via the transcription factor TonEBP (NFAT5), the expression of proteins that protect the cell and inhibit apoptosis (Burg et al., 2007).

Renal H^+/HCO_3^- transport (acid–base balance)

In individuals on a typical acid-producing diet, the kidneys must reabsorb essentially all the filtered HCO_3^- (> 4000 mmol/day) and add sufficient extra HCO_3^- to the plasma to regenerate the buffer anions consumed in buffering the daily acid load (normally ~ 50 mmol/day).

Acid–base transport

(See Chapter 24.)

Bicarbonate reabsorption

The bulk of filtered HCO_3^- (~ 80%) is reabsorbed in the proximal tubule, largely in the S1 and S2 segments. About half of the remainder is reabsorbed in the loop of Henle, the rest in the distal tubule and collecting duct. HCO_3^- reabsorption is indirect: H^+ and HCO_3^- ions are generated in tubular cells (facilitated by intracellular carbonic anhydrase (type II)); the H^+ ions are secreted into the lumen, whereas the HCO_3^- ions enter the plasma.

In the proximal tubule, H^+ is secreted mainly via apical NHE3 but also via apical H^+-ATPase. The secreted H^+ ions combine with filtered HCO_3^- ions to form H_2CO_3, which is rapidly converted to CO_2 and H_2O in the presence of apical carbonic anhydrase (type IV); CO_2 and H_2O diffuse into the cell; both moieties are able to use AQP1 water channels (Endeward et al., 2006). The HCO_3^- ions generated within the cell enter the interstitial fluid (and thence plasma) via the basolateral $Na^+HCO_3^-$ cotransporter (NBC-1, SLC4A4), which carries three HCO_3^- ions to one Na^+ ion. The net result of these processes is that a filtered HCO_3^- ion is removed while another one replaces it in plasma (Fig. 20.12).

Bicarbonate reabsorption in the loop of Henle takes place mainly in the TALH. Secretion of H^+ into the lumen is largely via NHE3, although H^+–ATPase makes a modest contribution. There is no apical carbonic anhydrase in the loop of Henle. Intracellularly

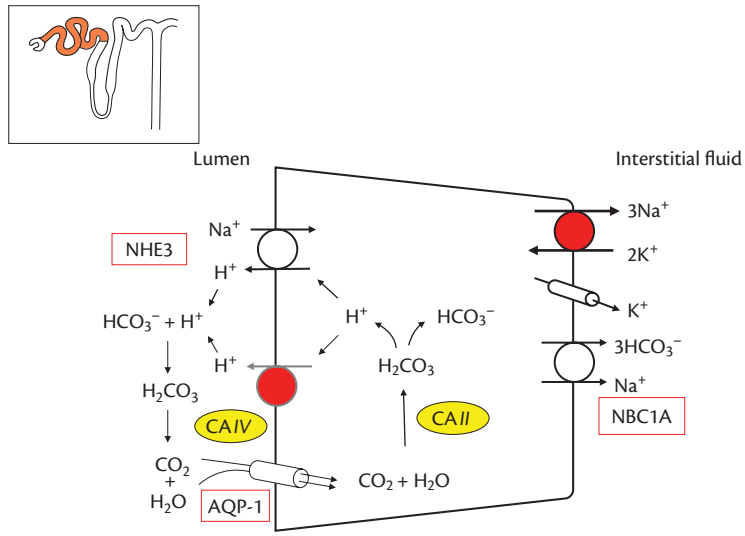

Fig. 20.12 HCO_3^- reabsorption in the proximal tubule is dependent on carbonic anhydrase (CA), types 2 and 4.

generated HCO_3^- enters the interstitial fluid via a basolateral Na-3 HCO_3 cotransporter, as in the proximal tubule, and possibly a Cl/HCO_3 anion exchanger (AE2; SLC4A2).

In the early DCT, there is evidence for some H^+ secretion through apical NHE2 and H^+-ATPase (Bailey et al., 2004), but the cells responsible for H^+ handling beyond the early distal tubule DCT2 to IMCD are the intercalated cells. As their name implies, these are interspersed among the majority cell types in each segment. Intercalated cells comprise approximately 30% of the cells in the distal nephron and come in three varieties, all of which contain carbonic anhydrase (type II) and generate H^+ and HCO_3^- ions: type A (the predominant form), type B (present only in the distal tubule and CCD), and non-A, non-B cells, which might be able to switch their function between the other two types according to the prevailing acid–base status.

Type A intercalated cells have an apical H^+-ATPase, which secretes H^+ into the lumen; HCO_3^- crosses the basolateral membrane using a Cl^-/HCO_3^- exchanger (AE1; SLC4A1A). The Cl^- ions entering the cell by this route are recycled largely through Cl^- channels (CLCNKB) and partly through a K^+Cl^- cotransporter (KCC4; SLC12A7). Although the H^+-ATPase is the major player, the apical membrane contains a second type of proton pump: H^+/K^+-ATPase, whose activity is upregulated during potassium depletion (Fig. 20.13).

Type B intercalated cells are essentially the reverse of type A intercalated cells: H^+ ions are pumped across the basolateral membrane via H^+-ATPase, and HCO_3^- ions enter the lumen via an anion exchanger, in this case pendrin (Fig. 20.14). Although intercalated cells have generally been regarded as being concerned solely with acid–base balance, the Cl^- ions entering via pendrin can exit the basolateral membrane through Cl^- channels, thus providing a mechanism for transepithelial Cl^- reabsorption. Moreover, as described above, recent studies suggest the existence of a thiazide-sensitive, Na^+-driven Cl^-/HCO_3^- exchanger (Leviel et al., 2010).

Addition of extra bicarbonate to plasma

As indicated above, the kidneys not only reabsorb virtually all filtered HCO_3^-, but also add further HCO_3^- to the renal circulation.

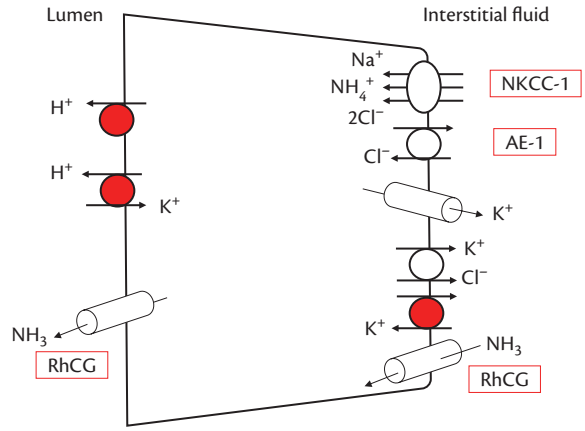

Fig. 20.13 An acid-secreting (type A) intercalated cell.

This is achieved by the generation of H^+ and HCO_3^- in tubular cells, by the same mechanism as for HCO_3^- reabsorption, and the addition of HCO_3^- to the peritubular plasma. The problem then becomes how to deal with the extra H^+ ions generated simultaneously with the extra HCO_3^- ions. There are two principal means of doing so.

Titratable acid excretion

Some of the extra H^+ ions are secreted into the lumen (via Na^+/H^+ exchange and H^+-ATPase), where they react with buffer anions (principally filtered HPO_4^{2-}); any buffer that escapes reabsorption effectively eliminates H^+ ions in the urine. The quantity of H^+ lost in this way, determined by back-titrating the urine with strong base to pH 7.4 (hence the term 'titratable acid'), normally amounts to approximately one-third of overall acid excretion. (A few free H^+ ions appear as such in the urine, but these are quantitatively insignificant since the minimum urine pH is ~ 4.5.)

Ammonium excretion

The other means of dealing with the extra H^+ ions requires the production of NH_4^+ ions in the proximal tubule. Proximal tubular cells

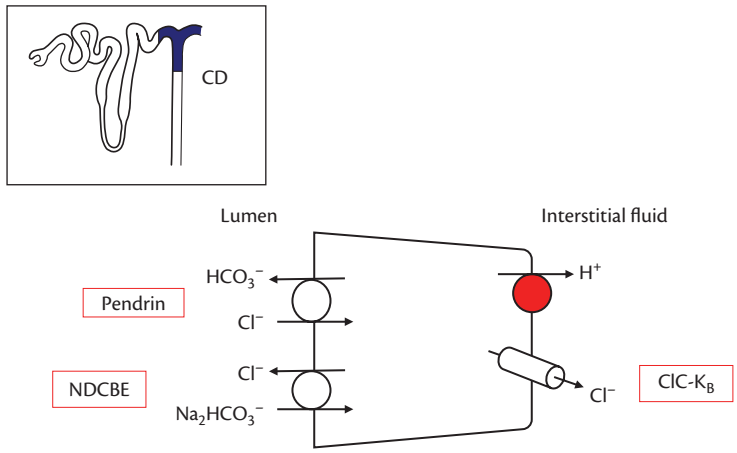

Fig. 20.14 A base-secreting (type B) intercalated cell, which also expresses the thiazide sensitive Na^+-driven chloride-bicarbonate exchanger (NDCBE).

take up the amino acid glutamine and deaminate it, through reactions catalysed by two enzymes (glutaminase and glutamate dehydrogenase), to NH_4^+ and α-ketoglutarate (Fig. 20.15). The NH_4^+ ions are secreted into the tubular lumen largely by substituting for H^+ on NHE3 (although some intracellular NH_4^+ dissociates into NH_3, which diffuses across the apical membrane to be 'trapped' in the lumen by recombining with secreted H^+). The α-ketoglutarate is largely metabolized to glucose, through a series of reactions that consume H^+ ions.

Most NH_4^+ ions secreted into the proximal tubule and delivered to the loop of Henle are reabsorbed in the TALH. This reabsorption is partly paracellular, driven by the lumen-positive transepithelial PD, and partly transcellular. Apical uptake of NH_4^+ is mainly via NKCC2, on which NH_4^+ can substitute for K^+, and basolateral exit is largely via a Na/H exchanger (NHE4; SLC9A4), on which NH_4^+ can substitute for H^+ (Weiner and Verlander, 2011).

As a result of events in the TALH, NH_4^+, in equilibrium with NH_3, accumulates in the medullary interstitium. The final, and critical, stage of NH_4^+ excretion is the transference of this interstitial NH_4^+/NH_3 to the lumen of the collecting duct. Until recently, this was thought to occur by simple diffusion of NH_3 across the basolateral and apical membranes, with 'diffusion trapping' of NH_4^+ in the lumen as a result of H^+ secretion. However, it is now clear that simple diffusion cannot account for the NH3 movement; instead, specific transport proteins are involved, notably the rhesus glycoprotein RhCG (Weiner and Verlander, 2011). Genetically engineered mice lacking RhCG have a much reduced capacity to excrete NH4$^+$, owing to reduced permeability to NH_3 of apical and basolateral membranes in the CD (Fig. 20.13). Moreover, increased expression of RhCG is seen in the OMCD (in both intercalated and principal cells) of rats subjected to metabolic acidosis, though as yet the mechanism of this apparent adaptation is not known (Wagner et al., 2011). Although rhesus glycoproteins are the major players, other transporters in the CD may have a subsidiary role in mediating NH_4^+ excretion. The basolateral membrane of A-type intercalated cells contains a Na^+-K^+-$2Cl^-$- cotransporter (NKCC1; SLC12A2), on which NH_4^+ can compete with K^+; similarly, NH_4^+ can compete with K^+ on a basolateral Na^+/K^+-ATPase,

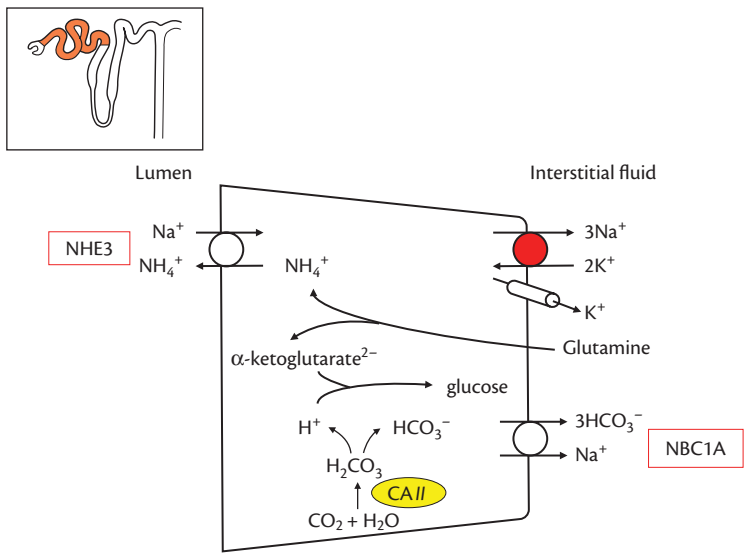

Fig. 20.15 Ammonium secretion by the proximal tubule.

but the physiological importance of these transporters in relation to acid–base balance is probably minor (Weiner and Verlander, 2011).

Major control mechanisms

Chronic acidosis (of non-renal origin) enhances H^+ secretion and HCO_3^- addition to the plasma, while chronic alkalosis results in HCO_3^- excretion. A variety of mechanisms are involved. First, changes in systemic pH are paralleled by changes in intracellular pH, affecting H^+ secretion directly. Second, NHE3 is directly upregulated by chronic acidosis. Third, chronic acidosis increases the trafficking of H^+-ATPase to the apical membrane and that of AE1 to the basolateral membrane, whilst the activity of β-intercalated cells is downregulated. In chronic alkalosis, the opposite occurs. Finally, chronic acidosis will increase ammoniagenesis by the proximal tubule.

In addition to the factors described above, acid–base disorders of respiratory origin affect intracellular PCO_2 and thereby influence the generation of protons and bicarbonate within tubular cells.

Stimulation of the RAAS by acidosis increases H^+ secretion by both the proximal and distal nephron. Direct actions of angiotensin II on NHE3 activity are described. Aldosterone can increases H^+-ATPase activity, both directly and due to increased lumen negative PD secondary to ENaC activity.

The metabolic alkalosis resulting from hypokalaemia is due to the combined action of several factors. First, hypokalaemia causes a compensatory loss of K^+ across the basolateral membrane and a reciprocal movement of H^+ into the cell. Second, hypokalaemia stimulates renal ammoniagenesis; third, hypokalaemia increases insertion of H^+-ATPase into the apical membrane of the α-intercalated cell (Bailey et al., 1998) and activates H^+/K^+-ATPase.

Renal handling of some specific solutes

Phosphate transport

(See Chapter 25.)

The kidney plays a central role in phosphate homeostasis. Inorganic phosphate is filtered as HPO_4^{2-} and $H_2PO_4^-$, normally in the ratio 4:1. Micropuncture studies in superficial nephrons indicate that approximately 80% of the filtered phosphate is usually reclaimed in the proximal tubule. The urinary excretion normally amounts to approximately 10% of the filtered load, suggesting that a small proportion is reabsorbed beyond the proximal tubule. There is some evidence for phosphate reabsorption in the distal tubule but this is controversial and an alternative possibility is that the proximal tubules of deep nephrons that are inaccessible to micropuncture have high reabsorption rates.

Proximal tubular reabsorption of phosphate is transcellular (Fig. 20.2A). Entry across the apical membrane uses one of two Na^+-phosphate cotransporters, NPT2a (SLC34A1) and NPT2c (SLC34A3): NPT2a is the major player, expression being subject to physiological regulation (Prie et al., 2011). Transport across the basolateral membrane is not defined at the molecular level, although there is functional evidence for a Na^+-phosphate cotransporter, a phosphate/anion exchanger, and possibly a phosphate channel.

Control of phosphate reabsorption

Dietary phosphate is a major factor in the control of absorption. A high phosphate intake lowers, and a low phosphate intake raises, the number of NPT2a cotransporters in the apical membrane.

PTH and glucocorticoids reduce the number of NPT2a cotransporters in the apical membrane and thereby increases phosphate excretion (Biber et al., 2009).

Phosphotonins are a group of phosphaturic factors (MEPE, SFRP-4, FGF-23) that inhibit reabsorption in the proximal tubule (Shirley et al., 2010) by reducing the abundance of NPT2a cotransporters in the apical membrane. The intracellular signalling events are not defined.

The active metabolite of vitamin D, calcitriol (1.25 dihydroxycholecalciferol) has profound effects on phosphate homeostasis. Evidence supports both stimulation and inhibition of tubular phosphate reabsorption. Inhibitory actions might be indirect, via phosphotonin; stimulatory effects might be direct via a response element in the NPT2a promotor (Biber et al., 2009).

Disturbances of acid–base balance affect phosphate excretion: alkalosis stimulates, whilst chronic acidosis inhibits, apical Na^+/phosphate co transporters, causing corresponding changes in excretion rates.

Calcium transport

(See Chapter 26.)

Around 60% of the filtered Ca^{2+} is reabsorbed in the proximal tubules, mainly in the PCT but also in the pars recta. No significant Ca^{2+} transport occurs in the thin descending or thin ascending limbs of Henle, owing to their low permeability to Ca^{2+}: the TALH reabsorbs approximately 25% of the filtered load. The remaining Ca^{2+} reabsorption takes place in the distal tubule (~ 10% of the filtered load); very little is reabsorbed in the collecting duct (Lambers et al., 2006a). Usually 1–2% of the filtered load of Ca^{2+} is excreted, the actual figure being closely regulated by the requirements for overall Ca^{2+} balance.

Proximal tubule

In the S1 segment the intratubular Ca^{2+} concentration increases slightly (by 10–20%), creating a small concentration gradient across the S2 epithelium. Together with the small lumen-positive transepithelial PD, this gradient is sufficient to drive passive paracellular Ca^{2+} reabsorption; a small proportion may be reabsorbed by solvent drag. A small component of proximal Ca^{2+} reabsorption is active and transcellular, but little information is available on the molecular mechanisms.

Thick ascending limb of Henle

At least half the Ca^{2+} reabsorption in the TALH is passive and paracellular, driven by the lumen-positive transepithelial PD. Loop diuretics or Bartter syndrome abolish this gradient and are calciuric. The remainder is transcellular, most likely due to passive entry through as-yet-unidentified apical Ca^{2+} channels, coupled with active exit across the basolateral membrane via Ca^{2+}-ATPase.

Distal tubule

Ca^{2+} is reabsorbed in both the DCT and CNT exclusively through a transcellular route (Fig. 20.8). These segments express the Na^+/Ca^{2+} exchanger (NCX1; SLC8A1) and a Ca^{2+}-ATPase in the basolateral membrane but the epithelial Ca^{2+} channel (ECaC or transient receptor potential vanilloid 5 (TRPV5) channel) in the apical membrane is rate-limiting (de Groot et al., 2008). DCT cells exhibit the highest Ca^{2+}-ATPase activity of any nephron segment, and in the DCT region it is the sole mode of basolateral Ca^{2+} efflux,

whereas in DCT and in CNT cells the basolateral membrane also contains Na^+/Ca^{2+} exchangers. Transcellular Ca^{2+} reabsorption is facilitated by calbindin D28k, an intracellular Ca^{2+} binding protein expressed predominantly in the DCT and CNT. By binding Ca^{2+}, calbindins help to maintain the extremely favourable electrochemical gradient for apical entry; they are also thought to help 'shuttle' Ca^{2+} from apical to basolateral membrane via a direct interaction with TRPV5 (Lambers et al., 2006b).

Regulation of calcium reabsorption

Ca^{2+} reabsorption in the proximal tubule is essentially unregulated; physiological control of Ca^{2+} excretion is exerted at the TALH and the distal tubule.

The principal hormone involved in the regulation of Ca^{2+} reabsorption is PTH. Although PTH may have a small inhibitory effect in the proximal tubule, it more than compensates for this by stimulating Ca^{2+} reabsorption in the TALH (at least in the cortical segment) and distal tubule. PTH appears to exert its effect on transcellular, rather than paracellular, reabsorption by stimulating apical Ca^{2+} uptake and basolateral Na^+/Ca^+ exchange activity.

The effects of the hormone calcitonin on renal Ca^{2+} reabsorption are somewhat paradoxical. Despite its generally hypocalcaemic action, calcitonin stimulates Ca^{2+} reabsorption in the TALH and distal tubule by cAMP-dependent mechanisms.

Calcitriol (1.25-dihydroxycholecalciferol) targets mainly intestine and bone, but may also affect Ca^{2+} handling in the kidney: there is evidence that it can stimulate Ca^{2+} reabsorption in the distal tubule, either directly or by potentiating the effect of PTH.

A Ca^{2+}/Mg^{2+} sensing receptor (see above) in the basolateral membrane of the TALH and distal tubule is activated by increased plasma concentrations and inhibits reabsorption of these cations.

Renal Ca^{2+} excretion is influenced by acid–base status: acidosis increases, and alkalosis reduces, Ca^{2+} excretion rates. Although this can be attributed partly to changes in the filtered load of Ca^{2+} (acidosis reduces the proportion of plasma calcium bound to albumin and thereby increases ultrafilterable Ca^{2+}), and partly to non-specific changes in proximal tubular reabsorption, these effects cannot account fully for the phenomenon. A specific inhibitory effect of acidosis on Ca^{2+} reabsorption in the distal tubule has been documented, and it is thought that the mechanism might involve pH sensitivity of the distal tubular apical Ca^{2+}channel.

Magnesium transport

(See Chapter 27.)

Mg^{2+} is largely an intracellular ion, found mostly in bone; only approximately 1% is in extracellular fluid. Typically, approximately 10 mmol of Mg^{2+} is consumed per day, with approximately 6 mmol being lost in the faeces and the remaining approximately 4% in the urine. In contrast to the situation with Ca^{2+}, bone does not appear to play a role in the control of Mg^{2+} levels, and there is little evidence for control of intestinal uptake, so Mg^{2+} balance depends entirely on the regulation of urinary excretion. Approximately 75% of serum Mg^{2+} is ultrafilterable, and overall tubular Mg^{2+} reabsorption usually amounts to approximately 97% of the filtered load. Intriguingly, the major site of reabsorption is the loop of Henle: only 15–20% of filtered Mg^{2+} is reabsorbed in the proximal tubule, whereas up to 70% is reabsorbed in the loop, mainly in the TALH; the remaining 10–15% is reabsorbed in the distal tubule.

There is no evidence for transcellular Mg^{2+} transport in either the proximal tubule or the TALH. In the proximal tubule S2 segment, both concentration and electrical gradients favour paracellular Mg^{2+} reabsorption, but the permeability is low. Mg^{2+} reabsorption is driven by the lumen-positive transepithelial voltage in the TALH (Fig. 20.6). Recent evidence indicates that the integrity of the cation-selective paracellular pathway is dependent on the interaction of at least two tight-junction proteins: claudin-16 and claudin-19 (Hou and Goodenough, 2010). Mutations in the gene for either protein result in the syndrome of familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (Haisch et al., 2011).

The final nephron segment in which Mg^{2+} is reabsorbed is the distal tubule, where its transport is transcellular (Ferre et al., 2011). Entry across the apical membrane may be mediated by the transient receptor potential channel melastatin (TRPM), members 6 and 7, but the concentration gradient for entry is much smaller than that for calcium. It is not yet known whether an intracellular binding protein exists for Mg^{2+}; nor has the mechanism of exit across the basolateral membrane been identified, though it is usually assumed to be a Na^+/Mg^{2+} exchanger (San-Cristobal et al., 2010).

Clearly, anything inhibiting normal reabsorptive processes in the TALH (e.g. loop diuretics) is likely to be magnesiuric, and anything stimulating reabsorption in the TALH (e.g. peptide hormones) antimagnesiuric, but the factors controlling Mg^{2+} excretion are ill understood. It appears that epidermal growth factor (EGF), released locally, has a stimulatory effect on TRPM6 activity, though how (or if) this is regulated remains to be determined. Other factors that can influence Mg^{2+} reabsorption in the distal tubule include the apical K^+ channel Kv1.1 and the basolateral K^+ channel Kir4.1 (KCNJ10), the latter often found as a heteromeric complex with Kir5.1. Mutations in the gene encoding Kv1.1 are associated with inappropriately high Mg^{2+} excretion. This channel normally hyperpolarizes the apical membrane, so its dysfunction reduces the driving force for Mg^{2+} entry (San-Cristobal et al., 2010). Mutations that disable the basolateral Kir4.1/5.1 channel, by preventing K^+ recycling, reduce the activity of the basolateral Na^+/K^+-ATPase and also result in hypomagnesaemia, though in this case the explanation is elusive.

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CHAPTER 21

Sodium transport and balance: a key role for the distal nephron

Laurent Schild

Introduction

Sodium (Na^+) is the most important contributor to the osmolality of the extracellular fluid (ECF), and hence is a major determinant of the ECF volume. The kidneys constantly maintain the ECF volume by regulating urinary Na^+ excretion. The urinary Na^+ excretion has to precisely balance the daily Na^+ intake to avoid changes in ECF volume. The intravascular volume of the ECF is critically dependent on changes in body Na^+ content and represents an important determinant of systemic blood pressure.

The arterial pressure of a normal adult is kept within a narrow range and rarely deviates by > 10–20%, even between diurnal and nocturnal periods. A number of pressure control systems are necessary to maintain such constancy in blood pressure; they include baroreceptors and neural reflex systems that respond within seconds or minutes to abrupt changes in blood pressure. This short-term regulation of blood pressure relies mainly on the heart, the blood vessels, and the adrenal medulla.

The kidney maintains blood pressure within hours or days by controlling the ECF volume. A. C. Guyton first described this renal control system for the long-term regulation of blood pressure, and postulated the existence of a unique mean arterial blood pressure called the equilibrium pressure, the pressure at which Na^+ intake and output are in balance (Guyton, 1992). If arterial pressure rises above the equilibrium pressure, then the urinary Na^+ excretion becomes greater than the net Na^+ intake, and circulating volume decreases until pressure returns to equilibrium. This pressure control never stops functioning to balance Na^+ intake and output to maintain blood pressure at equilibrium.

Of course, this leaves unexplained the reasons and the mechanisms that cause blood pressure to rise in the majority of the hypertensive patients. Elevated blood pressure may result from a high salt intake that exceeds the ability of the kidneys to eliminate Na^+. The prolonged ingestion of large quantities of salt increases blood pressure in the dog, rabbit, baboon, and chimpanzee. In humans, a relation between salt intake and blood pressure is well documented from epidemiological studies (Denton et al., 1995; Elliott et al., 1996; Meneton et al., 2005).

The effect of Na^+ on blood pressure is certainly complex and may include factors other than the Na^+ handling by the kidney. However, this chapter will focus mainly on regulated Na^+ absorption along the nephron, which influences Na^+ homeostasis and the maintenance of blood pressure.

Sodium handling by the nephron

(See Chapter 20.)

More than 99% of the filtered load of Na^+ is reabsorbed along the nephron and in the collecting tubule. The major fraction (90%) of the filtered Na^+ is reabsorbed along the proximal tubule and in the thick ascending limb (TAL). The remaining 8–10% of the filtered Na^+ is reabsorbed in the distal convoluted tubule (DCT), the connecting tubule (CNT), and the collecting duct; the Na^+ absorption in the distal nephron and the collecting duct is tightly regulated by aldosterone and vasopressin.

In the proximal tubule, the electroneutral Na^+/hydrogen (H^+) exchanger links Na^+ reabsorption to that of bicarbonate. In addition, Na^+ absorption is coupled with the uptake of solutes such as glucose, amino acids, phosphate, sulphate, and lactate by different cotransporter systems.

The thin descending limb of Henle is impermeable to Na^+ ions, but the TAL contributes to the reabsorption of 20–30% of the filtered Na^+. Two major transport pathways contribute to the Na^+ absorption in this segment, the electroneutral Na-K-2Cl cotransporter and the Na^+/H^+ exchanger (Fig. 21.1). A particularity of the TAL is the presence of a lumen positive electrical potential generated by the recycling of potassium (K^+) across the apical membrane; because of the selective cationic permeability of the tight junctions in the TAL, this lumen positive potential contributes to approximately 50% of Na^+ absorption along a favourable driving force for diffusion of Na^+ and divalent cations through the paracellular pathway.

In the DCT, Na^+ absorption is mediated by the electroneutral Na-Cl cotransporter (NCC) that is specifically expressed in the apical membrane of this nephron segment. Further downstream, the epithelial Na^+ channel (ENaC) is responsible for electrogenic Na^+ absorption. In the late portion of the distal DCT (DCT2), the expression of NCC and ENaC overlap. Further downstream in the CNT, the cortical (CCD) and medullary portions of the collecting duct (MCD), ENaC is found without NCC, and its expression follows a decreasing axial gradient from the CNT down to the MCD (Loffing and Kaissling, 2003). ENaC allows the electrogenic entry of Na^+ into the cell along a favourable electrochemical gradient (Fig. 21.1). The resulting depolarization of the apical membrane provides the driving force for K^+ secretion through the K^+ channels (ROMK) that co-localize with ENaC at the apical membrane of principal cells (Loffing and Kaissling, 2003; Loffing and Korbmacher, 2009). Thus, in the CNT and CCD, the electrogenic Na^+ absorption mediated by

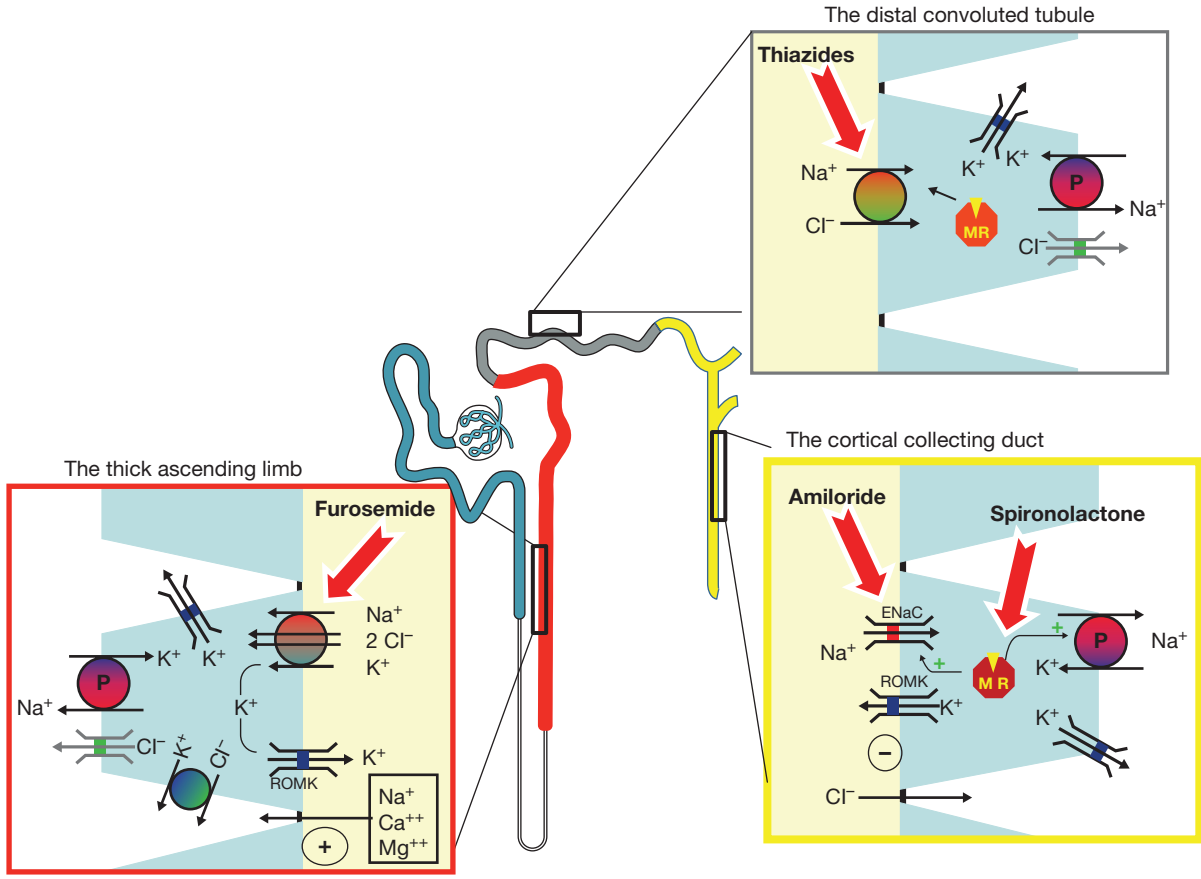

Fig. 21.1 Cellular mechanisms contributing to Na^+ absorption in the thick ascending limb (red) the distal convoluted tubule (grey), the connecting tubule, and the collecting duct (yellow). In the thick ascending limb, Na^+ absorption is essentially mediated by the Na-K-2Cl co-transporter (NKCC); this cotransport system serves Na^+ absorption and K^+ recycling across the apical membrane. In the distal convoluted tubule (DCT), the electroneutral Na-Cl cotransporter (NCC) is responsible for Na^+ absorption. In the principal cells of the connecting tubule and the collecting duct, Na^+ absorption is electrogenic via the epithelial sodium channel (ENaC). In these segments, the negative electrical potential in the lumen provides a favourable driving force for K^+ secretion. The transporters NKCC, NCC, and ENaC are the target of the diuretics furosemide, thiazides and amiloride respectively. Spironolactone is an antagonist of the mineralocorticoid receptor (MR).

ENaC is coupled with K^+ secretion, and is critically dependent on whole-body K^+ status (Frindt et al., 2011).

Although it represents only a small fraction of the filtered load, the Na^+ absorption in the distal nephron and the collecting tubule is under the tight control of the renin–angiotensin–aldosterone system (RAAS). Following a reduction of the effective circulating volume and/or a dietary salt restriction, the RAAS is activated, leading to a decrease in urinary Na^+ excretion. Aldosterone increases Na^+ absorption in the DCT, CNT, and CCD, where it increases the abundance of both transporters NCC and ENaC at the apical membrane of principal cells (Velazquez et al., 1996; Kim et al., 1998). Angiotensin II also enhances Na^+ absorption in the DCT (Wang and Giebisch, 1996). In addition, arginine vasopressin (AVP) stimulates Na^+ absorption in the DCT, CNT, and CCD (Ecelbarger et al., 2000; Pedersen et al., 2010). These nephron segments share in common the expression of the mineralocorticoid receptor (MR), the vasopressin receptor V2R, and the enzyme 11-β hydroxysteroid dehydrogenase type 2 (Bostanjoglo et al., 1998; Bachmann et al., 1999; Mutig et al., 2007).

The understanding of the detailed molecular and cellular mechanisms involved in the regulation of Na^+ excretion by the kidney has greatly progressed with the identification of the genetic basis of Mendelian disorders featuring alterations in Na^+ homeostasis and elevated blood pressure.

Human diseases

The importance of the RAAS in controlling renal Na^+ excretion, Na^+ balance, and blood pressure is largely supported by use of drugs that lower blood pressure such as diuretics, angiotensin converting enzyme inhibitors, or angiotensin II receptor antagonists. Beside this pharmacological evidence, recent genetic studies have identified renal and adrenal genes responsible for monogenic forms of hypertension.

The glucocorticoid-remediable aldosteronism (GRA) and the syndrome of apparent mineralocorticoid excess (AME) are two examples of Mendelian forms of elevated blood pressure (Lifton, 1996). GRA is a defect of the regulated synthesis of aldosterone associated with severe hypertension and elevated plasma aldosterone levels. The genetic defect is a gene duplication of the aldosterone synthase and 11β-hydroxylase, generating a novel gene that places the synthesis of aldosterone under the control of corticotrophin (ACTH), instead of its physiological secretagogue angiotensin II. The high plasma levels of aldosterone can be normalized by suppression of ACTH release with glucocorticoids.

AME is an autosomal recessive disorder due unphysiological stimulation of the MR by cortisol. This disorder is characterized by a moderate to severe hypertension with very low plasma aldosterone levels, but sensitive to MR antagonists such as spironolactone. The genetic defects of the AME syndrome are loss of function mutations in 11β-hydroxysteroid-dehydrogenase, a key enzyme present in aldosterone-sensitive kidney cells that protects the MR from stimulation by cortisol (Stewart et al., 1987). The MR has a high affinity for cortisol, which is present in the plasma at a higher concentration than aldosterone.

Other Mendelian disorders have established the critical role of the NCC or ENaC in the maintenance of salt balance. Pseudohypoaldosteronism type II (PHA-II also named familial hyperkalaemic hypertension or Gordon syndrome) is characterized by a salt-sensitive hypertension exquisitely sensitive to thiazide diuretics, associated with hyperkalaemia, and hyperchloraemic acidosis (Wilson et al., 2001). These clinical features strongly suggest that this disorder is due to an overactivity of the NCC in the DCT. Genome-wide linkage studies showed that PHA-II is genetically heterogeneous: they identified overlapping deletions in chromosome 1 located in the first intron of a gene encoding a serine-threonine protein kinase WNK1. Expression studies of WNK1 in lymphocytes of affected patients showed a fivefold increase in WNK1 expression compared with unaffected patients, suggesting a gain-of-function mutation of WNK1 (Wilson et al., 2001). The *WNK1* gene encodes two WNK1 isoforms, a ubiquitous long isoform L-WNK1, and a kidney-specific KS-WNK1, expressed predominantly in the DCT, and missing most of the kinase domain. In another PHA-II locus on chromosome 17, mutations were found in a gene encoding a WNK isoform, WNK4; these mutations target a highly conserved segment of negatively charged residues distal to the kinase domain. Although strong genetic evidence supports the notion that the mutations in the *WNK4* gene are the cause in PHA-II, there is no clear evidence that favours loss- or gain-of-function mutations of WNK4 kinase. WNK4 is localized in the aldosterone-sensitive distal nephron at the tight junctions and at the subapical membrane region of the DCT, in the cytoplasm of CNT and CCD (Wilson et al., 2001; Kahle et al., 2004). These genetic studies strongly suggest that the WNK1 and WNK4 kinases are important regulators of NCC in the distal nephron.

Liddle syndrome (or pseudoaldosteronism) is an autosomal dominant form of salt-sensitive hypertension associated with low plasma aldosterone, low plasma renin activity, hypokalaemia, and metabolic alkalosis. In the early 1960s, G. W. Liddle reported a case of pseudoaldosteronism, and described the syndrome as 'a disorder in which the renal tubules transport ions with such abnormal facility that the end result simulates that of a mineralocorticoid excess' (Liddle et al., 1963). Blood pressure in these patients could be normalized with amiloride and dietary salt restriction, but spironolactone was not effective. The genetic defects are mutations in the last exon of the *SCNN1B* and *SCNN1G* genes encoding the β and γ ENaC subunits that delete a conserved proline-rich motif in the cytosolic C-terminus (Shimkets et al., 1964). *In vitro* experiments could establish that these mutations are gain-of-function mutations, as postulated by G.W. Liddle, leading to an increase in the abundance and in the activity of ENaC at the cell surface (Schild et al., 1995; Firsov et al., 1996). The important role of NCC and ENaC in Na^+ homeostasis is further demonstrated by Mendelian disorders with mirror images of Liddle syndrome or PHA-II.

Pseudohypoaldosteronism type-1 (PHA-1) is a rare disease of mineralocorticoid resistance associating hyponatraemia, hyperkalaemia and metabolic acidosis with high levels of plasma aldosterone. Two forms of PHA-1 have been identified: an autosomal dominant form with usually mild symptoms restricted to the kidney, and associated with heterozygous mutations in the *NR3C2* gene encoding for the MR (Chang et al., 1996; Geller et al., 1998). The generalized PHA-I form, also called autosomal recessive PHA-I, is a multisystem disorder characterized by salt wasting from the kidney, the colon, the sweat gland, and a reduced capacity to reabsorb Na^+ in the airways leading to rhinorrhoea, pulmonary congestion, and recurrent pulmonary infections. Mutations have been identified in the *SCNN1A, SCNN1B*, and *SCNN1G* genes encoding for the α, β and γ ENaC subunits leading to loss of function of ENaC.

Patients with Gitelman syndrome exhibit hypokalaemic alkalosis, hypocalciuria, hypomagnesaemia, and low blood pressure. Gitelman syndrome is associated with loss-of-function mutations in the *SLC12A3* gene encoding the NCC transporter (Simon et al., 1996).

These genetic studies confirm Guyton's hypothesis that the kidney plays a central role in the maintenance of Na^+ balance and blood pressure. In addition, the identification of the genetic basis of Mendelian forms of hypertension and salt-losing nephropathies greatly helped to identify the distal nephron and the collecting tubules as the critical sites for the fine regulation of Na^+ absorption and for the maintenance of a Na^+ balance.

Cellular and molecular aspects of Na^+ transport in the ASDN

Na^+ absorption in the aldosterone-sensitive distal nephron (ASDN) and collecting duct results from the concerted activity of the NCC and the ENaC.

NCC function and regulation *in vitro*

The NCC cotransporter belongs to the solute carrier family 12 and allows the electroneutral entry of Na^+ with Cl^- from the tubule lumen into the cell. NCC is the pharmacological target for the thiazide diuretics.

The identification of mutations causing PHA-II in the WNK kinases led to the discovery of a novel regulatory pathway that controls NCC activity at the cell surface. *In vitro* co-expression experiments could demonstrate that WNK4 reduces the NCC activity; this NCC inhibition is due in part to a reduced NCC trafficking to the plasma membrane and a decrease in NCC abundance at the cell surface. WNK4 harbouring the PHA-II mutations (WNK4D561A) no longer inhibit NCC activity, leading to a hyperactivity of the transporter (Wilson et al., 2003; Yang et al., 2003). It could also be shown that WNK1 interacts with WNK4 and suppresses its inhibitory effect on NCC, resulting in an upregulation of NCC (Fig. 21.2) (Yang et al., 2005). Finally, a kidney isoform of WNK1 (KS-WNK1) lacking the kinase domain was found to be a negative regulator of WNK1, preventing inhibition of WNK4 (Delaloy et al., 2003; Subramanya et al., 2006). These *in vitro* experiments identify a kinase inhibitory pathway on NCC activity involving successively the KS-WINK1, the downstream WNK1, and the WNK4.

Except for the KS-WNK1 isoform, the regulation of the NCC but also the Na-K-2Cl (NKCC) cotransporters by WNK kinases is usually dependent on their catalytic activity. However, it is still

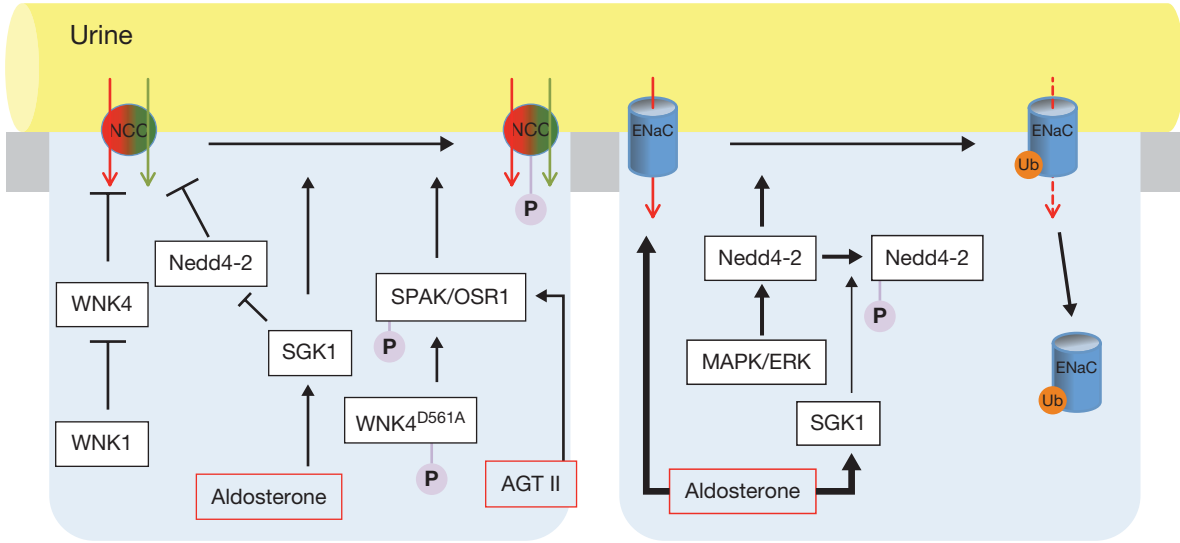

Fig. 21.2 Regulated Na^+ absorption mediated by the Na-Cl cotransporter (NCC; left) and epithelial sodium channel (ENaC; right). (Left) Phosphorylation of the NCC increases its transport activity at the cell surface. Kinases that contribute to the NCC phosphorylation include SPAK/OSR1 kinase under the control of angiotensin II, and the aldosterone-induced SGK-1 kinase. The NCC is negatively controlled by WNK4 kinase and ubiquitin-ligase Nedd4-2. (Right) ENaC activity at the apical membrane of principal cells is mainly controlled by aldosterone, which increases the number and the activity of the channel. The aldosterone-induced SGK-1 kinase plays a minor role is this regulation. The ubiquitin-ligase Nedd4-2 via a direct interaction with ENaC promotes the retrieval of active channels for the cell surface.

unknown if WNK4 directly phosphorylates NCC, and how WNK4 inhibits NCC.

Serine or threonine phosphorylation in the N-terminus of NCC increases the transporter activity without changing its abundance at the cell surface (Pacheco-Alvarez et al., 2006; Glover et al., 2009). Beside the WNKs, a number of kinases in the DCT have been identified as potential candidates for NCC phosphorylation and regulation. Among them, the STE20/SPS1-related proline alanine-rich kinase (SPAK) and the oxidative stress responsive protein type 1 (OSR1) may represent the missing link between WNK4 and NCC (Fig. 21.2). Serine or threonine residues in the N- terminus of NCC or NKCC are phosphorylation sites for OSR1 and SPAK and when phosphorylated increase the activity of NCC (Piechotta et al., 2002; Moriguchi et al., 2005; Vitari et al., 2005). *In vitro* it could be shown that WNK1 and WNK4 phosphorylate SPAK and OSR1 kinases (Moriguchi et al., 2005; Vitari et al., 2005). Other kinases that are components of the aldosterone-signalling pathway regulate Na^+ absorption in the ASDN. The serum and glucocorticoid-induced kinase1 (SGK1) is an early aldosterone-induced protein. The co-expression of SGK1 with WNK4 in heterologous expression systems decreases the inhibitory effect of WNK4 on NCC activity (Rozansky et al., 2009) (Fig. 21.2). Another potential signalling pathway for NCC regulation by aldosterone involves the ubiquitin ligase Nedd4-2 known to regulate ENaC at the cell surface. *In vitro* Nedd4-2 stimulates NCC ubiquitylation and reduces its activity at the cell surface. SGK1 prevents, in a kinase-dependent manner, the inhibition of NCC by Nedd4-2 (Arroyo et al., 2011).

NCC: regulation *in vivo* and contribution to sodium homeostasis

The genetic disruption of NCC in mice leads to mild perturbations in fluid and electrolyte homeostasis; no apparent hypokalaemic alkalosis is observed, nor hypovolaemia, or change in arterial blood pressure; however, hypocalciuria, and hypomagnesaemia, together with a sharp reduction in the number of DCT cells, could be demonstrated (Schultheis et al., 1998). To maintain Na^+ balance in the absence of a functional NCC transporter, compensatory mechanisms for Na absorption need to be evoked that likely include the upregulation of NKCC in the thick ascending limb or ENaC in the CNT.

The stimulation of the renin–angiotensin–aldosterone cascade by dietary Na^+ restriction increases Na^+ absorption in the DCT, NCC activity, and NCC phosphorylation. In mice lacking the aldosterone-induced kinase SGK1, a dietary Na^+ restriction attenuates NCC expression and phosphorylation (Vallon et al., 2009). The effects of dietary salt restriction on NCC in the DCT can potentially be mediated either by aldosterone or by angiotensin II. Angiotensin II independently of aldosterone, increases Na^+ absorption in the DCT together with NCC phosphorylation, and also increases the intracellular abundance of SPAK kinase (van der Lubbe et al., 2011). Thus, this upregulation of NCC following dietary salt restriction also involves the intermediary kinases SPAK/OSR1 (Chiga et al., 2008).

The central role of SPAK in the regulation of Na^+ balance and blood pressure is further supported by the study of a knock-in mouse model carrying a loss-of-function mutation in the kinase domain of SPAK; these mice exhibit marked hypotension on a normal salt diet, hypomagnesaemia, and hypocalciuria associated with reduced expression and phosphorylation of both NCC and NKCC cotransporters (Rafiqi et al., 2010). This phenotype contrasts with the mild phenotype observed in the NCC knockout mice.

The physiological role of WNK4 and WNK1 in mediating the aldosterone or angiotensin II effects on NCC has not yet been clearly established *in vivo*. No changes in the expression of WNK1 or WNK4 proteins were observed on dietary NaCl restriction or after aldosterone or angiotensin II infusion (van der Lubbe et al., 2011).

Nevertheless, WNK4 plays a central role in the regulation of NCC and in the pathogenesis of PHA-II. Mouse carrying two additional transgene copies of WNK4 wildtype, or the WNK4 mutant causing PHA-II (WNK4D561A/+), show opposite effects on blood pressure, and on K^+ or Ca^{2+} homeostasis (Lalioti et al., 2006). The mice expressing the WNK4 mutant recapitulate the essential features of the human PHA-II, including an elevated blood pressure sensitive to thiazides, hyperkalaemia, and hypercalciuria. Mice overexpressing WNK4 wild type exhibit a discrete phenotype similar to that observed in the NCC KO mice, with a slightly lower blood pressure compared with wild type, hypercalciuria, and hypokalaemia on a low K^+ diet. A knock-in transgenic mouse model, heterozygous for the mutation WNK4D561A/+ found in PHA-II, shows a constitutive activation of NCC as the primary cause of PHA-II (Yang et al., 2007). Indeed, during the first 3 months of life these mice developed an elevated blood pressure, hyperkalaemia with a reduced fractional excretion of K^+, and metabolic acidosis; these changes could be corrected by the NCC inhibitor hydrochlorothiazide. These changes also correlated with increased renal expression of phosphorylated NCC at the apical membrane of DCT cells, and enhanced phosphorylation of OSR1/SPAK kinases. This model also provides further evidence for the role of OSR1/SPAK kinases in the regulation of NCC.

It should be mentioned that the role of the kidney-specific KS-WNK1 kinase in the regulation of NCC remains uncertain, since the KS-WNK1−/− mouse model shows no clear phenotype similar to PHA-II (Hadchouel et al., 2010).

These *in vivo* experiments establish the critical role of WNK4 and SPAK/OSR1 interacting kinases in the pathogenesis of PHA-II. This is further supported by the use of a triple knock-in mouse approach, demonstrating that in PHA-II associated with the WNK4D561A mutation, the enhanced NCC phosphorylation is fully dependent on the integrity of the kinase domain of SPAK and OSR1 (Chiga et al., 2011).

The *in vitro* and *in vivo* experiments investigating the regulation of NCC by WNK kinases illustrate the complexity of the signalling cascades that modulate Na^+ absorption in the DCT (Fig. 21.2). From *in vitro* experiments we learn that WNK4 negatively regulates NCC independently of SPAK/OSR1 kinases and that the WNK4D561A mutant associated with PHA-II no longer inhibit NCC. *In vivo* experiments show that mice carrying the WNK4D561A mutation recapitulate the essential features of PHA-II; this phenotype correlates with an increase in NCC phosphorylation and NCC expression that requires the integrity of SPAK and OSR1 kinases. To reconcile these observations, one has to postulate a dual role for WNK4: WNK4 under the control of the WNK1 kinases inhibits NCC; alternatively, WNK4 can escape WNK1 control, as does the WNK4D591A mutant, and stimulate the SPAK/OSR1 kinases to activate NCC, as postulated in PHA-II (Fig. 21.2).

ENaC function and regulation *in vitro*

ENaC belongs to a family of cation channels called the ENaC/degenerins ion channel family (Kellenberger and Schild, 2002). This family comprises proton-gated acid sensing ion channels expressed in the mammalian central and peripheral nervous system, or touch-sensitive ion channels (degenerins) expressed in *Caenorhabditis elegans*. ENaC is a heteromultimeric channel made of three homologous α, β, and γ subunits; the α-ENaC subunit is absolutely required for channel activity, and the co-expression of three αβγ subunits is necessary for the maximal expression of ENaC-mediated current at the cell surface (Canessa et al., 1994). ENaC is highly selective to Na^+ ions, and is constitutively open when present at the apical membrane of principal cells. ENaC is the pharmacological target of the K^+-sparring diuretics such as amiloride or triamterene that act as pore channel blockers.

Each ENaC subunit is made of two α-helices that constitute the transmembrane part of the channel pore with the selectivity filter and the amiloride binding site; the transmembrane helices are separated by a large extracellular loop that makes up more than half of the mass of the protein. The amino- and carboxy-termini of the protein are intracellular (Canessa et al., 1994).

ENaC is constitutively open and fluctuates between an open and a closed state. A number of factors influence ENaC gating. An acute increase in intracellular Na^+ reduces the channel openings and channel current, likely to prevent a massive entry of Na^+ ions into the cell when luminal Na^+ concentration is increasing. Other intracellular factors associated with cellular stress such as a decrease in pH, increase in oxidative stress or a rise in Ca^{2+} ions decrease channel open probability (Palmer and Frindt, 1987; Chraibi and Horisberger, 2002; Kellenberger et al., 2005; Anantharam et al., 2006).

ENaC is activated by soluble proteases such trypsin, chymotrypsin, kallikrein, or elastase. When co-expressed with GPI-anchored proteases such as CAP-1, an orthologue of the human prostasin, or CAP-2, an orthologue of the human transmembrane protease serine 4 (TRPMSS4), ENaC activity was significantly increased (Vallet et al., 1997; Chraibi et al., 1998; Vuagniaux et al., 2000; Harris et al., 2007). In the kidney and in heterologous expression systems the α- and the γ-ENaC subunit are found in two molecular forms, a high- and a low-molecular-weight form, resulting from the cleavage of the subunits by proteases, possibly furin (Masilamani et al., 1999; Hughey et al., 2003; Ergonul et al., 2006; Harris et al., 2007; Frindt and Palmer, 2009). The molecular mechanisms by which proteases activate ENaC are still not completely understood.

Another important way to regulate ENaC-mediated Na^+ absorption in the ASDN is to control the number and the stability of ENaC channels at the cell surface. ENaC ubiquitylation is an important mechanism that determines the stability of the channel at the cell surface. Ubiquitylation is a general process that labels proteins with ubiquitin in the cell or at the cell surface and targets them for endocytosis and degradation. Nedd4-2 is a protein ubiquitin ligase that belongs to the HECT family (Rotin and Schild, 2008). It mediates their mono-ubiquitylation of β- and γ-ENaC subunits on binding specifically to conserved PY motifs present in their cytosolic C- terminus (Staub et al., 1997). The ubiquitylated ENaC at the cell surface undergoes clathrin-mediated endocytosis and degradation. *In vitro* Nedd4-2 efficiently suppresses ENaC activity. Mutations in the cytosolic PY motifs of ENaC subunits that are found in Liddle syndrome alter the interaction between Nedd4-2 and ENaC, leading to retention of active channels at the cell surface (Firsov et al., 1996; Schild et al., 1996). By contrast, deubiquitylation enzymes such as Usp2-45 increase ENaC activity (Ruffieux-Daidie et al., 2008).

In vitro experiments support the idea that aldosterone stabilizes ENaC at the cell surface by inhibition of ENaC ubiquitylation and endocytosis. Among the aldosterone-induced proteins identified *in vivo*, it was found that the phosphatidylinositide 3′-kinase (PI3K)-dependent kinase SGK-1 (serum- and glucocorticoid-regulated kinase 1), increases the abundance of

the active ENaC at the cell surface (Verrey et al., 2008). SGK1 phosphorylates Nedd4-2, promoting the interaction between Nedd4-2 and the 14-3-3 scaffolding proteins, preventing the Nedd4-2-dependent ubiquitylation of ENaC. As for SGK1, the 14-3-3 proteins are induced by aldosterone (Debonneville et al. 2001).

In addition to the SGK1/Nedd4-2 pathway that stabilizes ENaC at the cell surface, a number of kinases have been reported to modulate ENaC activity *in vitro*. The Raf-1-MAPK/ERK kinases act to inhibit the cell surface expression of ENaC by stimulating the interaction between Nedd4-2 and ENaC (Nicod et al., 2002; SHI et al., 2002; Falin and Cotton, 2007).

Finally, WNK4 partially inhibits ENaC activity *in vitro*, but not the WNK4 mutant causing PHA-II; this ENaC inhibition was independent of the active kinase domain (Ring et al., 2007a, 2007b). WNK1 increases ENaC activity via the activation of SGK that phosphorylates and inactivates Nedd4- 2 (Xu et al., 2005).

These studies performed *in vitro* in heterologous expression systems or in cortical collecting duct cell lines identify Nedd4-2 as a critical convergence point for the regulation of ENaC at the cell surface.

ENaC regulation *in vivo*

The physiologically most important regulator of ENaC is aldosterone. In the kidney, aldosterone increases the biosynthesis of α-ENaC, whereas the β- and the γ-ENaC subunits are constitutively expressed. The upregulation of the synthesis of α-ENaC by aldosterone leads to an increase in the expression of active ENaC channels at the cell surface. It is not yet clear whether the increase in the biosynthesis of the α-ENaC is sufficient for trafficking of the multimeric αβγ-ENaC channels at the cell surface or whether additional regulators of ENaC trafficking to the apical membrane are necessary.

A variety of genetic mouse models have been generated to study the physiological and pathophysiological roles of ENaC in the maintenance of whole-body salt balance, and in the control of blood pressure. Several ENaC KO mouse models recapitulate PHA-I. The constitutive inactivation of either α or β or γ leads to a severe renal phenotype, including increased Na^+ excretion, hyperkalaemia, and elevated plasma aldosterone levels (Hummler et al., 1997). Interestingly, the selective invalidation of ENaC in the different segments of the ASDN revealed the predominant role of ENaC in the CNT for the maintenance of the salt balance and K^+ homeostasis. Specific ENaC knockout in only the collecting duct did not result in any alteration in the Na^+ and K^+ homeostasis (Rubera et al., 2003). By contrast ENaC invalidation at the distal end of the DCT and in the CNT replicated the PHA-I phenotype (Christensen et al., 2010).

Mice deficient in the MR have normal prenatal development, but die soon after birth from dehydration and hyperkalaemia. This severe PHA-I phenotype of MR knockout mice further confirms the critical roles of NCC and ENaC in maintaining Na^+ balance (Berger et al., 1998). The severity of this phenotype contrasts with the milder renal phenotype of mice lacking the aldosterone-induced protein SGK1 (Fejes-Toth et al., 2008). The SGK deficient mice show higher natriuresis only on a low Na^+ diet, which is not related to decreased ENaC activity (Fejes-Toth et al., 2008). Furthermore, under chronic aldosterone treatment, ENaC activity is identical in the SGK deficient and the wild-type littermates. This suggests that *in vivo* SGK is not essential for the long-term regulation of ENaC by aldosterone. Interestingly the SGK knockout mouse model also reveals that other Na^+ transporters such as NCC located upstream of ENaC are also likely targets of SGK-1 kinase.

To gain more insight into the role of ENaC in regulating ECF volume and blood pressure, salt-sensitive mouse models of Liddle syndrome or pseudohypoaldosteronism were generated. Mice carrying Liddle's mutation in the *SCNN1B* gene with deletion of the C-terminus of β-ENaC have been generated; when maintained on a high-salt diet these mice recapitulate Liddle syndrome with an elevated blood pressure, low plasma levels of aldosterone and renin, hypokalaemia, and metabolic alkalosis (Pradervand et al., 1999). The deletion of the proline-rich motif in the C-terminus of β-ENaC, and impaired interaction with Nedd4-2, does not compromise the ability of the channel to respond to aldosterone (Dahlmann et al., 2003). Electrophysiological measurements of ENaC activity in microdissected tubules from Liddle's mice revealed that under normal dietary salt ENaC activity was not detectable, but under low dietary salt and with high plasma aldosterone, the affected mice show a dramatic increase in ENaC activity at the cell surface, which even more than in wild-type litter-mates. Thus, neither SGK1 nor Nedd4-2 appears to be critical limiting factors for the stimulation of ENaC-mediated Na^+ reabsorption by aldosterone.

Nedd4-2 is an important downregulator of ENaC, but also of NCC. The knock-out of the Nedd4-2 gene in mice results in an elevated blood pressure associated with low plasma aldosterone levels; effects that correlate with an increase in the expression of both ENaC and NCC (Shi et al., 2008).

Thus, *in vitro* and *in vivo* experiments on ENaC regulation have not yet unambiguously identified the molecular and cellular mechanisms involved in the aldosterone-signalling pathway.

Conclusion

In summary, the fine-tuning of Na^+ reabsorption in the ASDN and collecting duct is critical for the maintenance of Na^+ balance, ECF volume, and blood pressure. The identification of genes responsible for Mendelian forms of hypertension and the generation of transgenic mouse models, together with *in vitro* approaches, have provided us with an unprecedented understanding of the molecular and cellular mechanisms involved in hormonally regulated Na^+ absorption in the kidney.

Future research is needed to address the functional interactions between these newly identified regulatory pathways that control Na^+ absorption and the transport of other ions such as K^+ or calcium in these nephron segments. Another question of pathophysiological relevance raised by these recent studies on genetically modified mice models is how the kidney develops compensatory mechanisms to maintain Na^+ homeostasis when one regulatory pathway for Na^+ is deficient or defective.

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CHAPTER 22

Water homeostasis

David Marples and Søren Nielsen

Introduction

The kidney filters some 180 L/day of fluid from blood plasma: it follows that the vast majority of this filtrate needs to be reabsorbed. Most of this reabsorption occurs passively and constitutively, as water is drawn across the nephron wall by the osmotic gradient produced by active solute transport. In the proximal tubule and descending thin limb of Henle's loop, the combination of leaky tight junctions and the presence of specific water channels (aquaporin 1; AQP1) means that this reabsorption occurs rapidly, and in the presence of a very small osmotic gradient. In contrast, the low water permeability of the ascending limb and distal convoluted tubule allows salt transport to establish a substantial osmotic gradient, with the tubular fluid being substantially diluted as solute is removed. This is important when water needs to be lost without excessive salt depletion. The accumulation of salt in the medullary interstitium, and the dilute tubular fluid, together provide a driving force for water reabsorption from the collecting ducts, allowing the production of very concentrated urine. Whether the urine is dilute or concentrated is determined primarily by plasma levels of the antidiuretic hormone vasopressin, which alters the permeability of the collecting duct by causing the insertion of AQP2 water channels into the apical membrane of the tubular cells.

Arginine vasopressin (AVP) is a peptide hormone released from the posterior pituitary. The main trigger for its release is a rise in plasma osmolality. However, large changes in circulating volume and/or blood pressure can also cause AVP release, as can nausea and a number of other stimuli, including some drugs. Of course, antidiuresis cannot reverse a rise in plasma osmolality: it can only stop it getting worse. Ultimately, there needs to be the intake of additional water, and the major stimuli for thirst are similar to those for AVP release. However, the threshold for thirst is higher than that for AVP release.

Disorders of water balance can arise due to failures of normal organ function, regulatory mechanisms, or because of alterations in salt or water intake. The primary defect may be in the renal system, or may be a secondary consequence of problems elsewhere: for example, low effective circulating volume due to heart disease.

Body fluid composition

About half to two-thirds of body weight is made up of water, depending on body composition: muscle bulk increases the fraction, while fat decreases it. Of this water, two-thirds is intracellular, as a potassium-rich solution, while the remaining third is extracellular, and is predominantly a saline solution, with a range of other solutes depending on the compartment. Plasma makes up about a quarter of the extracellular fluid: typically about 5% of body weight. Typically water moves freely between compartments. Small solutes move freely between plasma and interstitial fluid, but movement across cell membranes is tightly controlled, as is movement into and out of some specialized compartments such as the cerebrospinal fluid.

Under normal circumstances plasma proteins cannot move out of the vasculature, and therefore exert an osmotic pressure, called the oncotic pressure, tending to draw water out of the interstitium back into the plasma, and opposing the effects of the hydrostatic pressure. The balance of these Starling forces result in turnover of fluid in normal capillary beds. These forces are exaggerated in the renal peritubular capillaries, where the hydrostatic pressure is lower than in most capillaries because of the resistance of the efferent arterioles, while the oncotic pressure is higher than usual, because of the essentially protein-free glomerular filtrate removed from the plasma. This facilitates the movement of the fluid reabsorbed from the filtrate back into the bloodstream.

Fluid balance

Under normal circumstances, water intake will be about 2.5 L/day, from a combination of drinking, water present in food, and water created by metabolism of food. Balancing this, there will be insensible losses from the lungs and skin, loss in the faeces, and loss in the urine, with the latter typically amounting to around 1.5 L/day, which equates to about 1 mL/min. A more detailed breakdown is shown in Table 22.1, but it is important to recognize that all these figures are very dependent on circumstance: a hot, dry environment will greatly increase losses through ventilation and perspiration, for example. Conversely, social circumstances, or compulsive polydipsia, can result in greatly increased fluid intake. The kidneys, under hormonal control, can reduce urinary loss to as little as about 400 mL/day, or increase it to 20 L/day, to maintain a balance.

Renal handling of water

The regulated excretion of water is handled by the kidney, but it has to coordinate this role with its excretion of waste substances and the regulation of salt balance. The first step of the process is glomerular filtration (see Chapter 43). About 20% of the plasma entering the kidney is filtered into the nephrons, with the exception that nearly all plasma proteins are retained within the blood vessels. All solutes with a molecular weight lower than about 7 kDa are freely filtered, while larger substances are progressively less freely filtered, until, at a molecular weight around 70 kDa (depending on the charge and shape of the molecule) there is virtually no filtration

Table 22.1 Normal water handling in adults in a temperate climate

Route	Volume (mL/day)	Modifying factors
Intake		
Oral fluids	1200	Social, cultural, environmental
Water in food	1000	Diet (fasting)
Metabolic water	300	Diet (fasting)
total	2500	
Output		
Urine	1500	Requirements for solute excretion, fluid intake (ADH)
Insensible (skin and lungs)	800	Exercise, environment
Sweat	100	Temperature, exercise
Faeces	100	Diarrhoea
Total	2500	

(Maddox and Brenner, 2000). This results in the entry of about 180 L/day of filtrate into the tubules, and a rise in the oncotic pressure of the blood leaving the glomeruli. This blood then flows through a portal system to the peritubular capillaries, where the consequent low hydrostatic pressure and high oncotic pressure facilitate fluid uptake from the peritubular space.

To reduce 180 L of filtrate to 1.5 L of urine, it is necessary for the tubules to reabsorb > 99% of the filtrate: even at maximal diuresis around 90% is being absorbed. This water is being drawn passively out of the tubule, down the osmotic gradient created by the active solute transport described in Chapter 20. For such reabsorption to be efficient, it is important that the water permeability of the epithelium is high, and this is achieved by the expression of specific water channels, called aquaporins (AQPs), in both the apical and basolateral membranes of the cells in water-permeant segments of the tubule.

AQP1 in the proximal tubule and descending limb

In the proximal tubule, where there is rapid reabsorption of both solutes and water, there is abundant expression of AQP1 in both the apical brush border and the basolateral membranes, with very little seen intracellularly (Nielsen et al., 1993), suggesting that following synthesis it is rapidly inserted into the membrane, with little or no regulation by shuttling from stores. This is consistent with the largely constitutive activity of the proximal tubule. Evidence from knockout studies in mice suggests that AQP1 is responsible for about 80% of the water permeability of the proximal tubule (Schnermann et al., 1998), and allows reabsorption of about two-thirds of the solute and water filtered to occur in this segment. By allowing water to move out of the tubules in response to a very modest osmotic gradient, it maximizes the efficiency of solute transport too, by limiting the back-flux that would otherwise occur through the leaky tight junctions between cells in this nephron segment. In knockout mice the osmotic gradient increased 3-4 fold, despite which water reabsorption fell by about half. These mice also had a substantially reduced GFR, presumably to limit the excess urinary water loss.

Furthermore, these mice had a substantially impaired urinary concentrating capacity: they could not produce urine concentrated to > 650 mOsm/kg, despite water deprivation that raised their plasma tonicity significantly (Ma et al., 1998). This is thought to reflect impairment of the countercurrent multiplication mechanism: AQP1 is also expressed in the descending thin limbs of long–loop nephrons (but probably not most short-loop nephrons), and this is thought to be important in allowing the concentration of solutes in the descending limbs. Water permeability in the descending limbs of AQP knockout mice was reduced by about 90% (Chou et al., 1999). (See Chapter 20 for details on the generation of the corticomedullary osmotic gradient.)

Despite these apparently important functional defects seen in AQP1 knockout mice, humans (and indeed mice) lacking AQP1 appear remarkably unaffected under normal circumstances (King et al., 2001). AQP1 is expressed on erythrocytes, and acts as a blood grouping antigen (Colton). Thus, Colton-null people lack AQP1 expression. This is exceptionally rare, but such people have no overt disease, although lab investigation does reveal a decrease in urinary concentrating capacity. Unlike in mice, their proximal tubular water reabsorption appears normal, and the defect is probably due to failure to generate a normal medullary osmotic gradient. This study also found the AQP1-null humans had a normal GFR, suggesting that, although AQP1 is expressed in human (but not rat) glomeruli (Maunsbach et al., 1997), this is of limited importance in the filtration process.

While we have described the reabsorption of solute and water in the proximal tubule as constitutive, it is certainly the case that there is hormonal modulation of this process (Reeves and Andreoli, 2007). As part of this modulation, there are changes in AQP1 expression in response to physiological changes (angiotensin levels, interstitial tonicity), and also in pathological conditions such as during ureteric obstruction (Li et al., 2003). These may contribute to the altered renal water handling seen in these conditions.

AQP7 in the late proximal tubule

A second aquaporin, AQP7, is expressed in the proximal straight tubule (Ishibashi et al., 2000; Nejsum et al., 2000). AQP7 is permeable to a number of other solutes, including glycerol and urea, as well as to water. In mice, knocking out this gene leads to glyceroluria, but not to a concentrating defect, although mice lacking both AQP1 and AQP7 have a more severe concentrating defect than those lacking AQP1 alone. These results suggest that AQP7 plays only a modest role in water reabsorption in the late proximal tubule, but is important in the reabsorption of glycerol (Hara-Chikuma et al., 2005). This may be significant for intrarenal glucose production.

Ascending limb of Henle's loop and distal convoluted tubule

These segments of the nephron have low water permeability, and little water reabsorption from the tubular fluid occurs. Consistent with this, there are no aquaporins expressed in these nephron regions. Indeed, the very active salt uptake in these regions tends to result in the production of a dilute tubular fluid (Moe et al., 2000). This is important for water balance in two ways: first, the salt extracted contributes substantially to the accumulation of salt in the renal medulla, thus providing an osmotic gradient for (regulated) water reabsorption from the collecting ducts, as discussed

below, and second, because it makes it possible to excrete a water load with relatively little salt loss. Drugs that block salt uptake, such as loop diuretics and thiazides, cause an increase in urine output, but actually blunt the ability to excrete free water (because salt is lost at the same time).

The connecting tubule and collecting duct—the site of regulated water permeability

Although only a small fraction (about 15%) of the filtered water reaches the later parts of the renal tubule, this still represents some 20–25 L of water per day. As noted in the previous paragraph, this fluid is markedly hypotonic, with a typical osmolality of around 100 mOsm/kg. H_2O. The water permeability of the connecting tubule and collecting duct are regulated by the antidiuretic hormone AVP, which causes shuttling of AQP2 water channels from an intracellular store to the apical plasma membrane of collecting duct granular cells. In the absence of these water channels this apical plasma membrane is highly impermeable to water (Nielsen et al., 1995), and large volumes of dilute urine can be excreted, allowing the clearance of a water load.

When vasopressin levels rise, the insertion of AQP2 water channels into the apical plasma membrane allows the entry of water from the tubular fluid (Nielsen et al., 1995). Because this fluid has been diluted by salt extraction in the ascending limb of Henle's loop and distal convoluted tubule, much of the water can be reabsorbed in the cortical collecting ducts (Ward et al., 1999), particularly if there is concomitant salt reabsorption occurring. In the cortex there is an abundant vasculature that can pick up this water, and the osmotic gradient is maintained by this high blood flow, which effectively clamps the interstitial tonicity to that of plasma. Thus only a modest amount of water flows down into the medullary collecting ducts, where further water can be extracted by the interstitial tonicity built up by the loop of Henle, allowing the production of small volumes of highly concentrated urine, without flooding the inner medulla with large amounts of water, which would flush out the salt and other solutes accumulated by the countercurrent multiplication process, etc. Depending on the levels of ADH, and the amount of solute that needs to be excreted, urine output can vary from about 0.5 to 20 L (Barlow and de Wardener, 1959).

In contrast to the regulated permeability of the apical plasma membrane, the basolateral membrane of the cells is consistently highly permeable to water, reflecting the constitutive expression of AQP3 and AQP4. Both provide exit pathways for water entering the cells through AQP2, but AQP3 is expressed predominantly in the cortical and outer medullary collecting ducts (Ecelbarger et al., 1995), while AQP4 is more abundant in the inner medulla (Terris et al., 1995). Mice with these channels knocked out give important confirmation of the relative contributions of the cortical and inner medullary collecting ducts to the control of urine volume: defects in AQP3 lead to a very marked polyuria, since cortical water reabsorption is severely impaired (Ma et al., 2000), while a lack of AQP4 only causes marginal polyuria, with a modest impairment of maximal concentrating capacity (Ma et al., 1997). However, interpretation of the results is complicated by the observation that knocking out AQP3 also caused a substantial decrease in AQP2 expression (Ma et al., 2000), perhaps because of a drop in intracellular concentration and/or cell swelling.

Cellular mechanisms of AQP2 regulation

As noted above, the major regulator of collecting duct water permeability is the posterior pituitary hormone vasopressin (arginine vasopressin in man, although the porcine equivalent, lysine vasopressin, is sometimes used clinically). AVP is carried in the bloodstream to the basolateral surface of the collecting duct principal cells, where it interacts with V_2 receptors, which are GPCRs linked to Gs (Lolait et al., 1992; Seibold et al., 1992). They consequently cause the production of the second messenger cAMP, and the activation of protein kinase A (PKA). Amongst the proteins phosphorylated by PKA is AQP2 itself, which undergoes phosphorylation of serine 256 in its C-terminus. This acts as a trigger for shuttling of AQP2 stored in vesicles to the cell surface (Nielsen et al., 1993b; Marples et al., 1995), although the details of how this is initiated remain to be established. Mutant forms of AQP2 where this phosphorylation site is abolished (e.g. S256A) are unable to traffic to the surface (Fushimi et al., 1997), while mutations which are effectively constitutively phosphorylated at serine 256 (S256D) traffic spontaneously to the cell surface in cell models of the collecting duct (Kamsteeg et al., 2000), suggesting that phosphorylation of AQP2 alone is sufficient to provoke the delivery process, but it remains possible that other targets of PKA also play an important part.

There is evidence for the involvement of both microtubule-based transport of AQP2-bearing vesicles towards the apical plasma membrane (Phillips and Taylor, 1989; Marples et al., 1998), and the reorganization of the actin cytoskeleton (Kachadorian et al., 1979; Ding et al., 1991), which may act as a barrier to vesicular movement, and may also act as a substrate for myosin-based transport of the vesicles (Chou et al., 2004). Targeting recognition proteins similar to those involved in the docking of synaptic vesicles also appear to be important in the delivery of the vesicles specifically to the apical plasma membrane (Nielsen et al., 1995b).

The retrieval of AQP2 from the cell surface also appears to be regulated: activation of protein kinase C increases the endocytosis, although this is not dependent on the phosphorylation of AQP2 itself; nor does it require the dephosphorylation of S256 (van Balkom et al., 2002). Conversely, cAMP decreases the rate of retrieval, slowing the background endocytic rate, and thus enhancing the effects of the increased insertion described above. The mechanisms behind this regulation of retrieval remain to be determined, but it is interesting that it may be prevented by phosphorylation of AQP2 at serine 269 (Moeller et al., 2010). The fate of the endocytosed AQP2 also remains unclear: while some cell models demonstrate that the AQP2 can be repeatedly recycled back into the exocytic pathway (Katsura et al., 1996; Gustafson et al., 2000), there is also evidence that it can be transported to multivesicular bodies, thought to be part of a degradative pathway (Nielsen et al., 1993b; van Balkom et al., 2009), and also that it is subsequently shed into the urine. This shedding of AQP2 does not appear to reflect loss of apical plasma membrane, or of whole cell fragments, but appears to be a specific process relating to vasopressin activity and AQP2 turnover (Wen et al., 1999). This loss of AQP2 may be an important component of the longer-term regulation that allows the body to adapt to chronic alterations in water balance.

While vasopressin is clearly the major trigger for AQP2 shuttling, it has been shown, at least in research models, that other stimuli can induce shuttling. For example, a rise in cGMP can lead to AQP2 phosphorylation via PKG, and cause its exocytosis (Bouley

et al., 2005). Similarly, inhibition of phosphatases has been shown to induce shuttling (Valenti et al., 2000). Drugs (such as sildenafil) which can activate these pathways may be useful in the management of some forms of nephrogenic diabetes insipidus (Sanches et al., 2012) (see Chapter 32).

The water permeability of the collecting ducts at any given time reflects the balance between AQP2 insertion and retrieval, together with the total amount of AQP2 available in the cell. Thus it is best thought of as a dynamic equilibrium, with increasing levels of AVP shifting the equilibrium towards greater plasma membrane AQP2, while falling levels (or other signals that increase endocytosis) shift the equilibrium towards intracellular AQP2, and thus decrease the water permeability.

The connecting tubule represents a transitional zone between distal convoluted tubule and the collecting duct proper. It expresses both V2 receptors and AQP2 (Kishore et al., 1996), and is able to shuttle AQP2 to the apical surface of the cells in response to AVP, like the collecting duct. However, unlike the cortical collecting ducts nearby, the connecting tubule can also insert a substantial amount of AQP2 into its basolateral membrane (Jeon et al., 2003), although this is not affected by AVP levels; the functional significance of this remains to be determined. It is clear that water reabsorption via AQP2 in the connecting tubule is functionally important (at least in mice), since mice lacking AQP2 altogether died of dehydration within a few days after birth (Yang et al., 2001), while those which expressed AQP2 in the connecting tubules but not the collecting ducts survived, albeit with severe polyuria (Rojek et al., 2006).

Long-term adaptation of the collecting duct

The shuttling mechanisms described above respond rapidly (within a few minutes) to changes in circulating AVP levels, thus providing a responsive system that can maintain good water balance despite substantial acute changes. It has become clear that there are also longer-term, adaptive changes, which allow the body to modulate the response to vasopressin. For example, chronic water restriction leads to an increase in AQP2 synthesis (Nielsen et al., 1993b), thus increasing the total store of AQP2 present in the principal cells. If a certain level of AVP results in the delivery of a certain fraction of total AQP2 to the plasma membrane, it is clear that increasing total AQP2 will result in higher plasma membrane permeability at any given AVP level. Thus a high degree of urinary concentration can occur at lower AVP levels. This has two advantages: the body needs less AVP to maintain a given degree of antidiuresis, and it retains a flexible response: further dehydration has the capacity to elicit a further response. Conversely, chronic water loading leads to a downregulation of AQP2 (Ecelbarger et al., 1997), reducing the collecting duct permeability at any given AVP level. Indeed, this decreased expression provides an opportunity to 'escape' partially from the water retention caused by SIADH. These changes provide a mechanism for the changes in human concentrating capacity brought about by alterations in body fluid balance (de Wardener and Herxheimer, 1957; Jones and de Wardener, 1956).

One signal driving the expression of AQP2 is vasopressin itself: Brattleboro rats, which lack significant levels of AVP, have substantially reduced AQP2 levels, which normalize following AVP infusion for 5 days (DiGiovanni et al., 1994). Similarly, infusion of AVP in normal rats increases AQP2 expression (Terris et al., 1996). Conversely, treatment with V2 receptor antagonists leads to a fall in AQP2 levels (Marples et al., 1998). Like the acute shuttling response to AVP, this also appears to be mediated by cAMP, and the 5′ untranslated region of the *AQP2* gene includes a cAMP-response element (Yasui et al., 1997). Thus dehydration will cause an increase in AQP2 expression at least partly by causing a rise in AVP levels, while water loading will suppress AVP release. However, it is clear that there are other signals that are at least as potent. The most direct evidence comes from 'vasopressin escape' experiments: Rats which are given a continuous infusion of AVP are nonetheless able to downregulate AQP2 expression when they are water-loaded for several days: this does not represent a loss of renal responsiveness to AVP, because their remaining AQP2 is still targeted to the apical plasma membrane (Ecelbarger et al., 1997). Further evidence comes from experiments in which rats deprived of water showed much greater increases in AQP2 expression than could be achieved by AVP infusion, suggesting that there are additional, possibly synergistic, signals (Terris et al., 1996). *In vitro* studies have suggested that the osmolality of the environment may be a significant factor, and a hypertonicity response element has been shown to be associated with the *AQP2* gene (Kasono et al., 2005; Hasler et al., 2005), but *in vivo* studies have not really supported this hypothesis (Marples et al., 1996, 1998). Another possible signal could be tubular flow rates, but again *in vivo* models are not really consistent with this (Marples et al., 1998). It is likely that a number of paracrine and/or systemic hormones may play a role, but remain to be identified.

Disordered regulation of aquaporins

A number of diseases are associated with impaired water balance, and these may cause or result from changes in aquaporin expression or function. We have seen that a genetic defect in *AQP1* leads to a subclinical defect in concentrating capacity (King et al., 2001): in contrast, mutations in *AQP2* (or the V2 receptor) lead to severe congenital nephrogenic diabetes insipidus (NDI) (Deen et al., 1994). No AQP3-null humans have yet been identified, but mouse models suggest that they too would suffer from severe NDI (Ma et al., 2000). These mutations are rare, but acquired forms of NDI are much more common: electrolyte disturbances such as hypokalaemia (Marples et al., 1996) and hypercalcaemia (Earm et al., 1998), drugs such as lithium (Marples et al., 1995), and pathological conditions such as urinary tract obstruction (Frøkiaer et al., 1996) have been shown to be associated with decreases in AQP2 expression, which explain at least part of the polyuria seen in these circumstances (see Chapter 32).

In contrast, overexpression of AQP2 can lead to water retaining states: for example, during pregnancy (Ohara et al., 1998), SIADH, and cirrhosis (Fujita et al., 1995), and congestive heart failure (Nielsen et al., 1997). In many cases, these seem to be due to excess AVP release due to non-osmotic stimuli (Schrier et al., 1998) (see Chapter 28).

Systemic control of water balance

Control of antidiuresis

As we have seen, in the absence of a central antidiuretic signal the kidney will produce and excrete a large volume of dilute urine. The main stimulus for antidiuresis is the hormone vasopressin, which is synthesized by the magnocellular neurons of the hypothalamus. Their cell bodies lie in the supraoptic and paraventricular nuclei of the hypothalamus, while their axons project to the posterior

pituitary (neurohypophysis). Vasopressin is a cyclic nonapeptide, but is synthesized as a preprohormone, which is then cleaved to yield AVP itself, together with neurophysin II and copeptin, within the secretory vesicles (Brownstein, 1983). Genetic defects in either the AVP or neurophysin components lead to autosomal dominantly inherited forms of central diabetes insipidus (Babey et al., 2011), suggesting that neurophysin plays a significant role in the packaging and/or release of AVP.

Osmotic stimuli

The main trigger for the release of AVP is plasma osmolality. The magnocellular neurons themselves are osmosensitive, but the main osmosensory cells appear to lie in the organum vasculosum laminae terminalis (OVLT), a region of the hypothalamus which lies functionally outside the blood–brain barrier (Bourque, 2008). Lesions in this region lead to both central diabetes insipidus and an impairment of thirst, suggesting that it is important for osmosensing in both systems, although different cells (with different osmotic thresholds) appear to be involved. Infusion of this region with hypertonic solutions leads to an increased neuronal firing rate, which in turn leads to increased AVP release. Conversely, hypotonic solutions are able to suppress the basal activity. The outcome is a system which produces essentially no AVP release below a plasma osmolality of about 280 mOsm/Kg H_2O, but a rapid rise thereafter, leading to a maximally antidiuretic level of AVP when plasma osmolality reaches about 295: thus the entire dynamic range of the system is triggered by a change of about 5%. Within this range, the relationship between osmolality and plasma AVP levels is roughly linear (Robertson et al., 1982).

There is some evidence that the action of central osmoreceptors can be modulated by input from peripheral sensors: gut osmosensors, as well as temperature sensors in the mouth and pharynx. These can result in the cessation of drinking, and a fall in AVP secretion, before there has been any detectable drop in plasma osmolality. Presumably this information is carried via vagal afferents, and signals passed to the hypothalamus via medullary nuclei (Bourque, 2008).

Non-osmotic stimuli

A second stimulus for antidiuresis is a low blood pressure or effective circulating volume. In contrast to the effects of osmolality, this response shows an exponential pattern: decreases of up to 5% in circulating volume or blood pressure have very little effect on AVP release, while once the levels have fallen by 10% the effect rapidly accelerates (Berl and Robertson, 2000). In addition to causing release of ADH in their own right, these stimuli appear to enhance the responsiveness to an osmotic challenge (in other words, they increase the rise in AVP seen for a given rise in osmolality). The signals for these stimuli come from atrial and vascular baroreceptors, signalling via medullary nuclei.

Table 22.2 Factors affecting AVP release

Factors increasing AVP release	Factors inhibiting AVP release
Physiological/pathophysiological	**Physiological/pathophysiological**
Hypertonicity/hyperosmolality	Hypotonicity/hypo-osmolality
Hypotension	Hypertension
Volume depletion (total or effective)	Volume expansion
Nausea	
Pain/stress	
Hypoxia and hypercapnia	
Hypothyroidism	
Hypoglycaemia	
Hormones: angiotensin II, bradykinin, histamine	
Circadian rhythm (AVP levels higher at night)	
Drugs	**Drugs**
μ-opioids and narcotics	κ-opioids
Alpha-adrenoceptor agonists (via BP?)	Beta-adrenoceptor agonists
Nicotine (in non-smokers)	Dopamine antagonists (via suppressed nausea?)
Barbiturates	Ethanol
General anaesthetics	Glucocorticoids
Antipsychotics and antidepressants	

Table 22.3 Causes of the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) (note that the drugs mentioned in Table 22.2 that stimulate AVP release will mimic this condition)

Malignancy	Central nervous system disorders	Pulmonary disease	Others
Pulmonary/mediastinal	Acute trauma/haemorrhage/stroke	Infections: bacterial, viral or fungal	Chronic inflammation
Gastrointestinal, including pancreas	Mass lesions (tumour, haematoma, abscess, hydrocephalus)	Cystic fibrosis	HIV
Genitourinary, including uterus, prostate and bladder	Inflammatory and demyelinating disease	Acute respiratory failure	Prolonged severe exercise
Leukaemia and lymphoma	Acute psychosis	Chronic obstructive pulmonary disease	Idiopathic
	Delirium tremens	Positive pressure ventilation	
	Pituitary stalk section		

A range of other stimuli and drugs (see Table 22.2) have also been shown to affect AVP release. The most powerful of these is nausea, which can cause very large rises in AVP secretion. Similarly, drugs which cause nausea can be potent stimuli. Other stimuli such as pain and stress may act by causing nausea, or there may be independent pathways. A rare, but potentially clinically important, cause of high AVP secretion is SIADH, which may be due to ectopic production, or to disease causing excessive release from the hypothalamic cells (Table 22.3).

Destruction of AVP

AVP has a half-life in the circulation of 10–35 minutes (Sharman and Low, 2008), so the system can respond fairly rapidly to changes in fluid balance. AVP is degraded mainly in the liver and kidneys, where peptidases break the cyclic structure and then progressively cleave the peptide. Some is also lost directly in the urine, and for the artificial analogue desmopressin this is the main route of loss: this explains its much longer duration of action.

Thirst

Ultimately, renal water retention can only stop a water deficit from getting worse: in order to reverse the problem further water intake is required. As noted above, a rising plasma osmolality stimulates thirst, at least partly through osmoreceptors located in the OVLT (Bourque, 2008). However, the sensation of thirst does not develop until plasma osmolality has reached a level at which antidiuresis is nearly maximal: typically about 290 mOsm/Kg H_2O (Robertson et al., 1982). The consequence is that water throughput is minimized. As with AVP release, thirst can also be stimulated by haemodynamic factors, but again these only become significant once quite large changes have been experienced.

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CHAPTER 23

Potassium homeostasis

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Introduction

Potassium (K^+), the most abundant cation in the organism, is involved in a large variety of physiological functions. The equilibrium between the concentration of K^+ in the extracellular space (low) and the intracellular compartment (high) is crucial for maintaining the electrical properties of excitable and non-excitable cells, because it determines the membrane resting potential. The high intracellular concentration of K^+ (120–140 mmol/L) also contributes to the intracellular osmolarity, a determinant of cell volume. It is therefore crucial to finely tune both extracellular and intracellular K^+ concentrations. To achieve this goal, there is a coordinated regulation between processes/mechanisms that store/release K^+ from internal stores (internal balance) and those that retain/excrete K^+ (external balance) (Fig. 23.1).

Internal potassium balance

K^+ compartments in the organism

The total K^+ content of the body is maintained constant around 50–55 mmol of K^+/kg of body weight (excepted during growth and pregnancy). The most important part (98%) of the body K^+ is intracellular and the remaining 2% is mainly present in the extracellular fluid (around 70 mmol; extracellular space). Skeletal muscle is the main store of intracellular K^+ (around 3000 mmol), whereas liver and red blood cells account for 200 mmol each. To maintain a constant equilibrium, intracellular storage compartments may either 'pump' from or release K^+ into the extracellular space; whereas the release of K^+ into the extracellular space is a passive process through K^+ channels and carriers, its pumping into muscle cells is active and mediated by the Na^+,K^+-ATPase. Therefore, regulatory factors (thyroid hormone, insulin, exercise, etc.) that modify Na^+,K^+-ATPase density in skeletal muscles have a direct impact on extracellular K^+ concentration by modifying the rate of K^+ entry into muscle cells.

Postprandial and fasting K^+ transfers

Because of its low K^+ level, the extracellular space is the compartment of the organism that is the most susceptible to K^+ concentration variations. Indeed, the amount of K^+ ingested daily (around 70–100 mmol) is similar to the plasma pool of K^+. Therefore, one challenge that the organism has to face is to maintain plasma K^+ constant (between 3.5 and 5 mmol/L) whatever the circumstances (after a meal or during fasting for instance). The inability to do so would modify the extracellular/intracellular K^+ ratio and affect physiological functions. A complex regulatory system, involving both internal (K^+ exchange from intracellular stores and extracellular space; internal balance) and external mechanisms (K^+ excretion in urine and stools; external balance), is therefore in charge of maintaining constant the plasma K^+ level. It is crucial that both internal and external K^+ balance mechanisms work in coordination to avoid harmful fluctuations of the extracellular K^+ level.

The absorption of a meal, which is primarily dictated by the need for an energy source (under the final form of glucose) is normally accompanied by a large intake of K^+ because natural products, from meat to vegetables, crops, or fruits are K^+-rich sources. Organisms have selected the same regulatory mechanism, which involves mainly insulin, to clear the plasma of both glucose and K^+ and avoid the harmful consequences of increasing their plasma concentration. Indeed, this hormone acts on skeletal muscles by increasing the density of glucose transporters (glut-4) and Na,K-ATPase at the cell surface that move glucose and K^+ into the cells respectively. During the fast period, this intracellular reserve of K^+ is then slowly released into the plasma compartment from where it is eliminated by the kidney, allowing the body to maintain a constant plasma K^+ value. This process is described by Youn and McDonough (2009) as an 'altruistic specialization to donate' intracellular K^+ to extracellular compartments.

External balance

Intestinal K^+ excretion

The colon contributes to K^+ homeostasis by its ability to either reabsorb or secrete K^+. Under a normal K^+ diet, < 10% of the K^+ intake is excreted in the faeces which, compared with kidney excretion, seems negligible. However, under special dietary conditions or pathological status like end-stage renal failure, the colonic contribution to K^+ homeostasis becomes more crucial. Colonic reabsorption of K^+ is mediated by the H^+,K^+-ATPase type 2 (HKA2, see below for details), which is expressed at the apical (luminal) side of the cells. Lack of HKA2 increases the faecal excretion of K^+ twofold under a normal diet and this enhancement is even stronger (fivefold) during K^+ restriction. The secretion of K^+ is mediated at the basolateral side by the Na^+,K^+-ATPase and the Na^+-K^+-$2Cl^-$ cotransporter NKCC1; at the apical side, potassium exits into the lumen via Ca^{2+}-dependent K^+ channels known as big K^+ (BK) channels. This secretion pathway is upregulated through a mechanism involving stimulation of BK activity by aldosterone and adrenaline when K^+-rich food is provided to animals.

Urine K^+ excretion

Renal excretion of K^+ results from glomerular filtration and transport of K^+ along the renal tubule. The daily filtered load of K^+ (~

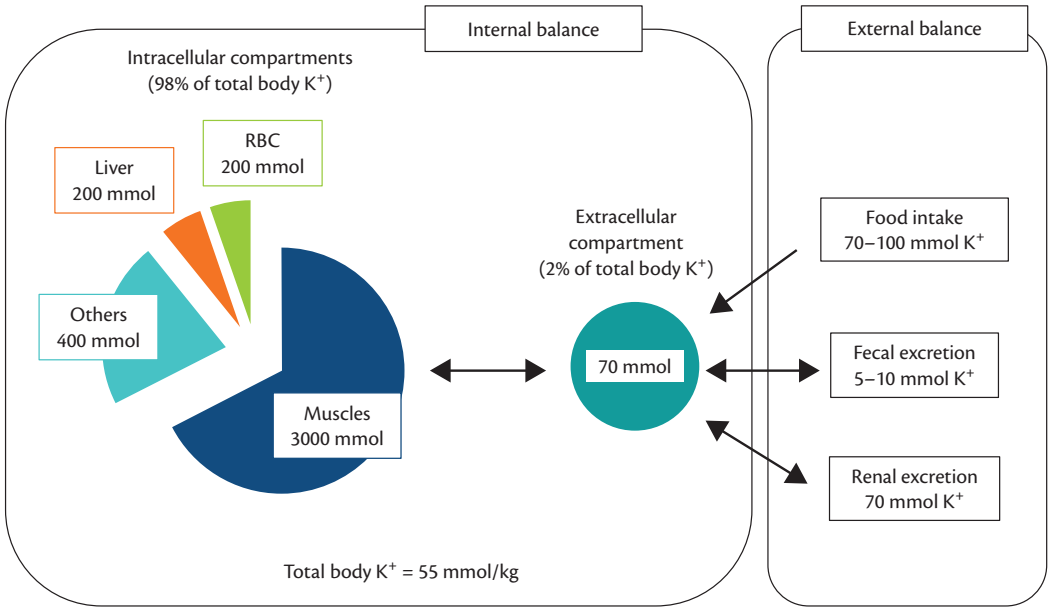

Fig. 23.1 Schematic representation of K^+ repartition in the different compartments of the organism. Ninety eight per cent of body K^+ is present in intracellular compartments, mainly in muscles. The priority of the organism is to maintain the extracellular K^+ concentration within a narrow range despite the variations of K^+ intake. For this purpose, K^+ may be pumped or released from internal stores (internal balance) or may be reabsorbed or secreted by kidneys and intestine (external balance).

750 mmol) far exceeds normal K^+ intake (~ 70–100 mmol/day), demonstrating a massive capacity for tubular K^+ reabsorption. Nevertheless, in some circumstances, for example, when glomerular filtration rate (GFR) is decreased or K^+ intake is high, the rate of K^+ excretion may exceed its rate of filtration, revealing the existence of a tubular secretion mechanism. However, whatever the condition and the net flux of K^+ within the kidney, urinary K^+ excretion results from both reabsorption and secretion processes that originate in specific segments of the nephron. As schematically shown in Fig. 23.2, the initial segments of the nephron reabsorb the bulk of filtered K^+, whereas the late portions either reabsorb or secrete K^+ so as to match homeostasis requirements. Superimposed on these processes are mechanisms that allow for the recycling of K^+ within the kidney medulla. Before analysing the cellular mechanisms of K^+ transport in the different segments of the nephron, we will review briefly the main properties of the proteins involved in the transport of K^+.

Potassium transporters

X,K^+-ATPases

Primary active transporters utilize the energy released by the hydrolysis of ATP to move ions against their electrochemical gradient. Membrane proteins able to achieve this goal therefore display the properties of ion transporters (ion binding and movement through a hydrophilic environment) and of enzymes (binding and hydrolysis of ATP), both properties being interconnected. These active transporters are referred to as pumps or as ATPases.

X,K^+-ATPases belong to a large family of proteins, the P-type ATPases, that share common topogenic motifs and use a similar mechanistic pathway to transport ions across the membrane. All P-type ATPases possess a core structure comprising three pairs of transmembrane domains and the connecting loops which together encompass the eight-amino acid signature of this family. A special feature of the X,K^+-ATPase subgroup is their requirement for an additional subunit (called the β-subunit) in addition to the P-type ATPase itself (the α-subunit) to form a mature and functional transporter.

The common mechanism of ion transport by P-type ATPases involves the transient phosphorylation of an aspartyl residue during the functional cycle, and the transition between two main and opposite conformations: the E1 conformation which exhibits a high affinity for intracellular Na^+ or H^+ (i.e. the ion that has to be moved out of the cell against its gradient) and ATP, and the E2 conformation which exhibits a high affinity for K^+ (which has to be moved into the cell against its gradient). The passage from one conformation to the other depends on the phosphorylation of the P-ATPase.

Two types of X,K^+-ATPase participate in the renal regulation of the K^+ balance, namely the Na^+,K^+-ATPase and the H^+,K^+-ATPase.

X,K^+-ATPases

The Na^+,K^+-ATPase is an ubiquitous plasma membrane enzyme that transports two K^+ into and three Na^+ out the cells by using the energy of the hydrolysis of one molecule of ATP. The Na^+,K^+-ATPase is composed of two obligatory subunits, the catalytic subunit (called α-subunit), a 10-transmembrane-spanning domain protein, and the β-subunit, a single transmembrane domain, type II glycoprotein with a large ectodomain. The Na^+,K^+-ATPase is inhibited by a family of compounds known as cardiac glycosides or digitalis (e.g. digoxin and ouabain).

The presence of the β subunit is necessary for the structural and functional maturation of the pump and influences the kinetic properties of the α subunit. The existing 4 α (α1 to α4) and 3 β (β1 to β3) isoforms exhibit a different tissue distribution and can assemble to produce Na^+,K^+-ATPase isozymes with different transport and pharmacological properties. The renal Na^+,K^+-ATPase consists of an α1β1 isozyme that is present all along the nephron at the

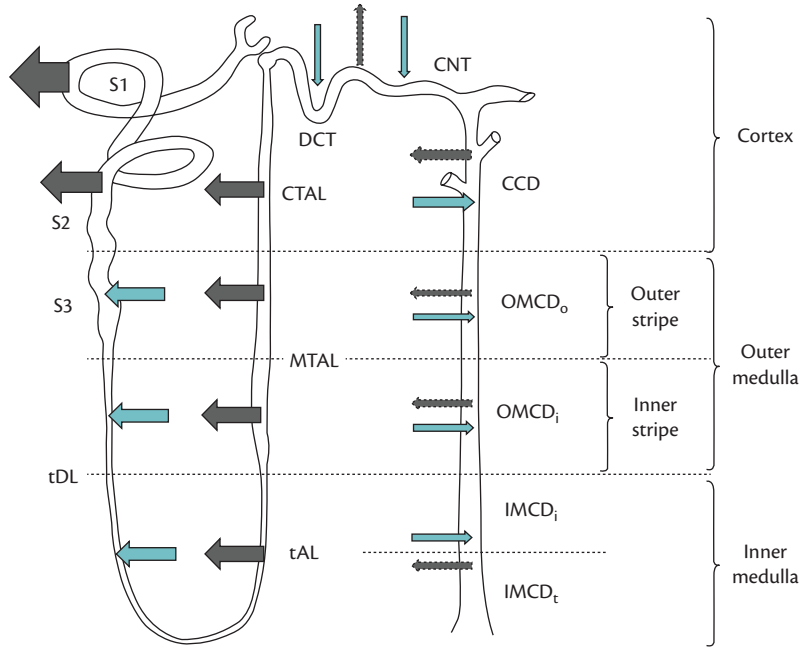

Fig. 23.2 Schematic representation of K^+ movements along the nephron. This diagram shows the nomenclature of nephron segments used in this chapter and the net movement of potassium in each of them: blue and grey arrows represent secretion and reabsorption of K^+ respectively. S1, proximal convoluted tubule; S2 and S3 cortical and medullary proximal straight tubule; tDL and tAL, thin descending and ascending limbs of Henle's loop; MTAL and CTAL, medullary and cortical thick ascending limb; DCT, distal convoluted tubule; CNT, connecting tubule; CCD and OMCD, cortical and outer medullary collecting duct (o and i subscripts refer to portions of OMCD located in the outer and inner stripes of the outer medulla); IMCD, inner medullary collecting duct (i and l subscripts refer to the initial and late half of IMCD).

basolateral side of the cells. This isozyme exhibits a high affinity for extracellular K^+ (around 1 mmol/L) and internal Na^+ (around 9 mmol/L) and a rapid turnover rate (50 transported charges/s at 20°C). These properties indicate that the concentration of intracellular Na^+ is the rate-limiting factor of the renal α1β1 Na^+,K^+-ATPase. The β3 subunit has also been observed in the kidney but its presence does not modify the main kinetic properties of the Na^+,K^+-ATPase. Na^+,K^+-ATPase activity is two to six times higher in the thick ascending limb of Henle's loop and the distal convoluted tubule than in proximal and terminal segments (Katz et al., 1979).

In addition to these obligatory subunits, members of a family of small, one-transmembrane domain proteins interact with the Na^+,K^+-ATPase and modulate its kinetic properties. These proteins, called FXYD in reference to a common amino-acid motif, exhibit specific expression profiles and affect differentially Na^+ and K^+ affinities. The two main FXYD family members expressed in the kidney are FXYD2 isoforms a and b (previously known as the γa and γb subunits of the Na^+,K^+-ATPase) and FXYD4 (known as the corticoid hormone-induced factor (CHIF)) (Sweadner et al., 2003). It seems established that FXYD2 isoforms are mainly expressed in the thick ascending limb (TAL) of Henle's loop, whereas FXYD4 is expressed exclusively in the distal part of nephron (Shi et al., 2001). The functional relevance of these regulatory subunits is still under investigation. There is evidence that FXYD4 plays a role in the regulation of the renal K^+ balance. Its expression is upregulated during K^+ loading and the association of FXYD4 with Na^+,K^+-ATPase decreases its affinity for external K^+. Combined together, these observations suggest that FXYD4 helps to excrete K^+ by allowing the Na^+,K^+-ATPase to function more efficiently when extracellular K^+ is high. However, when placed under a high-K^+ diet, FXYD4 null-mice exhibit only a mild phenotype featuring a higher urine volume, but no clear K^+ excretion defect (Aizman et al., 2002).

X,K^+-ATPases

Like the Na^+,K^+-ATPase, the H^+,K^+-ATPase is an heterodimer composed of an α and a β subunit. There are two isoforms of the α subunit that were originally distinguished by their tissue expression and their pharmacological properties. The H^+,K^+-ATPase type 1 (known as the gastric H^+,K^+-ATPase; HKA1) is mainly present in the parietal cells of the stomach where it serves to acidify the gastric content. It is associated with a specific β subunit (the HKβ) and is sensitive to pharmacological compounds like omeprazole and Schering 28080, but insensitive to cardiac glycoside (Sachs et al., 1995). The H^+,K^+-ATPase type 2 (known as the non-gastric or the colonic H^+,K^+-ATPase; HKA2) is abundantly expressed in the colon and is sensitive to both Schering 28080 and ouabain. In heterologous expression systems, all β subunits (subunits β1, β2, β3 of the Na,K-ATPase and the HKβ) may be coupled with the α subunit of the H^+, K^+-ATPase type 2 with no obvious modification of its kinetic properties. However, the nature of the β subunit associated with H^+, K^+-ATPase type 2 *in vivo* is not well defined and could be tissue-specific.

As opposed to HKA1, which specifically exchanges intracellular protons against extracellular K^+ ions, HKA2 seems more flexible in terms of the ions that it can transport. Indeed, since its first characterization, it has been established that the H^+ and K^+ fluxes are not equal and that another cation in addition to H^+ should be simultaneously transported to maintain electroneutrality. Further investigations have shown that HKA2 expression not only modifies

the intracellular content of Na^+, but also that the HKA2-mediated ^{86}Rb flux is Na^+-dependent with an affinity around 9 mmol/L. The *in vivo* consequences of the ability to transport Na^+ have not been elucidated yet.

Another particularity of the HKA2 is the presence of a truncated form (HKα2b) that lacks 108 amino acids at its N terminus. This truncated form arises from an alternative splicing that fuses the exon 1 to the exon 2. The short form of HKA2 is expressed with the long form both in colon and kidney, but here again its physiological relevance has not been elucidated.

Both types of H^+,K^+-ATPases are expressed in the distal part of the nephron (mainly cortical collecting duct (CCD), outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD)) and some reports suggest the presence of the H^+,K^+-ATPase type 2 also in the TAL and the connecting tubule (CNT) (for review, see Silver and Soleimani, 1999). As opposed to the Na^+,K^+-ATPase, both H^+,K^+-ATPases are located at the apical side of the cells. In the distal nephron, HKA1 seems restricted to the intercalated cells, whereas HKA2 has been identified in both principal and intercalated cells.

K^+ channels

K^+ channels are the most widely distributed ion channels and display a large diversity of biophysical characteristics. However, they all share a common tetrameric structure (homo- or heteromers) with each subunit showing a common core structure that consists of two transmembrane domains separated by a P-loop. There are four main families of K^+ channels: calcium-activated K^+ channels, inwardly rectifying K^+ channels (through which K^+ passes more easily in the inward direction), tandem pore domain K^+ channels that are constitutively open or display a basal activity, and voltage-gated K^+ channels that open or close in response to changes in the membrane voltage.

Members of all these families of K^+ channels are present in the kidney where they ensure a large variety of functions. At the basolateral side, they contribute to: (a) hyperpolarizing the cell membrane and thereby generating a driving force for other transport systems, (b) the maintenance of the cell volume, and (c) the recycling of K^+ ions accumulated by the Na^+,K^+-ATPase. On the opposite side, they participate in apical K^+ recycling in TAL cells (along with NKCC2) and in K^+ secretion in the distal part of the nephron. In view of the scope of this chapter, we will mainly focus on K^+ channels that have a direct impact on K^+ homeostasis, namely those expressed in the distal part of the nephron: renal outer medulla K^+ (ROMK) (Kir1.1), BK channel (or Maxi K channel), and Kir 4.1/Kir 5.1.

ROMK

The ROMK channel, the first K^+ channel to be functionally cloned, belongs to the family of KCNJ inwardly rectifying K^+ channels (Kir). Its structure is characterized by two transmembrane domains with intracellular NH_2- and COOH- termini. The extracellular loop that connects both transmembrane segments forms a P-loop that contains the K^+ selectivity filter. A functional channel requires the assembly of four ROMK monomers. There are three ROMK isoforms (from ROMK1 to ROMK3, or Kir1.1a to Kir 1.1c) that originate from different splicings, differ in the sequence of their NH_2- terminal domain, but exhibit similar biophysical properties. They also differ in their location along the nephron. ROMK1 has the most restricted localization, from DCT to OMCD. ROMK3 has the largest distribution, from the medullary TAL to the OMCD. ROMK2 displays a similar localization as ROMK3 but is not present in the TAL (Boim et al., 1995). The physiological relevance of the existence of these three isoforms is not well understood. The generation of ROMK-deficient mice finally confirmed the molecular signature of the small K^+ conductance described originally in the TAL and the CCD. It also provides the first model of Bartter syndrome type 2 (Lorenz et al., 2002).

The biophysical characteristics of ROMK channels expressed in heterologous systems correspond to those of the predominant apical K^+ current measured in TAL, namely a conductance of 35-pS with a high open probability (about 0.9) and a high sensitivity to intracellular pH (acidification leading to inhibition of ROMK). However, some discrepancies have also been observed. For instance, the 35-pS conductance is barely rectified and is regulated by ATP and sensitive to glibenclamide. These native properties suggest that ROMK is associated with proteins that may belong to the ATP-binding cassette protein family (ABC protein). It has been shown that expression in heterologous system of ROMK along with the sulfonylurea receptor, a subunit of the Kir6.1 K^+ channel, or with CFTR (two ABC proteins), leads to characteristics that correspond to those of the native channel. Interestingly, in CFTR-null mice the ROMK conductance is not regulated by intracellular ATP and is not sensitive to glibenclamide anymore, indicating that CFTR and ROMK interact functionally *in vivo*.

Big K^+ channel

The BK channel story started with the identification of an apical K^+ conductance of 90-pS that was activated by an elevated intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Hunter et al., 1984). In addition to its large conductance, this channel displays a low open probability and a high sensitivity to iberiotoxin. Because of these characteristics (low Po and activation by high $[Ca^{2+}]_i$), it has been proposed that this channel is not functional under basal conditions but more likely activates after the rise of $[Ca^{2+}]_i$ secondary to a depolarization, an hypo-osmotic stress or a membrane stretch. The molecular identification of this conductance was first done in 1993 by cloning mouse orthologues of the *Drosophila* gene *slo*. More recent studies have clarified the structure of BK channels. They consist of two subunits, the pore-forming α subunit (encoded by the *slo1* gene) and a β-regulatory subunit which is devoid of channel function but interferes with α subunit properties. In the kidney, there is one isoform of the α-subunit (*slo1*) and two of the β-subunit (β2 and β4). The BK channel conductance has been observed in the CNT and the CCD, both in principal and intercalated cells. As mentioned above, the characteristics of this channel do not support a constitutive activation. In conventional patch-clamp experiments on split-open tubules, BK channel activity is not observed. Other experiments have shown that BK activity is activated by fluid flow (Taniguchi and Imai, 1998), which was further confirmed by the demonstration that flow-stimulated K^+ secretion is absent in BK knockout mice (Pluznick et al., 2003). Renal epithelial cell are equipped with a cilium that acts as a 'flow-sensor' and it is now proposed that flow-induced bending of this cilium activates apical stretch-sensitive Ca^{2+}-channels and increases $[Ca^{2+}]_i$ which in turn activates BK.

Basolateral K^+ channels in the collecting duct: example of Kir4.1/Kir5.1

The presence of K^+ conductances in the basolateral membrane of CCD epithelial cells is required for (a) enabling K^+ recycling (which allows the Na^+,K^+-ATPase to function efficiently), (b) setting the basolateral membrane potential, and (3) determining the direction of the net basolateral K^+ flux. *In vivo* studies demonstrate the presence of at least three different K^+ conductances (20-pS, 40-pS, and 76-pS) in the basolateral membranes of native mouse CCD (Lachheb et al., 2008). Among them, the 40-pS conductance turns out to be the most abundant and to have biophysical properties similar to those of heterotetrameric Kir4.1/Kir5.1 K^+ channels. This K^+ channel, as its name implies, is formed of two proteins, Kir4.1 (encoded by the *KCNJ10* gene) and Kir5.1 (encoded by the *KCNJ16* gene). Kir4.1 was originally cloned from brain tissue on the basis of sequence similarity with ROMK channels. It shows a high expression level at the basolateral side of DCT, and CNT and CCD principal cells. The biophysical characteristics of Kir4.1 as determined in *Xenopus* oocytes revealed a channel with a high open probability and a small (around 25-pS) strongly rectified conductance. The channel displays a complex sensitivity to intracellular pH: acidification reduces its open probability, but increases its conductance.

In an effort to identify K^+ channels similar to ROMK, another member of this gene family, referred to as Kir5.1, was discovered and characterized. When expressed alone in *Xenopus* oocytes, Kir5.1 does not produce any ionic current. However, it specifically assembles with Kir4.1 and modifies its conductance properties. The most striking effects of Kir5.1 on the properties of Kir4.1 are the increase in the current amplitude, the stronger rectification and the appearance of a time-dependent component affecting the current amplitude. The channel formed by Kir4.1 and Kir5.1 is a heterotetramer and its biophysical properties depend on the ratio of both subunits and their position in the tetramer. Assembly of Kir5.1 with Kir4.1 also provides a higher sensitivity to intracellular pH. In the kidney, in contrast to Kir4.1, Kir5.1 has a broad localization along the nephron.

Kir4.1 and Kir5.1 genes are of particular interest since recent studies provide evidence for their involvement in human pathologies. Polymorphisms in *KCNJ10*, the human genes encoding Kir4.1, are associated with the SeSAME/EAST syndrome, a rare disease leading to neurological and renal dysfunctions (Scholl et al., 2009) whereas Kir5.1-null mice exhibit a renal phenotype exactly opposite to that of SeSAME/EAST patients (Paulais et al., 2011). These recent findings outline the importance of a functional basolateral K^+ channel in the distal part of the nephron.

K^+ carriers: NKCC and KCC

The electroneutral cation-Cl^- cotransporters belong to the solute carrier family slc (slc12 subfamily). These carriers utilize the Na^+ or K^+ gradient created by the Na^+,K^+-ATPase to move chloride ions across the plasma membrane. This family is divided into three large subgroups, the Na^+-Cl^- cotansporters (NCC), the K^+-Cl^- cotransporters (KCC), and the Na^+-K^+-Cl^- cotransporters (NKCC). For the purpose of this chapter we will focus on the K^+-dependent members of this family, the KCC and NKCC transporters that are expressed in the kidney.

KCC

K^+-Cl^- cotransporters were identified first as mediators of swelling-activated K^+ efflux in red blood cells. Further studies led to the cloning and molecular characterization of 4 isoforms of KCC (KCC1 to 4) encoded by the *slc12a4* to *slc12a7* genes. KCC transporters, as all slc12 family members, have 12 transmembrane-spanning domains and intracellular N- and C-terminal parts. More specifically, they exhibit a large and heavily glycosylated extracellular loop between transmembrane domains 5 and 6. KCC2 is a neuronal-specific isoform but the three other isoforms are present in the kidney: KCC1 is expressed all along the nephron; KCC3 is mainly found in the proximal tubules where it participates in the regulation of cell volume and KCC4 is confined to the basolateral membranes of TAL, DCT, and CCD α-intercalated cells. In addition to deafness, targeted disruption of the gene encoding KCC4 leads to renal tubular acidosis (Boettger et al., 2003).

NKCC

Two genes, *slc12a1* (NKCC2) and *slc12a2* (NKCC1), were found to encode for the Na^+-K^+-Cl^- cotransporters. These isoforms exhibit different tissue and cell patterns of expression. NKCC1 is present in almost all tissues and organs and, in polarized epithelia; it is located at the basolateral membrane. Conversely, NKCC2 is a kidney-specific isoform that displays a restricted expression at the apical side of TAL cells. NKCC uses the energy of the Na^+ gradient to move K^+ and Cl^- into the cells with a stoichiometry of $1Na^+$:$1K^+$:$2Cl^-$. NKCC resembles KCC with 12 transmembrane-spanning domains and intracellular N- and C-terminal extremities. However, NKCCs exhibit a large extracellular loop connecting transmembrane domains 7 and 8. NKCCs are also characterized by their sensitivity to loop diuretics such as bumetamide or furosemide. The molecular diversity of NKCCs is increased by the existence of alternate splicing and dual polyadenylation sites. Alternate splicing generates three variants (A, B, and F) that differ in the sequence of a 32 amino-acid cassette at the beginning of the second transmembrane domain. NKCC2A, B or F exhibit specific localization in the TAL with expression of the F form in the deep part of the medullary TAL, that of the A form extending all along the TAL to the macula densa, and that of the B form being mostly cortical. Interestingly, expression of the different isoforms in *Xenopus* oocytes revealed kinetic differences, in terms of Na^+, K^+, and Cl^- affinities for instance. However, their physiological relevance remains to be determined. In the TAL, the presence of NKCC2 enables the net reabsorption of Na^+ and Cl^-.

Human mutations in the NKCC2 gene that lead to a loss of function are related to Bartter syndrome type 1 (Simon et al., 1996) Other human polymorphisms in this gene have been associated with protection against hypertension (Ji et al., 2008); however the functional effect of these polymorphisms on NKCC2 activity remains to be determined.

Cellular mechanisms of potassium transport along the nephron

Proximal tubule

The first two-thirds of the proximal tubule (approximately the S1 and S2 segments) reabsorbs 30–70% of filtered K^+ (Malnic et al., 1964) whereas the last third (the S3 segment) secretes K^+ (Jamison, 1987). K^+ secretion along the S3 segment is thought to occur via passive diffusion through the paracellular pathway, owing to the presence of a high K^+ concentration in the medulla interstitium (Jamison, 1987). At least three mechanisms account for K^+

reabsorption along the initial proximal tubule: solvent drag and diffusion through the paracellular route and active transcellular reabsorption.

Active reabsorption of K^+ in the proximal tubule is supported by indirect *in vivo* pieces of evidence and by direct measurement of the electrochemical gradient in rabbit PCT perfused *in vitro*. K^+ transport proteins known to be present in proximal tubule cells include K^+ channels at the apical membrane, and Na^+,K^+-ATPase, K^+ channels and a K-Cl cotransporter at the basolateral membrane (Fig. 23.3A). Thus, in the absence of an active mechanism accounting for apical uptake of K^+, and given the transepithelial voltage prevailing along the proximal tubule (from slightly lumen-negative in the early S1 to slightly lumen-positive in the S2 and S3 segments (Fromter, 1984)), these cells appear equipped to secrete rather than to reabsorb K^+. Two mechanisms may circumvent this apparent paradox. Firstly, a H^+,K^+-ATPase activity has been described in the proximal tubule which, if efficient at the apical membrane, might account for active entry of K^+. Secondly, Weinstein has proposed an elegant model (Weinstein, 1988) in which he considers a fourth compartment constituted by the interspace limited by the lateral membranes of proximal tubule cells, the tight junction and the basement membrane (Fig. 23.3B). According to this model, the Na^+,K^+-ATPase located on lateral membranes pumps K^+ from the interspace into the cell, thereby providing a driving force for K^+ diffusion across the tight junction (assuming that K^+ diffusion across the basement membrane is slower). Intracellular K^+ then diffuses across the basal membrane. Whatever the mechanism, active K^+ reabsorption would account for only 20% of proximal tubule reabsorption.

Evidence for solvent drag comes from the observations that (a) K^+ reabsorption by proximal tubules is dependent on fluid reabsorption, and (b) experimentally inducing water secretion by increasing the osmolality of the luminal fluid induces K^+ secretion. Although proximal tubule reabsorbs approximately 60–70% of filtered fluid, solvent drag-mediated reabsorption is thought to account for only 20% of K^+ reabsorption. This stems from the fact that the bulk of water is reabsorbed via the transcellular route through K^+-impermeable aquaporins and not via the paracellular pathway.

Many pieces of evidence support passive diffusive reabsorption of K^+ in the late portion of the proximal convoluted tubule and S2 segment. In these portions of the proximal tubule, the favourable gradient for passive diffusion results from the slightly lumen-positive voltage and from the fact that K^+ concentration is slightly higher in

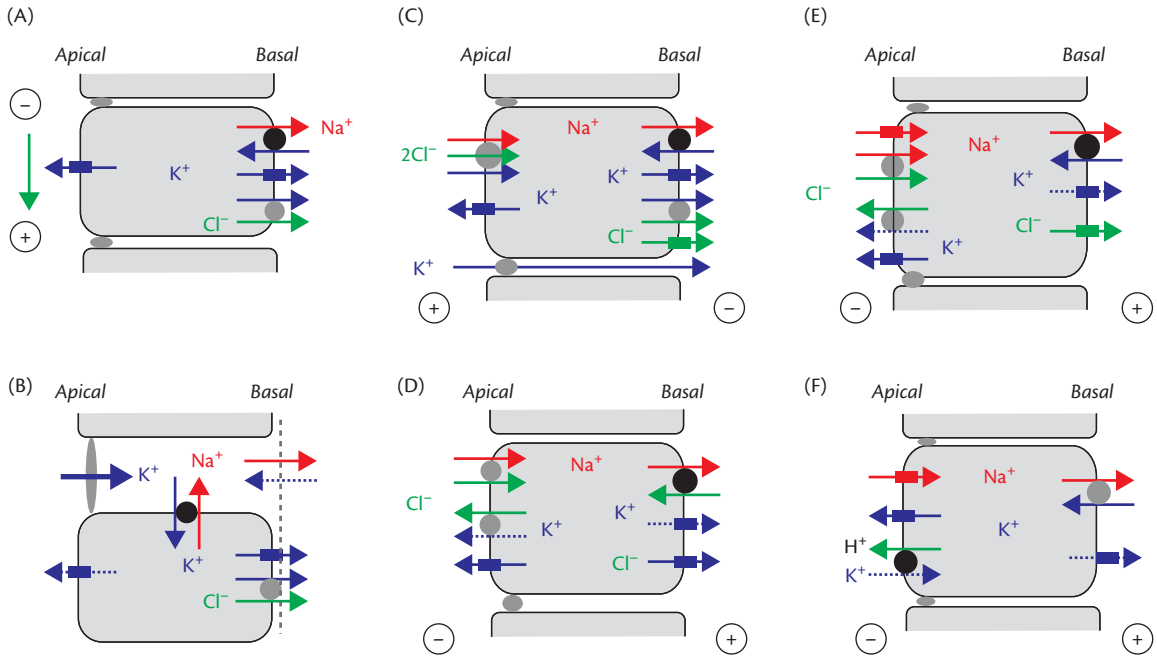

Fig. 23.3 Cell models of K^+ transport. Black circles represent primary active transporters (ATPases), grey circles are cotransport of counter-transport systems, and rectangles are channels. (A) Proximal convoluted tubule cell. Transepithelial voltage varies from slightly lumen-negative value in early convolutions to slightly negative in late convolutions. This model cannot account for the massive reabsorption of K^+ originating along the S1 segment, as it suggest that only K^+ secretion can occur. (B) Proximal convoluted tubule cell model developed by Weinstein to account for K^+ reabsorption. This model integrates an additional intercellular compartment limited by lateral membranes, intercellular junctions, and basement membrane, and considers that Na^+,K^+-ATPase is mainly expressed at the lateral membrane. (C) Thick ascending limb of Henle's loop. K^+ reabsorption in this segment may occur through the transcellular route (considering that some K^+ entering via NKCC2 leaves the cells through the basolateral membrane rather than being recycled across the apical membrane) and the para cellular route (owing to the lumen-positive transepithelial voltage generated by transcellular reabsorption of NaCl). (D) Initial distal convoluted tubule (DCT1). The lumen-negative transepithelial voltage favours K^+ secretion rather than its recycling across the basolateral membrane. (E) Late distal convoluted tubule (DCT2). The presence of a diffusive Na^+ entry pathway in parallel with electroneutral NCC depolarizes the apical membrane and increases the driving force for K^+ secretion. (F) Connecting tubule and collecting duct principal cell. The high lumen-negative transepithelial voltage brought about by diffusive apical entry of Na^+ generates a high driving force for K^+ secretion. Under K^+ restricted conditions, apical H^+,K^+-ATPase energizes K^+ reabsorption.

the tubular fluid than in plasma (owing to the primary iso-osmotic reabsorption of sodium bicarbonate and sodium chloride prevailing in the early proximal convoluted tubule). Paracellular diffusive reabsorption would account for 60% of proximal tubule K^+ reabsorption.

Although it is quantitatively important, it is worth mentioning that the rate of K^+ reabsorption along the proximal tubule is not a major determinant of the urinary excretion of K^+. As a matter of fact, the rate of K^+ reabsorption along the loop of Henle increases proportionally with K^+ delivery to this segment so that the fraction of K^+ leaving the loop of Henle is approximately 10% of filtered K^+, whatever the proximal tubule reabsorption rate.

Loop of Henle

The loop of Henle consists of the proximal straight tubule, the thin descending limb of Henle's loop, the thin ascending limb (only in juxtamedullary nephrons) and the thick ascending limb (TAL) with its medullary and cortical parts.

Thin segments of the loop of Henle show no apical K^+ conductance, indicating that any transepithelial transport occurs via the paracellular pathway which is highly permeable to K^+ (Imai et al., 1987). As the tubule fluid flows along the thin descending limbs, it enters regions with increasing concentrations of peritubular K^+ whereas the opposite occurs along the thin ascending limb. This peritubular K^+ gradient is responsible for passive secretion and reabsorption of K^+ along the thin descending and ascending segments respectively. Although these respective fluxes are not quantifiable, it is assumed that they are similar and therefore that the load of K^+ leaving the proximal tubule equals that delivered to the TAL.

As already mentioned, the TAL reabsorbs variable amounts of K^+, depending on the amount delivered (Greger, 1985). In some circumstances, for example, in response to loop diuretic treatment, it can also secrete K^+. TAL cells display both active and passive K^+ transport systems at both borders (Fig. 23.3C). At the apical membrane, they express an electroneutral Na^+-K^+-$2Cl^-$ cotransporter (NKCC2), which allows for active entry of K^+ and Cl^- coupled to passive entry of Na^+, as well as K^+ channels including ROMK. The basolateral membrane expresses Na^+,K^+-ATPase pumps, K^+ and Cl^- channels and possibly a K^+-Cl^- cotransporter These transport systems make possible the massive transcellular reabsorption of NaCl which is associated with depolarization of the basolateral membrane (due to the electrogenic diffusion of chloride) and hyperpolarization of the apical membrane (due to the electrogenic diffusion of K^+); they thereby generate a lumen-positive transepithelial voltage (Greger, 1985). In turn, the transepithelial voltage drives passive reabsorption of Na^+ and K^+ via the paracellular pathway. The K^+ ions that have accumulated within the cell above equilibrium by both the Na^+,K^+-ATPase and NKCC2 leave the cell via either apical or basolateral membranes. Thus, the net transcellular flux of K^+ depends on the balance between apical versus basolateral efflux. It should be recalled that apical recycling of a major fraction of K^+ entering the cell via NKCC2 is a requirement for sustained NaCl reabsorption (Greger, 1985): because K^+ concentration in the tubular fluid is approximately 30-fold lower than that of sodium and chloride, the activity of NKCC2 would stop rapidly unless K^+ is re-introduced in the tubular fluid. This is well illustrated by the fact that Bartter syndrome, a monogenic disease featuring inhibition of ion transport in the TAL, can be induced by loss-of-function mutations of either NKCC2 or ROMK, as well as mutations affecting the apical Cl^- conductance (Hebert, 2003). It is thus legitimate to assume that most K^+ reabsorption along the TAL takes place through the paracellular pathway.

Although the cellular mechanism of Na^+, K^+, and Cl^- reabsorption is identical in the medullary and cortical portions of the TAL, these two segments do not play the same role regarding ion balance. Due to the striking differences between blood circulation within the kidney cortex and medulla, most ions reabsorbed along the medullary thick ascending limb are recycled within the medulla whereas those reabsorbed within the cortex can be drained out of the kidney.

Distal convoluted tubule, connecting tubule, and cortical collecting duct

The nephron portion located downstream the cortical thick ascending limb and the macula densa, referred to as the distal tubule, is complex both by its architecture and its cell composition (see Fig. 23.2). Classically, it is subdivided into a distal convoluted tubule (DCT), a connecting tubule (CNT), and a cortical collecting duct (CCD), but (a) there are marked differences between superficial and juxtamedullary nephrons, the latter showing a much longer CNT, and (b) this subdivision does not reflect properly the axial functional heterogeneity of the distal tubule. The DCT is traditionally considered to be made of a single type of cells, but the cells from the initial and distal parts of this segment (respectively called DCT1 and DCT2) display different transport systems. Conversely, the CNT and CCD are both made of principal cells (called CNT cells in case of the CNT) and intercalated cells, and each of these cell types displays similar transport systems in the CNT and CCD. In addition, the quantitative contributions of these different structures to ion transport are not known because *in vivo* flux measurements by micropuncture studies either apply to combined structures (late DCT and early CNT) or cannot be done (late CNT and CCD). For these reasons, we find it more convenient to address the mechanisms of K^+ transport along the distal tubule at the level of the different cell types.

Globally, the distal tubule is the main site of K^+ transport adaptation. Under 'normal' conditions (of dietary K^+ ingestion and kidney function) the distal tubule secretes small amounts of K^+ to balance the load delivered at the DCT (~ 10% of the filtered load) with the dietary intake. It also shows a great ability to secrete large amounts of K^+ in response to K^+ overload. Conversely, it is able to reabsorb almost all the K^+ that is delivered by the TAL in case of severe K^+ restriction.

Cells from the DCT1 display Na^+,K^+-ATPase and K^+ and Cl^- channels at their basolateral pole, and K^+ channels (mainly ROMK) and K^+-Cl^- cotransporters in parallel with Na-Cl cotransporters (NCC) at their apical border (Fig. 23.3D). The K^+ ions which have accumulated in the cell by the Na^+,K^+-ATPase should preferentially leave the cell across the basolateral membrane, owing to the membrane depolarization brought about by conductive Cl^- exit. However, part of the Cl^- that has accumulated in the cells by NCC may recycle across the apical membrane by the K^+-Cl^- cotransporter and thereby drive K^+ secretion.

DCT2 cells have the same transport systems as DCT1 cells except that they also display an apical Na^+ channel (ENaC) which is responsible for part of Na^+ reabsorption (Fig. 23.3E). This electrogenic

Na^+ entry depolarizes the apical membrane and thereby facilitates K^+ secretion through apical K^+ channels. It also tends to generate a slightly lumen-negative transepithelial voltage which can drive K^+ secretion through the paracellular pathway.

CNT cells and CCD principal cells differ from DCT cells by the facts that (a) ENaC is the main, if not unique, pathway for apical entry of Na^+, (b) they express ROMK and BK channels at their apical membrane, and (c) there is no system for apical Cl^- entry (Fig. 23.3F). Thus, Na^+ reabsorption by principal cells strongly depolarizes the apical membrane which generates a driving force for K^+ exit. It also generates a lumen-negative transepithelial voltage, but the paracellular back diffusion of Na^+ and K^+ is negligible because the intercellular junctions are mostly impermeable to ions (tight epithelium). It is important to stress that, according to this mechanism, K^+ secretion by principal cells, the main pathway for K^+ secretion along the distal tubule, is dependent on Na^+ reabsorption and transepithelial voltage. Principal cells are also responsible for K^+ reabsorption during K^+ depletion because, under these conditions, they express the type 2 H^+,K^+-ATPase at their apical border.

Intercalated cells are classically considered responsible for the regulation of acid-base balance. Type A intercalated cells can secrete protons and ammonium whereas type B intercalated cells can secrete bicarbonate. Recently, it was also shown that type B intercalated cells mediate NaCl reabsorption (Leviel et al., 2010). Under basal conditions, intercalated cells are not equipped for transepithelial K^+ transport and are considered to be silent. However, because proton secretion by type A intercalated cells is mediated by the electrogenic V-type H-ATPase which generates a lumen-positive transepithelial voltage, activation of proton secretion during metabolic acidosis reduces the driving force for secretion by principal cells. Intercalated cells express type 2 H^+,K^+-ATPase at their apical border during K^+ depletion, and thereby may participate in K^+ reabsorption although no basolateral K^+ conductance has been described.

Medullary collecting duct

Based on topographical, morphological and functional criteria, the medullary collecting duct is divided into an outer and an inner medullary segment (OMCD and IMCD respectively), each of which is further subdivided into two sections, the outer and inner stripes for the OMCD ($OMCD_o$ and $OMCD_i$ respectively), and the initial (first 50%) and late (last 50%) portions for the IMCD ($IMCD_i$ and $IMCD_l$; see Fig 23.2).

The $OMCD_o$ consists of the same cell types as the CCD and it has the same K^+ transport systems and functional properties, except that the rates of Na^+ reabsorption and K^+ secretion are smaller. The $OMCD_i$ only displays passive paracellular K^+ transport, either secretion or reabsorption depending on the transepithelial electrochemical gradient. K^+ reabsorption along the $OMCD_i$ is the primary motor of K^+ recycling.

The IMCD secretes K^+ except during dietary K^+ depletion when it reabsorbs it (Backman and Hayslett, 1983). Besides the Na^+,K^+-ATPase, the basolateral membrane of IMCD cells display a Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) that accumulates K^+ within the cell, and K^+ and bicarbonate conductances. The apical membrane contains an amiloride-sensitive cation channel, with a similar conductance to Na^+, K^+ and ammonium that likely mediates part of K^+ secretion. The IMCD has a low transepithelial resistance which enables paracellular K^+ secretion. The molecular mechanism of K^+ reabsorption during dietary K^+ depletion remains unknown.

Potassium recycling

Medullary K^+ recycling makes it possible to highly concentrate K^+ in the inner medulla interstitium and to thereby excrete K^+ via secretion in the IMCD (Jamison, 1987). For example, during water deprivation, water reabsorption in the OMCD increases the concentration of K^+ in the luminal fluid and enables its passive reabsorption along the $OMCD_i$ and its secretion into the S3 segment of the proximal tubule and into the thin descending limb. The K^+ that has accumulated in the fluid of the thin descending limb is then reabsorbed passively along the thin ascending limb and accrues in the inner medulla interstitium. This prevents K^+ reabsorption along the IMCD and enables the excretion of the K^+ that was secreted upstream in the CNT and CCD. In the absence of this recycling, the K^+ ions that are concentrated in the urine during water deprivation would be reabsorbed in part along the IMCD, and K^+ excretion would be reduced. Note that K^+ reabsorption along the TAL may also contribute to K^+ recycling.

Regulation of urinary potassium excretion

The urinary excretion of K^+ is modulated by many factors which control K^+ secretion and/or K^+ reabsorption mostly beyond the DCT. Among these factors, which include dietary K^+ intake, plasma K^+ concentration, acid–base status, hormones, tubular flow rate, and tubular sodium concentration, aldosterone is considered to play a central role because it participates in a feedback regulatory loop: an increase in the plasma concentration of K^+ triggers the adrenal secretion of aldosterone which promotes K^+ secretion by the distal nephron. Aldosterone is also involved in the complex regulatory pathways triggered by several of the above listed factors that control urinary K^+ excretion.

Mineralocorticoids

Mechanism of action of mineralocorticoids

Aldosterone is an adrenal steroid that controls the balance of Na^+, Cl^-, K^+, and H^+ and therefore controls extracellular volume, blood pressure, acid–base balance, and plasma K^+ concentration. Because they modulate the metabolism of minerals, aldosterone and its derivatives are referred to as mineralocorticoids. The main renal effect of aldosterone is to induce Na^+ retention and K^+ secretion.

The mineralocorticoid receptor (MR) belongs to the family of nuclear receptors, a specific group of ligand-activated transcription factors. The MR displays similar affinities for aldosterone and glucocorticoids. Since the latter are present in the plasma at concentrations approximately 1000-fold higher than aldosterone, they should saturate the MR. The permanent activation of the MR by glucocorticoids is prevented by the presence of a type 2 11β-hydroxysteroid dehydrogenase (HSD2) which converts glucocorticoids into metabolites that have no affinity for the MR In the kidney, MR is expressed from the DCT to the OMCD, a nephron portion consequently defined as the aldosterone-sensitive distal nephron (ASDN). Specifically, MR is expressed in DCT cells and in the principal cells of the CNT and collecting duct. HSD2 is co-expressed

with MR in all these nephron segments except the DCT1 which therefore should be under permanent, positive MR control.

As for its targets, aldosterone modulates the expression of several genes that contribute to Na^+ reabsorption. Aldosterone has an 'early' effect enabling the over expression of regulators of Na^+ transport such as the kinase SGK1 (serum and glucocorticoid induced kinase) (Chen et al., 1999), which increases the abundance of Na^+,K^+-ATPase, NCC and ENaC at the cell surface. A 'late' effect of aldosterone is to directly induce the expression of these transporters and therefore to increase their total number (for references, see Verrey, 1999). Aldosterone also induces the hypertrophy and hyperplasia of ASDN (reviewed in Stanton, 1989), but this is an indirect effect that seems to be dependent on the stimulation of Na^+ reabsorption. All together, these early and late aldosterone effects stimulate a net transepithelial Na^+ flux. Increasing NCC activity has no direct effect on K^+ transport whereas stimulation of ENaC and Na^+,K^+-ATPase in the CNT/CCD increases the driving force for K^+ secretion. Therefore, the main (perhaps the only) mechanism by which aldosterone induces K^+ secretion is through the stimulation of the electrogenic Na^+ transport system in CNT/CCD principal cells. Indeed, neither BK channel expression nor ROMK expression is directly activated by aldosterone.

The aldosterone paradox

Hypovolaemia and hyperkalaemia are the main factors that induce aldosterone secretion by adrenals. Thus, the paradox is that to maintain homeostasis in these two conditions, aldosterone has to be able to either maximally increase Na^+ reabsorption while minimally increasing K^+ secretion during hypovolaemia, or maximally increase K^+ secretion while minimally increasing Na^+ reabsorption in response to hyperkalaemia. Conceptually, this can be achieved by two means: (1) by modulating the relative effects of aldosterone on electrogenic Na^+ reabsorption (which is coupled to K^+ secretion) on the one hand and on electroneutral NaCl reabsorption (which is uncoupled to K^+ secretion) on the other hand, and/or (2) by dissociating K^+ secretion from electrogenic Na^+ reabsorption in CNT/CCD principal cells. Whatever the mechanism, the solution to the paradox requires that some factor be differentially expressed during hypovolaemia and hyperkalaemia and modulate aldosterone effects. Angiotensin II is likely to play such a role because (a) its plasma level increases during hypovolemia but not hyperkalaemia, (b) its receptor is expressed along the whole ASDN, and (c) it increases Na^+ reabsorption in the DCT1 and inhibits ROMK throughout the ASDN (Kahle et al., 2008).

A key molecule in the modulation of aldosterone action by angiotensin II was identified in patients with Gordon syndrome (or pseudohypoaldosteronism type II, PHAII), a disease characterized by hypertension and hyperkalaemia, owing to constitutively active Na^+ reabsorption and inhibition of K^+ secretion. The disease is caused by mutations in genes encoding for 'with-no-lysine' (WNK) kinases (Wilson et al., 2001). One member of this family, WNK4, is mainly expressed in the distal nephron (DCT to OMCD) and is of particular interest; further investigations have demonstrated its role as a 'molecular switch' enabling the kidney either to reabsorb Na^+ or to secrete K^+. This kinase displays different conformations in response to aldosterone and/or angiotensin II. Under basal conditions, WNK4 is not phosphorylated and inhibits NCC, ROMK and ENaC (Kahle et al., 2003), impeding both Na^+ reabsorption and K^+ secretion. During hypovolaemia, angiotensin II promotes the WNK4-PHAII conformation also found in PHAII, which removes NCC and ENaC inhibition and promotes their activation by aldosterone, but which amplifies ROMK inhibition (Kahle et al., 2003). During hyperkalaemia, SGK1 phosphorylates WNK4 which, under this new conformation, maintains its inhibitory action on NCC whereas it activates ENaC and ROMK.

Potassium load

Because the amount of K^+ ingested daily is of the same order of magnitude as the total amount of K^+ in extracellular fluids, the first challenge the organism has to face is to maintain its plasma K^+ concentration constant after each meal. The kidneys are not only able to excrete the daily K^+ intake under normal feeding conditions, but they can also efficiently excrete a large overload of K^+ while maintaining the plasma K^+ concentration within tight limits. In addition, their ability to excrete an acute K^+ load is increased following chronic K^+ loading, a feature known as K^+ adaptation.

Classically, the regulation of renal K^+ excretion is considered to proceed via a feedback mechanism in which dietary K^+ ingestion increases the plasma K^+ level, which in turn stimulates pathways, in particular the secretion of aldosterone, that clear plasma K^+. Given the small magnitude of changes in plasma K^+ levels observed after a meal, an alternate, feedforward regulation mechanism has been proposed (Rabinowitz, 1996). According to this mechanism, the digestive tract senses the amount of K^+ ingested during a meal and sends messages towards target organs to stimulate their capacity to clear plasma K^+ and to thus anticipate any change in the plasma K^+ concentration. Feedback and feedforward mechanisms of K^+ balance regulation are not mutually exclusive.

Postprandial regulation of K^+ excretion

Following a 'normal' meal, the ingested load of K^+ is rapidly stored in muscles and liver so that its extracellular concentration increases only slightly (< 0.5 mmol/L). Insulin, the secretion of which increases rapidly during a meal, is the main effector of increased uptake of K^+ by liver and muscles, owing to its stimulatory action on Na^+,K^+-ATPase Thereafter, K^+ is slowly released from these storage organs into the extracellular compartment from where it is subsequently excreted, via the kidneys. Because changes in the plasma level of K^+ during this phase are too small to induce secretion of aldosterone and its kaliuretic effects, other regulation mechanisms were searched for.

Pieces of evidence supporting feedforward regulation of K^+ (Fig. 23.4) excretion were obtained in contexts resembling postprandial conditions. Initially, it was shown that a meal inducing a 0.5 mmol/L rise in plasma K^+ concentration increased the excretion of K^+, whereas intravenous administration of K^+ promoting the same increase in plasma K^+ level had no effect on K^+ excretion (Rabinowitz et al., 1985). This finding rules out the role of hyperkalaemia and aldosterone in the postprandial increase in K^+ excretion and points to the involvement of a factor of gastrointestinal origin. In a latter study (Lee et al., 2007), it was found that an intragastric infusion of K^+ did not alter the plasma level of K^+ but increased its excretion whereas intravenous injection of the same amount of K^+ raised both the plasma level of K^+ and its excretion, confirming that the postprandial excretion of K^+ is independent of hyperkalaemia but dependent instead on a factor present in the gut.

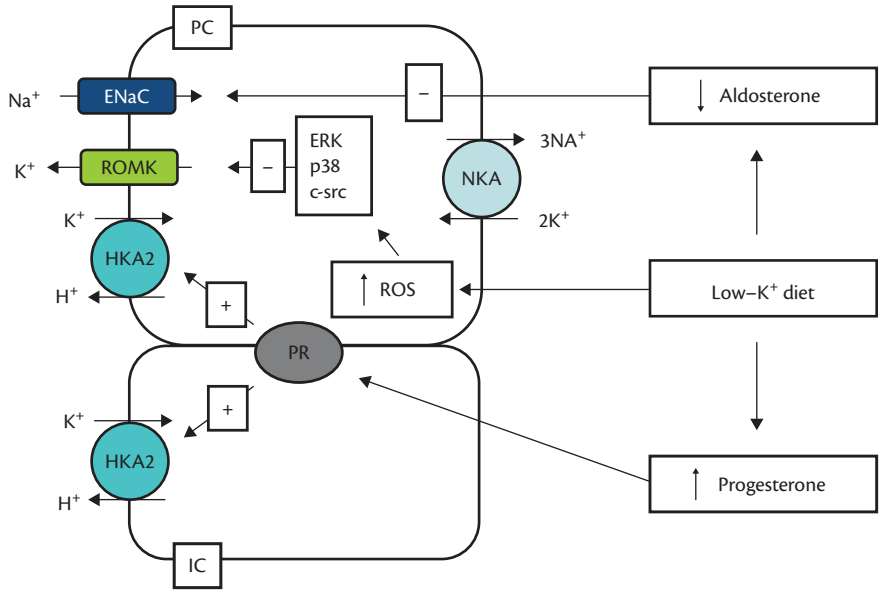

Fig. 23.4 Regulation of K^+ transport in the collecting duct during dietary K^+ depletion. Potassium depletion decreases ROMK activity in principal cells (PC) (a) through a decrease in aldosterone that reduces the electrogenic driving force for K^+ secretion and (b) by an increased production of reactive oxygen species (ROS) leading to ROMK endocytosis. In parallel, K^+ reabsorption is activated in principal and intercalated cells (PC and IC) through progesterone-induced expression of H^+,K^+-ATPase type 2 (HKA2).

Besides this gut factor, the nature of which remains unknown, other factors may participate in the feedforward regulation of postprandial renal K^+ secretion. Insulin increases urinary K^+ secretion (Hoekstra et al., 2012), but its mechanism of action is uncertain: insulin rapidly stimulates Na^+,K^+-ATPase in the CCD (Feraille et al., 1992), but a recent study in collecting duct cells shows that it also stimulates ROMK endocytosis (Cheng and Huang, 2011). Recently, it was shown that after a meal, the renal excretion of tissue kallikrein increased along with K^+ excretion, whereas the plasma levels of K^+ and aldosterone remained unchanged. Conversely, the postprandial plasma K^+ level increased in kallikrein-deficient mice. Furthermore, kallikrein was shown to inhibit H^+,K^+-ATPase activity in the collecting duct (El Moghrabi et al., 2010). Altogether these data suggest that kallikrein may contribute to the feedforward regulation of postprandial K^+ excretion by inhibiting K^+ reabsorption in the distal nephron. However, the mechanism responsible for the postprandial simulation of kallikrein release by distal tubule remains unknown.

Other factors may be involved in response to specific meals. In case of a protein-rich (and therefore K^+-rich) meal, glucagon is released by the pancreas and acts on the liver where it induces the massive production of its second messenger cAMP, which is then released in the blood. A glucagon-mediated increase in blood cAMP concentration is thought to trigger K^+ secretion along the distal nephron by promoting the renal hyperaemia and the increased GFR that are observed after a protein-rich meal, which per se increases K^+ secretion, but also through direct tubular effects on K^+ secretion (Ahloulay et al., 1996). In case of a salt-rich meal, the small intestine releases the natriuretic peptide uroguanylin. Uroguanylin has been reported to induce either kaliuretic (Fonteles et al., 1998) or antikaliuretic effects (Moss et al., 2010); the response seems to depend on two signalling mechanisms within the collecting duct that lead to either inhibition of ROMK or activation of BK.

Altogether, these factors contribute to the excretion of the dietary K^+ load on a day-to-day basis while maintaining the extracellular fluid concentration of K^+ almost constant. The amazing and unexplained feature of these regulatory mechanisms is that kidneys excrete the exact amount of K^+ ingested, and no more, even though muscles continually release K^+ in the extracellular fluid at each contraction.

Acute K^+ loading

In response to an acute K^+ load (e.g. a single K^+-enriched meal), the above regulatory mechanisms of K^+ excretion are triggered. However, because a large influx of K^+ in the extracellular fluid overcomes the storage capacity of muscles and liver, at least transiently, the extracellular concentration of K^+ increases more than after a regular meal. An increase in extracellular K^+ concentration stimulates renal K^+ excretion both directly and indirectly, in particular by increasing aldosterone secretion (Fig. 23.5).

Increasing the extracellular K^+ concentration stimulates Na^+,K^+-ATPase, which is the motor for K^+ secretion in CCD/CNT principal cells, by a substrate effect. However, this effect is likely limited because normal extracellular K^+ concentration stimulates Na^+,K^+-ATPase almost maximally. A high plasma K^+ concentration also reduces Na^+ and water reabsorption along the proximal tubule and loop of Henle and subsequently increases the delivery of Na^+ and the flow rate in the distal nephron, both of which stimulate K^+ secretion (see 'Flow rate and Na^+ delivery'). Hyperkalaemia is the most potent stimulus of aldosterone secretion by adrenal glands and, accordingly plasma aldosterone rises rapidly in response to an acute K^+ load. In the short term, aldosterone increases the abundance of ENaC and Na^+,K^+-ATPase at the apical and basolateral membrane respectively by recruiting pre-existing units (see 'Mechanism of action of mineralocorticoids').

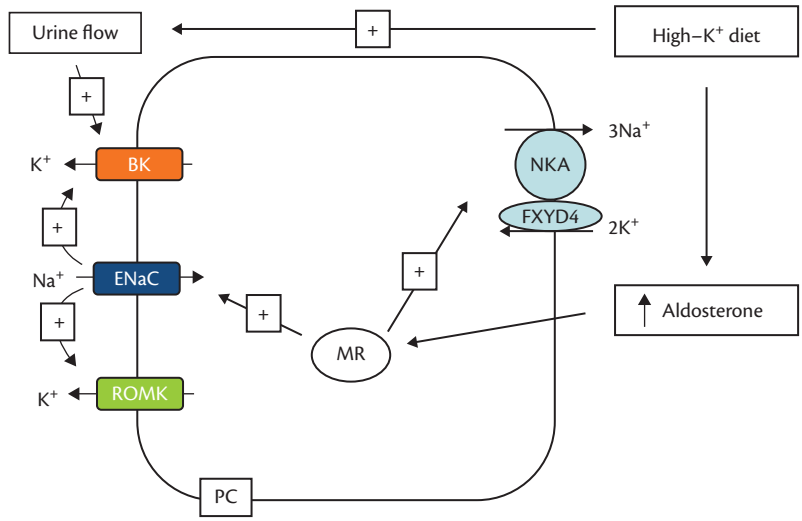

Fig. 23.5 Regulation of K^+ transport in the collecting duct during K^+ loading. Potassium loading induces increase of urinary K^+ secretion through two parallel mechanisms. Adrenal production of aldosterone is highly sensitive to increased plasma K^+ level. This hormone acts on principal cells (PC) by activating K^+ entry at the basolateral cells through activation of Na^+,K^+-ATPase and over-expression of its regulator FXYD4. Aldosterone also favours K^+ exit at the apical side by increasing the electrogenic driving force through activation of ENaC. In parallel of aldosterone action, the increase in urinary flow activates big K^+-channels (BK) through a Ca^{2+}-dependent pathway.

Chronic K^+ loading and K^+ adaptation

Chronic feeding a K^+-rich diet induces renal and extrarenal adaptations that make it possible to survive what would be a lethal K^+ overload if it were acute (Thatcher and Radike, 1947). The extrarenal adaptation mostly stems from an increased ability of muscles to accumulate extracellular K^+, owing to over-expression of Na^+,K^+-ATPase. The development of renal adaptations is mainly dependent on hyperaldosteronaemia but, when adapted, kidneys display an increased ability to secrete K^+ at any plasma levels of aldosterone and K^+. Renal adaptations lead to the increased ability of the CNT and CCD to secrete K^+ and to the recruitment of $OMCD_o$ to secrete rather than reabsorb K^+, which allows the kidney to excrete large acute overdoses of K^+. Adaptation of the CNT and CCD results from increased synthesis and membrane expression of Na^+,K^+-ATPase, FXYD4 (which decreases the Na^+,K^+-ATPase affinity for K^+) and ENaC, all of which increase the driving force for K^+ secretion as well as the redistribution of ROMK and BK channels to the apical membrane (with no change in the total amount of proteins), which increases the K^+ conductance. In addition, part of chronic K^+ loading-induced K^+ secretion in the CCD is independent of ENaC (Frindt and Palmer, 2009). These adaptations are associated with a marked enlargement of the surface area of the basolateral membrane of principal cells and enhanced density of mitochondria. This hypertrophic effect is in part due to hyperaldosteronaemia.

K^+ deprivation

Besides adjustments in their internal K^+ balance, organisms confront dietary K^+ restriction by reducing the intestinal and renal losses of K^+. The renal adaptation includes two synergistic mechanisms, the inhibition of K^+ secretion and the activation of K^+ reabsorption in the distal nephron.

Inhibition of K^+ secretion

Inhibition of K^+ secretion in the distal part of the nephron is the result of direct effects on ROMK and BK channels, and a decreased driving force. K^+ depletion decreases the circulating aldosterone level, ENaC activity, and hence the driving force for K^+ secretion in the CNT/CCD. Inhibition of ROMK involves a complex signalling cascade leading to endocytosis of ROMK channels. The initial event in this cascade is the production of reactive oxygen species (ROS). On the one hand, ROS activates ERK and p38 MAP kinases, which leads to endocytosis of ROMK. On the other hand, ROS activate c-src tyrosine kinases which, in turn, phosphorylate ROMK channels and facilitate their internalization (Babilonia et al., 2005). Interestingly, it has been shown that the production of ROS and its action on ROMK are not dependent on a hypokalaemic status.

Activation of K^+ reabsorption

Micropuncture studies have revealed that the distal tubules of rats placed under a low-K^+ diet reabsorb K^+ (Malnic et al., 1964). Identification of K^+ depletion-induced H^+,K^+-ATPase activity in the distal part of the nephron provided a molecular support to this reabsorption process. Later, this was confirmed by showing that K^+ reabsorption was inhibited by the H^+,K^+-ATPase inhibitor SCH28080. Among the two isoforms of H^+,K^+-ATPase that are expressed in the distal nephron, only type 2 (corresponding to the so-called colonic or nongastric HKA; HKA2 see 'H^+,K^+-ATPases') is overexpressed under conditions of K^+ restriction. The hormonal trigger of HKA2 stimulation was recently identified as the progesterone that is produced by adrenal glands (Elabida et al., 2011). The net production of this steroidogenesis intermediate increases because it is generated at a higher rate and consumed at a lower rate. The presence of the nuclear progesterone receptor (PR) in the distal part of the nephron (CCD, OMCD) and the sensitivity of the progesterone-induced HKA2 stimulation to the PR antagonist RU486 suggest the involvement of this receptor.

Hypertrophy

It was reported very early that dietary K^+ restriction increases kidney weight mainly as a result of enlargement of the OMCD. It is assumed that increasing the membrane surface area for ion

exchange increases the capacity for K^+ reabsorption. OMCD enlargement results from both hypertrophy (cell growth), which occurs during the first days of K^+-depletion, and hyperplasia (cell proliferation), which starts after a week of dietary restriction. The signals that triggers these effects are not fully characterized yet but growth factors such as the insulin-growth factor 1 (IGF-1) and the growth differentiation factor 15 (GDF15) are known to be involved. The renin–angiotensin system is also involved in the process of OMCD hypertrophy, since treatment with enalapril (an angiotensin converting enzyme inhibitor) impedes K^+ restriction-induced OMCD enlargement.

Flow rate and Na^+ delivery

In vivo micropuncture studies have shown that extracellular volume expansion, infusion of Na^+ salts with poorly absorbable anions, or administration of proximal tubule, and/or loop or distal tubule diuretics increases K^+ secretion along the late distal nephron (Duarte et al., 1971). Free-flow micropuncture confirmed that increasing fluid delivery to the distal tubule increases K^+ secretion (Kunau et al., 1974). Conversely, low GFR, Na^+ depletion, and extracellular volume contraction decrease K^+ secretion (Davidson et al., 1958). The common characteristic of these manoeuvres is that they alter the fluid flow rate in the distal nephron, and/or the Na^+ load delivered therein. In these experimental set-ups, it is difficult to determine which parameter among fluid flow rate, Na^+ load, and Na^+ concentration in the tubule fluid is responsible for the control of K^+ secretion.

Alternately, the possible effect of these parameters and their mechanism of action can be inferred from our current knowledge of the molecular and cellular mechanisms of K^+ secretion along the CNT and CCD. Increasing the concentration of Na^+ in the luminal fluid increases the driving force for Na^+ uptake via ENaC which in turn depolarizes the apical membrane and facilitates K^+ secretion. Increased Na^+ entry also enhances its intracellular concentration and the activity and abundance of Na^+,K^+-ATPase, which increases in turn intracellular K^+ concentration. This also favours K^+ secretion. An increased in tubular fluid flow rate is sensed by the apical cilium and translated into a rise in intracellular free calcium concentration. In turn, calcium activates BK channels. An increase in fluid flow rate also dilutes the secreted K^+ and decreases its luminal concentration, which increases the chemical gradient favourable to K^+ secretion. The Na^+ load delivered to the distal nephron is thought to modulate K^+ secretion through changes in Na^+ concentration and/or flow rate. All these parameters have synergistic effects on K^+ secretion because they affect the driving force and the membrane conductance respectively.

Acid–base balance

The acid–base status modulates K^+ transport beyond the DCT. Changes in acid–base status alter K^+ excretion both directly, by altering the activity of K^+ transport systems in the CNT/DCT and the driving force for K^+ secretion, and indirectly through changes in fluid/Na^+ delivery and aldosterone status. Direct and indirect effects alter K^+ transport in opposite directions. In the short term, direct effects prevail and metabolic acidosis decreases K^+ excretion whereas metabolic alkalosis increases it. Conversely, indirect effects prevail during chronic metabolic acidosis and lead to increased K^+ excretion and hypokalaemia.

Acute metabolic acidosis decreases K^+ excretion at any level of plasma K^+ concentration, and alkalosis does the opposite. A low pH reduces the activity of Na^+,K^+-ATPase and of ROMK in the CNT/CCD. It also inhibits the activity of ENaC and thereby hyperpolarizes the apical membrane which reduces the driving force for apical K^+ exit. Metabolic acidosis also promotes proton secretion by type A intercalated cells through the activation of V type H-ATPase. This electrogenic ATPase tends to reduce the lumen-negative transepithelial voltage generated by Na^+ reabsorption by principal cells, and thereby reduces the driving force for K^+ secretion. Finally, acidosis also increases the activity of H^+,K^+-ATPase and promotes K^+ reabsorption.

The increased K^+ excretion that is observed in chronic metabolic acidosis is accounted for by increased fluid delivery to the distal nephron and a high plasma aldosterone level. The low plasma bicarbonate concentration prevailing during metabolic alkalosis reduces the filtered load of bicarbonate, which in turn reduces the reabsorption of Na^+ and water in the proximal tubule. This proximal tubule defect increases fluid delivery to the distal nephron and induces volume depletion which triggers aldosterone secretion.

Other

Vasopressin

In vitro, vasopressin increases K^+ secretion by CNT/CCD principal cells via activation of its V2 receptors and the production of cAMP (Tomita et al., 1986). This effect is accounted for by increased activity of apical ENaC (which depolarizes the apical membrane) and of Na^+,K^+-ATPase (which increases intracellular K^+ concentration), the conjunction of which increases the electrochemical gradient favourable to K^+ secretion. Vasopressin also increases K^+ secretion by the distal nephron via activation of its V1 receptors located at the apical membrane (Amorim and Malnic, 2000). These receptors are coupled to Ca^{2+} which may therefore activate BK channels. *In vivo*, an increase in plasma vasopressin levels is mainly observed during the transition from diuresis to antidiuresis, a condition that does not alter K^+ excretion. As a matter of fact, the stimulatory effect of vasopressin on the electrochemical gradient favourable to K^+ secretion is likely blunted by the decreased fluid flow in the CNT/CCD that is associated with vasopressin-induced water reabsorption.

Adrenergic agents

Epinephrine decreases K^+ excretion by two means: (1) it stimulates K^+ uptake by muscle and liver and thereby decreases plasma K^+ concentration (Bia and DeFronzo, 1981), and (2) it exerts direct effect on the renal handling of K^+ (DeFronzo et al., 1983). This latter effect takes place beyond the DCT and is mediated mainly through activation of α-adrenergic receptors, which are located in type B intercalated cells. Activation of α-adrenergic receptors and the subsequent production of cAMP in type B intercalated cells stimulate bicarbonate secretion and generate a lumen-positive transepithelial voltage (Siga et al., 1996) that can in turn inhibit K^+ secretion by principal cells. It also increases H^+,K^+-ATPase activity and may thus enhance K^+ reabsorption.

The antikaliuretic effect of epinephrine in the distal nephron may also be mediated in part through activation of α_2-adrenergic receptors which antagonizes the effects of vasopressin and other cAMP-producing hormones in principal cells.

Diuretics

(See Chapters 28 and 29.)

Diuretics that primarily act on nephron segments localized from the proximal tubule to the distal convoluted tubule increase K^+ excretion whereas those acting on the CNT/CCD do not affect K^+ excretion. The kaliuretic effect of diuretics mostly results from inhibition of K^+ reabsorption at their site of action and/or increased secretion of K^+ along the CNT/CCD secondarily to increased delivery of Na^+ and water (flow) to these segments. In the long term, an increase in plasma aldosterone levels brought about by volume depletion also triggers K^+ secretion in the CNT/CCD.

Diuretics acting on the proximal tubule include osmotic agents (e.g. mannitol or urea) that prevent water reabsorption and inhibitors of carbonic anhydrase (e.g. acetazolamide) that inhibit Na^+ reabsorption coupled to proton secretion and consequently water reabsorption. Because K^+ reabsorption in the proximal tubule is tightly coupled to water reabsorption (see Chapter 21), proximal tubule diuretics reduce K^+ reabsorption along the proximal tubule (Velazquez and Giebisch, 1988). However, this effect is mostly compensated by increased K^+ reabsorption along the loop of Henle, and the kaliuretic effect of this class of diuretics mainly stems from flow-induced K^+ secretion along the CNT/CCD.

Through inhibition of NKCC2 in the TAL, loop diuretics (e.g. furosemide or bumetanide) primarily inhibit Na^+ reabsorption (natriuretic effect) which secondarily blunts the generation of the corticomedullary osmolarity gradient and thereby reduces water reabsorption along the medullary collecting duct (diuretic effect). Their kaliuretic effect results both from the inhibition of K^+ reabsorption along the thick ascending limb of Henle's loop (inhibition of apical K^+ entry and abolition of the transepithelial voltage) and from increased Na^+ delivery to the distal tubule (Greger, 1997).

Thiazides, the most widely used diuretics in the treatment of hypertension, mainly inhibit NCC in the DCT. This inhibition has no primary effect on K^+ transport in the DCT but it increases Na^+ delivery to the CNT/CCD and thus K^+ secretion therein (Velazquez and Giebisch, 1988). Thiazides also inhibit the electroneutral NaCl reabsorption in CNT/CCD intercalated cells (Leviel et al., 2010), but this has no effect on K^+ reabsorption.

Potassium-sparing diuretics (Horisberger and Giebisch, 1987) acting on the CNT/CCD include ENaC inhibitors (e.g. triamterene and amiloride) and antagonists of the aldosterone receptor (e.g. spironolactone). Inhibition of apical Na^+ entry in principal cells hyperpolarizes the apical membrane which reduces the driving force for K^+ secretion. Antagonists of aldosterone receptors antagonize the effects of aldosterone on ENaC and on K^+ secretion.

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CHAPTER 24

Renal acid–base homeostasis

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The kidney in systemic acid–base balance

The kidney has a central role in the maintenance and restoration of systemic acid–base balance. It does so in concert with other organs such as lung, bone, intestine, liver, or skeletal muscles. Daily metabolism produces approximately 1 mmol protons per kilogram body weight in a healthy adult on a standard diet and average physical activity, which needs to be buffered or eliminated. Elimination and buffering of these protons depend critically on the kidney. As depicted in Fig. 24.1, we absorb approximately 20 mmol acid from our diet and lose additional 10 mmol base equivalents from intestinal secretions which in total need to be replenished. The major acid load comes from daily metabolism liberating 15,000 mmol of volatile acid in the form of carbon dioxide (CO_2) that can be eliminated by ventilation, and 40 mmol of non-volatile acids that require buffering (mostly by bicarbonate) or direct renal excretion. The kidney contributes to buffering of acids by reabsorbing virtually all filtered bicarbonate and the *de novo* synthesis of bicarbonate from ammoniagenesis, leading to the excretion of approximately 40 mmol of ammonium into urine. In addition, the kidneys excrete free protons that have to be buffered by urinary buffers, so-called titratable acids (mostly phosphate and ammonium), thereby buffering and eliminating a total of 70 mmol of acid/day (Curthoys, 2008; Hamm et al., 2008).

Thus, the kidney contributes to acid–base balance with three major functions: (1) the reabsorption of filtered bicarbonate, (2) replenishing of bicarbonate buffers through ammoniagenesis, and (3) excretion of protons, involving ammonium and titratable acids as urinary buffers to increase the capacity to eliminate sufficient amounts of protons in a relatively small volume of urine.

Loss of these functions leads to various forms of metabolic acidosis seen in rare syndromes of inherited forms of renal tubular acidosis, more common forms of renal acidosis due to poisoning, hormone deficiencies, or as unwanted drug side effects, and are very common in patients with chronic kidney disease.

Acid–base handling along the nephron

The various nephron segments contribute in different ways to this task of the kidney. Proximal tubule segments are involved in bicarbonate reabsorption, ammoniagenesis, and determination of urinary excretion of titratable acids, whereas the thick ascending limb of the loop of Henle (TALH) reabsorbs mostly bicarbonate, and the collecting ducts excrete protons and ammonium, and together are the main sites of renal acid–base control and adaptation.

Bicarbonate reabsorption

Normal plasma bicarbonate concentrations are in the range of approximately 25 mmol/L; assuming a normal glomerular filtration rate of 120 mL/min, approximately 4300–4500 mmoles of bicarbonate are filtered into urine (tubular fluid). The normal urine is practically devoid of bicarbonate, which means that efficient mechanisms responsible for the reabsorption of all filtered bicarbonate operate in the proximal tubule, TALH, and initial portions of the distal convoluted tubule (DCT).

Proximal tubular bicarbonate reabsorption

The proximal tubule is the major site of bicarbonate reabsorption and accounts for about 80% of filtered bicarbonate. Both active transcellular and passive paracellular processes contribute to the efficient removal of bicarbonate from the ultrafiltrate. Removal of bicarbonate from urine and the secretion of protons result in a fall in urine pH from 7.4 (initial ultrafiltrate) to about pH 6.8 by the end of the proximal tubule (pars recta). The capacity of the proximal tubule to reabsorb bicarbonate is limited and is saturated if plasma bicarbonate levels exceed 26–28 mmol/L (Pitts et al., 1949). This threshold is explained by the maximal transport rates and abundance of proteins involved in bicarbonate reabsorption. Since bicarbonate reabsorption is achieved by Na^+/H^+ exchange and is thereby linked to Na^+ and volume status, extracellular volume contraction or expansion shifts the threshold for bicarbonate reabsorption by the proximal tubule, causing more or less bicarbonate to be reabsorbed.

Luminal mechanisms of bicarbonate transport

The reabsorption of bicarbonate in the proximal tubule is initiated by the secretion of protons into urine which is mediated by sodium/proton (Na^+/H^+)-exchangers (NHEs) and proton pumps located in the luminal brush border membrane (Fig. 24.2). About 80% of proton secretion is mediated by Na^+/H^+ exchange, whereas H^+ secretion by proton pumps contributes for about 20%. Several NHEs are expressed in the proximal tubule, NHE3 (SLC9A3) is the major isoform in adults (Orlowski and Grinstein, 2004). NHE3 contributes also as a major mechanism to sodium reabsorption in the proximal tubule. Protons for NHEs and proton pumps are provided by the activity of intracellular carbonic anhydrases (mostly carbonic anhydrase II (CAII)). The secreted protons combine in the luminal fluid with filtered bicarbonate to form CO_2 and water (H_2O). This reaction is catalysed by membrane anchored extracellular carbonic anhydrases (mostly carbonic anhydrase IV (CAIV)). CO_2 enters proximal tubule cells by diffusion. Whether passage through aquaporin 1 water channels contributes to facilitate CO_2 entry has not been fully clarified.

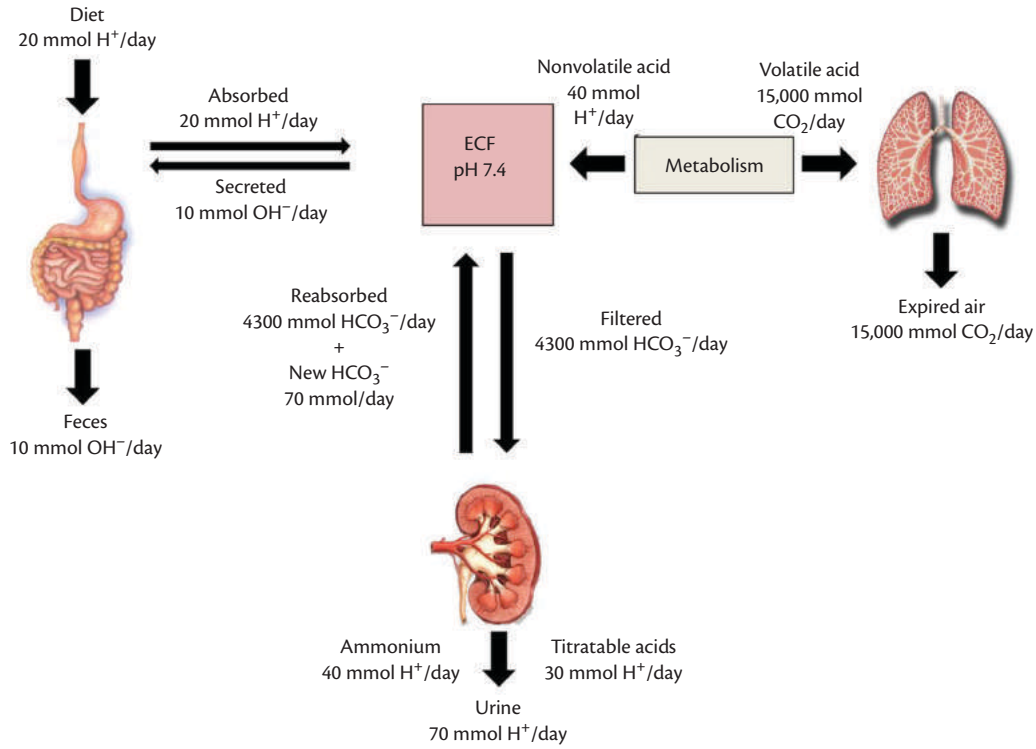

Fig. 24.1 Acid–base fluxes in a healthy adult of 70-kg body weight.
Adapted from Giebisch and Windhager (2009).

Activity of NHE3 and proton pumps at the luminal membrane is stimulated by acidosis, endothelin, and angiotensin II. Carbonic anhydrase inhibitors such as acetazolamide inhibit proximal tubular bicarbonate and sodium reabsorption by blocking CAII and CAIV activity.

Basolateral exit of bicarbonate

Bicarbonate formed by rehydration of CO_2 by intracellular carbonic anhydrases leaves proximal tubular cells across the basolateral membrane into blood via the electrogenic Na^+-bicarbonate (HCO_3) cotransporter NBCe1 (SLC4A4) (Romero et al., 1999, 2004). The transporter is stimulated by acidosis and angiotensin II (Geibel et al., 1990). Mutations in SLC4A4 underlie a severe form of inherited (autosomal recessive) proximal renal tubular acidosis (RTA, type II) often associated with ocular keratopathy, dental malformations, basal ganglia calcifications, and mental retardation (Igarashi et al., 1999).

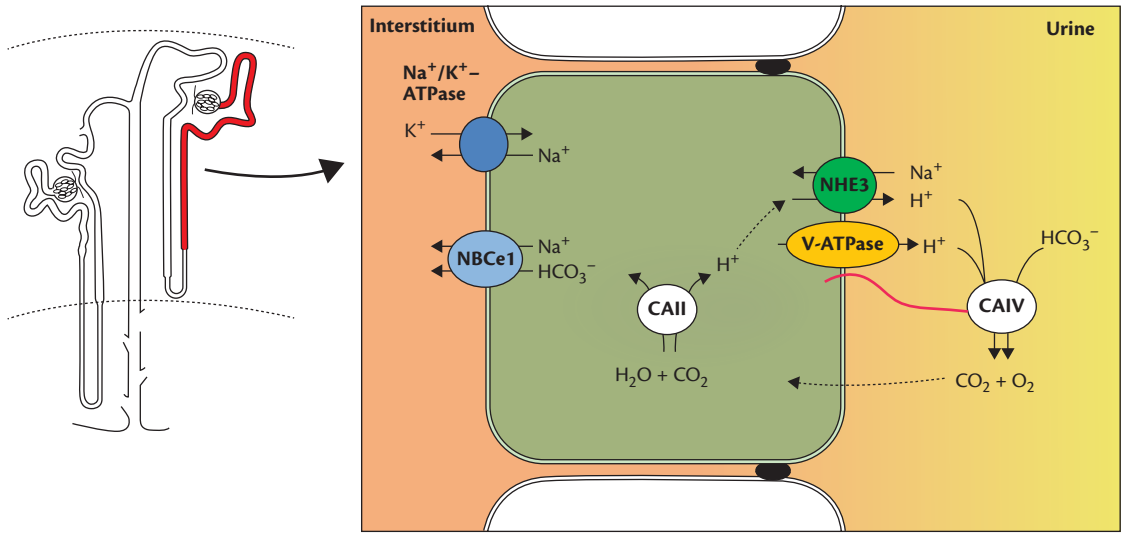

Fig. 24.2 Transcellular bicarbonate reabsorption in the proximal tubule. Absorption of filtered bicarbonate is initiated by the secretion of protons across the luminal brush border membrane of proximal tubular cells by the Na^+/H^+ exchanger NHE3 and by V-type H^+-ATPases. Luminal carbonic anhydrase IV (CAIV) catalyses the formation of CO_2 and water, CO_2 diffuses into the cells, where it is rehydrated by intracellular carbonic anhydrase II (CAII). The resulting protons are recycled across the luminal membrane, whereas the bicarbonate ions are transported into interstitium/blood by the basolateral NBCe1 Na^+-bicarbonate cotransporter.

Another form of RTA is caused by mutations in the gene encoding CAII. This autosomal recessive form of RTA (type III or mixed RTA) is characterized by loss of bicarbonate from the proximal tubule and a combined urinary acidification defect of the collecting duct (see below). Patients suffer also from osteopetrosis and mental retardation due to cerebral calcifications (Roth et al., 1992).

Paracellular movement of bicarbonate

The low rate of chloride reabsorption in the initial parts of the proximal tubule creates a lumen-negative potential that drives reabsorption of anions, including bicarbonate, through the paracellular pathway in the later parts of the proximal tubule. This movement of ions is further enhanced by solvent drag (movement of water and ions through the paracellular space) driven by the osmotic gradient between luminal tubular fluid and the interstitium, created by the active reabsorption of solutes. Solvent drag and lumen-negative potential provide mechanisms for massive bicarbonate reabsorption that do not directly use up energy in the form of ATP.

Bicarbonate reabsorption by the TALH and DCT

The filtered bicarbonate not reabsorbed by the proximal tubule is reabsorbed in the TALH and DCT. Thus, tubular fluid entering the collecting duct system contains only minute amounts of bicarbonate under conditions of acid–base balance. The mechanisms accounting for bicarbonate reabsorption in the TALH are similar to the proximal tubule, also involving NHE3 and proton pumps on the luminal membrane. The exit pathways for bicarbonate across the basolateral membrane are not well defined. The electroneutral Na^+-HCO_3 cotransporter NBCn1 is localized at the basolateral membrane, but is thought to mediate bicarbonate uptake from blood that is required for ammonium absorption by the TALH (Jakobsen et al., 2004).

The late section of the DCT is characterized by the presence of the first intercalated cells, which express a luminal proton pump (also called V-type H^+-ATPase) and a basolateral chloride-bicarbonate exchanger (AE1).

Ammoniagenesis

Ammoniagenesis occurs only in the proximal tubule and serves to eliminate protons, as well as *de novo* generation of bicarbonate from the metabolism of glutamine. Glutamine is extracted from peritubular capillaries, mediated in part by the glutamine transporter SNAT3 (SLC38A3) expressed in the basolateral membrane of proximal tubule cells (Moret et al., 2007). Filtered glutamine, reabsorbed by luminally localized amino acid transporters, may also contribute to ammoniagenesis, although the supply of glutamine is clearly not sufficient during the renal response to acid loading or acidosis. In proximal tubule cells, glutamine is imported into mitochondria and metabolized by mitochondrial phosphate-dependent glutamine and glutamate dehydrogenase to yield α-ketoglutarate. These processes liberate two NH_3 and one HCO_3^- ion per glutamine. Alpha-ketoglutarate can then be used for gluconeogenesis or further metabolized to yield an additional two HCO_3^- ions (Curthoys, 2008). The HCO_3^- synthesized during ammoniagenesis exits the cell via the basolateral NBCe1 bicarbonate transporter, whereas NH_3 either diffuses into urine and is trapped as NH_4^+ after protonation or binds intracellular protons and may be excreted into urine by the NHE3 exchanger instead of a proton. Some NH_3/NH_4^+ may also be transported into venous blood and must be detoxified by the liver. The buffering of intracellular protons by NH_3 contributes to the further elimination of protons and the replenishing of bicarbonate. Thus, complete metabolism of glutamine to glucose can thereby generate three HCO_3^- ions and two NH_3, which eliminates another two protons.

Ammoniagenesis and gluconeogenesis are stimulated during acidosis and by various hormones. Low intracellular pH leads to stabilization of mRNAs of various ammoniagenic and gluconeogenic enzymes (Curthoys, 2008; Ibrahim et al., 2008). Insulin and angiotensin II may stimulate some steps in the ammoniagenic pathway or the excretion of NH_3/NH_4^+ into urine (Chobanian and Hammerman, 1987). Prostaglandin F2-alpha ($PGEF_{2\alpha}$) on the other hand may inhibit ammoniagenesis (Sahai et al., 1995).

Most of the ammonium excreted into urine at the level of the proximal tubule is reabsorbed in the TALH by the NKCC2 cotransporter, the target of loop diuretics. NH_4^+ is then accumulated in the interstitium with a corticopapillary gradient and high concentrations of ammonium in the papilla (Wagner et al., 2011a). The high medullary concentrations of ammonium are required to maintain a gradient for ammonium secretion into urine along the collecting duct system. The ability of the medullary interstitium to accumulate high concentrations of ammonium may depend on the presence of specific negatively charged sulpholipids that bind ammonium with high affinity. Reabsorption of NH_4^+ by the TALH and accumulation in the medulla is increased during acidosis.

Acid–base excretion by the collecting duct system

The collecting duct system comprises the connecting tubule (CNT), the cortical collecting duct (CCD), the outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD). Acid–base transport occurs in all segments down to the initial part of the IMCD, whereas the last part of the IMCD does not participate in acid–base handling. The collecting duct system serves for fine-tuning of renal acid–base excretion and is critical for normal acid–base balance, as is evident from genetic disorders affecting transport proteins exclusively expressed in this segment (see below and Chapter 36). At least two types of intercalated cell mediate acid or alkali excretion, respectively. Type A intercalated cells secrete protons and ammonium, whereas type B intercalated cells excrete bicarbonate.

Proton and ammonium excretion

Type A intercalated cells generate bicarbonate from the hydration of CO_2 and the subsequent excretion of protons by V-type proton pumps (V-ATPases or H^+-ATPases) into urine (Fig. 24.3, upper panel) (Wagner et al., 2004). The role of H^+/K^+-ATPases in urinary acidification, beyond their function in potassium conservation, is unclear. The process of bicarbonate generation is catalysed by the intracellular carbonic anhydrase II (CAII). The newly formed bicarbonate is secreted into the interstitium/blood across the basolateral membrane by the anion exchanger 1 (SLC4A1, AE1) (Wagner et al., 2009). However, there may be additional anion exchangers involved, such as SLC26A7.

Type I or distal RTA (dRTA I) is caused by mutations in the AE1 (*SLC4A4*) gene (mostly autosomal dominant) or by mutations in the ATP6V1B1 (B1), or ATP6V0A4 (a4) V-ATPase subunits. Mutations in V-ATPase subunits are inherited in an autosomal

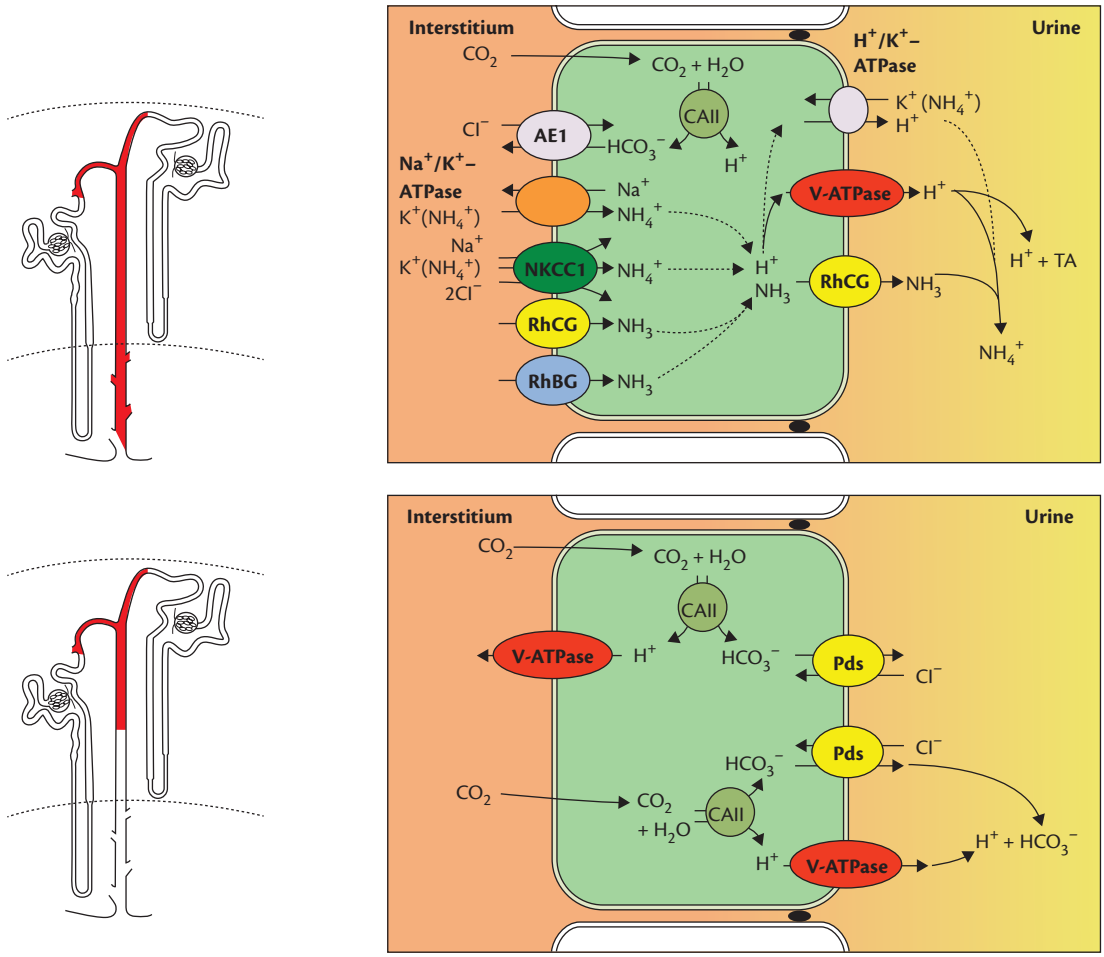

Fig. 24.3 Acid–base transport by intercalated cells in the collecting system.
(Upper panel) Type A intercalated cells generate bicarbonate catalysed by carbonic anhydrase II (CAII). The bicarbonate is exchanged for extracellular chloride by the basolateral anion exchanger AE1, whereas the proton is secreted into urine, mostly by V-type H^+-ATPases (V-ATPases). Intercalated cells also secrete NH_3 that is taken up from the interstitium by different transport routes, including Na^+/K^+-ATPases and NKCC1 where NH_4^+ substitutes for K^+, as well as by the RhBG and RhCG NH_3 transporters. At the luminal side, NH_3 is excreted into urine and is trapped after protonation to $NH4^+$.
(Lower panel) Type B and non-A/non-B intercalated cells are characterized by the expression and function of the luminal Pendrin (Pds) chloride/bicarbonate exchanger mediating bicarbonate secretion into urine. Bicarbonate secretion is driven by bicarbonate synthesis facilitated by carbonic anhydrase II (CAII) and proton secretion by V-ATPases. V-ATPases are localized on the basolateral side (type B intercalated cells) and/or luminal side (non-A/non-B intercalated cells), thereby mediating either net bicarbonate secretion or net chloride reabsorption, respectively.

recessive manner and are often associated with progressive bilateral sensorineural deafness due to expression of these V-ATPase subunits in the inner ear (Fry and Karet, 2007).

Proton secretion drives ammonium excretion along the collecting duct. Previously thought to be a passive process, ammonium excretion is now recognized to be mediated by specific transport proteins of the Rhesus (Rh) protein family (Weiner and Hamm, 2007; Wagner et al., 2009, 2011a). Two members are expressed in kidney: RhBG and RhCG, with RhBG found only in basolateral membranes, whereas RhCG is predominantly expressed in the luminal membrane, but also functional in the basolateral membrane. Both proteins are found not only in type A intercalated cells, but they have also been detected in type B intercalated cells and in principal cells. If RhCG is critical when maximal ammonium excretion is required, the role of RhBG is less clear, being potentially involved in the uptake of ammonium from the medullary interstitium. RhCG is remarkable, because it may function as a gas channel permeable only to NH_3, requiring intracellular de-protonation of NH_4^+ and then binding of protons in the urine by NH_3. The activity and expression of RhCG is upregulated during acidosis in parallel with enhanced urinary ammonium excretion (Weiner and Hamm, 2007).

Titratable acids

Titratable acids are alkali buffers binding and neutralizing protons in urine. The name refers to the process of analytical determination of titratable acids by titration of an acidified urine sample with alkali buffers. The major urinary titratable acids are inorganic phosphate (HPO_3^{2-} and H_2PO^{3-}) and citrate (Hamm et al., 1987). Other substances such as creatinine and uric acid can contribute to the buffering capacity of urine. The amount of available titratable acids depends mostly on their reabsorption in the proximal tubule, since both phosphate and citrate are freely filtered and actively reabsorbed to some extent by the proximal

tubule. The rate of reabsorption of phosphate by its major transporter NaPi-IIa, and citrate by NaDC1, is highly pH-sensitive (Aruga et al., 2000; Nowik et al., 2008). In the case of NaPi-IIa, protons directly block the transporter, increasing phosphate excretion during acidosis. NaDC1 expression is stimulated during acidosis, thereby reducing urinary citrate availability (with consequences for titratable acids and less ability to complex urinary calcium during acidosis). Increasing amounts of titratable acids, mostly phosphate, are mobilized by stimulated intestinal phosphate absorption and increased dissolution of bone matrix, releasing calcium, phosphate, and bicarbonate during prolonged periods of acidosis. Titratable acids are required to buffer protons along the collecting duct. Proton secretion by proton pumps acidifies urine, but this process is limited by the inability to pump protons against a proton gradient larger than 3.5 pH units (intracellular pH ~ 7.2 versus maximal urinary pH 4.5). Approximately 40 mmol of protons need to be excreted, whereas 1 L of unbuffered urine at pH 4.5 contains only 30 *micromoles*; without buffering, several hundred litres of urine would be required to excrete a sufficient amount of protons.

Bicarbonate excretion

HCO_3^- excretion is mediated by type B and non-A/non-B intercalated cells that are present in the late DCT, CNT, and CCD. These cells are characterized by the luminal expression of the chloride-bicarbonate exchanger pendrin (SLC26A4) secreting bicarbonate into urine in exchange for urinary chloride (Fig. 24.3, lower panel) (Royaux et al., 2001). Thus, pendrin may serve a second role in chloride absorption and blood pressure regulation (thereby providing a molecular link between plasma chloride and bicarbonate concentrations). Pendrin is critical for bicarbonate excretion, at least in mice, as indicated by *in vivo* balance and *in vitro* microperfusion studies with pendrin KO mice (Royaux et al., 2001). However, in humans with Pendred syndrome (due to mutations in pendrin/*SLC26A4*), no obvious renal phenotype has been reported to date. The activity of pendrin depends on the formation of bicarbonate by intracellular carbonic anhydrase II and even more on the activity of basolateral H^+-ATPases providing the driving force for bicarbonate secretion (Pech et al., 2006). In addition, pendrin activity may be stimulated by adrenergic agonists and aldosterone (Verlander et al., 2003; Azroyan et al., 2012; Pelzl et al., 2012; Mohebbi et al., 2013). Expression and luminal abundance are increased by alkali loading, metabolic alkalosis, aldosterone, and chloride depletion, whereas acidosis or hyperchloraemia may downregulate pendrin expression (Wagner et al., 2011b).

Regulation of renal acid–base handling

The kidneys adapt to changes in systemic acid–base status (acidaemia and alkalaemia), metabolic rates of acid or alkali production, dietary intake, or physical activity by excreting more acid equivalents and increasing ammoniagenesis or stimulating bicarbonate secretion, respectively. The exact mechanisms by which the kidney (and extrarenal organs) senses the changes in acid–base status are not clear (Brown and Wagner, 2012). However, several hormones regulate, at least in part, the adaptive process, which also involves pronounced morphological changes.

Hormonal adaption and regulation

Angiotensin II and aldosterone

Circulating levels of angiotensin II and aldosterone increase during metabolic acidosis (Schambelan et al., 1987; Gyorke et al., 1991). Blockade of the angiotensin-converting enzyme (ACE) or angiotensin II type 1 receptors delays the renal adaption to acidosis in healthy humans, as well as in various animal models (Henger et al., 2000). Angiotensin II acts on renal acid excretion by stimulating NHE3-, NBCe1- and V-ATPase-dependent bicarbonate absorption, as well as ammonium excretion in the proximal tubule, and by stimulating V-ATPase-mediated urinary acidification in the collecting duct (Geibel et al., 1990; Wagner et al., 1998; Nagami, 2002; Rothenberger et al., 2007). Angiotensin II may also increase phosphate reabsorption, thereby decreasing its delivery to the collecting duct and availability as a buffer (Riquier-Brison et al., 2010).

Aldosterone stimulates urinary acidification and type A intercalated cell function. It may act directly on V-ATPases and AE1 activity (Stone et al., 1983; Winter et al., 2004, 2011). Moreover, aldosterone stimulates sodium absorption by neighbouring principal cells through the amiloride-sensitive epithelial sodium channel (ENaC), creating a more lumen-negative potential that facilitates proton secretion by intercalated cells (Kovacikova et al., 2006). The latter mechanism underlies the urinary acidification test that uses a combination of loop diuretics (e.g. furosemide) and an aldosterone analogue (e.g. fludrocortisone), which increases sodium delivery from the upstream loop of Henle and stimulates sodium absorption by the collecting duct (Walsh et al., 2007). Inhibition of aldosterone's actions by mineralocorticoid receptor antagonists (e.g. spironolactone or eplerenone) reduces renal acid excretion. Acquired or inherited disorders of aldosterone synthesis or signalling, underlie type IV hyperkalaemic distal RTA (Sebastian et al., 1980; Karet, 2009).

Endothelin

The production and release of endothelin is stimulated during acidosis (Wesson et al., 1998). Endothelin enhances renal acid excretion directly by acting on various nephron segments and possibly indirectly by increasing aldosterone release (Wesson and Dolson, 1997; Khanna et al., 2004, 2005). Endothelin stimulates NHE3 activity in the proximal tubule and urinary acidification in distal nephron segments, which may involve V-ATPases (Eiam-Ong et al., 1992; Walter et al., 1995; Laghmani et al., 2001; Licht et al., 2004). In healthy humans, the function of endothelin in renal acid excretion is not entirely clear, and its effects may depend on salt balance (more active during salt depletion/restriction). Moreover, endothelin may *reduce* renal ammoniagenesis and acid excretion, at least during chronic acidosis. Since the above data were obtained under blockade of both major endothelin receptors (ET-A/ET-B receptors), which may have antagonistic effects (Pallini et al., 2012), experiments with selective agonists or antagonists are required to clarify the picture.

Other hormones and factors

A variety of other factors influence the renal capacity to excrete acid or base equivalents. Among these factors are the acid–base status, electrolyte intake and balance (most notably for chloride and potassium), volume status, and additional hormonal factors such as insulin, prostaglandins, norepinephrine, glucocorticoids, and many more (Hamm et al., 2008).

Mirroring the influence of pH on potassium balance, potassium depletion, and hypokalaemia are associated with metabolic alkalosis (Aronson and Giebisch, 2011). In the kidney, potassium depletion stimulates ammoniagenesis, proximal tubular bicarbonate reabsorption, as well as urinary acidification and acid excretion along the collecting duct. These effects involve glucocorticoids to some extent (Sicuro et al., 1998).

Morphological adaption and plasticity

Stimulation of renal acid or base excretion is associated with morphological changes that occur mostly in the proximal tubule and in the collecting duct. Chronic acid loading and acidosis induce hypertrophy of the kidney (Bento et al., 2005). The exact mechanisms governing such hypertrophy during acidosis are unknown. Subtle changes occur in the connecting tubule and cortical collecting duct where the proportion of the various subtypes of intercalated cells changes in response to acid or alkali loading. In particular, the relative abundance of type A intercalated cells is increased during acidosis, whereas the density of type B intercalated cells increases during alkalosis (Schwartz et al., 1985; Al-Awqati, 1996). Potential mechanisms for such plasticity include inter-conversion of type A and B intercalated cells (Al-Awqati, 2011), proliferation of the respective differentiated cell type (Duong Van Huyen et al., 2008; Welsh-Bacic et al., 2011), as well recruitment of new cells from a pool of progenitor cells. These processes may involve extracellular matrix proteins such as hensin (DMT1) (Al-Awqati, 2011), secreted factors such as GDF15 (Duong Van Huyen et al., 2008), Notch signalling (Jeong et al., 2009; Quigley et al., 2011), and transcription factors like Foxi1 or CP2L1 (Blomqvist et al., 2004; Yamaguchi et al., 2006). However, the exact regulation and role of these proteins in ontogenesis of the collecting duct, as well as plasticity, have not been clarified.

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CHAPTER 25

Phosphate homeostasis

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Renal handling of phosphate

Inorganic phosphate ions ($H_2PO_4^-$/ HPO_4^{2-}) (abbreviated as Pi) are almost freely filtered in the glomeruli. Thus Pi concentration of fluid in Bowman's space equals approximately the free phosphate concentration of plasma. From primary urine Pi is reabsorbed along the proximal tubules by a saturable process, thereby maximal rates vary considerably in response to phosphate intake and levels of different phosphaturic and antiphosphaturic factors. For individuals in phosphate balance, the daily urinary excretion of phosphate equals the net amount absorbed from the intestinal tract and usually represents 10–20% of the amount filtered (fractional excretion). In response to extremes of phosphate intake, the kidneys may excrete close to 100% or close to 0% of the filtered load. The transport maximum for phosphate (TmP) therefore is a variable rather than a constant parameter. The preferred description of the overall renal handling of phosphate is by the renal threshold of phosphate (TmP/glomerular filtration rate (GFR)); its normal range lies between 0.77 and 1.4 mmol/L (Walton and Bijvoet, 1975). Above a GFR of 40 mL/min TmP varies proportionally with GFR, so TmP/GFR is constant and is a reliable index of the tubular reabsorptive capacity. With advanced renal insufficiency (GFR < 40 mL/min), TmP is further decreased (e.g. due to secondary hyperparathyroidism) and fractional excretion of phosphate is increased. As the decrease in TmP is less than the decrease in GFR, TmP/GFR will rise and hyperphosphataemia results.

Reabsorption of filtered Pi occurs along the entire proximal tubule. Under normal conditions, reabsorption of Pi in proximal tubules shows both axial and internephron heterogeneity: highest rates are usually observed in S1 segments of juxtamedullary nephrons. Whether distal tubular segments contribute significantly to renal handling of Pi is still controversial and possible molecular mechanisms that eventually may be involved in a distal tubular reabsorption of Pi have not been characterized.

Proximal tubular Pi reabsorption

As demonstrated with isolated tubules and isolated brush border membrane vesicles, proximal tubular reabsorption of Pi across the luminal (brush border) membrane is dependent on the presence of sodium ions (Na/Pi cotransport) (Ullrich and Murer, 1982). Mechanistically, Na/Pi cotransport represents a secondary active, transcellular process that is driven by Na^+/K^+-ATPases localized at the basolateral membrane. The intracellular concentration of Pi (~1 mM) is below the thermodynamic equilibrium when taking into account the inwardly directed Na^+ gradient and the transmembrane potential of around −60 mV. Therefore, altered rates of apical Na^+/Pi cotransport are mostly achieved by a change of the number of (functional) transporter units. Exit of Pi occurs through the basolateral membrane by a mechanism that is poorly understood. No evidence for a paracellular pathway for Pi has yet been obtained.

Among the known and characterized mammalian Na^+/Pi cotransporters, three have been localized at the apical membrane of proximal tubular cells: two members of the SLC34 family, namely SLC34A1 (NaPi-IIa) and SLC34A3 (NaPi-IIc) and one member of the SLC20 family, namely SLC20A2 (PiT-2) (Biber et al., 2008; Picard et al., 2010). Although the presence of PiT-1 (Glvr-1) mRNA has been detected in mouse kidney (Tenenhouse et al., 1998), the localization of the PiT-1 protein remains to be determined.

Transport, by both NaPi-IIa and NaPi-IIc, is dependent on the presence of Na^+ ions and displays an apparent affinity constant for Pi of typically < 0.1 mM (divalent HPO_4^{2-} ions are preferentially transported) and an apparent affinity constant for Na^+ ions in the range of 40–60 mM. Arsenate is the only other substrate known to be transported by the type II Na^+/Pi cotransporters. Transport activity of NaPi-IIa is electrogenic, whereas for NaPi-IIc it is electroneutral. NaPi-IIa translocates one net positive charge per transport cycle and thus transport rates increase with membrane hyperpolarization. On the other hand, electroneutral transport by NaPi-IIc is insensitive to membrane potential. This functional distinction is reflected in their respective Na^+: Pi stoichiometries: 3 Na^+: 1 HPO_4^{2-} for NaPi-IIa and 2 Na^+:1 $HPO4^{2-}$ for NaPi-IIc. Loading of NaPi-IIa and NaPi-IIc proteins with substrates is proposed to be ordered. By biophysical analysis it was established that two Na^+ ions bind sequentially and cooperatively before one phosphate ion is bound. A third Na^+ binding precedes reorientation of the fully loaded carrier. For NaPi-IIc, one of the Na^+ ions, which confers electrogenicity to NaPi-IIa, can still interact with the protein but is not cotransported. Transport by NaPi-IIa and NaPi-IIc is blocked by the competitive inhibitor phosphonoformic acid or foscarnet (PFA) with a reported inhibition constant of approximately 0.4–0.6 mM. PFA itself is not transported (Virkki et al., 2007).

Both N- and C-termini, of type II Na/Pi-cotransporters are cytoplasmically oriented and 12 transmembrane domains (TMDs) are predicted by a current model of the secondary structure. It was established that the functional unit of NaPi-IIa is a monomer. Yet, by a yeast two-hybrid split ubiquitin approach and by freeze-fracture analysis of *Xenopus* oocyte membranes containing the expressed NaPi-IIa proteins, evidence has been obtained that dimerization of NaPi-IIa proteins may occur (Forster et al., 2002, 2006).

PiT-2 (SLC20A2) was originally identified as a retroviral receptor (Ram-1), and later was shown to be a Na^+-coupled Pi transporter (Collins and Ghishan, 2004). In contrast to SLC34 proteins, SLC20

proteins preferentially transport monovalent Pi ($H_2PO_4^-$) with a 2:1 Na^+: Pi stoichiometry, thus transport is electrogenic. The apparent affinities for Pi and Na^+ are typically ≤ 100 μM and approximately 50 mM, respectively (Bottger et al., 2006; Ravera et al., 2007; Virkki et al., 2007). To date, no specific inhibitors for SLC20 transporters have been reported; PFA is a poor inhibitor of SLC20 transporters (Villa-Bellosta et al., 2007). Similarly as for SLC34 transporters, 12 TMDs have been predicted, but with extracellular N- and C-terminal tails (Farrell et al., 2009).

How do different Na/Pi-cotransporters contribute to renal Pi reabsorption?

Knowledge about the relative roles of the different proximal tubular apical Na/Pi-cotransporters in renal handling of Pi has been obtained from the use of knockout mouse models (Miyamoto et al., 2011) and from analysis of hereditary human diseases with hypophosphataemia (Alizadeh and Reilly, 2010; Amatschek et al., 2010). Collectively, current information indicates that the relative roles of known renal Na/Pi-cotransporters (specifically of NaPi-IIa and NaPi-IIc) differ between man and mice. Whether PiT-2, localized in proximal tubules, significantly contributes to renal Pi reabsorption is currently not known and remains to be investigated.

In adult mice, NaPi-IIa appears to be the dominant Na/Pi cotransporter (Beck et al., 1998; Tenenhouse, 2005). The phenotype of NaPi-IIa knockout mice was described as hypophosphataemic, hyperphosphaturic, and Na^+/Pi cotransport in isolated renal membrane vesicles was reduced by approximately 70%. Most of the residual Na^+/Pi-cotransport was attributed to NaPi-IIc that is upregulated in these mice. In contrast, NaPi-IIc knockout mice do not show any phenotype related to Pi homeostasis. In these mice, renal Pi handling was normal, indicating that NaPi-IIc does not play a significant role in adult mice (Segawa et al., 2009; Miyamoto et al., 2011).

In contrast to mice, in humans NaPi-IIc appears to play a more important role. Various missense mutations and large deletions in the *SLC34A3* gene have been ascribed as the genetic cause of patients with hereditary hypophosphataemic rickets with hypercalciuria (HHRH) (Berwitz et al., 2006; Lorenz-Depiereux et al., 2006; Tencza et al., 2009; Amatschek et al., 2010; Miyamoto et al., 2011). These findings indicate that, in adult humans, the functional role of NaPi-IIc is more prominent compared to adult mice. In contrast, the role of the NaPi-IIa transporter in humans appears to be less important and remains somewhat controversial. Despite genetic variants that have been found within the *SLC34A1* gene, these polymorphisms within the NaPi-IIa gene seem not to be linked to renal phosphate anomalies (Lapointe et al., 2006). Of interest, in two siblings of a family with hypophosphataemic rickets with renal phosphate wasting, a duplication of a short amino acid stretch in NaPi-IIa was reported that was associated with hyperphosphaturia and a general Fanconi syndrome (Magen et al., 2010).

Regulation of proximal tubular Pi reabsorption

Regulation of proximal tubular reabsorption of Pi and consequently of urinary Pi excretion is primarily achieved by an alteration of the number of Na^+/Pi cotransporters residing in the apical membrane of proximal tubular cells (Fig. 25.1). Alterations in the number of

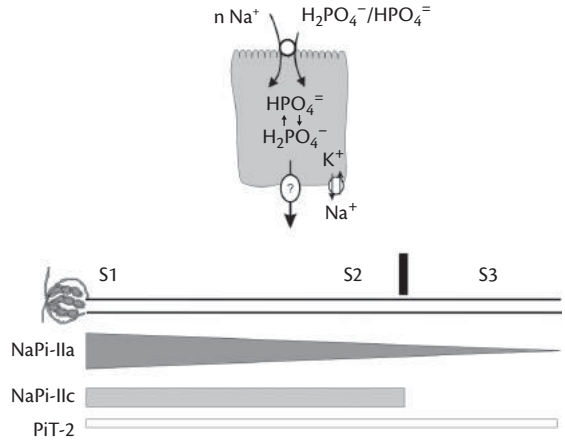

Fig. 25.1 Basis scheme of transepithelial transport of phosphate in proximal tubules and distribution of Na/Pi cotransporters along the proximal tubular segment.

Na^+/Pi cotransporters are achieved by regulated endocytosis on the one hand and by insertion of *de novo* synthesized proteins on the other hand. Currently, no direct modifications of known Na^+/Pi cotransporters are known that could change their transport characteristics (e.g. altered K_m values for Pi ions or Na^+ ions). Known factors (hormones and metabolic factors) that regulate renal reabsorption of Pi by altering the amount of Na^+/Pi cotransporters are listed in Table 25.1. Of note, the time course of changes in the amount of the different Na/Pi cotransporters (NaPi-IIa, NaPi-IIc, and PiT-2) varies significantly, indicating that different cellular mechanisms are involved or that certain factors act indirectly and are part of a regulatory network.

Parathyroid hormone

Besides its actions on calcium metabolism, PTH modulates the amount of apical Na^+/Pi cotransporters (Bacic et al., 2006; Lee and Partridge, 2009). PTH receptors are localized both at the luminal

Table 25.1 Hormonal and non-hormonal factors affecting renal excretion of phosphate

Excretion increased by	Excretion decreased by
Parathyroid hormone	Growth hormone
Dopamine	Insulin-like growth factor
Phosphatonins (FGF-23;	$1,25(OH)_2D3$
sFRP-4; MEPE)	Phosphate depletion
Glucocorticoids	metabolic alkalosis
Atrial natriuretic peptide	Volume contraction
Phosphate loading	Hypocalcemia
Metabolic acidosis	
Carbonic anhydrase inhibitors	
Estrogen	
Diuretics	

and basolateral membrane of proximal tubular cells. Upon activation, apically localized PTH receptors activate phospholipase C (PLC)/protein kinase C (PKC) pathway, whereas basolaterally localized PTH receptors activate a cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway (Forster et al., 2002, 2006; Gensure et al., 2005). By the use of different PTH analogues it was suggested that regulation of the NaPi-IIa protein occurs primarily via the cAMP/PKA pathway (Nagai et al., 2011).

In rodents, PTH leads to a rapid (within minutes) downregulation of the NaPi-IIa protein, while downregulation of NaPi-IIc protein is slower and is observed only after a few hours. In contrast, the regulation of PiT2 by PTH is less clear (Picard et al., 2010). A decrease of the amount of NaPi-IIa proteins occurs via enhanced clathrin-mediated endocytosis and internalized NaPi-IIa proteins accumulate transiently in early endosomes in the subapical compartment. Interestingly, internalized NaPi-IIa proteins are not recycled, but are sorted out of early endosomes and routed to the lysosomes for degradation (Bacic et al., 2004, 2006; Forster et al., 2006). A change of the amount of NaPi-IIc protein by PTH occurs by a slower rate and, although associated with clathrin-coated vesicles, NaPi-IIc protein does not accumulate in lysosomes (Segawa et al., 2007; Lanzano et al., 2011).

NaPi-IIa interacts with the NHERF1 protein (Na^+/H^+ exchange regulatory factor 1). This interaction is via the C-terminal amino acid motif TRL of the NaPi-IIa protein with the first (of two) PDZ domains of NHERF1 (Biber et al., 2004). The importance of NHERF1 for apical localization of NaPi-IIa proteins was revealed by the use of NHERF1 knockout mice, which demonstrate phosphaturia and hypophosphataemia due to reduced apical abundance of NaPi-IIa proteins (Weinman et al., 2005). Stimulation of either PKA or PKC signalling pathways by PTH results in a hyperphosphorylation of NHERF1 at residues Ser77 and Thr95 that are located within the first PDZ domain of NHERF1. Thus, it is conceivable that phosphorylation of Ser77 and Thr95 destabilizes the interaction of NaPi-IIa with NHERF1 and therefore allow higher lateral mobility along the microvilli and internalization of NaPi-IIa at intermicrovillar clefts (Weinman et al., 2010); no such mechanism has been demonstrated for regulation of NaPi-IIc by PTH.

Dopamine

In kidney, dopamine is synthesized from dihydroxyphenylalanine (DOPA) and acts via para-/autocrine mechanisms (Glahn et al., 1993; Friedlander, 1998). Activation of apically localized dopamine receptors D1 results, similar to PTH, in an internalization of the NaPi-IIa transporter (Bacic et al., 2005). Dopamine-induced activation of signalling cascades involving PKA and PKC results in phosphorylation of the first PDZ domain of NHERF1, resulting in a dissociation of the NaPi-IIa/NHERF1 complex (Weinman et al., 2010).

A possible role of dopamine in the adaptive response of renal Pi reabsorption (see below) has been suggested recently as the dopamine concentration in urine was increased after feeding mice with a diet of high Pi content (Weinman et al., 2011).

Phosphatonins

The term phosphatonin was introduced for factors that induce hypophosphataemia and an increase of renal Pi wasting but do not result in adequate production of 1,25 dihydroxyvitamin vitamin D_3, for example, in patients with oncogenic osteomalacia (TIO) or with autosomal dominant hypophosphataemic rickets (ADHR). The following phosphatonins have been identified (Berndt and Kumar, 2009).

Fibroblast growth factor 23

A positional cloning approach identified fibroblast growth factor 23 (FGF23) in ADHR patients (White et al., 2000) and later FGF23 was identified as the causal factor in tumour-induced osteomalacia (Shimada et al., 2001; Berndt and Kumar, 2009). Consequently, FGF23 has emerged as a major phosphaturic hormone (Prie et al., 2009; Bergwitz and Jüppner, 2010; Farrow and White, 2010).

Bone cells, osteoblasts, and osteocytes seem to be the major source of circulating levels of FGF23 and synthesis and excretion of FGF23 is regulated by the plasma levels of Pi and vitamin D_3 (Cheng and Hulley, 2010). The renal effects of FGF23 are twofold: on the one hand, FGF23 decreases reabsorption of Pi and on the other hand influences the level of vitamin D_3 by inhibition of 1,25α-hydroxylases and by stimulation of 24,25-hydroxylases (Bergwitz and Jüppner, 2010; Long and Kharitonenkov, 2011). FGF23 decreases the abundance of proximal tubular Na/Pi cotransporters NaPi-IIa, NaPi-IIc, and PiT2 as demonstrated with FGF23 null mice, overexpression and injection of FGF23, and by incubation of isolated proximal tubules with FGF23 (Farrow and White, 2010; Gattineni and Baum, 2010; Tomoe et al., 2010).

FGF23 regulates the abundance of apical Na/Pi cotransporters by activation of the fibroblast growth factor receptor FGFR1c. Interestingly, reduction of 1,25α-hydroxylase by FGF23 occurs via the FGF receptor FGFR3 and -4 (Gattineni and Baum 2010; Gattineni et al., 2011). Intracellular mechanisms of FGF23 leading to reduction of Na^+/Pi cotransporters are poorly understood, but may be via the mitogen-activated protein kinase (MAPK) cascade involving phosphorylation of extracellular signal-regulated kinase ERK1/2 (Farrow and White, 2010). A possible role of prostaglandins in FGF23 regulation of Na/Pi cotransporters has also been discussed (Gattineni and Baum, 2010).

Full bioactivity of FGF23 via the FGFR1c receptor requires the presence of the membrane associated isoform of Klotho. Co-activation of the FGFR1 by Klotho appears to be obligatory as Klotho null mice display the same phenotype as FGF23 null mice (Farrow and White, 2010). Of interest, in kidney, the primary site of expression of Klotho is the distal tubule (Kuro-o 2011), but expression of Klotho in proximal tubules has also been demonstrated (Huang and Moe 2011). The extracellular domain of Klotho is found in circulation, as a secreted isoform or as a proteolytic cleavage product. Klotho exhibits glycosidase-like activities and was shown to modify sugar residues, for example, of the calcium channel TRPV5 and thereby influencing the turnover of this channel (Cha et al., 2008). A similar action of circulating Klotho was postulated for the regulation of the NaPi-IIa protein (Huang and Moe, 2011).Thus, Klotho may be a phosphaturic factor in the absence of FGF23.

Secreted frizzled-related protein-4

In patients with tumour associated osteomalacia and renal Pi wasting, Secreted frizzled-related protein-4 (sFRP-4) is highly overexpressed (De Beur et al., 2002; Berndt and Kumar, 2009). Infusion of sFRP-4 into rats induces phosphaturia due to a reduced abundance of the type lla Na^+/Pi cotransporter. Similar observations were made with parathyroidectomized rats, indicating that the action of sFRP-4 is independent of PTH. In agreement with sFRP-4

being an antagonist of the WNT pathway, infusion of sFRP-4 into rats induced phosphorylation of β-catenin (Berndt et al., 2006). However, the role of sFRP-4 in phosphate homeostasis has been questioned based on genetic deletion in mice (Christov et al., 2011).

Matrix extracellular phosphoglycoprotein

Bone cells are the major source of matrix extracellular phosphoglycoprotein (MEPE) and expression of MEPE is increased in XLH patients and in Hyp mice, as well as in patients with oncogenic osteomalacia (Berndt and Kumar, 2009; Friedlander, 2010). Infusion of MEPE results in a reduction of the type IIa Na^+/Pi cotransporter and a correlation of increased levels of MEPE with phosphaturia has been demonstrated by a micropuncture study on proximal tubules after infusion of MEPE (Shirley et al., 2010). The cellular pathway involved in the regulation of Pi reabsorption by MEPE remains to be elucidated.

Fibroblast growth factor 7

FGF7 is overexpressed in oncogenic osteomalacia, yet its direct effect on renal Na^+/Pi cotransporters has not yet been demonstrated in *in vivo* situations. Nor is it known if FGF7 alters the metabolisms of vitamin D_3 (Gattineni and Baum, 2010).

Dietary intake of Pi

In addition to the above mentioned factors, the abundance of proximal tubular Na^+/Pi cotransporters and consequently phosphate excretion are influenced by dietary intake of Pi (Biber et al., 2008). The effects provoked by ingestion of different amounts of Pi can be subdivided into (sub-)acute (minutes, hours) and chronic (days) effects. Notably, the time courses of the changes in the abundance of the different Na^+/Pi cotransporters (NaPi-IIa, NaPi-IIc, and PiT2) differ markedly (Biber et al., 2008).

By mechanisms that are not yet understood, intake of a low-Pi or high-Pi diet alters, independently of PTH and other known factors, urinary excretion of Pi within a few hours that is paralleled primarily by an alteration of the amount of the NaPi-IIa cotransporter. A high-Pi diet acutely downregulates NaPi-IIa proteins by a mechanism similar as that described for PTH-mediated downregulation; that is, NaPi-IIa proteins are internalized and are degraded in lysosomes (Biber et al., 2008). The abundance of the NaPi-IIc and the PiT2 cotransporter is also influenced by altered dietary contents of Pi, yet the time courses of changes of NaPi-IIc and PiT2 abundances are slower and require several hours. The signal(s) that trigger(s) the alterations in the amount of NaPi lla transporters by altered intake of phosphate is (are) not known. Two possibilities have been postulated and discussed (Bergwitz and Jüppner, 2011): (1) proximal tubular cells may sense changes of luminal concentration of Pi (by an as yet unknown Pi sensor mechanism) directly and/or, (2) a respective signal is generated in the small intestine. With regard to the latter possibility, it is of interest that direct application of a Pi bolus into the duodenum of rats induced an increase of the fractional excretion of Pi already after 15 minutes that did not correlate with PTH or FGF23 and is not dependent on the innervation of the kidney. Based on this observation, a factor was postulated that eventually is released from the intestinal mucosa due to the altered amount of Pi within the intestinal lumen (Kumar 2009). This factor remains to be determined. In contrast, another study showed that Pi, when directly applied into the gut, alters PTH levels within minutes (Martin et al., 2005). Taken together, there is evidence that the gut may sense the amount of ingested Pi and may release (a) factor(s) influencing directly or indirectly renal excretion of Pi.

Intake of different Pi diets also alters renal vitamin D_3 metabolism, for example, a low-Pi diet stimulates both transcription and activity of the 1,25α-hydroxylase activity (Dusso et al., 2005). Compared to alterations of the NaPi-IIa cotransporter, this effect is slower and observed after approximately 1 day and is (in contrast to the response on NaPi-IIa protein) dependent on an intact pituitary gland (Tenenhouse et al., 1988).

Hypokalaemia

Phosphaturia associated with chronic hypokalaemia is explained by reduced Na^+/Pi cotransport activity in isolated brush border membranes. Hypokalaemia results in a decrease of the mRNAs of all Na^+/Pi cotransporters, but, paradoxically, only the amounts of NaPi-IIc and PiT-2 proteins were decreased while the abundance of NaPi-IIa proteins is increased (Breusegem et al., 2009). As hypokalaemia provokes metabolic alkalosis, alterations of apical Na^+/Pi cotransporters could, mechanistically, be similar to pathways involved in acid/base induced changes of Pi reabsorption.

Acid/base changes

Altered renal excretion of Pi under acidotic or alkalotic conditions respectively can be explained either by a change in extracellular Pi concentrations and altered filtered load of Pi or by alterations of the amount of renal Na^+/Pi cotransporters. Respiratory alkalosis causes a redistribution of phosphate into cells, resulting in hypophosphataemia, whilst metabolic acidosis increases bone release of Pi. In rats, metabolic acidosis induces acutely (within a few hours) retrieval of NaPi-IIa cotransporter protein and after chronic metabolic acidosis reduces the amount of NaPi-IIa mRNA. In contrast, in another study in mice and rats, although reduced amount of Na/Pi cotransporter mRNAs were observed after metabolic acidosis, the amount of NaPi-IIa and NaPi-IIc proteins was unchanged (Nowik et al., 2008). A direct interaction between protons and the transporter may explain the reduction of phosphate absorption due to reduced activity of normally expressed transporter proteins.

Oestrogen

In rats, oestrogen provokes phosphaturia due to a reduction in the amount of NaPi-IIa cotransporters and this effect was not correlated with levels of PTH (Faroqui et al., 2008; Guttmann-Rubinstein et al., 2010). As oestrogen treatment may induce production of dopamine and/or upregulation of dopamine receptors, an indirect effect of oestrogen cannot be ruled out.

Glucocorticoids

Several reports indicate that glucocorticoids, independent of PTH, regulate renal excretion of phosphate excretion (Levi et al., 1995 and references therein) due to changes of the abundance of the NaPi-IIa protein (Loffing et al., 1998). This effect appears to be associated with an altered composition of membrane lipids, such as, for example, glucosylceramide (Levi et al., 1995).

Volume expansion

Extracellular fluid volume expansion or contraction induces phosphaturia or decreases excretion of Pi. Increased phosphaturia induced by volume expansion may be explained by different mechanisms: (a) volume expansion leads to inhibition of proximal tubular reabsorption of sodium and water, and consequently to a dilution of the luminal Pi concentration; (b) volume expansion decreases the serum concentration of calcium and thus increases

secretion of PTH; and (c) the volume expansion-associated increase of phosphaturia is accompanied by a reduced rate of Na^+/Pi cotransport in isolated brush border membrane vesicles (Liput et al., 1989).

Genetic alterations leading to altered renal handling of Pi

Genetic defects that alter renal Pi handling can be localized either in NaPi cotransporter genes or in genes coding for factors/cofactors that regulate proximal tubular reabsorption of Pi.

As previously discussed, HHRH has been associated with mutations in the NaPi-IIc gene (Bergwitz et al., 2006; Tencza et al., 2009), whereas implication on the development of hypophosphataemia of mutants found in the NaPi-IIa gene remains controversial (Lapointe et al., 2006).

Several mutations in the *FGF23* gene itself or in genes coding for proteins involved in the synthesis, processing, and degradation of FGF23 were identified that cause disturbances in renal Pi reabsorption.

Mutations leading to elevated levels of FGF23: mutations within the proteolytic site (RXXR) of FGF23 prevent the degradation of FGF23 and thus result in elevated levels of FGF23. Such mutations are causal for autosomal dominant hypophosphataemic rickets. Elevated levels of FGF23 are achieved also by mutations in *PHEX*, a gene with homology to endopeptidases, as observed in patients with X-linked hypophosphataemia. However, if FGF23 is a substrate for PHEX remains controversial. Furthermore, high levels of FGF23 have been attributed to mutations in the dentin matrix acidic phosphoprotein 1 (DMP1) (Strom and Juppner, 2008).

Mutations leading to reduced levels of FGF23: patients with mutations in the *GALINT3* gene are hyperphosphataemic due to low levels of circulating FGF23. GALNT3 is glycosyl transferase that glycosylates FGF23 thereby making FGF23 more resistant to proteolytic cleavage (Ichikawa et al., 2009).

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CHAPTER 26

Calcium homeostasis

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Introduction

Calcium (Ca^{2+}) is the fifth most copious element in the human body, with approximately 1000 g present in adults. It plays an important role in skeletal mineralization and in a wide variety of biological functions. Dietary Ca^{2+} intake is essential for the body. Recommended dietary Ca^{2+} intake is 1000–1500 mg/day, depending on age (McCabe et al., 2004).

Ca^{2+} homeostasis is regulated by three key mechanisms: intestinal and renal reabsorption, and bone turnover. These, in turn, are adjusted by a set of interrelated hormones, including parathyroid hormone (PTH), 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), ionized Ca^{2+} itself, and their matching receptors in the gut, kidney, and bone.

Calcium distribution

Most of the total body Ca^{2+} (about 99%) is confined to the skeleton as Ca^{2+}-phosphate complexes, primarily as hydroxyapatite, where it guarantees skeletal strength and, at the same time, a constantly exchangeable store for the body (Wang et al., 2006). Ca^{2+} regulates a range of crucial functions, including extra- and intracellular signalling, muscle contraction, and nerve impulse conduction (Bootman et al., 2001). Total serum Ca^{2+} ranges from 2.2 to 2.6 mmol/L (8.8–10.4 mg/dL) in healthy subjects. It comprises free ions (51%), protein-bound complexes (40%), and ionic complexes (9%). To prevent Ca^{2+} toxicity, the concentration of serum ionized Ca^{2+} is closely maintained within the physiological range of 1.10–1.35 mmol/L (4.4–5.4 mg/dL). Non-ionized Ca^{2+} is bound to an array of various proteins and anions in both the extra- and intracellular pools. The main Ca^{2+} binding proteins are albumin and globulin in serum, and calmodulin and other Ca^{2+}-binding proteins in the cell. The major ionic complexes in serum are calcium phosphate, calcium carbonate, and calcium oxalate.

Calcium homeostasis

Ca^{2+} homeostasis is largely regulated through an integrated hormonal system that controls Ca^{2+} transport in the gut, kidney, and bone (Fig. 26.1). It involves two major Ca^{2+} -regulating hormones and their receptors: PTH and the PTH receptor protein (PTHrP) (Potts and Gardella, 2007), $1,25(OH)_2D_3$ and the vitamin D receptor (VDR) (Jurutka et al., 2001), as well as serum ionized Ca^{2+} and the Ca^{2+} -sensing receptor (CaSR) (Brown, 2007). Serum Ca^{2+} homeostasis is set to keep extracellular ionized Ca^{2+} levels in the physiological range. A decrease in serum Ca^{2+} inactivates the CaSR in the parathyroid glands, causing an increase in PTH secretion. PTH stimulates renal and bone Ca^{2+} reabsorption. In addition, PTH stimulates the synthesis of $1,25(OH)_2D_3$ (active vitamin D) in the kidney, which promotes intestinal Ca^{2+} absorption, and in a feedback loop inhibition of PTH secretion. The decrease in serum Ca^{2+} inactivates directly the CaSR in the kidney, leading to additional Ca^{2+} reabsorption and enhances the renal action of PTH. This integrated hormonal response re-establishes serum Ca^{2+} and shuts off the negative feedback loop; in contrast, an increase in Ca^{2+} level increases Ca^{2+} excretion and bone storage (Peacock, 2010).

Renal calcium handling

The kidney is the main organ that controls Ca^{2+} excretion. Every day, roughly 8 g of Ca^{2+} is filtered at the glomerulus, of which < 2% is excreted into the urine. Ca^{2+} is reabsorbed throughout the nephron: the principal sites are the proximal tubule, the thick ascending limb, and the distal tubule (Fig. 26.2).

The proximal tubule

Along the proximal tubule Ca^{2+} transport is, in essence, an iso-osmotic process, energetically passive, proceeding through the paracellular pathway. Nevertheless, renal micropuncture experiments, performed under experimental conditions in which the driving force for passive Ca^{2+} movement has been eliminated, demonstrate that 10–15% of the reabsorption is active, implicating a cellular pathway for this process (Ullrich et al., 1977; Petrazzuolo et al., 2010). Total proximal tubule reabsorption accounts for about 65% of total Ca^{2+} filtered at the glomerulus.

The loop of Henle

In the thin descending and ascending limbs of Henle, the permeability for Ca^{2+} is very low, and basically we can infer that significant net Ca^{2+} transport does not occur in these segments. Because these thin limbs of Henle do not transport Ca^{2+}, the thick ascending limb of the loop of Henle (TALH) is responsible for the Ca^{2+} reabsorption between the bend of the loop and the start of the distal convoluted tubule (DCT). Approximately 25% of the Ca^{2+} filtered at the glomerulus is reabsorbed in Henle's loop (Suki, 1979). Evidence has been provided indicating that Ca^{2+} transport is driven by the electrochemical gradient due to the recycling of potassium ions through the luminal membrane, compatible with a passive absorptive process (Bourdeau and Burg, 1979). These results have been confirmed by other investigators who provided evidence that PTH stimulates passive Ca^{2+} transport by increasing the electrical driving force and permeability for the paracellular pathway (Wittner et al., 1993).

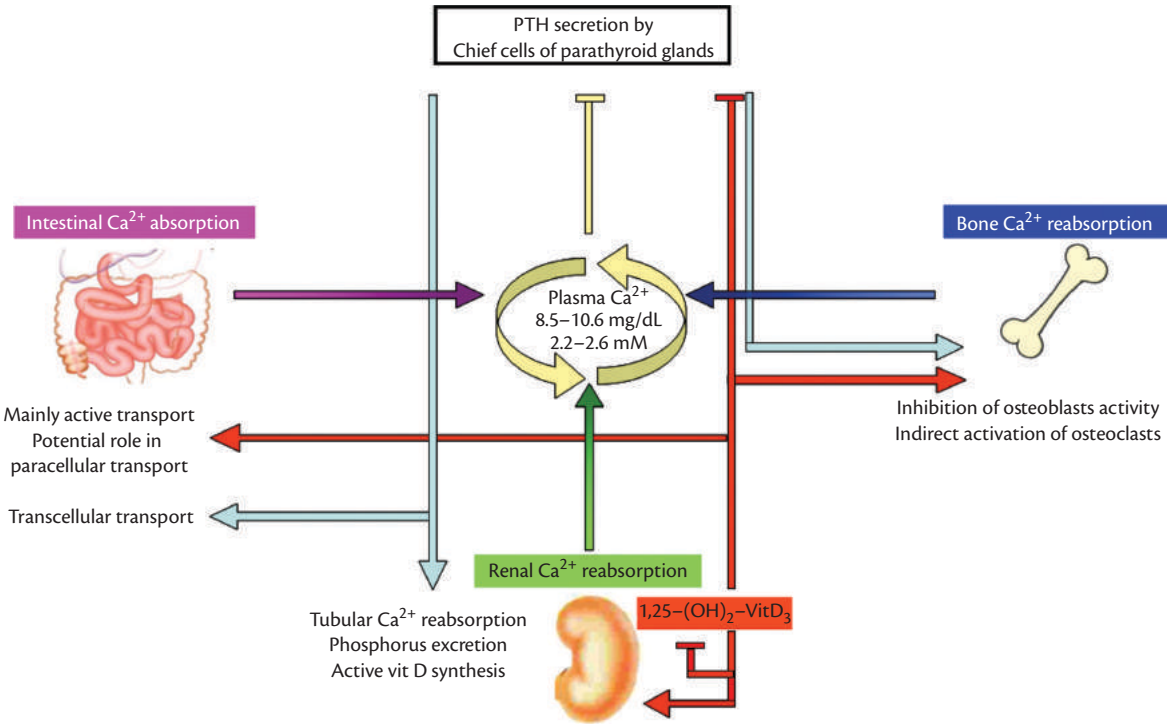

Fig. 26.1 Hormonal regulation pathway of calcium homeostasis. Ca^{2+} homeostasis is finely tuned by a complex hormonal system including PTH and vitamin D action on target organs, namely the gut, the kidney, and the bone. Parathyroid glands can sense the circulating level of Ca^{2+} through the Ca^{2+} sensing receptor and so modulate PTH secretion. Low Ca^{2+} levels stimulate PTH release. PTH promotes Ca^{2+} reabsorption in the kidney and in the bone, some evidence indicate it is active also in the intestinal Ca^{2+} absorption. PTH promotes active vitamin D synthesis in the kidney. Vitamin D increase active and, potentially, passive Ca^{2+} absorption in the gut. It potentiates also renal and bone reabsorption and modulates in a negative feedback PTH release.

However there are reports indicating an active Ca^{2+} transport component in cortical TALH segments (Imai, 1978; Friedman, 1988), although immunohistochemical studies on mouse and rat kidney sections have not detected the presence of proteins identified as Ca^{2+} transporters in the TALH (Loffing et al., 2001).

Interestingly, a new protein, named paracellin 1 (PCLN-1), expressed in human TALH tight junctions, probably plays a decisive role in the control of passive Ca^{2+}, and also Mg^{2+} reabsorption, since mutations of PCLN-1 are present in patients with the hypomagnesaemia with hypercalciuria syndrome (HHS) (Blanchard et al., 2001).

Overall, the TALH certainly plays a significant role in the process of Ca^{2+} reabsorption, mainly due to paracellular Ca^{2+} transport. However, the contribution of active Ca^{2+} transport deserves further investigation (Hoenderop et al., 2002a; Motoyama and Friedman, 2002).

Furosemide-induced calciuria

Loop diuretics reduce the lumen-positive transepithelial voltage and consequently diminish paracellular transport of Ca^{2+} and Mg^{2+} (Hebert, 1999). Patients with Bartter syndrome, caused by NKCC2 (Na^+-K^+-$2Cl^-$ co-transporter) mutations, manifest similar electrolyte disturbances to patients given loop diuretics such as furosemide (Simon and Lifton, 1966). Both acute and chronic (Rizzo et al., 2004) furosemide administration stimulate hypercalciuria associated with upregulation of the main Ca^{2+} transport proteins downstream in the DCT. Co-administration of chlorothiazide decreases furosemide-induced hypercalciuria, given acutely or chronically. Immunofluorescent staining studies reveal increased apical membrane protein abundance of the transient receptor potential vanilloid subtype 5 (TRPV5) channel, and intracellular Ca^{2+} binding protein calbindin-D_{28k}, along the DCT, even when both diuretics are given. Increased abundance of Ca^{2+} transport proteins in the DCT is an increased solute load-dependent effect in response to increased Ca^{2+} delivery, and serves as a compensatory adjustment in downstream nephron segments (Lee et al., 2007).

The distal tubule

The DCT and connecting tubule (CNT) account for about 15% of total renal Ca^{2+} transport. Along these segments Ca^{2+} reabsorption is inversely related to Na^+ reabsorption. In the DCT and CNT, the transepithelial potential difference opposes Ca^{2+} reabsorption, and the paracellular permeability of Ca^{2+} ions is very low. Thus, Ca^{2+} reabsorption is, mainly an active transcellular process regulated by PTH and $1,25(OH)_2D_3$ (Costanzo et al., 2000).

Along the DCT, active Ca^{2+} reabsorption is restricted to the late distal convoluted tubule (DCT-2). This segment shares similarities with the CNT; both segments express the Ca^{2+}-reabsorptive protein machinery, namely the TRPV5 channel, calbindin-D_{28k}, and the basolateral Na^+/Ca^{2+} exchanger (NCX1), and the plasma membrane Ca^{2+}-ATPase type 1b (PMCA1b) (Hoenderop et al., 1999; Loffing et al., 2001). DCT2 expresses the apical sodium channel, ENaC, as CNT, but DCT-2 solely expresses the Na^+-Cl^- cotransporter, NCC. Transepithelial transport of Ca^{2+} is a three-step process: Ca^{2+} crosses the apical membrane though TRPV5 (the

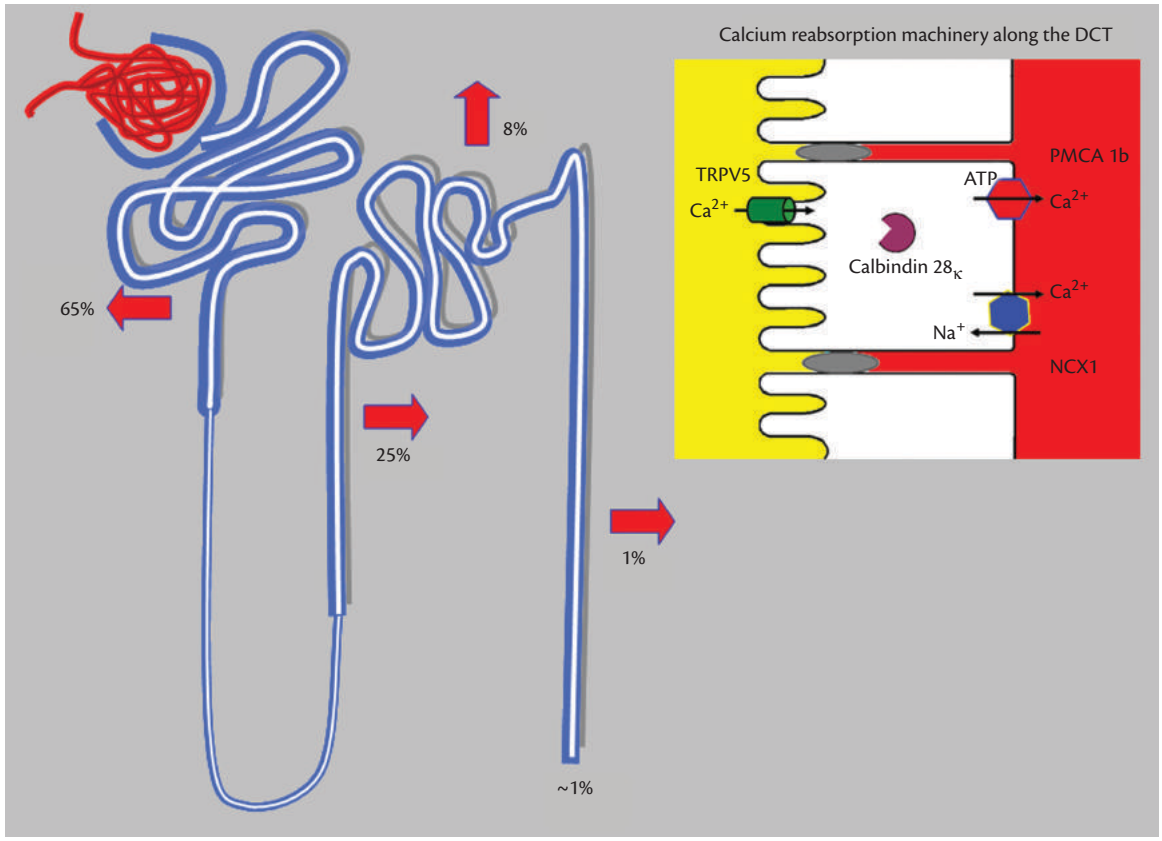

Fig. 26.2 Renal calcium handling. The kidney can reabsorb the majority of the filtered Ca^{2+}. About 65% of the filtered amount of Ca^{2+} takes a paracellular route along the proximal tubule. TAL reabsorption accounts for about 25% of the total filtered amount. This transport is mainly paracellular, but some evidences suggest also a transcellular component. The distal convolute tubule finely regulates Ca^{2+} by transcellular reabsorption. In the insert on the right it is illustrated the Ca^{2+} reabsorption machinery. Finally some studies report about 1% of filtered Ca^{2+} is reabsorbed along the collecting duct.

gatekeeper), is intracellularly buffered, mainly by calbindin-D_{28k}, and then diffuses into the interstitial space via NCX1 and PMCA1b Bindels, 2010) (Fig. 26.2).

Apical entry of calcium through TRPV5

The apical Ca^{2+} influx channel involved in transcellular Ca^{2+} reabsorption was identified by functional expression cloning using a cDNA library from rabbit primary CNT and the cortical collecting duct (Hoenderop et al., 2009). Injection of total mRNA from this isolate into *Xenopus laevis* oocytes induces Ca^{2+} uptake two to three times above background. Subsequently this was recognized to be due to a new epithelial Ca^{2+} channel, ECaC1, and later renamed TRPV5, and a member of the TRP channel superfamily (Hoenderop et al., 2009). TRPV5-null mice excrete 10-fold more Ca^{2+} than their wild-type littermates. Active Ca^{2+} reabsorption in DCT2 and CNT segments is severely impaired in TRPV5 knockout (KO) mice, in line with its postulated gatekeeper function (Hoenderop et al., 2003).

Intracellular buffering by calbindin-D_{28k}

Calbindin-D_{28k} dynamically controls TRPV5-mediated Ca^{2+} influx by physical interaction with the channel at the plasma membrane. Calbindin-D_{28k} moves towards the plasma membrane, where it is directly associated with TRPV5 at low intracellular Ca^{2+} concentrations (Lambers et al., 2006). Here it can buffer Ca^{2+} that enters the renal epithelial cell, thereby counteracting local accumulation of cytosolic-free Ca^{2+} and inactivation of the channel. After Ca^{2+} binding, calbindin-D_{28k} facilitates the transport of Ca^{2+} to the basolateral membrane and operates as a dynamic Ca^{2+} buffer. However, calbindin-D_{28k} KO mice have no abnormalities in renal Ca^{2+} handling; so it is possible that other Ca^{2+} binding proteins can replace its buffering role. Calbindin-D_{9k} has been proposed to have this function in a DCT cell line, but calbindin-D_{9k} KO mice also do not exhibit any obvious phenotypic abnormalities (Kutuzova et al., 2006). The role of renal calbindin-D_{9k} in compensating for impaired calbindin-D_{28k} function (and vice versa) needs further investigations (Schlatter, 2006).

The thiazide-induced calcium-sparing effect

Thiazide diuretics, coupled with their natriuretic effect, are able to reduce urinary Ca^{2+} excretion. This feature is also seen in Gitelman syndrome patients. Thiazide-induced Ca^{2+} reabsorption takes place mainly along the proximal tubule and is driven by the increase in proximal Na^+ reabsorption as a result of thiazide-induced contraction of the extracellular circulating volume (Bindels, 2010). An additional mechanism that has been proposed is a direct effect on Ca^{2+} reabsorption in the DCT-2 (Costanzo and Weiner, 1976). This hypothesis seems unlikely, because of the persistence of this anticalciuric effect of thiazides in TRPV5 null mice, and the parallel time-course of decreased Ca^{2+} excretion with the increased Na^+ reabsorption in the proximal tubule (Nijenhuis et al., 2005).

Intestinal calcium absorption

Dietary Ca^{2+} intake is essential for systemic Ca^{2+} homeostasis. Ca^{2+} absorption accounts for about 30% of total Ca^{2+} intake. Ca^{2+} absorption occurs in the small intestine by active (low dietary intake) and passive (high dietary intake) transport mechanisms. Active transcellular Ca^{2+} absorption is located largely in the duodenum and upper jejunum, whereas passive paracellular Ca^{2+} absorption occurs throughout the entire length of the intestine (Bronner et al., 1986; Christakos et al., 2011). Both transport systems are controlled by the circulating $1,25(OH)_2D_3$ (Fig. 26.3).

Active calcium absorption

Transcellular Ca^{2+} absorption requires Ca^{2+} flux through the apical Ca^{2+} channel, transient receptor potential vanilloid type 6 (TRPV6), Ca^{2+} buffering in the enterocyte by calbindin-D_{9k} and basolateral extrusion through PMCA1b (Wasserman, 1964) (Fig. 26.3).

TRPV6 and calbindin-D_{9k} co-localize in the intestine and their expression is induced at weaning, by low dietary Ca^{2+} intake and by $1,25(OH)_2D_3$ (Song et al., 2003); expression is reduced in vitamin D receptor (VDR) KO mice (Van Cromphaut et al., 2003). However, recent evidences from calbindin-D_{9k} KO and TRPV6 KO mice have challenged this traditional model (Kutuzova et al., 2006; Benn et al., 2008). Calbindin-D_{9k} KO mice show active intestinal Ca^{2+} transport similar to WT mice in response to a low Ca^{2+} diet or $1,25(OH)_2D_3$, suggesting a compensatory or alternative model for intestinal Ca^{2+} absorption. TRPV6 KO mice also maintain normal serum Ca^{2+} levels; in response to $1,25(OH)_2D_3$ intestinal Ca^{2+} transport is similar in WT and TRPV6 KO mice (Kutuzova et al., 2008; Thyagarajan et al., 2009). However, these KO mice fail to maintain Ca^{2+} homeostasis when challenged with a low-Ca^{2+} diet and they develop bones abnormalities (Thyagarajan et al., 2009; Lieben et al., 2010). In contrast, a mouse model of TRPV6 overexpression develops hypercalcaemia, hypercalciuria, and soft tissue calcification.

A vesicular-mediated transcellular route for Ca^{2+} absorption has been identified in chick enterocytes after stimulation with active vitamin D. Intracellular vesicles containing calbindin-D_{28k} seem to shuttle Ca^{2+} through the cell. In duodenal chick enterocytes a rapid Ca^{2+} efflux pathway has been described in response to acute stimulation with vitamin D. This mechanism is called transcaltachia and seems to be mediated by a membrane-associated rapid response steroid binding protein (MARRS) (Fleet and Schoch, 2010).

Passive calcium absorption

Paracellular Ca^{2+} absorption occurs throughout the entire intestine, especially in the distal segments (Fig. 26.3). The effectiveness of this pathway depends on the lumen-to-interstitium electrochemical gradient and the integrity of the intercellular tight junction complexes, and may be regulated by vitamin D (Fujita et al., 2008). In fact, VDR KO mice show low levels of claudin-2 and claudin-12. In addition, $1,25(OH)_2D_3$ has been shown to induce the expression of claudin-2 and claudin-12 *in vitro* in an intestinal epithelial cell line, resulting in facilitated paracellular Ca^{2+} conductance (Fujita et al., 2008). The vitamin D-dependent paracellular pathway could have a role in ameliorating the phenotype of the TRPV6 KO and the TRPV6/calbindin-D_{9k} double KO mice (Christakos, 2012).

Calcium and bone metabolism

Bone is the major Ca^{2+} storage of the body. Osteoblasts and osteoclasts connect bone turnover to systemic Ca^{2+} homeostasis. However, how this mechanism directly contributes to serum Ca^{2+} homeostasis has not been completely clarified. Bone reabsorption and formation may be the main pathways, but these processes are quite slow for quick responses to changes in serum Ca^{2+} (Fig. 26.4).

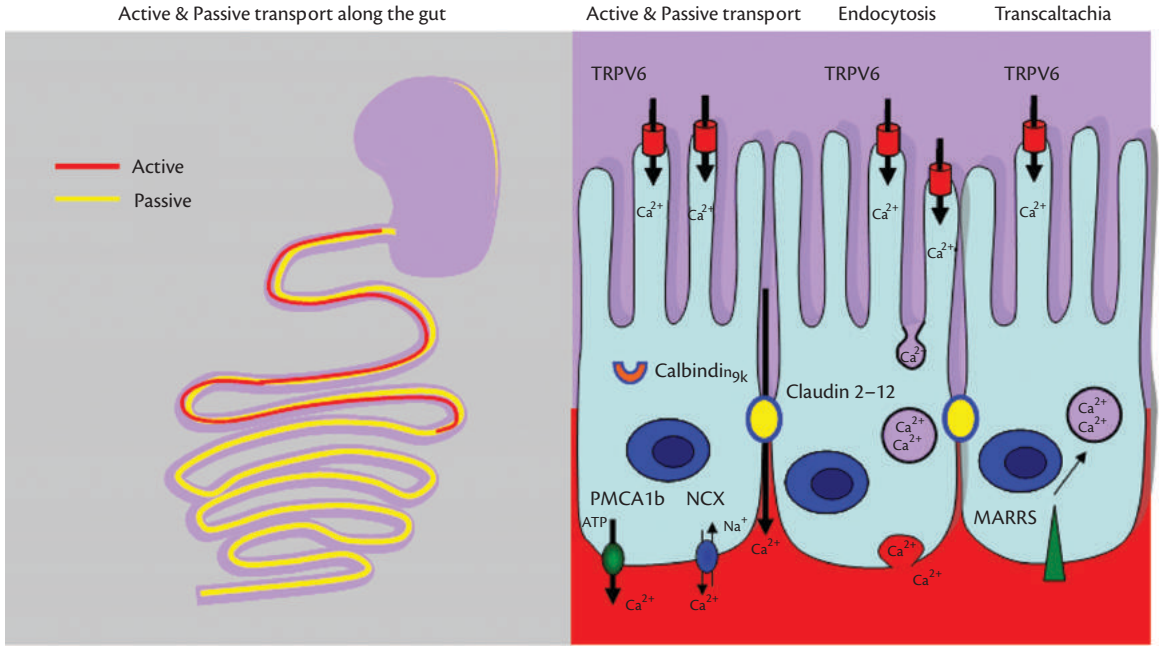

Fig. 26.3 Calcium handling by the gut. Calcium absorption along the gut requires both an active (transcelluar) and passive (paracellular) transport. During physiological feeding condition, active transport takes place, mainly, in the duodenum and in the first part of the jejunum, while passive transport occurs along the whole intestine (left side of the picture). On the right side, it is represented the molecular mechanism involved in the cellular Ca^{2+} absorption and release.

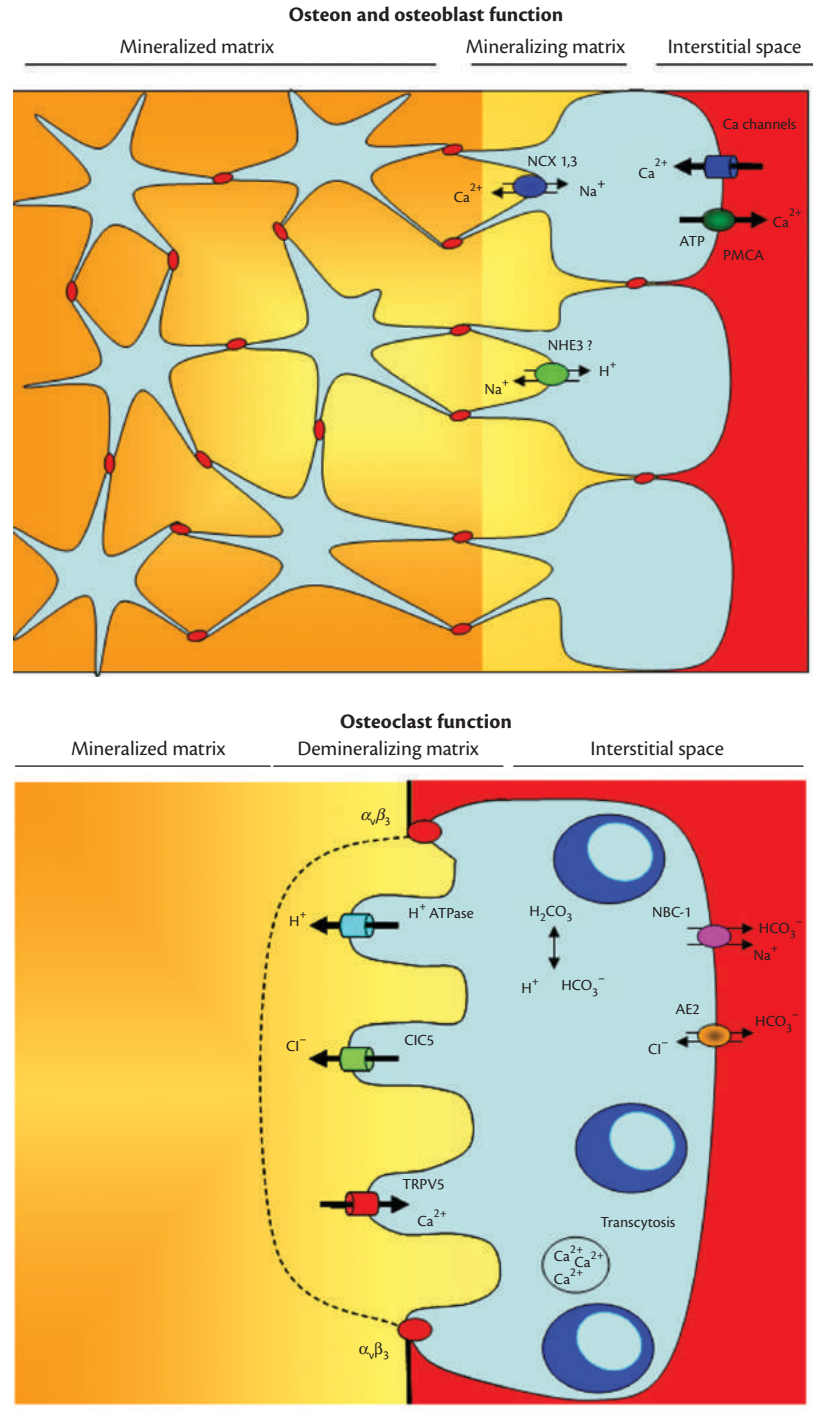

Fig. 26.4 Bone calcium handling: osteoblast and osteoclast function. (Upper) The upper part of the figure describes the bone forming unit, the osteon. Osteoblast contributes to both bone matrix deposition and its mineralization. Ca^{2+} release in the mineralizing area and proton removal are key steps for bone mineralization. NCX is crucial for Ca^{2+} release in the mineralizing matrix, while some evidences suggest a potential role for NHE in keeping mineral matrix alkalinization. (Lower) The lower part of the figure represents the osteoclast-induced bone matrix demineralization. Mature osteoclasts seal up to the mineral matrix through $\alpha_v\beta_3$ integrin. Acid secretion in the demineralizing area is mediated by H^+ATPase and CLC5 channel. This acid secretion is buffered by basolateral HCO^{3-} extrusion through AE2 or NBC1 proteins. Matrix acidification promotes the dissolvance of Ca^{2+} containing crystals. Ca^{2+} and proteins from the matrix are reabsorbed, mainly, by endocytosis.

Osteoblasts produce both the protein and mineral component of the extracellular matrix of the osteon, the bone-forming unit. Osteoblasts are permeable to circulating Ca^{2+} levels through several membrane Ca^{2+} channels. L-type voltage-sensitive Ca^{2+} channels (VSCCs) regulate the Ca^{2+} entry in the osteoblasts in response to multiple hormonal stimuli (Bergh et al., 2006). The two main Ca^{2+} extruding mechanisms expressed in the osteoblasts are the plasma membrane Ca^{2+}-ATPase (PMCA) present on the membrane facing the interstitial fluid, and the Na^+/Ca^{2+} exchanger (NCX) on the membrane facing bone matrix (Stains et al., 2002). This polar distribution suggests that PMCA is mainly involved in intracellular Ca^{2+} regulation. Many intracellular proteins in the osteoblast can buffer the large amount of Ca^{2+} being transported continuously through the cells. Calbindin-D_{28k} is not crucial for this functions and it may be replaced by other Ca^{2+}-buffering proteins (Turnbull et al., 2004). Therefore, Ca^{2+} efflux into the osteoid seems to be mainly mediated by the NCX type 1 and 3 (Stains et al., 2002). Phosphate accumulation in the matrix is another critical step for generating hydroxyapatite crystals. Pyrophosphate is the major source of phosphate. Its synthesis, transport and degradation to phosphate anions is finely regulated (Orimo, 2010). Lastly, removing protons from osteoid is crucial for the mineralization process. To date this acid-removing mechanism has not been clarified, but a potential role for the Na^+/H^+ exchanger (NHE) has been hypothesized (Redhead, 1988).

Osteoclasts provide bone demineralization and Ca^{2+} release into plasma. The key event for Ca^{2+} removal from bone is the acidification of the mineral matrix. This is achieved by proton secretion via a membrane H^+-ATPase. Electroneutrality is maintained by concomitant secretion of Cl^-, mainly through the CLC5 channel. Intracellular H_2CO_3 is the source for H^+ secretion and the bicarbonate produced from its dissociation is extruded on the basolateral side via a chloride-bicarbonate exchanger (AE-2) or the sodium-coupled bicarbonate co-transporter (NBC1). Ca^{2+} and degraded matrix proteins are translocated across the cell by vacuolar transcytosis (Nesbitt and Horton, 1997; Salo et al., 1997). This mechanism ensures removal of large amounts of Ca^{2+} from the mineral matrix to the extracellular space.

Direct calcium regulation of bone turnover

The link between bone storage and circulating Ca^{2+} levels is mediated by several Ca^{2+}-regulating hormones (see below). Recent evidence hypothesizes a direct effect of serum Ca^{2+} in the regulation of osteoblast and osteoclast activity, and so in bone turnover (Blair et al., 2011).

Osteoblast function seems, in part, to be regulated by the Ca^{2+} sensing receptor. Mice with conditional KO of the CaSR in osteoblasts show a severe deficit in mineralization of the skeleton. These mice have impaired post-natal growth and skeletal development. They suffer from rib and long bone fractures, and die within 3 weeks of birth (Chang et al., 2008). These findings suggest a pivotal role for the CaSR in the regulation of osteoblasts and the mineralization process. However, it is not completely clear if osteoclasts do express the CaSR. The finding that a low amount of CaSR is found in isolated osteoclasts might be related to contamination and dilution from other bone cell types during the isolation process; but the low sensitivity of the osteoclast to Ca^{2+} stimulation (5–20 mmol/L), is far from the typical for the CaSR (*c*.1.5 mmol/L).

Regulation by Klotho

Klotho is a beta-glucoronidase with multiple renal and extrarenal functions such as ageing, oxidative stress, and mineral metabolism. Klotho deficiency is associated with slight hypercalcaemia, bone demineralization, and hypercalciuria (Hu et al., 2010). The original explanation for these findings was a possible inhibitory effect of Klotho on $1,25(OH)_2D_3$ synthesis; the consequent hypervitaminosis D being responsible for the hypercalcaemia and hypercalciuria. However, a weak point of this theory was the reversal of hypercalcaemia and the bone phenotype, but not of the hypercalciuria, with the normalization of serum $1,25(OH)_2D_3$ levels (Imura et al., 2007). These data prompted a study of Klotho regulation of Ca^{2+} homeostasis as a primary modulator of TRPV5 activity. Indeed, Klotho mediates an increase in cellular surface expression of TRPV5 in DCT and CNT (Chang et al., 2005). Klotho null mice have a *primary* renal Ca^{2+} leak that contributes to a *secondary* increase in $1,25(OH)_2D_3$ synthesis with its consequences. In addition, Klotho increases the activity of the Na^+-K^+-ATPase α1-subunit; the increased Na^+ gradient created by increased Na^+-K^+-ATPase activity might drive the transepithelial transport of Ca^{2+} through the basolateral membrane via the Na^+/Ca^{2+} exchanger (NCX)-1 (Imura et al., 2007). These studies suggest a fundamental role for Klotho in the regulation of Ca^{2+} balance.

Regulation by the Ca^{2+}-sensing receptor

The extracellular CaSR allows all the tissues involved in Ca^{2+} homeostasis to monitor the blood Ca^{2+} level (Brown et al., 1993). When it senses even minor variations in extracellular Ca^{2+} from its normal level, the CaSR directly or indirectly modulates various homeostatic tissues to normalize extracellular Ca^{2+}. Key tissues expressing the CaSR include the PTH-secreting parathyroid glands, calcitonin (CT)-secreting thyroidal C cells, the intestine, bone, and kidneys. In non-parathyroid tissue, the CaSR determines how much Ca^{2+} moves into or out of the body through the intestine and kidneys, and how Ca^{2+} moves between the extracellular fluid and bone. These Ca^{2+} fluxes are regulated by PTH and CT, as well as by $1,25(OH)_2D_3$ (Chattopadhyay et al., 1996).

In the parathyroid glands, the CaSR represents the molecular mechanism by which parathyroid cells detect changes in blood ionized Ca^{2+} concentration, modulate PTH secretion accordingly, and so maintain serum Ca^{2+} levels within a narrow physiological range. Interestingly, in the kidney the CaSR regulates renal Ca^{2+} excretion and influences the transepithelial movement of water and other electrolytes.

CaSR in the proximal tubule

The CaSR is expressed in the subapical region of proximal tubular cells (Riccardi et al., 1998) where it is involved in the regulation of PTH-mediated phosphate (Pi) excretion (Ba et al., 2003). Studies carried out in proximal tubule-derived cell lines also suggest that 1α -hydroxylase activity is inhibited in the presence of high Ca^{2+} (Maiti et al., 2008).

Recent investigations performed on a murine model in which the full-length CaSR has been ablated have shown that the CaSR reduces the response to $1,25(OH)_2D_3$ independent of the actions of PTH (Egbuna et al., 2009). Thus, the CaSR exerts tight control on circulating $1,25(OH)_2D_3$ both at the level of its synthesis (in the

proximal tubule) and in modulating its effects (specifically on Ca^{2+} reabsorption by the distal tubule). Conversely, 1,25$(OH)_2D_3$, PTH and dietary phosphate modulate both the *CaSR* gene and CaSR protein expression in the proximal tubule, suggesting the existence of a local feedback loop for the regulation of Ca^{2+} and Pi excretion independent of systemic changes in calciotropic hormones. In addition, luminal Ca^{2+} concentration acting via the CaSR can also modulate sodium-dependent proton secretion and water reabsorption along the proximal tubule: increasing luminal Ca^{2+} or using a calcimimetic agent leads to increased water reabsorption (Capasso et al., 2013).

CaSR in the loop of Henle

The CaSR is localized at the basolateral side of TALH cells, where it directly modulates both paracellular and transcellular NaCl and divalent cations transport. Basolateral, but not urinary (luminal), increases in serum Ca^{2+} (or Mg^{2+}) concentrations reduce their own reabsorption (Quamme, 1982). During hypercalcaemia activation of the basolateral CaSR inhibits ROMK channels (Wang et al., 1997). Since apical K^+ recycling in the TAL is the rate-limiting step for Na^+-K^+-$2Cl^-$ co-transport and paracellular divalent cations reabsorption (Greger et al., 2001), CaSR activation induces Ca^{2+} loss. A novel role in the direct regulation of paracellular Ca^{2+} reabsorption along the TAL has also been demonstrated (Loupy et al., 2012).

Gain-of-function mutations of CaSR cause a Bartter-like phenotype (Vargas-Poussou et al., 2002). CaSR affinity for Ca^{2+} and Mg^{2+} in the TAL is also influenced by pH: as pH increases, the larger is its affinity for divalent cations (Quinn et al., 2004). This is yet another level of complexity whereby the CaSR activity is also influenced by extracellular pH.

CaSR in the distal convoluted tubule

The CaSR co-localizes with TRPV5 at the apical membrane and in subapical vesicles of DCT and CNT cells (Topala et al., 2009). In cell lines over-expressing TRPV5 and CaSR, the activation of this receptor increases the activity of TRPV5, activating Ca^{2+} reabsorption. CaSR also controls basolateral expression of NCX and PMCA, thus promoting the Ca^{2+} efflux pathway (Hoenderop et al., 2004; Topala et al., 2009). The role of the CaSR in calcium reabsorption is evident in Ca^{2+} overload along the DCT, for example, following administration of a loop diuretic.

CaSR in the collecting duct

The level of urine Ca^{2+} concentration is known to influence acid secretion and water reabsorption. This mechanism protects the kidney from forming stones. CaSR expression in principal and intercalated cells of the collecting duct is involved in this protection.

TRPV5 KO mice have hypercalciuria, but they do not form kidney stones (Renkema et al., 2009), probably because they exhibit marked urinary acidification with a low urine pH and increased urine flow rate. Mice lacking both TRPV5 and the B1 subunit of the H^+-ATPase develop severe nephrocalcinosis and die in the first 3 months of life, implying that the inability to acidify their urine leads to renal stones formation in the presence of hypercalciuria. Outer medullary collecting ducts dissected from TRPV5 null mice, when exposed to CaSR agonists, up-regulate H^+-ATPase and downregulate aquaporin 2 expression (Renkema et al., 2009). Hypercalciuria-induced polyuria via the CaSR is even more apparent in the inner medullary collecting duct (IMCD). In the IMCD the CaSR is expressed in principal cells and co-localizes with aquaporin 2 in the apical membrane. Isolated IMCD does not respond to vasopressin (DDAVP) when also exposed to a high Ca^{2+} concentration (Sands et al., 1997).

Regulation by 1,25-$(OH)_2D_3$

Vitamin D_3 (cholecalciferol) is essential for body Ca^{2+} homeostasis. Humans can absorb vitamin D_3 from the diet and synthesize it in the skin from its precursor 7-dehydrocholesterol in response to sunlight. Biologically active vitamin D_3 needs a double hydroxylation process occurring first in the liver and then in the kidney. In the liver 25-hydroxylation and in the kidney 1α-hydroxylation contribute to the synthesis of the biologically active 1,25-$(OH)_2D_3$. Mitochondria of renal proximal tubular cells produce the final 1,25-$(OH)_2D_3$, depending on the circulating Ca^{2+} level and PTH. When there is adequate dietary Ca^{2+} intake and a normal plasma Ca^{2+} concentration, 1α-OHase activity is low. However, when dietary Ca^{2+} intake is low and serum or plasma Ca^{2+} concentration decreased, the activity of this enzyme increases, promoting 1,25-$(OH)_2D_3$ synthesis and a rise in serum Ca^{2+} level.

The physiological effect of 1,25-$(OH)_2D_3$ is mediated by interaction with the nuclear vitamin D receptor (VDR) (Haussler et al., 1998). Notably, VDR expression is not confined to the tissues involved in Ca^{2+} homeostasis, specifically intestine, kidney, and bone, but it is present in many cell types and has other functions (Pike et al., 2010).

The effect of 1,25-$(OH)_2D_3$ on the intestine

Studies from VDR null mice show that 1,25-$(OH)_2D_3$ is mainly involved in the active Ca^{2+} transport in the intestine. After weaning, VDR null mice have reduced (40%) Ca^{2+} absorption and develop hypocalcaemia. This phenotype is rescued by either selectively reintroducing the VDR in only the intestine or by a high calcium/lactose diet (Lieben et al., 2011).

Transcellular Ca^{2+} transport in the small intestine is stimulated by 1,25-$(OH)_2D_3$ via a genomic action. Active vitamin D promotes the transcription of the Ca^{2+} transport proteins TRPV5, TRPV6, the calbindins, NCX1, and PMCA1b (Lieben et al., 2011). A potential role for 1,25-$(OH)_2D_3$ in modulating passive Ca^{2+} absorption in the intestine comes from the evidence that active vitamin D increases the expression of claudins 2 and 12 (Fujita et al., 2008).

The effect of 1,25-$(OH)_2D_3$ on the kidney

In the kidney 1,25-$(OH)_2D_3$ promotes Ca^{2+} reabsorption in the DCT. Several studies have tried to elucidate whether such an action is the consequence of a direct effect of 1,25-$(OH)_2D_3$ or if it is mediated predominantly by a concomitant PTH increase. Early studies of parathyroidectomized dogs supplemented with high doses of active vitamin D showed a direct effect of 1,25-$(OH)_2D_3$ on renal Ca^{2+} handling (Puschett et al., 1972). These observations have been confirmed by data showing that VDR KO mice have inappropriate hypercalciuria both in normo- and hypocalcaemic conditions. In addition, mice lacking the active form of vitamin D_3 (Cyp27b1 KO mice) have lower levels of TRPV5, Calbindin-D_{9k}, Calbindin-D_{28k}, and NCX1 in the kidney. This phenotype is rescued by 1,25-$(OH)_2D_3$ administration (Hoenderop et al., 2002b).

The effect of 1,25-$(OH)_2D_3$ on bone

Vitamin D deficiency leads to rickets. Skeletal abnormalities start after weaning and are strongly related to concomitant changes in Ca^{2+} and phosphate handling. High dietary Ca^{2+} intake (Dardenne et al., 2003) and selective introduction of VDR to the intestine of VDR null mice (Xue and Fleet, 2009) can rescue the vitamin D-induced skeleton changes. However, since the recent identification of the VDR in chondrocytes, osteoblasts, and osteoclasts, a strong research interest is growing in the role of 1,25-$(OH)_2D_3$ in the regulation of bone metabolism and thereby of Ca^{2+} homeostasis. Briefly, 1,25-$(OH)_2D_3$ appears to inhibit osteoblast differentiation and thus bone matrix mineralization (Shi et al., 2007); these findings are confirmed in an experimental model of hypervitaminosis D (St-Arnaud et al., 2000). In the osteoblast 1,25-$(OH)_2D_3$ promotes the synthesis of the receptor activator of nuclear factor kappa-B ligand (RANKL) and inhibits the transcription of the osteoprotegerin, activating, ultimately, the osteoclast. Both osteoblast inhibition and osteoclast indirect activation contribute to the 1,25-$(OH)_2D_3$-related increase in serum Ca^{2+} level (Lieben et al., 2011).

Regulation by parathyroid hormone

The parathyroid glands are the main organ finely tuning the blood Ca^{2+} level. Changes in blood Ca^{2+} are sensed by the CaSR expressed in the chief cells of the parathyroid and PTH secretion is adjusted accordingly; PTH is released in response to a low blood Ca^{2+} level. PTH is a ligand for the PTH/PTHrP receptor, an interaction that promotes Ca^{2+} reabsorption in the kidney, the bone and intestine.

In the proximal tubule, PTH stimulates the activity of 1α-OHase to increase the circulating level of active vitamin D (1,25-$(OH)_2D_3$). Although, the hypercalcaemic effect of PTH is related to its effect on vitamin D activity, experimental models have assessed the direct action of PTH in each of the main tissues involved in Ca^{2+} homeostasis.

The effect of PTH on the kidney

PTH/PTHrP receptor mRNA has been identified in several cell types of the rat kidney, including glomerular podocytes, convoluted and straight parts of the proximal tubule, cortical thick ascending limb and DCT, though not in the thin limb of Henle's loop or collecting duct. Immunohistochemical localization confirms its presence on the basolateral membrane (Lupp et al., 2010).

In the proximal tubule, PTH regulates vitamin D synthesis and phosphate transport, while Ca^{2+} reabsorption in this segment is mainly paracellular and seems to be independent of hormonal regulation (Friedman, 2000). Along the medullary and cortical part of the TAL, PTH enhances both transcellular and paracellular Ca^{2+} absorption (Friedman and Gesek, 1995), although the entry pathway for Ca^{2+} at this site has not been identified. PTH promotes active Ca^{2+} reabsorption in DCT by inducing the expression of the main Ca^{2+} regulating proteins expressed in the DCT. PTH administration to parathyroidectomized rats can restore the reduced expression of TRPV5, calbindin-D_{28k} and NCX1 along the DCT2 and CNT (van Abel et al., 2005). In addition, PTH can stimulate the PMCA activity by increasing its affinity for Ca^{2+}. This effect has not been observed after stimulation with vitamin D (Tsukamoto et al., 1992). The PTH signal is mediated by a process involving PKA and PKC-dependent pathways (Friedman et al., 1996) affecting Ca^{2+} channel activation, sorting (Bacskai and Friedman, 1990) and cellular hyperpolarization (Friedman and Gesek, 1994).

The effect of PTH on the intestine and bone

Along the intestine PTH/PTHrP receptor is expressed on both the apical and basolateral side of the epithelial cells, but not in the globet cells. Enterocytes increase Ca^{2+} absorption when cultured and stimulated with PTH (Gentili et al., 2003), showing that in the gut active vitamin D is not the only regulator of Ca^{2+} absorption (Nemere and Larsson, 2002). Bone is one of the main targets for PTH in the regulation of Ca^{2+} homeostasis. PTHrP is expressed in osteoblasts, while it is still uncertain whether the same receptor is present in osteoclasts. PTH stimulates osteoblasts to secrete RANKL and inhibits osteoprotegerin (OPG) a target decoy for RANKL. Osteoclasts are activated by PTH stimulation through the interaction of RANKL with RANK. OPG can interfere with this system, directly binding RANKL and so preventing the activation of osteoclasts. PTH normally induces both bone formation and bone reabsorption, increasing the total bone turnover. At the molecular level this is achieved by a fine tuning of the RANKL–OPG–RANK axis. Pathologically, PTH can induce severe bone reabsorption (primary hyperparathyroidism) or when given therapeutically new bone formation (intermittent PTH supplement therapy for osteoporosis) (Aslan et al., 2012).

Calcium regulation by calcitonin

CT is produced by the thyroidal C cells and is a serum Ca^{2+}-lowering hormone released in response to a hypercalcaemic stimulus. Its effect derives primarily from inhibition of osteoclast-mediated bone reabsorption. CT receptors are expressed in osteoclasts and their activation leads to cellular detachment from the mineral surface and inhibition of acid secretion. CT is used in the treatment of malignant hypercalcaemia, in osteoporosis, and Paget disease (Civitelli et al., 1988). CT KO mice are more sensitive to hypercalcaemic stimuli, confirming its counteracting role when compared with PTH and active vitamin D. In addition, CT KO mice have higher bone density, indicating a potential action of CT in regulating bone formation (Hoff et al., 2002).

CT has a direct effect on modulating renal Na^+ and K^+ excretion. Recent evidence shows that it can also directly influence renal Ca^{2+} excretion. The molecular mechanism of this process is not well understood, but experiments performed on TRPV5 KO mice seem to exclude any effect of CT on this Ca^{2+} channel (Hsu et al., 2010).

Calcium regulation by sex hormones

Humans show gender differences in Ca^{2+} handling. Nephrolithiasis is less common in women than men before 50 years of age, but this gender difference almost disappears over 50 years of age. Therefore, the menopause is typically associated with increased urinary Ca^{2+} excretion. These findings have been confirmed in rodents: male mice excrete more Ca^{2+} than females during the fertile period. Orchidectomy induces hypocalciuria in male mice and this is rescued by testosterone supplementation. Testosterone-associated urinary Ca^{2+} excretion is mediated by decreased expression of TRPV5, NCX1, PMCA1b and calbindin-D_{28k} (Hsu et al., 2010b). Oestrogens have the opposite effect on the Ca^{2+} reabsorption along the DCT (van Abel et al., 2002). Oestrogens modulate the

expression of TRPV5 directly in a vitamin D-independent manner, as shown in ovariectomized 1α-OHase KO mice. Oestrogens also affect duodenal Ca^{2+} transport via TRPV6. Oestrogen receptor KO mice have lower expression of both TRPV5 and TRPV6, and this is reversed by oestrogen supplementation (Van Cromphaut et al., 2003). Whether these changes are associated with decreased Ca^{2+} reabsorption has not been established. However, a recent clinical trial has shown that oestrogen supplements in postmenopausal women increase the risk of kidney stones (Maalouf et al., 2010), although this may be confounded by the additional use of oral calcium supplements.

Calcium regulation by thyroid hormone

There is a lot of evidence that thyroid hormone status influences Ca^{2+} metabolism (Capasso et al., 1987; Cross et al., 1990). Thyrotoxicosis can be accompanied by hypercalcaemia. The severity of thyrotoxicosis directly correlates with bone demineralization and altered biochemical markers of bone turnover (El Hadidy et al., 2011). Hyperthyroid rats have lower Ca^{2+} transport rates both at apical and basolateral membranes of enterocytes; the opposite is true in hypothyroid rats (Kumar and Prasad, 2003). Similar findings are described in the kidney (Kumar and Prasad, 2002).

Calcium regulation by magnesium and urinary pH

Magnesium can affect urinary Ca^{2+} excretion (Chesley and Tepper, 1958). How Mg^{2+} induces hypercalciuria is largely unknown. Systemic changes in pH can also influence Ca^{2+} homeostasis. Metabolic acidosis induces hypercalciuria, at least in part by increasing the filtered load of Ca^{2+} from increased free (ionized) calcium (Rizzo et al., 2000), and by decreasing renal Ca^{2+} reabsorption (Moe and Huang, 2006; Nijenhuis etal., 2006). This effect is also observed during a high dietary protein intake (Amanzadeh et al., 2003). Conversely, urinary alkalinization by potassium citrate or bicarbonate decreases urinary Ca^{2+} (Sebastian et al., 1994).

TRPV5 and TRPV6 function is modulated by variations in the urine pH within the physiological range (Bindels et al., 1994). An acid urine pH increases calciuria by inhibiting TRPV5/6-mediated Ca^{2+} reabsorption in the DCT. One study (Bonny et al., 2008) has shown that Ca^{2+} uptake by TRPV5 is directly inhibited by Mg^{2+} and a low pH. These findings may explain the interaction of Mg^{2+} with Ca^{2+} and urinary pH; moreover, they are consistent with the observation that urinary alkalinization and Mg^{2+} supplementation reduce kidney stones formation.

Calcium regulation by ciclosporin and tacrolimus

Abnormalities in mineral metabolism are common complications of organ transplantation. Treatment with ciclosporin and tacrolimus is associated with increased bone turnover and hypercalciuria, leading to osteoporosis (Stempfle et al., 2002). Tacrolimus significantly increases urinary Ca^{2+} excretion through downregulation of both mRNA and protein expression of TRPV5 and calbindin-D_{28k} (Nijenhuis et al., 2004). Ciclosporin can affect Ca^{2+} transport along the DCT by reducing the expression of the VDR and consequently inducing vitamin D resistance in this segment. This mechanism is supported by the inability of the kidney to retain Ca^{2+}, even in presence of elevated circulating levels of vitamin D (Lee et al., 2011).

Calcium homeostasis in hypertension

Essential hypertension is associated with hypercalciuria. Hypertensive patients have relative hypercalciuria in the presence of enhanced basal parathyroid function (McCarron et al., 1980). In addition, hypertensive patients have on average a 20% increase in Ca^{2+} excretion at any given level of urinary sodium (Strazzullo et al., 1983). Animal models of experimental hypertension confirm this association. The spontaneous hypertensive rat (SHR) has lower renal abundance of PMCA1b and an increase in expression of Calbindin-D_{28k} (Kamijo et al., 1996). The Milan hypertensive strain (MHS) of rat is also hypercalciuric. Recent data demonstrate that all the Ca^{2+} transport proteins are upregulated at both mRNA and protein levels along the distal tubules of MHS animals when compared with the normotensive strain (Petrazzuolo et al., 2010). Taken together, these data indicate that hypercalciuria in experimental hypertension results from decreased tubular reabsorption of Ca^{2+} along the DCT.

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CHAPTER 27

Magnesium homeostasis

Pascal Houillier

Introduction

Magnesium (Mg) is the fourth most abundant cation in vertebrates and the second most abundant intracellular cation. Magnesium is critical for a number of biological processes, the most important being the release of chemical energy: adenosine triphosphate (ATP) is formed by Mg-dependent oxidative phosphorylation. Magnesium is also involved in many enzymatic reactions within the cell: enzymes that use ATP use it as a metal chelate, MgATP. Magnesium is also required for glycolysis, DNA transcription, and protein synthesis. Finally, free magnesium (Mg^{2+}) plays a role in ion currents and membrane voltage stabilization.

Distribution in the body

Bone and soft tissue contain about 66% and 33% of body magnesium, respectively (1000 mmol, or 24 g in an adult human) (Rude, 1996; Ahmad and Sutton, 2000) (Fig. 27.1). Bony magnesium is mainly deposited on the bone crystal surfaces and is therefore rapidly exchangeable. Extracellular magnesium represents only 1% of the total body content. The normal plasma magnesium concentration is 0.70–1 mmol/L (1.7–2.4 mg/dL), and comprises 70–80% of free magnesium, the remainder being bound to proteins or complexed with bicarbonate, phosphate, and citrate (Le Grimellec et al., 1975).

The concentration of magnesium within the cells is 5–20 mmol/L. Most of intracellular magnesium is bound to metalloenzymes and phosphate, and intracellular magnesium is compartmentalized with the highest concentration in microsomes and mitochondria. Within the cytosol, most (80%) of the magnesium is complexed to ATP. Only 1–5% of cellular Mg is ionized (free). The smaller pool of free magnesium in the cell is in equilibrium with the bound magnesium. This equilibrium can buffer the changes in free magnesium concentration under conditions of magnesium excess or depletion. As a consequence, the cytosolic concentration of magnesium is maintained in the optimal range for many enzymatic reactions (Gunther, 1981), and the difference in concentration between the extracellular fluid and the cytosol is small. Nevertheless, magnesium can enter the cells down an electrical gradient owing to the relative intracellular electronegativity. Conversely, the exit of magnesium out of the cell is against the electrical gradient and, is therefore necessarily active.

Balance of magnesium

(For reviews, see Rude 1996; Ahmad and Sutton 2000; Quamme and de Rouffignac 2000.) The daily requirement for magnesium is estimated to be 8–16 mmol (16–32 mEq) (Jones et al., 1967). Magnesium is widely distributed in food, the main sources being meat, green vegetables, and grains. Magnesium is believed to be absorbed equally in jejunum and ileum through both active transport and facilitated diffusion (Brannan et al., 1976). The fraction of dietary magnesium absorbed is poorly defined (20–60%) and the role of calcium (Ca^{2+}) and vitamin D metabolites in its regulation is disputed. Substances such as phosphate and cellulose phosphate, which chelate magnesium, decrease its intestinal absorption. Under normal circumstances, only a small amount of endogenous magnesium is secreted across the intestinal epithelium.

In the steady state, the net amount of magnesium absorbed by the gastrointestinal tract is eliminated in the urine. The concentration of magnesium in the extracellular fluid is determined by the kidney, and the contribution of bone and cellular pool to the maintenance of extracellular magnesium concentration in states of deficiency is unclear.

Renal handling of magnesium

Renal magnesium handling is a filtration-reabsorption process. Seventy per cent of blood magnesium is in the free ionized form and an additional 10% is complexed to low-molecular-weight anions such as citrate, phosphate, and bicarbonate (Le Grimellec et al., 1975). The remainder is bound to plasma proteins, mainly albumin. Consequently, about 70–80% of blood magnesium is ultrafilterable.

Proximal tubule

Of the magnesium filtered load, 10–15% is reabsorbed in the proximal convoluted tubule (de Rouffignac et al., 1973, 1991; Le Grimellec et al., 1973; Quamme et al., 1978). Proximal magnesium reabsorption is therefore substantially less than sodium or calcium reabsorption. The available data consistently indicate a relatively low permeability of the proximal tubule to magnesium: the permeability to magnesium has been calculated to be 1.1×10^{-5} cm/s in this segment.

Of note, magnesium reabsorption in the proximal tubule of very young rats is proportionally much higher than in adult rats, reaching 70% of the filtered load, a value close to that of sodium and calcium reabsorption (Lelievre-Pegorier et al., 1983); the molecular basis of this difference is unclear. From the available data, it seems that proximal reabsorption of magnesium is unaffected by extracellular fluid volume expansion (Poujeol et al., 1976) or peptide hormones such as parathyroid hormone, calcitonin, or glucagon (de Rouffignac, 1990).

Loop of Henle

Micropuncture experiments indicate that a large part (60–70%) of filtered magnesium is reabsorbed in the loop of Henle

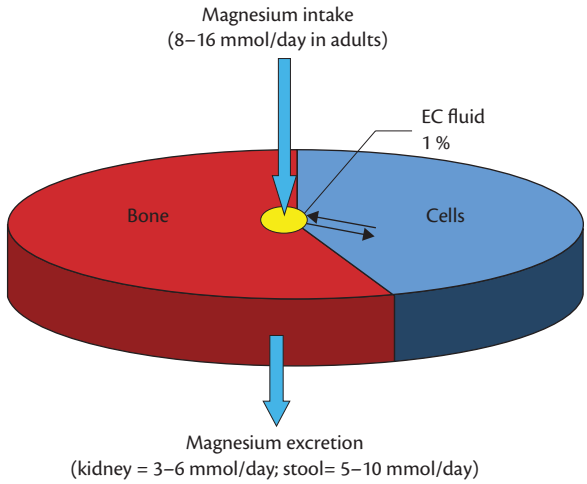

Fig. 27.1 Distribution of magnesium in the human body (normal).

(Fig. 27.2) (Brunette et al., 1974; Le Grimellec et al., 1973, 1974b; Bailly et al., 1984; Wong et al., 1986a; de Rouffignac et al., 1991). A low but significant fraction of filtered magnesium can be reabsorbed together with water in the descending limb in the concentrating kidney. However, evidence indicates that the bulk of reabsorption in the loop of Henle occurs in the thick ascending limb (TALH). Magnesium is reabsorbed in the cortical but not in the medullary part of the TALH of the rat and the mouse kidney (Wittner et al., 1988, 1993, 1996, 1997; Bailly et al., 1990; Di Stefano et al., 1989, 1990, 1992, 1993; Mandon et al., 1993; Wittner et al., 1991), whereas it is reabsorbed in both segments of the rabbit kidney.

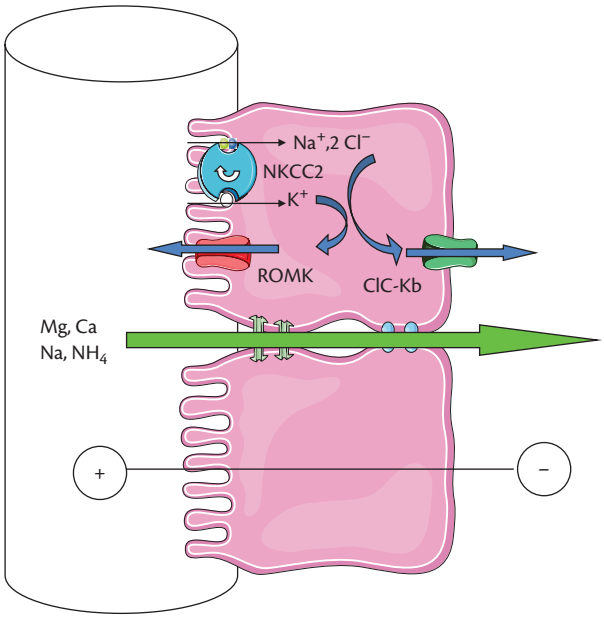

Fig. 27.2 Model of magnesium transport in the thick ascending limb of the loop of Henle (TALH). The epithelial cells of the cortical part of the TALH reabsorb NaCl via the apical cotransporter NKCC2. Most of the potassium that enters the cell recycles back to the lumen via the apical channel ROMK, thereby hyperpolarizing the apical membrane, while most of the chloride leaves the cell across the chloride channel CLCKB in the basolateral membrane, resulting in a depolarization of the membrane. The difference in voltage of the two membranes accounts for the lumen positive transepithelial potential difference, the driving force for the paracellular diffusion of magnesium. Alterations in claudin-16 and claudin-19 can cause a severe decrease in the paracellular pathway permeability, but the full molecular basis for the permeability to Mg of the paracellular pathway is remains elusive.

Theoretically, magnesium can cross the epithelium through a transcellular or a paracellular pathway, or both. The bulk of evidence indicates that the paracellular pathway is predominant, if not the exclusive, pathway for magnesium transport in the cortical TALH. Sodium chloride absorption in the TALH generates a lumen-positive transepithelial voltage (see Chapter 21), which is the driving force for magnesium reabsorption: magnesium flux is linearly related to the transepithelial voltage in this segment (Shareghi and Agus, 1982; Hebert and Andreoli 1984, 1986; Greger, 1985; Di Stefano et al., 1988). Accordingly, when the voltage is reduced to zero by the presence of furosemide in the lumen, transepithelial magnesium transport is not significantly different from zero (Shareghi and Agus, 1982). The reason why the medullary TALH, which is also characterized by a lumen-positive transepithelial voltage, does not transport a significant amount of magnesium is not obvious.

Although a lumen-positive transepithelial voltage is required for magnesium to be reabsorbed in the cortical TAL, this condition is not sufficient and the epithelium must also express a significant permeability to magnesium. Two related proteins clearly play a significant role in the process of magnesium reabsorption. Both claudin-16 and claudin-19 are expressed in the tight junction multiprotein complex. That claudin-16 may be a paracellular Mg^{2+} pore was originally suggested, based on the finding that mutations in *CLDN16*, the gene encoding claudin-16, were responsible for a severe impairment in Mg^{2+} reabsorption (Blanchard et al., 2001, Simon et al., 1999). When claudin-19 was found more recently to be co-expressed with claudin-16 in the tight junction, and mutations in *CLDN19* gene found to be responsible for a similar disturbance in Mg^{2+} reabsorption, the hypothesis was extended to claudin-19 (Konrad et al., 2006). However, an alternative hypothesis has been proposed, based on the finding that *in vitro* transfection of claudin-16 in epithelial cell lines provokes only a small increase in transepithelial magnesium permeability (Hou et al., 2005). The hypothesis is that the mutated claudin-16 has an indirect effect on magnesium reabsorption by decreasing the paracellular permeability to Na^+, thereby reducing the transepithelial voltage and driving force for magnesium reabsorption (Himmerkus et al., 2008).

The mechanism by which claudin-19 affects magnesium reabsorption is also unclear (Angelow et al., 2007; Hou et al., 2008). However, the effects of claudin-16 and of claudin-19, when co-expressed in LLC-PK1 cells, has been found to be additive (Hou et al., 2008): claudin-16 increases the permeability to Na^+, whereas claudin-19 decreases the permeability to Cl^-, thus increasing the permeability ratio, a prerequisite for the large diffusion potential in the TALH. Recent data suggest that a third claudin, claudin-14, may interact with claudin-16 in the mouse TALH and decrease the cation selectivity of the claudin-16-claudin-19 heteromeric channel (Gong et al., 2012). It is likely that one reason why the function(s) of the various claudins expressed in the tight junction of the TALH remain(s) elusive is that most of the experiments have not yet been performed on native tissue.

Distal convoluted tubule

The distal tubule reabsorbs 5–10% of filtered magnesium (de Rouffignac and Quamme, 1994), which is > 50% of the load delivered to this segment. It is very likely that this transport occurs, at least in part, in the distal convoluted tubule (DCT) Fig. 27.3 (de Rouffignac and Quamme, 1994). Because of the characteristics of the distal tubule, magnesium transport is transcellular and active (Quamme, 1997).

According to the current model of transcellular Mg transport in the distal tubule, free magnesium enters the cell across the apical membrane through the apical channel TRPM6, a member of the melastatin-like subfamily of the transient receptor protein (TRP) channel family (Voets et al., 2004). Because the luminal and cytosolic concentrations of free magnesium are of the same order of magnitude, the driving force for apical magnesium entry is likely to be the cytosol-negative membrane voltage. For this purpose, the shaker-related voltage-gated K^+ channel Kv1.1, which localizes with TRPM6 along the apical membrane of the DCT cell, is believed to play an important role in maintaining the polarity of the apical membrane (Glaudemans et al., 2009).

The exit out of the cell across the basolateral membrane is necessarily active, since it operates uphill against the electrochemical gradient. However, the molecular identity of the transporters involved in this process remains unknown, although CNNM2, a basolateral protein expressed in the DCT, has been proposed to be one of these transporters (Stuiver et al., 2011).

The reabsorption of NaCl by the DCT cell seems to be necessary to sustain magnesium reabsorption, since conditions characterized by a defect in NaCl transport in the DCT are commonly associated with renal loss of magnesium (see Chapter 40). In this process, the ATP-sensitive inward rectifier potassium channel 10 (Kir4.1), encoded by the *KCNJ10* gene, may allow potassium ions to recycle across the basolateral membrane and is likely to be important in sustaining basolateral sodium pump activity.

Comparisons of fluid delivered at the end of the distal tubule and final urine suggest that little quantitative transport of magnesium takes place beyond the DCT and connecting tubule (CNT).

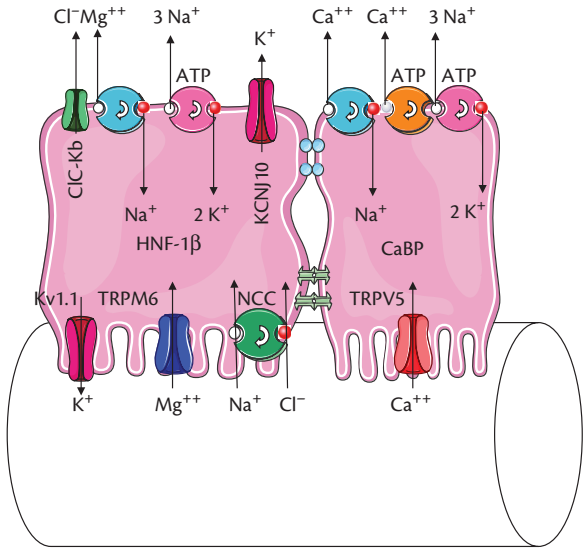

Fig. 27.3 Model of magnesium transport in the distal convoluted tubule (DCT). Magnesium absorption in the DCT occurs exclusively through the transcellular pathway. Magnesium enters the cell through the apical channel TRPM6; however, since the luminal and cytosolic concentrations of free Mg are similar, Mg absorption is not sustained by a concentration gradient. The apical potassium channel Kv1.1 is able to hyperpolarize the apical membrane and provide the driving force for magnesium diffusion into the cell. The mechanism(s) of basolateral magnesium exit out the cell is still unsettled; Mg is believed to leave the cell via a Mg/Na exchanger or a Mg-ATPase. Mg absorption is sustained by NaCl absorption, for which the apical electroneutral Na-Cl cotransporter NCC and the basolateral Na,K-ATPase and the potassium channel KCNJ10 are mandatory (see text for details).

Determinants of renal tubular magnesium transport

Hormones

No hormone has been identified as the regulator of blood magnesium concentration, despite the fact that many hormones have been shown to affect the renal magnesium transport, which is the main determinant of plasma magnesium concentration.

Parathyroid hormone (PTH) increases magnesium absorption both in the loop of Henle and in the DCT (Bailly et al., 1984). In the cortical TALH of the mouse, PTH increases the NaCl absorption, and subsequently the transepithelial voltage, but also the paracellular permeability for magnesium (Wittner et al., 1993), suggesting direct hormonal control of the function of proteins expressed in the tight junction. PTH also stimulates magnesium absorption in the distal convoluted tubule (Bailly et al., 1985, Harris et al., 1979), but the molecular mechanisms remain uncertain; in particular, *TRPM6* gene expression in the kidney is not affected by PTH (Groenestege et al., 2006).

As with PTH, calcitonin increases magnesium reabsorption in the cortical TALH and DCT. These effects are reproduced by arginine vasopressin, glucagon, and ß-adrenergic agonists, although the physiological role of these three hormones in magnesium homeostasis is unclear. Insulin also increases magnesium absorption, at least in the loop of Henle.

Finally, the epidermal growth factor (EGF) was recently identified as a hormone directly regulating the activity and the trafficking of the TRPM6 channel in DCT cells (Groenestege et al., 2007; Thebault et al., 2009). Similarly, the transcription factor HNF1ß also seems to be important for magnesium reabsorption in the distal nephron, possibly because it stimulates the expression of the FXYD2 protein, the γ-subunit of Na^+,K^+-ATPase (Adalat et al., 2009).

Prostaglandin E_2 (PGE_2), the major arachidonate metabolite synthesized in mammalian kidney, inhibits magnesium absorption in the renal tubule and increases urinary magnesium excretion (Schneider et al., 1973; Roman et al., 1984). The change in urinary magnesium excretion being paralleled by similar changes in sodium excretion, the hypothesis has been made that PGE_2 mainly acts in the TALH, primarily by decreasing NaCl absorption and thereby the driving force for magnesium reabsorption (Bailly, 1998). Because PGE_2 receptors are also expressed in the DCT cells, PGE_2 could act in this segment, but its effect has not been assessed, even though PGE_2 increases magnesium uptake in immortalized mouse DCT cells (Dai et al., 1998).

The mineralocorticoid receptor is present in DCT cells and chronic aldosterone administration results in renal magnesium wasting (Massry et al., 1967; Massry and Coburn, 1973). This effect has been explained by extracellular volume expansion induced by

the hormone and an attendant decrease in magnesium reabsorption. On the other hand, mineralocorticoids have been proposed to enhance the peptide hormone response of DCT cells and to indirectly increase magnesium absorption in this segment.

In contrast to calcium, little information exists on the effect of calcitriol, the most active metabolite of vitamin D, on renal tubular magnesium transport, despite the demonstration of expression of the vitamin D receptor in the distal nephron. *TRPM6* gene expression is not affected by 1,25-dihydroxyvitamin D (Groenestege et al., 2006). In contrast, it is increased in the presence of 17ßestradiol (Groenestege et al., 2006).

Non-hormonal factors

The factors influencing magnesium transport in the proximal tubule are poorly defined. As already mentioned, the transport of magnesium in the proximal tubule is not altered by inhibition of NaCl and water reabsorption (Poujeol et al., 1976). However, many factors have been shown to affect magnesium reabsorption in the loop of Henle. Magnesium absorption increases when magnesium concentration increases in the lumen (Quamme and Dirks, 1980; Wong et al., 1983) and decreases when peritubular magnesium concentration increases; an effect that is most likely to be mediated by activation of the Ca^{2+}/polycation-sensing receptor (CaSR) expressed in the basolateral membrane of the TALH cells. Hypercalcaemia decreases magnesium absorption in the loop of Henle (Le Grimellec et al., 1974a; Quamme, 1982). The effects of peritubular magnesium might explain the apparent maximal transport, or Tm, for magnesium in the kidney, and the decrease in urinary magnesium excretion that rapidly occurs in cases of magnesium depletion. It is noteworthy that a common variant (G allele of rs17251221) in the *CaSR* gene is associated with a higher serum magnesium concentration (O'Seaghdha et al., 2010); but whether it is associated with an increase in renal tubular magnesium absorption in unknown. The absorption of magnesium in the DCT also increases in response to magnesium depletion.

Systemic metabolic acidosis is associated with renal magnesium wasting (Martin and Jones, 1961; Lennon and Piering, 1970; Houillier et al., 1996); by contrast, bicarbonate infusion leads to a decrease in urinary magnesium excretion (Wong et al., 1986b). The acid–base status probably affects magnesium absorption both in the TALH and in the distal tubule (Shapiro et al., 1987; Houillier et al., 1996). In the latter segment, the luminal pH probably affects apical magnesium uptake by the magnesium channel TRPM6 (Dai et al., 1997).

Age and gender

Magnesium absorption in the TALH is higher in adult than young animals, whereas no such change has been described for NaCl transport. No difference has been observed between males and females (Wittner et al., 1997). Whether such an age-related change in transport exists in humans is unknown.

Adaptation to a low-magnesium diet

A decrease in the magnesium content of the diet is quickly followed by a fall in urinary magnesium excretion, without an initial change in blood magnesium concentration, indicating of an increase in renal tubular magnesium reabsorption (Shafik and Quamme, 1989). Micropuncture studies have shown that the adaptive increase in magnesium absorption takes place in the loop of Henle (Shafik and Quamme, 1989). Further studies have established that dietary restriction in magnesium is associated with increased magnesium (and calcium, but not sodium or chloride) reabsorption across the epithelium of the cortical TALH (Wittner et al., 2000). This is consistent with an adaptive increase in the paracellular pathway permeability to magnesium, but the molecular mechanisms underlying this change remain unknown. Similarly, dietary magnesium restriction is associated with an increase in magnesium reabsorption in the DCT (Quamme et al., 1980, Shafik and Quamme, 1989). Consistently, expression of the *TRPM6* gene and protein level increase during magnesium restriction (Groenestege et al., 2006), allowing the appropriate decrease in urinary magnesium excretion.

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CHAPTER 28

Approach to the patient with hyponatraemia

Ewout J. Hoorn and Robert Zietse

Introduction and epidemiology

Hyponatraemia counts as the most common electrolyte disorder in hospitalized patients. Its precise epidemiology, however, depends on the serum sodium used to define hyponatraemia, which varies from study to study. The prevalence of hyponatraemia in hospitalized patients is 15–30% when defined as a serum sodium < 136 mmol/L (which is the lower level of normal in most laboratories) and 2–3% when defined as a serum sodium < 125 mmol/L (Hoorn et al., 2006; Upadhyay et al., 2006). A decrease in serum sodium is usually associated with a decrease in serum osmolality (hypo-osmolality), which would normally suppress vasopressin (antidiuretic hormone) and cause a water diuresis. However, in the majority of patients with hyponatraemia, vasopressin levels are either detectable or increased (Anderson et al., 1985). There may be several reasons why vasopressin is present despite hypo-osmolality (Box 28.1). Therefore, hyponatraemia should prompt the question why vasopressin is present despite hypo-osmolality and what the source is of the electrolyte-free water that was retained. Hyponatraemia can be classified according to the time over which it developed, the presence of symptoms, the tonicity, and volume status (Table 28.1). Each of these classifications has their uses and limitations, depending on the clinical context. Ultimately, the clinical setting should dictate which classification is most useful to guide management. It is important to emphasize that these classifications are not mutually exclusive. For example, a patient can have acute and symptomatic hyponatraemia that is further characterized by hypotonicity and euvolaemia. This immediately provides useful information for management, because the presence of cerebral oedema is likely (acute, hypotonic, symptomatic) and the patient therefore requires emergency treatment with hypertonic saline.

Clinical features

There are three reasons why hyponatraemia is clinically important. First, hyponatraemia can be an early or even first sign of important underlying disease, such as adrenal insufficiency or lung cancer (van der Hoek et al., 2009; Hoorn et al., 2011a). Second, hyponatraemia can be complicated by two types of neurological disorders, including cerebral oedema and the osmotic demyelination syndrome (Fig. 28.1) (Arieff, 1986; Sterns et al., 1986). Third, hyponatraemia is invariably associated with increased morbidity and mortality rates in hospitalized patients (Wald et al., 2010), although it remains unclear whether these associations are mainly due to the underlying disease, direct effects of hyponatraemia, or a combination of both (Chawla et al., 2011; Hoorn and Zietse, 2011).

Symptoms associated with hyponatraemia in general are listed in Box 28.2. Acute hyponatraemia is usually more symptomatic than chronic hyponatraemia. Acute hyponatraemia (decrease to a serum sodium of ≤125 mmol/L in ≤ 48 hours) can cause cerebral oedema, because brain cells have insufficient time to adapt to their hypotonic environment. Severe symptoms such as seizures or coma are usually observed in acute hyponatraemia and reflect the presence of cerebral oedema. Milder symptoms such as nausea and vomiting, however, can also be the first signs of an increase in intracranial pressure due to cerebral oedema. In recent years, it has become clear that even patients with chronic hyponatraemia (present > 48 hours), when analysed more closely, also exhibit symptoms. These are usually more subtle neurocognitive or neuromotor symptoms, including gait disturbances, falls, and concentration deficits (Renneboog et al., 2006). Recently, hyponatraemia has been associated with osteoporosis and fractures, suggesting that hyponatraemia can also affect other organs besides the brain (Verbalis et al., 2010; Hoorn et al., 2011b). These indirect effects of hyponatraemia may also contribute to its association with morbidity and mortality. The osmotic demyelination syndrome is a complication of too rapid correction of chronic hyponatraemia and is therefore usually iatrogenic. Osmotic demyelination often has a 'biphasic' course; during the first phase patients are typically asymptomatic (2–6 days), while during the second phase patients develop neurological symptoms, including dysarthria, dysphagia, paresis, confusion, coma, and seizures. These symptoms may be irreversible and even progress to a 'locked-in' syndrome or death.

Investigations

Because hyponatraemia is so common in hospitalized patients, milder forms of hyponatraemia (usually serum sodium >130 mmol/L) may not require additional investigations, especially if obvious explanations are present (hyperglycaemia, postoperative state) and hyponatraemia is expected to be transient. In all other situations (usually serum sodium <130 mmol/L), hyponatraemia should prompt additional investigations. An algorithm for hyponatraemia is shown in Fig. 28.2, while the recommended laboratory investigations are shown in Box 28.3. A distinction can be made between essential investigations, important in all cases of hyponatraemia, and additional investigations, useful in certain settings. The 'core' diagnostic tests in a patient with

Box 28.1 Reasons for antidiuresis in hyponatraemia

1. Baroreceptor mediated (due to hypovolaemia or a low effective arterial blood volume)
2. Stimulation of central osmoreceptors (e.g. by drugs or cytokines)
3. Ectopic production (e.g. by a tumour)
4. Augmented renal effect of vasopressin (e.g. by certain drugs)
5. Activating mutation of the vasopressin-2 receptor (nephrogenic syndrome of inappropriate antidiuresis).

hyponatraemia include serum osmolality, serum glucose, serum creatinine, serum potassium, urine sodium and urine osmolality (Box 28.3). The serum osmolality is used to analyse whether the patient has hypotonic, isotonic, or hypertonic hyponatraemia (Table 28.1 and Fig. 28.2). Although the majority of patients have hypotonic hyponatraemia, that is, a low serum sodium with a low serum osmolality, it is important to identify patients with isotonic or hypertonic hyponatraemia, because these forms of hyponatraemia usually have different clinical implications. Substances that increase osmolality and decrease serum sodium ('translocational hyponatraemia'—osmotically driven fluid shifts) include glucose, mannitol, glycine, and maltose. Glycine is important because it is commonly used as an irrigation fluid during gynaecological or urological surgery. Entry of glycine into the systemic circulation can cause hypertonic hyponatraemia. The most typical example includes transurethral resection of the prostate (TURP) and this form of hyponatraemia is sometimes referred to as the post-TURP-syndrome (Agarwal and Emmett, 1994). It is important to emphasize the difference between the measured osmolality and the 'effective' osmolality (also tonicity). The effective osmolality can be calculated as $2 \times [\text{serum } Na^+ + K^+]$ + serum glucose and only includes 'effective' osmoles, that is, solutes that are more or less restricted to the extracellular fluid volume. Effective osmoles contribute to the movement of water between the intracellular and extracellular compartments. Comparing the measured osmolality to the effective osmolality can be important when, for example, serum urea is elevated. In this case, the measured osmolality may be increased (because urea is also measured), but because urea is not an effective osmole (it can enter cells), the effective osmolality may be low and water may have shifted to the intracellular compartment.

Table 28.1 Classifications of hyponatraemia

Classification	Criteria	Comment
Mild vs severe	Serum sodium concentration	Degree of hyponatraemia does not always correlate with symptoms
Acute vs chronic	Time in which hyponatraemia developed	Time of development is often unknown
Symptomatic vs asymptomatic	Presence or absence of (severe) symptoms	Overlap between symptoms occurring in acute or chronic hyponatraemia
Hypotonic, isotonic, hypertonic	The accompanying serum osmolality	Useful for diagnostic purposes
Hypovolaemic, euvolaemic, hypovolaemic	The assessment of volume status	Assessment of volume status is often unreliable

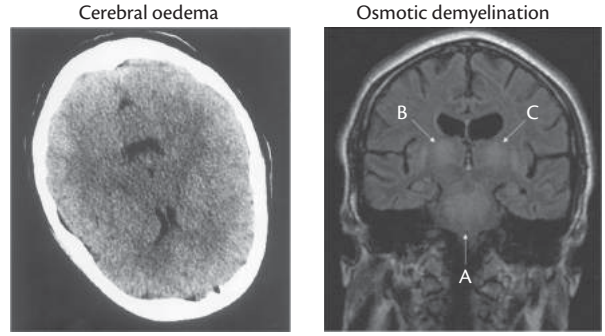

Fig. 28.1 Radiological images of the neurological complications of hyponatraemia. (Left) Computed tomography scan showing cerebral oedema in a patient with acute, desmopressin-induced hyponatraemia (111 mmol/L). From: Schreiber A, Kubitza S, Luft FC. A woman with postoperative hyponatraemia related to desmopressin acetate. *Am J Kidney Dis* 2004; 44: E3–E6. (Right) magnetic resonance image showing osmotic demyelination within the pons (A), thalami and basal ganglia (B and C) in a 42-year-old patient with chronic alcoholism who presented with severe hyponatraemia (serum sodium 105 mmol/L), which was corrected too rapidly (14 mmol/L in 18 hours). From: Abbott R, Silber E, Felber J, Ekpo E. Osmotic demyelination syndrome. *BMJ* 2005; 331: 829–30.

Pseudohyponatraemia

It is a myth that pseudohyponatraemia no longer exists since the advent of ion-selective electrodes (Turchin et al., 2003). In all venous samples, the laboratory applies a standard dilution. This dilution step assumes a normal distribution between the water and the solid phase (protein and lipids), which is normally 93% and 7%, respectively. If this distribution is disturbed, for example because there are elevated levels of protein, triglycerides, cholesterol or lipoprotein X, relatively more diluent will be added to the sample water phase. This can cause pseudohyponatraemia and constitutes a laboratory artefact. Clinical situations in which pseudohyponatraemia can occur include multiple myeloma, hypertriglyceridemia and hypercholesterolaemia. Therefore, finding a normal serum osmolality in a patient with hyponatraemia should always raise the

Box 28.2 Possible symptoms of hyponatraemia

- Headache
- Nausea and vomiting
- Lethargy
- Psychosis
- Ataxia and gait disturbances
- Non-cardiogenic pulmonary oedema
- Seizures
- Coma.

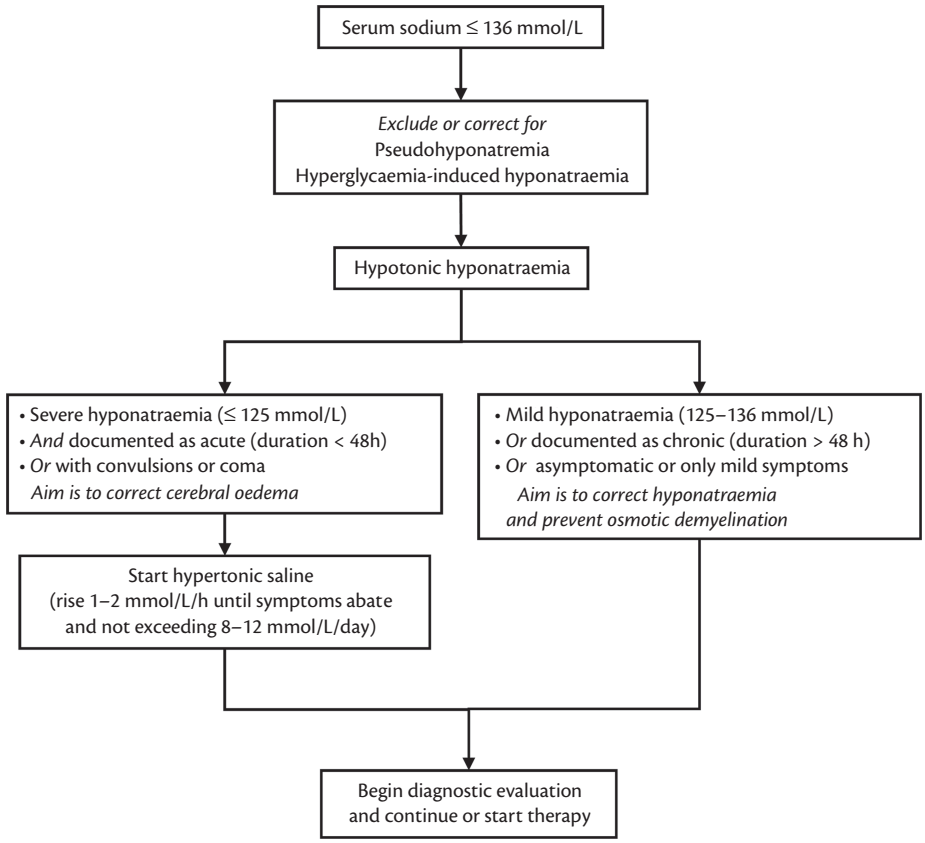

Fig. 28.2 Algorithm for hyponatraemia.

question whether pseudohyponatraemia is present. Another means to identify pseudohyponatraemia is to perform the measurement in an undiluted sample (direct potentiometry), for example using a blood gas analyser. If a low serum sodium concentration is found with indirect potentiometry, but a normal serum sodium with direct potentiometry, this is again suggestive of pseudohyponatraemia and should warrant a search for the underlying cause.

Box 28.3 Essential and optional investigations in hyponatraemia

All patients with hyponatraemia

- Serum glucose
- Serum potassium
- Serum creatinine
- Serum osmolality
- Urine sodium
- Urine osmolality.

Useful in certain settings

- Serum urea
- Serum uric acid
- Serum cortisol
- Serum thyroid stimulating hormone
- Fractional excretion of sodium, uric acid, and urea
- Urine chloride
- Urine potassium.

Hyperglycaemia-induced hyponatraemia

It is always important to analyse serum glucose in a patient with hyponatraemia. Glucose is an effective osmole and will therefore attract water from the intracellular compartment. Hyperglycaemia can, therefore, result in dilutional hyponatraemia. In fact, this relationship can sometimes be predicted with formulae, with serum sodium decreasing approximately 1.6–2.4 mmol/L for every 5.5 mmol/L increase in glycaemia (Hillier et al., 1999). However, several factors will affect this relationship, including oral intake, intravenous fluids, and ongoing osmotic diuresis. Still, in cases of severe hyperglycaemia, the degree of hyponatraemia can sometimes be completely attributed to hyperglycaemia and does not require therapy other than for hyperglycaemia. It is also important to differentiate hyperglycaemia-induced hyponatraemia from pseudohyponatraemia. In hyperglycaemia-induced hyponatraemia, the serum sodium is truly decreased, although the water shift is opposite to hypotonic forms of hyponatraemia. Because glucose is an effective osmole, it will contribute to serum osmolality and so hyperglycaemia-induced hyponatraemia is usually a hypertonic form of hyponatraemia. Because this will attract water from cells, it does not pose a risk of cerebral oedema. Nevertheless, it is important to regularly calculate the effective osmolality during the correction of hyperglycaemia, because a rapid decrease in effective osmolality can still cause cerebral oedema, for example,

if serum glucose decreases more than serum sodium rises (Hoorn et al., 2007).

Assessment of the extracellular fluid volume

The assessment of the extracellular fluid volume should always be performed in patients with hyponatraemia, because it offers a simple approach to detect cases with obvious hyper- or hypovolaemia. However, a caveat is that the assessment of the extracellular fluid volume in less clear-cut cases has a low sensitivity and specificity (Chung et al., 1987). This is especially true for the differentiation between hypovolaemia and euvolaemia. Therefore, the assessment of the extracellular fluid volume should not be the first and main determinant in the differentiation of hyponatraemia (Hoorn et al., 2005). This approach differs from what many of the 'traditional' algorithms for hyponatraemia propose, which still use the assessment of volume status as their first determinant. A systematic review on hypovolaemia concluded that more objective parameters such as capillary refill time, postural hypotension, and postural tachycardia have a higher sensitivity for diagnosing hypovolaemia, especially when these indices are combined (McGee et al., 1999).

Urine sodium and urine osmolality

The urine sodium and osmolality are very useful parameters in the differentiation of hyponatraemia, because they provide information on the underlying pathophysiology (Kamel et al., 1990). The urine sodium concentration can be considered a measure of the renin–angiotensin–aldosterone system, while the urine osmolality is a measure of vasopressin activity. A low urine sodium concentration usually indicates renal sodium retention by angiotensin II and aldosterone. Similarly, a high urine osmolality nearly always indicates active water reabsorption by the kidneys due to vasopressin or a vasopressin-like effect. These two concepts can be used to analyse the underlying cause of hyponatraemia (Table 28.2). For example, low urine sodium in combination with high urine osmolality suggests that both aldosterone and vasopressin are acting on the renal tubules. Clinical settings in which this can occur include true hypovolaemia or a low effective arterial blood volume (e.g. during heart failure or liver cirrhosis). The combination of finding a high urine sodium and a high urine osmolality is more challenging. This can reflect renal sodium loss with secondary vasopressin release due to hypovolaemia (e.g. with diuretic use). Conversely, this combination may be present in the syndrome of inappropriate antidiuretic hormone secretion (SIADH). During SIADH, urine osmolality is high due to the 'inappropriate' secretion of vasopressin, whereas urine sodium is normally excreted and therefore reflects dietary intake (and perhaps some degree of volume expansion). It is important to interpret urine sodium and osmolality in the context of the clinical setting. For example, urine sodium can also be low if the patient consumes a low sodium diet or when there is a water diuresis (when urine osmolality should also be low). The use of diuretics will also affect the urine sodium excretion, increasing it during active use, but decreasing it after recent discontinuation.

Table 28.2 Clinical settings of low or high urine sodium and osmolality

Urine sodium[a]	Urine osmolality[a]	Clinical setting
Low	High	Hypovolaemia with extrarenal sodium loss
		Low effective arterial blood volume (heart failure, liver cirrhosis, nephrotic syndrome)
		SIADH with low dietary salt intake
		Recent discontinuation of diuretics
Low	Low	Primary polydipsia and beer potomania
		Low solute intake ('tea and toast')
		Water diuresis during treatment of hyponatraemia
High	High	Active use of diuretics
		SIADH
		Adrenal insufficiency
		Renal or cerebral salt wasting
		Chronic kidney disease

a Although depending on other influencing factors, the threshold between low and high is approximately 30–40 mmol/L for urine sodium and between 100 and 280 for urine osmolality.

Urea and uric acid in hyponatraemia

The value of urea and uric acid in the differentiation of hyponatraemia is mainly based on their correlation with volume status. Expansion of the extracellular fluid volume will inhibit the renal tubular reabsorption of urea and uric acid. Therefore, a low serum concentration and high fractional excretion of uric acid and urea can be observed during, for example, hyponatraemia secondary to polydipsia or SIADH. Besides extracellular fluid volume expansion, the vasopressin V1 receptor may also contribute to this effect, because the induction of hyponatraemia with desmopressin (which only stimulates the V2 receptor) resulted in a significantly smaller fall in serum uric acid (Decaux et al., 1996). Recently, a fractional uric acid excretion > 13% was identified as the most sensitive parameter to diagnose SIADH in patients who were also using diuretics (Fenske et al., 2008).

Functional tests in hyponatraemia

In more complex cases of hyponatraemia, functional tests can be useful, including assessing the response to isotonic saline, a water load, or a vasopressin receptor antagonist. Assessing the response to isotonic saline can be used to differentiate hypovolaemic from euvolaemic hyponatraemia. The serum sodium concentration is expected to rise during the infusion of isotonic saline in hypovolaemic hyponatraemia, whereas no response or even a deterioration of hyponatraemia can occur in euvolaemic hyponatraemia (Steele et al., 1997). A water loading test can be used to differentiate true SIADH causing hyponatraemia from a reset osmostat, which is a subform of SIADH in which osmolality is regulated at a lower set-point (Fig. 28.3). During a water-loading test, 20 mL/kg water is administered and a 4-hour observation period is used to assess if this water is retained or normally excreted. Normal water excretion is defined as > 80% of the water load, which would suggest a reset osmostat. An important warning is that if the patient has true SIADH, part of the water load will be retained and hyponatraemia may worsen; therefore, close monitoring during the test is required. Vasopressin receptor antagonists, which have recently been introduced as a new treatment for hyponatraemia (see later), can also be applied diagnostically. Namely, a patient with true SIADH is expected to respond to a vasopressin receptor antagonist with the excretion of more dilute urine and therefore a rise in serum sodium. If this response is absent, this could suggest the

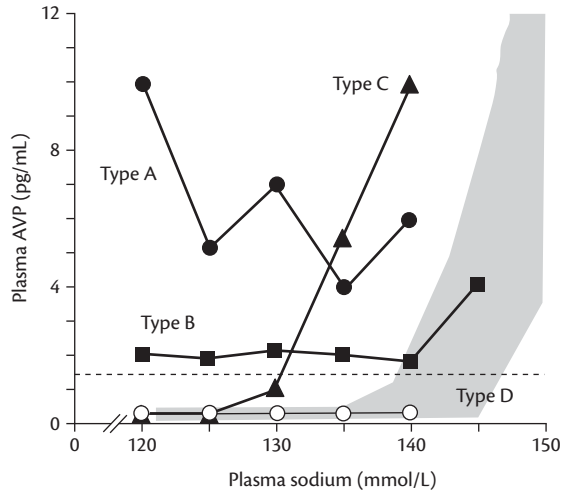

Fig. 28.3 Types of the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Patterns of plasma levels of arginine vasopressin (AVP) compared with plasma sodium levels in patients with the syndrome of inappropriate antidiuretic hormone secretion. Type A is characterized by unregulated secretion of AVP and can occur during ectopic production of AVP by neoplasia. Type B is characterized by an elevated basal secretion of AVP, despite normal regulation by osmolality. Type C is characterized by a 'reset osmostat,' meaning that the serum sodium is regulated at a lower concentration due to a lower osmotic threshold for vasopressin release. Type D is characterized by undetectable AVP despite the presence of SIADH; this can be observed in nephrogenic syndrome of inappropriate antidiuresis. The shaded area represents normal values of plasma AVP.

From: Robertson GL. Regulation of arginine vasopressin in the syndrome of inappropriate antidiuresis. *Am J Med* 2006; 119: Suppl 1: S36–42.

presence of an activating mutation of the vasopressin-2 receptor, the so-called nephrogenic syndrome of inappropriate antidiuresis (see later) (Decaux et al., 2007). Vasopressin is rarely measured in hyponatraemia because it is a difficult assay and because its activity is reflected by the urine osmolality. Still, measurement of vasopressin is sometimes useful in difficult cases of SIADH. The development of copeptin, a glycopeptide derived from same precursor peptide as vasopressin, has shown some diagnostic value in hyponatraemia, especially for primary polydipsia and volume depletion (Fenske et al., 2009).

Aetiology and pathogenesis

Diuretic-induced hyponatraemia

Diuretics are a common cause of hyponatraemia. This side effect is mainly observed with thiazide diuretics and, to a lesser extent, with potassium-sparing diuretics. Loop diuretics alone rarely produce hyponatraemia and can even be used as therapy for hyponatraemia, for example, in SIADH (Sonnenblick et al., 1993). The pathogenesis of thiazide-induced hyponatraemia remains incompletely understood and several mechanisms may contribute. The original theory that renal sodium loss causes hypovolaemia with secondary vasopressin release may be true for patients who use a combination of diuretics. However, patients who are treated with thiazides only and develop hyponatraemia, gain weight (Friedman et al., 1989). This suggests thiazide-induced hyponatraemia is primarily a water-retaining disorder. Whether this is mainly due to more central release of vasopressin or is primarily a renal effect is unknown. Increased fluid intake, for example because thirst is stimulated by elevated angiotensin II, is also believed to play a role. Others have proposed that the potassium depletion associated with thiazides reduces the set-point for baroreceptor-mediated vasopressin release. Finally, there also appears to be an individual predisposition to develop hyponatraemia with a thiazide, because it is reproducible during re-challenge (Friedman et al., 1989). The latter observation suggests that patients who develop thiazide-induced hyponatraemia, may have a contraindication for future use. When considering thiazide-induced hyponatraemia, it is also important to be aware of the many combination preparations that include a thiazide. These are mainly antihypertensive drugs that may also contain thiazide-like compounds, such as indapamide.

Syndrome of inappropriate antidiuretic hormone secretion

In SIADH, vasopressin secretion is considered 'inappropriate,' because neither hypertonicity nor hypovolaemia stimulates vasopressin release. Four patterns of abnormal vasopressin secretion are recognized (Fig. 28.3).

Antidiuresis causes progressive hyponatraemia until a renal defence mechanism called 'vasopressin escape' is activated. During escape from antidiuresis, the vasopressin V2 receptor and the water channel aquaporin-2 are downregulated, preventing further water reabsorption (Verbalis, 2006). The causes of SIADH are generally related to malignant diseases, pulmonary diseases, central nervous system disease, drugs, and miscellaneous causes (Table 28.3). A number of commonly used drugs are associated with SIADH, including antidepressants, antiepileptics, antipsychotics, and the vasopressin analogues desmopressin and oxytocin. A number of other drugs rarely cause hyponatraemia (reviewed in Liamis et al., 2008). The essential and supplemental criteria for the diagnosis of SIADH remain essentially the same as those originally proposed by Bartter and Schwartz in 1957 (Box 28.4) (Schwartz et al., 1957). Importantly, although SIADH is common, it should still be regarded as a diagnosis of exclusion, in which diuretic use, pituitary, adrenal, and thyroid insufficiency must be excluded. Although a urine osmolality > 100 mOsm/kg of water is used in the definition of SIADH, it usually exceeds the serum osmolality. A number of the miscellaneous causes of SIADH merit emphasis. For example, nausea, pain, and stress are non-specific, but strong stimuli for vasopressin release, and often contribute to postoperative hyponatraemia, especially when patients are also receiving hypotonic intravenous fluids (Chung et al., 1986). A rarer cause of SIADH is the so-called nephrogenic syndrome of inappropriate antidiuresis. In this genetic disorder, there is a gain-of-function mutation of the vasopressin V2 receptor causing constitutive activation of the V2R-AQP2 cascade, resulting in increased renal water reabsorption (Feldman et al., 2005). This diagnosis should be considered in a patient with chronic SIADH of unknown origin in whom vasopressin is undetectable and who does not respond to a vasopressin receptor antagonist. Another genetic susceptibility for hyponatraemia was identified in individuals with a polymorphism in the *TRPV4* gene, which encodes a calcium channel believed to be involved in 'osmosensing' (Tian et al., 2009). Finally, exercise-associated hyponatraemia, which is relatively common among marathon runners, is considered a form of SIADH, because besides over-hydration there is evidence for non-osmotic release of vasopressin, possibly

Table 28.3 Causes of the syndrome of inappropriate antidiuretic hormone secretion (SIADH)

Malignant diseases	Pulmonary disorders	Disorders of the central nervous system	Drugs	Other causes
Carcinoma	*Infections*	*Infections*	*Drugs that stimulate release of AVP or enhance its action*	*Hereditary* (gain of function mutations of the vasopressin V2 receptor)
Lung (small cell, mesothelioma)	Bacterial pneumonia	Encephalitis	Chlorpropamide	*Idiopathic*
Oropharynx	Viral pneumonia	Meningitis	SSRIs	*Transient*
Gastro-intestinal tract (stomach, duodenum, pancreas)	Pulmonary abscess	Brain abscess	TCAs	Exercise-associated hyponatraemia
Genitourinary tract (ureter, bladder, prostate, endometrium)	Tuberculosis	Rocky Mountain spotted fever	Clofibrate	General anaesthesia
Endocrine thymoma	Aspergillosis	AIDS	Carbamazepine	Nausea
Lymphomas	*Asthma*	Malaria	Vincristine	Pain
Sarcomas	*Cystic fibrosis*	*Bleeding and masses*	Nicotine	Stress
Ewing sarcoma	*Respiratory failure associated with positive pressure*	Subdural haematoma	Antipsychotic drugs	
		Subarachnoid haemorrhage	Ifosfamide	
		Cerebrovascular accident	Cyclo-phosphamide	
		Brain tumours	NSAIDs	
		Head trauma	MDMA ('ecstasy')	
		Hydrocephalus	*AVP analogues*	
		Cavernous sinus thrombosis	Desmopressin	
		Other	Oxytocin	
		Multiple sclerosis	Vasopressin	
		Guillain–Barré syndrome		
		Shy–Drager syndrome		
		Delirium tremens		
		Acute intermittent porphyria		

AIDS = acquired immunodeficiency syndrome; AVP = arginine vasopressin; MDMA = methylenedioxymethamphetamine; NSAIDs = non-steroidal anti-inflammatory drugs

mediated through cytokines (Almond et al., 2005; Siegel et al., 2007). Specific guidelines for exercise-associated hyponatraemia have been developed (Hew-Butler et al., 2008).

Endocrine causes of hyponatraemia

Endocrine causes of hyponatraemia include primary and secondary adrenal insufficiency and hypothyroidism. The mechanism and presentation of hyponatraemia due to primary or secondary adrenal insufficiency differ. Secondary adrenal insufficiency is characterized by hypocortisolism. Because cortisol normally suppresses vasopressin, hypocortisolism will increase central vasopressin release. Hypocortisolism, therefore, causes hyponatraemia that resembles SIADH. In primary adrenal insufficiency (Addison disease), however, there is a deficiency of both glucocorticoids and mineralocorticoids. In this setting, hyponatraemia is not only caused by hypocortisolism, but also by hypoaldosteronism, which leads to renal sodium loss. However, additional signs, including metabolic acidosis, hyperkalaemia, hypercalcaemia, and orthostatic hypotension, typically accompany mineralocorticoid deficiency. Although hyponatraemia is usually part of a more elaborate constellation of physical and biochemical findings, it can be the only or first presentation of adrenal insufficiency (Smith et al., 2004). Therefore, physicians should have an index of suspicion for adrenal insufficiency as a cause of hyponatraemia and have a low threshold for diagnostic testing using a random cortisol or stimulation test with adrenocorticotrophic hormone (Soule, 1999). Hypothyroidism can cause hyponatraemia, especially in patients with myxoedema (Curtis, 1956). Hyponatraemia in this context may be due to a decrease in cardiac output and glomerular filtration rate. A recent study showed that for every 10 mU/L rise in thyroid-stimulating hormone, serum sodium decreased 0.14 mmol/L (Warner et al., 2006). This suggests that in most cases, hypothyroidism has a very limited effect on serum sodium.

Cerebral salt wasting

Cerebral salt wasting (CSW) is an incompletely understood disorder (Singh et al., 2002). The best evidence for its existence comes from patients with subarachnoid haemorrhage in whom polyuria with a natriuresis can be observed (Berendes et al., 1997). It is thought that, if uncorrected, the loss of water and salt causes hypovolaemia with the subsequent release of vasopressin and therefore hyponatraemia. Brain natriuretic peptide has been implicated as the cause of the massive natriuresis. The difficulty is that most of the neurological disorders associated with cerebral salt wasting can also cause other forms of hyponatraemia, including SIADH and secondary adrenal insufficiency. Furthermore, biochemically, CSW and SIADH are remarkably similar, further complicating their differentiation (Table 28.4). Studies have indicated that SIADH is, in fact, more common than CSW following subarachnoid haemorrhage and CSW may therefore be overdiagnosed (Sherlock et al., 2006). Still, its recognition is important, because treatment is opposite to SIADH, since it relies on fluid resuscitation, rather than fluid restriction.

Box 28.4 Diagnostic criteria for the syndrome of inappropriate antidiuretic hormone secretion (SIADH)

Essential criteria

- Decreased effective serum osmolality (< 270 mOsm/kg of water)
- Urine osmolality > 100 mOsm/kg of water during hypotonicity
- Clinical euvolaemia
- Urinary sodium > 40 mmol/L with normal dietary salt intake
- Absence of adrenal, thyroid, pituitary, or renal insufficiency or diuretic use.

Supplemental criteria

- Serum uric acid < 0.24 mmol/L (< 4 mg/dL)
- Serum urea < 3.6 mmol/L (< 10 mg/dL)
- Failure to correct hyponatraemia after 0.9% saline infusion
- Fractional sodium excretion > 1%; fractional urea excretion > 55%
- Correction of hyponatraemia through fluid restriction
- Plasma vasopressin level inappropriately elevated relative to plasma osmolality
- Abnormal result on test of water load (< 80% excretion of 20 mL water/kg body weight over a period of 4 hours and/or failure to dilute urine osmolality to < 100 mOsm/kg of water).

Data are adapted from Schwartz et al. (1957) and Janicic and Verbalis (2003).

Heart failure, liver cirrhosis, and nephrotic syndrome

Heart failure, liver cirrhosis, and nephrotic syndrome are oedema-forming disorders in which an increase in total body water exceeds the increase in total body sodium (see Chapter 30). Despite hypervolaemia, the effective arterial blood volume is decreased in all three disorders, although for different reasons. In heart failure this is due to a low cardiac output, in liver cirrhosis it is due to systemic vasodilatation and arteriovenous fistulae, and in nephrotic syndrome it is due to loss of plasma oncotic pressure. A low effective arterial blood volume will activate both the renin–angiotensin–aldosterone system and the vasopressin axis. The release of vasopressin during a low effective arterial blood volume is mediated through baroreceptors. The activation of these systems explains why urine sodium is typically low and why urine osmolality is high (Table 28.2). Hyponatraemia usually develops in more advanced stages of heart failure (New York Heart Association classes III and IV) and liver cirrhosis (Child–Pugh B and C) and has a prevalence of approximately 20–30%. Hyponatraemia occurs less commonly in nephrotic syndrome and only develops when it is associated with severe intravascular volume depletion (usually serum albumin < 20 g/L). The development of hyponatraemia has been recognized as a poor prognostic sign in heart failure and liver cirrhosis, and has even emerged as an independent predictor for mortality (Gheorghiade et al., 2007; Kim et al., 2009). Interestingly, hyponatraemia was recently also found to be a predictor of long-term mortality, and admission for heart failure after hospital discharge in survivors of acute ST-elevation myocardial infarction (Goldberg et al., 2006). The explanation for these associations is probably not so much a direct effect of hyponatraemia, but rather hyponatraemia as a marker of the extent of the so-called neurohumoral response, and therefore the degree of decompensation. That hyponatraemia is a central feature of the neurohumoral response has clearly been demonstrated in heart and liver failure, in which hyponatraemia correlates with the activity of the renin–angiotensin and prostaglandin systems (Dzau et al., 1984). Of interest, hyponatraemia is also sometimes observed in pulmonary embolism and pulmonary hypertension (Forfia et al., 2008; Scherz et al., 2010); this is likely to be due to right-sided heart failure.

Table 28.4 Biochemical and haemodynamic parameters in the syndrome of inappropriate antidiuretic hormone secretion (SIADH) and cerebral salt wasting (CSW)

	SIADH	CSW
Serum sodium	Low	Low
Serum urea	Normal–low	Normal–elevated
Serum uric acid	Low	Low
Urine volume	Low	High
Urine sodium	> 40 mmol/L	>> 40 mmol/L
Blood pressure	Normal	Normal—postural drop
Central venous pressure	Normal	Low

Data are adapted from Sherlock et al. (2006).

Polydipsia and low solute intake

Hyponatraemia due to primary polydipsia, beer potomania, and low solute intake ('tea and toast') are similar in the sense that vasopressin activity is usually absent and urine osmolality, therefore, low (Table 28.2). In primary or psychogenic polydipsia patients consume quantities of water that exceed the water excretory capacity of the kidneys (15–20 L/day); it is most commonly seen during an acute psychosis in patients with schizophrenia. One study, however, suggested that in hyponatraemia due to polydipsia, water intake alone was usually not sufficient to explain the degree of hyponatraemia; it was proposed that apparent loss of solutes (possibly through a renal route) played a significant contributory role (Musch et al., 2003). In beer potomonia and subjects with low solute intake, fluid intake is usually less than in primary polydipsia, but solute excretion becomes the rate-limiting step for electrolyte-free water excretion (Thaler et al., 1998). In these settings, smaller amounts of fluid (e.g. 2–5 L/day) can already cause significant hyponatraemia. This mechanism may also play a role in hyponatraemia in anorexia nervosa, which has an estimated prevalence of 20% (Miller et al., 2005).

Extrarenal sodium loss with water retention

Hyponatraemia due to extrarenal sodium loss can be observed with gastrointestinal losses, burn wounds or 'third spacing' due to pancreatitis, bowel obstruction, or muscle trauma. Although the fluids lost are hypotonic, hyponatraemia can develop when there is hypovolaemia with ongoing intake or administration of hypotonic fluids. These patients have a total body sodium deficit that

exceeds their water deficit. In this setting, extracellular fluid volume contraction causes the release of vasopressin. This is mediated by baroreceptors located in the aortic arch, carotid sinus, cardiac atria, and pulmonary venous system. These patients typically have a very low urine sodium concentration and high urine osmolality (Table 28.2). One exception is vomiting, in which urine sodium is higher because alkalosis-induced bicarbonaturia causes natriuresis. A better parameter in this setting is urinary chloride, which is typically very low, because hydrogen chloride is lost with vomiting.

Renal insufficiency

Several causes of hyponatraemia are also associated with acute or chronic kidney disease, including extrarenal sodium loss, heart failure, liver cirrhosis, hepatorenal syndrome, renal artery stenosis, and salt-losing nephropathy. In these disorders, hyponatraemia is usually caused by baroreceptor-mediated vasopressin release, while renal insufficiency is often of prerenal origin. Therefore, the calculation of the fractional sodium excretion is useful, which is usually low (< 1%) except in salt-losing nephropathy. A more separate and rare entity is the hyponatraemic hypertensive syndrome, in which unilateral renal artery stenosis causes significant hypertension, hyponatraemia, hypokalaemia, polydipsia, and polyuria. The pathogenesis of hyponatraemia is ascribed to volume-mediated vasopressin release and polydipsia stimulated by high angiotensin II levels; hypokalaemia is also factor. This syndrome is most often seen in asthenic elderly women who smoke, but it has also been reported in children with fibromuscular dysplasia (Agarwal et al., 1999). Chronic kidney disease can also play a causal role in the pathogenesis of hyponatraemia. When the glomerular filtration rate is very low, or if patients are already undergoing renal replacement therapy, free water clearance is limited or absent. In this setting, hyponatraemia can easily develop if fluid restriction is not adhered to. A recent study showed that hyponatraemia in this setting was associated with poor outcome, because a lower pre-dialysis serum sodium was associated with an increased risk of death (Waikar et al., 2011). Hyponatraemia is also relatively common among patients undergoing peritoneal dialysis and may be related to hyperglycaemia, depending on the composition of dialysis fluid used, or a catabolic state with potassium depletion (Zevallos et al., 2001; Zanger, 2010).

Treatment and outcome

General principles

The treatment of hyponatraemia relies on the following principles: acute hyponatraemia should be treated immediately regardless of the cause, whereas treatment should be directed towards the underlying cause in chronic hyponatraemia, while avoiding rapid or over-correction. These opposite strategies are related to the fact that brain cells start adapting to the hypotonic environment within 1 or 2 days by extruding intracellular electrolytes and organic solutes, including myoinositol, phosphocreatine, and amino acids (Fig. 28.4). Because the time at which hyponatraemia developed is frequently unknown, the decision whether hyponatraemia is acute or chronic often depends on the assessment of symptoms, but must assumed to be chronic, if onset and duration are unclear. Although severe neurological symptoms such as seizures and coma should always point in the direction of acute hyponatraemia, more subtle symptoms can occur in both acute and chronic hyponatraemia (Box 28.2). Another challenge is that 'acute on chronic' hyponatraemia may occur, for example, when hypotonic fluids are administered to a chronically hyponatraemic patient. Therefore, a degree of uncertainty often remains when treating hyponatraemia, emphasizing the importance of careful follow-up to assess the serum sodium and evolution of symptoms. The current recommendations for correction rates are based on consensus and expert opinion, rather than evidence from randomized trials (Box 28.5) (Verbalis et al., 2007). Recently, however, a European guideline on the management of hyponatraemia was published (Spasovski et al., 2014). In general, the recommended correction rates have become slightly more conservative over the years. This probably stems from the recognition that osmotic demyelination can already occur with correction rates of 10–12 mmol/L/day, whereas cerebral oedema due to hyponatraemia can usually be treated effectively by raising the serum sodium by as little as 4–6 mmol/L. The susceptibility to cerebral oedema or osmotic demyelination is higher in certain patient groups (Table 28.5). Elderly women taking thiazides and hypoxaemic patients have a higher risk for both conditions.

Treatment of acute hyponatraemia

Acute hyponatraemia is most commonly seen in primary polydipsia, exercise-associated hyponatraemia, the use of drugs such as 3,4-methylenedioxymethamphetamine ('Ecstasy'), desmopressin, oxytocin, and thiazides, and excessive administration of hypotonic intravenous fluids (Arieff, 1986; Hsu et al., 2005). All these settings are usually characterized by the intake or administration of a large amount of electrolyte-free water in a short period of time, with vasopressin acting simultaneously to prevent excretion. Hypertonic saline remains the treatment of choice for acute hyponatraemia. By introducing a hypertonic solution into the extracellular space, water will be attracted from the intracellular space. This will reduce the cell swelling associated with acute hyponatraemia and is effective in treating cerebral oedema. Several formulae are available to help predict the rise in serum sodium when therapy with hypertonic saline is commenced. Although each formula has its strengths and limitations, we favour the Adrogué–Madias formula, because of its relative simplicity and its validation in clinical studies (Fig. 28.5) (Adrogue and Madias, 2000; Liamis et al., 2006). The Adrogué–Madias formula predicts what the rise in serum sodium will be when 1 L of a given solution is administered to a patient. It requires information on the amount of sodium present in the solution of choice, the serum sodium concentration of the patient, and an estimate of the patient's total body water. A simpler approach was recently proposed as initial emergency therapy for acute hyponatraemia, namely a bolus infusion of 3% sodium chloride (100 mL or 2 mL/kg, repeated up to two times) (Moritz and Ayus, 2010). Notably, whatever approach is used, the serum sodium concentration should be measured frequently during therapy with hypertonic saline (preferably every 2–4 hours).

Auto-correction and over-correction

It is essential to be aware of the possibility of auto-correction or over-correction during the treatment of hyponatraemia. Auto-correction usually occurs when the stimulus for vasopressin release suddenly abates, which is then followed by the rapid excretion of a dilute urine. During this process, the serum sodium concentration can rise quickly with a consequent risk of osmotic

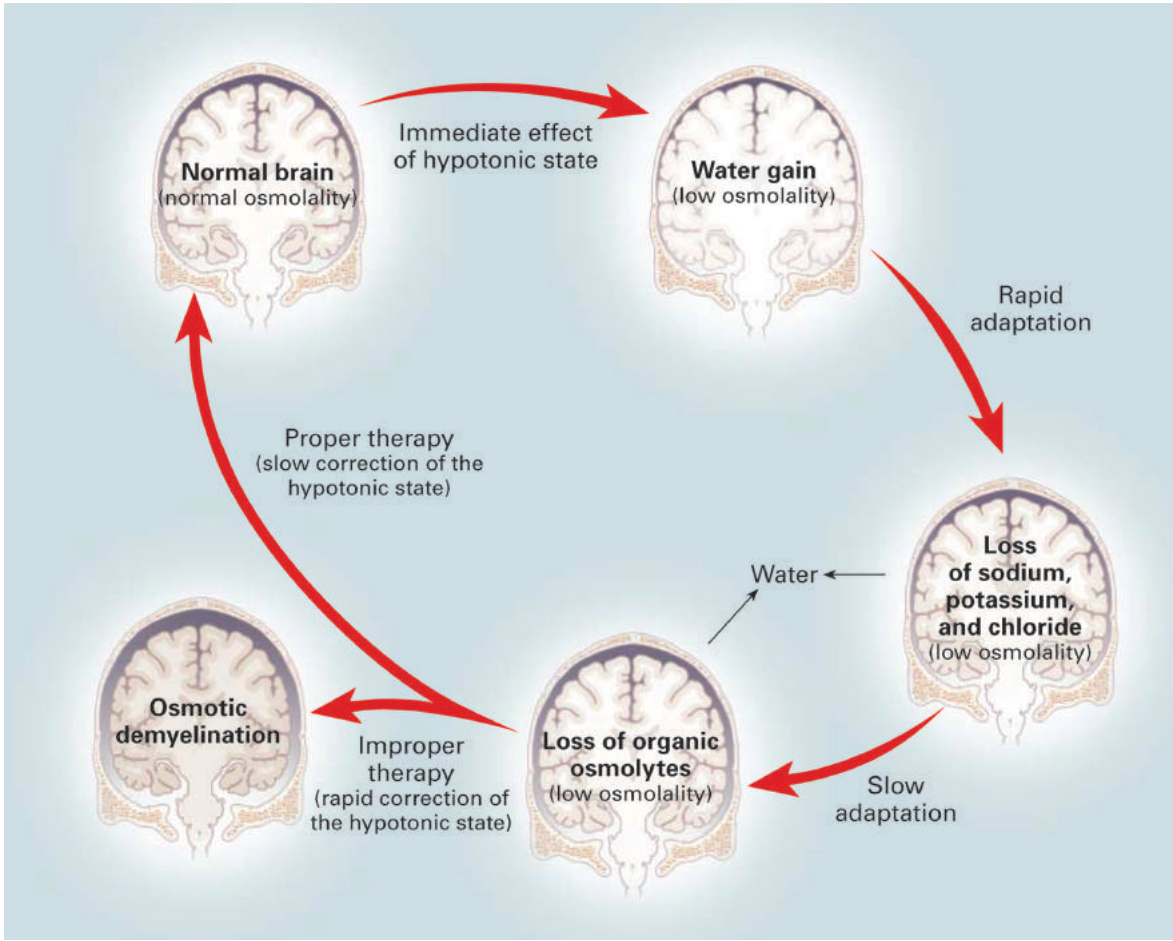

Fig. 28.4 Effects of hyponatraemia on the brain and adaptive responses. Within minutes after the development of hypotonicity, water gain causes swelling of the brain and a decrease in osmolality of the brain. Partial restoration of brain volume occurs within a few hours as a result of cellular loss of electrolytes (rapid adaptation). The normalization of brain volume is completed within several days through loss of organic osmolytes from brain cells (slow adaptation). Low osmolality in the brain persists despite the normalization of brain volume. Proper correction of hypotonicity re-establishes normal osmolality without risking damage to the brain. Overly aggressive correction of hyponatraemia can lead to irreversible brain damage.
From: Adrogué HJ, Madias NE. Hypnatraemia. *N Engl J Med* 2000; 342: 1581–9.

demyelination. Common examples include the treatment of hypovolaemic hyponatraemia, discontinuation of desmopressin (DDAVP), or the treatment of adrenal insufficiency with steroids. Conversely, over-correction usually occurs during treatment with hypertonic saline when the actual rise in serum sodium concentration exceeds the predicted rise (Mohmand et al., 2007). Auto-correction and overcorrection should be anticipated during the treatment of hyponatraemia by regularly monitoring the serum sodium concentration, urine osmolality, and urine output. If urine production increases and urine tonicity decreases, this suggests the onset of a water diuresis with the likelihood of a rapid rise in serum sodium. If the maximum correction rate is exceeded during auto-correction or over-correction, measures should be taken to curtail the rise in serum sodium concentration. Besides discontinuing the active therapy for hyponatraemia, additional measures include the infusion of hypotonic solutions or the administration of DDAVP (Perianayagam et al., 2008). In fact, some have proposed co-administering DDAVP during the correction of hyponatraemia to achieve a more controlled rise in serum sodium, especially in those patients with risk factors for osmotic demyelination (Table 28.5) (Sterns et al., 2010). In experimental animals, re-induction of hyponatraemia after rapid over-correction of hyponatraemia reduces mortality (Gankam-Kengne et al., 2009). Other experimental manoeuvres that have been reported to improve the outcome of osmotic demyelination in rodents include

Box 28.5 General principles of the correction rate of hyponatraemia

- The correction rate should be ≤ 10 mmol/L during the first 24 hours and ≤ 18 mmol/L during the first 48 hours of treatment.
- The maximum correction rates represent limits and should therefore not be the goal of treatment.
- Acute and/or symptomatic hyponatraemia may initially be corrected faster with 1–2 mmol/L/hour.
- If hyponatraemia is definitely chronic or if there are risk factors for the osmotic demyelination syndrome (see Table 28.5), slower rates of correction should be applied (≤ 8 mmol/L/day).

Table 28.5 Risk factors for neurologic complications of hyponatraemia

Cerebral oedema	Osmotic demyelination syndrome (during correction of hyponatraemia)
Postoperative state	Alcohol abuse
Use of desmopressin	Thiazide diuretics
Thiazide diuretics	Malnourishment
Children	Hypokalaemia
Psychogenic polydipsia	Hypoxia
Hypoxia	Burn victims
	Steroids for adrenal insufficiency
	Liver cirrhosis

Data are adapted from Lauriat and Berl (1997).

the administration of myoinositol, which increases the uptake of this osmolyte in brain cells (Silver et al., 2006), and minocycline, which decreases the permeability of the blood–brain barrier (Gankam-Kengne et al., 2010).

Treatment of chronic hyponatraemia

Because immediate treatment of cerebral oedema is not an issue during chronic hyponatraemia, therapy can be directed towards the underlying cause. The treatment modalities for the different causes of hyponatraemia are shown in Table 28.6. Some of the treatments are straightforward, such as discontinuation of the offending drug, or treatment with steroids or thyroid hormone in hyponatraemia due to hypocortisolism or hypothyroidism. The remaining treatments are less targeted in the sense that they do not suppress the stimulus for hyponatraemia. These treatments are directed to restricting the intake of electrolyte-free water or promoting its excretion and include fluid restriction, loop diuretics, urea, and demeclocycline. Fluid restriction and loop diuretics are most commonly used in the treatment of hyponatraemia due to SIADH, heart failure or liver cirrhosis, while urea and demeclocycline are

$$\text{Volume (L)} = \frac{\text{Desired } \Delta[\text{Na}]_s}{\Delta[\text{Na}]_s \text{ (with 1 L)}}$$

$$\text{Change in serum } \text{Na}^+ = \frac{(\text{infusate } \text{Na}^+ + \text{infusate } \text{K}^+) - \text{serum } \text{Na}^+}{\text{total body water} + 1}$$

Figure 28.5 Adrogué–Madias formula for predicting the effect on the serum sodium concentration with the administration of a given infusate. *The upper formula* can be used to predict the rise in serum sodium ($\Delta[Na]_s$) when 1 L of a given infusate is administered. The formula relies on subtracting the sodium concentration of the infusate ($[Na]_{inf}$) from the serum sodium concentration in the patient ($[Na]_1$) and dividing this number by total body water (TBW) plus one (because TBW will increase with 1 L when 1 L of the infusate is administered). The estimated TBW is calculated as a fraction of body weight. The fraction is 0.6 in children; 0.6 and 0.5 in nonelderly men and women, respectively; and 0.5 and 0.45 in elderly men and women, respectively. When potassium is added to the infusate, this concentration should be included in the formula ($[Na + K]_{inf}$). *The lower formula* can be used to calculate the volume of the infusate necessary to achieve the desired rise in serum sodium (Desired $\Delta[Na]_s$) by dividing this number with the calculated change in serum sodium concentration in the upper part of the formula ($\Delta[Na]_s$).
From: Adrogué HJ, Madias NE. Hyponatraemia. *N Engl J Med* 2000; 342: 1581–9.

Table 28.6 Treatment modalities for hyponatraemia

Treatment	Indication
Hypertonic saline	Acute and/or symptomatic hyponatraemia
Isotonic saline	Hyponatraemia associated with hypovolaemia
Vasopressin-receptor antagonists	SIADH, heart failure, liver cirrhosis[a]
Loop diuretics	SIADH, primary polydipsia, heart failure, liver cirrhosis
Urea	SIADH
Water restriction	SIADH, heart failure, liver cirrhosis, primary polydipsia
Dietary intake of salt and protein	Hyponatraemia due to low solute intake
Cause-directed therapy	E.g. thyroid hormone in hypothyroidism, steroids in adrenal insufficiency or discontinuation of the offending drug

[a] In Europe, vasopressin-receptor antagonists have only been registered for hyponatraemia secondary to SIADH.

CSW = cerebral salt wasting; SIADH = syndrome of inappropriate antidiuretic hormone secretion.

usually restricted to the treatment of SIADH. The recommended degree of fluid restriction should be determined by relating the urine sodium and potassium concentrations (which determine tonicity) to the serum sodium concentration (Fig. 28.6). Loop diuretics inhibit the generation of a concentration gradient in the renal medulla and promote the excretion of sodium and water. Urea causes an osmotic diuresis, which also promotes the excretion of electrolyte-free water. Demeclocycline is an antibiotic with nephrogenic diabetes insipidus as a side effect; this effect can be exploited during hyponatraemia to induce a water diuresis. Because of significant side effects and potential overcorrection, however, demeclocycline is not recommended. In many patients, especially the elderly, low solute intake plays a contributory role in the development of hyponatraemia. Therefore, fluid restriction or loop diuretics may be combined with increased dietary intake of sodium and protein (or alternatively sodium chloride tablets). It is also important to emphasize that hyponatraemia may resolve when the underlying disorder has been treated, for example, in SIADH due to infection: in this setting supportive treatment may be sufficient to prevent a further fall in the serum sodium. However, there will be causes of chronic hyponatraemia that remain difficult to treat and in which more targeted therapy would be desirable. Recently, a

$\frac{\text{Urinary sodium + urinary potassium}}{\text{Serum sodium}}$	Recommended fluid intake
>1	<500 mL/day
~1	500–700 mL/day
<1	1 liter/day

Fig. 28.6 Recommended fluid restriction. The recommend fluid intake can be calculated on the basis of the ratio between the urinary tonicity (urinary sodium plus urinary potassium concentration) and the serum sodium concentration. The recommended fluid intake is < 500 mL/day for a ratio > 1500–700 mL/day for a ratio near 1, and < 1 L/day for a ratio < 1.

more targeted approach has become available with the introduction of vasopressin receptor antagonists, which are discussed in more detail below.

Vasopressin receptor antagonists

Vasopressin receptor antagonists are non-peptide molecules that competitively inhibit one or more of the human vasopressin receptors V1a, V1b, or V2. The proposed molecular mechanism of vasopressin receptor antagonists is that they penetrate deeper and more selectively into the binding pocket of the vasopressin receptor type 2 than native vasopressin, but without activating the receptor (Decaux et al., 2008). Conivaptan is a combined V1a/V2 receptor antagonist for intravenous use, whereas tolvaptan, mozavaptan, and lixivaptan are orally active V2-selective receptor antagonists. All of these agents cause a free water diuresis without appreciable natriuresis or kaliuresis, and they are sometimes referred to as 'aquaretics'. This effect is mainly attributed to inhibition of the V2 receptor in the collecting duct, which prevents vasopressin from recruiting aquaporin-2 water channels to increase water reabsorption. Therefore, vasopressin receptor antagonists can be used to treat hypervolaemic or euvolaemic hyponatraemia, in which increased vasopressin is considered 'inappropriate'. Co-inhibition of the V1a receptor, which is located in vascular smooth muscle, could also be beneficial in reducing coronary vasoconstriction, myocyte hypertrophy, and vascular resistance in patients with heart failure, but definitive studies on this are lacking (Goldsmith, 2006). At present, some twenty clinical trials have tested these agents against placebo or conventional therapy in patients with liver cirrhosis, heart failure, or hyponatraemia secondary to SIADH (reviewed in Hoorn and Zietse, 2010). In all trials, vasopressin receptor antagonists effectively raised serum sodium and helped to correct hyponatraemia. In addition, a positive effect on some secondary endpoints was observed, including an improved mental state and reductions in body weight, dyspnoea, and ascites (Schrier et al., 2006; Konstam et al., 2007). However, the Efficacy of Vasopressin Antagonist in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial, which included 4133 patients hospitalized for heart failure, did not show a beneficial effect of tolvaptan on the primary outcomes of death or re-hospitalization for heart failure (Konstam et al., 2007). Thus, vasopressin receptor antagonists are effective for the correction of hypervolaemic or euvolaemic hyponatraemia, as was also confirmed by a meta-analysis (Rozen-Zvi et al., 2010), but they have not yet shown an effect on primary outcomes. Conivaptan and tolvaptan were recently approved for clinical use.

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CHAPTER 29

Approach to the patient with hypernatraemia

Robert Zietse

Introduction and epidemiology

Hypernatraemia is generally defined as a plasma sodium concentration of 146 mmol/L or higher. As plasma osmolality is largely determined by the plasma sodium concentration, hypernatraemia is a state of hypertonicity. For hypernatraemia to occur, the total amount of sodium in the extracellular volume (ECV) must be increased relative to the amount of water in the ECV. Similar to hyponatraemia, it is important to be aware of the fact that hypernatraemia is primarily a water balance problem. The causes of hypernatraemia are commonly categorized as being due to reduced water intake, increased water loss or (less commonly) a gain of sodium (Table 29.1). Although it occurs less frequent than hyponatraemia, hypernatraemia develops in some 1–2.6% of patients admitted to the hospital (Palevsky et al., 1996). In outpatients, it occurs most frequently in infants and the elderly, but in patients admitted to hospital it can occur in all age groups.

In most cases that present with hypernatraemia the cause will be apparent from the clinical setting. The main reason for its occurrence is uncorrected water loss. This is even more likely to be the case in patients who develop hypernatraemia outside of hospital. For hypernatraemia to develop, two mechanisms are usually required that work in concert: water loss (either renal or elsewhere) and the inability to correct this water loss with an appropriate water intake. Thus, in patients with increased renal water loss (e.g. diabetes insipidus (DI)), hypernatraemia only occurs when water intake is impaired and these patients rarely develop hypernatraemia when there is free access to water. Patients with free access to water may be polyuric, but will usually be able to drink sufficient amounts of water to keep the plasma sodium concentration within the normal range. Hypernatraemia will only ensue in the setting of unavailability of water, impaired thirst, physical barriers that impede water intake, or dependence on physicians for adequate hydration.

In the general population, hypernatraemia is a relatively infrequent occurrence. The incidence is estimated to be around 0.5–0.7 %, depending on the population studied (Bourdel-Marchasson et al., 2004; Funk et al., 2010). As water intake is the main line of defence against hypernatraemia, it is logical that the incidence is increased in groups that are unable to maintain an adequate water intake, such as infants or the elderly.

Groups at risk

Children

Hypertonic dehydration can occur in infants that receive inadequate breastfeeding due to breastfeeding difficulties associated with primiparity or prematurity (Konetzny et al., 2009), or are fed with a concentrated formula (Leung et al., 2009). Hypernatraemic dehydration in neonates is a serious, potentially devastating, and life-threatening disorder that can lead to severe neurological impairment (Unal et al., 2008). In infants with an extremely low birth weight, hypernatraemia and fluctuations in plasma sodium concentration are associated with an increased incidence of intraventricular haemorrhage (Barnette et al., 2010; Lim et al., 2011).

Renal tubular disorders leading to polyuria and subsequent hypernatraemia, such as Bartter syndrome and nephrogenic DI are extremely rare causes of hypernatraemia, but need to be considered if more likely causes have been excluded (Knoers, 1993; Bettinelli et al., 2000). In older children, hypernatraemia is less frequent, but can occur in the case of severe gastroenteritis (Robertson et al., 2007) or the onset of type 1 diabetes mellitus (McDonnell et al., 2005).

Elderly

In the general elderly population, the incidence of hypernatraemia is low (0.3%) and seems most often related to the use of drugs such as laxatives (Passare et al., 2004). The incidence rises sharply when the elderly need to be admitted to hospital (O'Connor et al., 2006); hypernatraemia at admission is associated with increased mortality. Hypernatraemia in older patients is also associated with cognitive impairment (Bruce et al., 2009). In a case–control study, abnormal subclavicular and thigh skin turgor, dry oral mucosa, and a recent change of consciousness were found to be independently associated with hypernatraemia (Chassagne et al., 2006). Apart from a reduced sense of thirst and impaired mobility, which are commonly held responsible for hypernatraemia in the elderly, an increase in insensible losses with ageing may in part explain the increased susceptibility to this disorder (Dmitrieva and Burg, 2011).

Patients in the intensive care unit

As patients in the intensive care unit (ICU) usually have no control over their water intake, but have to depend on the composition and volume of the infusate, hypernatraemia is a frequent occurrence (Hoorn et al., 2008; Funk et al., 2010; Lindner et al., 2010). The

Table 29.1 Causes of hypernatraemia

Reduced water intake	Increased water loss	Gain of sodium
Unavailability of water *Inability to gain access to water:* Impaired consciousness *Defective thirst mechanism:* Hypodipsia in the elderly Osmoreceptor dysfunction	*Renal water loss:* Central diabetes insipidus Nephrogenic diabetes insipidus Gestational diabetes insipidus Osmotic diuresis Diuretics *Gastrointestinal water loss:* Vomiting Osmotic diarrhoea *Cutaneous water loss:* Sweating Extensive burns	Infusion of fluids hypertonic to the urine Ingestion (or infusion) of excessive sodium in oliguria

incidence ranges from 3% to 11% and is independently related to mortality (Darmon et al., 2010).

Patients with uncontrolled diabetes mellitus

Diabetes mellitus primarily leads to hyperglycaemia. As glucose is an effective osmole in the setting of diabetes mellitus (glucose cannot enter most cell types without insulin) this leads to hypertonicity of the ECV, resulting in transport of water from the intracellular compartment. Initially, this leads to hyponatraemia. However, as the diabetic state persists, a glycosuria-induced osmotic diuresis leads to water loss, and hypernatraemia may develop.

Clinical features

Symptoms

The signs and symptoms of hypernatraemia generally result from functional impairment of the central nervous system and are much more likely to occur when hypertonicity develops rapidly. Therefore, the symptoms are less related to the plasma sodium concentration per se than to the movement of water out of brain cells driven by the osmotic gradient. In infants this leads to a characteristic high-pitched cry, flaccidity, and tachypnoea. In severe cases, patients may progress to a state of lethargy and even coma (Finberg and Harrison, 1955).

Depending on the aetiology, adults may present with polyuria and thirst. The latter, however, is frequently absent, since this is often part of the problem that led to the development of hypernatraemia. The elderly may have remarkably little symptoms, but a variety of non-specific symptoms such as generalized malaise and a reduced level of consciousness (in some patients progressing to coma) is frequently observed. At times, it can be difficult to distinguish the signs and symptoms related to hypernatraemia from those caused by the underlying and causative disease.

Signs

Signs may reflect central nervous system dysfunction, but examination is otherwise often unremarkable. Although hypernatraemia is frequently associated with some degree of volume depletion, hypovolaemia can be difficult to detect clinically. Barring overt hypotension resulting from hypovolaemia, orthostatic hypotension appears to be the measurement with the highest predictive value. Commonly used indices such as skin-fold turgor are relatively poor markers of volume status, especially in elderly patients with diminished skin elasticity (McGee et al., 1999).

Dangers

When caring for patients with dysnatraemias it is important to be aware of the threats to the patient, both those directly relating to the condition and those resulting from inadequate management (Table 29.2). As water can readily pass from the intracellular to the extracellular compartment, a rapid increase in effective plasma osmolality can result in a shift of water from the intracellular to the extracellular compartment (Fig. 29.1). This shift leads to shrinking of cells. The organ most affected by cell shrinkage is the brain. As its vessels are attached to the inner surface of the skull, these vessels are stretched on shrinkage, leading to the risk of rupture with subsequent haemorrhage. When osmolality is increased gradually, the cells have time to adjust by increasing the number of their intracellular osmolytes. This results in restoration of brain volume. However, this adaptation to chronic hypertonicity puts the brain (and the patient) at risk for over-correction, because a water shift into the brain will lead to cerebral oedema and death due to subsequent herniation of the brain (Alshayeb et al., 2011).

Investigations

History

In the adult outpatient, a careful history, in some instances directed towards the family, may reveal important clues related to the aetiology of the hypertonic state. The time-course, the presence or absence of thirst, and an estimate of the mobility of the patient may be indicative of certain diagnoses. In an awake, alert person the presence of hypernatraemia is highly suggestive of a thalamic lesion affecting thirst. Also, an estimation of urine volume (although frequently difficult to obtain), and the presence of other potential sources of water loss, can be helpful. The presence of previous problems, such as diabetes mellitus and the use of any form of pharmacological treatment, are also of importance.

In infants, one should ask about diarrhoea and vomiting, as well as an inadequate increase (or even decrease) in body weight, and failure to thrive.

Table 29.2 Dangers to the patient in acute and chronic hypernatraemia

Acute	Chronic
When hypernatraemia has occurred over a short period of time (< 48 hours) and has led to cerebral impairment, the patient is at risk for cerebral haemorrhage and prompt treatment should be instituted	When hypernatraemia has persisted for a period > 48 hours, brain adaptation to hypertonicity has occurred and overzealous treatment puts the patient at risk of cerebral oedema

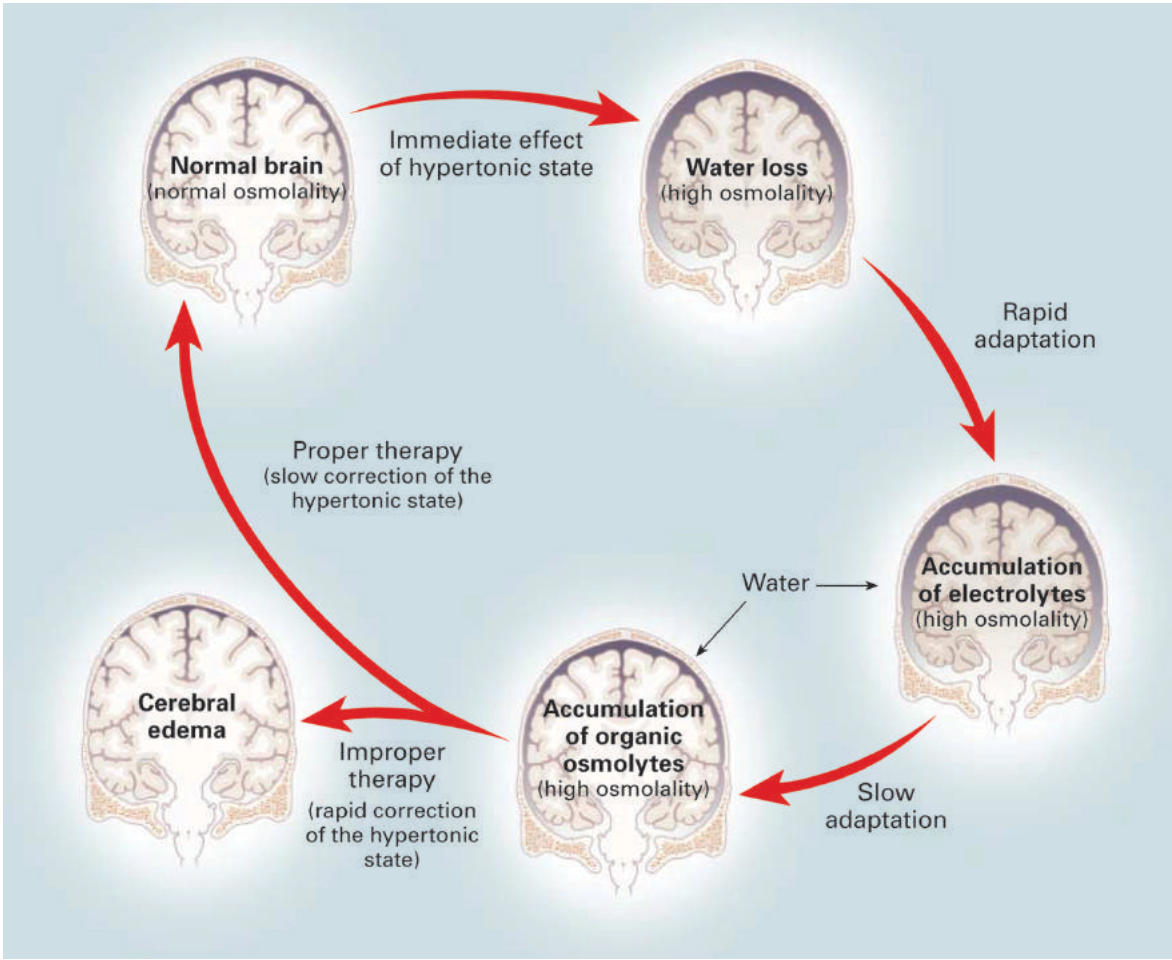

Fig. 29.1 Within minutes after the development of hypertonicity, water loss causes shrinkage of the brain and an increase in osmolality of the brain. Partial restoration of brain volume occurs within a few hours as electrolytes enter the brain (rapid adaptation). The normalization of brain volume is completed within several days through gain of organic osmolytes by brain cells (slow adaptation). Higher osmolality in the brain persists despite the normalization of brain volume. Proper correction of hypertonicity re-establishes normal osmolality without risking damage to the brain. Overly aggressive correction of hypernatremia can lead to brain oedema
From: Adrogué HJ, Madias NE. *N Engl J Med* 2000; 342:1493–1499.

Physical examination

The predictive value of findings at physical examination varies with the pathophysiological mechanism that has led to hypernatraemia. As a water deficit is the most frequent cause, one must look for signs of dehydration. Physical examination should include an assessment of the circulation, preferably by measuring blood pressure supine and in the upright position. Also the mental state should be examined.

Laboratory examination

Measurements that should be included in the initial investigation of a patient presenting with hypernatraemia are presented in Table 29.3.

Plasma sodium concentration

In general, it is worthwhile to repeat the initial measurement of serum sodium. This allows detection of any erroneous results. Moreover, it gives the physician some idea on the progression of hypernatraemia.

Plasma osmolality

Pseudohypernatraemia is extremely rare, but can be observed in hypoproteinaemic states due to the dilution step used in most routine laboratory methods (Lang et al., 2002). To confirm the hyperosmolar state, and to determine if other substances are involved in the hypertonic state (such as glucose), plasma osmolality should be measured.

As not all osmolytes in plasma contribute to the osmotic driving force between the extracellular and the intracellular compartments, it is important to calculate the effective plasma osmolality, excluding the contribution of urea to plasma osmolality. Since hyperglycaemia does lead to a water shift, effective plasma osmolality (POsm) can be calculated as:

$$\text{Effective POsm} = 2\times\left(\text{plasma}\ [\text{Na}]+\text{plasma}\ [\text{K}]\right)+[\text{glucose}]$$

Plasma vasopressin concentration

In patients presenting with hypernatraemia it is rarely necessary to measure vasopressin levels. However, in patients suspected of

Table 29.3 Blood and urine tests that should be performed in the initial evaluation of hypernatraemia

Blood	Urine
Osmolality	Osmolality
Sodium	Sodium
Potassium	Potassium
Calcium	
Glucose	
Urea	
Creatinine	

diabetes insipidus (DI) with an intermediate response to DDAVP (desmopressin), measuring the plasma vasopressin concentration can be helpful in distinguishing between central and nephrogenic DI (see Chapter 32). A high baseline vasopressin level indicates an intact pituitary, hence nephrogenic DI. Absent or low vasopressin concentrations are compatible with complete or partial central DI, respectively. If available, it is prudent to draw blood in all patients before the administration of DDAVP, allowing the subsequent determination of vasopressin levels in those patients with an intermediate response.

There are few reliable and commercially available vasopressin assays. In the circulation vasopressin is extensively bound to platelets and prolonged storage leads to falsely elevated plasma levels. Moreover, the measurement of plasma vasopressin concentration is technically challenging (Robertson et al., 1973). Vasopressin is synthesized as prepro-hormone and consists of a signal peptide, vasopressin, neurophysin II, and a C-terminal peptide of 39 amino acids, named copeptin. This latter peptide is much more stable than vasopressin and in healthy individuals copeptin and vasopressin levels are closely related over a wide range of plasma osmolalities (Balanescu et al., 2011). Although theoretically promising, the copeptin assay is a relatively new addition to the diagnostic armamentarium and few clinical studies using it have been reported. However, its measurement has been shown to increase the diagnostic performance of the water deprivation test in polyuria (Fenske et al., 2011).

Other plasma variables

In the initial examination of patients presenting with hypernatraemia, glucose, potassium, calcium, urea, and creatinine should also be measured (Table 29.3). Hypernatraemia frequently results from osmotic diuresis due to hyperglycaemia, necessitating the measurement of plasma glucose in such patients. For the same reason plasma urea should routinely be measured, as should creatinine to get an estimate of glomerular filtration rate. Plasma calcium should be measured routinely, because hypercalcaemia reduces the concentrating ability of the kidney. Hypokalaemia reduces medullary water reabsorption; therefore, plasma potassium should also be measured routinely in all patients presenting with hyperosmolar states.

Urine output

Patients with DI, osmotic diuresis, and/or salt loading may also present with polyuria. Patients with a true water deficit, either due to a low intake or extrarenal losses, are more likely to have oliguria. The differential diagnosis of a hypernatraemic and oliguric patient should also include salt loading in a patient with advanced renal failure.

Urinary osmolality

As hypernatraemia is primarily a disorder resulting from abnormal water homeostasis, urine osmolality is an important discriminating measurement (Table 29.4).

If urinary osmolality is low, lower than plasma osmolality, the patient has a water diuresis, which is inappropriate from a water balance standpoint. This indicates that the effect of vasopressin is absent and there is a defect in the vasopressin—renal collecting duct axis, that is, DI. The distinction between central and nephrogenic DI can be made by administering the arginine vasopressin (AVP) V2 receptor agonist DDAVP (1–4 micrograms intravenously). A sufficient increase in urine osmolality (defined as > 50% within 1–2 hours) indicates that the V2 vasopressin receptor and its downstream cascade function normally. This proves that the reason for the low urine osmolality is insufficient endogenous AVP secretion. A response of < 10% indicates that there is a resistance to the effects of vasopressin, confirming the diagnosis of nephrogenic DI. However, indeterminate increases between 10% and 50 % occur regularly. This may be due to washout of the medullary concentration gradient or downregulation of aquaporin-2 expression in the intramedullary collecting duct, resulting from chronic vasopressin deficiency.

If urinary osmolality is high, the body is efficiently trying to conserve water by maximizing the reabsorption of water in the collecting duct. There is a wide variation in the urine osmolality that can be maximally achieved and this maximum decreases with a reduction in the number of functioning nephrons due to age or renal failure. In general, a urine osmolality of 800 mOsmol/kg H_2O is considered an appropriate response to hypernatraemia, indicative of reduced water intake or extrarenal loss.

An intermediate urine osmolality (300–800 mOsmol/kg) is compatible with an osmotic diuresis. However, in some patients with DI, either central or nephrogenic, the defect is partial. Such a defect can also result in intermediate urinary osmolalities. Alternatively, severe volume depletion can lead to intermediate urine osmolalities in patients with central DI, as a result of residual water permeability of the collecting duct.

Urinary sodium concentration

In the absence of diuretics, sodium output in the urine is primarily useful for the estimation of volume status. Hypovolaemia leads to increased sodium reabsorption along the nephron, resulting in a urine sodium concentration of 20–30 mmol/L or lower. When hypernatraemia is due to (ongoing) osmotic diuresis or a positive sodium balance, urine sodium concentration will be (considerably) higher than 30 mmol/L.

Urinary sodium plus potassium concentrations

For hypernatraemia to develop, water must be lost in excess of sodium and potassium salts. Urine contains organic solutes such as urea that have no effect on water balance, but that do affect the osmolality of the urine. Quantitatively, it is more appropriate to compare 2 × (Urinary [Na] + [K]) to plasma osmolality. When a

Table 29.4 Diagnostic approach to hypernatraemia based urine parameters

	Reduced water intake	Diabetes insipidus	Osmotic diuresis	Extrarenal water loss	Sodium gain
Osmolality	U_{osm} maximal	$U_{osm} < P_{osm}$	$U_{osm} > P_{osm}$	U_{osm}maximal	U_{osm}maximal
Urine sodium	< 25 mmol/L	< 25 mmol/L	> 25 mmol/L	< 25 mmol/L	> 25 mmol/L
Urine output	Oliguria	Polyuria	Polyuria	Oliguria	Normal

patient excretes urine in which 2 × (Urinary [Na] + [K]) is lower than plasma osmolality, net (free) water is lost in the urine.

Electrolyte-free water excretion

When calculating the urinary excretion of water and solutes, it has been advocated to divide the excreted volume in two parts, one part isotonic fluid loss and one part loss of pure water (Rose, 1986). The latter is called electrolyte-free water and affects net water balance. However, when a patient excretes hypertonic urine, the amount of electrolyte-free water that is lost becomes negative, making interpretation a challenge. Also, this approach does not take the fluid input into account.

Tonicity balance

Although urine osmolality is important to determine whether vasopressin is acting or not, it is not particularly useful in quantitative terms. To determine the effects infusion fluids have had or will have, it is more appropriate to calculate the input and output of water and sodium + potassium separately (Fig. 29.2). This approach has been called the tonicity balance (Carlotti et al., 2001). Similar to the calculation of electrolyte free water, it requires the availability of data on both input and output. This generally limits the approach to dysnatraemias that occur during hospitalization. In patients with severe catabolism, the balance of other solutes also needs to be taken into account (Halperin and Bohn, 2002).

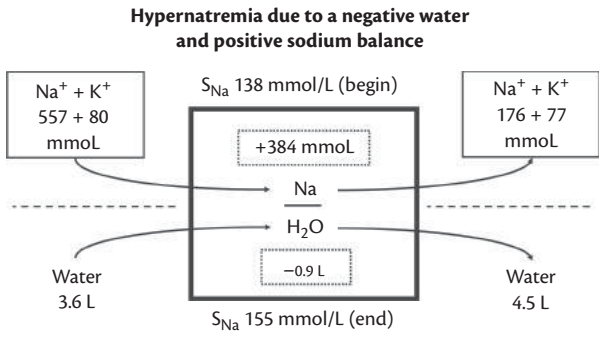

Fig. 29.2 Tonicity balance in hypernatraemia. The large darker rectangles represent total body water with the serum sodium concentration measured at the beginning and end of the observation shown on top and bottom of this rectangle, respectively. The quantities of sodium (Na^+) plus potassium (K^+) infused and excreted are shown in the two flanking rectangles, and the volumes of water (H_2O) infused and excreted are depicted below. The patient was a 50-year-old female (body weight 75 kg) who was admitted with respiratory insufficiency due to pneumonia. Hypernatremia developed in 4 days and was attributed to a combination of a negative water balance and a positive sodium balance due to the infusion of isotonic fluids and renal water loss from hyperglycaemia, hypercalcaemia, and hypokalaemia.
From: Hoorn EJ et al. *Nephrol Dial Translant* 2008; 23:1562–1568.

Water deprivation test

Although the increase in urine osmolality during fluid restriction certainly has merit in the work-up of patients with polyuria, it should not be performed in patients who are overtly hypernatraemic.

Aetiology and pathogenesis

Causes of hypernatraemia can be categorized as reduced water intake, renal or extrarenal water loss, and sodium gain (Table 29.1).

Unavailability of, or access to, water

In general, in the developed world, water is readily available. This does not hold true for all countries, now that water is becoming a precious commodity in many places. Even if water is available in principle, reduced mobility of the patient can prevent sufficient access. In patients with reduced levels of consciousness, fluid intake can become inadequate, leading to hypertonic dehydration. This can also be the case in the elderly who have inadequate social support or are admitted to understaffed nursing homes. In these patients fluid intake is further challenged by a decrease in thirst sensation that occurs with increasing age (Phillips et al., 1991). Mortality is high in elderly patients presenting with severe hypernatraemia, with the state of consciousness being the single most important prognostic factor (Chassagne et al., 2006). In general, increased fluid intake can prevent the disorder, but the 2003 heat wave in France has shown that over-zealous drinking (possibly combined with volume depletion) may lead to hyponatraemia in these patients (Kettaneh et al., 2010).

Breastfed neonates are completely dependent on their mother for adequate hydration. Neonatal hypernatraemic dehydration is present in 2% of hospitalized neonates (Moritz et al., 2005). It can present with lethargy, fever, and jaundice (Boskabadi et al., 2010). It results from inadequate feeding and is associated with a lower than normal number of feeds and with breastfeeding problems in the mother. This potentially devastating disorder can be prevented by frequent weighing (Unal et al., 2008).

Defective thirst mechanism

Thirst is an important physiological mechanism in the defence against dehydration. It can be stimulated by both increases in effective osmolality of the ECV and intravascular hypovolaemia. In elderly patients, water intake is often not increased in the face of increased fluid loss, which leads to hypernatraemia (Molaschi et al., 1997). This can be the result of a decreased sensitivity of the thirst mechanism (Adeleye et al., 2002). Thirst perception appears to be modulated by sodium balance, with a high salt diet (accompanied by low endogenous angiotensin II levels) increasing the osmotic threshold at which thirst occurs (Gordon et al., 1997). Low levels of

angiotensin II have been implicated in reducing thirst perception in the elderly (Yamamoto et al., 1988).

Central diabetes insipidus

(See also Chapter 32.)

Vasopressin is synthesized in the hypothalamus and is transported down the axons of the supraoptical-hypophyseal tract to be stored in and released from the pituitary. When this tract or the pituitary is damaged, vasopressin release can be diminished. Several forms of central DI can be distinguished.

Hereditary

An autosomal dominant form of central DI can result from mutations in the gene that encodes neurophysin II (Stephen et al., 2012). In this disease, loss of neurophysin II, a carrier protein for vasopressin, results in a progressive decrease in vasopressin levels. At birth patients are asymptomatic, but progressive polyuria develops with hypernatraemia later in childhood (Arima and Oiso, 2010) (Table 29.5).

Post-traumatic

DI occurs in a substantial percentage of patients presenting with severe head injury (blunt, but especially penetrating) (Hadjizacharia et al., 2008). Independent risk factors are a Glasgow coma score ≤ 8, cerebral oedema, and an Abbreviated (head) Injury Score ≥ 3.

Tumours and infections

Tumours that disrupt the supraoptical-hypophyseal tract can lead to central DI. This complication can occur in a variety of primary or secondary malignancies of the brain, as has been reviewed elsewhere (Verbalis, 2003). Several types of infections affecting the brain and or the meninges have been associated with central DI, including tuberculosis (Bajpai et al., 2008), influenza (Kobayashi et al., 2011), and histiocytosis (Schmitt et al., 1993).

Post-surgical

Disturbances in water excretion are a common cause of morbidity following trans-sphenoidal surgery. In a large series, the incidence of central DI in the postoperative phase was 18.3% (Nemergut et al., 2005). However, only 2% of patients required long-term treatment with DDAVP. Hyponatraemia, resulting from vasopressin release from the damaged pituitary, is observed in 25% of patients (Olson et al., 1997), and in 5% of patients the clinical course follows a biphasic or triphasic pattern, where central DI is interspersed with a period of SIADH (Hensen et al., 1999; Hoorn and Zietse, 2010).

Table 29.5 Forms of diabetes insipidus

Central diabetes insipidus	Nephrogenic diabetes insipidus
Hereditary	Mutations of the vasopressin V2 receptor
Post-traumatic	Mutations of aquaporin-2
Tumours and infections	Medullary renal disease.
Postsurgical	Hypercalcaemia or hypokalaemia
Ethanol consumption	*Drugs:*
Idiopathic	Lithium
Adipsogenic	Amphotericin B
	Demeclocycline
	Foscarnet
	V2-receptor antagonists

Adipsogenic

In some patients with central DI, reduced vasopressin release from the hypophysis is accompanied by the absence of thirst. This entity is known as adipsic DI and it is due to lesions in the hypothalamus, such as a craniopharyngioma (Crowley et al., 2007). Whereas thirst and subsequent drinking usually prevent overt hypernatraemia in patients with central DI, patients with the adipsic form can be markedly hypernatraemic. In most cases, the hormonal response to non-osmotic stimuli is intact (Smith et al., 2002). Following successful surgery, thirst sensation can recover (Sinha et al., 2011).

Nephrogenic diabetes insipidus

(See also Chapter 32.)

Hereditary

Congenital defects in either the vasopressin receptor (V2R) or aquaporin-2 (AQP2) can lead to nephrogenic DI (Knoers, 1993).

As the *V2R* gene is located on the X chromosome, the disease follows an X-linked recessive inheritance pattern. It accounts for the vast majority of patients with congenital nephrogenic DI (~ 90%) and is by nature a disease affecting males only. Female carriers are usually asymptomatic, but in some patients skewed inactivation of the X chromosome, favouring the mutated gene, can lead to partial disease (Faerch et al., 2010).

In most cases, AQP2-linked nephrogenic DI follows an autosomal recessive inheritance pattern (Nossent et al., 2008). In these patients, decreased expression of aquaporin leads to the concentrating defect, with osmosensing and vasopressin secretion being intact. As a result of renal water loss, circulating vasopressin levels and *V2R* gene expression may be increased. Activation of endothelial V2 receptors can lead to increase secretion of von Willebrand factor resulting in an increased risk for thromboembolism (Nossent et al., 2010).

Hereditary disorders that do not directly affect water transport in the collecting duct may have marked effects on the urinary concentration mechanism leading to hypernatraemia with hypotonic urine (Bockenhauer et al., 2010). In tubular disorders, such as Bartter syndrome and nephronophthisis, the reabsorption of sodium is diminished, resulting in isosthenuria (i.e. urine isotonic to plasma). Such patients may present with episodes of hypernatraemia unresponsive to DDAVP and the erroneous diagnosis of congenital nephrogenic DI. Severe hypokalaemia, that is frequently present, may further reduce renal water reabsorption.

Acquired

The most frequent cause of nephrogenic DI encountered in clinical practice is the use of drugs that inhibit the renal concentrating ability. A wide variety of drugs have been associated with nephrogenic DI (Table 29.5) and these have been extensively reviewed elsewhere (Garofeanu et al., 2005). Here, we will only discus the two most clinically relevant: lithium and amphotericin B.

By far the most prominent agent that causes DI is lithium, which is used to treat bipolar disorders, with a reported incidence of 40% of patients receiving the drug (Grunfeld and

Rossier, 2009). Lithium enters the tubular epithelial cell through the epithelial sodium channel (ENaC) and decreases aquaporin phosphorylation, thereby inhibiting translocation and insertion into the luminal membrane (Nielsen et al., 2008). Amiloride, an agent used as a potassium-sparing diuretic, because it blocks the ENaC, prevents the entry of lithium in the collecting duct epithelial cell and improves the renal concentrating ability (Kortenoeven et al., 2009). This putative effect of amiloride has been demonstrated in humans, where the drug increased both concentrating ability and AQP2 excretion in patients on lithium (Bedford et al., 2008).

Amphotericin B, decreases the ability of the kidney to concentrate the urine, due to both structural damage to the apical plasma membrane and to decreased AQP2 abundance (Zietse et al., 2009). Although hypokalaemia is frequently present in such patients and may contribute, the concentration defect also occurs when plasma potassium concentration is normal. In some patients, drug-induced DI can be associated with other tubular defects (Hoorn and Zietse, 2007).

Hypokalaemia

In rats, hypokalaemia has been shown to reduce the number of aquaporin water channels in the luminal membrane of the collecting duct (Marples et al., 1996). Although hypokalaemia is frequently associated with polyuria, it rarely leads to hypernatraemia. At present it has not been shown whether hypokalaemia-induced polyuria results from nephrogenic DI or from interstitial damage due to prolonged hypokalaemia.

Hypercalcaemia

In the kidney, the calcium sensing receptor (CaSR) is present both in the thick ascending limb (TAL) of the loop of Henle (basolateral membrane) and in the collecting duct (luminal membrane). In the collecting duct, the CaSR co-localizes with AQP2. *In vitro* data have shown that water permeability decreases at higher luminal calcium concentrations (Earm et al., 1998). Although these findings are compatible with nephrogenic DI, hypercalcaemia-induced polyuria is more likely to result from activation of the CaSR in the TAL, resulting in a loop diuretic-like effect (Hoorn et al., 2009).

Gestational diabetes insipidus

In pregnant patients presenting with hypernatraemia, the existence of diabetes mellitus should be excluded, because this is considerably more frequent than DI and can lead to grave consequences (Lee et al., 2010). Gestational DI is a rare disorder occurring in the third trimester of pregnancy (Sherer et al., 2003). It results from the circulating enzyme vasopressinase, which degrades circulating vasopressin, leading to non-renal DI. As vasopressinase is produced in the placenta, hypernatraemia is transient in this disorder (Aleksandrov et al., 2010). Treatment with vasopressin is not effective, but as the synthetic DDAVP is not cleaved by vasopressinase, DDAVP will readily correct the polyuria (Ananthakrishnan, 2009).

Osmotic diuresis

If plasma osmolality is greater than urine osmolality and the daily osmole excretion rate is > 1000 mOsmol/day an osmotic diuresis is present. The next step is to determine which solute is responsible for the observed increase in osmole excretion.

Hyperglycaemic hyperosmolarity is the best clinical example of a disorder in which osmotic diuresis causes hypernatraemia (Kitabchi and Nyenwe, 2006). When hyperglycaemia causes the amount of filtered glucose to exceed the reabsorptive capacity of the proximal tubule, significant glycosuria results. As glucose acts as an effective osmole in the tubular fluid, it prevents the reabsorption of water in the collecting duct. This reabsorption is further impaired by a relatively low osmolality of the renal medulla (which normally acts as the driving force for water reabsorption). During osmotic diuresis, urine contains a relatively low concentration of electrolytes (sodium plus potassium in the range of 50–80 mmol/L) and therefore the body loses more water than electrolytes, that is, loss of free water (Halperin and Bohn, 2002).

Apart from glucose, increased excretion of urea can also induce an osmotic diuresis, especially in the ICU. This can result from hyperalimentation, increased catabolism, gastrointestinal bleeding, or recovery from transient renal failure (Lindner et al., 2012).

Diuretics

In rare cases, loop diuretics can lead to hypernatraemia by interfering with the renal concentrating mechanism. The effect of loop diuretics is short-lived and hypernatraemia only occurs when water loss is not replaced by oral intake.

Gastrointestinal water loss

Prolonged diarrhoea and/or vomiting can lead to the loss of water from the body (Hartling et al., 2006). In secretory diarrhoeas, such as cholera, the fluid that is lost consists of Na and K salts and is isosmotic to plasma. Such fluid loss will readily lead to volume depletion, but will not cause hypernatraemia. In osmotic diarrhoeas, however, the main osmoles lost are organic compounds such as lactulose. As water is lost in relative excess of Na and K, hypernatraemia may develop. During prolonged vomiting, as occurs in oncology patients during chemotherapy, oral water intake is impaired, whereas insensible water loss continues, leading to hypernatraemia (Berk and Rana, 2006).

Cutaneous water loss

Excessive sweating could cause the body to lose large amounts of water that, if not replaced, would lead to hypernatraemia. Usually, however, this water loss leads to thirst and replenishment of lost fluids. Indeed, in collapsed marathon runners hyponatraemia is at least as likely to occur as hypernatraemia (Kratz et al., 2005).

Burn injury affects the skin integrity and its protection against fluid loss is lost (Pruitt, 1978). Therefore, severely burned patients need careful and extensive fluid resuscitation. This is even more important, because the presence of hypernatraemia in patients with burns adversely affects skin grafts (Namdar et al., 2011).

All states that increase the insensible loss of water through the skin (and from the respiratory tract) put the patient at risk of developing a hyperosmolar state. Preterm infants are especially at risk (Wada et al., 2008), as are patients with an increased body temperature and patients in hot and dry environments. In all these circumstances hypernatraemia will only develop if the patient is unable to drink freely.

Gain of sodium

In patients incapable of regulating their own fluid intake, hypernatraemia can develop for a variety of reasons (Lindner et al., 2009). In the ICU, uncorrected loss of water is certainly responsible for many instances of hypernatraemia. However, in a substantial number of cases the (hypervolaemic) hypernatraemia that develops

in the ICU results from a positive sodium balance (Hoorn et al., 2008). These patients commonly receive isotonic intravenous fluids, but pass relatively hypotonic urine. This indicates that hypernatraemia in the ICU is the result of inadequate fluid management and is, for the most part, preventable. As hypernatraemia in ICU patients is associated with adverse outcome, its incidence has been proposed as an indicator of the quality of medical care in the intensive care environment (Polderman et al., 1999).

Treatment and outcome

In general, inducing a positive net water balance treats hypernatraemia. In patients in whom the gastrointestinal tract is functional, this is best achieved with oral water intake. Intravenous water administration with glucose 5% solutions (dextrose; D5W) is limited by the amount of glucose that can be metabolized. If this maximal rate (estimated at 0.3 L/hour) is exceeded, hyperglycaemia may develop, leading to osmotic diuresis and further electrolyte free water loss.

The therapeutic approach to the patients should include the steps listed in Table 29.6.

Assess potential dangers to the patient. At presentation there are several questions one should ask before embarking on a specific treatment.

Question 1: is it acute or is it chronic?

In acute hypertonicity, the brain has a lower osmolality than plasma, which leads to shrinkage and ultimately (in rare cases) cerebral haemorrhage. In acute hypernatraemia the brain has not had the time to respond to the hypertonic state by increasing the amount of intracellular osmoles. Although it is uncertain when this correction is complete, it is estimated that it takes at least 48 hours for the brain to accumulate enough osmoles to achieve a new balance. Most patients with hypernatraemia present in the emergency department and the time-course is usually uncertain. Unless a patient has severe symptoms and a history that is compatible with the acute development of hypertonicity, it is prudent to assume that the hypernatraemia is chronic.

The main therapeutic implication of the distinction between acute and chronic is that in acute/symptomatic hypernatraemia plasma osmolality should be lowered rapidly to avoid cerebral complications. In chronic hypernatraemia, however, overly ambitious lowering of plasma osmolality would lead to cerebral oedema and death due to brain herniation.

Similar to recommendations in hyponatraemia, patients with hypernatraemia should be treated rapidly only if severely symptomatic due to the rapid (< 48 hours) development of hypernatraemia.

Question 2: is (profound) hypovolaemia present?

Frequently, water depletion is accompanied by the loss of sodium and or potassium salts, leading to true volume depletion. Plasma osmolality will only give an estimate on the amount of water that is lost relative to solute content, but will not indicate the amount of 'isotonic fluid' that is lost.

Question 3: what has caused the hypertonicity?

A major distinction should be 'is this sodium gain' (virtually only seen in patients admitted to the hospital/ICU) or 'is this net water loss' (nearly always). If considerable sodium gain is present and the patient is fluid overloaded, treatment with large amounts of hypotonic fluid may lead to pulmonary oedema. In such patients, natriuresis should be induced with (loop) diuretics, as discussed below.

In patients with hypernatraemia due to water loss, urine output should be measured to determine if (persistent) polyuria is present. As the expected urine flow would be low, in these patients (inappropriate) polyuria can be defined as a urine output > 30 mL/hour.

It is important to take the effect of osmolytes other than sodium on plasma tonicity into account. Especially, the contribution of glucose to measured osmolality should be assessed, since the treatment of hypernatraemia with concurrent hyperglycaemia poses specific challenges.

Correct ECV depletion if necessary

When a patient presents with circulatory shock, the first step in treatment should be treating with isotonic fluids, such as 0.9% saline, to restore ECV. The goal of this treatment is to restore tissue perfusion. As this 'isotonic' saline is hypotonic to the patient it will, depending on the volume and constitution of the urine, also modestly lower plasma osmolality. When the circulation has been restored more hypotonic solutions can be employed.

Set the [Na] target

In symptomatic patients, the first aim is to induce a shift of water towards the brain, by rapidly lowering plasma osmolality. Plasma sodium should be lowered by 1–2 mmol/L/h until symptoms disappear. Although there are no data from controlled trials, the overall correction rate should probably not exceed 8 mmol/L per 24 hours.

Start treatment to achieve the target

Replace water loss

Calculating the water deficit can provide an estimate of the amount of water that has been lost:

$$\text{Water deficit} = 0.6 \times \text{lean body weight} \times \left(\left(\text{plasma } [\text{Na}]/140\right) - 1\right)$$

Using this formula, estimation is made of the amount of water that is required to return plasma sodium concentration to 140 mmol/L. Following this, a period must be selected in which to restore water balance.

The effect a litre of a given infusate will have on plasma sodium concentration can be calculated using the formula developed by Adrogué and Madias (2000):

Table 29.6 Steps in treating hypernatraemia

Assess dangers:
Is it acute or is it chronic?
Is (profound) hypovolemia present?
What has caused hypertonicity?
Correct ECV if circulation is threatened
Set treatment target for [Na]
Select strategy to achieve target
Check progress frequently

$$\text{Change in serum } Na^+ = \frac{(\text{infusate } Na^+ + \text{infusate } K^+) - \text{serum } Na^+}{\text{total body water} + 1}$$

Fig. 29.3

In this approaches, estimation must be made of total body water. Usually the total body water is assumed to be 60% of lean body weight, which is not likely to be true in severely water-depleted patients. In cachectic and/or elderly patients, this percentage can easily be as low as 40%. Using these calculations may lead to over-estimation of water deficit with the subsequent danger of over-correction.

Both calculations are based on water loss and do not account for water that is lost concurrently with solutes, leading to volume depletion. In many patients dehydration is accompanied by true volume depletion.

When estimating the correction rates with these formulae, no correction is made for ongoing (renal) water loss. The effect a given infusate will have on plasma [Na] can be estimated by calculating a tonicity balance, where both input and output of sodium, potassium and water are calculated.

Stop ongoing renal water loss

The appropriate homeostatic response to hypertonicity is to increase the release of vasopressin from the pituitary, thereby increasing urinary osmolality and decreasing urine flow. In patients with urine flows that are inappropriately high (> 30 mL/h), the distinction should be made if there is a water diuresis (UOsm < POsm) or a solute diuresis (UOsm > POsm).

In patients with a water diuresis, vasopressin may be given in the form of DDAVP to correct a possible vasopressin deficiency. The advantage of DDAVP is that it is not subject to degradation by vasopressinase and can be used in pregnancy-related DI. If DDAVP does not reduce urine output, nephrogenic DI may be present.

In patients with a solute diuresis, the nature of the excreted solutes must be determined. The substances most frequently responsible for osmotic diuresis are glucose and urea.

Check progress

Calculation of correction rates is a useful basis and starting point for treatment, but these are frequently inaccurate. Many of the caveats previously described can affect the ability of even the best formulae to predict the response to treatment. This may be especially true in the ICU environment (Lindner et al., 2008), where the difference between predicted and measured sodium concentrations was as high as 15 mmol/L in some patients. This indicates that the response to treatment should always be followed closely (repeat measurements every 2–4 hours) and treatment should be adjusted in response to the actual measurements of plasma sodium.

Treatment in specific situations

Hypernatraemia in diabetes mellitus

In patients with diabetes, hyperglycaemia leads to a shift of water from the intracellular to the extracellular compartment. This dilution causes a reduction in the plasma sodium concentration. As hyperglycaemia leads to osmotic diuresis, water is lost in the urine and plasma sodium may rise. Both glucose and sodium contribute to the effective osmolality in such patients.

When glycaemia is corrected, plasma sodium concentration should rise, as water returns to the intracellular compartment. Effective osmolality (formula in p. 263) should be calculated at various time points and the goal of therapy is to prevent a significant fall is effective osmolality during the initial treatment to prevent cerebral oedema. This is especially important in children, because the brain is relatively large compared with the size of the skull at a younger age (Hoorn et al., 2007).

Hypernatraemia in the ICU

In the ICU, patients can lose water in a variety of ways and the most obvious treatment is to 'just add water' (Sterns, 1999). However, in a substantial number of patients sodium gain is present. In such patients, giving a water load can lead to fluid overload and pulmonary oedema. Excess sodium should be removed using (loop) diuretics. As diuretic-induced urine output is hypotonic to the patient, water should be given to lower the plasma osmolality. In patients with acute kidney injury and concomitant hypernatraemia, continuous renal replacement therapies can be used to gradually correct hypertonicity (Ostermann et al., 2010).

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CHAPTER 30

Approach to the patient with oedema

Jean-Marie Krzesinski and Eric P. Cohen

Introduction

Oedema was known to Hippocratic medicine as a sign of disease (Touwaide et al, 1999). The Greek οιδεμα was associated with many diseases, but an understanding of its pathophysiology would be delayed for many centuries. Modern concepts of oedema formation are now credited to Starling. These had their inchoate beginnings in the studies and work of Malpighi, the seventeenth-century discoverer of capillaries (Andreae and Fine, 1997). In the next century, Stephen Hales created experimental oedema. Anatomo-pathological correlations made by Richard Bright in the nineteenth century firmly linked renal disease with oedema (Bright, 1836). In the twentieth century, the advent of kidney biopsy and experimental nephrology further developed its understanding and treatment.

Oedema is also a cardinal sign of inflammation, but such oedemas are typically tense, localized and accompanied by the other signs of rubor, dolor, and calor. This chapter will not discuss inflammatory oedemas.

Clinical presentation

History and inspection

Oedema is tissue swelling. It often is first noticed in the feet or ankles, at the end of the day, or in the loose periorbital tissue, upon awakening. Some patients will sense oedema before it is palpable or visible to the doctor. Premenstrual oedema or that which can occur in pregnancy may not be abnormal, but most oedemas indicate disease.

Specific oedema locations are clear signs of particular organ disease, such as the pulmonary oedema of left ventricular failure or the ascites of cirrhosis.

The extent of oedema is semi-quantitatively assessed as 1+ for feet and ankles, 2+ for up to the knees, 3+ for all of the legs, and 4+ for the entire body, or anasarca. Changes in body weight over hours to days indicate fluid retention rather that a gain of tissue mass; daily body weight change can quantify oedema accumulation. In adults, < 3 kg of extracellular fluid (ECF) volume excess may not be detectable by physical examination. Conversely, detectable oedema implies body fluid gains of > 3 kg. One study reported that even 7 L of excess ECF excess was not detectable by physical exam (Ferraro et al., 1949).

Change after recumbency is a commonly noted feature of oedema. Lesser degrees of oedema may only be noticed in the feet and ankles at the end of the day, with apparent resolution upon waking, in the morning. While nocturia could explain some of that resolution, it is more likely that the distribution of oedema has shifted overnight from the feet and ankles to the trunk.

Palpation

Oedema may feel firm, even resistant to pressure. A brawny texture is felt in lymphoedema. Oedema of the nephrotic syndrome may feel soft and sponge-like. The characteristic of 'pitting' is a time-honoured physical finding of oedema (Fig. 30.1). Pressure by pushing with a fingertip on the oedematous area may leave a dent, or 'pit' that lasts for more than a few seconds. Pitting does not occur in lymphoedema. In subjects with hypoproteinaemia, the fingertip can create a pit more easily than for oedemas from other causes, and that pit resolves more quickly than the pit of oedema that occurs in other oedematous states such as heart failure (Henry and Altmann, 1978).

Physiology and pathophysiology

One can easily understand that oedema formation will depend on imbalances in the forces that determine transcapillary exchange of fluids in the microcirculation. Thus, an excess of arteriolar or venous intracapillary pressure, a deficiency of intracapillary oncotic pressure, or a reduction of lymphatic fluid reabsorption could lead to oedema (Fig. 30.2).

These imbalances do not immediately lead to oedema. First, minor imbalances in transcapillary forces may only lead to minor degrees of tissue expansion by ECF. Second, there appear to be compensatory effects that protect against oedema formation. In the case of elevation of venous pressures, those must reach 12 mmHg or more to cause oedema (Aukland, 1984). This depends on the initial transit of fluid from inside the capillaries to the interstitial, extracellular space, which leads to an increase in the extracellular tissue pressure, and a countervailing force on the exit of fluid from inside to outside of the capillary. The same is true for oedemas that may occur when there is hypoproteinaemia. Thus, minor degrees of hypoproteinaemia will not lead to oedema; one needs to reach levels of serum albumin near or below 2.5 g/dL for there is oedema solely on the basis of hypoproteinaemia (Kurnick, 1948). Finally, for any given elevation in venous pressure or reduction in serum proteins, the occurrence of oedema will depend on the total ECF volume as determined by dietary sodium intake.

These primary changes in the Starling forces are well-known correlates of oedema. It is worth considering the effect of primary

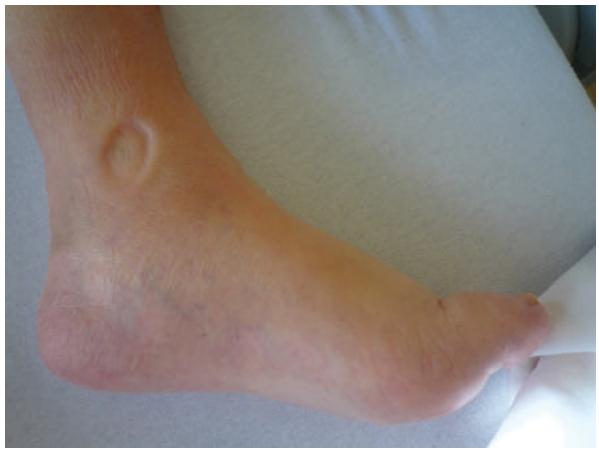

Fig. 30.1 Oedema. Photograph showing an indentation or 'pit' in the leg of a subject with oedema.

changes in renal sodium transport that cause enhanced sodium reabsorption, and which may result in hypertension. None of these cause oedema. The phenomenon of aldosterone escape is the best studied of these, in which a primary excess of aldosterone may cause hypertension but does not cause oedema. Hormonal or haemodynamic counter-regulatory mechanisms prevent primary aldosterone excess from leading to oedema, the so-called aldosterone escape (Hall et al., 1984). So, too, does enhanced renal sodium reabsorption as occurs in Gordon or Liddle syndromes lead to hypertension but not oedema. This underlines the concept of oedema as one that occurs because of the renal response to persistent arterial underfilling with activation of multiple sodium retentive mechanisms (Anand and Chugh, 1997).

Causes and pathophysiology of oedema

The causes of oedema may be inferred from its pathophysiology. Thus, heart failure, either left or right sided, liver failure, and renal disease are the 'usual suspects'. One's experience with one or the other cause will vary depending on one's time and place of practice.

Oedema in heart failure is the result of persistent underfilling of the arterial circulation, with renal counter-regulatory sodium reabsorption (Anand and Chugh, 1997). Indeed, in heart failure, the degree of sympathetic and/or renin–angiotensin system activation correlates with the stage of heart failure (Fitzpatrick et al., 1985). This mechanism is valid for left or right ventricular failure. The latter adds the additional element of the effect of elevated central venous pressures to reduce the glomerular filtration rate (GFR) and to promote renal sodium retention (Firth et al., 1988).

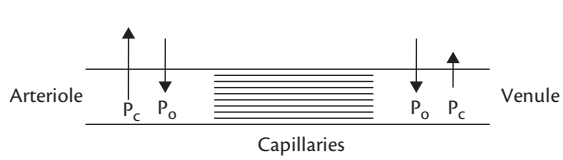

Fig. 30.2 Starling forces. A simplified illustration of the forces that affect transcapillary fluid exchange; the lymphatic circulation is omitted for clarity. The intracapillary capillary hydrostatic pressure (Pc) is higher than the intracapillary oncotic pressure (Po) at the arteriolar side, but Pc is lower than Po at the venular side of the microcirculation. Arteriolar filtration is thus balanced by venular reabsorption. Oedema may result from higher Pc or from lower Po.

The oedema of liver failure also results from underfilling, in this case from splanchnic arterial vasodilation and or the simple haemodynamic effect of portal hypertension and limitation to venous return. Secondary activation of the renin–angiotensin–aldosterone system is a prime mover of the sodium retention in cirrhosis as it is in heart failure (Schrier, 2007). To these mechanisms is added the effect of hypoalbuminaemia, a frequent occurrence in cirrhosis.

The salt and water retention of severe oliguric renal failure is obvious. In less-severe renal failure, reduction of the GFR is accompanied by elevation of the fractional excretion of sodium, such that in the steady state, sodium retention may not be apparent. But as GFR falls, in progressive decrements, there is a delay in tubular adaptation to that decline, such that progressive sodium retention can result in a cumulative fashion. There is impaired natriuresis in subjects with renal disease, such that there may be accumulation of a sodium load (Johnson et al., 2002). Multiple mediators are implicated in the hypertension of renal failure, including the renin–angiotensin–aldosterone system, nitric oxide deficiency, circulating inhibitors of sodium-potassium (Na-K)-ATPase, and many others. These may contribute to sodium retention and oedema of subjects with renal failure. Much as one overactive sodium transporter leads to hypertension but not oedema, it is likely that several mechanisms coexist to cause oedema in renal failure, rather than is a single one.

The association of oedema with hypoproteinaemia of the nephrotic syndrome is well known. Controversy persists as to the mechanisms of nephrotic oedema, specifically the accuracy of the classical construct of proteinuria → hypoproteinaemia → reduced plasma oncotic pressure → accumulation of interstitial oedema and transient *underfilling* of the circulation → reactive renal sodium retention (Palmer and Alpern, 1997; Schrier and Fassett, 1998). Primary renal sodium retention with an *overfilled* circulation has also been advocated as a cause of nephrotic oedema. Perhaps the clearest recent explanation of these competing ideas is that of Rodriguez-Iturbe et al, who state that when the nephrotic syndrome is accompanied by intrarenal inflammation, that inflammation can cause primary renal sodium retention (Rodriguez-Iturbe et al., 2002). This would hold for renal diseases such as diabetic or lupus nephropathy. In those cases, there is 'overfilling,' because the sodium retention and oedema are caused by renal sodium retention as well as by the hypoproteinaemia. But in non-inflammatory nephrotic syndrome, particularly minimal change disease, the simpler classical mechanism obtains, starting with proteinuria as stated above. These latter subjects would then be 'underfilled,' at least transiently.

The oedema of venous diseases is straightforward, being caused by the mechanical effect of elevated venous pressure that is transmitted to the capillaries, leading to transudation of fluid according to the Starling mechanism (Fig. 30.2).

The cause of oedema that may be caused by certain medications is less clear. Arteriolar vasodilation is said to a cause of oedema in some subjects who are using minoxidil or amlodipine, but confirmation is lacking. The oedema caused by thiazolidinediones could arise from primary renal sodium retention (Guan et al., 2005; Panchapakesan et al., 2009)

Testing for and imaging oedema

The chest X-ray findings of pulmonary oedema are well known, and ultrasound or computed tomography scan easily detect ascites.

Generally, oedema may be seen on X-ray imaging as tissue expansion, which is hypodense when compared to most tissue densities. This would not allow differentiation of inflammatory from non-inflammatory oedema. Generally, the most important part of testing and investigations of oedematous patients are the history and physical examination.

Differential diagnosis by history and physical examination

Findings in left-sided heart failure

Oedema in subjects with heart failure will depend on the severity of their heart disease, being generally absent for lesser degrees of heart failure. Classically, left heart failure will cause peripheral oedema only after it has caused pulmonary oedema. The presence of pulmonary oedema correlates with a body fluid excess of about 10% of the total body weight. A lower-than-normal blood pressure and a narrow pulse pressure may be present. There may be a high jugular venous pressure and also a hepatojugular reflux. One may feel the cardiac impulse in two interspaces, rather than just one, and there may be a third heart sound, along with rales or crepitations heard on listening over the lung bases. Oedema in subjects with heart failure is typically apparent in the dependant legs, its extent ascending upwards as the heart failure worsens. It may be perceived as being worse at the end of the day, when the oedema fluid has accumulated in the feet and legs, and 'better' in the morning, when that excess fluid has redistributed due to recumbency. There is, however, a lack of good correlation of the presence of oedema and the severity of heart failure (Stevenson and Perloff, 1989), perhaps in part because of variability in dietary sodium intake; subjects adherent to a low sodium diet may not form oedema, even when their ejection fractions are < 20% or when their pulmonary wedge pressures are > 22 mmHg.

Findings in right-sided heart failure

Oedema in subjects with right heart failure is the syndrome of cor pulmonale. It depends on severe lung disease, usually obstructive airways disease, although it could also be caused by pulmonary embolism. In classical cor pulmonale, there will be findings of emphysema or chronic bronchitis, and a loud pulmonic second heart sound. These patients will have jugular vein distension and positive hepatojugular reflux with hepatic enlargement accompanying peripheral oedema. Ascites may also be present. Here, as in left-sided failure, the primary stimulus to renal salt and water retention is arterial underfilling, which in the case of cor pulmonale has been attributed to hypercarbia and arteriolar dilation. Elevated venous pressures may also contribute, via their effect to lower the GFR and increase renal sodium retention.

Findings in kidney disease

Oedema in subjects with kidney diseases is classically worse in the morning. In subjects with nephrotic syndrome, this may be because the bodily fluid overload is most evident in the loose connective tissue around the eyes, where it can easily accumulate after recumbency. Oedema of the nephrotic syndrome may also be dependent, but will 'pit' more easily than will the pressure-dependent oedema of heart failure. In subjects with oedema that is solely due to kidney disease, findings of heart failure will be absent. With any kidney disease, heart failure may add to the tendency to fluid overload, and a single simple cause of oedema may no longer be apparent. When one organ dysfunction is combined with the other, lesser degrees of individual organ damage may cause oedema in combination that would not do so by themselves.

But oedema may not be a major feature of kidney disease. This was recognized over a century ago (Richet, 1993). Kidney diseases with no or minimal proteinuria, such as polycystic kidney disease or interstitial nephritis, may show substantial azotaemia, but little or no oedema.

Findings in liver disease

Oedema due to liver disease generally occurs only in those with severe liver disease that is clinically apparent. Jaundice, temporal wasting, and palmar erythema will be present; ascites typically precedes the formation of peripheral oedema. Yet, these patients may have superimposed heart or kidney disease, which may cause oedema not due to the liver disease.

Findings in starvation

Starvation alone should be evident by weight loss, and cachexia. Oedema of starvation is now quite rare in the developed world, but it was well documented during World War II. In one report from that time, the median total protein level in the serum was 4.5–5 g/dL in subjects with oedema, whereas non-oedematous subjects had total serum protein levels > 6.5 g/dL (Kurnick, 1948). Protein-losing enteropathy could cause a similar picture.

Findings in venous disease

Venous disease that causes oedema is usually accompanied by varicosities that are easily seen. In the absence of that finding, it is risky to ascribe oedema to venous disease alone. Moreover, the oedema of venous disease is usually asymmetric, a cardinal sign that is not the case for other diseases.

Treatment of oedema

Because oedema is a condition of sodium excess, restricting dietary sodium intake and using diuretics is the cornerstone of treatment. For generalized oedema, reversal of the underlying disorder will resolve the renal sodium retention. The latter is either an exaggerated response to a low effective arterial volume or a response of kidney tubules to damage. Pulmonary oedema is the only form of generalized oedema that is life threatening and requires immediate intravenous therapy within minutes. In all other oedematous states, removal of the excess fluid can proceed more slowly, over days to weeks. This is particularly true in patients with cirrhosis, who are at risk for hepatic coma or hepatorenal syndrome if they have a rapid diuresis.

Diet

Since ECF volume varies directly with sodium intake, dietary sodium restriction cannot be ignored. For patients with mild ECF volume expansion, a 'no added salt' diet may be appropriate, which is approximately 4 g of sodium/day, or approximately 160 mmol of sodium. This involves not adding salt during cooking and having no use of salt at the dining table. It is essential to avoid foods like potato chips, salted peanuts, or processed foods such as manufactured and cured meats. For more severe oedema, a very low sodium

chloride (NaCl) diet (2 g/day) should be prescribed. Water restriction per se is not needed, unless there is hyponatraemia.

Dietary compliance with sodium restriction can be checked by testing a 24-hour urine collection for sodium.

Medications

Prescription or over-the-counter medication may predispose to sodium retention or interfere with diuretic efficacy. Non-steroidal anti-inflammatory drugs (NSAIDs) promote renal sodium retention by reducing GFR and interfere with the efficacy of diuretics by competitively inhibiting the transport system of these diuretics at the proximal tubule, decreasing their concentration at their intratubular site of action. Vasodilators (minoxidil, hydralazine) can stimulate the sympathetic nervous system and renin-angiotensin system and thiazolidinediones promote ECF volume expansion by enhancing distal tubular sodium reabsorption. Dihydropyridine calcium channel blockers may induce dependant oedema through arteriolar vasodilation and increased intracapillary pressure.

Mechanical treatments

Elevation of the legs by placing them to above the level of the heart for 10–15 minutes, three to four times a day, stimulates interstitial fluid re-entry into the circulatory system, probably by reducing the venous pressures (Fig. 30.2). This can be useful in combination with sodium restriction in mild oedema. Sitting for long periods will increase swelling in the feet and ankles. Standing and/or walking at least every hour or two will help stimulate blood flow and reduce oedema formation. Use of compression elastic stockings will compress the leg vessels, promoting circulation and decreasing pooling of fluid due to gravity. In the case of oedema in preeclampsia, bed-rest is recommended. It will improve renal blood flow, reduce activity of the sympathetic nervous system and renin–angiotensin–aldosterone system, and thus mobilize oedema fluid from interstitial to intravascular space. Massaging the legs can help to stimulate the release of excess fluids, but should be avoided if the patient has blood clots in the veins.

A successful natriuretic response to head-out water immersion has been seen in children suffering from severe nephrotic syndrome caused by minimal change disease (Rascher et al., 1986). This technique can be tried in patients who have the hepatorenal syndrome. Repeated sessions of head-out water immersion could improve the sodium and water excretion and the renal function in hepatorenal syndrome (Yersin et al., 1995).

Aquapheresis, also known as ultrafiltration (UF), is a technique for removing excess fluid from the body. It involves the placement of a catheter in the bloodstream that continuously runs the patient's blood through a filter. Excess plasma water and electrolytes are removed from the blood through this filter, and the blood is then returned to the patient. This can be very useful in the treatment of refractory fluid overload in subjects with heart failure (Costanzo et al., 2007). But a recent randomized trial showed that diuretics alone were better and caused fewer side effects compared to UF in heart failure patients who had renal impairment (Bart et al., 2012). In subjects with heart failure whose serum creatinine is > 200 μmol/L, haemodialysis is needed rather than mere UF.

In refractory oedema due to severe nephrotic syndromes, isolated UF and hemofiltration have been tried with some success. In cirrhotic patients, re-infusion of ascites and paracentesis with albumin infusion may help in managing ascites refractory to diuretics. Hemofiltration is also useful to control ascitic fluid and oedema (Davenport, 2001).

The oedematous patient with severe renal failure may need to start regular dialysis treatments, which are very effective in treating fluid overload.

Diuretics

Once the decision to initiate diuretic therapy has been made, the initial choice of drug and dosage depends on the underlying cause of oedema and its severity (Brater, 1998).

Refilling phenomena

When diuretics are given, the fluid that is lost initially comes from the intravascular space. This results in a reduction in capillary hydraulic pressure; the plasma volume will be refilled by the mobilization of extracapillary oedema fluid into the intravascular space according to the familiar Starling forces.

The speed and magnitude of this refilling is variable. In patients with generalized oedema due to heart failure, nephrotic syndrome, or primary kidney sodium retention, the oedema fluid can be easily and quickly mobilized, since most capillary beds are involved. Thus, in patients with generalized oedema a diuresis of 1–2 L of oedema fluid or more in 24 hours can usually be achieved without an adverse reduction in plasma volume.

But in other patients, the decrease in effective circulating arterial volume is sufficient to significantly impair tissue perfusion. This most often occurs in two settings: when there is a low effective arterial circulating volume, as in heart failure with hypotension and after rapid fluid removal in cirrhosis (Pockros and Reynolds, 1986). When a cirrhotic patient has ascites but no peripheral oedema, refilling of the intravascular volume will depend on the peritoneal capillary bed alone, rather than on the capillaries and interstitial fluid of the whole body. In these cirrhotics, the maximum amount of fluid that can be mobilized is about 500 mL/day. If the diuresis proceeds more quickly than that, the ascitic fluid will be unable to completely replenish the plasma volume, resulting in azotaemia and possible precipitation of the hepatorenal syndrome. But in cirrhotics who also have peripheral oedema, the rate of diuresis can be more than 1 kg/day.

Pharmacology of diuretics

(See Chapter 33.)

For moderate to massive oedema (> 10% excess ECF volume), *loop diuretics* are most used, because they are the most potent diuretics and they act quickly. They are very useful for oedema caused by heart failure, cirrhosis, or nephrotic syndrome, or when renal failure is present. Loop diuretics can be given orally or intravenously. The onset of diuresis is earlier and the peak diuresis is greater with intravenous therapy because of greater and more rapid bioavailability.

There are no significant differences in efficacy between the different loop diuretics (furosemide, bumetanide, and torsemide) if they are given at equipotent doses (40 mg, 1 mg, and 10 mg, respectively). Each is active at all levels of renal function, and act quickly, even following oral administration. They have a sigmoidal dose–response curve characterized by a threshold drug dose to induce a diuresis, an ascending portion of the curve in which increased diuretic dose causes more and more sodium excretion, and a plateau at which further elevations in diuretic dose do not add to the diuresis

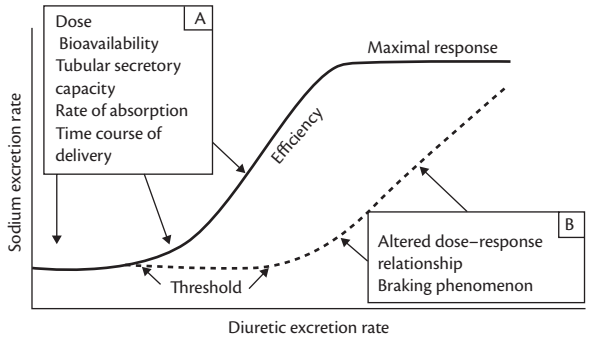

Fig. 30.3 Diuretic dose–response curve. Urinary sodium excretion according to loop diuretic use in normal conditions and when there is generalized oedema. The diuretic threshold depends on the dose, bioavailability, and tubular secretory capacity and the plateau on the braking phenomenon. CHF = congestive heart failure; HC = hepatic cirrhosis; NS = nephrotic syndrome; CRI = chronic renal insufficiency.

Adapted from NKF KDOQI clinical practice guidelines on HTA in CKD 2002 (guideline 12, Fig 56, reproduced with permission).

(Fig. 30.3). In subjects with normal renal function, a diuresis begins with 10 mg of intravenous furosemide given as a bolus with the maximal effect being seen with 40 mg. Going above this maximum will produce little or no further diuresis but may increase the risk of side effects. The doses that give this plateau are higher when either acute or chronic renal failure is present. For intravenous administration of furosemide the plateau dose could increase from 40 mg in subjects with normal renal function to 200 mg when the GFR is < 20 mL/min (Table 30.1) (Brater, 1998; Wilcox, 2002). For bumetanide, these doses range from 1 mg for subjects with normal renal function to 10 mg in subjects with severe renal failure (GFR < 20 mL/min).

The plasma half-life of loop diuretics is fairly short. Thus, in oedematous patients with normal renal function it is better to give these diuretics in multiple daily doses to counter the post-diuretic sodium retention. Kidney or liver disease needs to be considered. The half-life of furosemide is increased in renal failure. The half-lives of bumetanide and torsemide are increased when cirrhosis is present. But the efficacy of loop diuretics in patients with liver disease may be reduced due to competition at the secretion site at the proximal tubule between loop diuretics and bile salts.

Loop diuretics can be given orally, although seriously ill patients in the hospital or resistant to oral treatment may need them intravenously for a quicker response. The data on the maximally effective loop diuretic dose are based upon intravenous bolus therapy in patients with a relatively normal GFR and vary with the underlying disease (Brater, 1998). The doses required in congestive heart failure, cirrhosis, or nephrotic syndrome are shown in Table 30.1.

The intravenous equivalent dose for furosemide, but not for the other loop diuretics, is one-half the oral dose, because of its decreased oral availability. The effective diuretic dose is typically higher in patients with New York Heart Association (NYHA) stage III or IV heart failure, advanced cirrhosis, or renal failure. In these settings, decreased renal perfusion (and therefore decreased drug delivery to the kidney), diminished drug secretion into the lumen (due to the retention of competing anions in renal failure), and enhanced activity of sodium-retaining forces (such as the renin–angiotensin–aldosterone system and sympathetic nervous activity) combine to diminish the diuretic effect. The diuretic dose–response curve is shifted to the right and also shows a diminished maximal plateau (Fig. 30.3).

Selected hospitalized patients may benefit from a continuous intravenous infusion of a loop diuretic after an initial bolus dose, which can produce a greater diuresis than intravenous boluses (Table 30.2). It may also be safer, and less apt to cause ototoxicity.

The enhanced diuresis with a continuous infusion compared with bolus therapy is related to continuous inhibition of sodium chloride reabsorption in the loop of Henle. In contrast, bolus therapy is first associated with initially higher and then followed by lower rates of diuretic excretion; as a result, sodium excretion may be at near maximal levels for the first 2 hours but then gradually fall until the next dose is given. If the patient has received one or more intravenous boluses within the previous few hours, then an infusion can be started without a loading dose. Otherwise, an intravenous loading dose of 40–80 mg of furosemide is given over 5 minutes.

After the loading dose, the starting infusion rate with furosemide varies with the level of renal function. When the renal function is normal, an initial furosemide infusion rate of 5 mg/hour could be used. If the diuresis is not sustained, a second bolus can be given followed by a higher infusion rate of 10 mg/hour. But when there is substantial reduction in kidney function, it is suggested that after

Table 30.1 Ceiling doses for loop diuretics in mg/day

Medical conditions	Furosemide (mg)		Bumetanide (mg)		Torsemide (mg)	
	Intravenous	Oral	Intravenous	Oral	Intravenous	Oral
GFR > 50 mL/min	20–40	40–80	1–2	1–2	10–20	10–20
GFR 20–50 mL/min	120	240	3	3	50	50
GFR < 20 mL/min	200	400	10	10	100	100
Nephrotic syndrome	40–60	80–120	2–3	2–3	20–50	20–50
Cirrhosis with oedema	40	40	1	1	10	10
Congestive heart failure	40–80	40–80	1–2	1–2	10–20	10–20

Adapted from Brater (1998) and Wilcox (2002).

Table 30.2 Doses for continuous intravenous loop diuretic infusion

Diuretics	Intravenous bolus dose (mg)	Intravenous infusion (mg/h)[a]		
		GFR > 75 mL/min	GFR 25–75 mL/min	GFR < 25 mL/min
Furosemide	40	5 then 10	10 then 20	20 then 40
Bumetanide	1	0.5	0.5 then 1	1 then 2
Torsemide	20	5	5 then 10	10 then 20

[a] Before increasing the infusion speed, one needs first to inject a new IV bolus.

Adapted from Brater (1998) and Wilcox (2003).

the intravenous loading dose of 40–80 mg of furosemide, the initial furosemide infusion rate should be 20 mg/hour. If the diuresis is not sustained, a second bolus can be given followed by a higher infusion rate of 40 mg/hour. The equivalent bumetanide and torsemide dose is 1 mg/hour, increasing to 2 mg/hour for bumetanide and 10 mg/hour, increasing to 20 mg/hour for torsemide (Table 30.2). The vigorous diuresis produced by loop diuretics makes these especially useful for rapid reduction of oedematous fluid. However, other diuretics exist.

Thiazide diuretics decrease active re-absorption of sodium and chloride ions by inhibiting the sodium/chloride cotransporter in the distal convoluted tubule. They also increase potassium ion loss. These were originally synthesized as carbonic anhydrase inhibitors. Thiazide diuretics, like loop diuretics, are secreted into the tubular fluid by proximal tubule cells, then act at the distal convoluted tubule. While some thiazides have carbonic anhydrase inhibitory activity, the major site of action is to reduce tubular sodium reabsorption. Hydrochlorothiazide is the prototype for this class of drug. chlorthalidone, indapamide, and metolazone are long acting congeners. These drugs do not have the thiazide structure but are referred to as 'thiazide-like'.

The clinically available thiazides and thiazide-like agents have the same mechanism of action. They differ only in their plasma half-lives. Thiazides can be used to treat oedema associated with congestive heart, cirrhosis, renal insufficiency, and the nephrotic syndrome.

Oral doses of hydrochlorothiazide are well absorbed and reach peak effect in about 4 hours, and have a 6–12-hour duration of action. It is excreted unchanged in the urine with a half-life of 3–5 hours. The usual adult dose for oedema treatment is 25–100 mg per day, depending on patient response. Hydrochlorothiazide is not metabolized but is eliminated rapidly by the kidneys. At least 61% of the oral dose is eliminated unchanged within 24 hours.

The likelihood of side effects with the thiazides increases with the dose of the drug. These include hypokalaemia, hyponatraemia, hypochloraemia, hypercalcaemia, hypomagnesaemia, and hyperuricaemia. The average fall in serum potassium with use of thiazides is 0.6 mmol/L (Morgan and Davidson, 1980); a decrease in glucose tolerance can also be observed. Warning signs of fluid and electrolyte imbalance include dry mouth, thirst, weakness, lethargy, drowsiness, restlessness, muscle pains or cramps, muscular fatigue, hypotension, oliguria, tachycardia, nausea, vomiting, seizures, or confusion.

Hypokalaemia may be prevented or treated with potassium-rich foods, potassium supplements, or a potassium-sparing diuretic. 'Dilutional hyponatraemia' (see Chapter 28) most commonly occurs during hot weather in patients with chronic congestive heart failure or hepatic disease. Treatment includes withdrawal of the diuretic, fluid restriction, and potassium and/or magnesium supplementation. Administration of sodium chloride is usually not required, except in rare instances when the hyponatraemia is life-threatening. Caution is necessary when using thiazides in patients with hyperuricaemia or a history of gout.

The routine use of thiazide diuretics in an otherwise healthy pregnant woman with oedema is not appropriate. Thiazides cross the placenta and possible risks include fetal or neonatal jaundice, thrombocytopaenia, and possibly other adverse reactions that have occurred in the adult.

Metolazone is a thiazide-like diuretic used primarily to treat congestive heart failure and sometimes used together with loop diuretics. Approximately 65% of the amount ingested becomes available in the bloodstream. Its half-life is approximately 14 hours, similar to indapamide but considerably longer than hydrochlorothiazide. Metolazone is around ten times as potent as hydrochlorothiazide. Although most thiazide diuretics lose their effectiveness in renal failure, metolazone remains active even when the GFR is < 30–40 mL/min. This gives it a considerable advantage over other thiazide diuretics, since renal and heart failure often coexist and contribute to fluid retention.

For hypokalaemia resulting from thiazide or loop diuretics, the potassium loss can be mitigated with potassium supplementation (potassium salts or foods rich in potassium) or the use of *potassium-sparing* diuretics. There are two types of potassium-sparing diuretics: the renal epithelial sodium channel inhibitors *amiloride* and *triamterene* or the aldosterone antagonists *spironolactone* and *eplerenone* (see Chapter 21).

Amiloride and triamterene block the epithelial Na^+ channel. As a result, the driving force for K^+ secretion is eliminated and K^+ secretion is reduced; their diuretic effect is modest.

Aldosterone interacts with a cytoplasmic mineralocorticoid receptor to enhance the expression of the Na-K-ATPase and the Na^+ (and K^+) channel in the distal tubule; spironolactone and eplerenone block aldosterone by binding to the mineralocorticoid receptor. Their onset and duration of action are determined by the kinetics of the aldosterone response in the target tissue. Overall, spironolactone has a slow onset of action, requiring several days before its full effect is achieved. It decreases the reabsorption of sodium and water, while decreasing the secretion of potassium. This diuretic is useful for oedema treatment in cirrhosis or in combination therapy with loop diuretics in patients with refractory oedema, for instance in congestive heart failure. For nephrotic patients it can be used when treatment of the underlying disease, restriction of fluid and sodium

intake, and the use of other diuretics do not provide an adequate response.

Eplerenone has a lower affinity for the mineralocorticoid receptor compared with spironolactone. Nonetheless, it also blocks aldosterone-induced gene expression. However, eplerenone has little affinity for androgen or progesterone receptors. Therefore, it will not cause steroid hormone-like effects of spironolactone such as gynaecomastia, or hair growth. This diuretic, like spironolactone, has been shown to improve outcomes in patients with heart failure. The approach to diuretic therapy will vary according to the condition causing oedema.

Specific oedematous conditions

Cirrhosis

For patients with cirrhosis, the aldosterone antagonist spironolactone is the preferred initial regimen, based on the role for aldosterone in the fluid retention of cirrhosis, and also to avoid hypokalaemia. One can start at 25 mg/day but this dose may need to increase to 200 mg/day for optimal benefit. Spironolactone is combined with a loop diuretic when there is peripheral oedema in addition to the ascites. As mentioned above, the diuresis should not exceed 0.5 kg/day in the absence of oedema. When there is leg oedema, one can achieve a weight loss of 1 kg/day. In patients suffering from cirrhosis with large ascites and marked abdominal distension, paracentesis can be done in addition to low sodium diet and diuretics. Up to 10 L of fluid may be drained during the procedure. If fluid drainage is more than 5 L, patients should receive intravenous serum albumin right afterwards to prevent low blood pressure. As mentioned previously, head-out water immersion could be useful in some patients.

Heart failure

For patients with heart failure, the rate of diuresis is usually not a limiting issue, but one must monitor for signs of hypoperfusion (e.g. a rise in serum creatinine). In NYHA class II congestive heart failure, in addition to use of an angiotensin-converting enzyme (ACE) inhibitor and low sodium diet, thiazide diuretics may be enough to resolve oedema and ECF overload. But loop diuretics are typically used as first-line therapy in more severe heart failure (NYHA classes III and IV) along with the use of ACE inhibitors. This combination is needed as shown by Anand et al. (1990). The dose–response curves of these loop diuretics are shifted rightward because of delayed gut absorption and diminished nephron response (Brater et al., 1984) (Fig. 30.3). Rather than increasing the dose, it is sometimes more useful to give the loop diuretic several times a day to improve sodium excretion. This is in part due to increased tubular sodium reabsorption at different levels of the renal tubule (Loon et al., 1989; Rose, 1991; Wald et al., 1991) and also because of the half-life of loop diuretics such as furosemide. If worsening heart failure develops, there is even greater activation of the sympathetic nervous system and renin angiotensin aldosterone system. Escalation of the total daily dose of loop diuretics is then needed to overcome these stimuli to sodium retention.

In congestive heart failure hyponatraemia may occur because of non-osmotic arginine vasopressin (AVP) release. In these cases, vasopressin V2 receptor antagonists have been used and may attenuate water retention (Gheorghiade et al., 2007). However, long-term mortality and heart failure-related morbidity are not improved by use of vasopressin antagonists in heart failure (Konstam et al., 2007).

Nephrotic syndrome

For patients with nephrotic syndrome, diuretic treatment is needed to treat oedema unless and until immunosuppression and/or renin–angiotensin system antagonism are effective.

In the presence of normal renal function, diuretics will be needed such as furosemide (initial oral dose 1 mg/kg/day) given in two or three separate doses. The combination with spironolactone or amiloride will help when fluid retention is severe, but should be avoided in subjects with hyperkalaemia or hypotension (Deschenes et al., 2004). Thiazide diuretics may be added in some cases, because they act on a different site in the nephron (Garin, 1987; Fliser et al., 1994). However, such combined diuretic use may cause volume depletion, which should be carefully monitored for by assessment of symptoms, weight, heart rate, upright blood pressure, and laboratory testing.

Use of albumin has been much discussed for the treatment of oedema in the nephrotic syndrome. Its use is based on the premise that raising the serum albumin will 'pull' fluid from the extravascular to the intravascular space. Albumin may also increase diuretic delivery to the kidney by keeping furosemide within the vascular space, decreasing its catabolism and facilitating its secretion in the tubule lumen. Intravenous albumin at 1 g/kg can be given, followed by intravenous furosemide. But hypertension can occur in almost half of the patients treated, and respiratory and cardiac failure can develop (Dorhout Mees, 1996; Reid et al., 1996). Moreover it is reported that albumin may delay the response of nephrotic syndrome to steroids and may even induce more frequent nephrotic relapses, perhaps by causing severe glomerular epithelial damage (Yoshimura et al., 1992).

For children affected by nephrotic syndrome, the use of albumin for severe oedema may be analysed according to the two hypotheses proposed for the pathogenesis of nephrotic oedema. According to the underfill hypothesis, severe hypoalbuminaemia decreases intravascular oncotic pressure, leading to circulatory volume depletion and subsequent salt and water retention. In this condition, albumin infusion could be useful. On the other hand, the overfill mechanism proposes a primary renal defect in sodium excretion leading to salt and water retention and thereby hypervolaemia and oedema. These different mechanisms for oedema would demand different therapies, and Kapur et al recently reported this in a series of 30 children with nephrotic syndrome (Kapur et al., 2009). They showed that the fractional excretion of sodium could be used, along with other indicators of intravascular volume, to differentiate nephrotics who were volume contracted from those who were volume expanded. When the fractional excretion of sodium was < 0.2%, that is, when the nephrotic subject was volume contracted, intravenous albumin could be used, whereas it was not used in those with volume expansion. In the latter patients, the use of diuretics alone was effective and safe.

One randomized trial did show an increased urine volume with co-administration of albumin and furosemide, as compared with albumin administration alone or furosemide use alone, in 10 nephrotic patients with normal renal function. However, the 24-hour urinary sodium levels were not different between those on furosemide alone compared to those on furosemide in combination with albumin (208 vs 206 mmol) (Ghafari et al., 2011).

This confirms what was published more than10 years ago when Fliser et al showed that adding albumin to furosemide produced only a modest increase in sodium excretion compared with furosemide alone (Fliser et al., 1999), roughly equivalent to the amount of sodium contained in the colloid solution, suggesting that volume expansion was a likely explanation for the enhanced natriuresis. A similar lack of efficacy of furosemide plus albumin infusion has been shown in hypoalbuminaemic patients with cirrhosis (Chalasani et al., 2001)

Renal insufficiency

Although the oral bioavailability of loop diuretics is the same in renal insufficiency as in normal subjects, the dose of loop diuretic sufficient to cause a diuresis must be higher in subjects with lower GFR. That is because the diuretic gets to its tubular site of action through tubular secretion, and that tubular function is impaired in renal insufficiency.

The largest single dose of a loop diuretic giving the maximum natriuretic response in subjects with severe renal insufficiency is an intravenous bolus of 200 mg of furosemide or the equivalent of bumetanide or torsemide (Table 30.1). Some patients may require these large doses several times a day. Only side effects are gained by using larger doses. The maximum response is the excretion of about 20% of the filtered sodium. For furosemide, the usual maximal oral dose (the plateau dose) is twice the intravenous dose; this is not the case for bumetanide and torsemide for which the intravenous and oral doses are similar. In addition, the absorption of furosemide is not always 50% and can vary from a patient to another (between 10% and 100%); if there is some resistance in oedema treatment with this drug, one should use higher doses or change to another loop diuretic. In patients who have poor responses to intermittent doses of a loop diuretic, a continuous intravenous infusion can also be tried. For the latter, an initial loading dose is necessary to decrease the time needed to achieve drug concentrations at the intratubular site of action. A continuous infusion of bumetanide (Wilcox, 2002) enables a greater loss of sodium than the same total dose given as divided intravenous injections. The rate of the continuous injection is governed by the patient's renal function (Table 30.2).

The addition of thiazides to loop diuretics can be considered in oedematous subjects with renal failure, even though thiazides are commonly held to be ineffective when the GFR is < 50 mL/minute. That is because they act at a different site in the nephron, at the NaCl transporter in the distal tubule, thus potentiating the effect of loop diuretics that act on the Na-K-2Cl cotransporter just proximal to that site.

Idiopathic oedema

For patients with idiopathic oedema, if a diuretic needs to be prescribed, spironolactone is the drug of choice, because of the secondary hyperaldosteronism that can be found in these patients. But many patients with idiopathic oedema are actually already taking diuretics when first seen and may have 'diuretic-induced oedema'.

So, the initial approach is to try to discontinue the diuretic treatment for at least 2–3 weeks, after warning the patient that the oedema will probably worsen initially and reassuring her (it more usually affects women) that the diuretic can always be restarted. If the oedema does not improve after 4 weeks, spironolactone can then be initiated at a dose of 50–100 mg daily. When this oedema develops during the menstrual period, high capillary permeability is a possible mechanism and ephedrine could be tried (Edwards and Hudson, 1991).

Resistant oedema

For patients with resistant oedema from any cause, a complete review and assessment are needed. First, a high salt intake should be looked for because it can prevent net fluid loss even if there is an adequate urine output. To estimate NaCl intake, a 24-hour urine should be collected. A sodium value > 100 mmol/day suggests that non-compliance with sodium restriction is responsible for the apparent resistance to therapy. Use of other medication should be considered; NSAIDs should be discontinued, since diminished synthesis of vasodilator and natriuretic prostaglandins can impair diuretic responsiveness.

Posture is another factor that can influence diuretic responsiveness. Patients with heart failure, for example, are unable to increase cardiac output appropriately when upright. As a result, renal perfusion is reduced and urinary diuretic delivery diminished. Intermittent supine rest could improve the renal blood flow and the sodium and water excretion (Karnad and Abraham, 1986; Ring-Larsen et al., 1986)

Two additional factors can contribute to oedema that is refractory to usual oral or intravenous loop diuretic therapy: either decreased diuretic secretion into the tubular lumen or increased tubular sodium reabsorption. Loop diuretics are highly (≥ 95%) protein bound. As a result, they primarily enter the tubular lumen by secretion in the proximal tubule, not by glomerular filtration. Diuretic resistance can result from decreased renal perfusion in acute kidney injury from any cause, from heart failure due to the reduced cardiac output, or from cirrhosis due to renal vasoconstriction. In renal failure, acute or chronic, uraemic metabolites and metabolic acidosis may inhibit tubular secretion that may also cause diuretic resistance.

Decreased diuretic secretion may occur in two ways in the nephrotic syndrome. With hypoalbuminaemia, there is less delivery of the albumin-bound loop diuretic to the renal tubular epithelial cell for secretion and subsequent action. In addition, binding of the diuretic by filtered albumin within the tubular lumen may reduce loop diuretic efficacy; resistance may be overcome by increasing the dose of the drug.

A variety of other factors can account for persistent fluid retention in subjects prescribed diuretics, including variability of furosemide absorption at the intestinal site (substitution of bumetanide may obviate this problem), or delayed intestinal absorption of any diuretics when there is intestinal wall oedema (the use of the intravenous route could bypass this problem).

It is worth again noting that diuretics have a dose–response curve, with no natriuresis seen until a threshold rate of drug excretion is attained. If, for example, a patient does not respond to 40 mg of furosemide, the dose may not have exceeded this threshold. Thus, the single dose should be increased to 60 or 80 mg, rather than giving the same dose twice a day.

Loop diuretics have short half-lives. In the situation of active sodium retention (i.e. cardiac failure), loop diuretics must be given more frequently. Indeed, as the effect of loop diuretics wanes, the kidneys immediately begin to reabsorb sodium, which attenuates the diuretic effect. This process is called post-diuretic sodium chloride retention or diuretic braking phenomenon.

Combination therapy

Loop diuretics may be combined with an agent that works elsewhere in the renal tubule. When diuretics that work at different sites are used together, the response may be synergistic, ithat is, greater than the addition of the single responses to the individual diuretics. Combination therapy may include hydrochlorothiazide or metolazone. Thiazide administration should precede the loop diuretic by 2–5 hours, since the peak effect of the latter is at 4–6 hours.

Combination therapy must be given with careful monitoring, since a marked diuresis can occur in which daily sodium and potassium losses can be > 300 and 200 mmol, respectively (Oster et al., 1983; Fliser et al., 1994). This could lead to an excessive loss of potassium in the urine, leading to hypokalaemia and depletion of body potassium. These patients should take potassium supplements and/or to eat foods high in potassium. In certain instances, diuresis can be improved by adding a potassium-sparing diuretic, that is, one that does not cause depletion of potassium. Those diuretics include spironolactone, eplerenone, triamterene, and amiloride. Adding one of them may preclude the need for potassium supplements. But neither potassium-sparing diuretics nor potassium supplements are advisable in subjects with reduced kidney function. One can consider adding acetazolamide, which acts to reduce proximal tubular sodium and bicarbonate reabsorption, but that could induce a renal tubular acidosis.

To combat diuretic resistance, we thus advise restriction of sodium intake, use of escalating doses of loop diuretics up to ceiling levels, and adroit use of a second diuretic acting at a downstream site. In nephrotic syndrome, measures to reduce the proteinuria can also be very useful. UF is a last resort.

Ultrafiltration

Some oedematous patients with advanced heart failure or renal failure do not respond to any of the above modalities. In them, one could consider dialysis or UF. Peritoneal dialysis was first used for congestive heart failure in 1959 (Girard et al., 1959). Silverstein et al first proposed extracorporeal haemofiltration for fluid removal (Silverstein et al., 1974). Since then, this procedure has been much studied, either using haemodialysis with the UF component or by isolated haemofiltration in severe congestive heart failure (Agostoni et al., 1994; Dormans et al., 1996; Marenzi et al., 2001). Grapsa et al. (2004) reported that when patients with congestive heart failure have intractable oedema in spite of intravenous inotropes and diuretics, treatment by haemodialysis with UF yields subjective improvement, but a high death rate persists (Grapsa et al., 2004). A peripherally inserted UF device (Aquadex System 100) was recently approved by the US Food and Drug Administration for therapy in heart failure allowing UF at very low flows via a peripheral intravenous catheter with only < 40 mL of extracorporeal blood at any given time (Costanzo et al., 2005). In decompensated heart failure, UF with this method produces greater weight and fluid loss than intravenous diuretics and reduces cost. But, the rate of fluid removal can exceed 500–1000 mL/hour in this setting and careful monitoring is required to prevent volume depletion. A recent randomized trial showed that diuretics alone were better than UF in heart failure patients who had renal impairment (Bart et al., 2012). In subjects with heart failure whose serum creatinine is > 200 μmol/L, haemodialysis is needed, rather than just UF.

Care of patients treated by diuretics

Hypokalaemia may develop in half of all patients treated with thiazide diuretics, and require supplementation with potassium. Hypokalaemia is less common and less severe with use of loop diuretics (Morgan and Davidson, 1980), yet almost always occurs when loop diuretics are used with thiazides or metolazone. Hypotension may occur if diuretic use causes volume depletion. Weight must be measured and should decrease until the patients become free of oedema.

When changing the type or dose of diuretic, one must monitor the urine volume, the serum electrolytes (for hypokalaemia or hyponatraemia), and look for signs of a decline in tissue perfusion, such as weakness, asthenia, upright postural hypotension with dizziness, lethargy due to decreased cerebral blood flow, tachycardia, and unexplained rise in blood urea nitrogen and creatinine concentration. As long as these parameters remain constant, it can be assumed that diuretic therapy has not led to a significant impairment in perfusion to the kidneys or to other organs.

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CHAPTER 31

Approach to the patient with salt-wasting tubulopathies

Detlef Bockenhauer and Robert Kleta

Introduction

Sodium is the main ion constituent of the extracellular and intravascular fluid compartments, and it is through control of sodium reabsorption that the kidneys maintain volume homoeostasis and systemic blood pressure (see Chapter 21). The amount of sodium that is first filtered by the glomerulus and then reabsorbed in the tubule is quite staggering: assuming a glomerular filtration rate of 100 mL/min and a serum sodium concentration of 140 mmol/L, an average-sized person filters about 20,000 mmol of sodium per day, equivalent to the amount in 1.2 kg of cooking salt (Kleta and Bockenhauer, 2006). In the steady state, the amount of sodium excreted is equal to the amount ingested. An average Western diet contains about 8–10 g of salt per day; a low-salt diet would usually be around 2 g per day. Under physiological conditions, the tubules reabsorb about 99% of filtered sodium and without the kidneys' sophisticated ability to preserve salt (and water), human life would not exist. This enormous task is accomplished by a combination of distinct and sequentially oriented sodium or sodium-coupled transport systems along the nephron and the concerted and parallel action of some of these systems within the kidney (Fig. 31.1). Based on the molecular identity of the involved transporters, we typically distinguish four segments important for sodium and water reabsorption:

1. The proximal tubule (PT)
2. Thick ascending limb (TAL) of Henle's loop, including macula densa
3. Distal convoluted tubule (DCT) and connecting tubule
4. Collecting duct (CD).

Disorders affecting the individual segments lead to specific biochemical 'fingerprints', which can guide the diagnosis of these disorders (Table 31.1). Diuretics, or more correctly saluretics, typically target specific transporters involved in tubular sodium reabsorption and many of these disorders can be conceptualized by comparing them with the effects of the different saluretics. An overview of salt-wasting tubulopathies and their 'salient' clinical features is given in Table 31.2, including the underlying molecular basis and the corresponding diuretic (if any).

Disorders affecting salt transport in the proximal tubule

Salt wasting in the PT is typically in the form of a generalized PT dysfunction, that is, renal Fanconi syndrome, which is discussed separately (see Chapter 41) and will only be mentioned briefly (Fig. 31.2). Because of the molecular link of sodium transport with the various other transport pathways (see Chapter 20), patients typically exhibit multiple biochemical abnormalities, including metabolic acidosis, hypophosphataemia, glycosuria, and aminoaciduria.

There are only a few disorders affecting specific sodium transporters in the PT and these are not salt-wasting disorders per se. The main transporter for sodium in the PT is sodium-hydrogen exchanger 3(NHE3) and so far no human disease has been associated with mutations in NHE3. Interestingly, mice deleted for the encoding gene *Slc9a3* survive and exhibit only mild metabolic acidosis (Schultheis et al., 1998).

Mutations have been identified in proximal sodium-phosphate transporters. Mutations in the predominant form NaPi-IIa (*SLC34A1*) have recently been associated with a variant of an incomplete renal Fanconi syndrome (Magen et al., 2010), whereas mutations in NaPi-IIc (*SLC34A3*) are associated with hypophosphataemic rickets (Bergwitz et al., 2006; Lorenz-Depiereux et al., 2006; Ichikawa et al., 2006).

Diseases have also been linked with sodium-glucose transporters: mutations in *SGLT1* (*SLC5A1*) lead to glucose/galactose malabsorption with severe infantile-onset diarrhoea, highlighting the importance of this transporter in the intestine (Turk et al., 1991). In contrast, mutations in *SGLT2* (*SLC5A2*) lead to isolated renal glycosuria, in general without clinical symptoms (van den Heuvel et al., 2002). Whilst the loss of sodium and glucose (up to 150 g of glucose per day) may have been relevant in Palaeolithic times, it could actually be beneficial in the context of a current Western diet, potentially protecting against hypertension and diabetes (Francis et al., 2004); indeed, drugs that inhibit this transporter have been approved recently for control of hyperglycaemia in diabetes.

Thick ascending limb of Henle's loop: Bartter syndromes

Impaired salt transport in the TAL leads to Bartter syndromes. Currently, we recognize four different forms, based on molecular genetics (Table 31.2) (Simon et al., 1996a, 1996b, 1997; Birkenhager et al., 2001). A separate disease, familial hypocalcaemic hypercalciuria, due to dominant (activating) mutations in the calcium-sensing receptor (CaSR) can occasionally cause a biochemical urinary constellation resembling Bartter syndrome, and is sometimes referred to as Bartter type 5 (Watanabe et al., 2002). Similarly, a Bartter-like phenotype can be observed in some patients with mitochondrial

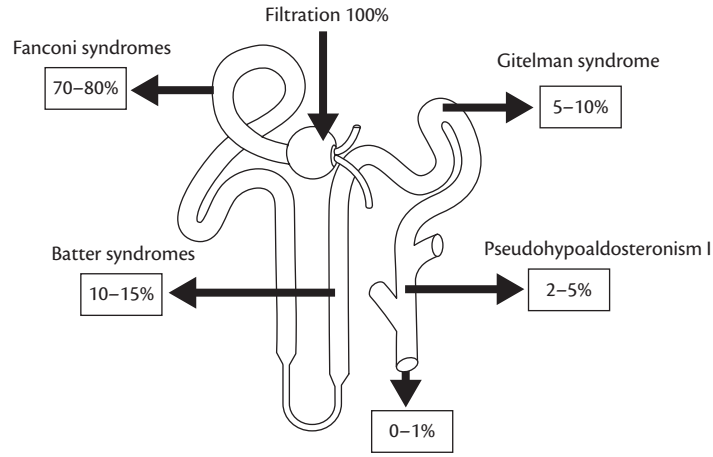

Fig. 31.1 A model of salt reabsorption along the nephron. The vast majority of filtered sodium is reabsorbed in proximal tubule. Segment specific human disorders affecting salt reabsorption are indicated.

cytopathies (Goto et al., 1990; Emma et al., 2006). However, as these are diseases with a phenotype beyond Bartter syndrome and have a separate molecular basis, only the four main variants, specifically affecting salt-transport in the TAL will be discussed here.

Pathophysiology and aetiology

Bartter syndrome type 1 is caused by mutations in NKCC2 (*SLC12A1*) (Simon et al., 1996a). This transporter is the target of loop diuretics and the symptoms are best compared with chronic furosemide administration (Reinalter et al., 2004). NKCC2 can only function if it is 'fully loaded', that is, if it has bound 1 sodium, 1 potassium and 2 chloride ions. The concentration of these ions in the tubular lumen is roughly the same as in plasma. Hence, the availability of potassium becomes the rate-limiting step for NKCC2 function. This critical availability of potassium is highlighted by Bartter syndrome type 2, which is caused by recessive mutations in ROMK (*KCNJ1*) (Simon et al., 1996b). ROMK is a potassium channel expressed in the apical membrane of TAL (Fig. 31.3) and ensures an adequate supply of potassium by recycling the potassium taken up by NKCC2 back into the tubular lumen. This may appear unnecessarily complicated at first sight: why transport potassium first into the cell only to have it leak back out through this potassium channel? However, there is an evolutionary reason for this arrangement: NKCC2 is electroneutral (it transports two cations and two anions). By having potassium leak back out through ROMK, the two transport molecules establish a voltage gradient (outside positive) across the apical membrane and, ultimately, across the epithelial cell layer (Fig. 31.4). This transepithelial potential then drives the paracellular reabsorption of calcium, magnesium and sodium mediated by claudins (Haisch et al., 2011). For this reason, patients with Bartter syndromes type 1 and 2 have hypercalciuria and hypermagnesuria: the failure to establish the transepithelial potential leads to losses of calcium and magnesium in the urine.

Whereas types 1 and 2 are due to defects in transport molecules on the apical membrane, types 3 and 4 concern the basolateral membrane. As detailed above, potassium taken up by NKCC2 recycles back into the tubular lumen. Sodium can exit the cell via the basolateral Na-K-ATPase, while chloride exits via the chloride channel CLCNKB, leading to net reabsorption of NaCl in this segment. Consequently, defects in CLCNKB impair salt reabsorption in the TAL and mutations in this channel have been found to underlie Bartter syndrome type 3 (Simon et al., 1997). CLCNKB is expressed not only in the TAL, but also in the DCT where it

Table 31.1 Biochemical 'fingerprints' of salt wasting tubulopathies based on affected tubular segment

Segment	PT	TAL	DCT	CD
Plasma Na	Normal	Normal	Normal	Low–normal
K	Low	Low (high neonatally Bartter type 2)	Low	High
HCO_3	Low	High	High	Low
Mg	Normal	Low–normal	Low	Normal
PO_4	Low	Normal	Normal	Normal
Urine K	High	High	High	Low
Ca	High	High (or normal in Bartter types 3, 4)	Low	Low
Tubular proteins	High	Normal	Normal	Normal

Table 31.2 Genetics, salient clinical features, and pharmacotyping of salt-wasting tubulopathies

Syndrome (inheritance)	Gene (protein)	Typical clinical features	Targeting diuretic
Bartter type 1 (AR)	*SLC12A1* (NKCC2)	Antenatal presentation with polyhydramnios and prematurity Nephrocalcinosis Urinary concentrating defect	Loop diuretics (furosemide)
Bartter type 2 (AR)	*KCNJ1* (ROMK)	Antenatal presentation with polyhydramnios and prematurity Nephrocalcinosis Neonatal hyperkalaemia Urinary concentrating defect	
Bartter type 3 (AR)	*CLCNKB* (CLCNKB)	Childhood presentation Most severe electrolyte abnormalities, often hypomagnesaemia	
Bartter type 4 (AR)	*BSND* (Barttin)	Antenatal presentation with polyhydramnios and prematurity Sensorineural deafness	
Gitelman (AR)	*SLC12A3* NCC	Hypocalciuria Hypomagnesaemia	Thiazides
EAST (AR)	*KCNJ10* Kir 4.1	Infantile-onset epilepsy; ataxia; sensorineural deafness; hypocalciuria Hypomagnesaemia	Thiazides
Pseudohypoaldosteronism type 1A (AR)	*SCNN1A, B, G* ENaC	Hypovolaemia, hyperkalaemia, acidosis, hyponatraemia, severe neonatal presentation	Amiloride
Pseudohypoaldosteronism type 1B (AD)	*MLR*	Hypovolaemia, hyperkalaemia, acidosis, hyponatraemia, acidosis	Spironolactone, eplerone

mediates chloride exit; therefore, there is some phenotypic overlap with Gitelman syndrome (see below). For proper surface membrane expression, CLCNKB needs a subunit, Barttin (encoded by *BSND*), and mutations in Barttin lead to Bartter syndrome type 4 (Birkenhager et al., 2001).

Clinical features

Despite the common category of 'Bartter syndrome', there is some clinical distinction between the four forms. An overview of the different forms is given in Table 31.2.

Hyperaldosteronism

All four forms are characterized by elevated renin and aldosterone levels, resulting in hypokalaemic hypochloraemic metabolic alkalosis (Table 31.1). This is associated with altered tubuloglomerular feedback in the macula densa. The macula densa senses tubular reabsorption of sodium-chloride: decreased reabsorption is interpreted as decreased delivery, that is, volume depletion (Schnermann et al., 2003). To enhance intravascular volume and glomerular filtration, macula densa cells produce locally prostaglandins, especially PGE2, which is secreted and then sensed by juxtaglomerular cells, which in turn produce and secrete renin (Yang et al., 2000; Peti-Peterdi et al., 2003). Prostaglandins are produced by cyclooxygenases, especially COX-2, explaining the success of treatment with COX-2-inhibitors (see 'Treatment') (Komhoff et al., 2000; Reinalter et al., 2002). In Bartter syndromes, this tubuloglomerular feedback is short-circuited: because the macula densa is part of the TAL and sodium-chloride reabsorption is defective, the patients produce high levels of prostaglandins, renin, and aldosterone. This excessive production of prostaglandins is likely to be the cause of the juxtaglomerular hyperplasia, which was a defining feature in the first description of this syndrome (Bartter et al., 1962). It is the consequent hyperaldosteronism and its action on the collecting duct that leads to the typical biochemical picture, because sodium is reabsorbed in exchange for potassium and protons in this nephron segment (see Fig. 31.5).

Transient neonatal hyperkalaemia

A specific feature of Bartter syndrome type 2 is the presence of hyperkalaemia in early postnatal life, often associated with hyponatraemia and acidosis (Finer et al., 2003; Brochard et al., 2009). The reason for this is the expression of ROMK in the principal cells of the CD, where it mediates potassium secretion. The biochemical picture in these patients mimics pseudohypoaldosteronism (see later) and can lead to misdiagnosis (Greenberg et al., 1995; Nozu et al., 2007). Usually, this picture disappears at the end of the first couple of weeks, as alternative pathways for potassium secretion mature and the typical biochemical features of Bartter syndrome evolve (Bailey et al., 2006).

Age of onset

In the original publication of the syndrome by Frederic Bartter and colleagues in 1962, two patients are described who first came

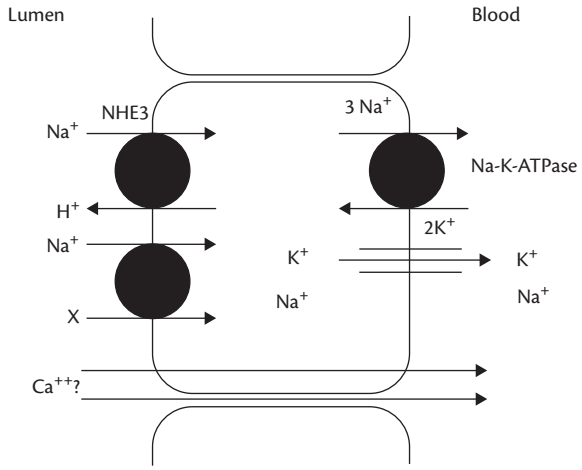

Fig. 31.2 Diagram of an epithelial renal proximal tubular cell. Sodium is reabsorbed via apical sodium exchangers and cotransporters. The electrochemical gradient for sodium uptake is provided by the basolateral Na-K-ATPase, which also provides a basolateral sodium exit pathway. This gradient is then utilized for uptake of various ions and small molecules (indicated by X) by luminal transport systems. Paracellular calcium reabsorption follows sodium passively, but the molecular identity of this transport pathway is yet unknown.

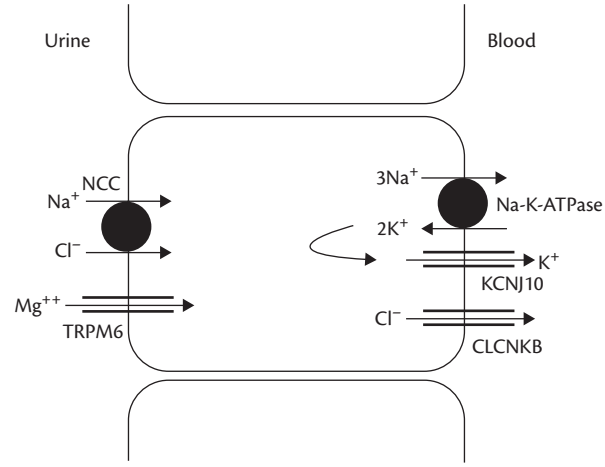

Fig. 31.4 Diagram of an epithelial cell in the distal convoluted tubule. Sodium is reabsorbed via the apical sodium-chloride cotransporter NCC, mutations in which cause Gitelman syndrome. The electrochemical gradient for sodium uptake is provided by the basolateral Na-K-ATPase, which also provides a basolateral sodium exit pathway. Activity is dependent on provision of potassium via the basolateral potassium channel KCNJ10, mutations in which cause EAST syndrome. Chloride exits the cell via the basolateral chloride channel CLCNKB.

to medical attention at 5 and 12 years of age, respectively (Bartter et al., 1962). This childhood-onset form is now often referred to as 'classic' Bartter syndrome, to distinguish it from the antenatal form that presents with severe polyhydramnios, often requiring repeated amniocentesis to relieve the pressure and typically resulting in premature birth. Molecular genetics has shown that the antenatal form is usually associated with mutations in *SLC12A1*, *KCNJ1*, or *BSND*, whereas classic Bartter syndrome is caused by mutations in *CLCNKB* (Peters et al., 2002). The likely explanation for this distinction is that the encoded proteins NKCC2, ROMK, and Barttin are each necessary and required for salt-reabsorption in the TAL. In contrast, CLCNKB has a very close homologue, CLCNKA (Jentsch et al., 2005; Briet et al., 2006). There is some controversy about expression of CLCNKA in the TAL and, unfortunately, due to the close homology there are no antibodies that

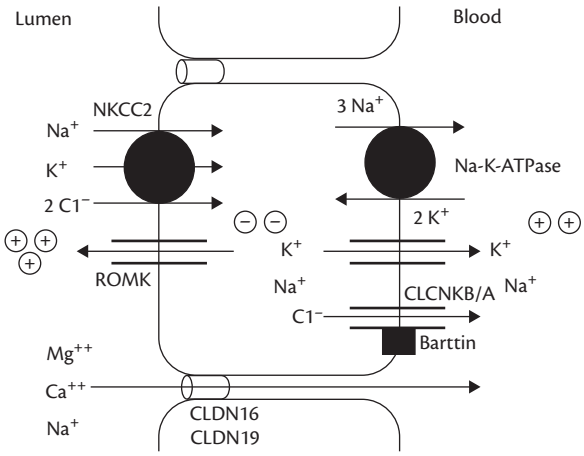

Fig. 31.3 Diagram of an epithelial cell in the thick ascending limb of Henle's loop. Sodium is reabsorbed via the apical sodium-potassium-2 chloride-cotransporter NKCC2, mutations in which cause Bartter syndrome type 1. Activity is dependent on provision of potassium via the apical potassium channel ROMK, mutations in which cause Bartter syndrome type 2. Together, these two molecules contribute to a transepithelial voltage gradient (lumen-positive, indicated by the number of + signs), which drives paracellular reabsorption of sodium, calcium and magnesium. The electrochemical gradient (indicated by font size for intracellular potassium and extracellular sodium and by + and − signs) for sodium uptake is provided by the basolateral Na-K-ATPase, which also provides a basolateral sodium exit pathway. Chloride exits the cell via the basolateral chloride channel CLCNKB, mutations in which cause Bartter syndrome type 3. Additional chloride exit is likely provided by CLCNKA (see text). Both channels require a subunit, Barttin, mutations in which cause Bartter syndrome type 4.

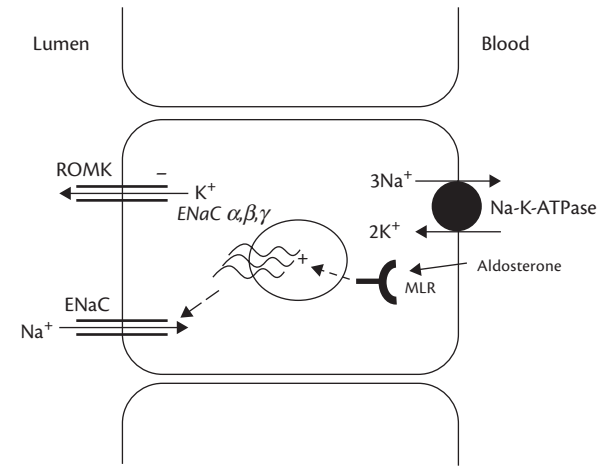

Fig. 31.5 Diagram of a principal cell in the collecting duct. Sodium is reabsorbed via the apical sodium channel ENaC, mutations (deactivating) in which cause the recessive form of pseudohypoaldosteronism type 1. This, in turn enhances potassium secretion through the apical potassium channel ROMK (and proton secretion via the proton pump in adjacent intercalated cells). Expression of ENaC is regulated via the mineralocorticoid receptor MLR, mutations in which cause the dominant form of pseudohypoaldosteronism type 1. The electrochemical gradient for sodium uptake is provided by the basolateral Na-K-ATPase, which also provides a basolateral sodium exit pathway.

can reliably distinguish between the two variants (Kramer et al., 2008). Yet, based on the clinical picture, some expression of CLCNKA in TAL is likely: because CLCNKA can provide an alternative basolateral exit pathway, the phenotype is milder with later onset of symptoms. Barttin is a required subunit for both channels, so if this protein is not functional, there is no chloride exit and the phenotype is severe with antenatal onset. Evidence for this hypothesis was provided by the discovery of a patient with severe antenatal onset who had inherited recessive mutations in both *CLCNKB* and *CLCNKA*, mimicking the phenotype of Bartter type 4 (Schlingmann et al., 2004).

As logical as the molecular explanation may seem, in clinical reality, separation between the antenatal and classical forms is not as clear-cut: some patients with types 1 and 2 present in child- or even adulthood, whereas occasional patients with type 3 have a severe antenatal presentation (Konrad et al., 2000; Brochard et al., 2009). Partly, this can be explained by differences in the genotype, as some mutations in *SLC12A1* or *KCNJ1* may retain some functionality of the protein (genotype–phenotype correlation), but in others there is no clear explanation (Pressler et al., 2006; Brum et al., 2007; Brochard et al., 2009).

Hypercalciuria and nephrocalcinosis

As detailed above, the combined action of NKCC2 and ROMK contribute to the generation of a transepithelial voltage gradient driving paracellular reabsorption of cations, including calcium. Consequently, Bartter syndrome type 1 and 2 are associated with hypercalciuria and nephrocalcinosis (Peters et al., 2002; Brochard et al., 2009). Patients with type 3 and 4 have intact NKCC2 and ROMK and so are still able to generate the voltage gradient and reabsorb calcium in the TAL. Hence, these forms typically exhibit neither hypercalciuria nor nephrocalcinosis. In fact, mutations in *CLCNKB* can sometimes mimic Gitelman syndrome, which is associated with hypocalciuria (Jeck et al., 2000; Zelikovic et al., 2003).

Hypomagnesaemia

About 60% of filtered magnesium is reabsorbed paracellularly in the TAL. Consequently, one would expect that patients with Bartter type 1 and 2 have severe hypermagnesuria and hypomagnesaemia, in addition to the observed hypercalciuria, as seen in patients with a defect in this paracellular pathway (familial hypomagnesaemia with hypercalciuria and nephrocalcinosis; see Chapter 40) (Simon et al., 1999; Konrad et al., 2006). However, this is usually not the case. Most patients with Bartter syndrome type 1 and 2 have, in fact, normal plasma magnesium levels. Likely, there is enhanced magnesium reabsorption in the DCT to compensate for the loss in the TAL, but why this is possible in Bartter syndrome and not in familial hypomagnesaemia with hypercalciuria and nephrocalcinosis is unclear. However, consistent with this hypothesis of a compensatory increase of magnesium transport in the DCT is the observation that patients with Bartter syndrome type 3 and 4 often have hypomagnesaemia (Jeck et al., 2000; Konrad et al., 2000; Peters et al., 2002). Since CLCNKB, together with Barttin, is also expressed in the DCT, these patients have impaired transport in both segments, leading to uncompensated magnesium losses.

Polyuria

Polyuria is a common feature of Bartter syndromes, especially in the antenatal forms (Bartter types 1 and 2), where fetal water and salt excretion exceeds the absorptive capacity of the placenta, leading to polyhydramnios. The TAL is water-impermeable and salt reabsorption leads to urinary dilution and establishes the medullary concentration gradient that drives water extraction in the CD (Bockenhauer, 2008). Consequently, a defect in salt transport in the TAL abolishes urinary dilution, as well as concentration, and patients typically exhibit isosthenuria (similar to plasma or serum). But, again, there are exceptions to the rule: some patients with Bartter syndrome exhibit a phenotype consistent with diabetes insipidus, so that the osmolality of urine is well below that of plasma (Bockenhauer et al., 2008, 2010). Occasionally, this can actually lead to a misdiagnosis of nephrogenic diabetes insipidus (Bettinelli et al., 2000). At first sight, it appears quite remarkable that patients with a genetic 'knockout' of the TAL, the urinary diluting segment, are able to dilute their urine so well. This feat is likely achieved by a dramatic compensatory increase of salt transport in the DCT, which is also water impermeable. Indeed, KCNJ1 knockout mice demonstrate marked hypertrophy of the DCT, as a morphological correlate of the increased transport activity (Cantone et al., 2008; Wagner et al., 2008). The apparent 'aquaporin-deficient' diabetes insipidus is more difficult to explain, but is likely to be related to the electrolyte abnormalities (hypokalaemia, hypercalciuria), since it also occurs in other disorders with this biochemical picture, and urinary concentration was normalized in one case once the biochemistry was corrected completely (Bockenhauer et al., 2010).

Deafness

Sensorineural deafness affecting all frequencies, necessitating cochlear implants early in life, is a unique feature of Bartter type 4. CLCNKB and CLCNKA together with the Barttin subunit are expressed in the stria vascularis of the inner ear and contribute to the generation of the high potassium concentration of the endolymph (Kramer et al., 2008). If only CLCNKB is mutated, CLCNKA can compensate in the inner ear and so patients with Bartter type 3 have no hearing problem. However, with Barttin mutations, both channels are non-functional and these patients suffer from deafness, in addition to Bartter syndrome.

Treatment

Salt supplementation

There is no curative treatment for Bartter syndrome (kidney transplantation is not seen as a therapeutic option) and management of these patients is entirely symptomatic. In those with an antenatal presentation the problems of prematurity often dominate, at least in the beginning. Salt supplementation is used to ameliorate electrolyte abnormalities, but limited by palatability and side effects, such as vomiting and gastric irritation. Moreover, with the fractional excretion of potassium often exceeding 100% in patients with Bartter syndrome, normalization of plasma potassium levels is virtually impossible to achieve. Bolus administration, especially when given intravenously, may normalize the plasma level in the short term, but will usually result in exaggerated swings of plasma potassium, whereas repeated doses distributed over the day will result in more even plasma levels. In infants, mixing of supplements in the milk formula can increase tolerability and provide a steady administration. Sodium supplements are usually helpful. The importance of salt replacement is emphasized by the marked craving for salt, which is apparent in most patients.

Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are a mainstay of the treatment of Bartter syndrome, at least during early childhood, when problems with salt and fluid intake and failure to thrive can be severe. Their beneficial effect is due to the suppression of prostaglandin synthesis, since the elevated prostaglandin levels are a defining feature of the disease, especially in the antenatal variant, which is historically sometimes also called hyperprostaglandin E syndrome (Seyberth et al., 1985). The prostaglandins likely have systemic effects, as well, which is why commencement of a NSAID typically results in a marked improvement of the patient's general condition with subsequent catch-up growth (Dillon et al., 1979). Even antenatal treatment by administration of indomethacin to the mother has been reported, though it is unclear if the improved outcome compared with the affected sibling was a result of drug treatment or just a reflection of the variability often seen in severity of the phenotype (Konrad et al., 1999). Typically, indomethacin is used (1–4 mg/kg/day, divided in four doses), but other non-steroidals can also be used, for example, ibuprofen (20–30 mg/kg/day, divided in three doses). Treatment can be limited by potentially severe side effects, especially gastric ulcers and bleeding (Dillon et al., 1979). Selective COX-2 inhibitors constitute an attractive alternative and their successful use has been reported (Kleta et al., 2000; Reinalter et al., 2002). However, recognition that these drugs are associated with an increased cardiovascular morbidity and the withdrawal of rofecoxib have dampened the initial enthusiasm by some, and the use of these drugs should be considered in each case individually (Dogne et al., 2006). NSAIDs are well recognized to have side effects on the kidney, as well and indeed, chronic NSAID administration has been suspected as causative in cases of Bartter syndrome who developed chronic kidney disease (Kim et al., 2000). Yet, causality is difficult to prove: in patients without Bartter syndrome who take NSAIDs because of pain or rheumatologic conditions, renal prostaglandin levels before NSAID use are presumably normal, and the subsequent depression of prostaglandins can impair renal perfusion and aldosterone production (Zawada et al., 1982). In contrast, patients with Bartter syndrome have elevated prostaglandin levels and NSAIDs are used to decrease these towards the normal range. Since the persistent activation of the renin–angiotensin system in Bartter syndrome has been postulated to contribute to chronic kidney disease, and even glomerulosclerosis, suppression of the system by a NSAID could actually be beneficial for the kidney (Su et al., 2000). NSAID treatment should be instituted after volume repletion to avoid potentially fatal nephrotoxicity (historically, indomethacin has been used to perform non-surgical nephrectomies).

Distal convoluted tubule: Gitelman and EAST syndrome

Pathophysiology and aetiology

The key transport molecule in the DCT is the sodium-chloride cotransporter NCC (*SLC12A3*), which can be inhibited by thiazides. The biochemical consequences of inherited defects of salt transport in the DCT are similar to chronic thiazide administration: hypokalaemic hypochloraemic alkalosis with hypomagnesaemia and hypocalciuria (Table 31.1). Gitelman syndrome is caused by loss-of-function mutations in NCC itself, whereas EAST syndrome (also called SeSAME syndrome) is caused by loss-of-function mutations in the basolateral potassium channel KCNJ10 (Simon et al., 1996c; Bockenhauer et al., 2009; Scholl et al., 2009). Similar to the interplay between NKCC2 and ROMK (see 'Bartter syndrome'), KCNJ10 recycles potassium to provide a steady supply of potassium for the basolateral Na-K-ATPase, which establishes the electrochemical gradient for reabsorption of not only sodium-chloride, but also for magnesium in the DCT (Bleich et al., 2009). Thus, the hypokalaemic hypochloraemic alkalosis is due to the aldosterone-mediated compensatory increase in sodium reabsorption in the CD, as in Bartter syndrome, but the hypomagnesaemia is characteristic for the impaired sodium transport in the DCT. The hypocalciuria is more complex: animal data suggest, that the decreased intravascular volume induced by the impaired sodium reabsorption leads to enhanced proximal sodium transport with calcium following passively (Nijenhuis et al., 2005). While this should apply equally to Bartter syndrome, the impaired calcium reabsorption in the TAL in Bartter type 1 and 2 apparently outweighs this proximal effect (see 'Hypercalciuria'). Clinically, Gitelman syndrome can be difficult to distinguish from Bartter syndrome type 3: as detailed above (Bartter syndrome, pathophysiology), CLCNKB is expressed in both the TAL and DCT, and signs and symptoms can overlap. Indeed, some patients with *CLCNKB* gene mutations may initially present with features of Bartter syndrome and later fit better with the clinical phenotype of Gitelman syndrome (Jeck et al., 2000).

Clinical features: Gitelman syndrome

Gitelman syndrome is often seen as the mild variant of Bartter syndrome: patients typically do not present until adolescence or even adulthood (Knoers and Levtchenko, 2008). Often, the diagnosis is made incidentally, when blood tests were obtained routinely for an unrelated problem. However, more severe cases with early onset and growth failure have been described (Riveira-Munoz et al., 2007). Moreover, even in those cases with late onset, the disease can have profound effects on quality of life (Cruz et al., 2001). Fatigue, palpitations, cramps, tetany, muscle weakness, and aches are frequently reported symptoms. In one study, about 50% of patients had a prolonged QT time (Foglia et al., 2004) and sudden cardiac arrest has been reported in isolated cases (Riveira-Munoz et al., 2007; Scognamiglio et al., 2007). Chondrocalcinosis is another potential complication attributed to chronic hypocalciuria and/or hypomagnesaemia, but this usually does not appear until later in adulthood (Cobeta-Garcia et al., 1998; Monnens et al., 1998; Ea et al., 2005).

Clinical features: EAST syndrome

While the biochemical features of EAST syndrome are indistinguishable from Gitelman syndrome, this severe disorder is dominated by the neurological manifestations, which are independent of the plasma and urine biochemistries (Bockenhauer et al., 2009; Reichold et al., 2010; Bandulik et al., 2011). EAST is an acronym for epilepsy, ataxia, sensorineural deafness, and tubulopathy, and it is the infantile-onset seizures and ataxia that affect patients the most. Electroencephalograms in general are non-diagnostic. Sensorineural deafness is mild in comparison with Bartter type 4 and is present in all EAST patients. Ataxia is severe and debilitating, affecting movement and speech from childhood. Intellectual abilities may not necessarily be compromised, but can be difficult to assess, because the ataxia may affect speech and writing, and the impaired expressive abilities result in the mistaken label of 'mental retardation'.

Treatment

Treatment is entirely symptomatic and consists mainly of electrolyte (potassium, sodium-chloride, and magnesium) supplementation. Indomethacin is rarely used, although a beneficial effect has occasionally been reported, but in an era before molecular diagnosis was possible (Liaw et al., 1999). These cases may, in fact, have had Bartter type 3, rather than Gitelman syndrome. As in Bartter syndrome, normalization of plasma values is difficult to achieve and magnesium supplementation is limited especially by diarrhoea, which may worsen the hypokalaemic alkalosis. Distribution of supplements over several smaller doses during the day can limit side effects and will likely provide more steady plasma levels. Drugs associated with prolonged QT interval or known to cause hypokalaemia (beta-adrenergic mimetics) should be avoided.

Collecting duct: pseudohypoaldosteronism type 1

Pathophysiology and aetiology

Impaired sodium reabsorption in the CD results in pseudohypoaldosteronism type 1 (PHA1) (Cheek and Perry, 1958). Although < 5% of total filtered sodium is reabsorbed in the CD, loss of sodium transport in this segment is associated with the clinically most severe form of salt wasting. Presumably, this is due to the absence of a further distal segment that can compensate by taking up the unreabsorbed sodium. Sodium is reabsorbed in the CD via the epithelial sodium channel (ENaC) (Fig. 31.5) and the autosomal recessive form of PHA1 is caused by loss-of-function mutations in one of the channel subunits (Table 31.2) (Chang et al., 1996). Expression of this channel is regulated by the mineralocorticoid receptor (MLR) and the dominant form is caused by loss-of-function mutations in MLR (Geller et al., 1998). ENaC is blocked by amiloride and MLR by spironolactone: consequently, the biochemical picture of PHA1 is similar to chronic use of these diuretics and characterized by severe hyperkalaemic acidosis and moderate to borderline hyponatraemia (see Table 31.1). Secondary forms of PHA1 are recognized (apart from the use of potassium-sparing diuretics) and can occur in urinary obstruction, acute kidney injury, or with pyelonephritis in infants (Uribarri et al., 1982; Rodriguez-Soriano et al., 1983; Pumberger et al., 1998; Schoen et al., 2002; Asano et al., 2006; Kashimada et al., 2008; Rogers, 2008).

Clinical features

In the recessive form, affected infants present in the early neonatal period with severe hypovolaemia and commonly circulatory shock (Dillon et al., 1980; Savage et al., 1982). Occasionally, presentation can be antenatal with polyhydramnios (Wong and Levine, 1998). Plasma aldosterone levels are markedly elevated, consistent with tubular unresponsiveness to the hormone. Since ENaC is expressed also in the lung and skin, children with recessive PHA1 typically have impaired fluid clearance from the lungs, leading to increased respiratory infections and even cystic fibrosis-like symptoms (Kerem et al., 1999; Schaedel et al., 1999; Riepe, 2009). Moreover, as in cystic fibrosis, affected patients have an increased sodium concentration in sweat, since sodium reabsorption from sweat is also mediated by ENaC (Hummler and Horisberger, 1999). Skin rashes are often seen, probably related to the impaired sodium clearance from sweat (Martin et al., 2005). Conversely, patients with cystic fibrosis can present in early childhood with hypokalaemia mimicking Bartter syndrome, due to the cutaneous salt losses with consequent hyperaldosteronism (Kleta et al., 1999). The dominant form of PHA1 is usually milder and typically recognized in infancy and early childhood with failure to thrive. The biochemical abnormalities are usually only mild (Riepe, 2009).

Treatment

Treatment consists mainly of salt supplementation in the form of NaCl and $NaHCO_3$. In the dominant form, supplementation can usually stop once children self-regulate their diet, presumably as they crave the necessary salt and eat accordingly. In the recessive form, supplementation is life-long and NaCl doses of > 50 mmol/kg/day are often required. Crisis with circulatory collapse is often precipitated by intercurrent infections, especially diarrhoea, and in our own practice we have used a portacath in some children to ensure easy venous access during these crises. The use of sodium-potassium exchange resins can also provide a more sustained means of giving sodium and removing potassium (Rosenberg et al., 1980; Saule et al., 1984; Porter et al., 2003).

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CHAPTER 32

Approach to the patient with polyuria

Daniel G. Bichet

Hypotonic and hypertonic urine in polyuric states

In the absence of a glucose-induced osmotic diuresis in uncontrolled diabetes mellitus, a hypertonic polyuric state, there are three major causes of hypotonic polyuria, each due to a defect in water balance, leading to the excretion of large volumes of dilute urine (urine osmolality usually < 250 mOsm/kg): primary polydipsia, central diabetes insipidus, and nephrogenic diabetes insipidus (NDI) (Fig. 32.1). Simple, inexpensive blood and urine measurements, together with clinical characteristics and magnetic resonance imaging (MRI) could distinguish between these three aetiologies (Chanson and Salenave, 2011).

Polyuria and nocturia, nocturnal polyuria in enuretic children

Polyuria could be constant during the day, but also present at night: the urine is normally most concentrated in the morning due to lack of fluid ingestion overnight and increased vasopressin secretion during the late sleep period (Trudel and Bourque, 2011). Neurons in the suprachiasmatic nucleus, the brain biological clock, send axonal projections towards the supraoptic nucleus, one of the hypothalamic nuclei producing vasopressin (Burbach et al., 2001), providing a possible anatomical substrate for the circadian modulation, an osmoregulatory gain during the late sleep period (Trudel and Bourque, 2011). As a result, the first manifestation of a mild to moderate loss of concentrating ability is often nocturia. However, nocturia is not diagnostic of a defect in concentrating ability since it can also be caused by other factors such as drinking before going to bed or, in men, by prostatic hypertrophy, which is characterized by urinary frequency rather than polyuria. Psychogenic polydipsic patients tend to ingest large amounts of fluid during the day but not at night, therefore nocturia is rarely seen in primary polydipsic patients (Barlow and de Wardener, 1959). The pattern of nocturnal polyuria in enuretic children is similar to that observed in acute sleep deprivation and enuresis in children might be related to the failure of sleep to cause a reflex reduction in arterial pressure and urine production (Denton, 2012; Mahler et al., 2012).

Plasma sodium and osmolality

Plasma sodium (Na^+) and osmolality are maintained within normal limits (136–143 mEq/L for plasma Na^+; 275–290 mOsmol/kg for plasma osmolality) by a thirst–arginine vasopressin (AVP)–renal axis (Bourque, 2008; Lechner et al., 2011). Thirst and AVP release, both stimulated by increased osmolality, is a 'double-negative' feedback system (Leaf, 1979). Even when the AVP component of this 'double-negative' regulatory feedback system is lost, the thirst mechanism still preserves the plasma Na^+ and osmolality within the normal range, but at the expense of pronounced polydipsia and polyuria. Thus, the plasma Na^+ concentration or osmolality of an untreated patient with diabetes insipidus with unlimited access to water may be slightly greater than the mean normal value, and a decrease in plasma Na^+ and osmolality might be observed in primary polydipsic patients, but these small increases have no diagnostic significance (Babey et al., 2011). Polyuric patients should be asked about their thirst and their way to quench it: cold water will quench thirst more effectively in severely polyuric and dehydrated patients, irrespective of their aetiology (central versus nephrogenic). Primary polydipsic patients may tend to absorb large quantities of water ice-cold or not. Glucose-induced osmotic diuresis is more frequent than any cause of non-osmotic polyuria. High plasma glucose levels with polyuria could also be observed in brain-dead patients with diabetes insipidus receiving glucose infusions at a rate exceeding 500 mL/h, which corresponds to the maximum (25 g/h) capacity for glucose metabolism.

The polyuria observed in post-obstructive diuresis is appropriate, representing an attempt to excrete the fluid retained during the period of obstruction (Bichet, 2011).

Mammals are osmoregulators—the cellular perception of tonicity

Mammals are osmoregulators: they have evolved mechanisms that maintain extracellular fluid (ECF) osmolality near a stable value. Yet, although mammals strive to maintain a constant ECF osmolality, values measured in an individual can fluctuate around the set-point owing to intermittent changes in the rates of water intake and water loss (through evaporation or diuresis) and to variations in the rates of Na^+ intake and excretion (natriuresis). In humans, for example, 40 minutes of strenuous exercise in the heat (Saat et al., 2005; Edwards et al., 2007), or 24 hours of water deprivation (Shirreffs et al., 2004) causes plasma osmolality to rise by more than 10 mOsm/kg. In a dehydrated individual, drinking the equivalent of two large glasses of water (~850 mL) lowers osmolality by approximately 6 mOsm/kg within 30 minutes (Geelen et al., 1996). Similarly, ingestion of 13 g of salt increases plasma osmolality by

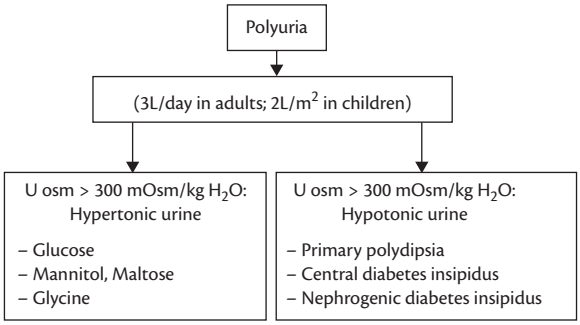

Fig. 32.1 Hypotonic and hypertonic polyuric states.

approximately 5 mOsm/kg within 30 minutes (Andersen et al., 2000). Although osmotic perturbations larger than these can be deleterious to health, changes in the 1–3% range play an integral part in the control of body-fluid homeostasis. Differences between the ECF osmolality and the desired set-point induce proportional homeostatic responses according to the principle of negative feedback (Bourque, 2008). ECF hyperosmolality stimulates the sensation of thirst, to promote water intake, and the release of vasopressin to enhance water reabsorption in the kidney. By contrast, ECF hypo-osmolality suppresses basal vasopressin secretion in rats and humans (Claybaugh et al., 2000).

As summarized elegantly by Bourque (2008) early studies provided clear evidence that 'cellular dehydration' (i.e. cell shrinking) was required for thirst and vasopressin release to be stimulated during ECF hyperosmolality: these responses could be induced by infusions of concentrated solutions containing membrane-impermeable solutes, which extract water from cells, but not by infusions of solutes that readily equilibrate across the cell membrane (such as urea). Verney coined the term osmoreceptor to designate the specialized sensory elements. He further showed that these were present in the brain and postulated that they might comprise 'tiny osmometers' and 'stretch receptors' that would allow osmotic stimuli to be 'transmuted into electrical' signals (Verney, 1947). Osmoreceptors are, therefore, defined functionally as neurons that are endowed with an intrinsic ability to detect changes in ECF osmolality and it is now known that both cerebral and peripheral osmoreceptors contribute to the body fluid balance (Fig. 32.2).

Although magnocellular neurons are themselves osmosensitive, they require input, by glutamatergic afferents, from the lamina terminalis to respond fully to osmotic challenges (Fig. 32.3). Neurons in the lamina terminalis are also osmosensitive and because the subfornical organ (SFO) and the organum vasculosum of the

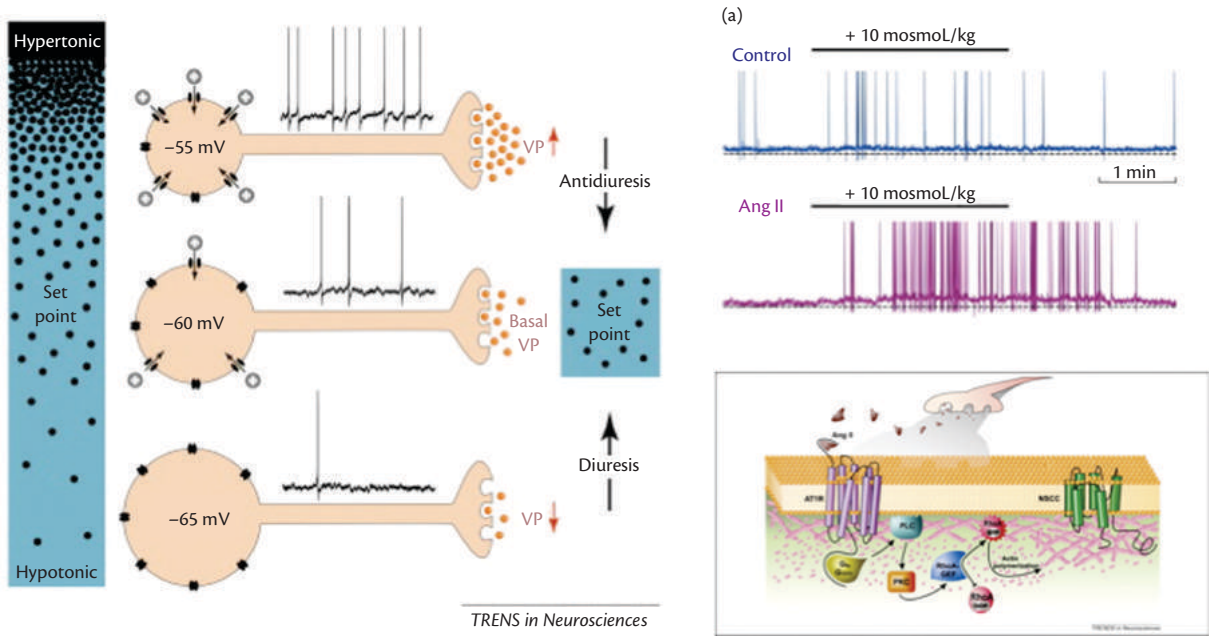

Fig. 32.2 (Left) Cell autonomous osmoreception in vasopressin neurons. Changes in osmolality cause inversely proportional changes in soma volume. Shrinkage activates non-selective cation channels (NSCCs) and the ensuing depolarization increases action potential firing rate and vasopressin (VP) release from axon terminals in the neurohypophysis. Increased VP levels in blood enhance water reabsorption by the kidney (antidiuresis) to restore extracellular fluid osmolality towards the set point. Hypotonic stimuli inhibit NSCCs. The resulting hyperpolarization and inhibition of firing reduces VP release and promotes diuresis. (Upper right) Whole-cell current clamp recordings from isolated MNCs and averaged data from multiple cells show that the depolarizing and action potential firing responses induced by a hypertonic stimulus are significantly enhanced in the presence of 100 nM Ang II. (Lower right) Hypothetical events mediating central Ang II enhancement of osmosensory gain. Ang II released by afferent nerve terminals (e.g. during hypovolaemia) binds to AT1 receptor (AT1R) coupled to G proteins such as Gq or/and G12/13. Activated G proteins signal through phospholipase C (PLC) and protein kinase C (PKC) to activate a RhoA-specific guanine nucleotide exchange factor (RhoA–GEF), such as p115RhoGEF or LARG (leukaemia-associated Rho guanine–nucleotide exchange factor) Activation of RhoA–GEF converts inactive cytosolic RhoA (RhoA–GDP) into active, membrane-associated RhoA–GTP by promoting the exchange of GDP to GTP. Activated RhoA induces actin polymerization and increases submembrane F-actin density to enhance the mechanical gating of non-specific cation channels.

With permission from Masha Prager-Khoutorsky and Charles W. Bourque: Osmosensation in vasopressin neurons: changing actin density to optimize function; *Trends Neurosci.* 2010 Feb; 33(2):76–83

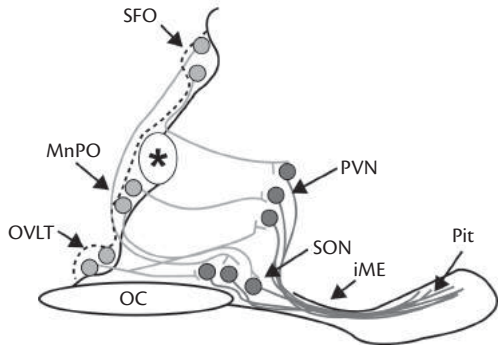

Fig. 32.3 Schematic representation of the osmoregulatory pathway of the hypothalamus (sagittal section of midline of ventral brain around the third ventricle in mice). Neurons (lightly filled circles) in the lamina terminalis (OVLT), median preoptic nucleus (MnPO), and subfornical organ (SFO)—that are responsive to plasma hyptertonicity send efferent axonal projections (grey lines) to magnocellular neurons of the paraventricular (PVN) and supraoptic nuclei (SON). The OVLT is one of the brain circumventricular organs and is a key osmosensing site in the mammalian brain (vide infra). The processes (dark lines) of these magnocellular neurons form the hypothalamo-neurohypophysial pathway that courses in the median eminence to reach the posterior pituitary, where neurosecretion of vasopressin and oxytocin occurs.
Modified from Wilson et al., Visualization of functionally activated circuitry in the brain. *PNAS*, 99: 3252–7, 2002.

lamina terminalis (OVLT) lie outside the blood–brain barrier, they can integrate this information with endocrine signals borne by circulating hormones, such as angiotensin II (Ang-II), relaxin, and atrial natriuretic peptide (ANP). While circulating Ang-II and relaxin excite both oxytocin and vasopressin magnocellular neurons, ANP inhibits vasopressin neurons. The non-osmotic pathways are more physiologically described now as 'osmoregulatory gain,' since Ang II amplifies osmosensory transduction by enhancing the proportional relationship between osmolality, receptor potential, and action potential firing in rat supraoptic nucleus neurons (Zhang and Bourque, 2008). Modifications in osmoregulatory gain induced by angiotensin explain why the changes in the slope and threshold of the relationship between plasma osmolality and vasopressin secretion are potentiated by hypovolaemia or hypotension and are attenuated by hypervolaemia or hypertension (Robertson and Athar, 1976).

Tonicity information is relayed by central osmoreceptor neurons expressing TRPV1 and peripheral osmoreceptor neurons expressing TRPV4

The osmotic regulation of the release of AVP from the posterior pituitary is primarily dependent, under normal circumstances, on tonicity information relayed by central osmoreceptor neurons expressing TRPV1 (Bourque, 2008) and peripheral osmoreceptor neurons expressing TRPV4 (Lechner et al., 2011).

The cellular basis for osmoreceptor potentials has been characterized using patch-clamp recordings and morphometric analysis in magnocellular cells isolated from the supraoptic nucleus of the adult rat. In these cells, stretch-inactivating cationic channels transduce osmotically evoked changes in cell volume into functionally relevant changes in membrane potential. In addition, magnocellular neurons also operate as intrinsic Na^+ detectors. The N-terminal variant of the transient receptor potential cation channel subfamily V member 1 (TRPV1) is an osmotically activated channel expressed in the magnocellular cells producing vasopressin (Sharif Naeini et al., 2006) and in the circumventricular organs, the OVLT, and the SFO (Ciura and Bourque, 2006). Since osmoregulation still operates in *Trpv*$1^{-/-}$ mice, other osmosensitive neurons or pathways must be able to compensate for loss of central osmoreceptor function (Ciura and Bourque, 2006; Sharif Naeini et al., 2006; Taylor et al., 2008). Afferent neurons expressing the osmotically activated ion channel, TRPV4 in the thoracic dorsal root ganglia that innervate hepatic blood vessels and detect physiological hypo-osmotic shifts in blood osmolality have recently been identified (Lechner et al., 2011). In mice lacking the osmotically activated ion channel, TRPV4, hepatic sensory neurons no longer exhibit osmosensitive inward currents and activation of peripheral osmoreceptors *in vivo* is abolished. In a large cohort of human liver transplantees, who presumably have denervated livers, plasma osmolality is significantly elevated compared with healthy controls, suggesting the presence of an inhibitory vasopressin effect of hyponatraemia, perceived in the portal vein from hepatic afferents (Lechner et al., 2011). TRPV1 (expressed in central neurons) and TRPV4 (expressed in peripheral neurons) thus appear to play entirely complementary roles in osmoreception. Lechner et al. (2011) have thus identified the primary afferent neurons that constitute the afferent arc of a well-characterized reflex in man and more recently also in rodents (McHugh et al., 2010). This reflex engages the sympathetic nervous system to raise blood pressure and stimulate metabolism (Tank et al., 2003; Boschmann et al., 2007). Of clinical interest, it has already been demonstrated that orthostatic hypotension and postprandial hypotension respond to water drinking (Jordan et al., 2000; Schroeder et al., 2002; Shannon et al., 2002). Moreover, water drinking in man can prevent neutrally mediated syncope during blood donation or after prolonged standing (Claydon et al., 2006). Finally, water drinking is also associated with weight loss in overweight individuals (Stookey et al., 2008). Other peripheral sensory neurons expressing other mechanosensitive proteins may also be involved in osmosensitivity (Coste et al., 2010).

Quantification of polyuria—volume, osmolality, Na^+, K^+, and Ca^{2+}

Polyuria is arbitrarily defined as a urine output exceeding 3 L/day in adults and 2 L/m^2 in children (Bichet, 2011). It is important to obtain a 24-hour urine collection with measurements of volume, osmolality, Na^+, potassium (K^+), and calcium (Ca^{2+}) to quantify precisely polyuric symptoms, since polyuria is difficult to measure in young infants and may even be confused with congenital chloride diarrhoea in patients referred with a suspected diagnosis of Bartter syndrome (Choi et al., 2009). Volume loss from the urinary tract versus the gastrointestinal tract may not be immediately discriminated in infants! Conversely, increased urinary frequency in men with prostatic hypertrophy might be confused with polyuria. The maximal attainable urine volume in normal individuals on a regular diet is > 10 L/day (e.g. 10 L of urine at a urine osmolality of 60 mOsm/kg of H_2O to excrete a 600 mOsm solute load). If the solute load to be excreted is increased due to increased protein intake (generating urea which accounts for two-thirds of urine solutes) or increased Na^+/K^+ intake, 1200 mOsm will need to be excreted, representing 20 L of urine with a urine osmolality of 60 mOsm/kg.

Once plasma Na^+, osmolality, and glucose measurements are obtained, the following steps should guide the investigation and treatment of polyuric states: measure 24-hour urine volume and record urine osmolality, Na^+, K^+, Ca^{2+}, and glucose. A low-salt, low-protein diet will *diminish* (see earlier) urine output in both central and NDI, but will not change maximal urine osmolality (Earley and Orloff, 1962). In central diabetes insipidus, the effectiveness of vasopressin replacement by desmopressin makes a low salt, low protein diet irrelevant for the treatment of this condition.

The approach to a polyuric patient will vary according to the age at presentation. Polyuric conditions in infants < 1 year of age are true emergency conditions, since young infants are unable to express their thirst and may suffer from severe dehydration and volume contraction. Repeated measures of plasma electrolyte, creatinine and urine volume and content may also be challenging in young infants.

Congenital (i.e. present at birth) and early polyuric states

Polyuria in an infant with polyhydramnios during the pregnancy leading to her/his birth and prematurity

The triad: polyuria/polyhydramnios/prematurity is a tell-tale sign of Bartter syndrome with abnormal conservation of water but also of Na^+, K^+, chloride (Cl^-), and Ca^{2+}. Bartter syndrome (OMIM 601678, 241200, 607364, and 62522) refers to a group of autosomal recessive disorders caused by inactivating mutations in one of four genes (*SLC12A1, KCNJ1, CLCNKB, CLCNKA* and *CLCNKB* in combination, or *BSND*) that encode the membrane proteins of the thick ascending limb of the loop of Henle (Puricelli et al., 2010; Bonnardeaux and Bichet, 2012). Since 30% of the filtered sodium chloride is reabsorbed in the thick ascending loop of Henle it is evident that the loss of function of these membrane transporters will induce alterations in the counter-current system (Fig. 32.5). In the

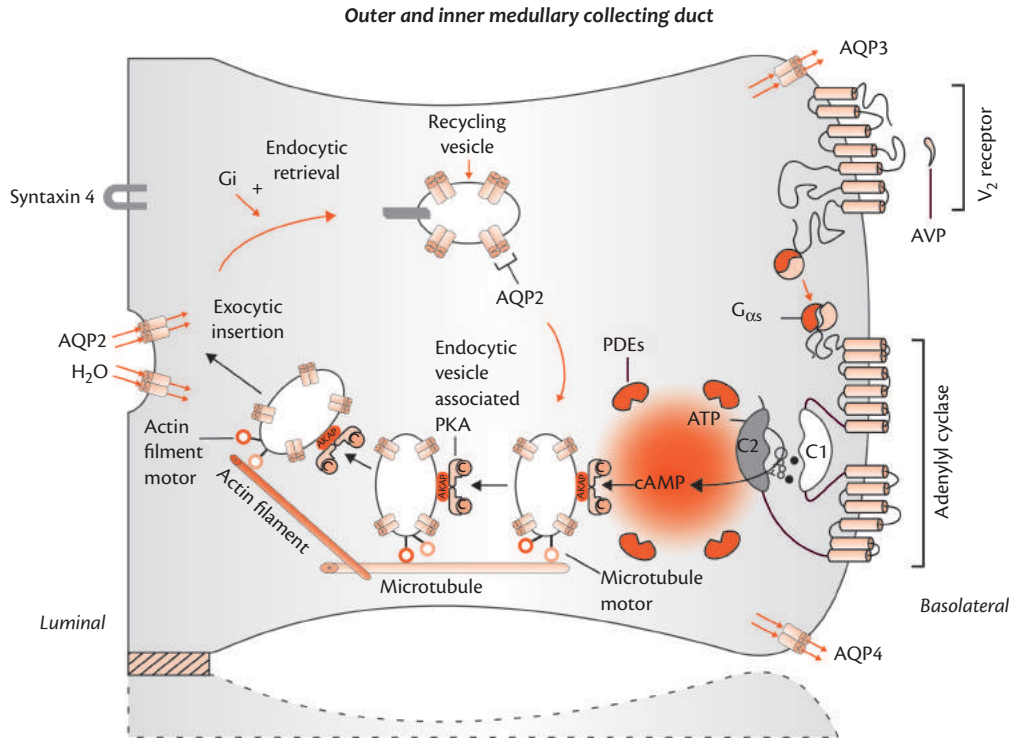

Fig. 32.4 Schematic representation of the effect of vasopressin (AVP) to increase water permeability in the principal cells of the collecting duct and representation of two types of 'pure' nephrogenic diabetes insipidus: loss of function of the AVPR2 protein or loss of function of the AQP2 protein. AVP is bound to the V_2 receptor (a G-protein-linked receptor) on the basolateral membrane. The basic process of G-protein-coupled receptor signalling consists of three steps: a hepta-helical receptor that detects a ligand (in this case, AVP) in the extracellular milieu, a G-protein ($G_{\alpha s}$) that dissociates into α subunits bound to GTP and $\beta\gamma$ subunits after interaction with the ligand-bound receptor, and an effector (in this case, adenylyl cyclase) that interacts with dissociated G-protein subunits to generate small-molecule second messengers. AVP activates adenylyl cyclase, increasing the intracellular concentration of cAMP. The topology of adenylyl cyclase is characterized by two tandem repeats of six hydrophobic transmembrane domains separated by a large cytoplasmic loop and terminates in a large intracellular tail. The dimeric structure (C_1 and C_2) of the catalytic domains is represented. Conversion of ATP to cAMP takes place at the dimer interface. Two aspartate residues (in C_1) coordinate two metal co-factors (Mg^{2+} or Mn^{2+} represented here as two small black circles), which enable the catalytic function of the enzyme. Adenosine is shown as an open circle and the three phosphate groups (ATP) are shown as smaller open circles. Protein kinase A (PKA) is the target of the generated cAMP. The binding of cAMP to the regulatory subunits of PKA induces a conformational change, causing these subunits to dissociate from the catalytic subunits. These activated subunits (C) as shown here are anchored to an aquaporin-2 (AQP2)-containing endocytic vesicle via an A-kinase anchoring protein. The local concentration and distribution of the cAMP gradient is limited by phosphodiesterases (PDEs). Cytoplasmic vesicles carrying the water channels (represented as homotetrameric complexes) are fused to the luminal membrane in response to AVP, thereby increasing the water permeability of this membrane. The dissociation of the A-kinase anchoring protein from the endocytic vesicle is not represented. Microtubules and actin filaments are necessary for vesicle movement towards the membrane. When AVP is not available, AQP2 water channels are retrieved by an endocytic process, and water permeability returns to its original low rate. Aquaporin-3 (AQP3) and aquaporin-4 (AQP4) water channels are expressed constitutively at the basolateral membrane.

large experience of Hans Seyberth and colleagues (Peters et al., 2002), polyuria was the leading symptom postnatally in 19 of 32 patients.

'Pure' polyuria, that is, loss of water only but normal conservation of Na^+, K^+, Cl^-, and Ca^{2+} in the first week of life

Most of the cases are 'pure' NDI secondary to mutations in the vasopressin V2 receptor gene (*AVPR2*, X-linked, OMIM 304800) or in the aquaporin-2 water channel gene (*AQP2*, autosomal recessive, OMIM 222000) and dominant (OMIM 125800) (Fig. 32.4).

The intensity of the polyuric manifestations will depend on the severity of the mutation identified: in both *AVPR2* and *AQP2* mutations, severe phenotypes (U Osm < 200 mOsm/kg) are observed with specific mutations and less severe phenotypes (U Osm~300 mOsm/kg) are observed with mild mutations (Bockenhauer et al., 2009; Guyon et al., 2009).

Very early (first week of life) polyuric states are usually nephrogenic but we and others have observed autosomal recessive central diabetes insipidus patients with early polyuria, dehydration episodes responding to DDAVP with specific mutations of the AVP gene (Bichet et al., 1998; Willcutts et al., 1999; Abu Libdeh et al., 2009; Christensen et al., 2013).

For complex (water + sodium + calcium) and pure (water only) early polyuric states a rapid molecular diagnosis cuts the 'diagnostic odyssey' that often involves false diagnostic leads and ineffective treatment (Fig. 32.6).

We are recommending the sequencing of the NDI and Bartter genes in all the affected patients. The genes involved are, with a few exceptions, relatively small and easy to sequence. This genomic information is key to the routine care of patients with congenital polyuria and, as in other genetic diseases, reduces health costs and provides psychological benefits to patients and families (Green and Guyer, 2011) (Fig. 32.6).

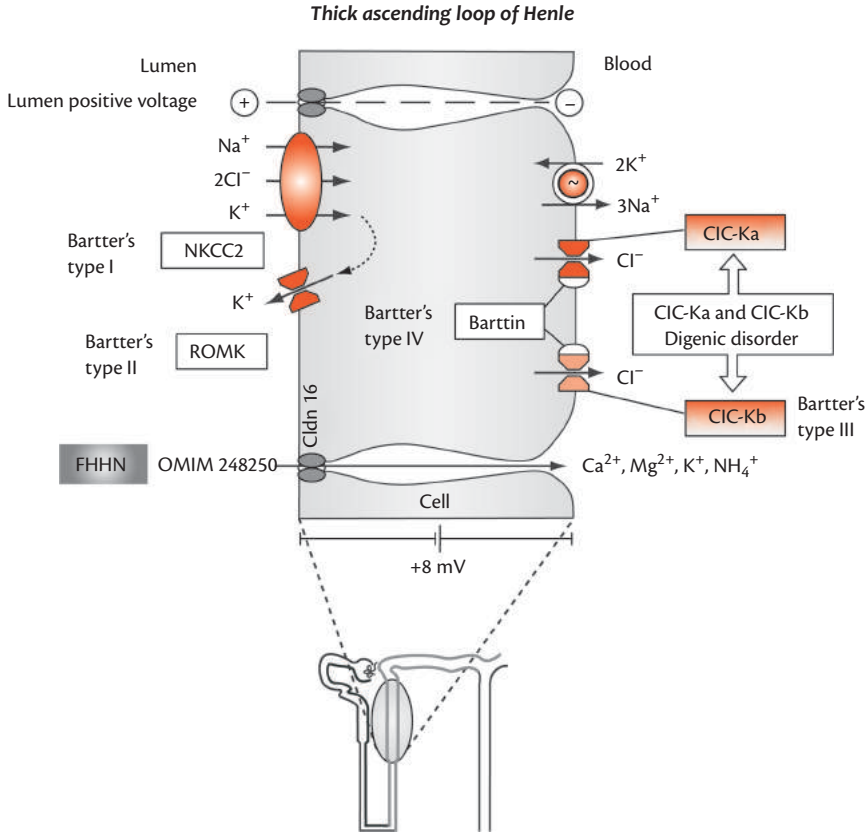

Fig. 32.5 Schematic representation of transepithelial salt resorption in a cell of the thick ascending limb (TAL) of the loop of Henle. Thirty per cent of the filtered sodium chloride (NaCl) is reabsorbed in the TAL and most of the energy for concentration and dilution of the urine derives from active NaCl transport in the TAL. Filtered NaCl is reabsorbed through NKCC2, which uses the sodium gradient across the membrane to transport chloride and potassium into cell. The potassium ions are recycled (100%) through the apical membrane by the potassium channel ROMK. Sodium leaves the cell actively through the basolateral Na-K-ATPase. Chloride diffuses passively through two basolateral channels, ClC-Ka and ClC-Kb. Both of these chloride channels must bind to the β subunit of barttin to be transported to the cell surface. Four types of Bartter syndrome (types I, II, III, and IV) are attributable to recessive mutations in the genes that encode the NKCC2 cotransporter, the potassium channel (ROMK), one of the chloride channels (CIC-Kb), and barttin, respectively. A fifth type of Bartter syndrome has also been shown to be a digenic disorder that is attributable to loss-of-function mutations in the genes that encode the chloride channels CIC-Ka and CIC-Kb. As a result of these different molecular alterations, NaCl is lost into the urine, positive lumen voltage is abolished, and calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and ammonium (NH_4^+) cannot be reabsorbed in the paracellular space. In the absence of mutations, the recycling of potassium maintains a lumen-positive gradient (+8 mV). Claudin 16 (CLDN16) is necessary for the paracellular transport of calcium and magnesium.

Modified from Bichet DG, Fujiwara TM: Reabsorption of sodium chloride—lessons from the chloride channels. *N Engl J Med*, 350:1281–1283, 2004.

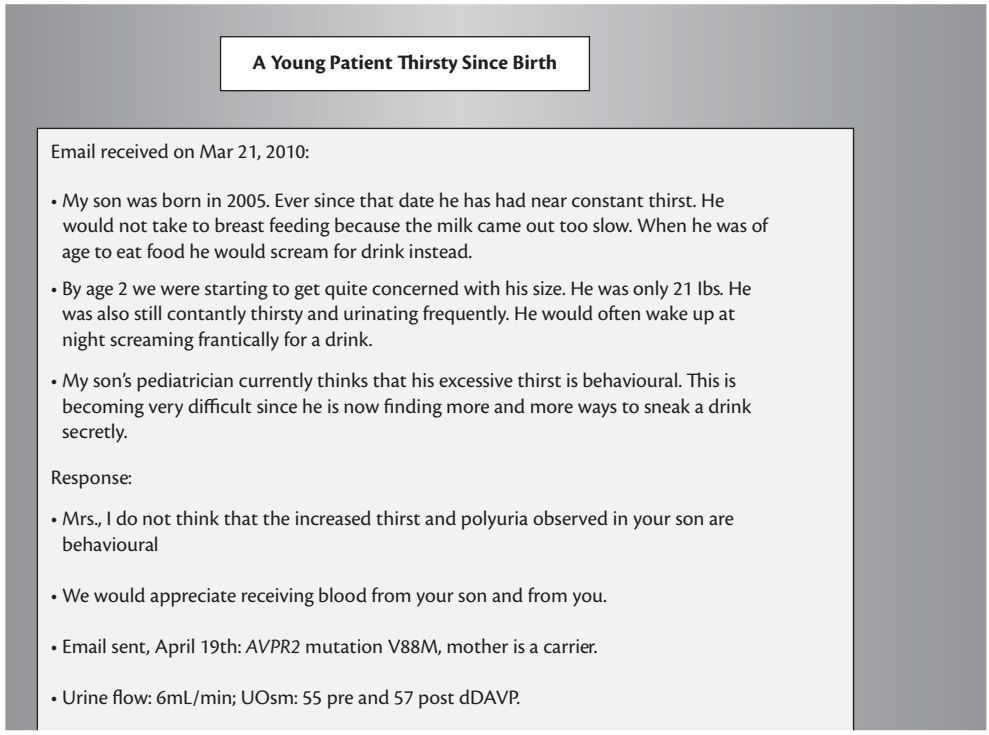

Fig. 32.6 A young patient thirsty since birth.

Other nephrogenic disorders with polyuric manifestations

Here, polyuria is appearing later in life, usually after the first year. Polyuria will be observed with variable severity in Bardet–Biedl syndrome (Marion et al., 2011), nephronophthisis (Hildebrandt et al., 2009), cystinosis, familial hypernatremia with hypervolemia and nephrocalcinosis, and the syndrome of apparent mineralocorticoid excess (Bockenhauer et al., 2010).

Acquired nephrogenic polyuric disorders

Acquired NDI is much more common than congenital NDI, but it is rarely as severe. The ability to produce hypertonic urine is usually preserved even though there is inadequate concentrating ability of the nephron. Polyuria and polydipsia are therefore moderate (3–4 L/day).

Among the more common causes of acquired NDI, lithium administration has become the most frequent cause; 54% of 1105 unselected patients on chronic lithium therapy developed NDI (Boton et al., 1987). Nineteen per cent of these patients had polyuria, as defined by a 24-hour urine output exceeding 3 L. The dysregulation of aquaporin-2 expression is the result of cytotoxic accumulation of lithium which enters via the epithelial sodium channel (ENaC) on the apical membrane and leads to the inhibition of signalling pathways that involve glycogen synthase kinase type 3 beta (Grunfeld and Rossier, 2009). The concentration of lithium in urine of patients on well-controlled lithium therapy (i.e. 10–40 mmol/L) is sufficient to exert this effect. For patients on long-term lithium therapy, amiloride has been proposed to prevent the uptake of lithium in the collecting ducts, thus preventing the inhibitory effect of intracellular lithium on water transport (Batlle et al., 1985).

Diabetes insipidus and pregnancy

Pregnancy in a patient known to have diabetes insipidus

An isolated deficiency of vasopressin without a concomitant loss of hormones in the anterior pituitary does not result in altered fertility, and with the exception of polyuria and polydipsia, gestation, delivery, and lactation are uncomplicated (Amico, 1985). Patients may require increasing dosages of DDAVP. The increased thirst may be due to a resetting of the thirst osmostat (Davison et al., 1988).

Increased polyuria also occurs during pregnancy in patients with partial NDI (Iwasaki et al., 1991). These patients may be obligatory carriers of the NDI gene (Forssman, 1945) or may be homozygotes, compound heterozygotes, or may have dominant *AQP2* mutations.

Syndromes of diabetes insipidus that begin during gestation and remit after delivery

Pregnancy may be associated with several different forms of diabetes insipidus, including central, nephrogenic, and vasopressinase-mediated forms (Hiett and Barton, 1990; Iwasaki et al., 1991; Brewster and Hayslett, 2005; Lindheimer, 2005).

Diagnostic work-up of polyuric states

Excepting the context of brain trauma, brain surgery, or long-term lithium administration where the diagnosis of polyuria is obvious, a logical approach to the patient with polyuria is to search for

arguments supporting known causes of polyuric states. Such arguments may be: (a) morphological (brain MRI), including the presence of a hypothalamic tumour or mass related to a granulomatous or inflammatory process; (B) hormonal, suggesting that the posterior pituitary involvement is not isolated but rather associated with other signs of anterior pituitary deficits; (C) systemic with the presence of a generalized inflammatory process or pituitary metastasis; or (D) hereditary with other members of the family affected with central or NDI.

An abrupt onset of polyuria in an adult would suggest acquired central diabetes insipidus. MRI of the hypothalamic structures and of the posterior pituitary should be obtained to assess the posterior pituitary normal 'bright spot,' a possible surrogate of the posterior pituitary vasopressin content, and any accompanying lesions. Clinical and biochemical indices of associated anterior pituitary/hormone deficiency should also be obtained (Maghnie et al., 2000) since additional deficits in anterior pituitary hormones were documented in 61% of patients, a median of 0.6 years after the onset of diabetes insipidus. The most frequent abnormality was growth hormone deficiency (59%) followed by hypothyroidism (28%), hypogonadism (24%), and adrenal insufficiency (22%). Seventy-five per cent of the patients with Langerhans cell histiocytosis had an anterior pituitary hormone deficiency that was first detected a median of 3.5 years after the onset of diabetes insipidus.

In this context, the dehydration test is rarely necessary and only recommended for patients with isolated polyuria, a normal pituitary stalk, and hypothalamic region on MRI and with no familial history of polyuria. If plasma osmolality and/or Na^+ concentration under conditions of *ad libitum* fluid intake are > 295 mOsm/kg and 143 mmol/L, respectively, the diagnosis of primary polydipsia is excluded (Robertson, 1981).

Water restriction tests are described in Bichet (2015). If severe polyuric symptoms and signs are documented, water should be restricted only to 2–4 hours during daytime in infants, plasma Na^+ should be available every 2 hours during testing and should not exceed 145–148 mmol/L in children and adults, since a maximal endogenous vasopressin stimulation (> 3.5 pg/mL) should occur at this level with a maximal urine osmolality response (> 800 mOsm/kg). If delays of > 60 minutes are encountered to obtain plasma Na^+ or urine osmolalities during dehydration tests, these tests should be done in other institutions where almost immediate laboratory reports are obtained after blood samplings.

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CHAPTER 33

Clinical use of diuretics

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Introduction

Oedema is usually a manifestation of expanded extracellular fluid (ECF) volume most typically caused by heart failure (HF), hepatic cirrhosis, nephrotic syndrome, or kidney dysfunction; it can also result from local factors or lymphatic obstruction. Surprisingly, primary renal NaCl retention does not lead to oedema, but instead to hypertension, because 'pressure natriuresis' occurs, preventing substantial ECF volume expansion. In contrast, when NaCl is retained because the *effective* arterial blood volume is reduced, oedema results. Regardless of its cause, symptomatic oedema often requires treatment with diuretics. Diuretics now comprise two classes, the natriuretics and the aquaretics, although diuretic treatment of oedema typically relies primarily on natriuretic diuretics.

In addition to their use for oedema, diuretic drugs are indicated for a wide variety of non-oedematous disorders. Treatment of hypertension, nephrolithiasis (see Chapter 30), and hyponatraemia (see Chapter 28) are discussed elsewhere. This chapter will focus on renal mechanisms of diuretic action and diuretic therapy of oedema.

The molecular targets of diuretic drugs are predominantly Na^+ transport pathways at the apical (luminal) surface of kidney tubule cells. When coupled with the basolateral Na/K-ATPase, these pathways permit the vectorial transport of sodium. A rational classification of diuretic drugs (see Table 33.1) is based on the primary nephron site of action.

Osmotic diuretics

Osmotic diuretics are substances that are freely filtered at the glomerulus, but are poorly reabsorbed. Inhibition of NaCl reabsorption by these drugs depends on the osmotic pressure exerted by the drug molecules in solution, not on interaction with specific transport proteins. Mannitol is the prototypical osmotic diuretic (Better et al., 1997). Because the relationship between the magnitude of diuretic effect and concentration of osmotic diuretic in solution is linear, all osmotic diuretics are small molecules. Other agents considered in this class include urea, sorbitol, and glycerol.

Although osmotic agents do not act directly on transport pathways, ion transport is affected. Following mannitol infusion, sodium, potassium, calcium, magnesium, bicarbonate and chloride excretion rates increase (Table 33.1). Sodium and water reabsorption rates are reduced by 27% and 12%, respectively (Seely and Dirks, 1969). Magnesium and calcium reabsorption is also reduced along the proximal tubule and loop of Henle.

The mechanisms by which mannitol produces a diuresis include (a) increasing the luminal osmotic pressure along the proximal tubule and loop of Henle, thereby retarding the passive reabsorption of water; and (b) increasing the renal plasma flow (RPF), thereby washing out medullary tonicity. Mannitol is freely filtered at the glomerulus and its presence in tubule fluid minimizes passive water reabsorption. When an osmotic diuretic is administered, the osmotic force of the non-reabsorbable solute in the lumen opposes the osmotic force produced by sodium reabsorption, and sodium reabsorption eventually stops. Perhaps surprisingly, mannitol has a greater effect on inhibiting Na and water reabsorption in the loop of Henle than in the proximal tubule. Further downstream, in the collecting duct, mannitol also can reduce sodium and water reabsorption (Buerkert et al., 1981).

During the administration of mannitol, its molecules diffuse from the blood stream into the interstitial space. In the interstitial space, the increased osmotic pressure draws water from the cells to increase extracellular fluid (ECF) volume, increasing renal plasma flow (Buerkert et al., 1981). Renal cortical and medullary blood flow rates increase following mannitol infusion (Buerkert et al., 1981). Single nephron glomerular filtration rate (GFR) increases in cortex, but decreases in medulla (Gennari and Kassirer, 1974) via unknown mechanisms. The net effect of mannitol on total kidney GFR has been variable, but most studies indicate that the overall effect is to increase GFR (Blantz, 1974).

The combination of enhanced renal plasma flow and reduced medullary GFR washes out the medullary osmotic gradient by reducing papillary sodium and urea content. Experimental studies indicate that the osmotic effect of mannitol to increase water movement from intracellular to extracellular space leads to a decrease in haematocrit and in blood viscosity. This fact contributes to a decrease in renal vascular resistance and increase in renal plasma flow (RPF). In addition, both prostacyclin (PGI_2) (Johnston et al., 1981) and atrial natriuretic peptide (Yamasaki et al., 1988) may participate in the effect of mannitol on RPF

Following infusion, mannitol distributes in ECF with a volume of distribution of approximately 16 L (Anderson et al., 1988); its excretion is almost entirely by glomerular filtration (Weiner, 1990). Of the filtered load, < 10% is reabsorbed by the renal tubule, and a similar quantity is metabolized, probably in the liver. With normal GFR, plasma half-life is approximately 2.2 hours. Marked accumulation of mannitol in patients can lead to reversible acute kidney injury (AKI) with vasoconstriction and tubular vacuolization (Dorman and Sondheimer, 1990; Visweswaran et al., 1997).

Proximal tubule diuretics (carbonic anhydrase inhibitors)

Carbonic anhydrase inhibitors increase urinary sodium excretion somewhat, but have a limited therapeutic role as diuretics, because

Table 33.1 Physiological classification of diuretic drugs

Natriuretics				Aquaretics
Proximal diuretics	Loop diuretics	DCT diuretics	Cortical collecting diuretics	Vaptans
Carbonic anhydrase inhibitors	*Na-K-2Cl transporter (NKCC2) inhibitors*	*Na-Cl cotransporter (NCC) inhibitors*	*Na channel blockers (ENaC inhibitors)*	*Vasopressin receptor blockers*
Acetazolamide	Furosemide	Hydrochlorothiazide	Amiloride	Conivaptan
	Bumetanide	Metolazone	Triamterene	Tolvaptan
	Torsemide	Chlorthalidone	*Aldosterone antagonists*	
	Ethacrynic acid[a]	Indapamide[b]	Spironolactone	
		others	Eplerenone	

Classification based on site and mechanism of action.

[a] Mechanism of ethacrynic acid differs from that of other loop diuretics. [b] Indapamide and metolazone may have other actions, as well.

DCT = distal convoluted tubule.

they are only weakly natriuretic during chronic use. Bicarbonate excretion rises by 25–30%, producing an alkaline diuresis (Table 33.1). Carbonic anhydrase inhibitors also increase potassium excretion, likely indirectly. The effect of carbonic anhydrase inhibitors on the proximal tubule ion transport facilitates an increase in tubular fluid flow rate and sodium and bicarbonate delivery to the distal nephron, where the lumen negative voltage (Malnic et al., 1966) and urine flow rate increase (Good and Wright, 1979).

The biochemical, morphological, and functional properties of carbonic anhydrase have been reviewed (Pastorekova et al., 2004). Normally the proximal tubule reabsorbs 80% of the filtered load of bicarbonate and 60% of the filtered load of sodium chloride via cellular mechanisms depicted in Fig. 33.1. Carbonic anhydrase inhibitors act primarily in this segment, yet their natriuretic potency is relatively weak. Several factors explain this observation. First, proximal sodium reabsorption is mediated by carbonic anhydrase-independent as well as carbonic anhydrase-dependent pathways. Second, the increased sodium delivered to distal nephron segments is largely reabsorbed. Third, carbonic anhydrase inhibitors generate a hyperchloraemic metabolic acidosis, further reducing the effects of subsequent doses of carbonic anhydrase inhibitor. Finally, metabolic acidosis increases the K_i for bicarbonate absorption by membrane impermeant carbonic anhydrase inhibitors by a factor of 100 to 500, suggesting that metabolic acidosis is associated with changes in the physical properties of the carbonic anhydrase protein (Shuichi and Schwartz. 1998). For these reasons, carbonic anhydrase inhibitors alone are rarely used as diuretic agents chronically; they do, however, play an important role in short-term treatment of diuretic resistance.

Systemic administration of carbonic anhydrase inhibitors reduces GFR by as much as 30%. Single nephron glomerular filtration rate (SNGFR) was 23% lower during acetazolamide infusion, partly because increased solute delivery to the macula densa activates the tubuloglomerular feedback (TGF) mechanism (Skott et al., 1989), although other factors are likely to contribute (Hashimoto et al., 2004).

Acetazolamide is well absorbed from the gastrointestinal (GI) tract. More than 90% of the drug is plasma protein bound. The highest concentrations are found in tissues that contain large amounts of carbonic anhydrase (e.g. renal cortex, red blood cells). Renal effects are noticeable within 30 minutes and are usually maximal at 2 hours. Acetazolamide is not metabolized, but is excreted rapidly by glomerular filtration and proximal tubular secretion. The half-life is approximately 5 hours and renal excretion is essentially complete in 24 hours (Weiner, 1990). In comparison, methazolamide is absorbed more slowly from the GI tract, and its duration of action is long, with a half-life of approximately 14 hours.

Generally, carbonic anhydrase inhibitors are well tolerated with infrequent serious adverse effects. Side effects of carbonic anhydrase inhibitors may arise from the continued excretion of electrolytes. Significant hypokalaemia and metabolic acidosis may develop. In elderly patients with glaucoma treated with acetazolamide (250–1000 mg/day), metabolic acidosis was a frequent finding (Heller et al., 1985). Even though carbonic anhydrase inhibitors do not increase urinary calcium excretion, they do increase the risk for nephrocalcinosis and nephrolithiasis, owing to their effects on urine pH and citrate excretion. Premature infants treated with furosemide and acetazolamide are particularly susceptible to nephrocalcinosis, presumably due to the combined effect of an alkaline urine and hypercalciuria (Stafstrom et al., 1992).

Loop diuretics

The loop diuretics inhibit sodium and chloride transport along the loop of Henle and macula densa (Fig. 33.1). Although these drugs also impair ion transport by proximal and distal tubules under some conditions, these effects probably contribute little to their action clinically. The loop diuretics available include furosemide, bumetanide, torsemide, and ethacrynic acid.

Loop diuretics increase water, Na^+, K^+, Cl^-, phosphate, magnesium, and calcium excretion rates (Table 33.1). The loop diuretic dose–response relationship is sigmoidal (Fig. 33.2), which has labelled them as 'threshold' drugs (Brater, 1997). Loop diuretics have the highest natriuretic and chloriuretic efficacy of any class of diuretics; they are sometimes called 'high ceiling' diuretics, for this reason. Loop diuretics can increase Na^+ and Cl^- excretion up to 25% of the filtered load. If administered during water loading, solute-free water clearance (C_{H_2O}) decreases and osmolar clearance increases, although the urine always remains dilute. During water restriction, loop diuretics impair the reabsorption of solute-free water $(T^C_{H_2O})$. During maximal loop diuretic action, the urinary Na^+ concentration is usually between 75 and 100 mmol/L (Puschett and Goldberg, 1968). Because urinary K^+ concentrations during

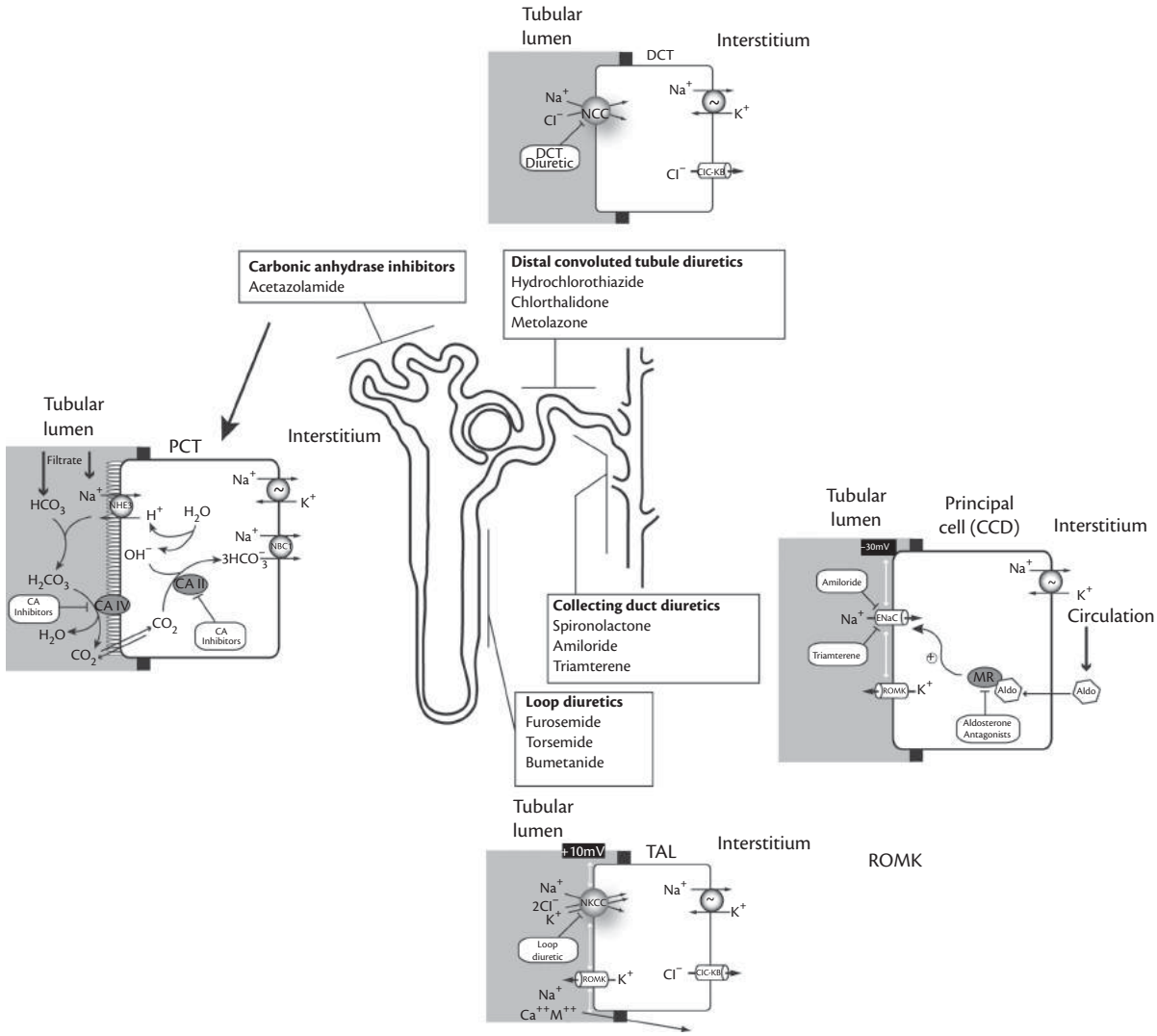

Fig. 33.1 Mechanisms and sites of diuretic action. The figure shows a cartoon of the nephron, with segments identified. Diuretics, classified as in Table 33.1, are shown. Functional models of diuretic actions are also shown, for each site of action.

furosemide-induced natriuresis remain relatively low, electrolyte free water (C_{H_2O}) excretion increases (Puschett and Goldberg, 1968). This effect of loop diuretics has been exploited to treat hyponatraemia, when combined with normal or hypertonic saline (Hantman et al., 1973; Decaux et al., 1981).

Na^+ and Cl^- transport

The predominant effect of loop diuretic drugs is to inhibit the electroneutral Na-K-2Cl cotransporter at the apical surface of thick ascending limb (TAL) cells (Fig. 33.1). This transporter is a member of the cation chloride cotransporter family (Hebert et al., 2004; Gamba, 2005); it is referred to as the Na-K-2Cl cotransporter, second isoform, (NKCC2), and is encoded by the gene *SLC12A1*. This protein uses the electrochemical gradient favouring Na^+ entry across the apical membrane to move Cl^- into the cell along with K^+, while K^+ diffuses back into the luminal fluid via a K^+ channel; thus, net reabsorption across this segment is primarily NaCl. The combination of K^+ movement across the apical membrane and Cl^- movement across the basolateral cell membrane generates a transepithelial voltage oriented in the lumen-positive direction (Greger and Schlatter, 1983), which drives absorption of Na^+, Ca^{2+} and Mg^{2+} via the paracellular pathway. It should be noted, however, that both the transcellular and the paracellular components of Na^+ transport are inhibited by loop diuretics, the former directly and the latter indirectly. The thick ascending limb is virtually impermeable to water. The combination of solute absorption and water impermeability determines the role of the thick ascending limb as the primary diluting segment of the kidney.

The predominant effect of the loop diuretics, furosemide, bumetanide, and torsemide, is to inhibit NKCC2 directly; the mechanisms of action of ethacrynic acid are not as clear. These drugs, however, have other important actions. Thick ascending limb cells have been shown to produce prostaglandin E_2 following stimulation with furosemide (Miyanoshita et al., 1989), perhaps via inhibition of prostaglandin dehydrogenase (Abe et al., 1977; Wright et al., 1976). Blockade of cyclooxygenase reduces the effects of furosemide to inhibit loop segment chloride transport in rats (Kirchner, 1985; Kirchner et al., 1986), and this effect appears to be important clinically, since non-steroidal anti-inflammatory drugs (NSAIDs)

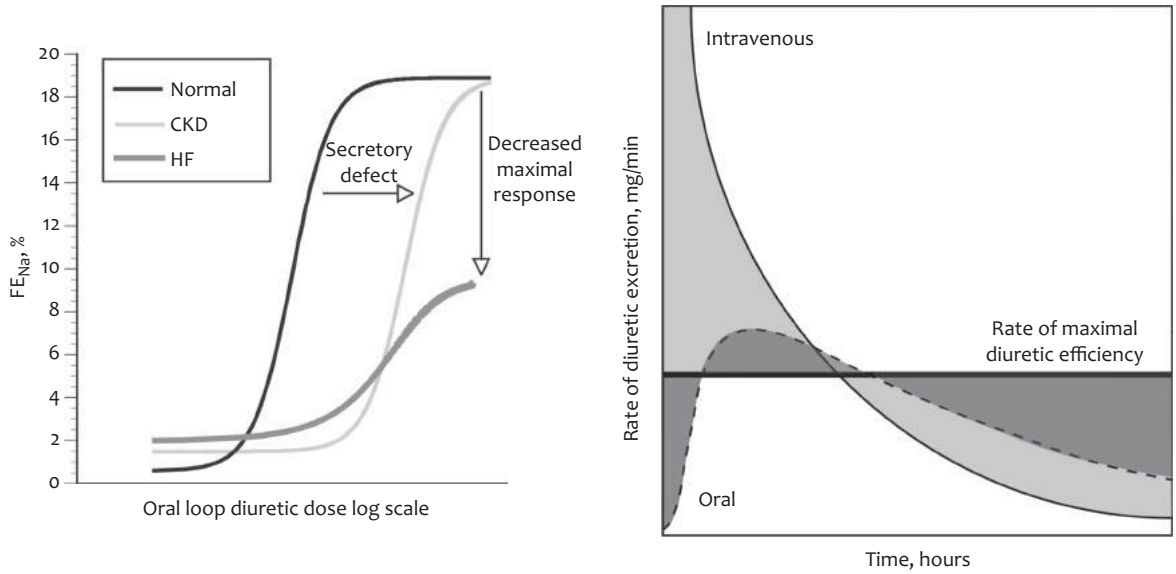

Fig. 33.2 Dose–response curve for loop diuretics. (A) Panel A shows the fractional Na excretion (FE_{Na}) as a function of loop diuretic concentration. Compared with normal patients, patients with chronic renal failure (CKF) show a rightward shift in the curve, owing to impaired diuretic secretion, but the maximal response is preserved. Note that when sodium excretion is expressed in absolute terms (not shown), the maximal diuretic effect in CKD is reduced. In contrast, patients with oedema demonstrate a rightward *and* downward shift in natriuretic effect, even when expressed as FE_{Na}. (B) Panel B compares the response to intravenous and oral doses of loop diuretics. The natriuretic threshold is shown. When given intravenously, peak diuretic concentrations are reached rapidly, but levels may decline more rapidly than during oral administration. As natriuresis depends on the time above the natriuretic threshold, and as the threshold is impacted by disease (A), the relationship between oral and intravenous efficacy is complex.

cause loop diuretic resistance. Increases in renal prostaglandins may also contribute to the haemodynamic effects of loop diuretics described below.

Ca^{2+} and Mg^{2+} transport

Loop diuretics increase the excretion of the divalent cations, calcium and magnesium, owing to their effects to reduce the transepithelial voltage. This stops passive paracellular calcium and magnesium absorption.

Renin secretion

Loop diuretics strongly stimulate renin secretion. Although a component of this effect results from ECF volume contraction (see below), loop diuretics also stimulate renin secretion directly, by inhibiting Na-K-2Cl cotransport, because NaCl entry into macula densa cells, via NKCC2, modulates renin release (Schnermann and Briggs, 2012). When loop diuretics are present, the cells cannot sense luminal NaCl and renin secretion cannot be suppressed. Interestingly, loop diuretics also may stimulate renin secretion by inhibiting NKCC1, the secretory form of the three ion co-transport mechanism present in the basolateral membrane (Schnermann and Briggs, 2012).

Prostaglandin production also participates in regulating renin secretion. Cyclooxygenase, COX-2, is expressed by macula densa cells and by interstitial cells in the kidney (Harris et al., 1994; Guan et al., 1997; Khan et al., 1998; Komhoff et al., 2000). This isoform is often found only after induction by inflammatory cytokines. Blockade of prostaglandin synthesis, either by non-specific cyclooxygenase inhibitors (Frölich et al., 1976) or by specific COX-2 blockers (Harding et al., 1997; Kammerl et al., 2001) reduces both the loop diuretic-induced natriuresis and the renin secretory response. These results have been corroborated in humans (Kammerl et al., 2001).

GFR and RPF flow tend to be preserved during loop diuretic administration (Hook et al., 1966), although GFR and RPF can decline if ECF volume contraction is severe. Loop diuretics reduce renal vascular resistance and increase RPF under experimental conditions (Ludens et al., 1968; Dluhy et al., 1970), probably resulting from the diuretic-induced vasodilatory prostaglandins (discussed earlier).

Another factor that may contribute to the tendency of loop diuretics to maintain GFR and RPF despite volume contraction is their effect on TGF. As noted earlier, the sensing mechanism that activates TGF involves NaCl transport across the apical membrane by the loop diuretic sensitive Na-K-2Cl cotransporter (Schnermann and Briggs, 2008). Under normal conditions, when the luminal concentration of NaCl reaching the macula densa rises, GFR decreases via TGF. Loop diuretic drugs block TGF by interfering with the sensing step of TGF (Wright and Schnermann, 1974). In the absence of effects on the macula densa, loop diuretics would be expected to suppress GFR and RPF by increasing distal NaCl delivery and activating the TGF system. Instead, TGF blockade permits GFR and RPF to be maintained.

Acute intravenous administration of loop diuretics increases venous capacitance (Dikshit et al., 1973), perhaps via prostaglandins (Bourland et al., 1977; Mukherjee et al., 1981), although direct effects in peripheral vascular beds may participate as well (Schmieder et al., 1987). Although venodilation and improvements in cardiac haemodynamics frequently result from intravenous therapy with loop diuretics, the haemodynamic response to intravenous loop diuretics may be more complex (Ellison, 1997b).

Johnston et al. reported that low dose furosemide increased venous capacitance, but that higher doses did not (Johnston et al., 1984). These investigators suggested that furosemide-induced renin secretion leads to angiotensin II-induced vasoconstriction, an effect that might overwhelm the prostaglandin-mediated vasodilatory effects. In two series, 1–1.5 mg/kg furosemide boluses administered to patients with chronic HF, resulted in transient *deteriorations* in haemodynamics (during the first hour), with declines in stroke volume index, increases in left ventricular filling pressure (Francis et al., 1985), and exacerbation of HF symptoms. These changes may be related to activation of both the sympathetic nervous system and the renin–angiotensin system by the diuretic drug. Evidence for a role of the renin–angiotensin system in the furosemide-induced deterioration in systemic haemodynamics includes the temporal association between its activation and haemodynamic deterioration (Francis et al., 1985), and the ability of angiotensin-converting enzyme inhibitors (ACEIs) to prevent much of the pressor effect (Goldsmith et al., 1989). The effects of renal denervation on sympathetic responses to furosemide were studied. These results confirm that the effects are mediated both by direct renal nerve traffic and indirectly by activation of the renin–angiotensin axis (Fitch and Weiss, 2000; Fitch et al., 2000).

The three loop diuretics that are used most commonly, furosemide, bumetanide, and torsemide, are absorbed quickly after oral administration, reaching peak concentrations within 0.5–2 hours. Furosemide absorption is slower than its elimination in normal subjects; thus the time to reach peak serum level is slower for furosemide than for bumetanide and torsemide. This phenomenon is called 'absorption-limited kinetics', as the rate of absorption is often slower than the rate of elimination (Brater, 1997). The bioavailability of loop diuretics varies from 50% to 90% (Table 33.2); furosemide bioavailability is approximately 50% (Shankar and Brater, 2003); when a patient is switched from intravenous to oral furosemide, it is therefore customary to double the dose to compensate for its poor bioavailability (Brater, 1997); in practice, however, there are many other variables that affect furosemide efficacy, and a fixed intravenous/oral conversion cannot be given (Brater, 1983). The half-lives of the loop diuretics available vary, but all are relatively short (ranging from approximately 1 hour for bumetanide to 3–4 hours for torsemide). The half-lives of muzolimine, xipamide, and ozolinone, which are not so widely available, are longer (6–15 hours).

Loop diuretics are organic anions that circulate tightly bound to albumin (> 95%), thus their volume of distribution is small except during extreme hypoalbuminaemia (Inoue et al., 1987). Approximately 50% of an administered dose of furosemide is excreted unchanged into the urine. The remainder appears to be eliminated by glucuronidation, probably by the kidney. Torsemide and bumetanide are eliminated both by hepatic processes and through renal excretion. The differences in metabolic fate mean that the half-life of furosemide is altered by kidney failure more than the half-lives of torsemide and bumetanide. Loop diuretics gain access to the tubular fluid almost exclusively by proximal secretion. The -uptake is mediated by the organic anion transporters OAT1 and OAT3, whereas the apically located multidrug resistance-associated protein 4 (Mrp-4) mediates secretion into the tubular fluid. Mice

Table 33.2 Effects of diuretics on electrolyte excretion

Diuretic	Na	Cl	K	Pi	Ca	Mg
Osmotic diuretics (Wesson and Anslow, 1948; Wesson, 1967; Seely Dirks, 1969; Eknoyan et al., 1970; Benabe and Martinez-Maldonado, 1986)	⇑ (10–25%)	⇑ (15–30%)	⇑ (6%)	⇑ (5–10%)	⇑ (10–20%)	⇑ (> 20%)
Carbonic anhydrase inhibitors (Puschett and Goldberg, 1968; Eknoyan et al., 1970; Cogan et al., 1979)	⇑ (6%)	⇑ (4%)	⇑ (60%)	⇑ (> 20%)	⇑ or ⇔ (< 5%)	⇑ (< 5%)
Loop diuretics (Earley and Friedler, 1964; Suki et al., 1965; Puschett and Goldberg, 1968; Eknoyan et al., 1970; Duarte et al., 1971; Hropot et al., 1985)	⇑ (30%)	⇑ (40%)	⇑ (60–100%)	⇑ (> 20%)	⇑ (> 20%)	⇑ (> 20%)
DCT diuretics (Demartini et al., 1962; Suki et al., 1965; Eknoyan et al., 1970; Hropot et al., 1985)	⇑ (6–11%)	⇑ (10%)	⇑ (200%)	⇑ (> 20%)	⇓	⇑ (5–10%)
Na channel blockers (Eknoyan et al., 1970; Duarte et al., 1971; Hropot et al., 1985)	⇑ (3–5%)	⇑ (6%)	⇓ (8%)	⇔	⇔	⇓
Collecting duct diuretics (Eknoyan et al., 1970)	⇑ (3%)	⇑ (6%)	⇓	⇔	⇔	⇓

Figures indicate approximate maximal fractional excretions of ions following acute diuretic administration in maximally effective doses. ⇑ indicates that the drug increases excretion; ß indicates that the drug decreases excretion; ⇔ indicates that the drug has little of no direct effect on excretion. During chronic treatment, effects often wane (Na excretion), may increase (K excretion during DCT diuretic treatment), or may reverse as with uric acid (not shown).

lacking OAT1, OAT3, or Mrp-4 are remarkably resistant to both loop and thiazide diuretics, illustrating the functional importance of these proteins (Eraly et al., 2006; Hasegawa et al., 2007; Vallon et al., 2008).

The most common adverse effects of loop diuretics result from their primary actions. Loop diuretics are frequently administered to treat oedematous expansion of the ECF volume. As noted above, oedema usually results from a decrease in the 'effective' arterial blood volume, but this volume is impossible to measure precisely. Overzealous diuretic usage or intercurrent complicating illnesses can lead to excessive contraction of the intravascular volume with orthostatic hypotension, renal dysfunction, and sympathetic overactivity. Patients suffering from HF are typically treated with both diuretics and ACEIs or angiotensin receptor blockers (ARBs); this combination is especially likely to worsen renal function in certain circumstances. AKI in such patients often responds to reduced diuretic doses and liberalization of dietary NaCl intake, permitting continued administration of the ACEIs/ARBs (Packer et al., 1987; Packer, 1989).

Other patients at increased risk for renal dysfunction during diuretic therapy include the elderly (Smith and Steele, 1983), patients with pre-existing renal insufficiency (Kaufman and Levit, 1985), patients with right-sided HF or pericardial disease, and patients taking NSAIDs. In a case–control study of NSAID use and renal failure, diuretic users had a 2.77 relative risk of AKI, compared with those who did not use the drugs (Huerta et al., 2005).

Disorders of Na^+ and K^+ concentration are among the most frequent adverse effects of loop diuretics. Hyponatraemia is less common with loop diuretics than with distal convoluted tubule diuretics (see later), but can occur. It is usually multifactorial, but involves both depletion of the 'effective' arterial volume and impairment of free water clearance. Additional factors that may contribute include the non-osmotic release of arginine vasopressin (Bichet et al., 1982), hypokalaemia, and hypomagnesaemia (Dyckner and Webster, 1982). Conversely, loop diuretics have been used to treat hyponatraemia when combined with hypertonic saline in the setting of the syndrome of inappropriate antidiuretic hormone (ADH) secretion (Hantman et al., 1973; Schrier, 1978). The combination of loop diuretics and ACEIs has been reported to ameliorate hyponatraemia in the setting of congestive HF (Dzau and Hollenberg, 1984). The value of adding a loop diuretic to treatment with a vasopressin V2 receptor antagonist for hyponatraemic syndrome of inappropriate ADH secretion has been suggested (Shimizu, 2003; Kazama et al., 2005).

Hypokalaemia occurs commonly during therapy with loop diuretics, although the magnitude is smaller than that induced by distal convoluted tubule diuretic (loop diuretics, 0.3 mmol/L versus DCT diuretics, 0.5–0.9 mmol/L) (Ram et al., 1981; Palmer, 1997). Loop diuretics increase the delivery of potassium to the distal tubule, because they block potassium reabsorption via the Na-K-2Cl cotransporter. In rats, under control conditions, approximately half the excreted potassium was delivered to the 'early' distal tubule. During furosemide infusion, the delivery of potassium to the 'early' distal tubule rose to 28% of the filtered load (Hropot et al., 1985). Thus, a component of the effect of loop diuretics on potassium excretion reflects their ability to block potassium reabsorption by the thick ascending limb, but during chronic diuretic therapy the degree of potassium wasting correlates best with ECF volume contraction and serum aldosterone levels (Wilcox et al., 1984). This suggests that when used chronically loop diuretics stimulate potassium excretion primarily because they increase mineralocorticoid hormones and increase distal Na^+ and water delivery into the aldosterone-sensitive distal nephron (ASDN).

Metabolic alkalosis is very common during chronic treatment with loop diuretics. Loop diuretics cause metabolic alkalosis via several mechanisms. First, they increase the excretion of urine that is bicarbonate free but contains Na^+ and Cl^-. This leads to contraction of the ECF around a fixed amount of bicarbonate buffer; a phenomenon known as 'contraction alkalosis'. Second, loop diuretics directly inhibit transport of Na^+ and Cl^- into thick ascending limb cells, which may stimulate H^+ secretion via Na^+/H^+ exchange (Good et al., 1984; Oberleithner et al., 1984; Good, 1985). Third, loop diuretics stimulate the renin–angiotensin–aldosterone system, increasing Na^+ reabsorption along the ASDN, which renders the tubule lumen more negative and increases H^+-ATPase activity (O'Neil et al., 1977). Aldosterone also activates the vacuolar H^+-ATPase in the outer medullary collecting tubule directly (Stone et al., 1983; Winter et al., 2004). Hypokalaemia itself also contributes to metabolic alkalosis by increasing ammonium production (Tannen, 1970), stimulating bicarbonate reabsorption by proximal tubules (Soleimani et al., 1987; Soleimani and Aronson, 1989), and increasing the activity of the H^+/K^+ ATPase in the distal nephron (Wingo and Straub, 1989; Okusa et al., 1992). Some of these effects may be offset because loop diuretics also strongly increase the expression and activity of pendrin, a chloride/bicarbonate exchanger expressed by type B intercalated cells (Quentin et al., 2004; Na et al., 2007).

Ototoxicity with deafness is the most common toxic effect of loop diuretics unrelated to their effects on the kidney. It appears likely that all loop diuretics cause ototoxicity, because ototoxicity can occur during use of chemically dissimilar drugs such as furosemide and ethacrynic acid (Maher and Schreiner, 1965; Nochy et al., 1976). The stria vascularis, which is responsible for maintaining endolymphatic potential and ion balance, appears to be a primary target for toxicity (Ikeda et al., 1997). A characteristic finding in loop diuretic ototoxicity is strial oedema, because an isoform of the Na-K-2Cl cotransporter in expressed there (Mizuta et al., 1997). Loop diuretics cause loss of outer hair cells in the basal turn of the cochlea, rupture of endothelial layers, cystic formation in the stria vascularis, and marginal cell oedema in the stria vascularis (Ryback, 1993).

Ototoxicity appears to be related to the peak serum concentration of loop diuretic and therefore tends to occur during rapid drug infusion of high doses. For this reason, this complication is most common in patients with uraemia (Star, 1997). It has been recommended that furosemide infusion be no more rapid than 4 mg/minute (Wigand and Heidland, 1971). In addition to those with renal failure, infants, patients with cirrhosis, and patients receiving aminoglycosides or cis-platinum may be at increased risk for ototoxicity (Star, 1997).

Distal convoluted tubule diuretics (thiazides)

The distal convoluted tubule diuretics represent a distinct and important class of diuretics; many are analogues of 1,2,4-benzothiadiazine-1,1-dioxide, but other structurally related diuretics, including the quinazolinones (such as metolazone) and

substituted benzopehenone sulphonamide (such as chlorthalidone) also appear to share the same mechanism of action. Although the term 'thiazide diuretics' is frequently used to describe this class, a more accurate descriptor is *distal convoluted tubule (DCT) diuretics*.

Acute administration of these drugs increases Na, K, Cl, HCO_3, phosphate, and urate excretion (Table 33.1). The increases in HCO_3, phosphate, and urate excretion are probably related primarily to carbonic anhydrase inhibition, and not to inhibition of the Na-Cl cotransporter (see below). As such, the effects of DCT diuretics to increase HCO_3, phosphate, and urate excretion may vary, depending on the carbonic anhydrase inhibiting potency. Chronically, as ECF volume contraction occurs, uric acid excretion declines and hyperuricaemia can occur (Toto, 1997). Further, bicarbonate excretion ceases, and continuing losses of chloride without bicarbonate, coupled with ECF volume contraction, may lead to metabolic alkalosis. In contrast to loop and proximally acting diuretics, DCT diuretics reduce urinary calcium excretion (see below).

DCT diuretics inhibit the clearance of solute free water when administered during water diuresis, because their site of action is a portion of the renal diluting segment. In contrast to loop diuretics, however, DCT diuretics do not limit water retention during antidiuresis.

Na^+ and water transport in the proximal tubule

Most DCT diuretics retain some carbonic anhydrase inhibiting activity (Goldfarb et al., 1991). Although this effect occurs during acute treatment (as during intravenous chlorothiazide administration), it probably contributes relatively little to overall natriuresis during chronic use (Kunau et al., 1975; Walter and Shirley, 1986). Yet this effect may play a role in the tendency for DCT diuretics to reduce the GFR by activating TGF (Okusa et al., 1989). The relative carbonic anhydrase inhibiting potency of some commonly used DCT diuretics (shown in parentheses) is chlorthalidone (67) > benthiazide (50) > polythiazide (40) > chlorothiazide (14) > hydrochlorothiazide (1) > bendroflumethiazide (0.07) (Friedman and Hebert, 1997, pp. 75–111).

NaCl absorption in the distal nephron

As the name indicates, the predominant site at which DCT diuretics inhibit ion transport is the DCT. The predominant action of these drugs is to inhibit the thiazide-sensitive Na-Cl cotransporter (NCC) encoded by *SLC12A3* (Fig. 33.1).

Like the loop diuretics, DCT diuretics are organic anions that bind to and inhibit NCC directly, although the specific site on which the drugs inhibit NCC continues to be debated (Tran et al., 1990; Monroy et al., 2000; Moreno et al., 2006). There is some evidence that thiazides inhibit solute transport in medullary collecting tubules of rats (Wilson et al., 1983) and in the cortical collecting ducts when animals are NaCl deprived (Terada and Knepper, 1990). Most recently, Leviel and colleagues found that thiazides inhibit a sodium-dependent chloride-bicarbonate exchanger (NDCBE) in the collecting duct of animals exposed to low NaCl diets (Leviel et al., 2010). There is now evidence that this pathway does contribute to the net effect of DCT diuretics (Soleimani et al., 2012).

Ca^{2+} and Mg^{2+} transport

When administered chronically, DCT diuretics reduce calcium excretion, but the mechanisms remain controversial. Acute administration of DCT diuretics has a variable effect on calcium excretion (Eknoyan et al., 1970; Popovtzer et al., 1975), probably reflecting the carbonic anhydrase inhibition along the proximal tubule. During chronic treatment, DCT diuretics reduce calcium excretion, and the effect likely occurs at several levels. First, the filtered calcium load may decrease slightly owing to ECF volume depletion and a decline in GFR. Second, proximal calcium reabsorption increases, as contraction of the ECF volume stimulates proximal Na^+ reabsorption, thereby stimulating calcium reabsorption secondarily. Third, DCT diuretics increase renal calcium reabsorption along the distal nephron (Costanzo and Windhager, 1978).

Stimulation of distal calcium reabsorption is accompanied by an increase in intracellular calcium activity, suggesting that a primary effect is to increase apical calcium entry (Gesek and Friedman, 1992). The drugs, however, may also enhance basolateral calcium uptake, as DCT cells express the Na/Ca exchanger and a Ca-ATPase at the basolateral cell membrane. The Na/Ca exchanger is electrogenic, and when the intracellular Na^+ concentration declines, the electrochemical driving force favouring calcium movement from cell to interstitium increases.

The roles of proximal and distal processes in effects of DCT diuretics on urinary calcium excretion continue to be debated (Reilly and Huang, 2011). Lee and colleagues confirmed an acute effect of DCT diuretics on distal calcium uptake, but also found that a large portion of the chronic effects of DCT diuretic required ECF volume depletion. They speculated that acute exposure to DCT diuretics activates calcium channels (TrpV5) along the distal tubule. During chronic exposure, however, they noted that ECF volume contraction reduces distal NaCl delivery, thereby reducing this effect (Lee et al., 2004). Nijenhuis and colleagues found that DCT diuretic reduced calcium excretion, even when TrpV5 was deleted genetically, suggesting a predominant role for enhanced proximal reabsorption (Nijenhuis et al., 2005). They suggested that distal processes contribute little to the overall effect. Yet there are compelling data indicating that effects along the DCT do play a role. In humans ECF volume is not altered substantially during chronic treatment. In animals with hypocalciuria resulting from deletion of salt transporting genes or regulators along the distal nephron, salt loading does not correct the hypocalciuria (McCormick et al., 2011). Similarly, in humans with Gitelman syndrome in which the thiazide-sensitive Na-Cl cotransporter is dysfunctional, saline infusion increases NaCl excretion, without correcting the hypocalciuria (Cheng et al., 2007). Finally, in mice with genetic disruption of parvalbumin, which disrupts NCC activity without causing ECF volume depletion, hypocalciuria is also observed (Belge et al., 2007). Overall, it appears that both proximal and distal processes contribute to the hypocalciuric effect of DCT diuretics (Reilly and Huang, 2011).

DCT diuretics enhance magnesium excretion, but the effects are generally much less profound than their effects on calcium excretion. This is in contrast to the effects of genetic NCC deletion or inactivity, as occurs in Gitelman syndrome, where hypomagnesaemia is a cardinal feature. Acute thiazide infusions have little effect on magnesium excretion (Duarte, 1968; Eknoyan et al., 1970; Quamme et al., 1975), whereas chronic administration may cause hypomagnesaemia (Hollifield, 1989; Douban et al., 1996; Quamme, 1997). One important pathway for magnesium reabsorption across the apical membrane of DCT cells is the transient receptor potential, TrpM6 (Schlingmann et al., 2002). The predominant mechanism

for DCT diuretic-induced magnesium wasting involves destruction of DCT cells, from apoptosis induced by DCT diuretics (Loffing et al., 1996, 2004). Several groups have reported that inactivation of NCC reduces the abundance of Trpm6, which would be expected to impair magnesium reabsorption (Nijenhuis et al., 2004, 2005).

DCT diuretics increase renal vascular resistance and decrease the GFR when given acutely. Okusa et al. (1989) showed that intravenous chlorothiazide reduced the GFR by 16% only when TGF was intact. During chronic treatment with DCT diuretics, mild contraction of the ECF volume develops, thereby increasing solute and water reabsorption by the proximal tubule. This effect reduces Na^+ delivery to the macula densa. This would be expected to return GFR toward baseline values during chronic treatment with DCT diuretics (Earley and Orloff, 1962; Walter and Shirley, 1986). Thus, when used chronically, DCT diuretics lead to a state of mild ECF volume contraction, increased fractional proximal reabsorption, and relatively preserved GFR (Earley and Orloff, 1962; Walter and Shirley, 1986).

When administered acutely, the effect of DCT diuretics on renin secretion is variable (McGuffin and Gunnells, 1969). If urinary NaCl losses are replaced, these drugs tend to suppress renin secretion (Brown et al., 1966), probably by increasing NaCl delivery to the macula densa (Okusa et al., 1989). In contrast, during chronic administration, renin secretion increases both because solute delivery to the macula densa declines (Walter and Shirley, 1986) and because volume depletion activates the vascular mechanism for renin secretion.

Like the loop diuretics, DCT diuretics are organic anions that circulate in a highly protein bound state. As a result, the predominant route of entry into tubular fluid is by secretion via the organic anion secretory pathway in the proximal tubule (Brater, 1997). DCT diuretics are rapidly absorbed across the gut, reaching peak concentrations within 1.5–4 hours (Brater, 1997). The amount of administered drug that reaches the urine varies greatly (for a review see Brater, 1997), as does the half-life. Shorter acting DCT diuretics include bendroflumethiazide, hydrochlorothiazide, tizolemide, and trichlormethiazide. Medium-acting DCT diuretics include chlorothiazide, hydroflumethiazide, indapamide, and mefruside. Long-acting DCT diuretics include chlorthalidone, metolazone, and polythiazide (Brater, 1997). These differences in half-life may have implications with regard to the efficacy of these drugs for the treatment of hypertension (Flack et al., 2011), and perhaps with regard to the incidence of hypokalaemia, which may be more common in patients taking the longer-acting drugs such as chlorthalidone (Dhalla et al., 2013).

DCT diuretics are used most commonly to treat essential hypertension; their use in this situation is beyond the scope of this chapter. DCT diuretics have become drugs of choice to prevent the recurrence of kidney stones in patients with idiopathic hypercalciuria. In several controlled and many uncontrolled studies, the recurrence rate for calcium stones has been reduced by up to 80% (Yendt and Cohanim, 1978; Laerum and Larsen, 1984; Ettinger et al., 1988). Relatively high doses of DCT diuretics are often employed for the treatment of nephrolithiasis (Breslau, 1997). Some studies suggest that the hypocalciuric effect of DCT diuretics wanes during chronic use, in the setting of absorptive hypercalciuria (Preminger and Pak, 1987). The observation that Gitelman syndrome, an inherited disorder of NCC inactivity, may present during adulthood with hypocalciuria suggests that compensatory mechanisms may not exist for the effects of DCT diuretics on calcium transport (Ellison, 2000). Reilly and colleagues reviewed DCT diuretic use for nephrolithiasis and suggested doses of indapamide at 2.5 mg/day, chlorthalidone at 25–50 mg/day, or HCTZ at 25 mg twice daily or 50 mg/daily (Reilly et al., 2010).

The ability of DCT diuretics to reduce urinary calcium excretion suggests that these drugs may prevent bone loss. Some (Ray et al., 1989; Felson et al., 1999), but not all (Heidrich et al., 1991; Cauley et al., 1993), epidemiological studies suggest that DCT diuretics reduce the risk of hip fracture and osteoporosis. A randomized controlled study confirmed that DCT diuretics reduce bone loss in women (Reid et al., 2000).

DCT diuretics are also employed to treat nephrogenic diabetes insipidus, causing a paradoxical decrease in urinary volume and flow rate. This action of DCT diuretics results from the combination of mild ECF volume contraction, owing to diuretic-induced natriuresis and suppression of GFR, due largely to diuretic-induced activation of TGF. The DCT, like the thick ascending limb, is nearly impermeable to water (Coleman et al., 1997). Solute reabsorption by NCC, therefore, contributes directly to urinary dilution. The central role of ECF volume contraction in the efficacy of DCT diuretics in diabetes insipidus was highlighted by the observation that dietary salt restriction is necessary to reduce urinary volume effectively (Earley and Orloff, 1962; Janjua et al., 2001). DCT diuretics may also increase the ADH-independent water permeability of the medullary collecting tubule (Cesar and Magaldi, 1999). DCT diuretic treatment increased the abundance of aquaporin-2, NCC and the alpha subunit of the epithelial Na channel (Kim et al., 2004), when administered to rats with lithium-induced nephrogenic diabetes insipidus. It was suggested that the upregulation of the abundance of the renal Na and water transporters might explain the antidiuretic effectiveness of DCT diuretics.

Electrolyte disorders, such as hypokalaemia, hyponatraemia, and hypomagnesaemia are common side effects of DCT diuretics. A measurable decline in serum K^+ concentration is nearly universal in patients given DCT diuretics, but most patients do not become frankly hypokalaemic. In the ALLHAT trial, mean serum K^+ concentrations declined from 4.3 to 4.0 and 4.1 mmol/L, after 2 and 4 years of treatment (ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002b). The clinical significance of diuretic-induced hypokalaemia continues to be debated. Unlike the loop diuretics, DCT diuretics do not influence K^+ transport directly (Velázquez and Wright, 1986), but instead increase tubule fluid flow and Na^+ concentration in the CNT and collecting duct. In addition, DCT diuretic-induced ECF volume contraction activates the renin–angiotensin–aldosterone system, further stimulating K^+ secretion. Evidence for the central role of aldosterone in diuretic-induced hypokalaemia includes the observation that hypokalaemia is more common during treatment with long-acting DCT diuretics, such as chlorthalidone, than with shorter-acting DCT diuretics, such as hydrochlorothiazide, or with the very short-acting loop diuretics (Ram et al., 1981; Dhalla et al., 2013). Another reason that DCT diuretics may produce more K^+ wasting than loop diuretics is the difference in effect on calcium transport. As discussed above, loop diuretics inhibit calcium transport by the thick ascending limb, increasing distal calcium delivery; in contrast, DCT diuretics stimulate calcium transport, reducing calcium delivery to sites of potassium secretion. Okusa and colleagues (1990) showed that high luminal concentrations of calcium

inhibit the functional activity of epithelial sodium channels (ENaC) in the distal nephron, thereby inhibiting potassium secretion. DCT diuretics also increase urinary magnesium excretion and can lead to hypomagnesaemia, which may also contribute to hypokalaemia (Rude, 1989; Dorup et al., 1993; Huang and Kuo, 2007). Some studies suggest that maintenance magnesium therapy can prevent or attenuate the development of hypokalaemia (Dorup et al., 1993), but this has not been supported universally.

Diuretics have been reported to contribute to more than one half of all hospitalizations for serious hyponatraemia. Hyponatraemia is especially common during treatment with DCT diuretics, compared with other classes of diuretics, and the disorder is potentially life-threatening (Ashraf et al., 1981). A recent case–control study suggested that hyponatraemia during thiazide treatment is more common than generally appreciated, but that in most cases, it does not prove morbid (Leung et al., 2011); on the other hand, some studies do suggest an association with mortality (Liamis et al., 2013).

Several factors contribute to DCT diuretic-induced hyponatraemia. First, DCT diuretics inhibit solute transport in the terminal portion of the 'diluting segment'. Second, DCT diuretics reduce the GFR, limiting solute delivery to the diluting segment and impairing solute-free water clearance. Third, DCT diuretics lead to ECF volume contraction, which increases proximal tubule solute and water reabsorption. Fourth, hyponatraemia has been correlated with the development of hypokalaemia (Fichman et al., 1971). Finally, susceptible patients may be stimulated to consume water during therapy with DCT diuretics. One report suggests that patients who are predisposed to develop hyponatraemia during treatment with DCT diuretics will demonstrate an acute decline in serum sodium concentration in response to a single dose of the drug (Friedman et al., 1989). Other studies suggest that risk factors for DCT diuretic-induced hyponatraemia include older age, lower body mass, and concomitant administration of selective serotonin reuptake inhibitors or benzodiazepines (Chow et al., 2003; Neafsey, 2004; Liamis et al., 2013).

DCT diuretics frequently cause mild metabolic alkalosis. The mechanisms are similar to those described above for loop diuretics, except that DCT diuretics do not stimulate Na/H exchange in the TAL.

Glucose intolerance has been a recognized complication of DCT diuretic use since the 1950s and appears to be dose related (Carlsen et al., 1990; Harper et al., 1995). In the ALLHAT trial there was a 1.8% increase in new onset diabetes at 4 years of treatment with chlorthalidone versus patients treated with calcium channel blockers (ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002a). This difference did not translate into adverse clinical outcomes in the diuretic group, but has generated a great deal of discussion. The pathogenesis of DCT diuretic-induced glucose intolerance remains unclear, but several factors have been suggested to contribute. First, diuretic-induced hypokalaemia may decrease insulin secretion by the pancreas, via effects on the membrane voltage of pancreatic beta cells. When hypokalaemia was prevented by oral potassium supplementation, the insulin response to hyperglycaemia normalized, suggesting an important role for hypokalaemia (Helderman et al., 1983). Hypokalaemia may also interfere with insulin-mediated glucose uptake by muscle, but most patients demonstrate relatively normal insulin sensitivity (Toto, 1997). ECF volume depletion may stimulate catecholamine secretion, but volume depletion during therapy with DCT diuretics is usually very mild. It has also been suggested that DCT diuretics directly activate calcium-activated potassium channels that are expressed by pancreatic beta cells (Pickkers et al., 1996). Activation of these channels is known to inhibit insulin secretion. Inhibiting the renin–angiotensin–aldosterone axis appears to reduce the development of new diabetes (Scheen, 2004). Drugs that inhibit this pathway might attenuate the effects of diuretics to impair glucose homeostasis, but this has not been tested directly. Other factors may contribute to glucose intolerance as well, including drug-specific factors (Ellison and Loffing, 2009).

DCT diuretics increase levels of total cholesterol, total triglyceride, and LDL cholesterol, and reduce HDL (Toto, 1997; Wilcox, 1999). Definitive information on the mechanisms by which DCT diuretics alter lipid metabolism is not available, but many of the mechanisms that affect glucose homeostasis have been suggested to contribute. Hyperlipidaemia, like hyperglycaemia, is a dose-related side effect, and one that wanes with chronic diuretic use. In the ALLHAT study, treatment with chlorthalidone resulted in total cholesterol 2.2 mg/dL (0.06 mmol/L) higher than did treatment with ACEIs (ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002a). In several large clinical studies, the effect of *low-dose* DCT diuretic treatment on serum LDL was not significant (Grimm et al., 1996). Furthermore, treatment of hypertension with DCT diuretics reduces the risk of stroke, coronary heart disease, congestive HF, and cardiovascular mortality.

Cortical collecting duct diuretics (distal potassium-sparing diuretics)

Diuretic drugs that act primarily in the cortical collecting duct (CCD) or the CNT (potassium-sparing diuretics) comprise three pharmacologically distinct groups: aldosterone antagonists (spironolactone and eplerenone), pteridines (triamterene), and pyrazinoylguanidines (amiloride). The site of action for all diuretics of this class is the CNT and the CCD, where they interfere with sodium reabsorption and indirectly potassium secretion. The recently introduced vasopressin V2-receptor antagonists (the 'vaptans') also act in the collecting duct and could be categorized as diuretics (Decaux et al., 2008). Because vasopressin-receptor antagonists are primarily used for the treatment of hyponatraemia secondary to the syndrome of inappropriate ADH secretion, HF, or liver cirrhosis, these compounds are discussed in other chapters.

The diuretic activity of amiloride, triamterene and aldosterone antagonists is weak acutely. Because these drugs are *relatively* weak natriuretic agents, they are used most commonly in combination with thiazides or loop diuretics, in combination or as a single preparation, to restrict potassium losses. In certain conditions, however, potassium-sparing diuretics are used as first-line agents (see below). Mineralocorticoid blocking drugs have become standard parts of the treatment of patients with systolic dysfunction HF, where these drugs reduce mortality of patients with severe (Pitt et al., 1999) or mild HF (Pitt et al., 2001; Zannad et al., 2011).

Amiloride, triamterene and spironolactone are weak natriuretic agents when given acutely (Table 33.1). Additionally, these agents decrease hydrogen ion secretion by the late distal tubule and collecting ducts. A common mechanism is likely to be involved in mediating the effects of all three diuretic agents on hydrogen ion secretion, as they all reduce the lumen-negative voltage in the distal

nephron and thus decrease the electrochemical gradient favouring hydrogen ion secretion.

Clearance studies in rats have demonstrated that amiloride decreases calcium excretion (Costanzo and Weiner, 1976). In these studies, amiloride produced both a decrease in the calcium clearance/sodium clearance ratio (C_{Ca}/C_{Na}), as well as a decrease in the fractional excretion of calcium. Amiloride is believed to stimulate calcium absorption through its ability to block sodium channels, thereby hyperpolarizing the apical membrane (Friedman PA, Gesek, 1995). Hyperpolarization of the apical membrane may stimulate TrpV5, as discussed above. Amiloride has also been reported to reduce magnesium excretion (Bundy et al., 1995; Douban et al., 1996) and to prevent the development of hypomagnesaemia during therapy with a DCT diuretic (Dyckner et al., 1988).

The site of action of potassium-sparing diuretics is the CNT and collecting duct. Although these segments reabsorb only a small percentage of the filtered Na^+ load, two characteristics render this segment important in the physiology of diuretic action. First, these segments are the primary site of action of the mineralocorticoid, aldosterone, a hormone that controls Na^+ reabsorption and K^+ secretion. Second, virtually all of the potassium that is excreted is due to the secretion of potassium by the connecting and collecting tubules. The apical membrane of cells in these segments expresses separate channels that permit selective conductive transport of sodium and potassium (Fig. 33.1). The low intracellular sodium concentration as a result of the basolateral Na/K-ATPase generates a favourable electrochemical gradient for sodium entry through sodium channels. Thus, sodium conductance depolarizes the apical membrane, resulting in a lumen-negative transepithelial potential difference, which tends to favour K^+ secretion.

Amiloride-sensitive sodium conductance is a function of the ENaC. The amount of sodium and potassium present in the final urine is tightly controlled by aldosterone action on ENaC. The cellular mechanisms that are responsible for these events have been extensively studied and reviewed (Thomas et al., 2008).

Mineralocorticoid receptor blockers

Spironolactone (Fig. 33.1) is an analogue of aldosterone that is extensively metabolized (Karim, 1978; Shackleton et al., 1986), having the principal effect of blocking aldosterone action (Fanestil, 1988; Menard, 2004). Spironolactone is converted by deacylation to 7a-thiospironolactone or by diethioacetylation to canrenone (Fanestil, 1988). In the kidney, spironolactone and its metabolites enter target cells from the peritubular side, bind to cytosolic mineralocorticoid receptors, and act as competitive inhibitors of the endogenous hormone. Spironolactone induces a mild increase in sodium excretion (1–2%) and a decrease in potassium and hydrogen ion excretion (Kagawa, 1960; Liddle, 1966). Its effect depends on the presence of aldosterone (Coppage and Liddle, 1960; Botero-Velez et al., 1994). In cortical collecting tubules perfused *in vitro*, spironolactone added to the bath solution reduced the aldosterone induced lumen negative transepithelial voltage (Gross and Kokko, 1977). By blocking sodium absorption in the collecting tubule, a decrease in lumen negative voltage reduces the driving force for passive sodium and hydrogen ion secretion (Gross and Kokko, 1977).

Spironolactone commonly causes troubling oestrogenic side effects. Eplerenone was developed as a competitive aldosterone antagonist that is more selective for mineralocorticoid receptors and therefore less likely to cause troubling side effects. Eplerenone was derived from spironolactone; in humans, it appears to be 50–75% as potent in inhibiting mineralocorticoid receptors (Weinberger et al., 2002; Brown, 2003). This structural modification significantly enhances the relative affinity of the drug for mineralocorticoid receptors over other steroid receptors.

Amiloride and triamterene

Amiloride and triamterene are both organic cations that act via the same mechanism (Fig. 33.1). Their actions on sodium and potassium transport, unlike spironolactone, are not dependent on aldosterone. Systemically administered amiloride produced a small increase in sodium excretion and a much larger decrease in potassium excretion (Duarte et al., 1971; Giebisch, 1978). Amiloride decreases potassium secretion by blocking ENaC in the apical membrane of CNT and collecting tubule cells (Koeppen et al., 1983; O'Neil et al., 1984), thereby decreasing the electrochemical gradient for potassium secretion, although in higher concentrations (>100 μmol/L), amiloride can inhibit Na^+/H^+ exchange along the proximal tubule. As used clinically, however, amiloride interacts specifically with ENaC (Garty and Benos, 1988; Garty, 1994).

The molecular mechanism by which amiloride blocks ENaC remains incompletely defined, but it appears that the drug occludes the pore of the sodium channel, ENaC (Garty, 1994; Garty and Palmer, 1997). Clearance and free-flow micropuncture studies using triamterene demonstrated results similar to studies with amiloride (Hropot et al., 1985), although its mechanism of action is not as clearly defined (Busch et al., 1996).

The bioavailability of spironolactone is approximately 90%. The drug is rapidly metabolized in the liver into a number of metabolites (see Karim, 1978; Shackleton et al., 1986). Spironolactone and its metabolites are extensively bound to plasma protein (98%). In normal volunteers, taking spironolactone (100 mg/day) for 15 days, the mean half-lives for spironolactone, canrenone, 7a-thiomethylspironolactone and 6b-hydroxy-7a-thiomethylspironolactone were 1.4, 16.5, 13.8, and 15 hours, respectively. Thus, although unmetabolized spironolactone is present in serum, it has a rapid elimination time. The onset of its physiological action, however, is extremely slow for spironolactone, with peak response sometimes occurring 48 hours or more after the first dose; effects gradually wane over a period of 48–72 hours. Spironolactone is used in cirrhotic patients to induce a natriuresis. In these patients, pharmacokinetic studies indicate that the half-lives of spironolactone and its metabolites are increased (Table 33.3). The half-lives for spironolactone, canrenone, 7a-thiomethylspironolactone and 6b-hydroxy-7a-thiomethylspironolactone are 9, 58, 24, and 126 hours respectively (Sungaila et al., 1992).

Eplerenone is rapidly absorbed, with peak serum levels at 1.5 hours (Brown, 2003). Its volume of distribution is 43–90 L, with approximately 50% protein bound. It is cleared primarily via the CYP4503A4 system to inactive metabolites with an elimination half-life of 4–6 hours (Brown, 2003). This is in contrast to spironolactone, where the half-life of the parent compound is short, but the half-life of metabolites is very long. The maximal plasma concentration and area under the curve are increased in people > 65 years of age and with kidney failure; eplerenone is not removed by haemodialysis (Brown, 2003).

Table 33.3 Pharmacokinetics of loop diuretics

		Elimination half-life (hours)			
	Bioavailability, % oral dose absorbed	**Healthy**	**Kidney disease**	**Liver disease**	**Heart failure**
Furosemide	50% (range, 10–100%)	1.5–2	2.8	2.5	2.7
Bumetanide	80–100%	1	1.6	2.3	1.3
Torsemide	80–100%	3-4	4-5	8	6

Data from Shankar, S.S. (2003). *Am J Physiol*, 284, F11–F21.

CCT diuretics can be used for the treatment of hypertension, primary aldosteronism, and secondary aldosteronism; they are also used to limit the kaliuretic effects of loop or DCT diuretics, and sometimes primarily to treat hypokalaemia due to renal potassium loss of various causes.

Spironolactone (or eplerenone) is the treatment of choice in patients with primary aldosteronism due to bilateral adrenal hyperplasia (Brown et al., 1972; Ganguly, 1998). The drug is also appropriate for cirrhosis with ascites (see below). A third use of spironolactone (or eplerenone) is in systolic HF, where mineralocorticoid antagonists have been shown to reduce morbidity and mortality (Zannad et al., 2011; Markowitz et al., 2012). Finally, there is growing interest in using spironolactone to treat resistant hypertension, even when demonstrable hyperaldosteronism is not present (Calhoun et al., 2008).

Triamterene or amiloride is generally used in combination with potassium-wasting diuretics (thiazide or loop diuretics), especially when maintenance of normal serum potassium concentrations is clinically important. In addition, amiloride (or triamterene) has also been used as initial therapy in potassium wasting states such as primary hyperaldosteronism (Ganguly and Weinberger, 1981; Griffing et al., 1982), Liddle syndrome (Botero-Velez et al., 1994), Bartter syndrome, or Gitelman syndrome (Okusa et al., 1987), although use in the latter situation has been disputed (Seyberth et al., 2011). Amiloride is recommended to treat lithium-induced nephrogenic diabetes insipidus (Batlle et al., 1985), where it blocks the pathway by which lithium gains entry into cells (Christensen et al., 2011). A small placebo-controlled cross-over trial (Bedford et al., 2008) and an animal study (Kortenoeven et al., 2009) confirmed these effects.

The most serious adverse reaction encountered during therapy with the CCD diuretics is hyperkalaemia. Serum K^+ should be monitored periodically, even when the drugs are administered with a potassium-wasting diuretic. Patients at highest risk are those with low GFR, patients with concurrent medication predisposing to hyperkalaemia, and individuals who take potassium supplements concurrently (Chapagain and Ashman, 2012). This problem has become more important, because of the wide use of aldosterone blocking drugs, together with ACEIs, ARBs, and beta blockers in patients with HF (Juurlink et al., 2004). Another group at risk for hyperkalaemia are the elderly receiving chronic treatment with spironolactone and who are intermittently treated with trimethoprim-sulfamethoxazole for a urinary tract infection (Antoniou et al., 2011a, 2011b). Surprisingly, however, another recent population-based study showed that despite a marked increase in the use of spironolactone, no increase was seen in hospital admissions for hyperkalaemia and that rates of outpatient hyperkalaemia actually fell; the authors ascribed these findings to more careful monitoring (Wei et al., 2010).

In patients with cirrhosis and ascites treated with spironolactone, hyperchloraemic metabolic acidosis can develop independent of changes in renal function (Gabow et al., 1979). Gynaecomastia may occur in men, especially as the dose is increased (Rose et al., 1977), but even at low doses (The Randomized Aldactone Evaluation Study, 1996); decreased libido and impotence have also been reported. Women may develop menstrual irregularities, hirsutism, or breast swelling and tenderness. Spironolactone induced agranulocytosis has also been reported (Whitling et al., 1997).

Triamterene and amiloride may also cause hyperkalaemia. The risk of hyperkalaemia is highest in patients with limited renal function (e.g. renal insufficiency, diabetes mellitus, and elderly patients). Additional complications included elevated serum blood urea nitrogen and uric acid, glucose intolerance, and gastrointestinal disturbances. Triamterene induces crystalluria or cylinduria (Fairley et al., 1986), which may contribute to or initiate formation of renal stones (Carr et al., 1990), and may cause AKI when combined with non-steroidal anti-inflammatory agents (Favre et al., 1982; Weinberg et al., 1985). Cortical collecting duct diuretics are contraindicated in patients with hyperkalaemia, individuals taking potassium supplements in any form, and in patients with severe renal failure with progressive oliguria.

Clinical use of diuretics

General concepts

Determinants of maximal diuresis

The change in urinary flow seen during the administration of a diuretic depends on many factors, including its mechanism of action, dose, kinetics of entry into the bloodstream, and delivery to its site of action. The maximal diuretic effect of these drugs is determined, largely by the transport protein and nephron site of action. For example, loop diuretics have a higher 'ceiling' action than DCT diuretics. This observation results from the fact that loop diuretics inhibit a transport pathway responsible for reabsorbing up to 25% of the filtered sodium load, while DCT diuretics inhibit a pathway responsible for reabsorbing only 5–10%. Similarly, mineralocorticoid antagonists have a mild natriuretic effect due to the fact that they suppress a pathway responsible for reabsorbing only 3% of the filtered Na load. There are, of course, exceptions to this rule. The carbonic anhydrase inhibitors, which reduce proximal tubule reabsorption are only weakly natriuretic, due to adaptive changes in the loop of Henle and DCT (Lorenz et al., 1999). Within classes, drugs

vary in potency (effect/amount of drug required). For example, a lower dose of bumetanide is required to elicit the same effect as furosemide, even though the maximal natriuretic effect of each is similar.

Diuretic efficacy is also dependent on the kinetics of drug entry into the bloodstream. The dynamics of drug absorption may be perturbed in certain clinical situations, and this might result in a diminished effect. This is exemplified by the pharmacokinetics of furosemide. In normal individuals, the rate of furosemide absorption from the GI tract is not rapid, and a reservoir of drug can persist long after the diuretic is administered (Brater, 1997). This reservoir makes the effective half-life of the drug longer than the actual plasma half-life. In certain oedematous states, however, absorption from the gut may be slowed, so that furosemide absorption never reaches the diuretic threshold, rendering it ineffective (Vasko et al., 1985). To compensate for this, giving a high dose of the drug, or switching to a different diuretic with better absorption, such as torsemide or bumetanide, may facilitate a brisker diuresis (Murray et al., 2001). Another approach is to switch to an intravenous loop diuretic preparation, which is, of course, 100% bioavailable.

The effectiveness of a diuretic is also dependent on its rate of delivery to its site of action. In the cases of loop and DCT diuretics, these sites are at the luminal surface of the tubules. Brater established that diuretics such as furosemide have an *excretion rate of maximal efficiency*, that is, a rate of diuretic delivery that is associated with a maximal natriuretic response (Kaojarern et al., 1982). This concept helps to explain why an orally administered dose of furosemide can be more effective than an equivalent single intravenous dose in individuals with normal GI absorption (Fig. 33.2). When a dose of furosemide is given as an intravenous bolus, the rate of diuretic excretion is very high early on in the time-course, substantially greater than the rate of maximal efficiency. This rate tapers down over time, but the curve quickly dips below the maximal efficiency rate. In contrast, oral administration of the same dose of diuretic reaches the bloodstream more gradually, because of the processes described above regarding absorption. Thus, a reservoir of furosemide in the GI tract may keep the circulating level above the natriuretic threshold and close to the rate of maximal diuretic efficiency for a longer period.

As described above, most diuretics reach their sites of action via tubular secretion, primarily along the proximal tubule. These transport processes are relatively non-specific, and a single transporter type can facilitate the movement of a variety of similarly charged molecules into the tubular lumen. Accordingly, any exogenous or endogenous substance that competes with a diuretic for one of these transport processes can potentially limit the efficient arrival of that diuretic to its site of action. For instance, cimetidine, an organic cation, has been shown to inhibit the tubular secretion of amiloride (Somogyi et al., 1989). Other substances, such as NSAIDs, probenecid, penicillins, and uraemic anions all compete with loop and thiazide diuretics for tubular secretion (Rose et al., 1976; Brater, 1978). In certain disease states, competition between different drugs or endogenous substances for transport to the tubular lumen may lead to diuretic resistance. The prototypical example of such a condition is chronic kidney disease (CKD), in which diuretic delivery to the urine is impaired (Brater, 1978). In CKD, impaired drug delivery shifts the diuretic dose–response curve to the right, and a higher dose is required to achieve a diuretic effect (Fig. 33.2). This could potentially unmask the competitive effects of two different pharmacologic agents on an organic ion transport process, since a slight decrease in the rate of transport of the diuretic to the urinary space could make the tubular diuretic concentration fall beneath its threshold of effectiveness.

Diuretic adaptation and resistance

Typically, the brisk increase in urinary solute and water excretion that is seen just after diuretic therapy is initiated wanes over several days of treatment (Fig. 33.3). This phenomenon occurs because certain renal and systemic adaptations take place in response to diuretic therapy. These adaptations are essential for chronic diuretic use, since continued depletion of the ECF volume would prove harmful; yet when they occur before the achievement of the desired

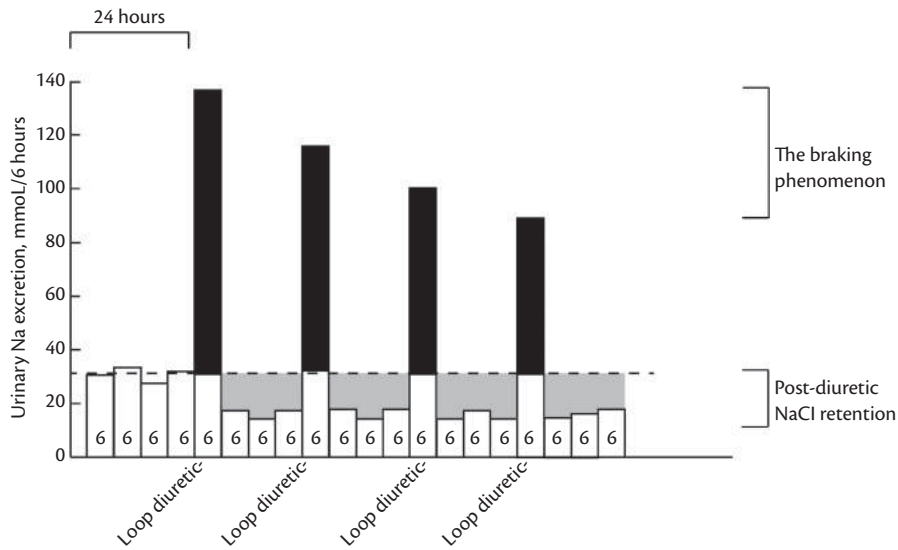

Fig. 33.3 Effects of intermittent diuretic dosing. The figure shows urinary Na^+ excretion, in 6-hour intervals, at baseline, and after dosing with a loop diuretic. Note that urinary Na^+ excretion at baseline is near to dietary intake (dashed line). After each dose of diuretic, Na^+ excretion rises transiently, and then declines below baseline. The phenomena of 'Post-diuretic NaCl retention' and 'Braking' are shown.

ECF volume, they are causes of diuretic resistance. These adaptations can be classified into immediate, short-term, and chronic (Okusa and Ellison, 2008).

Immediate adaptation refers to the instantaneous secondary changes in sodium transport along the nephron *during* diuretic-induced natriuresis. An example of an immediate diuretic adaptation would be the increased sodium reabsorption along the loop of Henle during acetazolamide use. As discussed, this effect is a major factor that limits the natriuretic effectiveness of carbonic anhydrase inhibitors. Coadministration of a loop diuretic with a carbonic anhydrase inhibitor can limit sodium reabsorption along the TAL and counteract the immediate diuretic adaptations.

Short-term adaptation refers to stimulation of sodium reabsorption along the nephron once the diuretic concentration declines beneath the natriuretic threshold (Fig. 33.3). This phenomenon is often referred to as 'post-diuretic NaCl retention,' and has been attributed to three factors. First, short-term changes occur in response to an acute decrease in ECF volume. These effects are both renal and systemic and involve the activation of the renin–angiotensin–aldosterone axis and sympathetic nervous system, changes in GFR, and suppression of atrial natriuretic peptide secretion (reviewed in Ellison and Wilcox, 2008). The net effect of these responses is to enhance renal NaCl retention in an effort to increase ECF volume. Second, the decline of a diuretic drug concentration to a level beneath the natriuretic threshold induces rebound effects at its direct site of action. For example, in the case of loop diuretics, the number of NKCC2 cotransporters expressed at the apical surface of the TAL increases in response to a reduction in intracellular chloride concentration (Gagnon et al., 2004). While a loop diuretic is present in the lumen of the TAL, NaCl transport is inhibited despite any increase, but once the loop diuretic concentration declines, the increased transport capacity is unmasked. Third, post-diuretic NaCl retention occurs as a consequence of changes in sodium chloride reabsorption along nephron segments downstream of the diuretic's molecular site of action. For example, the number of thiazide-sensitive cotransporters in the DCT increases within 60 minutes of loop diuretic administration (Chen et al., 1990). This effect is likely to be a consequence of the increase in luminal sodium chloride concentration, which activates molecular mechanisms that stimulate NCC synthesis and delivery to the DCT apical surface.

Chronic adaptation, often termed the 'braking phenomenon' (Fig. 33.3), refers to the decline in natriuresis following each dose of diuretic, when the diuretic is administered repetitively. The braking phenomenon is likely due to a combination of factors. These factors include those that contribute to post-diuretic NaCl retention, such as the chronic intermittent stimulation of the sympathetic nervous and renin–angiotensin–aldosterone systems from ECF volume contraction. But other, more long-term changes also take place. One of the most significant of these is the effect of chronic diuretic therapy to induce structural changes in the epithelium lining the nephron. Specifically, chronic diuretic therapy can lead to both hypertrophy and hyperplasia of sodium chloride reabsorbing cells (Kaissling et al., 1985; Ellison et al., 1989). These effects act together to enhance the sodium chloride reabsorbing capacity of the nephron. For instance, loop diuretic infusions of 7 days increase the number and size of distal convoluted cells substantially (Kaissling et al., 1985; Ellison et al., 1989). Accordingly, the same treatment increases the number of active thiazide-sensitive NaCl cotransporters in the DCT (Kaissling and Stanton, 1988; Stanton and Kaisslin, 1988; Ellison et al., 1989). These changes increase the sodium chloride transport capacity (Ellison et al., 1989); this can undermine the therapeutic effectiveness of loop diuretics and contribute to diuretic resistance. Since chronic loop diuretic therapy increases the fraction of thiazide sensitive NaCl reabsorption, combining a low-dose thiazide with a loop diuretic can be a highly effective approach to counteracting resistance (see below).

Approach to the treatment of oedema

The treatment of generalized oedema consists of four key interventions: optimizing treatment of the underlying disorder, dietary sodium restriction, measures to mobilize fluid from oedematous tissues, and diuretic drug therapy. The focus here will be on the contribution of diuretics to this approach.

Oral diuretic therapy

When frank oedema is present, diuretics are usually necessary to reach therapeutic goals, even though dietary salt restriction is an essential element of the treatment regimen. In general, the goal of diuretic therapy in patients with ECF volume overload is to facilitate an efficient negative NaCl balance without compromising EABV substantially. The rate of fluid removal is dictated both by the urgency of need and safety. In some patients with HF, fluid readily moves from the interstitium to the intravascular compartment, and up to 2 L of oedema fluid can be removed per day without complications; in the outpatient setting, however, the goal is much less. In other situations, the rate of refilling can be slower, as in cirrhosis without peripheral oedema, where a negative fluid balance on the order of up to only 750 mL/day can be safely achieved without depleting intravascular volume (Pockros and Reynolds, 1986). Thus, in all outpatient and most inpatient situations where diuretic therapy is required, gentle but consistent fluid removal is recommended.

In the outpatient setting, the goal of therapy should be to find the minimum dose of diuretic that consistently ensures a natriuretic response. Loop diuretics are typically the initial treatment of choice for patients who present with significant generalized oedema, even though DCT diuretics may also be effective, when oedema is mild. Patients with normal GFR who are naïve to the effects of a loop diuretic can develop a natriuresis with as little as 10 mg of furosemide per day. In contrast, those with CKD typically require a higher initial dose to achieve natriuresis (Brater, 1998). In either case, the clinician can inquire about the urine output within hours after taking an oral dose of loop diuretic to gauge the therapeutic threshold (Ellison and Wilcox, 2008). In addition, patients on diuretic therapy should weigh themselves on a daily basis. If the patient does *not* perceive a significant difference in urine output or if the patient's weight does not change within a few days of starting diuretic therapy, it is unlikely that the prescribed diuretic dose is generating a negative fluid balance.

The choice of oral diuretic is not dictated by strong evidence. Furosemide has been used as first-line treatment for many years. It is effective and inexpensive, but it does suffer from limited bioavailability; furthermore, the bioavailability varies between patients, between days, and with food (Table 33.3) (Brater, 1997). In contrast, both bumetanide and torsemide exhibit excellent and more consistent bioavailability. Bumetanide has a very short half-life,

whereas torsemide's half-life is longer (Table 33.3). Based purely on pharmacokinetic parameters, torsemide's diuretic profile should make it the most effective among the three; unfortunately, the data to support this speculation are limited. Nevertheless, a comprehensive review comparing the three primary loop diuretics noted that several studies suggested better outcomes with torsemide compared with furosemide (Wargo and Banta, 2009). They found less evidence supporting the superiority of one or other loop diuretic in other clinical conditions. The cost differential between the three diuretics has declined, since all are now available generically, but bumetanide remains more expensive than furosemide. Typical starting doses for HF are 20–40 mg twice daily for furosemide, 0.5–1.0 mg twice daily for bumetanide, and 5–10 mg daily for torsemide. For CKD, it is often necessary to start with a higher dose, for reasons discussed above.

Intravenous diuretics

When a patient is hospitalized for oedema, it is often useful to use loop diuretics intravenously to obviate problems associated with limited bioavailability and guarantee efficacy. Typically, the loop diuretics are administered as bolus doses, but in some cases continuous infusions have been recommended. When given intravenously, differences in bioavailability become irrelevant, but important pharmacokinetic differences do persist. Importantly, the apparent half-life of furosemide becomes shorter, because 'absorption limited kinetics', resulting from the GI depot discussed above, is no longer relevant. Furthermore, as shown in Table 33.3, the half-life of bumetanide remains shorter, leaving more time for post-diuretic NaCl retention, when it is administered as a bolus; once again, torsemide has the longest half-life and the most favourable pharmacokinetic profile. Based on these differences, there seems little rational basis to switch from furosemide to bumetanide when given intravenously, although torsemide may still be more effective.

Many smaller trials have suggested benefits of continuous infusions. In one prospective randomized crossover trial that studied modes of diuretic administration in patients with HF, continuous furosemide infusion preceded by a loading dose produced a greater diuresis and natriuresis than a 24-hour dose equivalent of furosemide given in boluses intermittently (Lahav et al., 1992). No significant differences in side effects were noted between the two groups. Similar findings were reported from a study of patients with CKD in which bumetanide was administered either by bolus or infusion (Rudy et al., 1991). In this case, side effects were also reduced by the continuous infusion. The effectiveness of continuous loop diuretic infusion (Table 33.5) likely results from the fact that a constant supply of diuretic is being maintained in the blood stream. This serves to clamp urinary diuretic levels at a concentration above the diuretic threshold, close to the concentration of maximal diuretic efficiency (Fig. 33.2). Moreover, continuous therapy has the benefit of minimizing the adaptive effect of post-diuretic NaCl retention, and should facilitate negative fluid balance more effectively (Ellison, 1997a, pp. 209–32).

In contrast, a recent well-designed randomized controlled trial of furosemide in HF (Felker et al., 2011) compared bolus versus continuous infusion, and lower versus higher doses, of loop diuretic for patients hospitalized with acute decompensated HF. The results showed no significant difference in efficacy or safety endpoints for bolus versus continuous infusion. Patients assigned to intravenous bolus therapy were more likely to require an increased dose at 48 hours; as a result, the total dose of furosemide over 72 hours was higher in the bolus group compared with the continuous infusion group, a difference that was not quite significant (592 vs 480 mg, $P = 0.06$). In this study, the higher-dose furosemide regimen (2.5 × the daily home dose) produced greater net fluid loss, weight loss, and relief from dyspnoea, but also more frequent, though transient, worsening of renal function. There was an almost significant trend toward greater improvement in patients' global assessment of symptoms in the high-dose group ($P = 0.06$); the mean change in the serum creatinine was < 0.1 mg/dL (9 micromol/L) in both groups.

It should be emphasized that the continuous infusion protocol used in this study did not include a bolus diuretic dose at the beginning of treatment, as recommended by Brater and others (Brate, 1998; Ellison and Wilcox, 2008). In post hoc studies derived from the same dataset (Shah et al., 2012), several additional insights emerged. Although the comparison between bolus and continuous infusion was neutral overall, when baseline diuretic dose was taken into consideration, there was an interaction effect. In this case, those who presented on a lower baseline diuretic dose responded more favourably to continuous infusion (in terms of weight loss), whereas those on a higher basal dose (> 120 mg furosemide) responded better to bolus administration. These differences are likely to result from the induction of the adaptive processes described above in those individuals whose home diuretic doses were higher.

While these data suggest that the initial approach to diuretic treatment can include bolus diuretics, several caveats emerge. First, the HF trial did not examine patients who were truly resistant to loop diuretics, only those who presented to the hospital with decompensated HF. For those who fail initial bolus therapy, it may still be reasonable to try a continuous approach. Second, the trial did not examine patients with substantial CKD, a situation in which continuous infusions may be more effective, or at least safer. Third, the maximum dose, up to 600 mg/day, may not have achieved the maximal recommended dose during continuous infusion (960 mg/day or 40 mg/hour (Breater, 1998)), especially because the protocol did not necessarily deliver the maximal dose to all resistant individuals.

When switching back from intravenous to oral furosemide, however, it is often recommended to double the dose, because the average bioavailability of furosemide is approximately 50%, but this is only a guideline, since the actual relative efficacy cannot be predicted. Clearly, since bumetanide and torsemide are more completely absorbed, their oral and intravenous doses should be closer, under most circumstances.

Combination diuretic therapy

Diuretic resistance can often be treated with two classes of diuretic used simultaneously. Controlled trials (Chemtob et al., 1989) suggest little or no benefit from giving two agents of the same class (e.g. ethacrynic acid and furosemide). In contrast, adding a proximal tubule diuretic or a distal convoluted tubule diuretic to a loop diuretic is often dramatically effective. Distal convoluted tubule diuretics added to loop diuretics are synergistic (the combination is more effective than the sum of the effects of each drug alone) (Brater, 1985; Heller et al., 1985; Loon et al., 1989; Ellison, 1991; Knauf et al., 1995; Knauf and Mutschler, 1997).

Distal convoluted tubule diuretics do not alter the pharmacokinetics or the bioavailability of loop diuretics. The addition

of a distal convoluted tubule diuretic to a loop diuretic enhances NaCl excretion via several mechanisms (for a review, see Ellison, 1999). The most important mechanism is probably by inhibiting NaCl transport along the distal tubule, where tubular Na^+ and Cl^- uptake is stimulated by the loop diuretic. During prolonged loop diuretic use, distal nephron cells become hypertrophic and hyperplastic (Kaissling et al., 1985; Kaissling and Stanto, 1988; Ellison et al., 1989) and there is an increase in the abundance of Na/K-ATPase pumps (Scherzer et al., 1987; Barlet-Bas et al., 1990), NCC (Abdallah et al., 2001), and the capacity to reabsorb Na^+ and Cl^- (Stanton and Kaisslin, 1988; Ellison et al., 1989). Thus, when microperfused with a standard NaCl load, distal tubules from animals treated chronically with loop diuretics reabsorb Na^+ and Cl^- more rapidly than tubules from control animals (Ellison et al., 1989). Because distal convoluted tubule diuretics inhibit NCC even under these stimulated conditions, the effect of these diuretics will be greatly magnified in patients in whom high doses of loop diuretics have led to hypertrophy and hyperplasia. Wilcox and co-workers (Loon et al., 1989) showed that the natriuretic effect of chlorothiazide in humans was enhanced following treatment with furosemide for 1 month, suggesting that daily oral furosemide treatment, even in modest doses, may induce adaptive distal changes.

When a second class of diuretic is added, the dose of loop diuretic should not be altered, because the shape of the loop diuretic dose–response curve is not affected by addition of other classes of diuretic. Thus, the loop diuretic should be given in an effective or ceiling dose (Table 33.4). The choice of distal convoluted tubule diuretic is arbitrary. Many clinicians choose metolazone because its half-life is longer than some classic thiazide diuretics, but direct comparisons between metolazone and classic thiazides have shown little difference in natriuretic potency during combination use (Garin, 1987; Channer et al., 1994; Fliser et al., 1994).

Distal convoluted tubule diuretics may be added in full doses (Table 33.6) when a rapid and robust response is needed, but this is likely to lead to complications and an extremely close follow-up is mandatory. Patients should be monitored closely when combination therapy is begun, because fluid and electrolyte depletion, sometimes massive, occurs commonly. Serious side effects have been noted frequently (Jentzer et al., 2010). One reasonable approach is to establish a therapeutic target weight and start with a low dose of DCT diuretic. The dose can then be escalated if necessary until the clinical goals are achieved. When the target weight is attained, the distal convoluted tubule diuretic can often be prescribed only three times weekly or the dose adjusted to maintain the ECF volume at the desired level.

Another approach to combination therapy may be a short fixed-dose course. Comparison was made of adding a thiazide-type diuretic to furosemide for either a fixed 3-day period or adjusting the dose to achieve targeted volume losses during 5–7 days. Both regimens were equally effective in reducing ECF volume and symptoms. Surprisingly, natriuresis and diuresis continued even after the thiazide-type diuretic was discontinued during the fixed regimen (Channer et al., 1994). For outpatients requiring combined therapy, one approach is to add a modest dose of distal convoluted tubule diuretic, such as 2.5–5 mg/day of metolazone, for 3 days only. Higher doses or longer time periods are effective, but may increase risk. Because distal convoluted tubule diuretics are absorbed more slowly than loop diuretics (peak levels at 1.5–4.0 hours for distal convoluted tubule diuretics compared with 0.5–2.0 hours for loop diuretics), it is sensible to recommend that the distal convoluted tubule diuretic be taken 0.5–1 hour prior to the loop diuretic, although this suggestion has not been tested.

Cortical collecting duct diuretics, such as amiloride, spironolactone, or eplerenone, can be added to a regimen of loop diuretics, but their natriuretic effects are generally less dramatic than those of distal convoluted tubule diuretics (Levy, 1977; Ramsay et al., 1980). The combination of spironolactone and loop diuretics has not been shown to be synergistic, but can prevent hypokalaemia, while maintaining renal Na^+ excretion. One situation in which cortical collecting duct diuretics may be preferred agents in combination is in patients with cirrhosis. A combination of furosemide and spironolactone or eplerenone is now considered the preferred regimen for cirrhotic ascites (Runyon, 2004), where some guidelines suggest maintaining a ratio of 40 mg furosemide/100 mg spironolactone. Potassium-sparing distal diuretics also reduce Mg^{2+} excretion, making hypomagnesaemia less likely than when combined with loop diuretics.

Mineralocorticoid receptor blockade reduces mortality of patients with systolic dysfunction, whether severe (spironolactone) HF (173) or mild (eplerenone) (Zannad et al., 2011). While this effect has been attributed to direct cardiac or vascular effects (Pitt et al., 1999; Rossignol et al., 2011), renal effects are also

Table 33.4 Ceiling doses of loop diuretics

	Furosemide (mg)		Bumetanide (mg)		Torsemide (mg)	
	IV	**PO**	**IV**	**PO**	**IV**	**PO**
GFR 20–50 mL/min	80	80–160	2–3	2–3	50	50
GFR < 20 mL/min	200	240	8–10	8–10	100	100
Severe AKI	500	NA	12	NA		
Nephrotic syndrome	120	240	3	3	50	50
Cirrhosis	40–80	80–160	1	1–2	10–20	10–20
Heart failure	40–80	160–240	2–3	2–3	20–50	50

Note: ceiling dose indicates the dose that produces the maximal increase in fractional sodium excretion. Larger doses may increase net daily natriuresis by increasing the *duration* of natriuresis without increasing the maximal rate.

GFR = glomerular filtration rate.

Table 33.5 Continuous diuretic infusion

Diuretic	Loading dose	GFR < 25 mL/min	GFR 25–75 mL/min	GFR >75 mL/min
	mg	mg/hour	mg/hour	mg/hour
Furosemide	40	20–40	10–20	10
Torsemide	20	10–20	5–10	5
Bumetanide	1	1–2	0.5–1	0.5

Data adapted from Brater, D. C. (1998). Diuretic therapy. *N Engl J Med*. 339, 387–395.

likely to participate. Barr et al. (1995) randomized 42 patients with New York Heart Association class II–III congestive HF to either 50–100 mg/day of spironolactone or placebo added to a regimen of loop diuretics and ACEIs. Spironolactone increased Na^+ excretion, urinary Na/K ratio, and serum Mg^{2+} concentration, and reduced ventricular arrhythmias. Others have reported similar results (Dehlström and Karlsson, 1993; Van Vliet et al., 1993). Nevertheless, hyperkalaemia is a concern when adding spironolactone to ACEI therapy, especially in those patients with renal insufficiency. In one study, potentially life-threatening hyperkalaemia during spironolactone treatment was found to be predicted by renal insufficiency, diabetes, older age, dehydration, and concomitant use of other medications that may cause hyperkalaemia (Schepkens et al., 2001). Yet results of some other large trials suggest that spironolactone or eplerenone is often tolerated with GFR>60 ml/min/1.73 m^2 (Eschalier et al., 2013).

Combination diuretic therapy is often indicated for hospitalized patients in an intensive care unit who need urgent diuresis in the setting of obligate fluid and solute loads. Two intravenous drugs are available to supplement loop diuretics: chlorothiazide (500–1000 mg once or twice daily) and acetazolamide (250–375 mg up to four times daily). Chlorothiazide has relatively potent carbonic anhydrase-inhibiting capacity in the proximal tubule and also blocks NCC in the distal convoluted tubule. It has a longer half-life than some other thiazides. Both chlorothiazide and acetazolamide can act synergistically with loop diuretics. Acetazolamide is especially useful when metabolic alkalosis complicates the treatment of oedema, since this may make it difficult to correct hypokalaemia or to wean a patient from a ventilator (Miller and Berns, 1977). The use of acetazolamide can correct alkalosis without the need to administer saline. In other situations, combination diuretic therapy may be targeted at the underlying disease process.

Table 33.6 Combination diuretic therapy (to add to a ceiling dose of a loop diuretic)

Distal convoluted tubule diuretics:
Metolazone 2.5–10 mg P.O. daily[a]
Hydrochlorothiazide (or equivalent) 25–100 mg orally daily
Chlorothiazide 500–1000 mg intravenously
Proximal tubule diuretics:
Acetazolamide 250–375 mg daily or up to 500 mg intravenously
Collecting duct diuretics: Spironolactone 100–200 mg daily
Amiloride 5–10 mg daily

a Metolazone may be given for a limited period of time (3–5 days) or the response should be monitored very closely, once ECF volume has declined to the target level. Only in patients who remain volume expanded should full doses be continued indefinitely.

Ultrafiltration

During the past 20 years, there has been ongoing interest in using mechanical processes to reduce ECF volume, when pharmacological therapy proves insufficient. Plasma ultrafiltration, with or without accompanying haemodialysis, may be used to remove extracellular fluid. Agostoni and colleagues (Agostoni et al., 1994; Marenzi et al., 2001) randomized patients with congestive HF to equal volume removal by ultrafiltration or furosemide. The extracellular fluid volume remained contracted following ultrafiltration, but rebounded to baseline after the intravenous diuretic treatment was discontinued. The extracellular fluid volume rebound following loop diuretic usage was associated with a brisk rise in plasma renin and angiotensin II levels. These observations led to the development of methods for fluid removal without the need for central lines. Positive outcomes were suggested by smaller studies (Costanzo et al., 2005, 2007), but a more recent large randomized controlled trial for patients with acute decompensated HF found that diuretic approaches were just as successful, with fewer adverse effects than ultrafiltration (Bart et al., 2012). Another recent retrospective analysis reached similar conclusions that ultrafiltration led to worsening renal function (Dev et al., 2012). Until contradictory information becomes available, therefore, ultrafiltration will generally be reserved for situations in which patients need dialysis, as well as fluid removal.

Diuretics in special situations

Acute kidney injury

Oliguric AKI generally implies that urine output is < 400–500 mL/24 hours. It is associated with a markedly worse prognosis than AKI without oliguria (Anderson et al., 1977). Loop diuretic therapy has been proposed to serve as a potential treatment for AKI, supported by studies suggesting that loop diuretics increase the degree of oxygenation of the renal medulla (Heyman et al., 1994). In addition, since volume overload is commonly an indication for dialysis in patients with AKI, it was thought that loop diuretics might improve outcomes by minimizing the number of patients that require acute dialysis. Finally, it was also proposed that in many cases, loop diuretics could increase urinary flow and wash casts out of the kidney tubules.

Multiple small randomized controlled trials used loop diuretic therapy as an intervention to treat AKI (Brown et al., 1981; Allison and Shilliday, 1993). The results from these studies were negative; in each case, although loop diuretics were able to increase the urine

output above the defined oliguric threshold, they did not improve patient mortality or reduce the need for dialysis. It is important to note however, that these trials were small and statistically underpowered. More recently, Mehta and colleagues (2002) conducted a large-scale multicenter retrospective analysis of the outcomes of all patients hospitalized in intensive care units with AKI who were seen in nephrology consultation over a 6-year period. In this study, diuretic treatment was associated with an increased risk of death and lack of recovery of renal function. Although these findings suggest that high-dose furosemide therapy *might* be harmful to patients with AKI, it is important to note that these observational studies are subject to confounding-by-indication. Indeed, a recent meta-analysis of nine acute renal failure trials encompassing 849 patients was unable to replicate the association between loop diuretic therapy and higher patient mortality (Ho and Sheridan, 2006). There has also been interest, however, in the effects of fluid balance on mortality and morbidity in intensive care units. This led to a study testing 'conservative versus liberal' approaches to fluid balance in the intensive care unit (the FACTT) (Wiedemann et al., 2006). While this study did not assess AKI specifically, it provided the opportunity to test the effects of fluid strategies in which treatment approaches were determined randomly. A post hoc analysis of FACTT patients who developed AKI found substantially higher mortality in patients randomized to more liberal fluid administration. The conservative arm of this study involved much more aggressive use of diuretics, leading the authors to conclude that diuretic use in patients with AKI in the intensive care unit is not likely to be harmful, and may be beneficial. This suggestion warrants further evaluation (Schrier, 2009, 2010). In the meantime, it seems reasonable to consider the use of loop diuretics to maintain ECF volume in patients with AKI safe, and potentially beneficial. Their use simply to increase urine output, however, cannot be supported.

Cirrhosis

A reasonable initial daily negative fluid balance in a cirrhotic patient with ascites should total approximately 250–750 mL/24 hours. Given the overactivity of the renin–angiotensin system in cirrhotic ascites, aldosterone receptor antagonists are the first-line diuretic of choice. Spironolactone is typically prescribed initially at a dose of up to 100 mg orally per day. If the patient does not appear to respond to aldosterone receptor antagonist monotherapy, a loop diuretic such as furosemide may be added, usually starting at 20–40 mg orally per day. The American Society for the Study of Liver Disease recommends that the ratio of furosemide to spironolactone be 40 mg/100 mg (Runyon, 2004). In the setting of tense ascites requiring large volume paracentesis, diuretic therapy may need to be adjusted to account for any fluid shifts that might occur following the bulk removal of peritoneal fluid. Aquaretic therapy may eventually be useful to facilitate a water diuresis in the cirrhotic patient with oedema and hyponatraemia, and studies are currently being conducted to confirm the safety and efficacy of this novel treatment modality.

Nephrotic syndrome

Loop diuretics are the treatment of choice for oedema in the nephrotic syndrome, due to the fact that other diuretic classes are less capable of facilitating a clinically significant natriuretic effect. Massive proteinuria, the hallmark of the nephrotic syndrome, diminishes loop diuretic efficacy. When Brater and colleagues measured the diuretic efficiency of furosemide in nephrotic rats, sodium reabsorption was decreased relative to urinary furosemide excretion, compared with non-nephrotic controls (Voelker et al., 1989). This finding illustrates that, compared with their efficacy in some oedematous disorders; loop diuretics are less capable of provoking a natriuresis in the nephrotic syndrome. The authors suggested that this observation may be due to the fact that a large fraction of the furosemide that enters the loop of Henle during diuretic therapy remains bound to albumin and is, therefore, unable to inhibit Na-K-2Cl cotransport. Yet work by the same group later showed that albumin binding to loop diuretics in the tubule lumen is *not* a major contributor to diuretic resistance: agents that reduce diuretic binding to albumin had no substantial effect on diuretic efficacy in nephrotic patients (Agarwal et al., 2000). Hypoalbuminaemia also may act to diminish the effectiveness of loop diuretics. Once loop diuretics are absorbed into the bloodstream, they become largely bound to albumin. A low serum albumin level diminishes the total blood concentration of loop diuretic and increases its volume of distribution. Therefore, the renal circulation will convey less diuretic to the nephron, and less will be extruded by basolateral-to-apical proximal tubule organic anion transport into the tubule lumen for delivery to the TAL. This scenario provides the rationale for infusing albumin together with loop diuretics to patients with substantial hypoalbuminaemia, a suggestion that has received some support in the literature (Mattana et al., 1996; Blendis and Wong, 1999; Fliser et al., 1999; Gentilini et al., 1999; Brater et al., 2001). Yet there is little evidence that such an approach is useful, if the serum albumin concentration exceeds 2 g/dL (Brater et al., 2001).

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CHAPTER 34

Approach to the patient with hypo-/hyperkalaemia

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Hypokalaemia

Hypokalaemia is a common electrolyte disorder that may have a wide range of presentations. Patients may have no symptoms, exhibit neuromuscular symptoms ranging from weakness to frank paralysis, exhibit polyuria, have impairment of glucose control if they have diabetes mellitus, have exacerbation of their hypertension if hypertensive, or present with sudden death. The frequency of hypokalaemia (serum potassium concentration (S_K) < 3.5 mmol/L) largely depends on the patient population. Hypokalaemia is present in < 1% of healthy adults not receiving pharmacologic agents. However, as many as 20% of hospitalized patients on a general internal medicine service (Widmer et al., 1995) and as many as 50% of patients treated with either loop or thiazide-type diuretics (Bloomfield et al., 1986) will exhibit hypokalaemia.

Hypokalaemia frequently occurs either as a consequence or as a complication of other diseases. Thiazide and loop diuretics directly increase renal potassium excretion (Table 34.1) and result in hypokalaemia, but with excessive diuresis they also can present with prerenal azotaemia, which predisposes to hyperkalaemia. Carbonic anhydrase inhibitions, used for refractory glaucoma and for both prophylaxis and treatment of 'mountain sickness,' directly increase renal potassium excretion, but the ensuing potassium depletion and metabolic acidosis can prevent continued potassium loss (Maren et al., 1954). Individuals with secondary hyperaldosteronism, whether due to congestive heart failure, hepatic insufficiency, or nephrotic syndrome, frequently exhibit hypokalaemia. Patients that have increased NaCl delivery to the collecting duct and distal nephron, whether resulting from high dietary NaCl intake or from the use of diuretics are at high risk for hypokalaemia.

Classification of hypokalaemia

Treating hypokalaemia should begin by identifying the cause. Broadly, hypokalaemia may be due to pseudohypokalaemia, redistribution, extrarenal potassium loss, or renal potassium loss. Pseudohypokalaemia is a rare laboratory artefact and in its absence, one should determine whether the hypokalaemia is associated with normal or decreased total body potassium content. In the latter case, the hypokalaemia corrects with sufficient replacement of the potassium deficits, which may be substantial. Hypokalaemia with normal total body potassium stores reflects potassium redistribution from the extracellular to the intracellular space. In such individuals, even ample potassium replacement therapy usually fails to fully correct the S_K. Individuals with hypokalaemia and normal cellular potassium will exhibit an increase in potassium excretion commensurate with intake that is usually due to enhanced renal potassium clearance. Thus, the assessment of persistent hypokalaemia must include not only ongoing potassium losses, but also potassium intake as well.

Pseudohypokalaemia

Pseudohypokalaemia is a rare laboratory artefact from abnormal cellular uptake of potassium in the blood sample between collection and measurement, which is seen in acute myelogenous leukaemia and when blood is stored for prolonged periods at room temperature, and results in low plasma potassium measurements (Sodi et al., 2009). However, acute myelogenous leukaemia can also cause 'true' hypokalaemia through inappropriate renal loss of potassium (Perry et al., 1983).

Redistribution

The intracellular fluid contains > 98% of total body potassium, with the predominant stores in skeletal muscle (see Chapter 23). The hormones insulin, catecholamines, and aldosterone stimulate cellular redistribution-induced hypokalaemia. Insulin stimulates active cellular potassium uptake and can lead to hypokalaemia (Unwin et al., 2011). This frequently occurs acutely with treatment of diabetic ketoacidosis. Decreased end-organ responsiveness to insulin in adult-onset diabetes may contribute to the frequently observed hyperkalaemia.

Potassium redistribution is frequently due to catecholamines and sympathomimetic agents, including β_2-adrenergic agonists, dopamine, and dobutamine. These agents directly stimulate cellular potassium uptake and increase insulin release, which indirectly stimulate potassium uptake (DeFronzo, 1992; Kamel et al., 1996). Sympathomimetic-induced redistribution leading to hypokalaemia is important in acute myocardial ischaemia and the treatment of severe asthmatic attacks. Myocardial ischaemia commonly increases sympathetic tone, whether as a direct result of the ischaemia, decreased cardiac output, or pain and anxiety from the ischaemia. The resulting hypokalaemia increases the risk of ventricular arrhythmias and sudden cardiac death. Theophylline, used to treat asthma, can lead to potassium redistribution and hypokalaemia and impair respiratory muscle function with development of CO_2 retention. Beta-agonist therapy of pregnant women with premature labour can provoke hypokalaemia.

Table 34.1 Causes of renal potassium loss

1. Drugs
A. Diuretics
i. Thiazide diuretics
ii. Loop diuretics
iii. Osmotic diuretics
B. Antibiotics
i. Penicillin and penicillin analogues
ii. Amphotericin B
iii. Aminoglycosides
C. Other drugs
i. Cisplatin
ii. Ifosphamide
iii. Carbonic anhydrase inhibition
2. Hormones
A. Aldosterone
i. Primary
ii. Secondary
B. Glucocorticoid-remediable hypertension
C. Glucocorticoid-excess states
3. Magnesium deficiency
4. Intrinsic renal transport defects
A. Bartter syndrome
B. Gitelman syndrome
C. Liddle syndrome
5. Bicarbonaturia
A. Distal renal tubular acidosis
B. Treatment of proximal renal tubular acidosis
C. Correction phase of metabolic alkalosis
6. Acquired tubular transport defects
A. Recovery from acute tubular necrosis
B. Lysozymuria associated with leukaemia

States of rapid cellular proliferation such as acute leukaemia, high-grade lymphomas, granulocyte-macrophage colony-stimulating factor treatment of refractory anaemia, the initial treatment of pernicious anaemia with vitamin B_{12} (Lawson et al., 1970), or acute anabolic states can result in hypokalaemia from cellular potassium uptake. This can cause acute hypokalaemia that may lead to arrhythmias and sudden death (Lawson et al., 1972).

Hypokalaemic periodic paralysis and thyrotoxicosis

A rare, but dramatic, cause of potassium redistribution and hypokalaemia is hypokalaemic periodic paralysis (Kamel et al., 1996; Knochel, 1992). Affected individuals have normal S_K levels between attacks, but experience intermittent acute episodes of weakness, which may progress to paralysis, associated with redistribution-induced hypokalaemia. Attacks occur typically upon awakening from sleep or after resting, and may be precipitated by a large carbohydrate or salt meal, or by alcohol intake. Genetic abnormalities underlie the aetiology of most individuals with hypokalaemic periodic paralysis. Most hereditary cases exhibit an autosomal dominant distribution but an X-linked recessive form occurs and sporadic cases, presumably reflecting *de novo* mutations, have also been identified. Certain cases are due to a genetic defect in dihydropyridine-sensitive calcium channel (Ptacek et al., 1994) and others are due to defects in specific sodium channels (Jurkat-Rott et al., 2000).The attacks are best treated with oral potassium chloride if the patient is able to ingest it; otherwise intravenous potassium should be used. Because the weaknesses is due to potassium redistribution, upon resolution of the attack, S_K levels increase and, if over-aggressive potassium supplementation is continued, may result in significant increases in the S_K, potentially to dangerous levels. Carbonic anhydrase inhibitors (acetazolamide 250 mg four times daily), β-blockers or spironolactone may prevent attacks. Similar attacks with muscle weakness, hypokalaemia and thyrotoxicosis are observed in individuals of Asian descent (Knochel, 1992).

Other drugs and agents

Chloroquine poisoning (Clemessy et al. 1995), severe verapamil overdose (Minella and Schulman, 1991; Oe et al., 1998), and barium intoxication (Knochel, 1992; Rosa et al., 1992) have been reported to cause hypokalaemia. Clinical studies show little evidence of hypokalaemia with calcium channel antagonists (Freed et al., 1991), but several of these agents can accentuate the effect of catecholamines to induce hypokalaemia (Mimran et al., 1993a, 1993b).

'Pa Ping paralysis' from barium poisoning has been reported in Chinese patients ingesting food or wine with high barium concentrations (Bowen et al., 2010) and simulates familial hypokalaemic periodic paralysis. Ionized barium is a potent potassium channel blocker that impairs cellular potassium exit and repolarization of excitable tissue. Hypokalaemia with cardiac arrhythmias, skeletal muscle paralysis, and depolarization of excitable tissues is a predictable consequence of barium poisoning (Knochel, 1992).

Non-renal potassium loss

Gastrointestinal and sweat potassium losses can occasionally be sufficiently large to result in hypokalaemia. Normally, these sources of net fluid and potassium loss are small, but prolonged exertion in hot, dry environments, or chronic diarrhoea, can lead to severe potassium loss and hypokalaemia (Knochel et al., 1972).

Hypokalaemia is a predictable consequence of prolonged loss of gastric contents, from vomiting or nasogastric suctioning. Most potassium loss is indirect due to the concomitant metabolic alkalosis, which increases renal potassium excretion (Kassirer and Schwartz, 1966).

Diarrhoea, whether infectious or due to laxative abuse, can cause profound gastrointestinal potassium loss. The presence of hypokalaemia with non-anion (normal) gap metabolic acidosis should raise the possibility of diarrhoea as the aetiology of the hypokalaemia. Patients with acquired immune deficiency syndrome can develop refractory diarrhoea and hypokalaemia. Patients with laxative abuse frequently deny this condition, as do diuretic abusers, and both conditions can present a particularly difficult diagnostic

challenge. Calculation of the urine anion gap (as an indirect measure of urinary ammonium excretion) may be helpful in identifying a diarrhoea-induced (with increased urinary ammonium and negative anion gap) aetiology of hypokalaemic metabolic acidosis.

Renal potassium loss

The most common cause of hypokalaemia is increased renal potassium excretion, usually from drugs or, in rare conditions, intrinsic renal defects. Diuretics are the most common cause and Table 34.5 (later in the chapter) summarizes drugs frequently provoking hypokalaemia.

Drugs

Many medicines increase renal potassium excretion, including diuretics, certain antibiotics, and anti-neoplastic agents, and toxins. Both thiazide and loop diuretics increase urinary potassium excretion; (Siegel et al., 1992; Stacpoole et al., 1992), but loop diuretics generally have a shorter pharmacologic half-life, enabling adaptive renal potassium conservation. All diuretics, except the potassium-sparing diuretics, increase potassium excretion increasing collecting duct luminal flow rate and luminal sodium delivery and high dietary sodium chloride intake exacerbates the kaliuretic effects of diuretics.

Some antibiotics, anti-neoplastic drugs, and toxins can increase urinary potassium excretion by several mechanisms. High-dose penicillin and some penicillin analogues, such as carbenicillin, oxacillin and ampicillin, increase distal tubular delivery of a non-reabsorbable anion, which increases urinary potassium excretion (Gill et al., 1977). Polyene antibiotics, particularly amphotericin B, create cation channels in the apical membrane of collecting duct cells, which increases potassium secretion and results in impaired potassium conservation (Kamel et al., 1996). Cisplatin may induce hypokalaemia via an increase in renal potassium excretion (Jones and Chesney, 1995), and ifosfamide causes a Fanconi-like syndrome with hypokalaemia in up to 4% of patients who receive this drug (Ho et al., 1995). Toluene inhalation, which often results from 'glue sniffing', can also cause hypokalaemia, presumably by increasing renal potassium excretion (Mujais and Katz, 1992). Aminoglycoside antibiotics can cause hypokalaemia either in the presence or absence of overt nephrotoxicity. Potassium supplementation protects against experimental aminoglycoside nephrotoxicity (Thompson et al., 1990) and potassium depletion enhances aminoglycoside nephrotoxicity (Dobyan et al., 1982; Cronin and Thompson, 1991). Most antibiotics do not cause hypokalaemia, and trimethoprim and pentamidine can cause hyperkalaemia.

Hormones

Endogenous hormones are important causes of hypokalaemia. Aldosterone is an important hormone that regulates total body potassium homeostasis, and excess aldosterone activity frequently leads to hypokalaemia.

Hyperaldosteronism can be either primary or secondary. Primary hyperaldosteronism results in hypertension (Holland, 1995), in part due to the sodium-retaining effects of aldosterone and partly through direct effects of aldosterone on vascular endothelium and on vascular smooth muscle cells, and through central nervous system-induced mechanisms. In addition, the associated hypokalaemia may also contribute by sensitizing the vasculature to neurohumoral regulators of blood pressure. An aldosterone-producing adrenal adenoma (APA) is a potentially surgically curable cause of primary hyperaldosteronism, but this condition should be distinguished from bilateral adrenal hyperplasia, which is not amenable to surgical correction. With the current use of the aldosterone:renin ratio (ARR) as a screening tool for the identification of primary hyperaldosteronism, an APA is now recognized as being present in only a minority of patients with primary hyperaldosteronism (Weiner and Wingo, 2010). Angiotensin II stimulates adrenal gland aldosterone synthesis, and conditions that increase plasma angiotensin II concentration typically cause secondary hyperaldosteronism. This may occur in a variety of conditions that stimulate renin secretion, including intravascular volume depletion, congestive heart failure, acute and chronic liver dysfunction, and nephrotic syndrome. Activation of the renin–angiotensin–aldosterone system is a consistent finding in malignant hypertension (Holten and Peterson, 1955), renovascular hypertension (Simon et al., 1972), and renin-secreting tumours (Brown et al., 1973).

Certain genomic defects lead to excessive aldosterone production. In glucocorticoid-remediable aldosteronism, an adrenocorticotropin (ACTH)-regulated gene promoter is linked to the coding sequence of the aldosterone synthase gene, the rate-limiting enzyme for aldosterone synthesis (Lifton et al., 1992). Consequently, aldosterone synthase is regulated by ACTH rather than angiotensin-II, and excessive aldosterone production ensues. Congenital adrenal hyperplasia from either 11β-hydroxylase or 17β-hydroxylase enzyme deficiency, results in, excessive hypothalamic corticotropin-releasing hormone (CRH) secretion and persistent adrenal synthesis of 11-desoxycorticosterone, a potent mineralocorticoid (White et al., 1987). Phenotypically 17β-hydroxylase deficiency inhibits sex hormone metabolism and, leads to incomplete development of sexual characteristics, whereas 11β-hydroxylase deficiency results in increased androgen production, leading to early virilization of males and females.

Rarely glucocorticoids function as mineralocorticoids, causing hypokalaemia and hypertension. The glucocorticoid, cortisol, has a high affinity for the mineralocorticoid receptor, but in selectively mineralocorticoid-responsive cells normally is metabolized intracellularly by the enzyme 11β-hydroxysteroid dehydrogenase type 2(11β-HSDH2) which converts cortisol to cortisone, and cortisone does not bind to the mineralocorticoid receptor (Funder et al. 1988). The importance of this enzyme is illustrated by such rare conditions as the syndrome of apparent mineralocorticoid excess, which results from absence of 11β-HSDH2 activity (Mune et al. 1995; Ferrari et al. 1996a, 1996b). Children with this syndrome exhibit early onset severe hypertension, a high incidence of cerebral infarction, and electrolyte features of mineralocorticoid excess, including hypokalaemia (Oberfield et al., 1979; New et al., 1986). Certain natural products and drugs such as glycerrhetinic acid and carbenoxolone inhibit 11β-HSDH2, allowing cortisol to exert mineralocorticoid-like effects (Farese et al. 1991).

Magnesium depletion

Magnesium depletion is found in many clinical circumstances associated with potassium depletion (see Chapter 27). Between 10% and 40% of individuals with potassium deletion also exhibit magnesium depletion (Watson and O'Kell, 1980; Whang et al., 1992). Simultaneous magnesium and potassium depletion are observed frequently with diuretic administration, diabetic ketoacidosis, chronic alcoholism, and with the recovery from acute tubular

necrosis. This is also true in certain cases of aminoglycoside toxicity and cisplatin toxicity, hypokalaemia associated with lysozymuria in acute leukaemia, and in individuals with Gitelman syndrome (see below).

Hypomagnesaemia is frequently observed with hypokalaemia and may contribute to its development. In humans, magnesium depletion is associated with hypokalaemia (Kelepouris and Agus, 1998) and may induce renal potassium wasting (Shils, 1969). In addition, dietary magnesium depletion causes a selective potassium loss from cardiac as well as skeletal muscle, and intracellular potassium deficiency may not be restored by potassium administration alone in the presence of magnesium deficiency (Whang, 1987; Rodriguez et al., 1989). These observations suggest co-administration of magnesium may further improve potassium handling when both are deficient. Potassium loss in magnesium deficiency may also be related to a relative increase in the activity of renal potassium channels and, hence, continued urinary potassium excretion (Hille, 1992). Magnesium oxide 250–500 mg by mouth four times daily is the preferred method of replacement.

Renal transport defects

Genetic diseases of hypokalaemia are rare, but have advanced our understanding of renal physiology. In 1962, Bartter described the association of hypokalaemia, hypomagnesaemia, hyper-reninaemia, and metabolic alkalosis (Bartter et al., 1962). Further phenotypic refinement led to the recognition of two syndromes: Bartter syndrome and Gitelman syndrome (Gitelman et al., 1966).

Patients with Bartter syndrome feature hypercalciuria and present generally at an early age with severe volume depletion and evidence of 'failure to thrive'. This condition most commonly results from defects in either the renal Na-K-2Cl cotransporter gene, *NKCC2* (Simon et al., 1996a), the Kir1.1 potassium channel (ROMK) or a basolateral Cl^- channel (ClC-1). These three proteins are necessary for sodium reabsorption in the thick ascending limb of the loop of Henle (Simon et al., 1996b). Gitelman syndrome features hypocalciuria, hypomagnesaemia, milder clinical manifestations, and it generally presents at a later age. This syndrome most commonly is due to mutations affecting the thiazide-sensitive NaCl cotransporter (Simon et al., 1996c).

Hypotension and intravascular volume depletion due to renal sodium-wasting are common features in both. Hypokalaemia results, in part, from renal defects in regulation of potassium excretion resulting from failure of sodium absorption in the loop of Henle or distal convoluted tubule, which results in increased distal sodium delivery and luminal flow rates, and increased renal potassium excretion at normal S_K values. Concomitant intravascular volume depletion and secondary hyperaldosteronism further exacerbates the hypokalaemia. Treatment of the hypokalaemia frequently involves oral administration of large amounts of potassium chloride, but some degree of hypokalaemia frequently persists.

Liddle syndrome is associated with hypertension, hypokalaemia, metabolic alkalosis, and suppressed renin and aldosterone levels (Liddle et al., 1963). Defects in the collecting duct epithelial sodium channel, ENaC, are responsible for this condition, which leads to excessive sodium reabsorption and presumed volume expansion, hypertension, and suppression of renin and aldosterone (Schild et al., 1995). Mutations in the β and γ, but not the α, subunits have been reported (Bubien et al., 1996; Gao et al., 2001).

Bicarbonaturia and other poorly reabsorbable anions

Bicarbonaturia and increased excretion of poorly reabsorbable anions stimulate potassium secretion in part by reducing luminal chloride ion concentration which facilitates KCl secretion by the distal nephron and collecting duct (Ellison et al., 1985; Wingo, 1989). This occurs with intravenous infusion of the $NaHCO_3$, Na_2HPO_4, Na_2SO_4, $MgSO_4$, and semi-synthetic penicillin such as carbenicillin and related compounds. The obligatory loss of cations, to preserve electroneutrality, results in renal potassium wasting. Bicarbonaturia can result from metabolic alkalosis, distal renal tubular acidosis (RTA) or treatment of proximal RTA. In each case, increased distal tubular luminal bicarbonate delivery increases potassium secretion (Malnic et al., 1971).

Diagnosis of hypokalaemia

Evaluation of the patient with hypokalaemia should begin with a thorough history and physical examination. Fig. 34.1 provides a logical algorithm. One should first consider and exclude pseudohypokalaemia due to potassium uptake by abnormal leucocytes and consider hormones, drugs or conditions that result in redistribution of potassium from the extra- to the intracellular space. If none of these possibilities is present, then the hypokalaemia likely represents total body potassium depletion resulting from potassium loss via the kidney, gastrointestinal tract, or skin.

Diuretics are a frequent cause of hypokalaemia due to renal loss of potassium. Hypomagnesaemia-induced hypokalaemia may causes renal potassium wasting, and can occur with aggressive diuresis. Gastrointestinal potassium loss occurs from diarrhoea, vomiting, nasogastric suction, or a gastrointestinal fistula. Many cases are apparent from the history and the clinical setting, but less obvious causes include surreptitious vomiting and laxative abuse, which are frequently diagnostic challenges. Erosion of the dental enamel, metabolic alkalosis, and low urinary chloride content are all features of chronic vomiting and clues to its diagnosis. In patients with self-induced diarrhoea from catharctics, the history of laxative use may be difficult to obtain. Habitual use of anthracene laxatives, such as senna, cascara, and aloe, leads to melanosis coli (Wittoesch et al., 1958), and the diagnosis can be supported by sigmoidoscopy. Phenolphthalein is a cathartic and has been previously used in laxatives that turns pink or purple in the presence of a strong alkali. The development of a pink-purple colour to the stool after the application of NaOH or KOH suggests the diagnosis. Excessive potassium loss from the skin can result from prolonged exposure in hot environments where sweat loss is high, and this diagnosis should be apparent from the history.

Type 1 (distal) and II (proximal) RTA (see Chapter 36), diabetic ketoacidosis, and ureterosigmoidostomy also cause renal potassium loss. These conditions may present with frank acidosis (Wrong and Davies, 1959). Bartter and Gitelman syndromes are rare genetic disorders that exhibit hypokalaemia and usually exhibit metabolic alkalosis with a normal or low blood pressure. However, a much more frequent cause for this constellation of findings is surreptitious diuretic abuse. A urine screen for diuretics is an important component of the evaluation of the patient with possible Bartter or Gitelman syndrome in order to exclude surreptitious diuretic use. Bartter and Gitelman syndrome can be differentiated from each other by assessment of urinary calcium excretion, which is high with Bartter syndrome and suppressed in Gitelman syndrome.

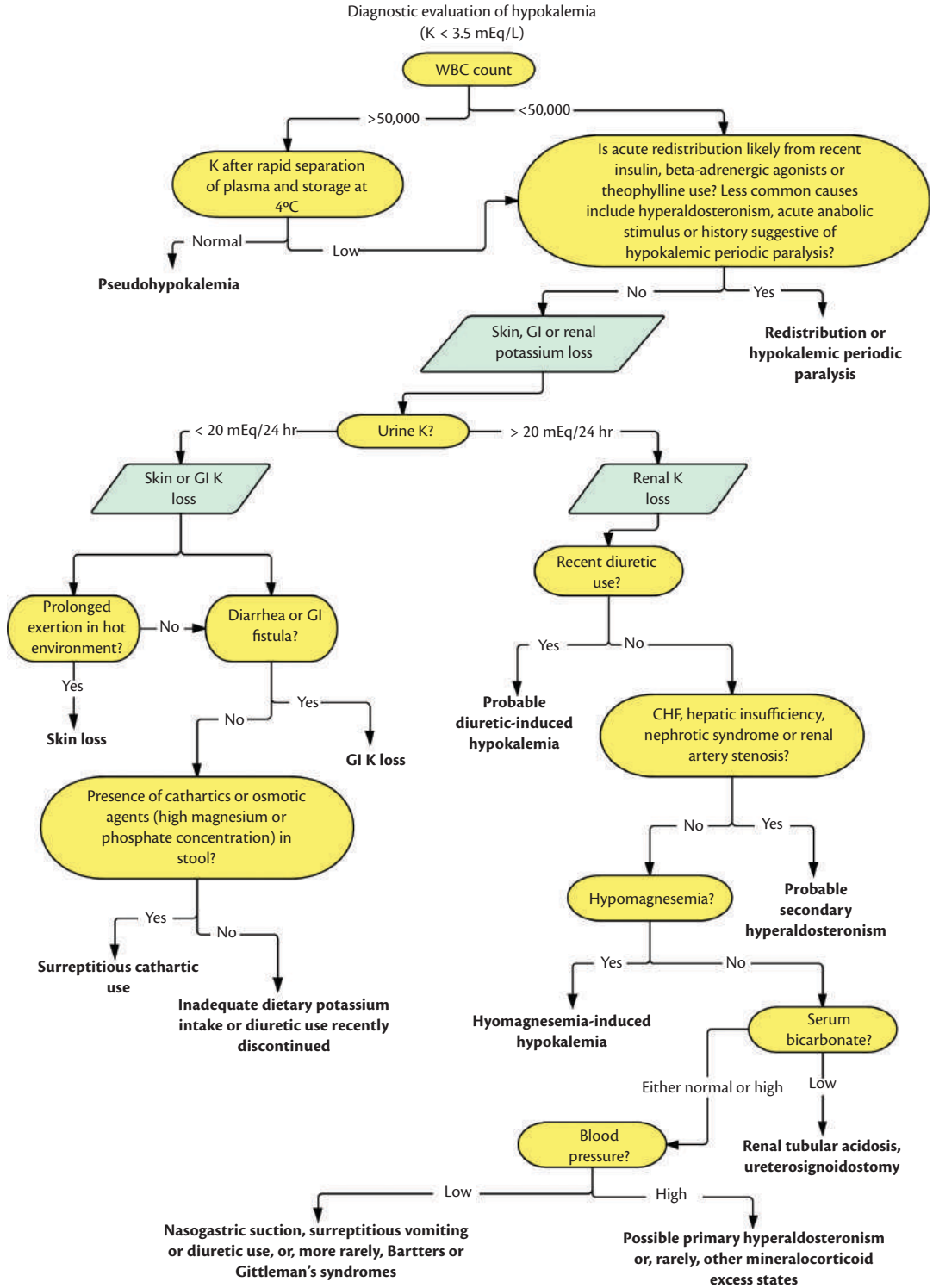

Fig. 34.1 A method for the evaluation of hypokalaemia.
Modified from Weiner and Wingo (1997).

Poor potassium intake in combination with elevated sodium chloride intake and primary hyperaldosteronism are two likely diagnoses in patients with persistently low or borderline low S_K concentrations and hypertension, particularly if metabolic alkalosis is present (Hilden and Krogsgaard, 1958; Wrong, 1961). The absolute plasma aldosterone concentration in combination with the plasma aldosterone to plasma renin activity ratio has been used to differentiate these possibilities. In patients with conditions other than primary hyperaldosteronism, the plasma aldosterone level is normal and the ARR is normal, usually 10:1. In typical primary hyperaldosteronism, the plasma aldosterone level is at least > 10 ng/mL and the ARR is elevated, typically 50:1, or greater (depending on units).

However, assays reporting renin concentration, rather than activity, will have different normal renin values and consequently different values for the ARR (higher ratio and threshold for renin activity). For complete details of the evaluation and treatment of primary hyperaldosteronism refer to textbook chapters that deal with the diagnosis of hyperaldosteronism (Weiner and Wingo, 2010). Rare causes of hypokalaemia include liquorice-like compounds, which inhibit metabolism of cortisol to cortisone by 11β-OH-steroid dehydrogenase type 2, and exhibit a clinical picture of mineralocorticoid excess (Blachley and Knochel, 1980).

Treatment of hypokalaemia

The primary short-term risks of hypokalaemia is cardiovascular, and the most important effect in the short term is to predispose to cardiac arrhythmias. However, the primary risk of too rapid potassium replacement is the development of hyperkalaemia, with resultant ventricular fibrillation. Thus, the risks associated with hypokalaemia must be balanced against the risks of therapy when determining the appropriate approach to the patient. Whenever possible, replacement therapy should be administered orally, which allows endogenous gastrointestinal potassium sensors to monitor potassium repletion therapy (Morita et al., 2000; Lee et al., 2007; Oh et al., 2011). Occasionally, incorrect therapy of hypokalaemia (e.g. using D_5W-containing solutions, see below) can lead to paradoxical worsening of the hypokalaemia.

Situations that require emergent therapy are rare but may include the patient with severe hypokalaemia that requires emergent surgery, and the concern is heightened if the patient has known coronary artery disease or is receiving digitalis. Some retrospective studies have suggested that the incidence of intraoperative complications attributable to hypokalaemia is low (Hirsch et al., 1988; Wong et al., 1993), and that occurrence of complex ventricular arrhythmias appears to correlate better with a history of long-term digoxin therapy or congestive heart failure than with plasma potassium levels (Hirsch et al., 1988). A second generally accepted indication for emergent therapy is patient with an acute myocardial infarction and significant ventricular ectopy. In such cases, administration of 5–10 mmol of KCl over 15–20 minutes and repeated as needed may be used to increase S_K above 3.0 mEq/L. Continuous monitoring of the serum level and the electrocardiogram (ECG) are necessary to reduce the risk of hyperkalaemia. Finally, hypokalaemia is frequently associated with some degree of skeletal muscle weakness and with severe hypokalaemia frank paralysis can ensue with respiratory compromise which requires urgent treatment.

The choice of parenteral versus oral therapy usually depends on the ability of the patient to take oral medicine and a normally functioning gastrointestinal tract (Weiner and Wingo, 1997). If the patient is unable to take oral potassium safely, KCl may need to be given intravenously. When given intravenously, KCl replacement can be given safely at a rate of 10 mmol/h, typically for an individual dose of 40 mmol. One study has found that 20 mmol/h of KCl causes the S_K to increase by an average of 0.25 mmol/L per hour (Kruse and Carlson, 1990). If more rapid replacement is necessary, then 40 mmol/h can be administered through a central catheter provided continuous ECG monitoring is in use. However, such rates are rarely needed and oral replacement therapy is safer and is the preferred route of administration.

The choice of parenteral fluids used for potassium administration can affect the response. Intravenous D-glucose administration increases serum insulin levels, which can stimulate cellular potassium uptake. As a result, administering KCl in D_5W can paradoxically lower S_K (Kunin et al., 1962). Thus, parenteral KCl should be provided in normal saline. If large concentrations of KCl are necessary and are added to the parenteral fluid, then KCl may be administered in half normal saline to avoid administration of a hypertonic solution, but the potassium concentration in the replacement fluid should not exceed 40 mmol/L.

Hypokalaemia usually can be treated successfully with oral therapy. If diuretic therapy is required, for example, in the treatment of hypertension or heart failure, concomitant use of a potassium-sparing diuretic, such as amiloride or triamterene, should be considered and the dietary sodium and potassium content reassessed. KCl is the preferred potassium salt in most patients, except for patients with metabolic acidosis, because it minimizes renal potassium losses. In metabolic acidosis with hypokalaemia, potassium citrate is preferred.

Hypomagnesaemia can lead to renal potassium wasting, and refractoriness to potassium replacement (Kamel et al., 1996). Correction of the hypokalaemia may not occur until the hypomagnesaemia is corrected (Shils, 1969). Patients with diuretic-induced hypokalaemia who are refractory to oral potassium chloride administration should be tested for hypomagnesaemia, and magnesium replacement therapy begun if indicated. The coexistence of other electrolyte abnormalities, particularly hypophosphataemia, should be also sought.

Hyperkalaemia

Hyperkalaemia, when severe, has predictable effects on cardiac electrical conduction which make this condition a potentially lethal disorder; however, from a clinical perspective many cases of hyperkalaemia are asymptomatic. The assessment of hyperkalaemia includes exclusion of laboratory error and pseudohyperkalaemia, determination of the urgency for treatment, and institution of appropriate therapy. Long-term treatment requires identification of the aetiology and prevention of recurrence.

Classification of hyperkalaemia

Hyperkalaemia reflects impaired potassium clearance relative to potassium intake or an altered distribution between intra- and extracellular potassium, but chronic stable hyperkalaemia without a change in potassium intake indicates renal adaptation albeit at an abnormal plasma potassium concentration. To evaluate a patient with hyperkalaemia, one should consider four broad groups of aetiologies: pseudohyperkalaemia and laboratory artefacts, excessive intake, redistribution, and impaired renal potassium clearance. A careful history and physical examination in combination with selected laboratory tests is sufficient to differentiate most cases.

Laboratory artefacts and pseudohyperkalaemia

The method of collection and sample handling can significantly affect the estimate of the patient's true blood potassium concentration. Pseudohyperkalaemia refers to reported potassium values that do not accurately reflect the potassium activity in the patient's blood and usually extracellular space. Frequently, potassium concentration is measured in blood that has been allowed to clot and centrifuged to obtain the serum. Potassium release from any of the cellular elements of blood can artificially elevate the S_K concentration.

The most common cause of pseudohyperkalaemia is haemolysis, which is usually noted by the laboratory due to a pink tinge to

the serum resulting from release of haemoglobin from the damaged red blood cells. Small needle size and excessive aspiration are frequent causes of haemolysis. Haemolysis that does not produce visible colour change to the serum should not significantly increase S_K concentration. Centrifuging the specimen before the clot has formed completely can increase the susceptibility of red blood cells to membrane damage during centrifugation. This can lead to leakage of potassium from erythrocytes and to development of pseudohyperkalaemia. In addition, exercising muscle releases potassium. Ischaemia, as with an excessively tight tourniquet, can increase S_K in some cases by > 2 mEq/L (Skinner and Adelaide, 1961). Even minimal exercise, such as 'fist squeezing' during the phlebotomy procedure, can result in sufficient skeletal muscle potassium release to invalidate the potassium measurement.

Release of potassium from leucocytes and platelets can also cause pseudohyperkalaemia. Leucocytosis (Bronson et al., 1966; Bellevue et al., 1975; Lichtman and Rowe, 1982), > 70,000/cm^3, or thrombocytosis (Hartmann and Mellinkoff, 1955; Harman et al., 1958; Paice et al., 1983), > 1,000,000/cm^3, can frequently lead to pseudohyperkalaemia. The resulting change in S_K generally is proportional to the severity of the leucocytosis or thrombocytosis, and can occur with less severe platelet or leucocyte values. With platelet counts between 500,000/cm^3 and 1,000,000/cm^3, 34% of patients exhibit pseudohyperkalaemia (Graber et al. 1988). Pseudohyperkalaemia should also be suspected if there is a family history of hyperkalaemia, or if conditions associated with significant leucocytosis or thrombocytosis are present. Rarely, pseudohyperkalaemia has been reported in association with rheumatoid arthritis (Ralston et al., 1988) and mononucleosis (Ho-Yen and Pennington, 1980). Occasional families have abnormal red blood cell membrane potassium permeability, which leads to excessive potassium leakage rates and pseudohyperkalaemia (Stewart et al., 1979; James and Stansbie, 1987; Dagher et al., 1989). Recognizing pseudo-hyperkalaemia is important, because it is purely a laboratory artefact and does not require specific therapy. Inappropriate treatment of pseudohyperkalaemia can result in serious hypokalaemia and increase the risk of hypokalaemia-related complications.

Pseudohyperkalaemia can be excluded by simultaneously measuring plasma and S_K concentrations. Plasma potassium can be measured by obtaining a heparinized blood specimen, using a 'green-top', or heparinized, tube. If the S_K is abnormal and exceeds the plasma potassium by > 0.3 mmol/L, pseudohyperkalaemia is strongly suspected, and subsequent potassium measurements should be determined using plasma samples.

Excess intake or potassium release

The normal kidney can excrete hundreds of mmoles of potassium per day (Rabelink et al., 1990), and early studies demonstrated the ability of the *normal* kidney to adapt chronically to even greater potassium loads (Schwartz, 1955). Thus, excessive potassium ingestion is an infrequent cause of hyperkalaemia *in the absence of other contributing factors*. However, if renal potassium excretion is impaired, whether through drugs, renal insufficiency, or other causes, then excess potassium intake can produce hyperkalaemia. It is important to recognize that the renal mechanisms which enable excretion of large amounts of potassium are much more sensitive to oral potassium intake than to intravenous potassium administration possibly due to gastrointestinal potassium sensors. Thus, the risk of acute hyperkalaemia with potassium supplementation is much greater with intravenous compared to oral potassium administration.

Potassium intake

Although abnormal renal potassium clearance is generally necessary for the development of persistent hyperkalaemia, excessive potassium intake is often an aggravating factor. Essentially all foods contain potassium, although the relative amounts of potassium differ greatly (Table 34.2). Common causes of hyperkalaemia are potassium supplements and salt substitutes. For example, as many as 4% of patients receiving potassium chloride supplements develop hyperkalaemia (Lawson, 1974). Salt substitutes frequently contain 10–13 mmol/g, which is equivalent to 283 mmol/tablespoon

Table 34.2 Foods rich in potassium

Greatest potassium content (> 1000 mg (25 mmol)/100 g:	Sun-dried tomatoes-
	Lima beans, mature
	Molasses
	Wheat bran, crude
Very large potassium content (>500 mg (12.5 mmol)/100 g):	Plums, dried (Prunes)
	Dates
	Peanuts, raw
	Cashews, raw
	Dried figs
	Oat Bran, raw
	Spinach, raw
	Walnuts, black dried
Large potassium content (>250 mg (6.2 mmol)/100 g)	Plantains
Vegetables	Avocados
	Potatoes
	Sweet potatoes
	Winter squash
	Beets
	Carrots
	Broccoli
	Cauliflower
	Tomatoes
	Summer Squash
Fruits	Bananas
	Purple Passion fruit
	Kiwis
	Cantaloupe
	Mangos
	Oranges
	Grapefruit
Meats	Lamb
	Lean Pork
	Steak
	Veal

(Sopko and Freeman, 1977). Many enteral nutrition products contain 40 mmol/L KCl or more; and administration of 100 mL/h of such products can result in a potassium intake of approximately 100 mmol/day. Some studies estimate that 50% of all cases of hyperkalaemia are related to potassium supplements (Shapiro et al., 1971; Paice et al., 1983; Shemer et al., 1983; Borra et al., 1988). Oral alimentation solutions are another source of dietary potassium (Table 34.3). Patients with renal failure who are receiving complete nutritional support through enteral nutritional supplements frequently develop hyperkalaemia. For intravenous hyperalimentation fluids, the potassium content recommended in normal individuals usually should be reduced when administered to patients with renal insufficiency, and frequent monitoring of the serum/plasma potassium concentration is recommended.

Medicines are another source of potassium. Potassium supplements may provoke hyperkalaemia, especially if administered intravenously. They are ordered frequently for patients receiving diuretics who may also be receiving other drugs that predispose to hyperkalaemia. Potassium supplements may be prescribed with increasing frequency for conditions other than hypokalaemia, due to the recognition that potassium supplementation decreases blood pressure (Smith et al., 1992; Whelton et al., 1997) and may improve mineral balance and skeletal calcium metabolism in post-menopausal women (Sebastian et al. 1994). This has the potential for an increased incidence of hyperkalaemia, particularly in patients with chronic kidney disease (CKD) or if the S_K is not monitored. Penicillin and citrate salts are other medicines that supply potassium. Penicillin G is supplied as a potassium salt, supplying 1.7 mmol/1,000,000 units, but may be supplied as sodium penicillin G. Citrate therapy is a common method to supply alkali to patients with a variety of conditions including advanced renal insufficiency, RTA, and nephrolithiasis. Citrate can be supplied either as a sodium salt, potassium salt, or as a sodium-potassium salt (Table 34.4). Because some citrate preparations can provide large amounts of potassium, significant hyperkalaemia may develop if one does not monitor S_K and note the method of alkali replacement therapy.

Tissue necrosis

Tissue necrosis can lead to hyperkalaemia, depending on the mass and the rapidity of the cell lysis. Common examples include rhabdomyolysis, ischaemic extremities or bowel and haemorrhage, particularly retroperitoneal haemorrhage. Rhabdomyolysis can result from crush injury, seizures, electrical shock, cocaine ingestion, sepsis, ischaemia, blunt or penetrating trauma, and excessive exertion. Of note, potassium deficiency can cause or predispose to rhabdomyolysis, resulting in potassium liberation into the extracellular fluid compartment, which results in either normokalaemia or hyperkalaemia, despite total body potassium deficiency. Rhabdomyolysis is a cause of acute kidney injury that impairs renal potassium excretion potentially lead to lethal hyperkalaemia. Large amounts of potassium and nucleic acids are liberated in the treatment of rapidly proliferating lymphomas. Without prior hydration and volume expansion to preserve urine output, hyperkalaemia and uric acid acute kidney injury may ensue (Arrambide and Toto, 1993).

Table 34.3 Potassium content of common enteral supplements

	Calories/cc	Potassium (mmol/L)	Sodium (mmol/L)	Osmolality (mOsm/kg)
Ensure®	1.06	42	35	520
Ensure Plus®	1.50	43	40	680
Glucerna®	1.00	40	40	355
Osmolite®	1.06	40	40	300
Pulmocare®	1.50	50	57	475
Suplena®	2.00	29	35	780
Ultracal®	1.06	41	40	310
Vivonex TEN®	1.00	24	27	630

Table 34.4 Potassium content of various citrate-based alkali preparations

	Potassium (mmol/mL)	Sodium (mmol/mL)	Citrate/citric acid (mmol/mL)
Polycitra-K®	2	–	2
Bicitra® (Shohl's solution)	–	1	1
Polycitra®	1	1	2

Redistribution

Several common clinical conditions are known to cause redistribution. These include membrane-depolarizing anaesthetics, and extracellular hypertonicity if due to 'effective osmoles'. In addition, less common causes of hyperkalaemia include drugs and conditions that affect membrane voltage.

Acid–base disturbances

Patients without endogenous renal function exhibit little change in S_K with acute or sustained $NaHCO_3$ infusion and substantial change in acid–base status, which suggests that the effect of acid–base disturbances on S_K is principally due to effects on renal potassium clearance (Toussaint and Vereerstraeten, 1962). In general, metabolic acidosis due to 'organic acids', such as β-hydroxybutyric acid or lactic acid, has little direct effect on S_K concentration. In diabetic ketoacidosis, hyperkalaemia is generally due to the lack of insulin-stimulated cellular potassium uptake and to the presence of glucose as an ineffective extracellular osmole (discussed in more detail below) and not the concomitant metabolic acidosis.

Hyperosmolality

Hyperosmolality can cause hyperkalaemia as a predictable effect of potassium redistribution from intracellular compartment into the extracellular space. Hyperosmolality, when caused by 'effective osmoles', can increase S_K by 1–2 mmol/L (Goldfarb et al., 1975; Makoff et al. 1970, 1971; Viberti, 1978). Hyperglycaemia, if occurring in the absence of either sufficient insulin or tissue responsiveness to insulin, and mannitol, often used in neurosurgical patients, are common causes of hyperosmolality. Hypertonicity causes cell shrinkage, leading to stimulation of net cellular potassium efflux. Hyperosmolality from solutes that rapidly cross plasma membranes, such as urea, does not alter cellular volume and does not cause hyperkalaemia. In diabetic ketoacidosis the hyperglycaemia and attendant hyperosmolality may also contribute to the hyperkalaemia in addition to the insulinopenic state.

Drugs

Drugs may interfere with the hormonal systems that regulate the distribution of potassium between the intra- and extracellular fluid compartments. Typically, they do so through predictable effects on hormone systems that regulate potassium homeostasis and renal transport mechanisms know to be involved in renal potassium excretion. Aldosterone, insulin, and β-adrenergic agonists are known to affect the transcellular potassium distribution. The normal response to increased potassium intake is increased aldosterone synthesis, and aldosterone stimulates cellular potassium uptake.

Many drugs in common use affect aldosterone synthesis or action and aldosterone's action has a major effect on the S_K. Aldosterone synthesis is regulated in large part by renin-stimulated angiotensin II production acting through the angiotensin II, type 1 (AT1) receptor. Thus, drugs that (a) decrease renin secretion, such as β-adrenergic antagonists and atrial natriuretic peptide (ANP) analogues; (b) block renin action (direct renin inhibitors); (c) inhibit angiotensin converting enzyme (ACE); (d) block AT1 receptors (ARBs); (e) decrease adrenal aldosterone synthesis; or (f) inhibit aldosterone action are frequent causes of hyperkalaemia (Atlas and Maack, 1992). Heparin is a dose-dependent inhibitor adrenal aldosterone production. It can cause hyperkalaemia in a small percentage (~ 7%) of patients, particularly when given intravenously at high doses (Oster et al., 1995). Low-molecular-weight heparin has less effect on aldosterone synthesis and is less likely to cause hyperkalaemia. Spironolactone and eplerenone, clinically important mineralocorticoid receptor antagonists, inhibit aldosterone action at the cellular level. Increased use of spironolactone and eplerenone, in response to evidence that they decrease mortality in many patients with congestive heart failure, has resulted in a substantial increase in the number of patients being admitted with hyperkalaemia. Additionally, the progesterone agonist drospirenone used in some birth control pills can cause hyperkalaemia (Cremer et al., 2010). Cationic amino acids, such as arginine and lysine, can cause hyperkalaemia, probably by exchanging with cellular potassium. Toxic levels of cardiac glycosides, such as the medicine digoxin, or from poisoning with related compounds, can lead to hyperkalaemia as a predictable effect of inhibiting Na^+,K^+-ATPase and thereby decreasing cellular K^+ uptake (Weizenberg et al., 1985; Wenger et al., 1985). Fluoride intoxication can rarely cause of death due to hyperkalaemia (Baltazar et al., 1980; Bradberry and Vale, 1995).

Effects on membrane voltage

Rarely, drugs and certain conditions can cause membrane depolarization that can lead to hyperkalaemia. The most common example is the skeletal muscle relaxant succinylcholine (Sterns et al., 1981). Hyperkalaemic periodic paralysis represents a rare form of period paralysis associated with weakness frequently provoked by exercise. Defects in the gating of skeletal muscle sodium channels has been identified as a cause of this condition (Lehmann-Horn et al., 1991). Genetic analysis has revealed mutations and polymorphisms at the SCN4A locus for this disorder and the closely related condition paramyotonia congenita (McClatchey et al., 1992). These two, rare genetic muscle disorders share common features of myotonia and episodic weakness. In hyperkalaemic periodic paralysis, patient symptoms and signs are worsened by increased S_K In paramyotonia congenita muscle cooling exacerbates symptoms (Plassart et al., 1994). Exercise frequently provokes attacks in hyperkalaemia periodic paralysis and β-adrenergic agonists have been reported to improve attacks (Wang and Clausen, 1976; Bendheim et al., 1985).

Renal potassium excretion

The normal kidney's ability to excrete potassium is sufficiently large that chronic hyperkalaemia is rarely observed unless either renal function or the renal mechanism of active potassium secretion is impaired (Schwartz, 1955). For example, daily ingestion of 400 mmol of KCl, approximately four- to ten-fold greater than the usual daily intake, increases S_K by < 1 mmol/L if renal function is normal and potassium excretion mechanisms are intact (Rabelink et al., 1990). Persistent hyperkalaemia which is not due to laboratory artefact or redistribution thus almost always involves alteration in renal potassium clearance.

Essentially all regulation of renal potassium excretion occurs in the renal collecting duct, and it proximal extension the initial collecting tubule. This is the site of action of many medicines which affect collecting duct potassium secretion (Table 34.5). Many drugs affect potassium clearance by chronically inhibiting potassium secretion, by either inhibiting aldosterone synthesis production or action, or by inhibiting the cellular processes necessary for collecting duct potassium secretion (see below).

Mineralocorticoids play an important role regulating extracellular potassium concentration through both effects on cellular potassium redistribution and by enhancing the maximum capacity for potassium secretion by the aldosterone-sensitive distal nephron. A major mechanism regulating aldosterone production is angiotensin II-stimulation of adrenal cortical cells through the AT1 receptor. Accordingly, any drug that affects angiotensin II production and/or activation of AT1 receptor can contribute to the development of hyperkalaemia. Medicines such as direct renin inhibitors, ACE inhibitors and ARBs directly or indirectly inhibit the action of the AT1 receptor and can provoke hyperkalaemia. Heparin can

Table 34.5 Drugs that frequently cause hyperkalaemia

Common	Non-steroidal anti-inflammatory drugs
	Amiloride
	Triamterene
	Spironolactone & eplerenone
	Potassium-sparing diuretics
	Ciclosporin
	Tacrolimus (FK506)
	Heparin
	Angiotensin-converting enzyme inhibitors
	Angiotensin receptor blockers
	Pentamidine
	Sulfamethoxazole-trimethoprim (high-dose therapy)
Less common	Cationic amino acids, arginine, lysine
	β-Adrenergic antagonists
	Succinylcholine
	Digitalis poisoning
	Fluoride poisoning
	Lithium toxicity

inhibit aldosterone synthase, the rate-limiting enzyme for aldosterone synthesis, and thereby, in a dose-dependent mechanism can contribute to hyperkalaemia.

Without prior adaptation, sodium reabsorption in the collecting duct is essential for maximum rates of collecting duct potassium secretion. This occurs because potassium secretion normally requires sodium absorption.

Several medications inhibit collecting duct sodium reabsorption. The potassium-sparing diuretics amiloride and triamterene are specific inhibitors of the apical sodium channel, ENaC (Hartmann and Mellinkoff, 1955), as are the antibiotics trimethoprim and pentamidine (Velazquez et al., 1993; Schlanger et al., 1994; Kleyman et al., 1995) (Lachaal and Venuto, 1989; Briceland and Bailie, 1991; O'Brien et al., 1997). High-dose trimethoprim, typically used with sulfamethoxazole to treat *Pneumocystis jirovecii* (formerly *carinii*) pneumonia, increases the S_K an average of 1.1 mmol/L (Greenberg et al., 1993b), and can cause life-threatening hyperkalaemia if not recognized (Greenberg et al., 1993a, 1993b; Hsu and Wordell, 1995; Marinella, 1995). Those with renal insufficiency and the elderly may develop hyperkalaemia even with conventional doses of sulfamethoxazole-trimethoprim (Perazella and Mahnensmith, 1996; Perlmutter et al., 1996). Lithium therapy has been reported to cause hyperkalaemia (Mercado and Michelis, 1977), which may be related to its effect on collecting duct function (Eiam-Ong et al., 1993). Severe intravascular volume depletion can cause hyperkalaemia if more proximal renal epithelial sodium reabsorption is nearly complete.

Arachidonic acid metabolites play an important role in collecting duct potassium secretion. This is in part due to their action to decrease renin release, thereby decreasing aldosterone production (Larsson et al., 1974). Arachidonic acid metabolites also regulate potassium channels; and non-steroidal anti-inflammatory drugs (NSAIDs) reduce their production and decrease potassium channel activity (Ling et al., 1992; Macica et al., 1996), thereby decreasing potassium secretion and, potentially, causing hyperkalaemia.

Renal potassium excretion requires intact basolateral Na^+,K^+-ATPase function, both to enable continued sodium reabsorption and to enable renal collecting duct cell potassium uptake and secretion. Digoxin, and its analogues, inhibit Na^+,K^+-ATPase, and through this action can cause hyperkalaemia (Citrin et al., 1972; Reza et al., 1974). In addition, Na^+,K^+-ATPase is critical for cellular potassium uptake and the maintenance of high intracellular potassium content in non-renal cells. Accordingly, digoxin, and other digitalis glycosides, can cause hyperkalaemia in predisposed patients, such as those with advanced CKD (Papadakis et al., 1985). Digoxin-induced hyperkalaemia most commonly occurs in conditions of super-therapeutic digoxin levels, but can contribute to hyperkalaemia in patients predisposed because of other co-morbid conditions or medications. Another class of pharmacologic agents with similar actions are bufadienolides, naturally occurring cardioactive steroids that have digoxin-like effects, which have been associated with several deaths (Brubacher et al., 1995).

Calcineurin inhibitors are widely used immunosuppressive medications, and have multiple effects that can contribute to hyperkalaemia. They inhibit basolateral Na^+,-K^+-ATPase and they can inhibit apical sodium reabsorption, and may have effects on the apical potassium channels involved in collecting duct potassium secretion. Accordingly, calcineurin inhibitors can, particularly in patients with impaired renal function, contribute to hyperkalaemia.

In addition to specific drugs that affect specific components of potassium secretion, decreased renal function, whether due to acute kidney injury (AKI) or chronic kidney disease (CKD) is frequently observed in individuals with hyperkalaemia. Generally, basal rates of potassium excretion are well preserved as long as the patient does not have severe reduction in urinary output (oliguria < 400 mL/day).

Obstructive uropathy

Obstructive uropathy frequently presents with hyperkalaemia. This occurs through multiple mechanisms. First, there is decreased flow through renal tubules, leading to decreased potassium secretion (Batlle et al., 1981; Pelleya et al., 1983; Perez et al., 1983). Chronic obstruction also induces a degree of collecting duct dysfunction involving altered expression of proteins involved in sodium reabsorption and potassium secretion (Batlle et al., 1981; Pelleya et al., 1983). Patients with obstructive uropathy-induced hyperkalaemia may remain hyperkalaemic for several weeks following relief of the obstruction, which reflects the delayed resolution of the collecting duct dysfunction.

Intrinsic renal parenchymal disease

Both AKI and CKD are associated with hyperkalaemia (Acker et al., 1998). Diabetic nephropathy and interstitial nephritis are frequent pathological lesions associated with involvement of the medullary interstitium that predisposes to hyperkalaemia. In some patients with modest CKD, the degree of hyperkalaemia is disproportionate to the degree of intrinsic renal disease and hypoaldosteronism secondary to subnormal plasma renin activity has been proposed to explain the acidosis and hyperkalaemia (Schambelan et al., 1972; Perez et al. 1977). Intravascular volume expansion, which may not be detectable by usual physical examination techniques, likely contributes to the hypoaldosteronism; volume expansion increases atrial natriuretic peptide (ANP) release that inhibits aldosterone synthesis (Williams, 2005). The aldosterone analogue, fludrocortisone may improve the hyperkalaemia, but is not recommended as a general therapeutic approach because it stimulates fluid retention, hypertension, and can accelerate the progression of CKD. In this syndrome, frequently referred to as hyperkalaemic RTA, diuretic and alkali therapy generally achieves better results (Rastogi et al., 1985).

Genetic hyperkalaemic syndromes

Two rare syndromes exhibit severe, persistent hyperkalaemia, but with widely divergent effects on sodium balance and blood pressure. The first, pseudohypoaldosteronism type 1, is characterized by severe renal sodium chloride wasting, dehydration, hypotension, metabolic acidosis, and hyperkalaemia with normal or elevated plasma aldosterone levels (Cheek and Perry, 1958; Bosson et al., 1986). These patients usually present in infancy and require large sodium intake. There may be both autosomal dominant and autosomal recessive patterns of inheritance (Bosson et al., 1986). Defects in the mineralocorticoid receptor (Bosson et al., 1986) and mutations of a subunit of the epithelial sodium channel (Chang et al., 1996) have been reported.

The second syndrome is referred to as pseudohypoaldosteronism type 2 or Gordon syndrome. In 1986, Gordon described 28 patients with refractory hypertension, hyperkalaemia, and normal renal function as assessed by GFR. Both renin and aldosterone values were suppressed, but these patients responded to sodium restriction and diuretic therapy (Gordon, 1986a, 1986b; Gordon and

Hodsman, 1986). In this syndrome patients frequently present during adolescence or as young adults with severe hypertension. Other features included short statue, intellectual impairment, and muscle weakness. This condition is known to be due to genetic abnormalities in specific intracellular signalling proteins (WNK kinases), resulting in overexpression of the apical sodium chloride cotransporter (NCC) in the distal convoluted tubule. Overexpression causes excessive NaCl reabsorption, leading to intravascular volume expansion and resultant hypertension. Excessive sodium chloride reabsorption also decreases sodium delivery to the collecting duct, which is proposed to blunt collecting duct potassium secretion and lead to the hyperkalaemia. Thiazide diuretics, which are inhibitors of NCC, are a highly effective therapy. (For a more detailed review of WNK kinases, see Hoorn et al. 2011).

Diagnosis of hyperkalaemia

The evaluation of hyperkalaemia should begin with the exclusion of pseudohyperkalaemia or a laboratory artefact. The severity of the hyperkalaemia and the risk of cardiotoxicity should be assessed with an ECG. Electrocardiographic changes of hyperkalaemia are potentially an ominous sign and warrant urgent corrective therapy. Comparison with a previous ECG should be done if one is available. If ambiguous, a repeat ECG immediately after calcium gluconate infusion may be informative. Acute cellular potassium release, either from tissue necrosis, rhabdomyolysis, and membrane-depolarizing states, such as succinyl choline and hyperkalaemia periodic paralysis, are usually apparent from the clinical setting. Strenuous exercise frequently leads to mild hyperkalaemia resulting from skeletal muscle potassium release. This will typically resolve spontaneously and in the absence of ECG changes, usually does not require specific therapy. After the acute stabilization of the patient, diagnosis of the factors which contributed to the development of hyperkalaemia should be preceded. As discussed above, hyperkalaemia, if not due to pseudohyperkalaemia or acute redistribution, almost always reflects contributions of impaired renal potassium excretion, either from drugs or decreased renal function, and potentially involving multiple medicines in association with decreased renal function. Excessive potassium dietary intake is also an important contributing cause to consider.

Common causes of hyperkalaemia and drugs that impair renal potassium excretion and frequently offending agents are listed in Table 34.5.

Treatment of hyperkalaemia

If true hyperkalaemia is present, the potassium content of intravenous fluids and enteral intake should be assessed, and all medicines should be reviewed. With mild hyperkalaemia, these simple measures may be sufficient. For more serious hyperkalaemia, specific treatment is necessary. Therapies for hyperkalaemia include (a) minimize the cardiac effects of hyperkalaemia, (b) induce potassium uptake by cells resulting in a decrease in plasma potassium, and (c) remove potassium from the body.

Stabilize membrane potential and antagonize cardiac effects

Intravenous calcium administration specifically antagonizes the effects of hyperkalaemia on the myocardial conduction system and on myocardial repolarization (Schwartz, 1978). Intravenous calcium is the most rapid way to treat hyperkalaemia, and is effective even in normocalcaemic patients. Effects can be seen on the ECG within seconds following the administration, and last for 20–30 minutes. A second dose can be given if no effect is seen. Because of the rapid onset of its effect, intravenous calcium administration should be the initial, and temporizing, treatment for individuals with ECG abnormalities related to hyperkalaemia. Rare exceptions include instances of hyperkalaemia induced by cardiac glycosides, or related toxins, in which digoxin-specific antibody treatment is more specific (see below). In some instances, continuous infusion may be helpful if more definitive therapies are delayed.

Several precautions should be observed when using intravenous calcium. First, calcium should not be administered in solutions containing $NaHCO_3$ because $CaCO_3$ precipitation can occur. Second, hypercalcaemia which occurs during rapid calcium infusion can potentiate the myocardial toxicity of digitalis. Although it has been traditionally taught that calcium infusion should not be used in cases of digoxin overdose, more recent evidence indicates that treatment with intravenous calcium was not associated with a greater mortality or potentially fatal dysrhythmias when used for patients with life-threatening hyperkalaemia and digoxin toxicity (Levine et al., 2011).

Although sodium salts, including $NaHCO_3$, have some of the same effects to stabilize membrane potential and antagonize the effects of hyperkalaemia on the cardiac conduction system, they are not as consistent in their action as intravenous calcium. They may also worsen hyperkalaemia if administered as hypertonic solutions due to their effects on serum osmolality. Their use is not routinely recommended in the acute setting to oliguric patients unless deemed necessary for coexisting metabolic acidosis.

Accelerate cellular potassium uptake

The second most rapid method to treat hyperkalaemia is to alter potassium distribution by increasing cellular uptake. Insulin is the most consistent and frequently used hormone to promote cellular potassium uptake, but β_2-adrenergic agonist can be used also. Insulin rapidly stimulates cellular potassium uptake by extrarenal cells, primarily hepatocytes and myocytes (Andres et al., 1962). Insulin, 10 U, should be administered intravenously to ensure rapid and consistent bioavailability, and will begin to affect S_K levels within 10–20 minutes, with effects lasting for 4–6 hours (Clausen and Hansen, 1977). Glucose is generally co-administered to avoid hypoglycaemia, but should not be given to hyperglycaemic individuals. Glucose-induced hyperglycaemia can lead to further increases in the potassium concentration due to hypertonicity-induced potassium redistribution. If glucose is not administered, frequent rechecks of the serum glucose level should be performed because of the possibility of insulin-induced hypoglycaemia. In patients with CKD, delayed insulin clearance resulting from the CKD, can lead to more persistent effects of insulin on glucose levels; these patients should also have frequent reassessment of the serum glucose in order to detect possible insulin-induced hypoglycaemia.

β_2-agonist administration is a second method to stimulate cellular potassium uptake and treat hyperkalaemia. Intravenous albuterol (salbutamol), 0.5 mg, rapidly stimulates prompt potassium uptake, and can decrease S_K by approximately 1 mmol/L (Montoliu et al., 1987). Although this agent is not approved for intravenous use in the United States, nebulized β_2-agonists can be used. Albuterol, when administered by nebulizer at a dose of 10 or 20 mg decreases S_K by 0.6 and 1.0 mmol/L, respectively, with an immediate onset of action and maximal effect at 90–120 minutes (Allon et al.,1989).

The primary limitations of β_2-agonist therapy are tachycardia when given intravenous (Montoliu et al., 1987) and lack of response in 20–33% of patients when given by nebulizer (Allon et al., 1989; Liou et al. 1994). In addition, albuterol may decrease potassium removal during subsequent haemodialysis (Allon and Shanklin 1995). Of note, the dose of albuterol required is two- to eightfold greater that usually given by nebulizer and 50–100 times the dose administered by metered dose inhalers (Greenberg 1998). In severe hyperkalaemia, combined therapy with insulin and albuterol may be more effective than either alone (Allon and Copkney, 1990).

Importantly, these medications have only temporary effects on S_K. Within 4–6 hours they are removed from the body by normal metabolism, and their effect is no longer present. Accordingly, their use is indicated only when needed as a temporizing approach before more definitive measures, typically to increase potassium removal, are instituted. Bicarbonate administration cannot be justified in the absence of renal function as a primary treatment for hyperkalaemia, but may be beneficial if there is a coexistent metabolic acidosis. Although early studies demonstrated a substantial benefit of bicarbonate therapy, most of these patients had significant acidosis and residual renal function, in which case bicarbonate administration increases renal potassium excretion. However, in patients without endogenous renal function the changes in S_K with intravenous bicarbonate are small and inconsistent (Blumberg et al., 1988; Allon and Shanklin, 1996; Kim 1996). Generally, sodium bicarbonate therapy should be reserved for those patients with intact renal function, metabolic acidosis, and either intravascular volume contraction or normal intravascular volume. In such patients administration of 5% dextrose solutions with the addition of 150 mmol/L sodium bicarbonate (3 amps of sodium bicarbonate in 1L D_5W) may correct the acidosis, promote a kaliuresis, and correct the hyperkalaemia.

Enhance potassium removal

Removal of potassium from the body is the definitive treatment for hyperkalaemia requires. Table 34.6 summarizes the available options for potassium removal.

In many patients, renal potassium elimination may be adequate for treatment of hyperkalaemia. With chronic, mild hyperkalaemia stimulation of renal potassium excretion with either loop or thiazide diuretics may suffice. Diuretics are usually less effective for acute hyperkalaemia because the rate of potassium excretion usually will not be adequate, and most patients with hyperkalaemia have underlying renal insufficiency as a contributing factor (Acker et al., 1998). If a rapidly reversible cause of renal failure is present, such as obstructive uropathy, treatment of the underlying condition and close assessment of the potassium level in association with continuous ECG observation may be adequate.

A second mode of potassium elimination is with the resin, sodium polystyrene sulphonate. This resin exchanges sodium for potassium in the gastrointestinal tract, and allows potassium elimination in the stool. In general, 1 g of sodium polystyrene sulphonate removes approximately 0.5–1.0 mmol of potassium in exchange for 2–3 mmol of sodium. This agent can be administered either orally or per rectum as a retention enema. The rate of potassium removal is relatively slow, requiring approximately 4 hours for full effect. When given orally, sodium polystyrene sulphonate is generally administered with 20% sorbitol to avoid constipation. If given as an enema, sorbitol should usually be omitted because several case reports suggest an association between rectal administration of sodium polystyrene sulphonate with 20% sorbitol and subsequent colonic perforation (Lillemoe et al., 1987; Gerstman et al., 1992; Rashid and Hamilton, 1997). Animal models suggest that the sorbitol is responsible for the colonic perforation, possibly due to mucosal dehydration related to fluid loss into the colon lumen (Lillemoe et al., 1987).

Dialysis should be considered for potassium removal when renal function in absent and hyperkalaemia is persistent or severe despite medical therapy. In this setting, and if vascular access is immediately available (arteriovenous fistula, haemodialysis catheter, etc.) and haemodialysis is also readily available, this modality provides rapid potassium removal but dialysis does not obviate the need for medical treatment until dialysis has commenced. With severe hyperkalaemia, there is an urgency to reduce the plasma potassium concentration, but precipitous reduction can precipitate cardiac arrhythmias (Feig et al., 1981). Thus, the use of a 0 or 1 mmol/L K^+ dialysate generally should be avoided to prevent precipitating hypokalaemia. Depending on the patient, their history of cardiac disease and the degree of hyperkalaemia a 3 mmol/L K^+ dialysate during the first 1–2 hours of dialysis followed during the remaining time with a 2 mmol/L K^+ dialysate, is likely to be both safe and effective. Continuous dialytic modalities, such as peritoneal dialysis or chronic venovenous haemodialysis are effective for chronic hyperkalaemia, but do not remove potassium sufficiently quickly for use in life-threatening hyperkalaemia.

Table 34.6 Treatment of hyperkalaemia

Mechanism	Therapy	Dose	Onset	Duration
Antagonize membrane depolarization	Calcium	Calcium gluconate, 10% solution, 10 mL intravenously (IV) over 10 minutes	1–3 minutes	30–60 minutes
Increase cellular potassium uptake	Insulin	Regular insulin, 10U IV; add dextrose, 50%, 50 mL IV if plasma glucose < 250 mg/dL	30 minutes	4–6 hours
	β_2-adrenergic agonist	Nebulized albuterol (salbutamol), 10 mg	30 minutes	2–4 hours
Remove potassium	Sodium polystyrene sulphonate	Kayexalate, 60 g orally, in 20% sorbitol, or Kayexalate, 60 g per retention enema, without sorbitol	1–2 hours	4–6 hours
	Haemodialysis	Blood flow and dialysate flow as tolerated; avoid excessive K gradient (e.g. 1 or 0 mmol/L [K^+] dialysate) which can cause cardiac ectopy and hypokalaemia).	Immediate	Until dialysis completed

It is important for the clinician to recognize that although dialysis is the most rapid method to treat most cases of hyperkalaemia, other treatments should not be delayed while waiting to initiate dialysis. In many instances, dialysis initiation can be substantially delayed such as during non-routine dialysis hours or if vascular access for haemodialysis is not already present. Thus, whereas haemodialysis removes more potassium than either restoration of renal function after chronic obstruction or with administration of sodium polystyrene sulphonate, the time required to institute therapy is frequently greater, and the patient may progress to life-threatening hyperkalaemia if other therapies are not instituted while awaiting dialysis.

Specific therapies may be quite valuable and depend on the causes of hyperkalaemia. In cases of digitalis or related toxicity, digoxin-specific antibody Fab fragments administration may be beneficial (Smith et al., 1982; Marchlinski et al., 1991). However, impaired excretion of the Fab fragments occurs in patients with concomitant renal insufficiency. In these patients, because of delayed clearance of the Fab fragments, there can be delayed release of digoxin and recurrence of digoxin toxicity. Repeat doses of the Fab fragments may be necessary in this condition. Because serum levels are not interpretable in the presence of antidigoxin antibodies, the decision as to whether to provide additional doses must be based on clinical indications of recurrence of digoxin toxicity. Thus, substantial adjustment may be required by the clinical setting. Relief of urinary tract obstruction may effectively treat the associated hyperkalaemia, but the rate of potassium excretion may be variable, which requires frequent measurement of plasma potassium. Preparation of this chapter supported by funding from the NIH (R01 DK082680 to CSW, and DK045788 to IDW) and from the Department of Veterans Affairs (I01 BX001472-01 to CSW, and I01-BX00818 to IDW).

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CHAPTER 35

Approach to the patient with metabolic acidosis or alkalosis

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Introduction

The concentration of hydrogen ions (H^+) in all body compartments is maintained at a very low level, because H^+ may bind very avidly to histidine residues in proteins. Binding of H^+ to proteins changes their charge to a more positive valence, which might alter their shape, and possibly their functions. Since most proteins are enzymes, transporters, contractile elements, and structural compounds, a change in their function could pose a major threat to survival. The concentration of H^+ in body fluids is exceedingly tiny (in the nmol/L range) and, moreover, is maintained within a very narrow range. In the extracellular fluid (ECF) compartment, the $[H^+]$ is 40 ± 2 nmol/L, while in the intracellular fluid (ICF) compartment, the $[H^+]$ is approximately 80 nmol/L. These are all the more impressive, because an enormous quantity of H^+ is produced and removed by metabolism each day. In more detail, acids are obligatory intermediates of carbohydrate, fat, and protein metabolism. For example, since adults typically consume and oxidize 1500 mmol of glucose per day, at least 3000 mmol (3,000,000,000 nmol) of H^+ are produced, as pyruvic and/or L-lactic acids in glycolysis. Their oxidation to carbon dioxide (CO_2) and water (H_2O) removes both the H^+ and conjugate bases almost as quickly as they are formed. However, in an adult eating a typical Western diet, a net of 70 mmol (70,000,000 nmol) of H^+ are added daily to the body. This implies that there are very effective control mechanisms that minimize fluctuations in $[H^+]$.

Acid–base balance

An analysis of acid–base balance must consider not only acid balance, but also the balance for bases or alkali (see Chapter 24).

Acid balance

There are three major components to consider in the physiology of acid balance (Halperin et al., 1987). First, H^+ are produced during the metabolism of sulphur-containing amino acids. Second, H^+ are removed from the body largely because they react with bicarbonate ions (HCO_3^-), forming CO_2 and H_2O. The CO_2 is eliminated via the lungs. The net result of these reactions is a deficit of HCO_3 in the body that is equal to the H^+ gain. Since H^+ cannot be excreted with the sulphate anions, and sulphate anion cannot be metabolized to regenerate HCO_3^-, the third component of the process to achieve acid balance is to generate new HCO_3^- to replace the HCO_3^- lost in titrating these H^+. This is done through metabolism of the amino acid glutamine in the cells of the proximal convoluted tubule (PCT) to yield ammonium (NH_4^+) and α-ketoglutarate anion. Metabolism of α-ketoglutarate anion to neutral end-products (CO_2 or glucose) yields HCO_3^- that are added to the body. Nevertheless, for a net gain of HCO_3^-, NH_4^+ must be made into end products of metabolism by being excreted in the urine (Fig. 35.1). H^+ are also produced when dietary phosphates enter the body as monovalent inorganic phosphate (H_2PO_4). One does not need urinary NH_4^+ excretion to restore acid balance, because at usual urine pH of about 6, filtered HPO_4^{2-}anions are excreted in the urine as $H_2PO_4^-$.

Base-balance

The diet also provides alkaline salts; the best example is the ingestion of citrus fruits that contain potassium (K^+) plus citrate anions. Metabolism of these citrate anions occurs rapidly in the liver, the net result is the production of HCO_3^-. Removal of this HCO_3^- load is achieved by production of a variety of new endogenous organic acids (e.g. citric acid) (Fig. 35.2). The H^+ of these acids titrate HCO_3^- and base balance is maintained by excreting the conjugate base of these acids, for example $citrate^{3-}$, in the urine as their sodium (Na^+), K^+, and/or calcium (Ca^{2+}) salts (Cheema-Dhadli et al., 2002). This disposal of alkali with the excretion of organic anions serves also to minimize the risk of forming calcium-containing kidney stones.

Metabolic acidosis

Metabolic acidosis is a process that causes a fall in the concentration of HCO_3^- in plasma (P_{HCO3}) and a rise in the concentration of H^+. Metabolic acidosis represents a diagnostic category with many different causes (Table 35.1). The risks for the patient are those due to the underlying disorder that caused the metabolic acidosis, the ill effects due to the binding of H^+ to intracellular proteins in vital organs (e.g. the brain and the heart), and possible dangers associated with the anions that accompanied the H^+ load (e.g. chelation of ionized calcium (Ca^{2+}) by citrate anions in a patient with metabolic acidosis due to ingestion of a large amount of citric acid) (DeMars et al., 2001).

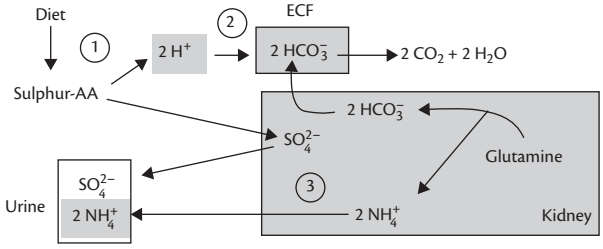

Fig. 35.1 H^+ balance during the metabolism of sulphur-containing amino acids. Renal events are represented in the shaded area. When sulphur-containing amino acids are converted to SO_4^{2-} anions in the liver, 2 H^+ are produced (*site 1*). These H^+ will react with HCO_3^- and this produces a deficit of HCO_3^- in the body (*site 2*). To achieve H^+ and HCO_3^- balance, an equivalent quantity of new HCO_3^- must be regenerated and this occurs when the kidney excretes 2 NH_4^+ per SO_4^{2-} anion (*site 3*).

Our goal is to provide a logical bedside approach to the management of the patient with metabolic acidosis (Kamel and Halperin, 2006). The first step is to determine if an emergency is present and to anticipate and prevent threats that may develop during therapy (Fig. 35.3). The concepts that provide the underpinning of our clinical approach are outlined, followed by a discussion of specific causes of metabolic acidosis.

Clinical approach to the patient with metabolic acidosis

Concept: the P_{HCO3} is the ratio of the content of HCO_3^- in the ECF compartment to the ECF volume (Equation 35.1).

$$\left[HCO_3^-\right] = \frac{\text{Content of } HCO_3^- \text{ in the ECF compartment}}{\text{ECF volume}} \qquad (35.1)$$

It is important to distinguish between acidaemia, or a lower plasma pH, and acidosis. Acidaemia may not be present in a patient who has metabolic acidosis. The P_{HCO3} may not be appreciably low, even though there is a marked decrease in the content of HCO_3^- in the ECF compartment, if there is a large decrease in the in the ECF volume. Clinical examples of this have been described in a patient with severe diarrhoea (Zalunardo et al., 2004), and in some patients with diabetic ketoacidosis (DKA) (Napolova et al., 2003). To make a diagnosis of metabolic acidosis in this setting, a quantitative estimate of the ECF volume is needed to assess its *content* of HCO_3^-.

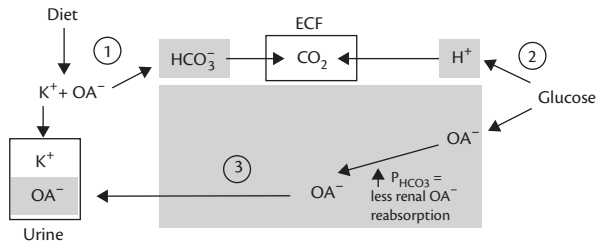

Fig. 35.2 Overview of base balance. Step 1 is the production of HCO_3^- from dietary K^+ salts of organic anions. In step 2, organic acids were produced and their H^+ titrated these new added HCO_3^-. In step 3, the renal component of the process, is shown in the shaded rectangle. The organic anions (OA^-) are made into end-products of metabolism by being excreted in the urine.

To obtain a quantitative assessment of the ECF volume we use the haematocrit (Napolova et al., 2003), whereas others use the concentration of total proteins in plasma (Love and Phillips, 1969). The assumptions are that the patient does not have a pre-existing anaemia or a low total plasma protein concentration. The haematocrit is the ratio of total red blood cell (RBC) volume (2 L in a normal adult) to blood volume (5 L in a normal adult) (Equation 35.2). For example, if the initial haematocrit is 60%, this implies that the plasma volume is contracted by more than 50%:

$$\begin{aligned}&\text{Normal :}\\&0.40 = 2\text{ L RBC volume} / 5\text{ L Blood volume}\left(3\text{ L plasma} + 2\text{ L RBC}\right)\\&\text{Patient :}\\&0.60 = 2\text{ L RBC volume} / 3.3\text{ L blood volume}\left(1.3\text{ L plasma} + 2\text{ L RBC}\right)\end{aligned} \qquad (35.2)$$

Concept: H^+ must be removed by the bicarbonate buffer system (BBS) to avoid their binding to intracellular proteins.

Buffering of H^+ load should not only diminish the degree of acidaemia, but also, more importantly, minimize the binding of H^+ to proteins in cells of vital organs (i.e. brain and heart). Binding of H^+ to proteins could change their charge, shape, and possibly their functions. To minimize binding of H^+ to proteins, H^+ removal should be carried out by the BBS, the bulk of which is in the ICF compartment and the interstitial space of skeletal muscle (Vasuvattakul et al., 1992). As shown in Equation 35.3, the BBS is driven by 'pull' (i.e. by a lower PCO_2 primarily in the interstitial space and ICF of skeletal muscle).

$$H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow H_2O + CO_2 \qquad (35.3)$$

Acidaemia stimulates the respiratory centre, which leads to a fall in the arterial PCO_2. While the arterial PCO_2 sets the lower limit on the PCO_2 in capillaries, it does not guarantee that the capillary PCO_2 in skeletal muscle will be low enough to ensure effective buffering of H^+ by the BBS. Because the free-flowing brachial venous PCO_2 reflects the capillary PCO_2 in skeletal muscles, it should provide a means of assessing the effectiveness of the BBS in patients with metabolic acidosis (Fig. 35.3) (Gowrishankar et al., 2007). The capillary PCO_2 in skeletal muscles will be higher if the rate of blood flow to muscles is low for example as a result of decreased effective arterial blood volume (EABV). In this setting, if muscle oxygen consumption remains unchanged, more oxygen will be extracted from, and more CO_2 will be added to, each litre of blood. The higher PCO_2 in muscle capillaries will diminish the effectiveness of their BBS to remove extra H^+; hence, acidaemia may become more pronounced, with the risk that more H^+ will be titrated by intracellular proteins, including critical enzymes in vital organs (Fig. 35.5).

At usual rates of blood flow and metabolic work at rest, the brachial venous PCO_2 is approximately 6 mmHg > arterial PCO_2 (Geers and Gros, 2000). If the blood flow rate to muscles is low, their venous PCO_2 will be > 6 mmHg greater than arterial PCO_2. Enough saline should be administered to increase the blood flow

Table 35.1 Causes of metabolic acidosis

Acid gain: with the retention of new anions in plasma
1. L-lactic acidosis
A. Due predominantly to overproduction of L-lactic acid
i. Hypoxic lactic acidosis:
Inadequate deliver of O_2 (cardiogenic shock, shunting of blood past organs, e.g. sepsis, or excessive demand for oxygen, e.g. seizures)
ii. Increased production of L-lactic acid in absence of hypoxia:
Overproduction of NADH and accumulation of pyruvate in the liver (e.g. metabolism of ethanol plus a deficiency of thiamine)
Decreased pyruvate dehydrogenase activity (e.g. thiamine deficiency, inborn errors of metabolism)
Compromised mitochondrial electron transport system (e.g. cyanide, riboflavin deficiency, inborn errors affecting the electron transport system)
Excessive degree of uncoupling of oxidative phosphorylation (e.g. phenformin or metformin)
B. Due predominantly to reduced removal of L-lactate: liver failure (e.g. severe acute viral hepatitis, shock liver, drugs)
C. Due to a combination of reduced removal and overproduction of L-lactic acid
Antiretroviral drugs (inhibition of mitochondrial electron transport plus hepatic stenosis)
Metastatic tumours (especially large tumours with hypoxic areas plus liver involvement)
2. Ketoacidosis (diabetic ketoacidosis, alcoholic ketoacidosis, hypoglycaemic ketoacidosis including starvation (ketoacidosis), ketoacidosis due to a large supply of short-chain fatty acids (i.e. acetic acid from fermentation of poorly absorbed carbohydrate plus inhibition of acetyl-CoA carboxylase)
3. Renal insufficiency (metabolism of dietary sulphur-containing amino acids and decreased renal excretion of NH_4^+)
4. Metabolism of toxic alcohols (e.g. formic acid from metabolism of methanol, glycolic acid, and oxalic acid from metabolism of ethylene glycol)
5. D-lactic acidosis
6. Pyroglutamic acidosis
Loss of $NaHCO_3$
1. Direct loss of $NaHCO_3$
A. Via the GI tract (e.g. diarrhoea, ileus, fistula)
B. Via the urine (proximal renal tubular acidosis or low carbonic anhydrase II activity)
2. Indirect loss of $NaHCO_3$ (low urinary NH_4^+ secretion):
A. Low glomerular filtration rate
B. Renal tubular acidosis
i. Low availability of NH_3 (urine pH ~ 5):
Problem in PCT causing low ammoniagenesis: alkaline ICF pH in PCT (e.g. hyperkalaemia) and/or problem in the renal medullary NH_3 transfer
ii. Defect in net distal H^+ secretion (urine pH often ~7):
H^+ ATPase defect or alkaline α-intercalated cells (a number of autoimmune disorders or disorders with hyper γ-globulinaemia, e.g. Sjögren syndrome)
H^+ back-leak (e.g. amphotericin B)
HCO_3 secretion in the collecting ducts (e.g. a molecular defect in the Cl^-/HCO_3^- exchanger leading to its mis-targeting to the luminal membrane of α-intercalated cells (e.g. some patients with Southeast Asian ovalocytosis)
iii. Problem with both distal H^+ secretion and medullary NH_3 transfer (urine pH ~6):
Diseases involving the renal interstitial compartment (e.g. sickle cell disease)

rate to muscle to achieve a brachial venous PCO_2 that is no more than 6 mmHg greater than arterial PCO_2.

Initial steps in the clinical approach to the patient with metabolic acidosis:

1. Determine if emergencies are present, anticipate and prevent dangers that may arise during therapy (Fig. 35.3).
2. Determine whether H^+ have been buffered adequately by the BBS in skeletal muscle.
3. Determine whether the basis of the metabolic acidosis is due to added acid and/or a deficit of base ($NaHCO_3$) (Figs 35.4 and 35.6).

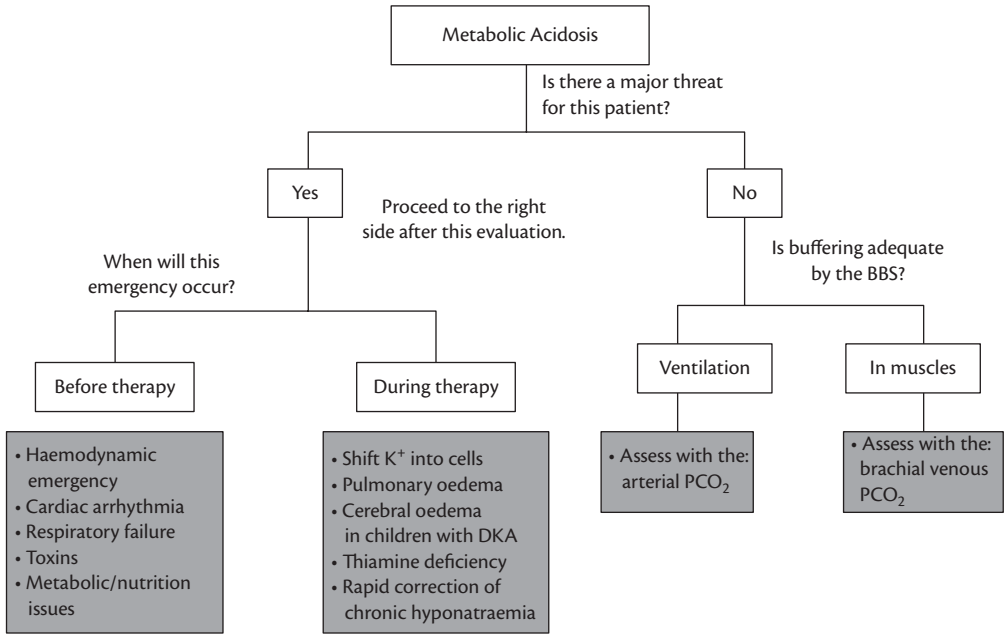

Fig. 35.3 Initial steps in the clinical approach to a patient with metabolic acidosis.

Metabolic acidosis due to added acids

Concept: addition of H^+ can be detected by the appearance of new anions. These new anions may remain in the body, and/or be excreted in the urine or diarrhoea fluid.

Acid–base balance is maintained if the new anions are metabolized to neutral end products, or if they are excreted in the urine along with H^+ or NH_4^+. On the other hand, there is a net gain of H^+ in the body if these anions are retained in the body or are excreted as their Na^+ or K^+ salts.

The accumulation of new anions in plasma can be detected from a calculation of the anion gap in plasma ($P_{Anion\ gap}$) (Emmett and Narins, 1977; Emmett, 2006; Kraut and Madias, 2007) (Equation 35.4). The major cation in plasma is Na^+, and the major anions are Cl^- and HCO_3^-. The term $P_{Anion\ gap}$ is used for the difference between the plasma concentration of Na^+ (P_{Na}) and the concentrations of Cl^- (P_{Cl}) and HCO_3^- (P_{HCO3}), which reflect the usual excess of the other unmeasured anions in plasma over that of the other unmeasured cations in plasma. This difference is largely due to the net anionic valence on plasma proteins, principally plasma albumin ($P_{Albumin}$). If the difference is larger than the 'normal' value of the $P_{Anion\ gap}$, then other anions are present in plasma. Of note, however, because of difference in laboratory methods (e.g., measurement of P_{Cl}), there is a large difference in the mean value for the $P_{Anion\ gap}$ reported by clinical laboratories. Furthermore, regardless of the laboratory method used, there is a wide range within the normal values of the $P_{Anion\ gap}$. The clinician should know what are the normal values $P_{Anion\ gap}$ for his/her clinical laboratory. Nevertheless, it is difficult to know what is the individual patient base line $P_{Anion\ gap}$ considering the wide range of normal values. When using this calculation to detect the presence of new anions in plasma, one must adjust the baseline value of the $P_{Anion\ gap}$ for the $P_{Albumin}$. As a rough estimate, the the $P_{Anion\ gap}$ rises or falls by approximately 2.5 mEq/L for every 10g/L rise or fall in the $P_{Albumin}$.

$$P_{Anion\ gap} = P_{Na} - \left(P_{Cl} + P_{HCO3}\right) \quad (35.4)$$

Another approach to detect new anions in plasma was recommended by Stewart (1981); it is based on calculation of strong ions difference. This approach is rather complex and offers only a minor advantage over the $P_{Anion\ gap}$ in that it includes a correction for the net negative charge on $P_{Albumin}$.

Use of delta AG/delta HCO_3^-

It is widely held that the rise in the concentration of new anions, as reflected by a higher value for the $P_{Anion\ gap}$, should be equal to the fall in the P_{HCO3}. This relationship is used to detect the presence of coexisting metabolic alkalosis (the rise in $P_{Anion\ gap}$ is *larger* than the fall in P_{HCO3}) and/or the presence of both an 'acid over-production type' and a '$NaHCO_3$ loss' type of metabolic acidosis (the rise in $P_{Anion\ gap}$ is *smaller* than the fall in P_{HCO3}).

There are several pitfalls in using the $P_{Anion\ gap}$ and this relationship that must be recognized (Halperin and Kamel, 2010).

Failure to adjust for changes in the ECF volume: this is an important step, as illustrated in the following paragraphs.

Clinical example: a patient with diabetic ketoacidosis (DKA) has a fall in the P_{HCO3} from 25 to 10 mmol/L and the expected rise in his $P_{Anion\ gap}$ of 15 mmol/L. This patient had a normal ECF volume of 10 L before DKA developed, but as a result of the glucose-induced osmotic diuresis, his current ECF volume is 7 L. Although, the fall in the P_{HCO3} and the rise in the concentration of ketoacid anions in plasma (as reflected by the rise in $P_{Anion\ gap}$) are equal, the deficit of HCO_3^- exceeds the amount of ketoacids added to the ECF compartment. The deficit of HCO_3^- is 180 mmoles ([10 L × 25 mmol/L] – [7 L × 10 mmol/L]), but the quantity of extra new ketoacid anions in the ECF is only 105 mmoles (7 L × 15 mmol/L). Thus, there is another important explanation for the deficit of HCO_3^- when ketoacids were added, which is that some of the ketoacid anions were excreted in the urine with K^+ and/or Na^+ (indirect form of $NaHCO_3$ loss) and

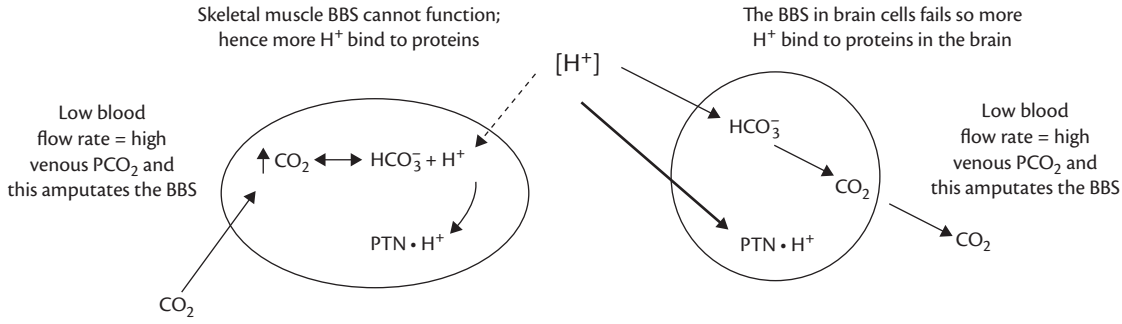

Fig. 35.4 Consequences of failure of BBS in a patient with metabolic acidemia and a low effective arterial blood volume (EABV). The oval represents a skeletal muscle cell and the circle depicts a cell in the brain. The goal is to remove, as many H^+ as possible by the BBS in skeletal muscle in a patient with acidaemia to minimize H^+ binding to its proteins. This requires a low PCO_2 in muscle ICF and interstitial space. If the patient has a contracted EABV, the PCO_2 in ICF and interstitial space in muscle (which is reflected by muscle capillary blood PCO_2) may not be low enough for effective buffering of H^+ by its BBS. As a result, the degree of acidemia may become more pronounced and more H^+ should bind to proteins in the intracellular fluids in other organs, including the brain.

is not reflected by the increase in $P_{Anion\ gap}$ The $P_{Anion\ gap}$ did not reveal the actual quantity of H^+ that were added during DKA and the fall in P_{HCO3} did not reflect the actual magnitude of the deficit of HCO_3^-. However, on re-expansion of the ECF volume with saline, the degree of deficit of HCO_3^- will become evident. In addition, the fall in the $P_{Anion\ gap}$ will not be matched by the rise in the P_{HCO3}.

Failure to correct for the net negative valence attributable to $P_{Albumin}$: when calculating the $P_{Anion\ gap}$, one must adjust for changes in the concentration of albumin as it is the most abundant unmeasured anion in plasma. We emphasize that adjustments should be made for a fall or an increase in the $P_{Albumin}$.

Detect new anions in the urine

The presence of new anions in the urine can be detected with the calculation of the urine anion gap ($U_{Anion\ gap}$) (Equation 35.5) (Halperin et al., 1992). For this calculation, the concentration of NH_4^+ in the urine (U_{NH4}) is estimated from the urine osmolal gap ($U_{Osm\ gap}$), as discussed in the next section. The nature of these new anions may sometimes be deduced by comparing their filtered load to their excretion rate. For example, when there is a very large quantity of new anions excreted in the urine compared with the rise in the $P_{Anion\ gap}$, suspect that this anion is secreted in PCT, for example, the hippurate anion from metabolism of toluene (Carlisle et al., 1991), or is freely filtered and poorly reabsorbed by the PCT, for example, the absorption of ketoacid anions may be inhibited by salicylate anions. On the other hand, a very low excretion of new anions suggests that they were avidly reabsorbed in the PCT, for example, L-lactate$^-$

$$\text{Urine anion gap} = (U_{Na} + U_K + U_{NH4}) - U_{Cl}. \qquad (35.5)$$

Detect toxic alcohols

The presence of alcohols in plasma can be detected by finding a large increase in the plasma osmolal gap ($P_{Osmolal\ gap}$) (Worthley et al., 1987; Kraut and Xing, 2011) (Equation 35.6). This large increase in $P_{Osmolal\ gap}$ occurs because alcohols are uncharged compounds, have a low molecular weight, and because large quantities have been ingested.

$$P_{Osmolal\ gap} = \text{Measured } P_{Osm} - \left(2\left(P_{Na}\right) + P_{Glucose} + P_{Urea}, \text{ all in mmol units}\right) \qquad (35.6)$$

Clinical approach to patients with metabolic acidosis due to added acids

The steps to follow are illustrated in Fig. 35.5. The causes of metabolic acidosis are listed in Table 35.1.

Hyperchloraemic metabolic acidosis

This type of metabolic acidosis is due to the loss of $NaHCO_3$. In this type of metabolic acidosis, very few new anions are present in plasma, hence the term 'non-anion gap metabolic acidosis'. As the fall in the P_{HCO3} is matched by a rise in the P_{Cl}, this type of metabolic acidosis is also called hyperchloremic metabolic acidosis. There are two major groups of causes for this type of metabolic acidosis (Table 35.1), one is the direct loss of $NaHCO_3$ and the other is an indirect loss of $NaHCO_3$. The direct loss of $NaHCO_3$ may be via the gastrointestinal (GI) tract (e.g. patients with diarrhoea) or the urine in patients at the start of a disease process that causes proximal renal tubular acidosis (pRTA) (see Chapter 36). The indirect loss of $NaHCO_3$ may be due to a low rate of excretion of NH_4^+ that is insufficient to match the daily rate of production of sulphuric acid produced from the metabolism of sulphur-containing amino acids, (e.g. in patients with chronic renal failure, patients with distal renal tubular acidosis (dRTA)) (Fig. 35.1). Indirect loss of $NaHCO_3$ may also be due to an over-production of an acid (e.g. hippuric acid formed during the metabolism of toluene) with the excretion of its conjugate base (hippurate anions) in the urine at a rate that exceeds the rate of excretion of NH_4^+ (Carlisle et al., 1991).

> *Concept:* in a patient with chronic metabolic acidosis, the expected rate of excretion of NH_4^+ should be > 200 mmol/day (Simpson, 1983).

To generate new HCO_3^-, glutamine, must be metabolized in the cells of the PCT to yield NH_4^+ and the α-ketoglutarate anion. Metabolism of α-ketoglutarate to neutral end-products (CO_2 or glucose) produces HCO_3^- ions that are added to the body. Nevertheless, for a net gain of HCO_3^-, NH_4^+ must be made into an end-product of metabolism by being excreted in the urine.

> *Concept*: a low rate of excretion of NH_4^+ could be due a decreased production of NH_4^+ or a decreased NH_4^+ transfer into the urine. The later could be due to a decreased renal medullary interstitial NH_3 and/or a decreased net H^+ secretion in the distal nephron.

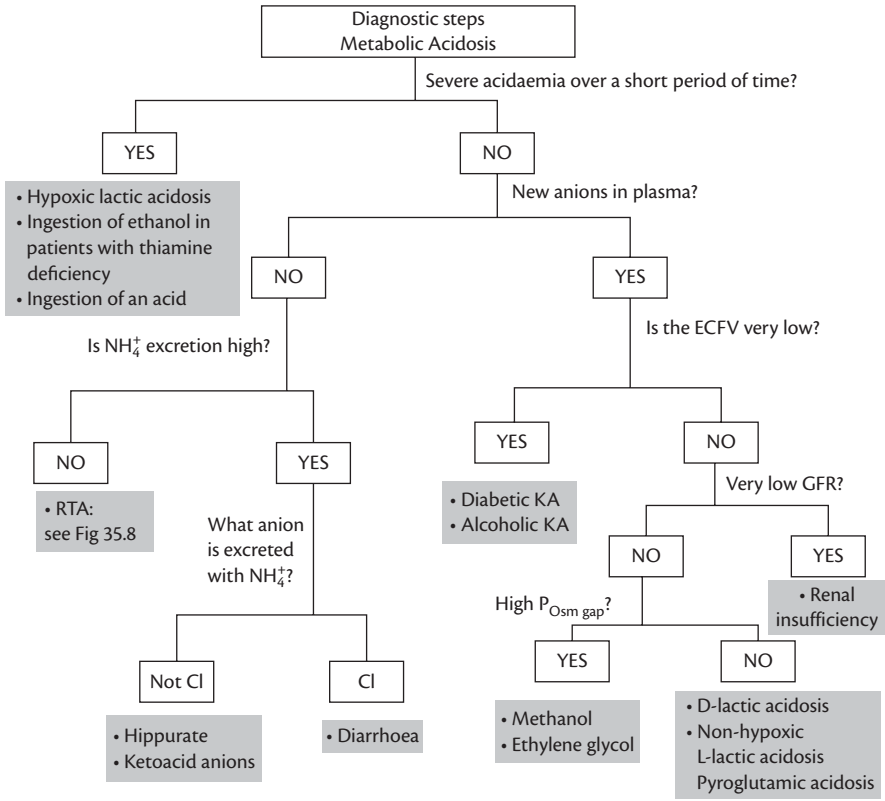

Fig. 35.5 Steps in the clinical approach to the diagnosis of the cause of metabolic acidosis.

Production of NH_4^+

Several factors influence the rate of production of NH_4^+; it is important to recognize that there is a 1–2-day lag period before acidaemia stimulates renal ammoniagenesis. Hypokalaemia also stimulates ammoniagenesis, because it is associated with an intracellular acidosis (Tannen, 1980). The opposite is true for hyperkalaemia. There is an upper limit on the rate of NH_4^+ production in cells of the PCT set by the rate of regeneration of ATP in these cells (Halperin et al., 1984). ATP is utilized in PCT cells primarily to provide the energy for the reabsorption of filtered Na^+. Patients with a low GFR filter less Na^+ and they have a lower rate of reabsorption of Na^+ in the PCT, and hence a lower rate of production of NH_4^+. Patients with isolated proximal renal tubular acidosis (pRTA) have a lower rate of NH_4^+ production, it was suggested that an alkaline PCT cell pH may be its underlying pathophysiology (Halperin et al., 1989).

Transfer of NH_3 into the urine

(See Eladari and Chambrey, 2010; Weiner and Verlander, 2013.) NH_4^+ produced in cells of the PCT is secreted into its lumen, at

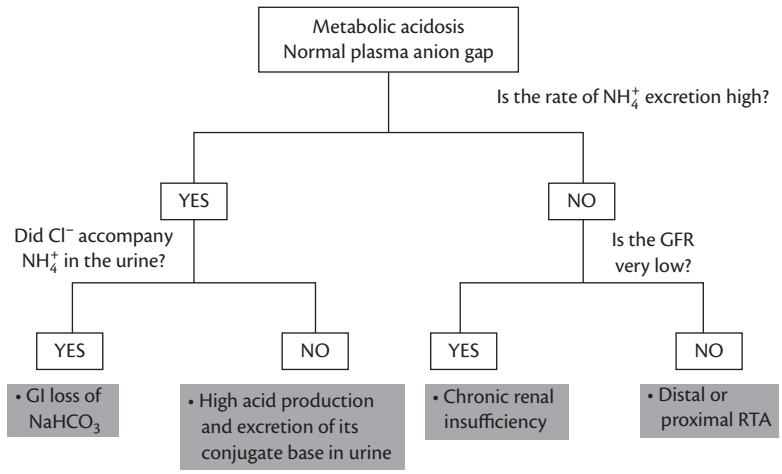

Fig. 35.6 Initial steps in the clinical approach to a patient with metabolic acidosis and a normal anion gap in plasma.

least in part, by replacing H^+ on the sodium/hydrogen exchanger-3 (NHE-3), making it a Na^+/NH_4^+ exchanger. Reabsorption of NH_4^+ in the medullary thick ascending limb of the loop of Henle occurs when NH_4^+ replaces K^+ on the $Na^+/K^+/2Cl^-$cotransporter. This provides the 'single effect' for the medullary recycling of NH_4^+ required for the establishment of a high concentration of NH_4^+ in the medullary interstitium. Excretion of NH_4^+ in the urine requires the transfer of NH_3 from the medullary interstitial compartment across the membrane of the collecting duct, which occurs non-erythroid Rh glycoproteins, Rhbg and Rhcg, that function as gas channels, plus the secretion of H^+ in the lumen of the medullary collecting duct (MCD).

H^+ secretion in the late distal nephron is mediated primarily by an H^+-ATPase, but it may also occur via an H^+/K^+-ATPase.

Assess the rate of excretion of ammonium in the urine

If a direct assay for urine NH_4^+ is not available, the calculation of the $U_{Osmolal\ gap}$ provides the best indirect estimate of the U_{NH4}, because it detects all NH_4^+ salts in the urine (Dyck et al., 1990) (Equation 35.7). We no longer use the urine net charge (or urine anion gap) for this purpose (Kamel and Halperin, 2006). We use the $U_{NH4}/U_{Creatinine}$ ratio in a spot urine sample to assess the rate of excretion of NH_4^+. In a patient with chronic metabolic acidosis and the usual rate of excretion of creatinine of 10 mmol/day, the expected renal response is a $U_{NH4}/U_{Creatinine}$ ratio in a spot urine sample of > 20.

$$U_{Osmolal\ gap} = \text{Measured } U_{Osm} - \text{calculated } U_{osm}$$

$$\text{Calculated } U_{Osmolality} = 2\,(U_{Na} + U_K) + U_{Urea} + U_{Glucose}\text{, in mM units}$$

$$\text{Concentration of } NH_4^+ \text{ in the urine} = U_{Osmolal\,gap}/2 \qquad (35.7)$$

Determine why the rate of excretion of ammonium is low

The urine pH is *not* a reliable indicator for the rate of excretion of NH_4^+ (Richardson and Halperin, 1987). On the other hand, the basis of a *low* rate of excretion NH_4^+ may be deduced from the urine pH. A urine pH that is approximately 5 suggests that the defect is a decreased availability of NH_3 in the medullary interstitial compartment—low urinary pH due to reduced urinary NH_4^+ content and buffering capacity. A urine pH that is > 7.0 suggests that its basis is a defect in diminished net distal H^+ secretion in the late distal nephron—raised urinary pH due to reduced H^+ secretion (Kamel et al., 1997).

Assess distal H^+ secretion

Hydrogen ion secretion in the distal nephron can be evaluated using the PCO_2 in alkaline urine (U_{PCO2}) during bicarbonate loading (Halperin et al., 1974). A U_{PCO2} that is < 70 mmHg in a second-voided alkaline urine implies that H^+ secretion in the distal nephron is likely to be impaired. In patients with low net distal H^+ secretion, the U_{PCO2} can be high if there is a lesion causing a back-leak of H^+ from the lumen of the collecting ducts (e.g. use of amphotericin B) or distal secretion of HCO_3^-, as may occur in some rare patients with South Asian ovalocytosis (SAO) who have a second mutation in the HCO_3^-/Cl^- anion exchanger (AE1) that may lead to its mis-targeting to the luminal membrane of the α-intercalated cell (Kaitwatcharachai et al., 1999). In this setting, the secretion of HCO_3^- causes the luminal pH to increase, liberating H^+ from $H_2PO_4^-$, which raises the urine PCO_2 in alkaline urine to > 70 mm Hg (Kaitwatcharachai et al., 1999). A caveat with this test is that the U_{PCO2} is also influenced by the renal medullary concentrating ability and therefore the presence of nephrocalcinosis (see Chapter 36).

Assess PCT cell pH

In patients with metabolic acidosis associated with a low capacity to reabsorb filtered HCO_3^- (e.g. disorders with defects in H^+ secretion in the PCT; pRTA), the fractional excretion of HCO_3^- after infusing $NaHCO_3$ may be measured to confirm this diagnosis (Kamel et al., 1997). In our opinion, this is not needed in most cases, because the results are often not clear (e.g. in a patient with a contracted ECF volume or low P_K) and the test can impose a danger (e.g. worsening hypokalaemia in a patient with an already low P_K). These patients will be detected clinically by failure to correct their metabolic acidosis, despite being given large amounts of $NaHCO_3$.

Rate of citrate excretion

This is a marker of pH in cells of the PCT (Simpson, 1983). The rate of excretion of citrate in children and adults consuming their usual diet is approximately 400 mg (~ 2.1 mmol)/day. The rate of excretion of citrate is very low during most forms of metabolic acidosis, a notable exception is in patients with isolated pRTA, suggesting that its basis is an alkaline PCT cell pH (Halperin et al., 1989).

General comments about alkali therapy in patients with metabolic acidosis

The issue of the use of $NaHCO_3$ in the treatment of patients with metabolic acidosis is controversial. In patients with hyperchloraemic metabolic acidosis, administration of $NaHCO_3$ may be necessary, since there are no anions in the ECF that can be metabolized to produce HCO_3^-. It is interesting to note that some patients with severe diarrhoea due to cholera developed pulmonary oedema when given an amount of saline that was not sufficient to restore their effective arterial blood volume (EABV) (Greenough et al., 1976). Paradoxically, pulmonary oedema in these patients could be averted by the administration of $NaHCO_3$. Perhaps, the explanation is that these patients developed worsening acidaemia with the administration of saline, which lacks HCO_3^- and potential HCO_3^-, and hence may have developed constriction of peripheral veins and an acute increase in central blood volume. In a patient who has metabolic acidosis due to a low rate of excretion of NH_4^+, metabolic acidosis will persist unless $NaHCO_3$ is given. In this case, one must give enough $NaHCO_3$ to titrate H^+ that have accumulated, then maintain the patient on enough alkali to match the daily rate of production of H^+ from metabolism of sulphur containing amino acids (usually 20–40 mmol/day in an adult).

In patients with acute metabolic acidosis due to added organic acids, there are arguments for and against the use of $NaHCO_3$ (Stacpoole, 1986; Narins and Cohen, 1987; Forsythe and Schmidt, 2000; Sabatini and Kurtzman, 2009; Kraut and Madias, 2012) with strong feelings on both sides, but there is a lack of convincing clinical data.

Arguments for the use of $NaHCO_3$

First, it seems intuitively obvious that at some point during acidaemia, too many H^+ may bind to intracellular proteins and compromise vital cellular functions. For example, *in vitro* studies showed that myocardial contractility and binding of adrenaline to its receptors are decreased when the fall in pH is large (Mitchell et al., 1972; Huang et al., 1995); these adverse effects may be reversed by

lowering the concentration of H^+. Notwithstanding, the administration of $NaHCO_3$ does not seem to enhance the contractility of the ischaemic myocardium in dogs *in vivo* (Mazer et al., 1996). The administration $NaHCO_3$ appeared to be beneficial in the setting of hypoxic L-lactic acidosis in rats, induced by ventilation with a hypoxic gas mixture (Halperin et al., 1996). The survival period in these rats was extended, even though $NaHCO_3$ led to an enhanced rate of production of L-lactic acid. If these data apply to humans with hypoxia-induced L-lactic acidosis, the administration of $NaHCO_3$ might be viewed as a temporary measure to allow for more direct interventions to deal with the underlying cause for the metabolic acidosis to be employed. Of note, a large dose of NaHCO3 was administered in these rats, which is not likely to be feasible in the clinical setting, because of the risk of pulmonary oedema.

Arguments against the use of $NaHCO_3$

When $NaHCO_3$ is administered, HCO_3^- reacts with H^+ and CO_2 is produced. Some have argued that this represents an important deleterious effect of HCO_3^-, because this CO_2 can enter cells and cause a paradoxical acidification of the ICF. This is a circular argument, since if the source of the H^+ that is titrated by the administered HCO_3^- is H^+ bound to intracellular proteins, the ICF should be alkalinized and not acidified. Alternatively, if the source of these H^+ is from the production of ATP in the process of anaerobic glycolysis, stimulation of L-lactic acid production, and ATP generation, by alkali could be beneficial (Halperin et al., 1994). However, a possible danger would be if some of the CO_2 produced were retained due to impaired lung function or inadequate mechanical ventilation.

Another argument against the use of $NaHCO_3$ to treat patients with a severe degree of metabolic acidosis is that the administration of alkali to rats pretreated with HCl failed to yield an acute and significant restoration of non-bicarbonate buffers (Kamel, 1996). This, however, could have been because these rats were on fixed ventilation and there may have been a rise in tissue PCO_2.

A fall in concentration of calcium (Ca^{2+}) in plasma can occur with the administration of $NaHCO_3$, because the addition of HCO_3^- leads to rapid production of carbonate (CO_3^{2-}) and the precipitation of $CaCO_3$. It has been suggested that a fall in the concentration of ionized calcium in the myocardial interstitial fluid compartment can depress myocardial contractility; but if this were a problem, it should have been obvious in situations where carbonate-bicarbonate (carb-bicarb) buffer (i.e. CO_3^{2-}) was used.

Hypokalaemia could be another problem if the administered $NaHCO_3$ causes an acute shift of K^+ into cells, especially if there is already total body K^+ depletion, as seen in patients with DKA, dRTA, secretory diarrhoea, and in glue sniffers. If P_K is low, the use of $NaHCO_3$ should be delayed until the P_K has been raised toward the normal range.

Volume overload is only a problem in patients with metabolic acidosis, which has an expanded ECF volume (e.g. patients with renal failure, patients with cardiogenic shock, or those in whom large amounts of alkali are used, e.g. hypoxic lactic acidosis). Hypernatraemia may occur if a large volume of a hypertonic $NaHCO_3$ solution is given.

In patients with metabolic acidosis due to overproduction of H^+ and anions that can be metabolized to produce HCO_3^- (e.g. L- or D-lactate, or ß-hydroxybutyrate$^-$ (ß-HB$^-$)), and are given a large infusion of $NaHCO_3$, the final P_{HCO3} can be higher than normal if these anions are retained and metabolized to HCO_3^-. The clinical significance of 'rebound metabolic alkalosis' is primarily when patients are being weaned from mechanical ventilation, since alkalaemia depresses ventilation, and the impact of the increase in filtered HCO_3^- on the renal excretion of K^+, which can exacerbate hypokalaemia.

Recommendations

One must individualize the decision for each patient, balancing potential beneficial versus adverse effects (see later discussion of individual causes of metabolic acidosis). If the decision is made to administer $NaHCO_3$, other issues include how much to give and how fast it should be given. It is important to recognize that as the P_{HCO3} falls, even more of the added H^+ are bound to intracellular proteins (Fig. 35.4). Therefore, when $NaHCO_3$ is given, the hope is that much of it will titrate these intracellular H^+, and that they will disappear as CO_2 and water, and the increment in the P_{HCO3} will be small. Moreover, CO_2 removal by the lungs must keep up with the increment in CO_2 production.

A decision needs to be made on the initial target P_{HCO3} when a patient has an extremely low baseline P_{HCO3} (e.g. 3 mmol/L). A reasonable target in this setting is either to double the P_{HCO3} by aiming for an absolute value of 5–6 mmol/L. If this rise in P_{HCO3} was achieved, and the arterial PCO_2 remained unchanged, its pH will rise by 0.3 units. Nevertheless, the amount needed to achieve this goal may be rather large, depending on the ongoing rate of H^+ production and amount of added H^+ that was buffered by intracellular proteins.

Specific causes for metabolic acidosis

The list of causes of metabolic acidosis is provided in Table 35.1.

Ketoacidosis

(See Schreiber et al., 1994; Halperin et al., 2002.) The process of production of ketoacids in the liver can be divided into two major steps; first, the formation of acetyl-CoA and second, the conversion of acetyl-CoA to ketoacids (Halperin et al., 2010). There are three substrates from which acetyl-CoA can be made rapidly enough in in hepatic mitochondria to lead to an appreciable rate of formation of ketoacids. The major physiologic function of the metabolic process involving ketoacids is to supply the brain with a water-soluble, fat-derived fuel when its major fuel in the fed state, glucose, is in short supply. The only important physiologic substrate for hepatic ketogenesis is free fatty acids derived from storage fat.

In prolonged fasting the $P_{Glucose}$ is low; hence there is a low concentration of insulin in blood delivered to the liver. In the patient with DKA, there is lack of insulin due to damage of ß-cells of the pancreas. In either case, the relative lack of insulin provides the signal to activate the enzyme lipase, which catalyses the release of free fatty acids from triglycerides in adipose tissues.

The second substrate for ketoacid formation is ethanol. To permit the liver to remove the maximum quantity of ethanol that is produced from fermentation in the colon, to avoid a disturbance in cerebral function, ketoacids must be the final product of its metabolism. This biochemistry however, may lead to a serious degree of ketoacidosis when a large quantity of ethanol is ingested and insulin levels are low.

The third substrate for ketoacids production in the liver is a group of short-chain organic acids, the most abundant of which is acetic acid, that are produced during the fermentation of poorly

absorbed carbohydrates (fibre or fructose) by bacteria in the colon (Davids et al., 2004).

A general rule of regulation of coupled oxidative phosphorylation is that the availability of adenosine diphosphate (ADP), which depends on the rate of utilization of adenosine triphosphate (ATP) to perform biological work, sets an upper limit on the rate of fuel oxidation (Flatt, 1972). Biological work can be mechanical, electrical (ion pumping) and/or biosynthesis. While there are two major fates for acetyl-CoA, formation of ketoacids becomes its major removal pathway when these two pathways are inhibited. Fatty acids synthesis is inhibited because insulin is required for the conversion of acetyl-CoA to fatty acids by activating the enzyme acetyl-CoA carboxylase. Oxidation of long chain fatty acids (e.g. palmitate) in hepatic mitochondria produces acetyl-CoA and converts nicotinamide adenine dinucleotide (NAD^+) to its more reduced form, NADH. Hence, one limiting factor for the rate of hepatic ketoacid formation could be the availability of mitochondial NAD^+. NADH is converted back to NAD^+ during coupled oxidative phosphorylation, which converts ADP into ATP. The availability of ADP depends on the rate of utilization of ATP to perform biologic work. Because the liver, unlike muscle, does not perform mechanical work, and since in the absence of protein ingestion there is not a large enough supply of amino acids to have high rates of gluconeogenesis, the rate of oxidative phosphorylation in the liver would be quite low during conditions associated with ketoacidosis. When the oxidation of acetyl-CoA in the citric acid cycle and its conversion to long chain fatty acids are inhibited, acetyl-CoA is converted to ketoacids.Two molecules of acetyl CoA condense to form acetoacetyl CoA. Acetoacetyl CoA is metabolized to acetoacetic acid in the HMG-CoA pathway. The major ketoacid that is produced by the liver is ß-hydroxybuutyric acid, which is formed from acetoacetic acid in a reaction driven by a high mitochondrial ratio of $NADH/NAD^+$. Nevertheless, the liver needs to produce a high enough quantity of ketoacids for consumption by the brain and the kidney. The observed rates of production of ketoacids during prolonged fasting (~ 1500 mmol/day) would suggest that there are other ways for the liver to bypass the limitation by availability of ADP. One such process is *uncoupled oxidative phosphorylation*, in which H^+ re-enter mitochondria via un-coupler proteins and hence are not linked to the conversion of ADP to ATP.

Removal of ketoacids

There are two major sites of ketoacid removal, the brain and the kidneys. The brain oxidizes approximately 750 mmol of ketoacids per day; almost half the quantity of ketoacids produced when ketogenesis is most rapid during prolonged fasting. If the rate of generation of ADP declines in the brain, because of less biological work (e.g. due to coma, intake of sedatives or ethanol, or effects of anaesthesia), fewer ketoacids can be oxidized and the degree of acidaemia will become more severe.

The kidneys remove approximately 400 mmol of ketoacids per day. If renal work (largely the reabsorption of filtered Na^+) is at its usual rate, the kidneys will oxidize approximately 250 mmol of ketoacids per day. Because more ketoacids are filtered than reabsorbed, approximately 150 mmol of ketoacid anions are excreted per day during the ketoacidosis of prolonged fasting. Since most of these anions are excreted along with NH_4^+, acid-base balance is maintained. H^+ will accumulate if ketoacid anions are excreted in the urine with a cation other than NH_4^+ (or H^+).

In DKA, the filtered load of Na^+ declines (due to prerenal failure secondary to the loss of Na^+ in the glucose-induced osmotic diuresis). Accordingly, renal removal of ß-HB^- and H^+ declines, because the rates of ß-HB^- oxidation and NH_4^+ excretion are both reduced. From an energy point of view, oxidation of ß-HB^- and glutamine are equivalent in terms of ADP utilization.

To summarize, unless there is a much larger degree of uncoupling of oxidative phosphorylation during DKA, the rate of production of ketoacids is not substantially higher than in subjects with ketosis of prolonged fasting. The reason a severe degree of acidaemia develops in a patient with DKA is likely to be due to factors that compromise the rate of removal of generated ketoacids

Diabetic ketoacidosis

DKA is the metabolic consequence of insufficient actions of insulin, and it is characterized by the accumulation of glucose and ketoacids in the body. The precipitating illness and the complications of this metabolic disturbance can be life threatening. DKA may be the first presentation of undiagnosed type 1 diabetes mellitus in children. In patients with known type 1 diabetes mellitus, the precipitating causes include gastroenteritis, pancreatitis, infections, and conditions where counter-regulatory hormones may be present in excess (e.g. thyrotoxicosis, surgery, stress, pregnancy, and hyperadrenocorticism). Failure to take insulin can be an important aetiological factor in patients with repeated episodes of DKA. The clinical manifestations of DKA are the expected consequences of the major biochemical changes, hyperglycaemia, glucosuria, and ketoacidosis. Early symptoms represent exacerbations of the classic features of diabetes mellitus that is poorly controlled: thirst, polydipsia, polyuria, weakness, lethargy, and malaise. Hyperglycaemia causes an osmotic diuresis with loss of Na^+ and water, resulting in ECF volume contraction, low blood pressure, postural hypotension, and tachycardia. In contrast, a higher $P_{Glucose}$ can also lead to a higher ECF volume, by drawing water out of cells in patients with end stage renal disease. Metabolic acidosis results in an increased rate and depth of breathing (air hunger, Kussmaul respiration). The conversion of acetoacetic acid to acetone imparts the characteristic fruity odour to the breath.

Not all the clinical findings, however, are completely explained in terms of these biochemical aberrations. The state of consciousness does not correlate well with the concentration of ketoacids in blood. A much better correlation was found between the level of consciousness and the plasma hyperosmolality. This, however, may also reflect a larger degree of osmotic diuresis and natruresis with a very contracted EABV and hence, possibly a higher PCO_2 in cells of the brain which results in more H^+ bind intracellular proteins. Another feature of DKA that remains unexplained is hypothermia, even in the presence of infection. This together with the fact that leucocytosis is a common finding in these patients may diminish one's suspicion of an underlying infection. Anorexia, nausea, vomiting, and abdominal pain are frequent non-specific GI complaints, especially in children. These symptoms, together with abdominal tenderness, decreased bowel sounds, guarding, and leucocytosis, may be severe, mimicking an acute abdominal emergency. Rebound tenderness is usually absent. The cause for the abdominal pain is not entirely clear, but in some cases it may be related to hypertriglyceridaemia and pancreatitis. Signs and symptoms of the disorder that precipitated DKA may dominate the clinical picture.

Laboratory evaluation

Hyperglycaemia, ketonaemia, glucosuria, and ketonuria are the four hallmarks of the laboratory diagnosis of DKA.

Hyperglycaemia: the degree of hyperglycaemia varies markedly—the $P_{Glucose}$ usually exceeds 250 mg/dL (14 mmol/L). Higher $P_{Glucose}$ values are seen if there is a large reduction in the GFR due to marked intravascular volume depletion (usually with oliguria), or if the patient has consumed a large quantity of carbohydrates, for example, in the form of sweetened soft drinks to quench thirst (usually with polyuria).

Ketoacids: in DKA, serum ketones are usually strongly positive in a dilution of 1 in 8. However, only acetoacetate and acetone yield a positive reaction with the nitroprusside test (Acetest) used for clinical screening for ketoacids. If there is an increase in the NADH/NAD^+ ratio, as occurs with hypoxia or due to metabolism of ethanol, ketoacids will be predominantly in the form of ß-HB acid, which is not detected by Acetest; a specific enzymatic analysis is necessary to measure ß-HB acid.

Sodium: owing to glucose-induced osmotic diuresis, patients with DKA have a large deficit of Na^+ (usually 3 to 9 mmol/kg body weight).

Plasma Na^+ concentration: much attention is given to the possibility that glucose will draw water out of cells and thereby lower the P_{Na} by dilution. This, however, occurs only when the addition of glucose to the body is as a solution that is hyperosmolar to plasma. In contrast, when glucose is added as a solution that has an osmolality similar to or lower than that of plasma, there is no shift of water from cells. In this circumstance, the P_{Na} will be lower than seen with hypertonic glucose addition for an identical rise in $P_{Glucose}$ (Davids et al., 2002). Therefore, calculation of the expected fall in P_{Na} for a given rise in $P_{Glucose}$ or of the expected rise in P_{Na} with a fall in $P_{Glucose,}$ based on a shift of water should not be done, because the assumptions made may not be valid. Thus, it is incorrect based on these calculations to justify the use of hypotonic saline to avoid the development of hypernatremia during therapy, since this may increase the risk for the development of cerebral oedema in children with DKA. In our view, the plasma effective osmolality ($P_{Effective\ Osm}$) (Equation 35.8) must not be permitted to fall in the first 15 hours of treatment.

$$\text{Effective } P_{osm} = 2\left(P_{Na}\right) + P_{Glucose}\left(\text{in mmol/L}\right) \quad (35.8)$$

Potassium: despite a deficit of K^+ that is usually in the range of 4–6 mmol/kg body weight, the P_K is usually increased to the mid-5 range, because K^+ has shifted from the ICF to the ECF compartment due primarily to the lack of action of insulin.

P_{HCO3}: in patients with DKA, the P_{HCO3} is low, because many H^+ were added to the ECF along with ß-HB^- anions. There is also an indirect loss of Na^+ and HCO_3^- early in the course of DKA, because there is a lag period before there is a large increase in the rate of NH_4^+ excretion. As a result, ketoacid anions are excreted in the urine with Na^+ and/or K^+.

PCO_2: acidaemia in arterial blood stimulates the respiratory centre and leads to a predictable degree of decrease in the arterial PCO_2 (a fall of approximately 1.2 mm Hg (0.16 kPa) in the arterial PCO_2 for every 1 mmol/L reduction in P_{HCO3}) (Pierce et al., 1970). As discussed above, while the arterial PCO_2 sets a lower limit on the PCO_2 in capillaries, it does not guarantee that the PCO_2 in skeletal muscle capillary blood, which reflects the PCO_2 in their ICF and the interstitial space, will be low enough to ensure effective buffering of H^+ by the BBS. Since most patients with DKA have a decreased EABV, the rate of blood flow to muscles will be low and hence their capillary PCO_2 will be higher, which diminishes the effectiveness of their BBS to remove extra H^+. As a result, the degree of acidaemia may become more pronounced and more H^+ may be titrated by proteins in the ECF and intracellular fluids in other organs, including the brain. Owing to autoregulaion of cerebral blood flow, it is likely that the PCO_2 in brain capillary blood will change minimally with all but a severe degree of contraction of EABV. Hence the BBS in the brain will continue to titrate this H^+ load. Nevertheless, considering its limited quantity of BBS, and that the brain will receive a relatively larger proportion of blood flow, there is a risk that more H^+ will bind to intracellular proteins in the brain. If cerebral autoregulation fails, because of a severe degree of intravascular volume depletion, the PCO_2 in capillary blood in the brain should rise and its BBS will fail and an even larger H^+ load will bind to proteins in brain cells.

GFR: since patients with DKA often have a very low EABV, their GFR will be reduced and the concentrations of creatinine in plasma ($P_{creatinine}$) will be elevated. There may be errors in the measurement of creatinine, depending on the method used. Higher $P_{creatinine}$ values are reported with the picric acid method, if the level of acetoacetate in plasma is elevated, whereas lower $P_{creatinine}$ values are reported with severe hyperglycaemia, if the enzymatic assay for creatinine is performed on the Kodak analyser.

Treatment of the patient with DKA

DKA is a medical emergency. Mortality is influenced by a number of factors, including precipitating causes, the age of the patient, the level of consciousness, and the severity of the biochemical abnormalities. In children, the leading cause of morbidity and mortality is the development of cerebral oedema (see later in chapter). Other causes of death are infection, vascular thrombosis, and shock. Early diagnosis, a better design of therapy, and dealing with the underlying causes of DKA may reduce mortality.

Treat a haemodynamic emergency if present

A true haemodynamic emergency is uncommon in children with DKA. A large bolus of intravenous saline can be a risk factor for the development of cerebral oedema, because it increases the hydrostatic pressure and diminishes the colloid osmotic pressure in capillaries in the blood–brain barrier (BBB). If a haemodynamic emergency is present, enough saline should be given to restore haemodynamic stability. In the absence of a haemodynamic emergency, we use the brachial venous PCO_2 as a guide to the rate of infusion of saline. Enough saline should be administered to lower the brachial venous PCO_2 to a value that is no more than 6 mmHg above the arterial PCO_2 (Geers and Gros, 2000). This is important to allow effective buffering of H^+ by the BBS to occur in muscle and decrease binding of H^+ to intracellular proteins in vital organs.

Avoid a large fall in the $P_{Effective\ osm}$

To prevent this fall in the $P_{Effective\ osm}$, the effective osmolality of the infusate should be equal to that of the urine in this polyuric state. A solution of isotonic saline with 40 mmol KCl per litre (when addition of K^+ is needed) has an effective osmolality of close to 400

mOsm/L. Hypotonic saline should *not* be used to treat this hyperosmolar state, because this can be dangerous, since it may increase the risk of developing cerebral oedema (see later in this chapter).

Replace the Na^+ deficit

A guide to the total amount of Na^+ that is needed is obtained by estimating the deficit of Na^+ in the ECF compartment on presentation from the P_{Na} and a quantitative assessment of the ECF volume (using the haematocrit). Hence, over-expansion of the ECF volume, which is a common occurrence during therapy in these patients, can be avoided.

Stop ketoacid production

Insulin plays a central role in arresting ketogenesis, but this is usually not an urgent aspect of therapy, because the maximum possible rate of ketogenesis is only approximately 1 mmol/min (Flatt, 1972). In our view, the only emergency action of insulin needed is its effect to decrease the P_K by accelerating a shift of K^+ into cells in a patient with a significantly abnormal ECG. The hypoglycaemic effects of insulin are minimal early in therapy. Rather, the $P_{Glucose}$ will fall initially as a result of re-expansion of the ECF volume (dilution) and glucosuria. Six to 8 hours after therapy begins, insulin will lower $P_{Glucose}$ by increasing the rate of oxidation of glucose, because competing fat fuels are no longer available, and by promoting the synthesis of glycogen.

A bolus of insulin should *not* be used in children, because it may lead to brain cell swelling (Carlotti et al., 2003). Insulin therapy has potentially detrimental side effects that should be anticipated and avoided. The major ones are hypokalaemia (at 1–3 hours) and hypoglycaemia (at 6–10 hours). The former risk will be discussed below; the latter is minimized by infusing glucose when the $P_{Glucose}$ falls to approximately 250 mg/dL (~ 15 mmol/L).

K^+ therapy

KCl should be added to each litre of fluid infused, once insulin is given, if the P_K is < 5 mmol/L. If the initial P_K is < 4 mmol/L, the patient is profoundly K^+ depleted. Because the P_K will fall after insulin administration, do not administer any insulin for the first 1–2 hours until P_K is raised to around 4 mmol/L with aggressive replacement of K^+. These patients are particularly at risk for the development of severe hypokalaemia later during therapy as the rate of excretion of K^+ in the urine increases significantly (Carlotti, 2013).

$NaHCO_3$ therapy

Severe acidaemia may be associated with decreased cardiac contractility, diminished responses to both endogenous and administered catecholamines, and a predisposition to cardiac arrhythmias (Mitchell et al., 1972). In addition, severe acidaemia may interfere with binding of insulin to its receptor, and hence may diminish its action to slow the rate of production of ketoacids (Sonne et al., 1981). Most patients with DKA do not require administration of $NaHCO_3$, because administered insulin will slow the rate of production of ketoacids, and HCO_3^- will be produced when ketoacids are oxidized. The consensus of opinion is not to administer $NaHCO_3$ to adult patients with DKA unless the plasma pH is close to 6.90. We suggest that this decision in patients with DKA should be individualized and is not based solely on an arbitrary blood pH value. Therapy with $NaHCO_3$ should be considered in the initial treatment of a subset of patients who are at risk for developing a more severe degree of acidaemia, particularly those who are haemodynamically unstable, before a dangerous fall in plasma pH develops (Kamel and Halperin, 2015). In patients with a very low P_{HCO3}, a quantitatively small additional H^+ load will produce a proportionately larger fall in the P_{HCO3} and plasma pH. The rate of ketoacid oxidation will be diminished if there is a lower rate of work in the brain (e.g. coma, intake of sedatives, including ethanol) and the kidneys (e.g. patients with a very low GFR). The target of $NaHCO_3$ therapy would be at least to avoid a significant fall in the P_{HCO3}. To achieve this goal, the rate of infusion of $NaHCO_3$ should match the expected rate of production of ketoacids by the liver. Based on data from subjects with starvation ketosis, this is approximately 60 mmol/hour (Kamel et al., 1998). We think that this a reasonable start, which can be re-evaluated based on serial measurements of the P_{HCO3}. We would give $NaHCO_3$ at a rate of 1 mmol/min as an infusion in a solution with a similar tonicity to the calculated $P_{Effective\ osm}$.

In a multicentre, case-controlled retrospective study in paediatric patients with DKA, Glaser et al found that patients and who were treated with $NaHCO_3$ had a significantly greater risk of developing cerebral oedema (Glaser et al., 2001). In our opinion, $NaHCO_3$ should not be administered to children with DKA, unless acidaemia is very severe and haemodynamic instability is unresponsive to the usual manoeuvres to restore blood pressure.

Phosphate therapy

Patients with DKA are catabolic; they have large deficits of phosphate and of K^+. Because the plasma phosphate levels decline markedly once insulin acts, there is a rationale to administer phosphate to patients with DKA. On the other hand, there is no evidence that this alters the course of recovery of patients with DKA and there is the danger of development of hypocalcaemia due to precipitation of calcium with the administered phosphate.

Search for and treat an underlying illness

Always look for an underlying illness (e.g. an infection) that initiated this metabolic emergency and for complications that may arise during therapy such as venous thrombosis or aspiration pneumonia.

Cerebral oedema during therapy in children with DKA

The incidence of cerebral oedema (CE) during therapy for DKA in children remains unacceptably high (0.5–1% of admissions in excellent medical centres) (Sperling, 2006). It is most important to recognize that CE is usually discovered 3–13 hours after treatment begins. This suggests that important risk factors for CE develop during therapy, and hence the current treatment may not be ideal (Brown, 2004). Understanding the pathophysiology of brain cell swelling may provide a framework to address this problem (Carlotti et al., 2003):

- Brain cells swell when there is a higher effective osmolality in brain cells as compared with that in plasma in capillaries near the BBB.
- The interstitial compartment of the ECF of the brain will expand if there is a higher capillary hydrostatic pressure, a lower plasma colloid osmotic pressure, and/or an increase in capillary permeability.

Based on these considerations, we suggest that the following may be risk factors for the development of CE:

An increase in number of effective osmoles in brain cells

The Na^+/H^+ exchanger 1 (NHE-1), which is normally inactive in cell membranes, becomes activated by a high H^+ concentration in the ICF and a high insulin concentration in plasma. Following an intravenous bolus of insulin in the presence of a severe degree of acidaemia due to accumulation of monocarboxylic acids, NHE-1 in brain cell membranes could become activated (Van der Meulen, Klip, and Grinstein, 1987). This will lead to a gain of Na^+ and/or K^+ in, and a loss of H^+ from, the ICF compartment. This increases the number of effective osmoles in cells, because the bulk of H^+ exported from the cell were bound to ICF proteins (Kamel and Halperin, 2015).

A fall in the $P_{Effective\ Osm}$

This could occur if there is a rapid fall in $P_{Glucose}$ and/or a gain of electrolyte-free water (EFW).

A rapid fall in the $P_{Glucose}$

A major factor, which leads to a rapid fall in the $P_{Glucose}$, is glucosuria due to a rise in GFR following EABV expansion. When the concentrations of ketoacids in plasma decline, glucose becomes the primary brain fuel. Furthermore, more glucose may be oxidized in skeletal muscle, because there are fewer circulating FFA owing to actions of insulin to inhibit hormone-sensitive lipase in adipocytes. Another metabolic pathway for the removal of glucose is its conversion to glycogen in the liver and/or skeletal muscle.

Gain of EFW

There are several possible sources of EFW in this setting, including the administration of hypotonic saline, and/or D_5W to prevent neuroglucopaenia when the $P_{Glucose}$ falls. Another two sources of EFW that are less obvious are:

- *Gastric emptying*: patients with DKA often consume large volumes of fluid to quench their thirst. This ingested fluid may be retained in the stomach, because hyperglycaemia slows stomach emptying. This, however, will represent a gain of water when absorbed, if water has been ingested or after glucose is metabolized, if fruit juice or sugar-containing soft drinks have been consumed (Carlotti et al., 2009). Rapid absorption of a large volume of water may result in an appreciable fall in arterial $P_{Effective\ Osm}$, to which the brain is exposed and may not be detected by the measurement of venous $P_{Effective\ Osm}$.
- *Desalination of administered isotonic saline*: large volumes of saline are usually administered to patients with DKA. As the excretion of glucose diminishes, and in the presence of vasopressin, this salt load may be excreted in a hypertonic form in the urine, thus generating retained EFW.

Increase ECFV in the brain

A large bolus of saline may lead to an increase the interstitial volume of the brain ECF compartment and lead to CE, since it causes an increase in the capillary hydrostatic pressure and a decrease in colloid osmotic pressure. There is evidence to suggest that the BBB may be leaky in patients with DKA.

Clinical implications

We suggest the following modifications to the current management of children with DKA (Kamel and Halperin, 2015):

1. Do not administer an intravenous bolus of insulin.
2. Prevent a fall in $P_{Effective\ Osm}$. The $P_{Effective\ Osm}$ must not be permitted to fall in the first 15 hours of treatment. The goal of fluid therapy should be to raise the P_{Na} by ½ of the fall in $P_{Glucose}$ in mmol/L. To prevent a fall in the $P_{Effective\ osm}$, the effective osmolality of the infusate should be equal to that of the urine in this polyuric state. A solution of isotonic saline with 40 mmol KCl per litre has an effective osmolality of close to 400 mOsm/L. As children with DKA often present with near-normal P_{Na}, a degree of hypernatraemia will develop; this, however, is needed to prevent a fall in the $P_{Effective\ osmolality}$
3. Use of D_{10}-0.9 % NaCl instead of D_5W to minimize the amount of EFW retained after glucose is metabolized.
4. Monitor for signs of gastric emptying. This is suggested by the absence of a large fall in $P_{Glucose}$ when glucosuria is large, a large increase in the urine flow rate with little fall in the $P_{Glucose}$, or a sudden fall in the $P_{Effective\ osm}$. The later occurs if water without sugar was ingested.
5. Avoid overzealous saline administration: a large bolus of saline should be given only if there is a haemodynamic emergency. The goal of saline therapy should be to maintain haemodynamic stability and, as discussed, above to increase blood flow rate to muscle sufficiently to have a brachial venous PCO_2 that is close to 6 mmHg (0.8 kPa) higher than arterial PCO_2.

Alcoholic ketoacidosis

Alcoholic ketoacidosis (AKA) is seen following binge drinking of large amounts of ethanol, complicated by vomiting (usually due to alcohol-induced gastritis) and poor food intake (Halperin et al., 1983; Wrenn et al., 1991; Kamel, 1997). The lack of food intake and the EABV depletion due to vomiting (causing the release of α-adrenergics) lead to suppression of insulin secretion (Porte, 1969). Ethanol is metabolized in the cytosol of hepatocytes to produce acetic acid. NAD^+ is made into NADH in the metabolism of ethanol by alcohol dehydrogenase and acetaldhyde dehydrogenase. Acetic acid enters the mitochondria and is converted to acetyl-CoA. Oxidation of acetyl-CoA in the citric acid cycle converts NAD^+ to NADH. NADH is converted back to NAD^+ during coupled oxidative phosphorylation, which converts ADP into ATP. The availability of ADP depends on the rate of utilization of ATP to perform biologic work. As acestyl CoA accumulates, two molecules of acetyl CoA condense to form acetoacetyl CoA. Acetoacetyl CoA is metabolized to acetoacetic acid in the HMG-CoA pathway. Because of the high mitochondrial ratio of NADH/NAD^+ due to metabolism of ethanol, the majority of acetoacetic acid is converted to β-hydroxybutyric acid. Therefore, the rate of ethanol metabolism and ketoacids production may be diminished due to decreased availability of NAD^+. Nevertheless, a severe degree of ketoacidosis may develop, as the rate of ketoacids by the brain is diminished because of the sedative effect of alcohol (Flatt, 1972; Schreiber et al., 1994).

Establishing the diagnosis of AKA may not be straightforward. One reason for this is that there are frequently coexisting acid–base disturbances that may result in the blood pH being normal or even alkalaemic in a substantial number of patients. Metabolic alkalosis commonly occurs as a result of the vomiting, and respiratory alkalosis may occur due to stimulation of ventilation by alcohol withdrawal or because of aspiration pneumonia. Another difficulty in making the diagnosis in patients with alcoholic ketoacidosis is that the NAD^+/NADH ratio in the liver is often even more reduced due in part to the metabolism of ethanol. In this setting, more of the ketoacids produced will be in the form of ß-HB acid and hence the

nitroprusside screening test for ketones may be falsely low (see earlier). The $P_{Glucose}$ is usually only modestly high, rather than being markedly elevated as in DKA. At times it is difficult to distinguish AKA from methanol or ethylene glycol poisoning as the primary cause of acidosis as all of them can cause an elevated value for the plasma $P_{Osmolal\ gap}$, a high $P_{Anion\ gap}$, and a near-normal $P_{Glucose}$. If the ECF volume is not markedly contracted, one should suspect methanol or ethylene glycol intoxication. A direct assay for methanol and ethylene glycol is needed to establish the diagnosis.

Treatment of AKA is usually straightforward. Isotonic saline is required to correct the marked degree of EABV depletion. If the $P_{Glucose}$ is low, a small quantity of glucose should be added to raise the $P_{Glucose}$ to the high-normal range. Reducing α-adrenergic activity with the expansion of the EABV and the higher $P_{Glucose}$ should stimulate insulin secretion and thereby diminish the rate of ketoacid production. Treatment with $NaHCO_3$ is rarely required, because the degree of acidaemia is usually mild and the net production of ketoacids can be reversed quickly with appropriate intravenous fluid therapy. Thiamine must be given with the initial therapy. Attention must also be paid to K^+ and phosphate depletion, which are common in this disorder.

L-lactic acidosis

A rise in the concentration of L-lactate$^-$ and H^+ in plasma can be caused by an increased rate of production and/or a decreased rate of removal. Although both of these pathways are involved in most cases, usually one will predominate (Luft, 2001). In virtually every condition of an increased production of L-lactate$^-$ from glucose, there is a rise in the concentration of ADP in cells (Halperin et al., 2010). When work is being done, and there is a problem converting ADP to ATP in mitochondria (e.g. hypoxia, uncoupling of oxidative phosphorylation, inhibition of electron transport), a high ADP concentration in the cytosol will drive the glycolytic pathway, which regenerates ATP (Equation 35.9). Since the rate of glycolysis can greatly exceed the rate of oxidation of pyruvate in mitochondria, accumulation of pyruvate and the rise in NADH/NAD^+ drives the conversion of pyruvate anions into L-lactate$^-$ in a reaction catalysed by the enzyme lactate dehydrogenase (LDH). While approximately 32 mmol of ATP are regenerated from oxidation of 1 mmol of glucose, only 2 mmol of ATP are regenerated from 1 mmol of glucose in anaerobic glycolysis. Nevertheless, it provides the cell with a rapid way to regenerate some ATP. But the price to pay is high, as 1 mmol of L-lactic acid is produced per 1 mmol of ATP formed in this process. Although it seems 'obvious' that H^+ are produced during flux through the glycolytic pathway, H^+ are actually formed during the hydrolysis of ATP when work is performed; this is the initial step that generates ADP and augments the rate of glycolysis:

$$\begin{aligned}
&\text{Work} + \text{n ATP}^{4-} \rightarrow \text{n}\left(\text{ADP} + \text{P}_\text{i}\right)^{5-} + \text{n H}^+ \\
&\text{Glucose} + \text{n}\left(\text{ADP} + \text{P}_\text{i}\right)^{5-} \rightarrow \text{n L} - \text{lactate}^- + \text{n ATP}^{4-} \\
&\text{Sum: Work} + \text{Glucose} + \text{n}\left(\text{ADP} + \text{P}_\text{i}\right)^{5-} \rightarrow \\
&\qquad \text{n L} - \text{lactate}^- + \text{n ATP}^{4-} + \text{n H}^+
\end{aligned} \tag{35.9}$$

(n equals any whole number).

H^+ can accumulate very quickly when the concentration of ADP rises in cells. This marked rise in the concentration of H^+ will cause one of the key enzymes in glycolysis, phosphofrucotokinase-1, to lose all of its activity. While this minimizes the drop in intracellular pH, there is a huge price to pay, since this may lead to *an energy crisis*, especially in cells of vital organs (e.g. brain).

A high rate of glycolysis may occur in the presence of an adequate supply of oxygen if there is a defect in the electron transport system or if there is a very high rate of uncoupling of oxidative phosphorylation, because in these conditions ADP cannot be converted back to ATP quickly enough.

The major clinical scenario causing L-lactic acidosis is cardiogenic shock in which inadequate delivery of oxygen to the tissues impairs rapid regeneration of ATP. This type of L-lactic acidosis is known as type A L-lactic acidosis; all other causes are lumped together as type B L-lactic acidosis. We don't find this classification helpful, since cardiogenic shock is such an obvious clinical diagnosis. Furthermore, it ignores the fact that among patients with type B L-lactic acidosis are those in which the underlying pathophysiology is also due to overproduction of L-lactic acid for reasons other than hypoxia.

Clinical settings with L-lactic acid overproduction

Inadequate delivery of O_2

The commonest clinical setting for rapid overproduction of L-lactic acidosis is cardiogenic shock. Other examples of conditions that lead to an inadequate delivery of O_2 to tissues include acute airway obstruction, haemorrhagic shock, and carbon monoxide poisoning. In patients with sepsis, there can be circulatory disturbances that lead to tissue hypoxia (both decreased delivery of oxygen and impaired extraction of oxygen). In addition to *an energy crisis* due to failure to regenerate ATP, when L-Lactic acidosis is associated with a decreased EABV and a high capillary PCO_2, the BBS will fail to remove enough H^+; hence more H^+ will bind to intracellular proteins in vital organs (e.g. the brain), which may further impair their functions.

The aim of therapy is to increase blood flow and delivery of oxygen to vital organs by whatever means are necessary—no other therapy will save the patient if the cardiac output cannot be significantly improved. Therefore, the crucial issue in therapy is to improve the ability of the patient to regenerate ATP in vital organs, rather than correction of the metabolic acidosis per se. Measures to improve haemodynamics to restore adequate cardiac output and tissue perfusion (e.g. ionotropic agents) are critical, as are means to ensure that blood has an adequate content of oxygen. The use of $NaHCO_3$ during severe hypoxia may be of no value, because of the large magnitude of the H^+ load. Nevertheless, $NaHCO_3$ may gain some time to improve myocardial function in cases in which hypoxia is marginal and potentially reversible, but this issue remains controversial. The Na^+ load accompanying the HCO_3^- poses a major limit to this type of therapy in patients with cardiogenic shock and pulmonary oedema.

Excessive demand for oxygen

L-lactic acidosis due to excessive demand for oxygen occurs during seizures or extreme exercise. Another example is the mini-seizures causing L-lactic acidosis in some patients given isoniazid, a drug commonly used to treat tuberculosis. This may be due to the rapid development of vitamin B_6 (pyridoxine) deficiency, because of the formation of an isoniazid-vitamin B_6 complex. Pyridoxine is a cofactor for the reaction catalysed by the enzyme glutamic acid decarboxylase, in which glutamate is converted to the inhibitory neurotransmitter γ-amino butyric acid (GABA). Therefore, a deficiency of GABA could result in increased excitability and increased muscle twitching, and at times seizures (Chin et al., 1979). Patients on chronic haemodialysis are at increased risk, because they tend

to be deficient in vitamin B_6 due to removal of this vitamin by haemodialysis (Siskind et al., 1993).

Clinical settings with increased production of L-lactic acid in absence of hypoxia

Ethanol intoxication

The degree of L-lactic acidosis is usually mild (~ 5 mmol/L), because it reflects the higher NADH/NAD^+ ratio due to ongoing production of NADH from ethanol metabolism, which is largely restricted to the liver. A more severe degree of L-lactic acidosis suggests that there is L-lactic acid overproduction caused by hypoxia (e.g. shock following GI bleeding), thiamine deficiency, seizures (alcohol withdrawal, delirium tremens, and or a central nervous system (CNS) lesion), and/or L-lactic acid under-utilization due to severe liver disease from an acute alcoholic hepatitis superimposed on chronic liver disease (e.g. fatty liver, cirrhosis).

Thiamine deficiency and ethanol intoxication

A severe degree of lactic acidosis may develop rapidly in these patients (Shull and Rapoport, 2010). Thiamine (vitamin B_1) is a key cofactor for pyruvate dehydrogenase (PDH). The site of L-lactic acid production is likely to be the liver, because it is the site where there is accumulation of pyruvate owing to diminished activity of PDH and a high NADH/NAD^+ ratio (due to metabolism of ethanol). Nevertheless, for a severe degree of L-lactic acidosis to develop there must be a high flux in glycolysis. This occurs when the rate of hydrolysis of ATP to ADP and inorganic phosphate to perform work exceeds the rate of regeneration of ATP from ADP in oxidative phosphorylation. As pyruvate accumulates, it gets converted to L-lactate in a reaction catalysed by lactate dehydrogenase, in which NADH is converted to NAD^+. The conversion of NADH to NAD^+ in the cytosol limits its availability for mitochondrial oxidative phosphorylation and the regeneration of ATP. Thus the concentration of ADP will rise and anaerobic glycolysis will be stimulated in the liver to make ATP.

While acidaemia may be severe, damage to the brain is the major concern in these patients. The brain must regenerate ATP as fast as it is being used to perform biologic work. Ketoacids are the preferred fuel if they are present, because they are derived from storage fat and hence proteins from lean body mass are spared as a source of glucose for the brain during prolonged starvation. After successful treatment of the disorder, and as ketoacids become unavailable, the brain must regenerate most of its ATP from the oxidation of glucose, which will be limited by the diminished activity of PDH due to a lack of thiamine. Perhaps of greater significance is the likelihood of an increased demand for ATP regeneration in this setting (e.g. due to delirium tremens or the use of salicylates that may uncouple oxidative phosphorylation in the brain). Hence the concentration of ADP will rise and anaerobic glycolysis will be stimulated in the brain to make ATP. As a result, there will be a sudden rise in the production of H^+ and L-lactate anions in areas of the brain where the metabolic rate is more rapid and/or ones that have the lowest reserve of thiamine. Treatment is obviously to administer thiamine early in the course of therapy *before* the ketoacids concentration in plasma falls.

Riboflavin deficiency and the use of tricyclic antidepressants

The active metabolites formed from vitamin B_2 (riboflavin), flavine mononucleotide (FMN) and flavine adenine dinucleotide (FAD), are components of the mitochondrial electron transport system, which is the principal pathway to regenerate ATP (Luzzati et al., 1999). Riboflavin deficiency may cause L-lactic acidosis. Riboflavin must be activated via an ATP-dependent kinase to produce FMN and FAD. Tricyclic antidepressant drugs (e.g. amitriptyline and imipramine) inhibit this kinase (Pinto et al., 1981). The activity of this kinase is also decreased in hypothyroidism and L-lactic acidosis may be seen in patients with myxoedema crisis.

Uncoupling of oxidative phosphorylation

In coupled oxidative phosphorylation, H^+ are pumped out from the mitochondrial matrix through the inner mitochondrial membrane using the energy derived from the oxidation of fuels. These H^+ re-enter the mitochondrial matrix via special H^+ channels in the inner mitochondrial membrane that are linked to the conversion of ADP to ATP. In contrast, if H^+ re-enter the mitochondrial matrix through another H^+ channel that is *not* linked to the conversion of ADP to ATP, this is called uncoupled oxidative phosphorylation (Hanstein, 1976). Phenformin is a biguanide drug that is no longer in use because it caused a high incidence of L-lactic acidosis in patients with type 2 diabetes mellitus. This drug has a large hydrophobic end, which allows it to cross the lipid-rich mitochondrial membrane rapidly. In doing so, it brought H^+ into the mitochondria quickly unlinked to the conversion of ADP to ATP. Metformin is another biguanide drug, but since it does not have a large hydrophobic tail, it is only a very weak uncoupler of oxidative phosphorylation, and is rarely (in the absence of severe renal impairment in which the drug can accumulate sufficiently to be) a cause of L-lactic acidosis (Lalau, 2010; Salpeter et al., 2010). Acetyl salicylic acid is also an uncoupler of oxidative phosphorylation (Miyahara and Karler, 1965).

Clinical settings with primary slow removal of L-lactic acid

This type of L-lactic acidosis does not have the same urgency as the type with primary overproduction of L-lactic acid, because it is not caused by a problem in regenerating ATP. In addition, the rate of H^+ accumulation is usually much slower. A chronic steady state of L-lactic acidosis is often present and the causes are a low rate of removal of L-lactic acid usually related to problems with the liver due to hepatitis, replacement of normal liver cells (e.g. by tumour cells or large fat deposits), or destruction of liver from prior hypoxia (e.g. 'shock liver'). In patients with a malignancy and hepatic metastases, the mechanisms that contribute to the L-lactic acidosis are the replacement of a substantial number of liver cells with tumour cells to impair L-lactic acid removal or production of metabolites by tumour cells such as the amino acid tryptophan, which may inhibit the conversion of pyruvate to glucose in the liver, and/or the fact that ischaemic tumour cells will produce more L-lactic acid.

Administration of $NaHCO_3$ to these patients may have detrimental long-term effects, because the load of alkali may increase L-lactic acid production from glucose by de-inhibiting an important rate-controlling enzyme in glycolysis in malignant cells (phosphofructokinase-1). A considerable amount of lean body mass may be lost if the source of pyruvate is glucose that is made from amino acids (gluconeogenesis) (Fields et al., 1981).

Antiretroviral drugs

L-lactic acidosis has been reported in patients with HIV infection treated with various anti-retroviral agents. The agent most

frequently associated with L-lactic acidosis is zidovudine, but didanosine, stavudine, lamivudine, and indinavir have also been implicated. There are two possible mechanisms whereby anti-retroviral agents may cause L-lactic acidosis. First, they block the electron transport system. This may lead to mitochondrial myopathy manifested by ragged-red fibres and mitochondrial DNA depletion (Brinkman et al., 1999). Second, these drugs may lead to replacement of liver parenchyma with storage fat (steatosis) (Coghlan et al., 2001). This view is supported by the fact that in some of these patients the consumption of a small dose of ethanol, which results in a higher NADH concentration in hepatocytes and the diversion of pyruvate to L-lactate, led to a significant increase in the severity of L-lactic acidosis (Gopinath et al., 1992).

Methanol intoxication

Methanol is methyl alcohol (molecular weight 32); it is used as antifreeze, an additive to gasoline, and a solvent in the manufacture of various drugs. Methanol itself is not toxic, but its metabolic product, formaldehyde, is the major cause of toxicity (Oh et al., 2005), because it rapidly binds to tissue proteins.

Methanol is converted to formaldehyde by alcohol dehydrogenase in the liver, but a high concentration of methanol is required for rapid rates of oxidation. Formaldehyde is rapidly converted to formic acid by aldehyde dehydrogenase. In each step, NAD^+ is converted to NADH. The metabolic acidosis of methanol poisoning is associated with an increased $P_{Anion\ gap}$ due to the accumulation of formate and L-lactate anions; the L-lactate level greatly exceeds that of formate. The L-lactic acidosis results from inhibition of cytochrome oxidase by formate and also from the conversion of pyruvate to L-lactate in the liver due to an increased $NADH/NAD^+$ ratio caused by methanol metabolism. HCO_3^- is regenerated when lactate and formate anions are metabolized to neutral end products; folic acid is a cofactor in formate metabolism.

Early on, symptoms of intoxication (e.g. ataxia, and slurred speech) dominate the clinical picture. Later, when methanol is converted to formaldehyde by retinol dehydrogenase, blurred vision and blindness may develop. Abdominal pain, malaise, headache, and vomiting are other findings. Fundoscopic examination may reveal papilloedema. Methanol intoxication should always be considered in the differential diagnosis of metabolic acidosis with an increased $P_{Anion\ gap}$, particularly if the ECF volume is not very contracted. This diagnosis should be suspected when there is an elevated $P_{Osmolal\ gap}$ (see earlier) and confirmed by a direct assay for methanol in the blood.

Ethylene glycol intoxication

Ethylene glycol (molecular weight of 62) is widely used as an antifreeze, as a solvent in the manufacture of paint and plastics, and in the formulation of printers' inks, stamp pad inks, and inks for ballpoint pens. Ethylene glycol is converted to glycoaldehyde by alcohol dehydrogenase in the liver, the affinity of this enzyme for ethylene glycol is close to 100 times lower than for ethanol; thus, the rate of metabolism of ethylene glycol is rapid only when its concentration is high. Glycoaldehyde is further metabolized to glycolic acid by hepatic aldehyde dehydrogenase, which is the major acid that accumulates in ethylene glycol poisoning (Oh et al., 2005). One per cent or less of glycolic acid is converted to oxalic acid, mainly by the action of the enzyme lactae dehydrogenase. Virtually all oxalate produced is precipitated as calcium oxalate, contributing to acute renal failure and hypocalcaemia. The major end product of glycolic acid metabolism is glycine via transamination with alanine; vitamin B_6 is a cofactor.

Ethylene glycol itself causes CNS symptoms such as inebriation, ataxia, and slurred speech. At this stage, the $P_{Osmolal\ gap}$ is high. After a latent period of about 4–12 hours, patients develop nausea, vomiting, hyperventilation, elevated blood pressure, tachycardia, tetany, and convulsions. At this point, metabolic acidosis with an increased $P_{Anion\ gap}$ is present. The tetany is most likely caused by hypocalcaemia, which is thought to be the result of deposition of calcium oxalate crystals. Renal failure is common and usually develops 36–48 hours after the ingestion of ethylene glycol; glycoaldehyde appears to be the main toxin.

The principles of treatment of methanol or ethylene glycol poisoning are virtually identical. They include administration of ethanol to achieve blood concentrations of about 20 mmol/L to reduce metabolism, and removal of these toxic alcohols and their metabolites by haemodialysis. One could administer fomepizole, an inhibitor of hepatic alcohol dehydrogenase, instead of ethanol. The major difference in treating ethylene glycol poisoning is that when acute oliguric renal failure is present, ECF volume overload or pulmonary oedema may limit the amount of $NaHCO_3$ that can be administered, so early institution of dialysis may be important.

Salicylate intoxication

The major issue with an overdose of aspirin is the toxicity related to the effect of salicylate anions in cells (Oh et al., 2005; Halperin et al., 2010). This may result from direct toxic effects of salicylate on cell functions. It is also possible that this organic acid uncouples oxidative phosphorylation (Miyahara and Karler, 1965). If an increased consumption of O_2 and production of CO_2 occurs near the respiratory centre, this could stimulate alveolar ventilation and may explain the respiratory alkalosis that is commonly seen in these patients. A modest degree of uncoupling of oxidative phosphorylation can increase the production of ketoacids in the liver. In severe intoxications, the degree of uncoupling of oxidative phosphorylation may be excessive. If this compromises the rate of conversion of ADP to ATP, anaerobic glycolysis is stimulated and a severe degree of L-lactic acidosis develops. Hypoglycaemia is common in patients with salicylate intoxication, which likely reflects increased utilization of glucose by the brain (due to uncoupling of oxidative phosphorylation) and/or impaired gluconeogenesis (Oh et al., 2005). Toxicity caused by the monovalent salicylate anion occurs when its concentration is 3–5 mmol/L; if the $P_{Anion\ gap}$ is elevated by a much greater amount, accumulation of ketoacid or L-lactate anions is likely to be present.

Treatment is initially aimed at preventing the accumulation of salicylates in brain cells. Salicylic acid (H•SA) is a weak organic acid that is transported across cell membranes in its undissociated form. To appreciate the effect of acidaemia on salicylate toxicity, consider the following example in which the total salicylate concentration in the ECF is 7 mmol/L. Because of its low pK (~ 3.5), only a very tiny fraction is in the H•SA form at normal blood pH value of 7.40 (i.e. H•SA = 0.3 μmol/L). H•SA diffuses across cell membranes until its concentration is equal inside and outside cells. In the cell, the concentration of total salicylate depends on the ICF pH. As ICF pH is normally close to 7.1, the pK of salicylic

acid/salicylate is ~3.5, at H•SA concentration of 0.3 umol/L, the concentration of salicylate anion in cells will be close to 3.5 mmol/L. If the pH in ECF drops to 7.1, the concentration of H•SA will rise from 0.3 to 0.6 μmol/L. Because H•SA diffuses across cell membranes to achieve equilibrium, the intracellular total salicylate concentration will rise from 3.5 to 6.0 mmol/L. Hence, alkalinizing the ECF tends to decrease salicylate accumulation in cells. By the same token, alkalinizing the urine may reduce salicylate reabsorption by PCT cells and markedly enhance its excretion.

Dialysis should be instituted if the salicylate blood level exceeds 6 mmol/L (90 mg/dL). If the salicylate blood level exceeds 4 mmol/L (60 mg/dL), dialysis should be considered, particularly if further absorption is anticipated. In patients with an unexplained decreased level of consciousness, dialysis should be started, even at lower levels of salicylate in blood, because of the poor prognosis. Haemodialysis is more efficient for the removal of salicylate, but peritoneal dialysis may be considered if there will be a long delay before haemodialysis can be initiated. In the absence of severe toxicity, the therapeutic efforts in salicylate intoxication are to decrease the concentration of H•SA in blood and to promote the urinary excretion of salicylate with the administration of $NaHCO_3$. Notwithstanding, aggressive therapy with $NaHCO_3$ should be avoided, because patients may become very alkalaemic due to coexistent respiratory alkalosis. Furthermore, patients with salicylate intoxication may have increased capillary permeability and be at risk for pulmonary oedema and cerebral oedema following excessive fluid administration.

Acetazolamide, a carbonic anhydrase inhibitor, may be useful in the therapy for salicylate intoxication to increase excretion of salicylate anions in the urine. Its mechanism of action is controversial. The traditional view is that acetazolamide increases the excretion of the salicylate anions by raising the pH in the lumen of the PCT, thereby decreasing the concentration of the undissociated H•SA. However, acetazolamide causes an acid disequilibrium pH (by slowing the conversion of H_2CO_2 to CO_2 and H_2O) in the lumen of the PCT, yet it still promotes salicylate excretion.

Caution is needed because acetazolamide competes with salicylate anions for binding to plasma albumin, which may increase the free concentration of H•SA in blood. In addition, acetazolamide may induce acidaemia by increasing the excretion of HCO_3^- in the urine. There is some experimental evidence in humans to suggest that 250 mg of acetazolamide has a tubular effect that lasts for approximately 16 hours (Bayoumi et al., 1993). Therefore, one could use a low dose of acetazolamide instead of alkali therapy in a patient with a high blood pH (i.e. > 7.5) and a modestly elevated blood level of salicylate.

Glue sniffing

It was thought that glue sniffing causes dRTA, but the high rate of excretion of NH_4^+ in response to the metabolic acidosis in many of these patients means that they do not have a major defect in renal acid excretion. Patients who sniff glue for its intoxicating properties absorb a significant quantity of toluene (methylbenzene). Toluene is metabolized via a series of reactions in the liver to hippuric acid (Carlisle et al., 1991). Despite the production of the hippurate anion, the $P_{Anion\ gap}$ is not significantly elevated, because the kidneys, via filtration and especially via tubular secretion, efficiently excrete hippurate. As a result, there is development of a hyperchloraemic metabolic acidosis. Together with the hippurate anion, Na^+ and K^+ are excreted in the urine, leading to ECF volume contraction and hypokalaemia. The presence of NH_4^+ and hippurate in the urine could be detected by the presence of an appreciable $U_{Osmolal\ gap}$ (see above).

When the inhalation of toluene stops, ultimately the production of hippuric acid will be diminished, but there can be a lag of 1–3 days, because of the large volume of distribution of toluene. With regard to treatment, hypokalaemia and ECF volume contraction need to be corrected with the administration of KCl and saline. If metabolic acidosis is severe, consider giving $NaHCO_3$, because there is no appreciable amount of hippurate anions present in the body that can be metabolized to HCO_3^-. The major caveat to the use of $NaHCO_3$ is coexisting K^+ depletion, which could be severe, leading to the risk of a cardiac arrhythmia. Thus the P_K must be raised first to the mid-3 mmol/L range before $NaHCO_3$ is given.

D-lactic acidosis

Certain bacteria in the GI tract may convert carbohydrate (cellulose and fructose) into organic acids (Halperin and Kamel, 1996; Uribarri et al., 1998). Three factors that make this possible are: slow GI transit (blind loops, obstruction), change of the normal gut flora (usually prior antibiotic therapy), and the supply of carbohydrate substrate to these bacteria (foods containing cellulose or fructose). The most prevalent organic acid that is produced in this process is D-lactic acid. Although humans lack the enzyme D-lactate dehydrogenase, metabolism of D-lactate occurs via the enzyme D-2-hydroxyacid dehydrogenase. Therefore, humans metabolize this D-isomer more slowly than L-lactate, but since the rate of production of these organic acids is not rapid, the degree of acidaemia is usually not severe, unless there is also a defect in the excretion of NH_4^+. There are three additional points that should be noted with respect to D-lactic acidosis. First, the usual clinical laboratory test for lactate is specific for the L-lactate isomer and so the laboratory measurement for lactate will not be elevated in this setting. Second, GI bacteria produce amines, mercaptans, and other compounds that may cause the clinical signs and symptoms related to CNS dysfunction (personality changes, gait changes, confusion, etc.). Third, some of the D-lactate anions may be lost in the GI tract or in the urine (if the GFR is not too low) and the rise in the $P_{Anion\ gap}$ may not be as high as expected for the fall in the P_{HCO3}.

Treatment should be directed at the GI problem. The oral intake of fructose and complex carbohydrates should be decreased. Antacids and oral $NaHCO_3$ should be avoided, because they may lead to a higher intestinal luminal pH and a higher rate of production of organic acids and other toxic products of fermentation. Insulin may be helpful by lowering the rate of oxidation of fatty acids and hence permitting a higher rate of oxidation of these organic acids. Poorly absorbed antibiotics (e.g. vancomycin) can be used to change the bacterial flora in the GI tract.

Pyroglutamic acidosis

Pyroglutamic acidosis (PGA) was previously thought to represent a rare inborn error of metabolism in the glutathione synthesis pathway (a defect in 5-oxoprolinase or in glutathione synthase). A number of cases of PGA have been reported, particularly in critically ill patients (Dempsey et al., 2000; Mizock and Mecher, 2000). Of interest, the majority of these cases occur in women, the reason for this is not clear. Glutathione (GSH) is made up of three amino acids: glutamate, cysteine, and glycine. It is the sulfhydryl moiety of cysteine that endows this compound with its ability to detoxify

reactive oxygen species (ROS). In this process, the reduced form of glutathione (GSH) is converted to its oxidized form (GS-SG). Key to understanding the basis for the accumulation of PGA is that GSH inhibits the enzyme γ-glutamylcysteine (γ-GC) synthase, which catalyses the first step in the cycle that converts glutamate to γ-GC (Fig. 35.7). Hence, when ROS accumulate, as in a patient with sepsis, the concentration of GSH declines, and its inhibitory effect on γ-GC synthase is removed. This results in accelerated formation of γ-glutamyl phosphate. If the patient is cysteine deficient, γ-GC will not be formed, instead, γ-glutamyl phosphate will be transformed to PGA by the enzyme cyclotransferase (Emmett, 2014). A number of drugs have been identified as causes of PGA accumulation. *N*-acetyl-*p*-benzoquinonimide (NAPBQ I), a highly reactive metabolite of acetaminophen, depletes GSH. Thus, the feedback inhibition of the enzyme γ-GC synthase is removed, and PGA develops as above (Fenves et al., 2006). Certain drugs (e.g. the antibiotic flucloxacillin and the anticonvulsant, vigabatrin) inhibit 5-oxoprolinase, which converts PGA to glutamate (Fig. 35.6).

The major danger in patients with PGA related to sepsis is tissue damage by ROS. While $NaHCO_3$ may be needed, treatment of sepsis is the key issue. Drugs that may cause PGA should be discontinued. *N*-acetyl cysteine should be given in patients with acetaminophen overdose and is also likely beneficial in patients with PGA related to sepsis. Malnutrition is thought to be a risk factor for the development of PGA and so nutritional support must be provided. Riboflavin deficiency may be important in this regard, because FMN and FAD are cofactors in the reaction catalysed by glutathione reductase, which mediates the conversion of oxidized GS-SG to GSH.

Chronic renal failure

When the GFR is markedly reduced, the following changes can be expected.

NH_4^+ excretion: the excretion of NH_4^+ declines markedly when there is a low availability of ADP in PCT cells (lower filtered load of Na^+, so less renal work). The quantity of H^+ retained can be as high as 30–40 mmol/day, if dietary protein intake is not reduced and excretion of NH_4^+ is very low.

Diet alkali: the alkali load is derived mainly from dietary fruit and vegetables (e.g. K^+ plus organic anions (OA^-)), and it is normally eliminated as K^+ plus OA^- in the urine if the K^+ can be excreted. Once acidaemia develops, the excretion of alkali, as potential HCO_3^-, declines and the dietary HCO_3^- load may be retained. In this setting, these HCO_3^- ions would titrate many of the H^+ from H_2SO_4 produced from the metabolism of sulphur-containing amino acids, and thereby diminish the net H^+ load that must be eliminated each day by the excretion of NH_4^+. Because patients with renal insufficiency are usually placed on a low K^+ diet, they eat less alkali and, as a result, are more likely to become acidaemic.

The $P_{Anion\ gap}$ usually does not rise appreciably until the GFR has fallen to < 20 mL/min. The high $P_{Anion\ gap}$ in patients with renal insufficiency does not represent the production of an unusually high amount of new acids; rather it is due to the low GFR and accumulation of SO_4^{2-} and phosphate anions.

Experimental evidence from studies in rats strongly suggests that acidaemia is a catabolic signal in uraemia, although evidence from human data is less robust (Weinstein et al., 2004). $NaHCO_3$ supplementation has also been shown to slow the progression of chronic kidney disease (Yaqoob, 2010). It is now recommend that acidaemia in patients with chronic kidney disease should be corrected. After the P_{HCO3} is corrected, the dose of $NaHCO_3$ needed to maintain P_{HCO3} in the normal range is usually < 30–40 mmol/day (i.e. enough to titrate the acid load produced from the metabolism of dietary sulphur-containing amino acids). This salt load should not represent a problem to most patients with chronic kidney disease.

Diarrhoea

There are two major sites where HCO_3^- are added to the lumen of the GI tract and are possible sites for its loss (Field, 2003).

Secretion of HCO_3^- by the pancreas and small intestine

This secretion is stimulated by the load of H^+ from the stomach and is approximately 100 mmol/day. Hence, there is only a modest daily net deficit of $NaHCO_3$ if most of these upper GI secretions are lost.

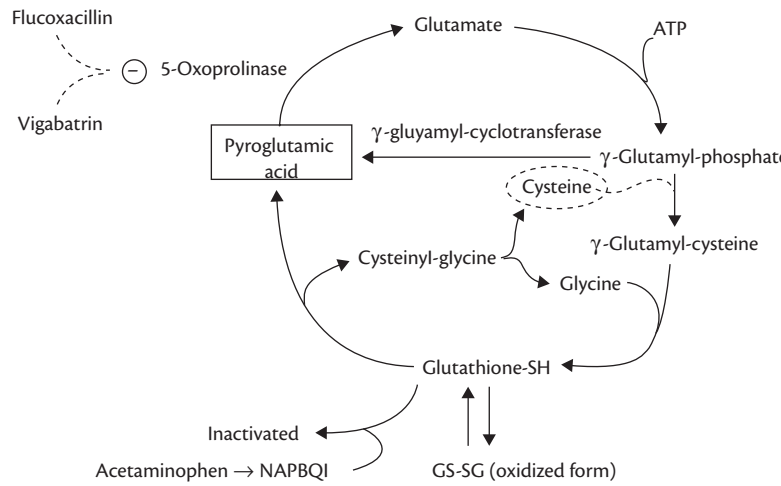

Fig. 35.7 Production of pyroglutamic acid. When there are low levels of reduced glutathione (e.g. due to depletion by NAPBQI, a highly reactive metabolite of acetaminophen, or when ROS accumulate, as in a patient with sepsis), its inhibitory effect on γ-GC synthase is removed. This results in accelerated formation of γ-glutamyl phosphate. If the patient is cysteine deficient, γ-GC will not be formed, instead, γ-glutamyl phosphate will be transformed to PGA by the enzyme cyclotransferase. In addition, if 5-oxyprolinase is inhibited (e.g. by flucloxacillin, vigabatrin), pyroglutamic acid will accumulate.

Therefore, metabolic acidosis in these patients is likely to be mild, unless the duration of these losses is prolonged and/or there is also another disorder that diminishes the rate of excretion of NH_4^+ in the urine. Metabolic acidosis may also develop if the fluid rich in $NaHCO_3$ is retained in the lumen of the intestine (e.g. ileus).

Secretion of HCO_3^- by the late small intestine and the colon

Two luminal transport mechanisms are involved in this process, an NHE and a Cl^-/HCO_3^- anion exchanger (AE). Normally, the maximum transport capacity of NHE is less than that of AE. If there is a large delivery of Na^+ and Cl^- to the colon, due to osmotic or secretory diarrhoea, the net effect, as more Cl^- is exchanged with HCO_3^- than Na^+ is exchanged for H^+, is a loss of $NaHCO_3$ in stool, which may lead to the development of acidaemia. On the other hand, if there is a large loss of NaCl in stool and a large decrease in the ECF volume, a significant degree of acidaemia is less likely to occur.

In some patients who have diarrhoea, there may be overproduction of organic acids in the colon, such as D-lactic acid. The degree of metabolic acidosis may be more severe, if there is also a low rate of excretion of NH_4^+ due to a low the GFR resulting from a contracted EABV.

For treatment, one must first identify and treat emergencies that may be present on admission (e.g. haemodynamic instability), as well as anticipate and avoid those that might develop with therapy (e.g. hypokalaemia). It is interesting to note that some patients with severe diarrhoea due to cholera developed pulmonary oedema when given an amount of saline that is not sufficient to restore their EABV (Greenough et al., 1976). Paradoxically, pulmonary oedema in these patients was cured with the administration of $NaHCO_3$. The explanation may be that these patients developed worsening acidaemia with the administration of saline, which results in venoconstriction and an acute increase in central blood volume. Thus, administration of $NaHCO_3$ (or Na^+ with an anion that can be metabolized to produce HCO_3^-, such as lactate) may be necessary in patients with diarrhoea, since there are no retained anions that can be metabolized to produce HCO_3^-. Enhancing the absorption of NaCl secreted by the intestinal tract diminishes the volume of diarrhoea fluid. This can be achieved by giving oral rehydration therapy (ORT) with an equimolar solution of glucose and NaCl. In more modern versions of this solution, a form of potential HCO_3^- is added by, for example, replacing 25 to 50 mmol of Cl^- with citrate anions that can be metabolized to HCO_3^-.

Proximal renal tubular acidosis

(See Chapter 36.)

Proximal RTA may occur as an isolated defect or as part of more generalized PCT cell dysfunction (Fanconi syndrome with glucosuria, phosphaturia, uricosuria, citraturia, and aminoaciduria) (Haque et al., 2012). The major causes of pRTA in adults are paraproteinaemias (e.g. patients with multiple myeloma) and use of carbonic anhydrase inhibitors (e.g. acetazolamide, the antiepileptic drug topiramate). In contrast, cystinosis and the use of ifosfamide are the most common causes of pRTA in children. Hereditary isolated pRTA is a rare autosomal recessive disease that can present with ocular abnormalities such as band keratopathy, cataract and glaucoma. Mutations in the gene encoding for the Na $(HCO_3)_3^{2-}$ cotransporter (NBC1) have been identified in these families (Igarashi et al., 2002).

The initial mechanism for the acidosis in patients with pRTA is the loss of HCO_3^- in the urine. Once a steady state supervenes, chronic metabolic acidosis is sustained, because the rate of NH_4^+ excretion is lower than expected for the presence of acidaemia (Kamel et al., 1997).

Because the high rate of excretion of citrate decreases the urinary concentration of ionized Ca^{2+}, calcium stones are not usually seen in patients with pRTA. Patients on acetazolamide or topiramate may develop calcium phosphate stones.

From a therapeutic standpoint, the acidaemia in these patients is usually mild and complications due to the acidosis are minor, which argues against alkali therapy in adults. In addition, if $NaHCO_3$ is given, the P_{HCO3} rises temporarily, and urinary excretion HCO_3^- will also increase. A large increase in delivery of Na^+ and HCO_3^- to the cortical distal nephron can augment the secretion of K^+, resulting in hypokalaemia and also increases the risk of forming calcium phosphate kidney stones. In contrast, alkali therapy is useful in children to prevent growth retardation.

Distal renal tubular acidosis

(See Chapter 36.)

The hallmark of distal RTA is a low rate of excretion of NH_4^+ in a patient with chronic metabolic acidosis, a normal value for the $P_{Anion\ gap}$, and a GFR that is not markedly reduced (Carlisle et al., 1991). Having defined these components, the next step is to find out why the rate of excretion of NH_4^+ may be lower than expected in this setting. We use the urinary pH at this point to separate patients into three categories; those with a primary problem with NH_3 availability (urine pH close to 5), those with a defect in net distal H^+ secretion (urine pH close to 7), and those in which there is a structural lesion in the renal medulla that compromises both medullary NH_3 availability and distal H^+ secretion (the urine pH is usually close to 6).

Subtypes of disorders causing low NH_4^+ excretion

Low availability of NH_3

The usual causes of a low production of NH_4^+ are an alkaline PCT cell pH due to hyperkalaemia, and a low availability of ADP in PCT cells due to a very low GFR (Fig. 35.8). Less common causes include an alkaline PCT cell pH due to a genetic disorder or a disease process that causes a defect in the exit step for HCO_3^-, decreased availability of glutamine due to malnutrition, and/or high levels of other fuels that PCT cells can oxidize in place of glutamine to regenerate ATP (e.g. during total parenteral nutrition).

Low net distal H^+ secretion

Autoimmune disorders (such as Sjögren syndrome, systemic lupus erythematosus, hyper-γ-globulinaemia) are the most common causes of dRTA with a high urinary pH in adults. RTA in patients with Sjögren syndrome seems to be due to a defect in H^+ secretion in the distal nephron. In some of these patients, absence of the H^+-ATPase pump in intercalated cells of the collecting tubule was found on immunocytochemical analysis of tissue obtained by renal biopsy (Cohen et al., 1992). It is not known how the immune injury leads to the loss of H^+-ATPase activity. It has also been suggested that the defect may be due to autoantibodies against carbonic anhydrase II or the basolateral Cl^-/HCO^-_3 anion exchanger, as well as decreased number of intercalated cells. Amphotericin B may cause a low *net* H^+ secretion in the distal nephron, because of a back-leak of H^+ into α-intercalated cells. In rare patients with Southeast Asian

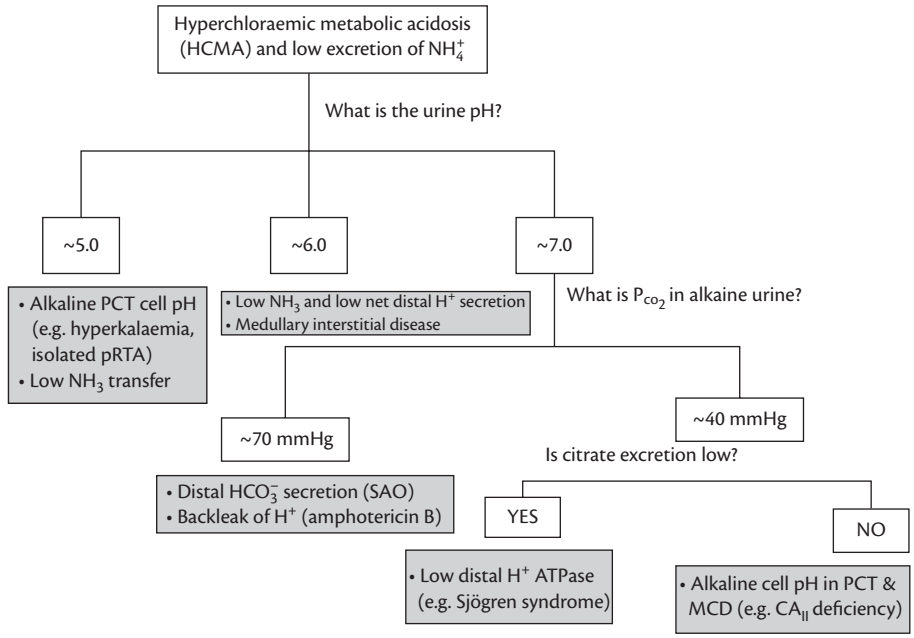

Fig. 35.8 Steps in clinical approach to the patient with metabolic acidosis and a low rate of excretion of ammonium.

ovalocytosis (SAO), a second mutation in the anion exchanger AE1, results in some of the AE1 being targeted abnormally to the luminal membrane of α-intercalated cells.

Alkali therapy is usually needed in patients with dRTA and a urinary pH > 6.5, as they are unable to excrete enough NH_4^+ to regenerate the HCO_3^- consumed by the dietary acid load. Bicarbonaturia, however, should be minimized, because it might predispose to excessive renal K^+ loss and $CaHPO_4$ kidney stone formation (see earlier for pRTA). Therefore, the dose of $NaHCO_3$ should be as small as possible and distributed throughout the day. After the P_{HCO3} is corrected, the dose of $NaHCO_3$ needed to maintain P_{HCO3} in the normal range is usually less than 30 to 40 mmol/day (i.e. enough to titrate the acid load produced from the metabolism of dietary sulphur-containing amino acids).

Lesions involving both distal H^+ secretion and NH_3 availability

The list of causes of disorders that affect the renal medullary interstitial compartment is long and includes infections, drugs, infiltrations, precipitations, inflammatory disorders, and sickle cell anaemia, among others. Because of the medullary interstitial disorder, these patients may also have a reduced urinary concentrating ability. Hyperkalaemia may be present if the disease process also involves the distal cortical nephron. Nevertheless, when the P_K returns to the normal range, the acidaemia persists (see below).

Administration of alkali is needed to correct the acidaemia. The issues concerning alkali therapy were discussed above in the subgroup with diminished net H^+ secretion and apply in this setting too.

Distal RTA with hyperkalaemia

The term *type IV RTA* is used to describe the constellation of findings of hyperkalaemia and metabolic acidosis due to a low rate of excretion of NH_4^+. Nevertheless, there are two distinct ways that hyperkalaemia and a low rate of excretion of NH_4^+ may coexist (Halperin et al., 2010).

Hyperkalaemia is responsible for the low rate of excretion of NH_4^+

Hyperkalaemia may cause a low rate of NH_4^+ excretion by inhibiting either its production (hyperkalaemia is associate with an alkaline PCT cell pH) and/or the transfer of NH_3 in the loop of Henle (K^+ competes with NH_4^+ on the $Na^+/K^+/2Cl^-$ cotransporter). In these patients, the urinary pH is low (~ 5). If this is the case, the patient should have a sufficient increase in the rate of excretion of NH_4^+ to correct the metabolic acidemia after the P_K returns to the normal range.

Hyperkalaemia is not the major reason for the low rate of excretion of NH_4^+

In this subgroup of patients the low rate of excretion of NH_4^+ is not causally linked to the hyperkalaemia, as is indicated by the fact that the rate of excretion of NH_4^+ remains low after the P_K has returned to the normal range. In general, the basis of the low rate of excretion of NH_4^+ in this subgroup is a combination of low NH_3 availability and low distal H^+ secretion; hence, the urine pH would be close to 6. For chronic hyperkalaemia to be present, the disease process must involve the late cortical distal nephron, the major site of K^+ secretion.

Metabolic alkalosis

Metabolic alkalosis is an electrolyte disorder that is accompanied by changes in acid–base parameters in plasma, namely an elevated P_{HCO3} and plasma pH. Most patients with metabolic alkalosis have a deficit of NaCl, KCl, and/or HCl (Fig. 35.9), each of which leads to a higher P_{HCO3}. The following concepts are central to our understanding of why metabolic alkalosis develops (Halperin et al., 2010). They also provide the basis for our clinical approach to this diagnostic category, and to the design of optimal therapy.

Concept: the concentration of HCO_3^- is the ratio of the content of HCO_3^- in the ECF compartment (numerator) and the ECF volume (denominator).

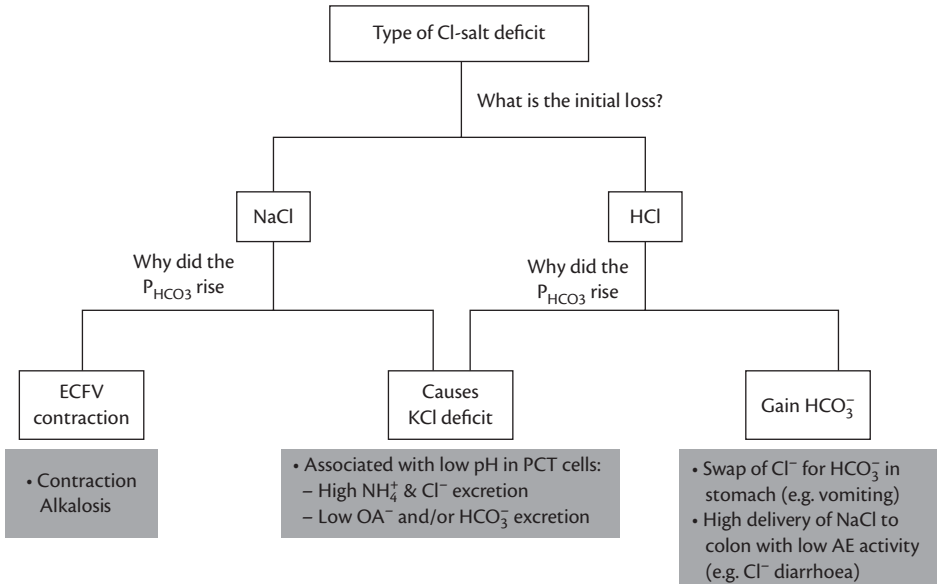

Fig. 35.9 Pathophysiology of metabolic alkalosis caused by a deficiency of chloride salts.

A rise in the concentration of HCO_3^- might be due to an increase in its numerator (addition of HCO_3^-) and/or a decrease in its denominator (diminished ECF volume). A quantitative estimate of the ECF volume is critical to determine the quantity of HCO_3^- in the ECF compartment and to determine the basis of the metabolic alkalosis.

Concept: electroneutrality must be present in every body compartment and in the urine.

This implies that terms such as 'Cl^- depletion alkalosis' are misleading; deficits must be defined as HCl, KCl, and/or NaCl.

Concept: knowing the balances for Na^+, K^+, and Cl^- allows one to decide why the P_{HCO3} has risen and what changes have occurred in the composition of the ECF and ICF compartments.

Although balance data are not available in most patients, a quantitative assessment of ECF volume using the haematocrit can help to reach a tentative conclusion about the contribution of individual deficits of the different Cl^--containing compounds to the development of metabolic alkalosis.

Concept: critical to the understanding of the pathophysiology of metabolic alkalosis is that there is no tubular maximum for HCO_3^- reabsorption in the kidney.

Angiotensin II and the usual pH in PCT cells are the two major physiologic stimuli for $NaHCO_3$ reabsorption in this nephron segment. Both of these stimuli must be removed for $NaHCO_3$ to be excreted. Contrary to the widely held impression, there is no renal tubular maximum for the reabsorption of HCO_3^-. Rather HCO_3^- ions are retained unless their reabsorption is inhibited (low angiotensin II (AII), because of expansion of the EABV and/or an alkaline PCT cell pH or a high plasma HCO_3^- which diminishes HCO_3^- reabsorption by PCT) (Rubin et al., 1994). $NaHCO_3$ loading will not cause metabolic alkalosis, because it expands the EABV and raises the P_{HCO3}. Nevertheless, $NaHCO_3$ may be retained when there is a significant decrease in its filtered load due to an appreciable fall in the GFR.

A disorder which results in a deficit of NaCl or HCl, which can cause a higher P_{HCO3}, may also lead to a secondary deficit in KCl and hypokalaemia. A deficit of K^+ is associated with an acidified PCT cell pH and can initiate and sustain a high P_{HCO3} as a result of renal new HCO_3^- generation (higher rate of excretion of NH_4^+), reduced excretion of dietary HCO_3^- in the form of organic anions, and enhanced reabsorption of HCO_3^- in the PCT.

Concept: alkalaemia suppresses the respiratory centre and this leads to hypoventilation

Diagnostic tests

Quantitative estimate of the ECF volume: it is critical to have a quantitative estimate of the ECF volume to determine the content of HCO_3^- in the ECF compartment and why there was a rise in the P_{HCO3}. We use the haematocrit for this purpose (see 'Metabolic acidosis').

Balance data for Na^+, K^+, and Cl^-: although these data are not available in most patients, they can be inferred if one knows the new ECF volume and the P_{Na}, P_{Cl} and P_{HCO3}. One cannot know the balances for K^+ from these calculations, but one can deduce their rough magnitude by comparing the differences in the content of Na^+ with that of Cl^- and HCO_3^- in the ECF compartment.

Arterial PCO_2: hypoventilation due to metabolic acidosis raise the arterial PCO_2 by 0.7 mmHg for every 1 mmol /L rise in P_{HCO3} (Adrogue and Madias, 2010).

Clinical approach

A list of causes of metabolic alkalosis is provided in Table 35.2. Four aspects of the clinical picture in a patient with metabolic alkalosis merit careful attention and include the medical history (e.g. vomiting, diuretic use), the presence of hypertension, the EABV status, and the P_K. Our clinical approach to a patient with metabolic alkalosis is outlined in Fig. 35.10. The first step is to rule out the common causes of metabolic alkalosis such as vomiting and use of diuretics. Although this may be evident from the history, some

Table 35.2 Causes of metabolic alkalosis

Causes usually associated with a contracted 'effective' arterial blood volume
Low UCl
Loss of gastric secretions (e.g. vomiting, nasogastric suction)
Remote use of diuretics
Delivery of Na^+ to CDN with non-reabsorbable anions plus a reason for Na^+ avidity
Post-hypercapnic states
Loss of HCl via lower GI tract (e.g. congenital disorder with Cl^- loss in diarrhoea, acquired forms of DRA)
High UCl
Recent diuretic use
Endogenous diuretic effect (occupancy of the Ca-SR in the thick ascending limb of the loop of Henle, inborn errors affecting transporters of Na^+ and/or Cl^- in the nephron, e.g. Bartter syndrome or Gitelman syndrome)
Causes associated with an expanded ECF volume and possibly hypertension
Disorders with primary enhanced mineralocorticoid activity causing hypokalaemia
Primary hyperaldosteronism
Primary hyper-reninaemic hyperaldosteronism (e.g. renal artery stenosis, malignant hypertension, renin-producing tumour)
Disorders with cortisol acting as a mineralocorticoid (e.g. apparent mineralocorticoid excess syndrome, liquorice ingestion, ACTH producing tumour)
Disorders with constitutively active ENaC in the CDN (e.g. Liddle syndrome)
Large reduction in GFR plus a source of $NaHCO_3$

CaSR = calcium-sensing receptor; CDN = cortical distal nephron (including the late distal convoluted tubule, the connecting tubule and the cortical collecting duct); DRA = downregulated Cl/HCO_3 exchanger in adenoma/adenocarcinoma.

patients may deny the intake of diuretics or self-induced vomiting; examining the urinary electrolytes is particularly helpful if you suspect these diagnoses.

An excellent initial test is to examine the concentration of Cl^- in the urine (U_{Cl}) (Fig. 35.10). A very low U_{Cl} is expected when there is a deficit of HCl and/or NaCl, but the *recent* intake of diuretics causes the excretion of Na^+ and Cl^- in the urine. The U_{Na} may be high if there is a recent episode of vomiting. If the U_{Cl} is not low, assessment of EABV and blood pressure helps separate patients with disorders of high epithelial sodium channel (ENaC) activity in the distal cortical nephron (the EABV is not low, presence of hypertension) from those with Bartter's or Gitelman syndromes (EABV is low, absence of hypertension). Serial measurements of U_{Cl} in spot urine samples are helpful to separate patients with Bartter or Gitelman syndromes (persistently high U_{Cl}) from those with diuretic abuse (intermittently high U_{Cl}).

Common causes for metabolic alkalosis

Vomiting or nasogastric suction

The diagnosis is obvious if the patient has a history of prolonged vomiting or nasogastric suction. The difficulty arises if the patient denies vomiting. Nevertheless, there are several helpful clues to make the diagnosis, for example, the patient is particularly concerned with body image, has a profession where weight control is a very important factor (ballet dancer, fashion model, or beautician), has an eating disorder, and/or has a psychiatric disorder that might lead to self-induced vomiting. The physical examination may also provide some helpful clues. These may include a calloused lesion on the back of the finger or knuckles, which are often inserted into the mouth to induce vomiting, and erosion of dental enamel from repeated exposure to HCl.

The EABV is often contracted. Hypokalaemia is always present and the deficit of KCl is a major factor in the pathophysiology of the metabolic alkalosis in these patients (Kassirer and Schwartz, 1966; Halperin and Scheich, 1994). Alkalaemia suppresses the respiratory centre and this leads to hypoventilation. A primary respiratory acidosis may be present if respiratory muscle weakness results from hypokalaemia. On the other hand, a primary respiratory alkalosis may be present if, for example, the patient develops aspiration pneumonia.

The urinary electrolytes are very helpful when this diagnosis is suspected—the key finding is an extremely low U_{Cl}. If there has been recent vomiting, the U_{Na} may be high due to bicarbonaturia (the urine pH will be > 7.0), which increases the excretion of Na^+ (Kamel et al., 1990).

Diuretics

The key findings in patients with metabolic alkalosis due to the use of diuretics are low EABV, hypokalaemia, and intermittently high concentrations of Na^+ and Cl^- in the urine (when the diuretic acts) (Fig. 35.10). Hypokalaemia is more likely to occur in patients who have a low intake of K^+. A large deficit of NaCl is most commonly seen in patients who have a low intake of NaCl (e.g. in the elderly). The use of diuretics might be denied at times, especially in patients concerned about their body image or those seeking medical attention. To help distinguish these patients from those with Bartter or Gitelman syndromes (which are genetic, rare causes of hypokalemia and metabolic alkalosis) (Simon and Lifton. 1998), measure the urinary electrolytes using multiple random spot urine samples. If there is doubt, an assay for diuretics in the urine may be helpful, but make sure that the assay is performed in a urine sample that contains a high concentration of Na^+ and Cl^-.

Some cationic agents (e.g. drugs such as gentamicin or cis platin or cationic proteins) may bind to the calcium sensing receptor in the thick ascending limb of the loop of Henle and lead to a picture that mimic Bartter syndrome (see Chapter 35).

Less common causes of metabolic alkalosis

Conditions with high mineralocorticoid activity

Clinical clues to conditions with high mineralocorticoid activity as the cause of metabolic alkalosis usually include hypokalaemia and hypertension. Hypokalaemia is of major importance in causing metabolic alkalosis in these patients. The specific disorders are listed in Table 35.2.

Because of the high mineralocorticoid activity or a constitutively active ENaC, principal cells of the cortical distal nephron (CDN) are poised to reabsorb Na^+. Initially Na^+ and Cl^- are retained, and hence the ECF volume will be expanded. Subsequently, K^+ will be lost in the urine with Cl^-, if reabsorption of Na^+ in CDN is electrogenic (i.e. without Cl^-) and if principal cells have open K^+ channels in their luminal membrane $^-$. Hypokalaemia is associated with

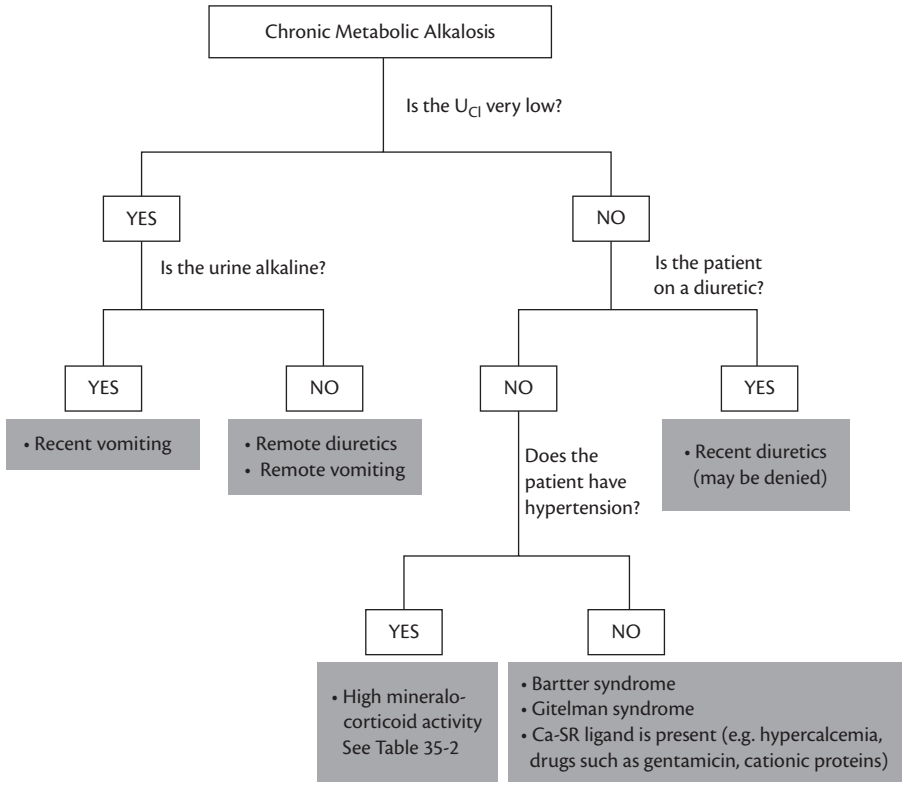

Fig. 35.10 Steps in the clinical approach to a patient with metabolic alkalosis. Ca-SR, calcium sensing receptor.

an acidified PCT cell, which results in the excretion of more NH_4^+ and also the retention of dietary alkali. Overall, the body continues to have a surplus of Na^+, but some of the retained Cl^- are excreted in the urine (with NH_4^+) and are replaced in the body with HCO_3^-. The ICF compartment has a deficit in K^+. The cations that are retained in the ICF are likely to be Na^+ and possibly some H^+ (Gowrishankar et al., 1996).

Metabolic alkalosis associated with the milk-alkali syndrome

Although milk and absorbable alkali to treat duodenal ulcers are not used much nowadays, this form of metabolic alkalosis still continues to occur, but the setting has changed (Beall etal., 2006; Felsenfeld et al., 2006). Its cardinal features are still a dietary source of alkali and absence of suppression of the stimuli for the PCT to retain this alkali load. The intake of calcium supplements, commonly in the form of $CaCO_3$ tablets, is now a common cause of hypercalcaemia, particularly in elderly women. Traditional Betel nut chewing with chalk is another example. Hypercalcaemia develops primarily because more calcium is absorbed in the GI tract (especially if the intake of calcium exceeds that of dietary phosphate). When more Ca^{2+} binds to its receptor in the medullary thick ascending limb of the loop of Henle, it acts as a loop diuretic that leads to an excessive excretion of NaCl and KCl. Both the high levels of AII due to decreased EABV and the intracellular acidosis in PCT cells associated with hypokalaemia lead to the retention of ingested alkali (Lin et al., 2002). The combination of a contracted EABV and direct effects of hypercalcaemia can also cause a marked reduction in the GFR, which itself further reduces the filtration and excretion of HCO_3^-. Therapy consists of stopping the intake of calcium and alkali, and replacing the deficits of NaCl and KCl.

Metabolic alkalosis associated with a post-hypercapnic state

In the course of chronic hypercapnia, the high PCO_2 causes acidosis in the cells of the PCT. This leads to an enhanced excretion of NH_4Cl in the urine and stimulates reabsorption of HCO_3, and results in increased P_{HCO3} (Schwartz et al., 1961). If the patient has a contracted EABV when the hypercapnia resolves, $NaHCO_3$ will still be retained because of the high AII levels maintaining reabsorption of $NaHCO_3$ by PCT cells. Expansion of the EABV will lower AII levels and cause the excretion of the excess $NaHCO_3$.

Metabolic alkalosis associated with the intake of non-reabsorbed anions

If a patient has a contracted EABV and takes a Na^+ salt with an anion that cannot be reabsorbed by the kidney (e.g. Na^+ carbenicillinate), the patient may develop hypokalaemia and metabolic alkalosis. In the cortical distal nephron, the actions of aldosterone cause Na^+ to be reabsorbed in conjunction with K^+ secretion, because of the low delivery of Cl^- and hence hypokalaemia develops. The rise in P_{HCO3} is the result of the NaCl and the KCl deficits.

Metabolic alkalosis associated with magnesium depletion

Patients with Mg^{2+} depletion may have hypokalaemia and metabolic alkalosis. The usual clinical settings for this deficiency include malabsorption, chronic alcoholism, chronic use of proton pump inhibitors, use of loop diuretics, or the administration of drugs that may bind the calcium sensing receptor in the loop of Henle (e.g. cisplatin, or aminoglycosides). These patients must be distinguished from those with primary hyperaldosteronism and those with Bartter or Gitelman syndrome who may also have Mg^{2+} deficiency.

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CHAPTER 36

Approach to the patient with renal tubular acidosis

Stephen B. Walsh

Introduction

Classification

The first description of what would later be termed renal tubular acidosis (RTA) was by an English paediatrician, Reginald Lightwood, whose report was an abstract from a meeting (Lightwood, 1936) in 1936 that did not mention acidosis, but a 'calcium infarction of the kidneys' in infants who died of a dehydrating, salt-wasting disease. Later the same year, Butler, Wilson, and Farber of the Massachusetts General Hospital described 'Dehydration and acidosis with calcification of renal tubules' (Butler et al., 1936). The paper was chiefly concerned with infants, but the appendices described two adults, one of whom had recently had a parathyroid adenoma removed for primary hyperparathyroidism, the first report of RTA and nephrocalcinosis secondary to hypercalcaemic renal damage. Subsequently, Lightwood, Payne, and Black added acidosis and a urinary acidification defect to the syndrome suffered by the sick infants with nephrocalcinosis (Lightwood et al., 1953). In this paper, and others appearing at the same time, including those from Stapleton (Stapleton, 1949, 1954), it was acknowledged that hypercalciuria was often a feature of these patients.

New cases of the so-called Lightwood syndrome failed to appear through the 1950s and 1960s, and it was also reported that the urinary acidification defect tended to disappear, as affected children grew older (Buchanan and Komrower, 1958). This finding raised the possibility that its cause had been removed from the environment: vitamin D or calcium excess, or mercury in teething powders was suggested. The Lightwood syndrome came to be regarded as an unexplained historic accident of the 1940s that no longer existed, and the interest of tubular physicians tended to shift more to older children, where renal acidosis with a urinary acidification defect seemed to be a commoner and more persistent problem.

Albright, a colleague of Butler's at the Massachusetts General Hospital described clearly what we would now term 'distal renal tubular acidosis' (dRTA) in his book with Reifenstein (Albright and Reifenstein, 1948, p. 393), detailing the resultant bone disease and nephrocalcinosis and the role of alkali treatment, and labelling it as a form of 'tubular insufficiency without glomerular insufficiency'; he attributed the acidosis to renal tubular inability to make ammonia and an acid urine.

Albright did not use the term 'renal tubular acidosis', the term was probably first used by Pines and Mudge in 1951 (although one of the adults that they reported had a ureterocolic anastomosis, and so a tubular defect was an unlikely explanation for their acidosis) (Pines and Mudge, 1951)

Wrong and Davies introduced the concept of an acidification defect in the absence of a systemic acidosis, the so-called incomplete syndrome, and also quantified the threshold pH that patients with a dRTA were unable to acidify their urine below; 5.3 (Wrong and Davies, 1959). Although acidosis was recognized in the Fanconi syndrome, it was not initially described as a form of RTA, as it was rare and features other than the acidosis dominated the clinical picture. The acidosis was attributed to a loss of base in the urine, either bicarbonate, or a base that would have been converted to bicarbonate, if it were retained. The terms 'proximal renal tubular acidosis' and 'distal renal tubular acidosis' were coined by Rodriguez-Soriano and colleagues in their classification published in 1969 (Rodriguez-Soriano et al., 1969).

The nomenclature was confused somewhat when Curtis Morris introduced a numerical system for their classification (Morris and McSherry, 1972). Morris labelled distal or classical RTA as 'type 1' RTA, proximal RTA (pRTA) as 'type 2', and designated 'type 3' as a paediatric form of RTA that was a mixture of types 1 and 2, which most nephrologists regarded as a severe form of type 1, requiring rather more alkali than most type 1 patients. Morris' 'type 3' disappeared from the clinical spectrum, later to be replaced by the different cause of carbonic anhydrase deficiency, but its existence meant that hyperkalaemic renal tubular acidosis had to be designated 'type 4'.

Distal renal tubular acidosis (dRTA, type 1 RTA)

Aetiology

Distal RTA is caused by a failure of the acid-secreting α-intercalated cell in the cortical collecting duct. It has a number of causes, primary and secondary:

Primary

Mutations of the anion exchanger AE1, normally present on the basolateral surface of the α-intercalated cell (Bruce et al., 1997). These may be autosomal dominant or recessive. Mutations of the a4 or B1 subunit of the vH^+-ATPase, normally present on the apical surface of the α-intercalated cell (Karet et al., 1999). These are all autosomal recessive.

Secondary

In association with autoimmune disease, notably Sjögren syndrome (Shearn and Tu, 1968), but also systemic lupus erythematosus (Tu and Shearn, 1967), rheumatoid arthritis (Pasternack et al., 1970), autoimmune thyroid disease (Mason and Golding, 1970), primary biliary cirrhosis (Golding and Mason, 1971) and hypergammaglobulinaemia (McCurdy et al., 1967). Due to a number of toxins, such as ifosfamide (Boddy et al., 1996), toluene (Batlle et al., 1988), lithium carbonate (Boton et al., 1987), and amphotericin B (McCurdy et al., 1968). Nephrocalcinosis (Butler et al., 1936) (which can cause, as well as be caused by, dRTA: it can be secondary to medullary sponge kidney (MSK), vitamin D intoxication and sarcoidosis). It has also been attributed to other conditions, but the evidence in these cases is less secure:

- Wilson disease (Hoppe et al., 1993)
- Sickle cell disease (Goossens et al., 1972)
- Renal transplantation (Wilson and Siddiqui, 1973)
- Chronic urinary tract obstruction (Van der Heijden et al., 1985)
- Liver cirrhosis (Shear et al., 1969).

Epidemiology

As a rare syndrome, there is little published literature on the epidemiology of dRTA. Little is known about the prevalence of the mutations of the a4 or B1 subunits of the apical proton pump (vH^+-ATPase), other than that they are rare. The autosomal dominant AE1 mutations, which are generally found in Western populations, are also rare. Autosomal recessive AE1 mutations, found in tropical populations, predominantly in Southeast Asia, are more common there. dRTA is endemic in Northeast Thailand (Nilwarangkur et al., 1990), present in as much as 2.8% of the population (Nimmannit et al., 1996), predominantly in the Lao-Thai people of that region, in whom just one of the disease-causing AE1 mutations approaches polymorphic frequency (Yenchitsomanus et al., 2003). Since this hereditary tropical dRTA is prevalent in malarious areas, and because AE1 is also a major constituent of the red cell membrane, some have hypothesized that it may confer some protection against malaria (Wrong et al., 2002; Walsh et al., 2009; Khositseth et al., 2012).

Acquired causes of dRTA are also rare, and the literature on the epidemiology of acquired dRTA is even sparser than for hereditary dRTA. The commonest cause of acquired dRTA is autoimmune disease, predominantly Sjögren syndrome. The reported prevalence of urinary acidification defects (i.e. dRTA) is quite high: 33% of patients with primary Sjögren in one series (Siamopoulos et al., 1992), the majority of these were 'incomplete dRTA'—in other words, had no systemic acidosis, but needed urinary acidification testing to reveal their acidification defect. Earlier studies showed similar high incidences of dRTA: Talal et al. found 50% of Sjögren patients had dRTA, of which half were incomplete dRTA (Talal et al., 1968); Shearn and Tu found incomplete dRTA in 30% of a series of 10 Sjögren patients without metabolic acidosis (Shearn and Tu, 1968). Pertovaara et al. found dRTA in 18 of 55 (32.7%) patients with primary Sjögren (Pertovaara et al., 1999). Ren and co-workers found RTA in 73% (95 of 130 patients) (Ren et al., 2008); they found more complete dRTA than incomplete (66 vs 25), and they may have overdiagnosed complete dRTA according to their methodology.

As bone demineralization/osteoporosis is a part of dRTA, some investigators have looked for occult urinary acidification defects in patients with 'primary' osteoporosis. Weger et al found incomplete dRTA in 9 of 48 subjects (19%), even after 11 subjects with dRTA were excluded, since they had an obvious cause for their acidification defect (Weger et al., 1999). The same group found incomplete dRTA in 10 of 46 subjects (22%) investigated for 'primary' osteoporosis by using the both the furosemide (see 'Investigations') and ammonium chloride tests (Weger et al., 2000). Furthermore, Deutschmann and co-workers found incomplete dRTA in 12 of 285 (4.2%) women and 15 of 92 (16.3%) men with osteoporosis (Deutschmann et al., 2002).

Clinical features

dRTA is the classical and commonest form, and involves a failure of acid secretion in the distal nephron, more specifically by the acid secreting α-intercalated cells of the distal convoluted tubule and the cortical collecting duct. It is characterized by an inability to acidify the urine to a pH of < 5.3, which can lead to:

- variable metabolic acidosis
- osteomalacia and/or bone demineralization subsequent to the acidosis
- nephrocalcinosis (calcium salt deposition in renal parenchyma)
- renal calculi (calcium phosphate stones due to high calcium, low citrate, alkaline urine).
- variable hypokalaemia due to urinary potassium wasting
- variable salt-losing nephropathy due to urinary sodium wasting.

The metabolic acidosis can be very variable, ranging from very severe (serum bicarbonate < 10 mmol/L) to completely normal. DRTA with a normal serum bicarbonate is termed 'incomplete dRTA'; the other clinical features may still be present

Investigations

The diagnosis of dRTA is often difficult, because the routine biochemical tests done in patients with kidney stones or bone disease are usually fairly unremarkable (Table 36.1). Hypokalaemia is often present (Sebastian et al., 1971) and may be severe enough to cause paralysis (Zimhony et al., 1995) or cardiac arrhythmias (Palkar et al., 2011); this is usually in the setting of a coincident problem worsening the hypokalaemia (e.g. a diarrhoeal illness). There

Table 36.1 Distal renal tubular acidosis: investigations

Routine serum biochemistry	Hypokalaemia Low serum bicarbonate
Urine biochemistry	Hypocitraturia Hypercalciuria
Stone biochemistry	Calcium phosphate stones
Radiology	AXR: radiolucent stones, nephrocalcinosis CT KUB: radiolucent stones, nephrocalcinosis DXA: reduced bone mineral density
Dynamic test	Inability to acidify urine < pH 5.3 in urinary acidification test (ammonium chloride or furosemide plus fludrocortisone test)

may be a severe metabolic acidosis (with the serum bicarbonate < 10 mmol/L), but there may be no systemic acidosis at all. Urinary biochemistry will reveal hypocitraturia; this is a strong clue that dRTA may be present. Hypercalciuria is also usually present. An inappropriately high urine pH (> 5.3) in the face of a metabolic acidosis is diagnostic of dRTA; however, the urine pH must be measured immediately with a glass electrode pH meter (dipstick urine pHs are unreliable) since delay will allow diffusive loss of CO_2 from urine, raising the pH. This usually means that an accurate urine pH measurement is often difficult in routine clinical practice. Analysis of any stones will show the calculi are composed mainly or exclusively of calcium phosphate, rather than the commoner calcium oxalate. Radiologically, the main features are of calcium deposition. A plain abdominal film may detect visible calculi at any point between the renal pelvis and the bladder. It may also reveal medullary nephrocalcinosis: deposition of calcium phosphate in the medullary parenchyma. Both of these radiological signs will also be visible on computed tomography (CT), because CT is very good at visualizing calcium. Dual X-ray absorptiometry (DXA) scanning can show reduced bone mineral density, although this may be attenuated by alkali treatment (Domrongkitchaiporn et al., 2002).

As mentioned previously, the serum bicarbonate may be normal ('incomplete dRTA'), which makes a definitive diagnosis of dRTA by urine pH measurement alone difficult, since you cannot determine whether the urine pH is inappropriately high. The solution to this problem is to do a urinary acidification test. This can be done either by the administration of oral ammonium chloride capsules (100 mg/kg) to produce a metabolic acidosis, followed by hourly urine pH measurements for at least 6 hours: a failure to acidify the urine to a pH < 5.3 is diagnostic of dRTA (Wrong and Davies, 1959) Ammonium chloride capsules can be difficult to procure, and often provoke nausea and vomiting, which can invalidate the test. An alternative is to give furosemide with fludrocortisone (40 mg and 1 mg respectively) and measure urine pH every hour afterwards for at least 4 hours (Walsh et al., 2007). Again, failure to acidify the urine to a pH of < 5.3 is diagnostic of dRTA. In this case, no acidosis is produced; the stimulus to urinary acidification is due to the increased delivery of sodium ions to the cortical collecting duct and enhanced absorption there by principal cells. The addition of the mineralocorticoid fludrocortisone is a more consistent stimulus to collecting duct acidification that the original furosemide (alone) test mentioned earlier.

Aetiology and pathogenesis

As already mentioned, dRTA is due to failure of the acid-secreting α-intercalated cell in the cortical collecting duct. Acid secretion is driven by the hydration of carbon dioxide, catalysed by carbonic anhydrase 2. This produces carbonic acid, which rapidly dissociates to form a free proton and a bicarbonate ion. The proton is extruded apically by the apical proton pump, vH^+-ATPase, while the bicarbonate ion is reclaimed systemically by being transported by the basolateral anion exchanger 1(AE1, band 3, SLC4A1). The hereditary forms of this disease can be caused by a defect in vH^+-ATPase or by a defect in the basolateral anion exchanger, AE1.

AE1

The role of AE1 in hereditary dRTA was first postulated by Wrong, Unwin, and Tanner in 1996 (Wrong et al., 1996), and this was confirmed a year later by Bruce and co-workers (Bruce et al., 1997). Mutations in *SLC4A1* can cause autosomal dominant (AD) dRTA (Bruce et al., 1997; Karet et al., 1998; Weber et al., 2000; Sritippayawan et al., 2003), as well as autosomal recessive (AR) dRTA (Tanphaichitr et al., 1998; Vasuvattakul et al., 1999; Bruce et al., 2000; Ribeiro et al., 2000). The phenotypes of hereditary AE1-associated AD and AR dRTA are determined by *SLC4A1* point mutations or deletions that affect AE1 folding and tertiary structure, without significantly changing the anion transport properties of the protein. These structural alterations do not seem to affect the dimerization of these mutant proteins as hetero- or homodimers. They do, however, affect the trafficking of the mutant homodimers, from the ER and the trans-Golgi network to the cell membrane. The trafficking of the mutant/wild type (wt) heterodimer determines the dominant or recessive nature of the phenotype. In AD dRTA the mutant kAE1 in the heterodimer exerts a 'dominant negative' effect, inducing a trafficking defect on the dimerized wt kAE1. In AR dRTA, the wt kAE1 in the heterodimer corrects the defective trafficking of the mutant kAE1, resulting in a 'dominant positive' effect and thus normal cell surface expression of kAE1 in the heterozygote. In two cases (R901X and G609R) the dominant mutants can exit the ER and is partially mis-sorted to the apical membrane; this and the reduced basolateral expression of AE1 lead to the dRTA phenotype.

vH^+-ATPase

Mutations of two genes encoding two subunits (B1 and a4) of the vH^+-ATPase have been reported, mainly by Karet and co-workers, to cause AR dRTA (Karet et al., 1999; Smith et al., 2000; Stover et al., 2002; Ruf et al., 2003). The respective genes are *ATP6V1B1*, which encodes the B1 subunit of the catalytic V_1 domain and *ATP6V0A4*, which encodes the a4 subunit in the Vo domain of the vH^+-ATPase.

Bilateral hearing loss is an early feature of ATP6B1 mutations, due to expression of the ATP6B1 subunit in the cochlea, where it is responsible for the acidification of endolymph (Karet et al., 1999). Although the initial report of the ATP6VOA4 mutations claimed that ATP6VOA4 was expressed only in the α-intercalated cell, and that hearing was preserved (Smith et al., 2000), it was subsequently demonstrated by the same group that affected patients developed late onset deafness, and that there is expression of ATP6VOA4 in the cochlea (Stover et al., 2002).

Carbonic anhydrase II mutations cause a syndrome incorporating both dRTA and pRTA. Because of this, some authors have reclassified it as 'type 3 renal tubular acidosis' since the original type 3 RTA is no longer seen. Carbonic anhydrase catalyses the hydration of CO_2 into carbonic acid from which H^+ and HCO_3^- dissociate. Carbonic anhydrase II (CAII) is the enzyme that performs this function in the α-intercalated cell, where it is necessary for the generation of protons to acidify the urine; and also in the brush border of the proximal tubule cell, where it is crucial for the reabsorption of bicarbonate. This syndrome also includes osteopetrosis and cerebral calcification with subsequent cognitive impairment (Sly et al., 1985; Hu et al, 1992) and is very rare, with most cases reported from the Maghreb region of North Africa (Fathallah et al., 1997).

Sjögren syndrome

Sjögren syndrome is an autoimmune disorder that has been estimated to affect as much as 1–2% of the adult female population (Kabasakal et al., 2006). It is characterized by the 'sicca syndrome,' which is due to an infiltration of inflammatory cells into the lacrimal and salivary glands, resulting in a lack of secretory ability. Indeed,

Sjögren has been dubbed an 'inflammatory epitheliitis' (Tzioufas et al., 2012). This is a useful term, because epithelial cells appear to be central targets for a cell-mediated autoimmune response in the lacrimal and salivary glands, as well as the renal tubulointerstitial compartment. In the salivary glands the plasma cells are predominantly T cells (CD4+) when mild and predominantly B cells when severe (Tzioufas et al., 2012).

The prevalence of renal involvement in primary Sjögren syndrome (pSS) has been estimated at approximately 30%. The predominant lesion is a tubulointerstitial nephritis (TIN) again related to cellular autoimmunity. It is characterized by a focal or diffuse lymphoplasmocellular infiltrate of mononuclear cells with variable tubular atrophy, often with interstitial fibrosis. The histopathological appearance of the TIN is strikingly similar to that seen in the salivary gland. In the kidney too the majority of invading cells are CD4+ (Skopouli, 2001). The functional consequence of this are:

1. Distal tubular dysfunction, most commonly dRTA and less commonly nephrogenic diabetes insipidus
2. Reduced renal excretory function, with a reduced glomerular filtration rate (GFR)
3. Proximal tubular dysfunction with the Fanconi syndrome has been reported infrequently (Walker et al., 1971).

Glomerular involvement may also occur in pSS. This is an unusual complication, occurring late in the disease, if at all (Skopouli, 2001), and appears to be related to humoral autoimmunity. Palpable purpura, low C4, and circulating cryoglobulin are correlated with the development of a glomerulonephritis in Sjögren (Skopouli et al., 2000), suggesting that the pathogenesis involves the deposition of monoclonal immunoglobulin (Ig)-M and polyclonal IgA and IgG in glomeruli.

Treatment and outcome

General

The mainstay of treatment for dRTA is supportive and is primarily treatment of metabolic acidosis with sodium bicarbonate replacement. Correction of the metabolic acidosis may have a number of beneficial effects. In children, it can reverse osteopenia and restore normal bone growth, even in those with severely stunted skeletal development (McSherry et al., 1978; Caldas et al., 1992). It may reduce the risk of forming stones or nephrocalcinosis (Coe et al., 1980; Preminger et al., 1985), and theoretically might reduce urinary potassium loss. The aim of alkali supplementation is to normalize the serum bicarbonate (i.e. > 22 mmol/L). As there is no bicarbonate wasting in dRTA (as opposed to pRTA), the amount of oral sodium bicarbonate needed to do this is relatively modest (around 500 mg to 1g twice daily). Potassium citrate may be substituted for those with problematic calcium phosphate stone disease (as it will help to increase the urinary citrate concentration) or problematic hypokalaemia. Citrate is metabolized to bicarbonate and is an oral alkali supplement in its own right. Many patients end up requiring oral potassium supplements, especially if oral potassium citrate isn't tolerated or available.

Specific

For secondary dRTA, treatment of the underlying disease is the key to treatment. This has been studied in pSS. There are no clinical trials for treating renal pSS, but there are numerous case reports. The main agents that are described in the literature are corticosteroids alone (Bailey and Swainson, 1986; Akposso et al., 2000; Komatsu et al., 2003; Kawamoto et al., 2005; Kobayashi et al., 2006), or in combination with azathioprine (Kaufman et al., 2008) or cyclophosphamide (Goules et al., 2000; Mukai et al., 2001), and a few reports on the successful use of rituximab (Maripuri et al., 2009). Resolution of the functional renal lesions has been repeatedly described, although a return of normal renal acidification has not been formally tested (Kaufman et al., 2008).

Proximal renal tubular acidosis (pRTA, type 2 RTA)

Aetiology

pRTA is caused by a failure of bicarbonate reabsorption along the proximal tubule. It is, in all but two specific hereditary cases, associated with generalized proximal tubular dysfunction, causing the renal Fanconi syndrome. It has a several causes:

Hereditary

- AR NBCe1 (bicarbonate transporter) loss-of-function mutation (Igarashi et al., 1999)
- Carbonic anhydrase II mutation
- Sodium phosphate transporter (Na-PiIIa) loss of function mutation (Magen et al., 2010)
- Cystinosis (Drablos, 1951)
- Tyrosinaemia (Gentz et al., 1965)
- Hereditary fructose intolerance (Morris et al., 1968)
- Galactosaemia (Golberg et al., 1956)
- Wilson disease (Bearn et al., 1957)
- Lowe syndrome (Bockenhauer et al., 2008)/Dent disease (Wrong et al., 1994)
- Fanconi–Bickle syndrome (Manz et al., 1987)
- Mitochondrial cytopathies (Niaudet and Rotig, 1997).

Acquired

- Multiple myeloma/light chain disease (Maldonado et al., 1975)
- Drugs (tenofovir, ifosfamide (Burk et al., 1990), carbonic anhydrase inhibitors e.g. acetazolamide, topiramate (Mirza et al., 2009))
- Heavy metals (lead, cadmium (Kazantzis et al., 1963), mercury)
- Paroxysmal nocturnal haemoglobinuria (Riley et al., 1977).

Epidemiology

pRTA is rarer than dRTA, and there is even less documentary evidence on its epidemiology. However, the prevalence of some of the acquired causes can be inferred. Myeloma is often thought of as one of the commonest causes of renal Fanconi syndrome, but published series of these patients in the literature are small: the largest series was published by the Mayo clinic and it comprised 32 patients over 34 years (Ma et al., 2004). Tenofovir is an antiretroviral agent that can cause Fanconi syndrome and acute kidney injury (AKI). It was described in 22 patients by Woodward et al. which represented approximately 1.5% of patients exposed to the agent at the referring centre (Woodward et al., 2009).

Clinical features

The key feature of pRTA is a failure of proximal tubular cells to reabsorb bicarbonate, which leads to bicarbonaturia. It is important to realize that there are other, less efficient bicarbonate reclaiming mechanisms in the more distal tubule (in the loop of Henle and the collecting duct). Therefore, as bicarbonaturia causes the serum bicarbonate to fall, the filtered load of bicarbonate also falls, until it reaches a level at which all of the filtered load can be reclaimed by these less efficient mechanisms. This tends to happen at a serum bicarbonate concentration of approximately14 mmol/L. This has two consequences: first, it means that the acidosis of pRTA is never particularly severe, as it can be in dRTA; second, it means that bicarbonaturia is not a consistent feature of pRTA, since it will naturally resolve as bicarbonate is lost, and, conversely, will reappear if bicarbonate is replaced (the basis of the bicarbonate infusion test used to confirm the diagnosis). With the exception of isolated mutations of NBCe1 or carbonic anhydrase II mutations, pRTA is always accompanied by signs of generalized proximal tubular dysfunction, which is known as the renal Fanconi syndrome and comprises the pentad of:

- glycosuria
- phosphaturia
- uricosuria
- aminoaciduria
- low-molecular-weight ('tubular') proteinuria.

These features are not invariable, and with the exception of phosphate, unlikely to have any clinical consequences per se. Phosphaturia, if ongoing, may lead to hypophosphataemia and subsequent leeching of phosphate from bone stores, leading eventually to bone demineralization, osteopenia (Brenner et al., 1982), and reduced bone growth in children (Lemann et al., 2000). This is reversible with oral phosphate replacement therapy.

Investigations

Hypokalaemia may be present, but is variable depending on the amount of bicarbonate in the urine. Bicarbonate is a non-absorbable anion in the collecting duct, where it will increase the luminal electronegativity as sodium cations are reabsorbed by ENaC in principal cells. This increased electronegativity will favour the secretion of potassium ions, increasing potassium excretion and leading to hypokalaemia. Therefore, hypokalaemia may not be a problem at all in untreated pRTA, but may become pronounced after bicarbonate replacement is given. As mentioned previously, there may be a metabolic acidosis that is relatively mild (>12 mmol/L) and there may be hypophosphataemia and occasionally hypouricaemia, if there is also a renal Fanconi syndrome (Table 36.2).

Urinary examination may be more revealing. The urine dipstick may reveal glycosuria, although this is not invariable in the Fanconi syndrome, but if it is present in a non-diabetic, it is a strong clue that proximal tubular dysfunction is present. The urine dipstick is usually only a test for albuminuria, rather than low molecular weight proteinuria and is therefore often negative. A laboratory protein/creatinine ratio will detect tubular proteinuria; thus a negative urine dipstick test for protein and a positive protein/creatinine ratio should raise suspicion of the presence of tubular proteinuria. Urinary phosphate excretion (usually measured as a FE_{PO4} or TmP/GFR) and urinary urate concentration can be elevated in the Fanconi syndrome. Aminoaciduria is usually difficult and expensive to measure (by chromatography), and is not done routinely. Direct measurement of tubular protein excretion is more straightforward, but is sometimes not locally available. If it is, measurement of retinol binding protein or β_2-microglobulin excretion may be helpful in detecting proximal tubular dysfunction.

Urinary pH can vary, as indicated above. It will be alkaline when there is bicarbonaturia, but appropriately acid when the bicarbonate threshold is reached and acid excretion challenged with ammonium chloride. When on bicarbonate replacement therapy, there will be bicarbonaturia and alkaline urine. It is unlikely that there will be much difficulty differentiating pRTA from dRTA, given that Fanconi syndrome almost invariably accompanies pRTA. However, the nature of the urinary acidification defect can be tested if there is any uncertainty. In dRTA, the urinary pH will never be below 5.3, even if the patient is challenged with an oral acid load or furosemide and fludrocortisone. In pRTA, once the serum bicarbonate has fallen low enough for all of the filtered bicarbonate to be reabsorbed, the urine can be acidified by the α-intercalated cells normally, and the urine pH may fall below 5.3. A challenge with intravenous sodium bicarbonate (0.5–1 mmol/kg/h) to raise the serum bicarbonate to 18–20 mmol/L will cause bicarbonate to reappear in the urine and the urine pH will rise rapidly to approximately 7.5. Furthermore, the measured urinary bicarbonate will increase, and a calculation of the fractional excretion of bicarbonate can be done, which will rise to 15% or more (Rose and Post, 2001).

Radiography is likely to be unhelpful in pRTA and Fanconi syndrome, since apart from Dent and Lowe syndromes, nephrocalcinosis and nephrolithiasis are not typical features. Bone density may be decreased on DEXA scanning if phosphaturia has been significant for some time.

Table 36.2 Proximal renal tubular acidosis: investigations

Serum biochemistry	Metabolic acidosis: mild (bicarbonate > 12 mmol/L) if present Hypokalaemia: possible if bicarbonaturia ongoing Possible hypophosphataemia Possible hypouricaemia
Urine biochemistry	Urine pH: high if serum bicarbonate >14 mmol/L Glycosuria Phosphaturia (TmP/GFR < 80%) Uricosuria Aminoaciduria (chromatography) Tubular proteinuria (retinol binding protein/creatinine ratio)
Radiology	Nephrocalcinosis unlikely unless Lowe or Dent syndromes DEXA may show osteopenia
Dynamic testing	Bicarbonate challenge: IV HCO_3 to normalize serum bicarbonate to 18–20 mmol/L. Rapid rise in urine pH to ~7.5

Aetiology

Hereditary

Mutations of the bicarbonate transporter SLC4A4 result in an isolated pRTA without renal Fanconi syndrome, but associated with short stature and ocular abnormalities, presumably reflecting the distribution of this transporter in ocular tissue and possibly bone (Igarashi et al., 2001).

Myeloma

Almost certainly the most common cause of pRTA is secondary to a monoclonal gammopathy, which like other forms of myeloma-related kidney disease may otherwise be latent (Maldonado et al., 1975). Filtered light chains are endocytosed by proximal tubular cells by the megalin/cubulin pathway for disposal (Sanders et al., 1988). Some varieties of light chain appear to be more toxic than others, which may be due to the variable domain of these 'toxic' light chains being more resistant to lysosomal proteases, and therefore cleared less efficiently (Leboulleux t al., 1995). Accumulation of fragments of these variable domains and subsequent crystallization is apparently responsible for the development of the Fanconi syndrome, at least in a mouse model of myeloma-associated Fanconi syndrome (Decourt et al., 1999).

Other acquired causes are increasing in relevance as causes of pRTA, for example, the antiretroviral Tenofovir. Tenofovir is a nucleotide analogue reverse transcriptase inhibitor that inhibits viral replication by being incorporated into the growing DNA strand and stopping elongation. However, they also inhibit DNA polymerase γ, the enzyme involved in mitochondrial DNA (mtDNA) synthesis. Mitochondrial toxicity is almost certainly the mechanism of Tenofovir-associated proximal tubular injury; biopsy specimens show abnormal mitochondria (Woodward et al., 2009; Herlitz et al., 2010), and Tenofovir exposure is associated with mtDNA depletion (Cote et al., 2006). In fact, the proximal tubule is very vulnerable to mitochondrial dysfunction, because it has limited anaerobic respiratory capacity (Bagnasco et al., 1985). Indeed, Fanconi syndrome is a common phenotype in diseases that are caused by mtDNA mutations (Niaudet and Rotig, 1997; Martin-Hernandez et al., 2005); other drugs that are mitochondrial toxins (e.g. ifosphamide, valproate, aminoglycosides) also cause Fanconi syndrome (Izzedine et al., 2003).

Treatment and outcome

The aim of treatment of pRTA and the renal Fanconi syndrome is chiefly protection of the skeleton. This is achieved in two ways, by correction of the metabolic acidosis and also by replacement of any phosphate losses, if these are prominent. Correction of the acidosis is desirable, because it will reverse osteomalacia/rickets (Brenner et al., 1982) and restore normal bone growth in growth-delayed children (1981). However, achieving a normal serum bicarbonate concentration is much more difficult than in dRTA, since oral bicarbonate replacement will result in bicarbonaturia and higher bicarbonate requirements. The bicarbonaturia will also stimulate a kaliuresis and thus tend to cause hypokalaemia, as discussed previously. Therefore, the dose of sodium bicarbonate required to normalize the serum bicarbonate concentration is much higher than in dRTA, and may be as much as 4–6 g twice daily. If hypokalaemia is problematic, potassium replacement may be necessary, in which case, some of the alkali load could be given as potassium citrate or bicarbonate.

Correction of hypophosphataemia, if present, will reverse incipient or overt osteomalacia. Replacement of phosphate (e.g. phosphate Sandoz 2 tablets BD) reverses biochemical abnormalities (often including raised alkaline phosphatase, secondary hyperparathyroidism and inappropriately low serum calcitriol levels) (Clarke et al., 1995), symptoms (e.g. bone pain) and increases bone mineral density. Replacement of vitamin D (not calcitriol) may also be necessary (Clarke et al., 1995). Caution is needed in not prescribing too much of either supplement: phosphate can stimulate PTH secretion and vitamin D-induced hypercalcaemia may lead to nephrocalcinosis.

Hyperkalaemic renal tubular acidosis (hypoaldosteronism, type 4 RTA)

Introduction

A number of hyperkalaemic conditions that are variably associated with a mild metabolic acidosis were classified by Morris and McSherry (1972) as 'type 4 renal tubular acidosis, and so merit some consideration here. They are entirely different from dRTA and pRTA, and are never part of the same differential diagnosis. They are typified by real or apparent hypoaldosteronism, and the cardinal feature is hyperkalaemia.

Aetiology

- Hypoaldosteronism
- Diabetes and renal impairment
- Adrenal insufficiency
- Non-steroidal anti-inflammatory agent-associated TIN
- Calcineurin inhibitors
- ACE inhibitors
- Angiotensin receptor blockers
- Heparin
- Pseudohypoaldosteronism type 2 (Gordon syndrome)
- Congenital isolated hypoaldosteronism
- Aldosterone resistance
- Potassium sparing diuretics (e.g. spironolactone, amiloride)
- Trimethoprim and pentamidine (also inhibit ENaC)
- Pseudohypoaldosteronism type 1.

Clinical features

Hyperkalaemia

This is usually mild, unless combined with another factor that tends to increase the serum potassium (e.g. ACE inhibitors).

Metabolic acidosis

This is variable, mild when present and is a hyperchloraemic (i.e. normal anion gap) acidosis. It is thought to be due to reduced ammonium excretion.

Investigations

Serum biochemistry may reveal a mild hyperkalaemia and a mild metabolic acidosis. Also, the plasma renin activity (or renin concentration) and serum aldosterone level should be measured. The following should be considered in diagnosis:

- Low plasma renin activity/renin concentration and serum aldosterone:
- Diabetes and renal impairment
- Adrenal insufficiency
- Non-steroidal anti-inflammatory agent associated with TIN
- PHA 2/Gordon syndrome
- Calcineurin inhibitors
- High plasma renin activity/renin concentration and low serum aldosterone:
- ACE inhibitors
- ARBs
- Heparin
- Congenital isolated hypoaldosteronism
- High plasma renin activity/renin concentration and serum aldosterone:
- Potassium-sparing diuretics (e.g. spironolactone, amiloride)
- Trimethoprim and pentamidine (also inhibit epithelial sodium channels (ENaC))
- Pseudohypoaldosteronism type 1.

Aetiology

The hallmark of hyperaldosteronism is a hypokalaemic metabolic alkalosis, and the hyperkalaemia and acidosis seen in type 4 dRTA is a mirror image of this. Aldosterone causes increased expression of ENaC on the apical surface of principal cells in the cortical collecting duct (CCD). ENaC reabsorbs sodium from tubular fluid, which depolarizes the apical membrane of the principal cell leading to a negative transepithelial electrical potential difference along the CCD. This negative potential favours the secretion of cations (either protons or potassium) from the α-intercalated cell. This process is increased when there is more aldosterone acting on the CCD, resulting in hypokalaemia (from potassium losses in the urine) and alkalosis (from increased secretion of hydrogen ions, which can form ammonium ions in the urine). In conditions of reduced aldosterone action, there is less lumen electronegativity, so less driving force for potassium secretion (causing a tendency to hyperkalaemia) and less proton secretion (causing a tendency toward acidosis and reduced ammonium excretion).

Treatment and outcome

Hypoaldosteronism (but not aldosterone resistance) respond well to fludrocortisone (0.05–0.2 mg/day for adrenal insufficiency, 0.2–1 mg/day for other causes). However, it will worsen hypertension and fluid overload, so if these are present, a loop or thiazide diuretic can be used instead.

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CHAPTER 37

Approach to the patient with hypercalcaemia

Dennis Joseph and Theresa A. Guise

Introduction

Definition of hypercalcaemia

The normal range of total serum calcium concentration (0.25 × mg/dL = mmol/L), corrected for albumin, is 2.2–2.6 mmol/L (8.7–10.2 mg/dL) (Kratz et al., 2011). Hypercalcaemia is defined as an increased calcium concentration in the blood, usually > 2 standard deviations (SDs) above the normal mean in a given laboratory. Forty per cent of serum calcium is bound to protein, primarily albumin. Hence, pseudohypercalcaemia (with normal levels of ionized calcium) may be present in the setting of hyperalbuminemia (due to dehydration) or a paraproteinemia (due to multiple myeloma). Alternatively, hypercalcaemia can be present in hypoalbuminemia with normal total serum calcium levels, but elevated ionized calcium levels. Alterations in acid–base balance also affect the concentration of ionized calcium; ionized calcium increases by 0.05 mmol/L (0.2 mg/dL) when pH decreases by 0.1.

Hypercalcaemia results from an abnormality in the calcium flux between the extracellular fluid and the main calcium regulatory compartments: bone, gastrointestinal tract (GIT), and kidney. Thus, excessive bone resorption, increased gastrointestinal calcium absorption, reduced renal excretion of calcium, or a combination of these, are responsible for hypercalcaemia.

Symptoms and signs

Mild hypercalcaemia (calcium < 3 mmol/L (12 mg/dL)) may be asymptomatic and is usually discovered incidentally on routine blood tests. However, marked symptoms occur with acute increases in serum calcium levels > 3 mmol/L. The severity of symptoms depends on the degree of hypercalcaemia, the rapidity of rise in serum calcium concentration and co-morbidities present in the patient (Stewart, 2005). The common symptoms associated with hypercalcaemia are listed in Table 37.1.

Pathophysiology

Normal calcium metabolism

The adult human body contains about 1100 g of calcium (Barrett et al., 2011). Ninety-nine per cent of this is present in the skeletal framework formed by bones and teeth in the form of calcium phosphate or hydroxyapatite ($Ca_5[PO_4]_3[OH]$).

The daily dietary intake of elemental calcium in a healthy adult consuming a Western diet is roughly 1 g. About 30% (300 mg) of the ingested calcium is absorbed through the small and large intestine. Active transport occurs via transient receptor potential vanilloid type 6 (TRPV6) and an intracellular protein known as calbindin, and is principally regulated by 1,25-dihydroxyvitamin D (Barger-Lux et al., 1989). Additionally, passive transport of calcium occurs paracellularly in proportion to the calcium intake. The GIT is also a site of constant calcium secretion approximating about 150 mg daily, resulting in a net calcium intake of 150 mg per day. The newly acquired 150 mg of dietary calcium enters the blood stream and is filtered by the kidney, where 98% of calcium is reabsorbed in the proximal convoluted tubule, while the remaining excess calcium is eventually excreted in the urine. The plasma calcium concentration also remains in a steady state in dynamic equilibrium with the calcium pool in the bones. Thus, the kidneys, bone, and GIT are the main regulators of plasma (or extracellular) calcium homeostasis, which is tightly controlled by the hormones parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D. The calcium-sensing receptor (CaSR) is a G protein-coupled receptor that senses extracellular levels of calcium ions (Brown et al., 1993). It is present in the parathyroid gland, distal nephron of the kidney, and in bone. In the parathyroid gland, the CaSR controls calcium homeostasis by regulating the release of PTH. PTH acts to increase the concentration of calcium in the blood by increasing bone resorption and distal tubular reabsorption of calcium. In addition, it indirectly enhances gastrointestinal calcium absorption by increasing the activity of 1α-hydroxylase enzyme, which converts 25-hydroxyvitamin D (25-OH vitamin D) to 1,25-dihydroxyvitamin D, the active form of vitamin D. PTH enhances the release of calcium from bone by indirectly activating osteoclasts through osteoblast expression of receptor activator of nuclear factor kappa B ligand (RANKL). Calcitonin (a polypeptide hormone produced by the parafollicular cells (C cells) of the thyroid gland) acts to decrease calcium concentration by directly inhibiting bone resorption and increasing renal excretion of calcium. The physiological role of calcitonin in humans remains uncertain.

Causes of hypercalcaemia

Hypercalcaemia occurs when there is a mismatch between the entry of calcium into plasma (from bone or gut) and its removal through bone deposition and renal excretion. The most common causes of hypercalcaemia are primary hyperparathyroidism and malignancy, which together account for > 90% of cases of hypercalcaemia. These conditions can easily be differentiated by measurement of intact

Table 37.1 Clinical features of hypercalcaemia

Neurologic and psychiatric	Lethargy, drowsiness
	Confusion, disorientation
	Disturbed sleep, nightmares
	Irritability, depression
	Hypotonia, decreased deep tendon reflexes
	Stupor, coma
Gastrointestinal	Anorexia, vomiting
	Constipation
	Peptic ulceration
	Acute pancreatitis
Cardiovascular	Arrhythmias
	Synergism with digoxin
	Hypertension
Renal	Polyuria, polydipsia
	Hypercalciuria
	Nephrocalcinosis
	Impaired glomerular filtration

serum PTH levels, which are elevated or inappropriately normal in primary hyperparathyroidism, and suppressed in hypercalcaemia of malignancy.

Other causes of hypercalcaemia associated with increased PTH levels include tertiary hyperparathyroidism, usually due to chronic renal failure, lithium-induced hypercalcaemia, and familial hypocalciuric hypercalcaemia (FHH). Suppressed levels of PTH are seen in hypercalcaemia due to chronic granulomatous diseases, including sarcoidosis, acute kidney injury, immobilization, thyrotoxicosis, thiazide diuretic use, and rarer causes such as hypervitaminosis D, milk-alkali syndrome, hypervitaminosis A, and adrenal insufficiency.

Conditions with elevated PTH levels

Primary hyperparathyroidism

The routine measurement of serum calcium with the use of automated multichannel biochemical screening has led to a marked rise in the incidence of primary hyperparathyroidism. Most cases occur after age 45 (Wermers et al., 2006) and women are twice as likely to be affected as men, likely due to increased bone resorption occurring at the menopause, which unmasks primary hyperparathyroidism.

The causes of primary hyperparathyroidism are poorly understood. The better recognized causes include a history of ionizing radiation to the neck (Beard et al., 1989), chronically low calcium intake (Paik et al., 2012), chronic lithium exposure, and rare genetic abnormalities such as the multiple endocrine neoplasia syndromes (Marx et al., 2002).

The majority of cases of primary hyperparathyroidism are due to a single adenoma, while the rest are due to multiple gland hyperplasia. Parathyroid carcinoma is a very rare cause of primary hyperparathyroidism (Wynne et al., 1992). The diagnosis of primary hyperparathyroidism is made when PTH concentrations are high or inappropriately normal in the setting of high serum calcium, when other causes of hypercalcaemia with elevated PTH (especially FHH) have been excluded. Other laboratory findings may include a urinary calcium to creatinine clearance ratio > 0.02, and reduced serum phosphate levels.

Surgical resection is the definitive treatment for primary hyperparathyroidism. However, since many patients are asymptomatic and may not progress to symptomatic primary hyperparathyroidism, periodic monitoring of calcium levels is an option. Indications for parathyroidectomy are serum calcium concentration of 0.25 mmol/L (1.0 mg/dL) or more above the upper limit of normal, a creatinine clearance < 60 mL/min, recurrent nephrolithiasis, bone density at the hip, lumbar spine, or distal 1/3 radius that is more than 2.5 SDs below peak bone mass (T score < −2.5), and/or previous fragility fracture and age < 50 years (Bilezikian et al., 2009). Calcimimetic agents (such as cinacalcet) that interact with the CaSR to lower PTH, may be used for primary hyperparathyroidism when surgery is not possible or high risk (Peacock et al., 2005).

Lithium-induced hypercalcaemia

Hypercalcaemia induced by lithium is a subtype of primary hyperparathyroidism and results from lithium's effect of decreasing parathyroid gland sensitivity to calcium, shifting the set-point of the Ca-PTH curve to the right (Mallette et al., 1989). This condition is frequently irreversible, even with discontinuation of lithium in those who have been on chronic therapy for more than ten years, and may require parathyroidectomy.

Tertiary hyperparathyroidism

Stimuli such as calcitriol deficiency and hyperphosphatemia, as in chronic kidney disease, or long-term administration of phosphate and vitamin D preparations in X-linked hypophosphatemic rickets (Makitie et al., 2003), or chronic hypocalcemia, cause increased mitotic activity in parathyroid cells, leading to parathyroid gland hyperplasia and secondary hyperparathyroidism. With prolonged secondary hyperparathyroidism, the parathyroid glands can autonomously produce PTH, a condition known as tertiary hyperparathyroidism. Medical management options in this condition include calcimimetics such as cinacalcet. The main indications for parathyroidectomy for tertiary hyperparathyroidism in dialysis patients include severe refractory hypercalcaemia, progressive hyperparathyroid bone disease, intractable pruritus, or progressive extraskeletal calcification or calciphylaxis.

Familial hypocalciuric hypercalcaemia

FHH is a benign cause of hypercalcaemia characterized by mild hypercalcaemia and hypocalciuria. It is a genetically heterogeneous autosomal dominant disorder with three variants: types 1, 2, and 3. FHH1 is caused by inactivating mutations in the gene for the CaSR located on the long arm of chromosome 3 (Pollak et al., 1993). The CaSR mutation decreases the sensitivity of the parathyroids and kidney to calcium, resulting in mildly elevated or inappropriately normal PTH levels and increased tubular reabsorption of calcium. FHH2 and FHH2 are caused by mutations in *GNA11* and *AP2S1* respectively (Nesbit et al., 2013a, Nesbit et al., 2013b). Most patients with FHH have urinary calcium to creatinine clearance ratios < 0.01. Distinguishing FHH from primary hyperparathyroidism can be difficult because there is considerable overlap

in the urinary calcium to creatinine clearance ratios in these two conditions; however a calcium to creatinine clearance ratio > 0.02 makes FHH very unlikely (Fuleihan Gel, 2002). The presence of family history, lifelong mild hypercalcaemia or mutational analysis can provide important clues to differentiate FHH from primary hyperparathyroidism. FHH has a benign natural history and patients and their families should be counseled on the benign nature of this condition and, consequently, the importance of avoiding parathyroid surgery.

Conditions with suppressed PTH levels

Hypercalcaemia of malignancy

Up to 10–30% of all patients with advanced cancer can have hypercalcaemia during the course of their disease (Horwitz and Stewart, 2003) and it carries a poor prognosis. It is seen in both solid tumours and haematologic malignancies. There are several basic mechanisms of cancer-associated hypercalcaemia. The most common cause is tumour cell secretion of parathyroid hormone-related peptide (PTHrP), also known as humoral hypercalcaemia of malignancy (HHM) (Stewart, 2005). Direct osteolytic activity at sites of skeletal metastases is responsible in 20% of cases. Rarely, certain lymphomas and ovarian tumours have been described in association with tumour secretion of 1,25-dihydroxyvitamin D (Seymour et al., 1994; Hibi et al., 2008). Ectopic secretion of PTH by non-parathyroid tumours is exceedingly rare and reported in only a handful of patients (Yoshimoto et al., 1989; Nussbaum et al., 1990; Strewler et al., 1993; Rizzoli et al., 1994; Nielsen et al., 1996; Iguchi et al., 1998).

Parathyroid hormone-related peptide

PTHrP and PTH have a 70% structural homology for the first 13 amino acids of the amino-terminal portion that accounts for the biological activity of the peptide. PTH and PTHrP bind to a common PTH/PTHrP receptor and share similar biologic activities (Abou-Samra et al., 1992), such as activating adenylate cyclase in renal and bone systems, increasing renal tubular reabsorption of calcium and osteoclastic bone resorption, and reducing renal phosphate uptake. It has a multifunctional role in cancer, including mediating hypercalcaemia, promoting the development and progression of osteolytic bone metastasis, regulating the growth of cancer cells (Luparello et al., 1993; Luparello et al., 1995; Li et al., 1996), and functioning as a cell survival factor (Chen et al., 2002). HHM occurs most commonly with squamous cell carcinoma, but can occur with other solid tumours (e.g. renal, breast, ovarian, and urothelial carcinomas) and leukaemia.

1,25-dihydroxyvitamin D

A major mediator of hypercalcaemia in Hodgkin's disease, non-Hodgkin's lymphoma, and other hematological malignancies, is extrarenal secretion of 1,25-dihydroxyvitamin D. Normally, immune cells of the lymphocyte and macrophage lineage produce small amounts of 1,25-dihydroxyvitamin D where it acts as a local cytokine (Edfeldt et al., 2010; Nelson et al., 2010). The mechanisms responsible for hypercalcaemia in the setting of elevated 1,25-dihydroxyvitamin D levels are increased intestinal absorption of calcium and osteoclastic bone resorption.

Local osteolytic hypercalcaemia

Local osteolytic hypercalcaemia (LOH) is by definition, a syndrome of malignancy-associated hypercalcaemia resulting from the direct action of locally acting osteolytic factors released in conjunction with tumour deposits adjacent to bone. LOH accounts for about 20% of the patients with malignancy associated hypercalcaemia and is frequently encountered in patients with multiple myeloma, lymphoma, leukaemia, and breast cancer (Stewart et al., 1980; Kinder and Stewart, 2002). Patients with LOH have widespread osteolytic bone metastasis and the tumour cells within the skeletal space secrete several cytokines in a paracrine manner that induce osteoclastogenesis.

Treatment of hypercalcaemia in malignancy

In addition to vigorous hydration with isotonic saline solution to increase the urinary excretion of calcium, the primary treatment strategy involves treatment of the underlying tumour. Bisphosphonates have emerged as the cornerstone of the pharmacological treatment of hypercalcaemia of malignancy. Intravenous bisphosphonates, such as pamidronate, ibandronate, and zoledronate have proven to be effective in treating malignancy associated hypercalcaemia through their ability to reduce osteoclastic bone resorption by inhibiting osteoclastic bone resorption. Denosumab is a human immunoglobulin G2 monoclonal antibody against RANKL. This drug mimics the endogenous effects of osteoprotegerin (OPG), a protein produced by osteoblasts, which acts as an alternative receptor for RANKL, thereby modulating the RANK/RANKL-induced osteoclast activity. By inhibiting osteoclastic bone resorption, it is also effective in malignancy-associated hypercalcaemia (Bech and de Boer, 2012; Boikos and Hammers, 2012). Haemodialysis or peritoneal dialysis with a low calcium dialysate provides an effective strategy for patients where other treatment modalities have failed and also for individuals with renal failure or cardiac disease who cannot tolerate large fluid infusions or bisphosphonates (Koo et al., 1996).

Granulomatous disease

Hypercalcaemia has been described in association with most granulomatous disorders. Among them, sarcoidosis (Adams et al., 1983; Insogna et al., 1988), tuberculosis (Gkonos et al., 1984; Cadranel et al., 1990), and histoplasmosis (Murray and Heim, 1985), are probably the most common. Hypercalcaemia of granulomatous disease is mediated through extrarenal secretion of 1,25-dihydroxyvitamin D. Activated mononuclear cells (particularly macrophages) in granulomas are resistant to the normal feedback control of calcitriol production, probably as a result of interferon-gamma (Dusso et al., 1997). In addition to vigorous hydration, specific treatment options include treatment of the underlying granulomatous disorder (such as glucocorticoid therapy in the setting of sarcoidosis) and restriction of dietary calcium and vitamin D intake.

Miscellaneous causes

Rarely hypercalcaemia occurs due to increased stimulation of bone resorption (as with thyrotoxicosis (Iqbal et al., 2003), immobilization (Stewart et al., 1982), or vitamin A toxicity (Villablanca et al., 1993)), enhanced active calcium reabsorption in the distal tubule (as with thiazide diuretics), and increased oral calcium intake in the setting of metabolic alkalosis (as with the milk—alkali syndrome (Beall and Scofield, 1995)).

Diagnostic approach

Hypercalcaemia should be confirmed by measurement of total serum calcium. If a paraproteinaemia or abnormal albumin levels

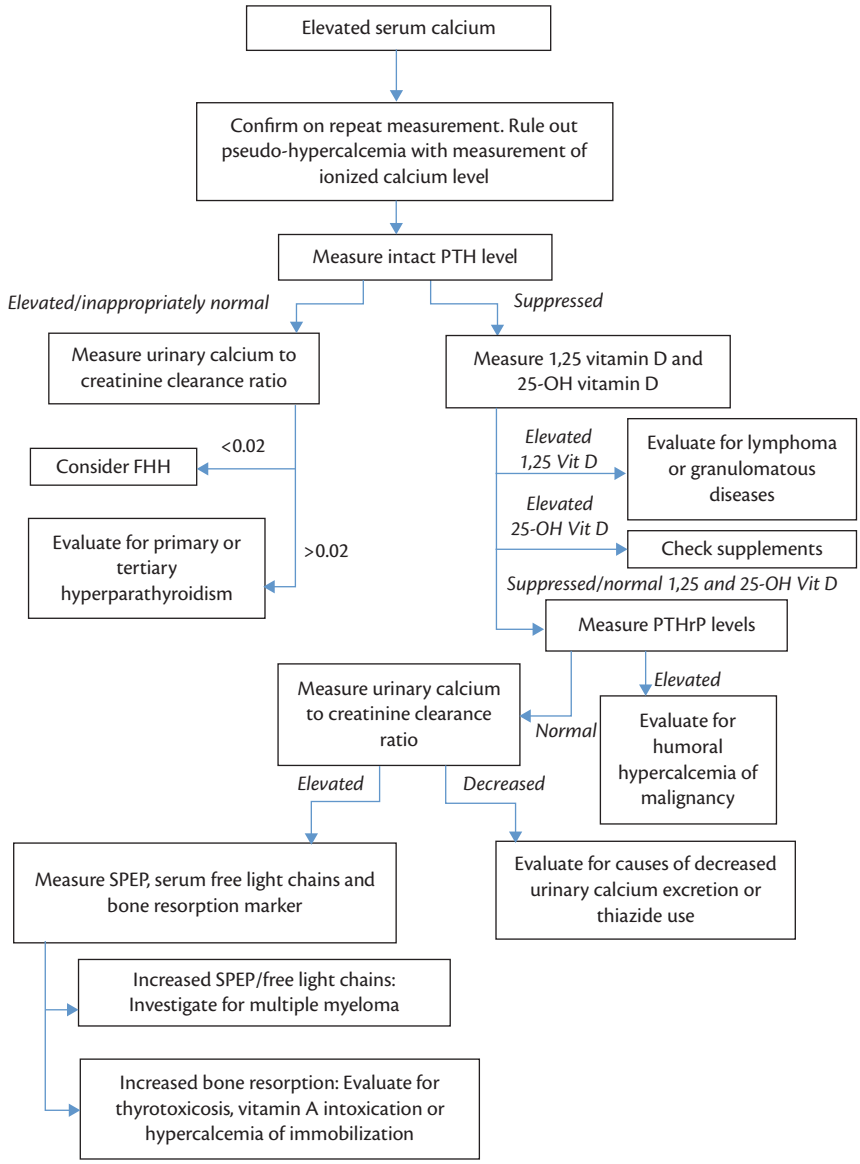

Fig. 37.1 Algorithm for dxiagnosis.

are suspected, ionized calcium levels should be obtained to rule out pseudohypercalcaemia. Alternatively, total serum calcium values can be adjusted for hypoalbuminemia using the following formula: corrected calcium (mg/dL) = measured calcium (mg/dL) + (0.8 × [4.0 – albumin (mg/dL]) (Pelosof and Gerber, 2010).

This first step in the work-up of hypercalcaemia is to obtain an intact serum PTH level. Conditions associated with increased PTH levels include primary and tertiary hyperparathyroidism, and FHH. In addition to obtaining a family history, urinary calcium to creatinine clearance ratio should be measured to distinguish these. Mutational analysis of the *CASR, GNA11*, and *AP2S1* genes should be considered in patients with urinary calcium to creatinine clearance ratio less than 0.02 to identify FHH and avoid unnecessary parathyroid surgery. Tertiary hyperparathyroidism is usually associated with PTH levels that exceed 800 pg/mL (88 pmol/L). It is important to note that inappropriately normal PTH levels in the setting of hypercalcaemia also indicate primary hyperparathyroidism. It is not unusual to have coexistent primary hyperparathyroidism with an additional cause of hypercalcaemia. Hence, the finding of elevated or inappropriately normal PTH levels should not preclude additional work-up, if clinical suspicion for other conditions is high.

The appropriate parathyroid response to hypercalcaemia is demonstration of suppressed PTH levels. In this setting, PTHrP and 1,25 dihydroxyvitamin D levels should be measured to identify the mediator of hypercalcaemia. Up to 80% of patients with HHM have an elevated intact PTHrP level measured by two-site immunoradiometric assays (IRMAs) (Burtis et al., 1990). In some cancer cells, tumour-specific processing of PTHrP occurs, resulting in the secretion of biologically active amino-terminal fragments of PTHrP. These active amino-terminal fragments may not be detected in the IRMAs that measure intact PTHrP, but could be measured by radioimmunoassays for the amino-terminal end of PTHrP (Rankin et al., 1997). However, it should be noted that

the diagnosis of HHM is often made on clinical grounds and clinical judgment should over-rule any discrepancies in PTHrP assay. If 1,25 dihydroxyvitamin D levels are elevated, the patient should be evaluated for lymphoma, hematological malignancies, or granulomatous disorders. Hypercalcaemia can also occur in patients with markedly elevated levels of 25-OH vitamin D, and this should also be measured.

If PTHrP, 25-OH vitamin D, and 1,25 dihydroxyvitamin D levels are normal, a 24-hour urinary calcium to creatinine clearance ratio should be obtained to rule out decreased urinary excretion of calcium. Also, known as the fractional excretion of calcium, this is calculated using the formula: (urinary calcium × serum creatinine)/(urinary creatinine × serum calcium). An additional step would be to rule out conditions causing increased bone turn-over such as multiple myeloma with serum protein electrophoresis and serum free light chain assay, and measurement of bone turnover markers such as urinary excretion of cross-linked N-telopeptides of type I collagen or serum C-terminal collagen crosslink. Additional investigations and focused history should be done to identify the cause of increased bone turnover—such as thyrotoxicosis, vitamin A toxicity, or immobilization.

The algorithm in Fig. 37.1 illustrates the diagnostic approach to hypercalcaemia. Though supportive measures such as hydration are the initial step in the treatment of hypercalcaemia, recognizing the pathophysiology responsible for an elevated serum calcium level and treatment of the underlying cause are key to managing hypercalcaemia.

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CHAPTER 38

Approach to the patient with hypocalcaemia

Agnès Linglart and Anne-Sophie Lambert

Introduction

Calcium is so essential for normal cellular functions—nerve impulse conduction, muscle fibre contraction, blood coagulation, cell differentiation, control of secretory mechanisms, and post-receptor second messenger transduction—that evolution has narrowed the tolerated range for extracellular ionized calcium to 0.5 mmol/L (2.25–2.75 mmol/L), and developed a complex system to regulate and maintain calcium levels, as well as bone mineralization, with few redundant mechanisms. Discovery of hypo- or hypercalcaemia always reflects a pathological process challenging the fine balance of calcium absorption, parathyroid hormone (PTH) secretion and action, vitamin D production and action, cellular compartmentalization of calcium ions, and renal function. Therefore, when faced with a patient with hypo/hypercalcaemia, newborn or elderly, we must consider two things: (1) therapy to restore the calcium level to normal and (2) investigations to determine the cause of hypo/hypercalcaemia.

Dietary calcium is mainly absorbed in the duodenum and proximal jejunum through a passive paracellular mechanism and an active transcellular process involving the calcium channel TRPV6, calbindins, and membrane exchangers such as Na^+/Ca^{2+} (NCX1) and PMCA1b. The dihydroxy form of vitamin D, 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D), is the most powerful stimulus to calcium absorption, enhancing expression of channels and transporters (Hoenderop et al., 2005). In the kidney, calcium reabsorption depends on the additional action of the calcium sensing receptor (CaSR), as well as PTH. Vitamin D (25-(OH)D) is generated through a series of enzymatic steps from cholesterol, or it is absorbed from the diet, and then further hydroxylated to 1,25-$(OH)_2$D, which in turn is able to bind the nuclear receptor VDR (vitamin D receptor) to activate various target genes, including *PTH, FGF23*, a phosphaturic factor, and *CYP24*, encoding the enzyme 24-hydroxylase, which inactivates 1,25-$(OH)_2$D. Activation of PTH1R, the PTH/parathyroid hormone-related protein receptor, increases serum calcium levels through the stimulation of 1,25-$(OH)_2$D synthesis and tubular reabsorption of calcium by the kidney (see Chapter 26). Ionized calcium is the main ligand for the CaSR: on binding calcium, CaSR inhibits PTH secretion by parathyroid cells and inhibits calcium reabsorption in the loop of Henle (thick ascending limb) (Bergwitz and Juppner, 2010).

In children, normal growth and bone mass accrual require a large amount of mineral, so determinants of blood calcium level are tightly controlled to ensure normal skeletal growth. Indeed, periods of rapid growth can reveal hypocalcaemia, if normal calcium handling is disturbed (impaired digestive absorption, bone resorption, or renal reabsorption) (Gilsanz and Nelson, 2003).

In summary, molecular defects, severe and prolonged environmental injuries, or dysregulation of organs and tissues involved in calcium balance, can all result in hypocalcaemia.

Is it really hypocalcaemia?

Measurement of calcium

Only 1% of the total body calcium circulates between fluids and tissues, 99% being trapped in hydroxyapatite. In physiological conditions, the extracellular calcium (2.2–2.60 mmol/L in serum) is 45–50% ionized calcium, 40% protein-bound calcium (mainly albumin), and 10% in diffusible complexes. Thus, the total calcium value can be influenced by metabolic disorders or dysproteinaemias. In theory, the measurement of ionized calcium is the most accurate way of evaluating serum calcium. However, since it requires collection and handling under anaerobic conditions with immediate measurement, total calcium is often used as a surrogate measure. Examples of patients requiring ionized calcium to be measured are the critically ill; especially patients receiving citrated blood, those with advanced renal failure, and neonates. In disorders affecting the albumin concentration, formulas can be devised to correct the measured total calcium (deduct 0.25 mmol/L from total calcium for each 10 g/L decrease in albumin). While diseases associated with acidosis or alkalosis can affect free calcium levels (acidosis decreases protein-bound calcium and increases free calcium), ionized calcium should not be corrected for pH using formulae. The formulae devised to estimate ionized calcium or to correct total calcium have no real advantage over uncorrected calcium; if needed, ionized calcium should be measured stringently, as above, and at the patient's pH.

Definition of hypocalcaemia

Hypocalcaemia is defined as a serum calcium below the normal range, that is, < 2.2 mmol/L or < 1.15 mmol/L of total calcium or ionized calcium, respectively. Calcium values do not vary with age and this definition applies to children and adults. Hypomagnesaemia should be excluded in patients with hypocalcaemia, since its clinical features can be similar: it may lead to hypocalcaemia, but its causes and treatment are different.

Clinical evaluation

Diagnosis of hypocalcaemia

Symptoms correlate with the severity of hypocalcaemia, ranging from asymptomatic to acute and life threatening. The evolution of hypocalcaemia (acute versus chronic) affects its clinical presentation, with better tolerance of chronic and slowly evolving hypocalcaemia. Hypocalcaemia is more symptomatic in children, especially during early life and adolescence, because of the increased need for calcium for growth.

Symptoms of hypocalcaemia

- Mainly neuromuscular irritability manifesting as paraesthesiae, cramps, tetany, Chvostek's sign (spasm of the circumoral muscles in response to a gentle tap of the facial nerve) and Trousseau's sign (carpal spasm in response to the inflation of a blood pressure cuff to 20 mmHg above the patient's systolic pressure), seizures of various types, laryngospasm, prolonged QT interval on electrocardiogram (ECG), and dysrhythmias.
- Memory loss, difficulty thinking, problems at school, poor physical and mental performance; cerebral calcification of the basal ganglia (Fig. 38.1) may be present in patients with long-standing hypocalcaemia.
- In neonates, the presence of hypocalcaemia is often revealed by tremor or seizures. Weight gain is not usually affected, but development of cognitive function is often delayed.
- Except for ectopic calcification, all signs and symptoms resolve with the restoration of a normal, or almost normal, calcium level.

In critically ill patients, attention should be focused on acute causes, such as pancreatitis, rhabdomyolysis, tumour lysis syndrome, or therapies with calcium chelators or resorption inhibitors. In non-critically ill patients, consider previous neck surgery or irradiation, autoimmune disease, digestive malabsorption, alcoholism, renal or liver disease, or an iatrogenic cause. In young adults and children, rickets suggests severe vitamin D deficiency or resistance; other symptoms such as candidiasis (Fig. 38.1), dysmorphic features, or chondrodysplasia (Fig. 38.1) will suggest a form of primary hypoparathyroidism due to a defect in parathyroid gland development or function, or PTH resistance. In neonates, maternal hypercalcaemia (due to primary hyperparathyroidism) or maternal vitamin D deficiency can explain most hypocalcaemic episodes (Thakker, 2003; Mallet et al., 2010; Holt, 2012).

Laboratory investigation

Biochemistry

The careful investigation of hypocalcaemia aims to evaluate:

- severity and consequences of hypocalcaemia
- total serum calcium
- if possible, ionized calcium with albumin
- serum phosphate

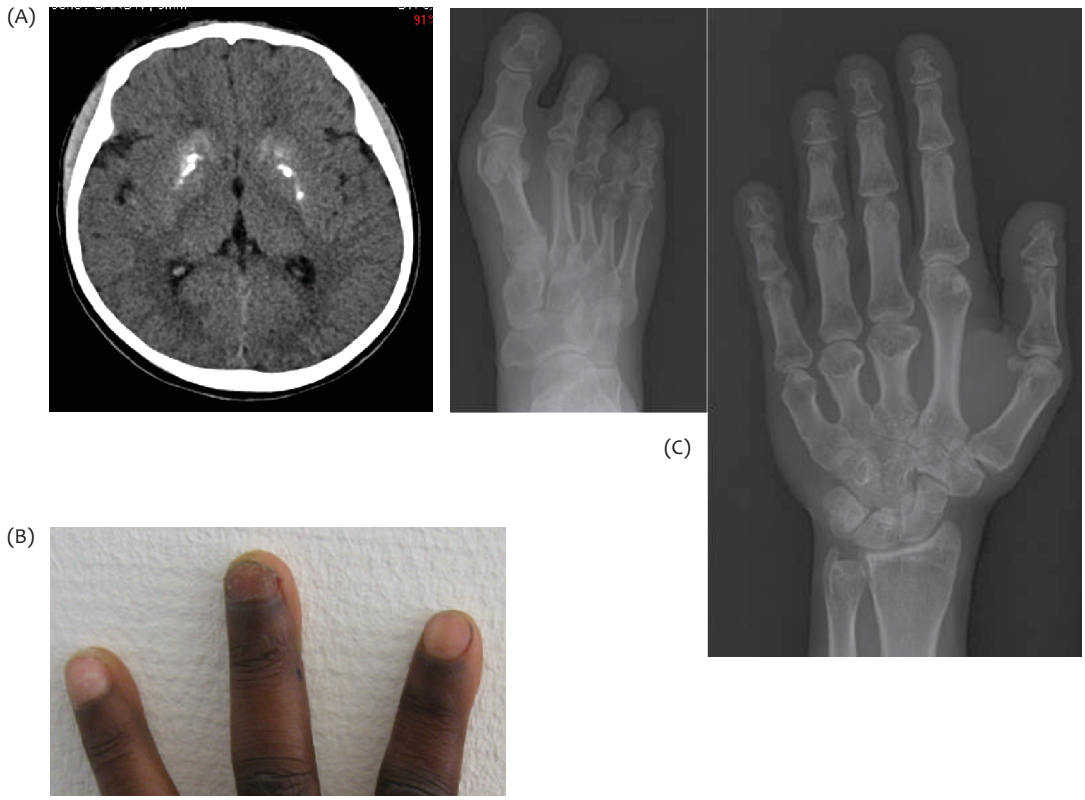

Fig. 38.1 (A) Cerebral calcification of lenticular and caudate nuclei in a 13-year-old girl with recently diagnosed hypoparathyroidism (unknown cause, no 22 q1.1 deletion, no GCMB, PTH or CaSR mutation). (B) Candidiasis affecting one nail in an 8-year-old boy with hypoparathyroidism and autoimmune polyglandular syndrome type 1 (APECED). (C) Brachymetacarpy (3rd, 4th, and 5th digit) and brachymetatarsy (3rd and 4th digits) in a patient with pseudohypoparathyroidism type IA and a mutation in the *GNAS* gene.

Table 38.1 Main causes of hypocalcaemia and their typical biochemical pattern; genes or genomic regions involved in hypocalcaemia are in parentheses

Cause	Calcium and phosphate	Urinary calcium excretion	PTH	25-(OH)D and 1,25-$(OH)_2$D	Comments
Hypoparathyroidism	Low calcium	Low at diagnosis	Low	Normal or low 25-(OH)D	Low alkaline phosphatases
Defect in parathyroid gland embryogenesis	High phosphate	Low at diagnosis	Low	Low-normal or low 1,25-$(OH)_2$D	Hungry bone syndrome may occur
DiGeorge syndrome (22q11 deletion or *TBX1*)	Low calcium	Elevated	Low	Normal or low 25-(OH)D; low-normal or low 1,25-$(OH)_2$D	
Sanjad–Sakati syndrome (*TBCE*)	High phosphate	Low at diagnosis	Low	Normal or low 25-(OH)D; low-normal or low 1,25-$(OH)_2$D	
Kenny–Caffey (*TBCE*, *FAM111A*)	Low calcium			Normal or low 25-(OH)D	
Hypoparathyroidism, deafness and renal dysplasia, HDR (*GATA3*)	High phosphate			Low-normal or low 1,25-$(OH)_2$D	
Mitochondrial diseases like Kearns–Sayre syndrome	Low calcium				
Isolated hypoparathyroidism (*GCMB*, X-linked, autosomal recessive)	High phosphate				
Defect in PTH production or secretion					
Isolated hypoparathyroidism (PTH)					
Autosomal dominant hypoparathyroidism (activating mutation of the calcium sensing receptor CaSR, G11)					
Destruction of the parathyroid glands					
Auto-immune polyendocrinopathy type 1 or APECED (AIRE)					
Auto-immune hypoparathyroidism (anti-CaSR antibodies)					
Surgery					
Infiltration (neoplasia, granulomas) or irradiation					
Pseudohypoparathyroidism	Low calcium	Low	Elevated	Normal or low 25-(OH)D; low-normal or low 1,25 $(OH)_2$D	Low alkaline phosphatase
Type 1A, 1B or 1C (*GNAS*)	High phosphate	Low	Elevated	Normal or low 25-(OH)D; low-normal or low 1,25-$(OH)_2$D	
Acrodysostosis (*PRKAR1A*)	Low or low-normal calcium				
	High phosphate				
Vitamin D deficiency or resistance	Low calcium	Low	Elevated	Low 25-(OH) D; low-normal or 1,25-$(OH)_2$D	High alkaline phosphatase
Insufficient vitamin D intake or sunlight exposure, digestive malabsorption, deficient steroid metabolism (antiepileptics, liver diseases)	Low phosphate	Low	Elevated	Normal or high 25-(OH)D; low 1,25-$(OH)_2$D	High alkaline phosphatase
Vitamin D-resistant rickets type I (CYP24A1 mutations)	Low calcium	Low	Elevated	Normal or high 25-(OH)D; high 1,25-$(OH)_2$D	High alkaline phosphatase
Vitamin D-resistant rickets type II (vitamin D receptor mutations)	Low phosphate	Low	Elevated	Normal 25-(OH)D; 1,25-$(OH)_2$D	Alopecia in 50% of the cases
Oncogenic osteomalacia induced by FGF23 secreting tumours	Low calcium				High alkaline phosphatase, osteomalacia
	Low phosphate				
Renal failure	Low calcium	Low	Elevated	Normal 25-(OH)D; 1,25-$(OH)_2$D	Elevated creatinine
	High phosphate				High alkaline phosphatase

(*Continued*)

Table 38.1 Continued

Cause	Calcium and phosphate	Urinary calcium excretion	PTH	25-(OH)D and 1,25-$(OH)_2$D	Comments
Iatrogenic					
Phosphate supplements	Low calcium High phosphate	Low Low	Elevated Elevated	Normal 25-(OH) D; normal or high 1,25-$(OH)_2$D	Low bone resorption markers
Bisphosphonates and inhibitors of bone resorption	Low calcium Low phosphate			Normal 25-(OH) D; normal or high 1,25-$(OH)_2$D	
Miscellaneous					
Acute pancreatitis					
Cell lysis (rhabdomyolysis, tumour)					
Toxic shock syndrome					

- QT interval on ECG
- differential diagnosis
- serum magnesium
- aetiology
- serum creatinine
- serum PTH—even a few hours after the initial treatment of acute hypocalcaemia, PTH levels are still reliable in determining its cause
- serum (bone) alkaline phosphatase
- serum 25-(OH)D should be measured before vitamin D administration—in urgent cases, a blood sample should be drawn and serum frozen, or kept at 4°C and protected from light until measured
- in rare situations, a 1,25-$(OH)_2$D level is required as part of the work-up of hypocalcaemia (to distinguish vitamin D-resistant rickets (VDRR), VDRR type I with low levels of 1,25-$(OH)_2$D from VDRR type II with high levels of 1,25-$(OH)_2$D)—sampling conditions are similar to those for 25-(OH)D above
- other measurements depend on the clinical context, for example, pancreatic enzymes or liver function tests.

Typical biochemical features of the most common causes of hypocalcaemia are set out in Table 38.1.

Interpretation of the laboratory tests

First, hypomagnesaemia must be excluded. PTH is the most important determinant of the serum calcium level. Hypocalcaemia may be divided into hypocalcaemia caused by PTH insufficiency or hypoparathyroidism (low serum calcium and PTH levels) or hypocalcaemia from other causes with a normal parathyroid gland response, usually described as secondary hyperparathyroidism (low serum calcium and high PTH levels). The latter is typically due to renal insufficiency, vitamin D deficiency (or resistance), or PTH resistance (Table 38.1).

Causes of hypocalcaemia

In adults, hypocalcaemia is often the consequence of an acquired disease. Although usually transient in most patients, hypocalcaemia following thyroid surgery may persist in some patients following complete removal of the parathyroid glands (Bilezikian et al., 2011). Hypocalcaemia may be associated with chronic renal failure, acute diseases (e.g. pancreatitis, rhabdomyolysis, or toxic shock syndrome) or use of therapies such as bisphosphonates, plicamycin (mithramycin), or calcitonin. Conditions leading to severe malabsorption of both calcium and vitamin D may also cause hypocalcaemia. Isolated hypoparathyroidism revealed by hypocalcaemia can occur in adults, when an autoimmune disease affecting the parathyroids should be suspected; if not due to this, genetic investigation should be carried out to test for DiGeorge syndrome or activating mutations of the *CaSR* (autosomal dominant hypoparathyroidism or ADH), and extended to family members (Pollak et al., 1994).

In children, vitamin D deficiency, with or without rickets, is the most likely cause of hypocalcaemia. Apart from this, hypocalcaemia is often due to a genetic defect in the secretion or action of PTH. Hypoparathyroidism in childhood occurs in DiGeorge syndrome, autoimmune polyendocrinopathy-candiasis-ectodermal dystrophy (APECED) syndrome, Kearns–Sayre syndrome, and autosomal dominant hypocalcaemia (ADH); pseudohypoparathyroidism is due to genetic or epigenetic defects at the imprinted *GNAS* locus (Linglart et al., 2013). In rare cases, hypocalcaemia and rickets diagnosed between 1 and 3 years of age may be due to vitamin D resistance from loss of function mutations of the renal 1α-hydroxylase (*CY27B1*) (Glorieux and St-Arnaud, 1998) or of the vitamin D_3 receptor gene (*VDR*) (Hochberg et al., 1992).

In neonates, hypocalcaemia is often related to intrauterine growth retardation or to maternal conditions such as maternal vitamin D deficiency or maternal primary hyperparathyroidism and hypercalcaemia. Transient hypoparathyroidism, (hypocalcaemia and a low level of PTH) may occur because of immaturity of the parathyroid glands. In the absence of maternal disease, hypoparathyroidism due to lack of parathyroid gland development should be investigated, for example, DiGeorge syndrome, *GCMB* (autosomal dominant or recessive), *GATA3* or *TBCE* mutations, or activating mutations of the *CaSR* (ADH) (Carpenter, 2003).

Management of hypocalcaemia

Acute or severe hypocalcaemia

In emergency situations such as neuromuscular spasms, seizures, or cardiac dysrhythmias, calcium gluconate 10% should be given intravenously (Table 38.2). If there is no intravenous access, rectal

Table 38.2 Treatment of hypocalcaemia

Therapy	Molecule	Situations	Protocol of administration	Comments
Parenteral infusion of calcium	Calcium gluconate 10%	Life-threatening emergency	Intravenous 0.5 mL/kg up to 10 mL, slowly over 10–15 minutes to avoid bradycardia Intrarectal	Repeatable once, under cardiac monitoring Repeatable once
		Symptomatic hypocalcaemia or total calcium < 1.75 mmol/L		Verify serum calcium every 12 hours
		Neonates	Intravenous diluted in 5% dextrose or 0.9% sodium chloride	Stop the intravenous when calcium ≥ 2.2 mmol/L
		Children	80 mg/kg/day	
		Adults	50 mg/kg/day or 1000 mg/m^2/day 50 mL/h of the solution: 100 mL of calcium gluconate 10% (10 ampules) in 1 L of 5% dextrose or 0.9% sodium chloride	
Oral calcium supplements	Calcium carbonate or calcium citrate	Relay of intravenous infusion of calcium gluconate		Maximum 2000 mg/day in adults
		Asymptomatic hypocalcaemia or total calcium > 1.75 mmol/L		Divided in 2 or 3 doses daily
		Neonates	Promote breastfeeding; 200 mg/day of supplements are possible	May be removed in children after a few months if calcium requirements are achieved through diet
		Children	5-years-old: 500 mg/day; 10-years-old: 1000 mg/day	
		Adults	1000–1500 mg/day; 2000 mg if proton-pump inhibitors associated	
25-(OH) vitamin D	Cholecalciferol (vitamin D_3) Ergocalciferol (vitamin D_2)	Vitamin D deficiency	Many protocols exist and cannot be all described here. 100,000 IU orally repeated after 1 week	For store replenishment Half-life 20–45 days
Active vitamin D analogues	Alfacalcidol Calcitriol	Hypoparathyroidism Acute hypocalcaemia		
		Neonates	Alfacalcidol: 2 to 6 mcg/day; calcitriol: 1 to 3 mcg/day	
		Children	Alfacalcidol: 1 to 1.5 mcg/day; calcitriol: 0.5 to 0.75 mcg/day	
		Adolescence	Alfacalcidol: ~ 1 mcg/day; calcitriol: ~ 0.5 mcg/day	
		Adults	Alfacalcidol: 1 to 1.5 mcg/day; calcitriol: 0.5 to 0.75 mcg/day	

administration is possible. Whatever the age, symptomatic (e.g. neurological symptoms, cardiac dysrhythmias) and/or profound (e.g. total calcium < 1.75 mmol/L without hypoalbuminaemia) hypocalcaemia require intravenous calcium infusion. Various protocols and doses are available (Table 38.2). Infusion should start with 1000 mg/m^2 body surface area per 24 hours. The duration of infusion depends on the severity and duration of hypocalcaemia: chronic and severe hypocalcaemia requiring 3–6 days; hungry bone syndrome requiring up to several weeks; acute hypocalcaemia requiring only a few hours. Intravenous infusion should be stopped when total calcium reaches 2.2 mmol/L, since it will usually drop by 0.2 mmol/L in the following hours. In most cases, intravenous infusion is followed by oral calcium supplements. Hypomagnesaemia should be corrected or hypocalcaemia will not resolve. Vitamin D therapy is almost always given with intravenous calcium, except for hypocalcaemia in severe illnesses such as pancreatitis or rhabdomyolysis. The type of vitamin D given (e.g. D_2, D_3, 25-(OH)D_3, 1,25(OH)$_2D_3$) depends on the origin of the hypocalcaemia and dosage depends on the severity and duration of hypocalcaemia (Table 38.2) (Mallet et al., 2010; Holt, 2012; Kelly and Levine, 2013).

Therapy for chronic hypocalcaemia

In most situations, chronic hypocalcaemia is treated with calcium supplements and vitamin D analogues (see Table 38.2 for doses and protocols) (Thakker, 2003). In children, calcium supplements are

often stopped if calcium requirements can be achieved through a normal diet.

Patients with vitamin D deficiency should receive an acute oral load of cholecalciferol (vitamin D_3) or, if not available, ergocalciferol (vitamin D_2) over a few weeks as a daily dose of vitamin D. As the hydroxylation of calciferol may take 2–3 days, a short course of calcitriol or alfacalcidol may also be given. Patients with digestive malabsorption, impaired liver function, or alcoholic hepatitis should receive parenteral vitamin D (fat-soluble preparations).

Most cases of hypocalcaemia are associated with defective 1,25-$(OH)_2$D synthesis and will resolve with the administration of 1α-hydroxylated vitamin D and oral calcium supplementation (Thakker et al., 1998). Alfacalcidol (1α-hydroxycholecalciferol) and calcitriol (1,25 dihydroxycholecalciferol) are commonly used (Table 38.2). Due to their different half-lives, alfacalcidol is given once a day and calcitriol twice a day. In patients with poor liver function calcitriol is preferred, because it does not require 25-hydroxylation (enzymatic conversion in the liver). The treatment goals depend on the underlying disease. In children and adults with hypoparathyroidism therapy should restore low-normal calcium levels (2–2.2 mmol/L total calcium). Normalization of calcium is to be avoided, because it can cause hypercalciuria in the absence of PTH-stimulated calcium reabsorption in the distal renal tubule. In ADH, it may be particularly difficult to increase serum calcium without causing hypercalciuria (Lienhardt et al., 2001). In children, 1α-hydroxylated vitamin D analogues are mandatory because of the high calcium requirement for skeletal growth. The highest doses are used in infancy, early childhood, and during adolescence. After puberty, calcium requirements fall and patients with hypoparathyroidism can maintain adequate, though subnormal, calcium levels with vitamin D and calcium supplements. It is noteworthy that patients with pseudohypoparathyroidism usually do not present with hypercalciuria following vitamin D analogue therapy; treatment should aim to decrease their PTH level and maintain normal serum calcium levels (Linglart et al., 2013). Patients affected with VDRR type I are easily controlled with low doses of alfacalcidol or calcitriol with calcium supplements. Treatment should restore normal calcium and phosphate levels within days, PTH levels within weeks, alkaline phosphatase levels within months, and rickets, leg bowing, and growth over years. In both children and adults, administration of alfacalcidol or calcitriol is closely monitored by serum and urine calcium measurements to avoid hypercalciuria, nephrocalcinosis, and nephrolithiasis (Bilezikian et al., 2011; Lienhardt and Linglart, 2012; Mitchell et al., 2012).

In contrast, VDRR type II is the only cause of hypocalcaemia that cannot be corrected by giving 1α-hydroxylated vitamin D. In these extremely rare patients, calcium is given intravenously for months or years, together with high doses of oral calcium (Hochberg et al., 1992; Tiosano and Gepstein, 2012).

Recombinant PTH (recPTH 1-34 or recPTH 1-84) has been successfully given to children and adults with hypoparathyroidism (Linglart et al., 2011; Cusano et al., 2013). Although it is not approved by the regulatory authorities (and so is off licence) recPTH may be an alternative to current therapy in patients with refractory hypoparathyroidism who experience life-threatening complications of their disease (Linglart et al., 2011; Winer et al., 2012). In our experience, doses of recPTH1-34 vary between 0.1 and 0.6 micrograms/kg/day to ensure a normal calcium level.

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CHAPTER 39

Approach to the patient with hypo-/hyperphosphataemia

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Introduction

Phosphate is the most abundant anion in the human body and has an indispensable role in numerous biological functions, including energy metabolism, bone formation, signal transduction, and as a constituent of phospholipids and nucleic acids. Total body phosphorus content in an average adult is 600–700 g (8.5–10g/kg body weight), distributed in the skeleton (85%) mostly in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), in soft tissues (14%) and only 1% in extracellular fluid, including serum levels. Despite constituting only a small part of the total store, serum phosphate (Pi) levels are subject to a dynamic fine tuning involving several hormones to maintain a normal range from 0.81 to 1.45 mmol/L (2.5–4.5 mg/dL) in adulthood and higher levels during infancy and childhood (Table 39.1) (Levine and Kleeman, 1994). Phosphate homeostasis is regulated by a complex interplay of different tissues modulating the renal tubular reabsorption, the intestinal absorption, and the bone resorption.

Hypophosphataemia

Hypophosphatemia refers to serum Pi concentrations of < 0.81 mmol/L (2.5 mg/dL). Hypophosphatemia usually results from one or a combination of the following factors (Fig. 39.1): (1) increased excretion of Pi in the urine, (2) decreased dietary Pi intake, (3) decreased GI absorption of Pi, or (4) translocation of Pi from the extracellular to the intracellular space. The major causes of hypophosphatemia are shown in Table 39.2.

Increased excretion of phosphorus in the urine

Several pathophysiologic conditions increase excretion of Pi in the urine. Some of these are characterized by elevated levels of circulating parathyroid hormone (PTH) or fibroblast growth factor 23 (FGF23). Because PTH and FGF23 decrease Pi reabsorption by the kidney (see Chapter 25), elevations of the hormones increase urinary excretion (Table 39.2). Decreased tubular reabsorption of Pi may also occur without increased levels of PTH or FGF23 and may be due to changes in the reabsorption of salt and water or to renal tubular defects specific for the reabsorption of certain solutes or Pi. Hypophosphataemia may also occur in the diuretic phase of acute tubular necrosis or in post-obstructive diuresis, presumably due to a combination of high levels of PTH and decreased tubular reabsorption of salt and water.

Inherited disorders—familial hypophosphatemia

In spite of the low incidence of inherited hypophosphataemic disorders (a few familial cases in most of the syndromes), the identification of the genes inducing these syndromes has dramatically increased our understanding of Pi homeostasis. The emerging importance of SLC34A3 (NaPi-IIc) (Bergwitz et al., 2006; Ichikawa et al., 2006; Lorenz-Depiereux et al., 2006) and FGF23 (Jonsson et al., 2003; Shimada et al., 2004) as mediators of Pi regulation, and the identification of the kidney–intestine–bone hormonal axis managing the complex signalling interplay of Pi homeostasis are some of the breakthroughs.

Rickets is the common denominator in most inherited hypophosphataemic syndromes during infancy and childhood. Rickets is a disease of the growth plate, and hence only children are affected, while osteomalacia is present in both children and adult hypophosphataemic patients (Greenbaum, 2011)

In an attempt to describe with clarity the variety of disorders involving hypophosphataemia we will classify them based on the pathophysiological mechanisms leading to the clinical features. In this chapter, we will emphasize the disorders associated with FGF23, NaPi-IIa, and NaPi-IIc in regulation of general Pi homeostasis and refer to other sources to discuss the syndromes related to PTH and vitamin D malfunction, which are complex disorders that alter the homeostasis of other ions, including calcium.

Hypophosphatemia caused by defective renal Na/Pi transporters activity

The most obvious target causing hereditary hypophosphataemic disorders are genetic mutations inducing loss of function of the renal sodium-dependent Pi cotransporters (Na/Pi), with subsequent renal Pi wasting as the cause of the hypophosphataemia.

Hereditary hypophosphataemic rickets with hypercalciuria

Hereditary hypophosphataemic rickets with hypercalciuria (HHRH) (OMIM 241530) is an autosomal recessive inherited disorder characterized by early childhood onset presenting rickets, short stature, bone pain, and muscle weakness. First identified in a consanguineous Bedouin kindred in 1985 (Tieder et al., 1985), it was not until 2006 that genetic mutations were identified in the *SLC34A3* gene (NaPi-IIc) (Bergwitz et al., 2006; Ichikawa et al., 2006; Lorenz-Depiereux et al., 2006), one of the Na/Pi transporters mediating reabsorption in the renal proximal tubule. Homozygous individuals display the full

Table 39.1 Serum phosphate levels range reference in normophosphataemia, hypophosphataemia, and hyperphosphataemia

	Age	Serum phosphate range mmol/L (mg/dL)
Normal range		
Infancy	0–5	1.45–2.67 (4.5–8.3)
Childhood	6–17	1.19–1.81 (3.7–5.6)
Adulthood	> 18	0.81–1.45 (2.5–4.5)
Hypophosphataemia in adults		
Adult	Mild	0.65–0.81 (2–2.5)
	Moderate	0.32–0.65 (1–2)
	Severe	< 0.32 (< 1)
Hyperphosphataemia in adults		
Adult	Moderate	1.48–1.93 (4.6–6)
	Severe	> 1.93 (>6)

From Marwaha et al. (2010).

clinical spectrum of symptoms and show frequently compound heterozygous *SLC34A3* mutations, meaning that they carry different mutations in the maternal and paternal alleles. Heterozygous carriers often show some of the symptoms, mostly hypercalciuria with milder hypophosphataemia and elevation of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) (Jaureguiberry et al., 2008).

Laboratory findings consist of hypophosphataemia due increased renal Pi clearance, leading to rickets or osteomalacia. Compensatory upregulation of circulating $1,25(OH)_2D$ levels enhances intestinal absorption of calcium, which then causes hypercalciuria despite normal serum calcium levels (Tieder et al., 1985). PTH and FGF23 levels are generally normal or low (Ichikawa et al., 2006). Nephrolithiasis seems to be more common in HHRH than originally described, presumably induced by the increased urinary calcium and Pi excretion (Alizadeh Naderi and Reilly, 2010).

Treatment of different hereditary hypophosphataemic conditions depends on the underlying genetic defect. In the case of HHRH, where the patients show elevated levels of $1,25(OH)_2D$, treatment consists of Pi salts supplementation since the administration of vitamin D analogues could cause worsening hypercalciuria and associated nephrolithiasis (Tieder et al., 1992).

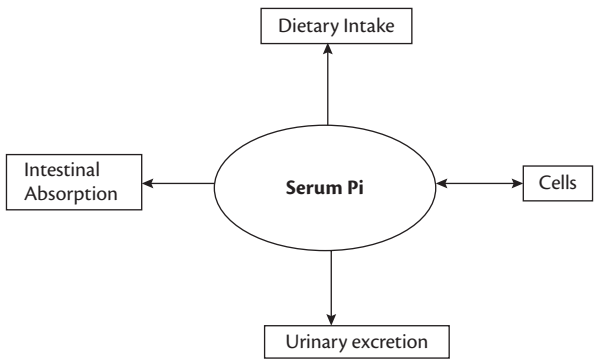

Fig. 39.1 The major determinants of serum inorganic phosphate (Pi) concentration. Serum phosphate concentration is determined by dietary intake of Pi, shift of Pi into and out of cells, intestinal absorption and urinary excretion of Pi.

Other syndromes

While NaPi-IIa has a dominant role in Pi homeostasis in the mouse (as demonstrated by the NaPi-IIa$^{-/-}$ mouse (Beck et al., 1998)), mutations identified in hypophosphataemic patients have been associated more often with NaPi-IIc, rather than NaPi-IIa. These findings have raised a controversy about the relative importance of each of these transporters in human Pi homeostasis. Correlation of hypophosphataemic syndromes and mutations in *SLC34A1* (NaPi-IIa) have not been reliably established until recently. Although different heterozygous NaPi-IIa variations have been described in patients with urolithiasis or osteoporosis, and persistent idiopathic hypophosphataemia (Prie et al., 2002), and in a large cohort of hypercalciuric stone-forming kindred (Lapointe et al., 2006), in both cases these genetic variations do not seem to be the cause of the abnormalities in these patients (Bastepe and Juppner, 2008). Magen et al. described two siblings with autosomal recessive Fanconi syndrome, severe renal Pi wasting, and hypophosphataemic rickets, carrying genetic mutation in NaPi-IIa (Magen et al., 2010), in the form of an in-frame duplication of 21 base pairs that induce loss of function (Alizadeh Naderi and Reilly, 2010).

It is interesting to note that mutations in the main intestinal Na/Pi transporter, SLC34A2 or NaPi-IIb, have been reported as the cause of pulmonary alveolar microlithiasis (PAM) (Corut et al., 2006; Huqun et al., 2007), but these patients do not exhibit hypophosphataemia or any other symptoms of unregulated Pi homeostasis. Moreover, NaPi-IIb deficiency is embryonically lethal in knockout mice (Ohi et al., 2011), but obviously not in humans, which raises questions about species specific mechanisms involved in Pi homeostasis, at least in the embryo.

Finally, mutations in the sodium/hydrogen exchanger regulatory factor 1 (NHERF1) have been implicated in the occurrence of nephrolithiasis and osteoporosis (Karim et al., 2008). NHERF1 is a PDZ protein involved in the stabilization of NaPi-IIa, and presumably also NaPi-IIc, in the apical membrane of proximal tubules (Shenolikar et al., 2002; Villa-Bellosta et al., 2008). The loss of function of NHERF1 would imply decreased activity of the Na/Pi transporters with impaired renal Pi reabsorption (Levi and Breusegem, 2008; Giral et al., 2011)

Familial hypophosphataemia caused by increased FGF23 signalling pathway

Several inherited hypophosphataemic disorders have been associated with abnormally elevated circulating levels of FGF23, occurring due to its impaired degradation or increased secretion from osteocytes. FGF23 was first identified in the serum of patients with tumour-induced osteomalacia (TIO) as a circulating phosphaturic factor that regulates Pi reabsorption and $1,25(OH)_2D$ synthesis in the renal proximal tubule (Shimada et al., 2001; White et al., 2001). (For a more detailed discussion of the FGF23 actions see Chapter 25 or the excellent reviews by Rowe (2004) and Juppner (2007).)

X-linked hypophosphataemic rickets

The most common form of hereditary rickets is X-linked hypophosphataemic rickets (XLH) (OMIM 307800) with an

Table 39.2 Causes of hypophosphataemia

Increased urinary excretion	Decreased gastrointestinal absorption	Miscellaneous causes/increased translocation
A. Primary hyperparathyroidism	A. Abnormalities of vitamin D metabolism 1. Vitamin D-deficient rickets 2. Familial a. Vitamin D-dependent rickets b. X-linked hypophosphataemia	A. Leukaemia, lymphoma
B. Secondary hyperparathyroidism	B. Malabsorption	B. Diabetes mellitus: during treatment for ketoacidosis
C. Renal tubular defects (Fanconi syndrome	C. Malnutrition-starvation	C. Severe respiratory alkalosis
D. Diuretic phase of acute tubular necrosis		D. Recovery phase of malnutrition
E. Post-obstructive diuresis		E. Alcohol withdrawal
F. Extracellular fluid volume expansion		F. Toxic shock syndrome
G. Familial 1. X-linked hypophosphataemia 2. Autosomal dominant hypophosphataemic rickets 3. Autosomal recessive hypophosphataemic rickets 1; autosomal recessive hypophosphataemic rickets 2 4. Mutations in NaPi-IIa		G. Severe burns
H. Acquired 1. Oncogenic hypophosphataemic osteomalacia or tumour-induced osteomalacia 2. McCune–Albright syndrome/fibrous dysplasia 3. Post-transplant hypophosphataemia		

incidence of 1:20,000 individuals. XLH syndrome is caused by loss of function mutations in the *PHEX* gene (*P*hosphate-regulating gene with *H*omologies to *E*ndopeptidases on the *X*-chromosome), a membrane-bound member of the neutral endopeptidases expressed mainly by osteoblasts in bone and odontoblasts in teeth. Several investigations have proved that FGF23 is not the direct substrate for PHEX (Liu et al., 2003; Benet-Pages et al., 2004; Sitara et al., 2004) as was first believed, implying the existence of an unidentified intermediate substrate that controls FGF23 expression (Pettifor, 2008). It has been proposed that PHEX loss of function may cause overexpression of FGF23 through a role in osteocyte maturation (see later for DMP1 in ARHR1) (Farrow and White, 2010). Thus, increased circulating levels of intact FGF23 (the active form) would induce hypophosphataemia by two mechanisms: (1) Na/Pi transporter inactivation with subsequent renal Pi wasting, and (2) reduction of circulating $1,25(OH)_2D$ levels that decrease intestinal Pi absorption.

Autosomal dominant hypophosphataemic rickets

Autosomal dominant hypophosphataemic rickets (ADHR) (OMIM 193100) is a very rare disease that is caused by mutations in the cleavage site of FGF23 (RXXR), increasing its resistance to proteolysis and therefore the half-life of the intact FGF23 hormone. It is important to note that two different commercial assays to measure FGF23 levels are currently available: one detects the full active form (intact peptide) and the other the C-terminal region (which would include active and inactive forms) (Juppner, 2007). In theory, increased stability of FGF23 would induce higher levels of the hormone causing phosphaturia and reduced $1,25(OH)_2D$ levels in a similar way to XLH. However, the clinical spectrum of ADHR is highly variable, possibly due to individual adaptability of patients to control FGF23 levels, as discussed below.

Autosomal recessive hypophosphataemic rickets

Mutations in two different genes have been found in autosomal recessive hypophosphataemic rickets (ARHR) resulting in two subtypes:

- ARHR1 (OMIM 241520) patients present with mutations in the gene that encodes the *D*entin *M*atrix *P*rotein 1 (*DMP1*), a member of the SIBLING (small integrin-binding ligand N-linked glycoprotein) family (Bastepe and Juppner, 2008).
- ARHR2 (OMIM 613312) was described recently as a syndrome induced by loss of function mutations in the *Ecto*-*n*ucleotide *P*yrophosphatase/*P*hosphodiesterase 1 (*ENPP1*) gene (Levy-Litan et al., 2010; Lorenz-Depiereux et al., 2010).

DMP1 is a key regulatory protein required for bone growth and development. DMP1 has been also proposed to suppress production or secretion of FGF23 by an unclear mechanism. Inactivating mutations in DMP1 presumably induce increased circulating levels of FGF23 that cause the hypophosphataemic symptoms. As already mentioned, PHEX loss of function may also cause overexpression of FGF23 through an effect on osteocyte maturation (cf. DMP1 in ARHR1).

ENPP1 is a cell surface enzyme that generates inorganic pyrophosphate (PPi), a physiological inhibitor of hydroxyapatite crystal

deposition and osteoblasts differentiation. Loss of function mutations of ENPP1 are the cause of generalized arterial calcification of infancy (GACI) syndrome. However, several ARHR patients were recently identified with ENPP1 mutations in which a hypophosphataemic phenotype seems to be protective against the calcifications observed in CAGI patients. It is believed that an increased level of FGF23 induced by an unknown mechanism also causes the hypophosphatemia observed in ARHR2 patients.

Related acquired disorders—tumour-induced osteomalacia and McCune–Albright syndrome

Although TIO and McCune–Albright syndrome (MAS) are not genetic disorders, we include them in this section because they show many similar features with the inherited syndromes associated to FGF23 gain of function.

TIO is a rare paraneoplastic syndrome caused by small endocrine tumours that secrete high levels of the phosphaturic hormones or 'phosphatonins' including FGF23, MEPE and frizzled related protein-4 (FRP-4). FGF23 is the most extensively studied. However, serum levels of FGF23 are found to be elevated in most but not all TIO patients.

The length of time from onset of symptoms until diagnosis is often long, resulting in multiple fractures, height loss and bone pain, and muscle weakness. Curiously, there are also a high percentage of patients that show symptoms without presenting with a localized tumour. Although the disease has been described mainly in adult subjects, paediatric patients have also been reported. The diagnosis is based on the impaired renal Pi reabsorption, making it necessary to differentially diagnose from the inherited disorders (XLH, ADHR, and AHRH). Once inherited and other acquired disorders have been eliminated, it is important to locate the tumour causing the disease. The treatment of choice is the resection of the tumour, which results in rapid improvement in most cases. When the tumour cannot be localized, treatment is similar to inherited hypophosphataemias, including Pi supplementation and calcitriol (for review, see Chong et al., 2011).

MAS is a rare disease that results from gain-of-function mutations occurring during early development in the gene encoding the α subunit of a stimulatory G protein, GNAS. The mutations in the *GNAS* gene may affect many different tissues, but when they occur in bone induce fibrous dysplasia. These patients develop hypophosphatemia induced by renal Pi wasting due to elevated circulating FGF23, presumably produced by the bone cells with mutations in the *GNAS* gene by an unknown mechanism.

Klotho

Recently, a case of hypophosphataemic rickets and hyperparathyroidism was associated with a *de novo* translocation of the α-Klotho gene (Brownstein et al., 2008). Klotho is a protein found in both transmembrane and soluble forms that is required for FGF23 signalling through binding with the FGF receptors (FGFR). The mutation of Klotho in this patient causes increased circulating levels of α-Klotho that result in marked elevation of plasma FGF23 and PTH levels. The induced hypophosphataemia is more severe than in usual XLH patients and could be caused by the action of the elevated phosphaturic hormones, FGF23 and PTH, but also from the direct action of Klotho on the renal NaPi transporters.

Clinical features and laboratory findings

XLH, ADHR, and ARHR syndromes show a similarity in clinical presentation, including growth retardation, skeletal deformities of the lower and upper extremities, and metaphyseal widening (Alizadeh Naderi and Reilly, 2010). Although it is considered that elevated FGF23 plays a central role in hypophosphataemia and disturbed vitamin D metabolism, it is unclear whether the bone disease is a direct or indirect effect of FGF23. Affected patients develop rickets, a disorder in which abnormal mineralization of bone and growth plate cartilage results in diminished bone strength, deformity, short stature, and bone pain. Rapidly growing bones of the lower extremities generally show the most striking abnormalities.

As a consequence of the specific genes affected, there are differences in clinical presentation for each disorder. For example, XLH manifests frequently during late infancy and therapy can improve, but usually not completely resolve, the symptoms (Petersen et al., 1992; Makitie et al., 2003). However, clinical manifestations vary in severity: while males manifest the full spectrum of symptoms, females show a more diverse response from asymptomatic hypophosphataemia to a severe syndrome identical to that present in males (Graham et al., 1959).

In ADHR, the incomplete penetrance leads to a more variable age of clinical onset. Young ADHR patients present with similar clinical features to XLH, but adolescent or adult female patients present with milder symptoms such as muscle weakness, bone pain and fractures, indicative of a late onset (Econs and McEnery, 1997). Remittance of symptoms has even been described in a number of older subjects (Econs and McEnery, 1997). In contrast to ADHR, affected individuals with ARHR present late during childhood and even into adulthood.

Several striking features are helpful in differentiating TIO from the other hypophosphataemic syndromes: clinical features include severe muscle weakness, marked bone demineralization and severe osteomalacia. In addition, $1,25(OH)_2D$ concentrations are markedly suppressed.

Typical laboratory findings in TIO include hypophosphataemia and low or inappropriately normal circulating $1,25(OH)_2D$, normal serum calcium and 25(OH)D, and high or inappropriately normal FGF23 levels. Values in the normal range of $1,25(OH)_2D$ or FGF23 are inappropriate in the context of hypophosphataemia, because the physiological homeostatic mechanisms would try to compensate by increasing $1,25(OH)_2D$ (increasing intestinal absorption) and decreasing FGF23 levels (reduced Pi excretion).

Hypophosphataemia develops within the first few months of life and is the first indicator in young infants with a family history of hypophosphataemia. Definitive evidence of renal Pi wasting is critical for diagnosis of heritable hypophosphataemic disorders, thereby ruling out the nutritional form of rickets mediated by intestinal malabsorption. Renal Pi wasting can be determined by measurement of the percentage tubular reabsorption of Pi (TRPi) or measuring the serum Pi threshold (tubular threshold maximum corrected for glomerular filtration rate, or TmPi/GFR). Both of these values are far below the normal range in inherited hypophosphataemic patients.

The diagnosis normally includes FGF23 measurements since elevated circulating levels are the cause of the underlying pathogenesis. A recent report found that FGF23 values were surprisingly not consistently elevated in patients with ADHR and that serum concentrations fluctuated between normal and elevated values, depending

on whether or not the individual subject was hypophosphataemic (symptomatic) (Imel et al., 2007; Sun et al., 2012). Although FGF23 concentrations are not invariably increased in all the patients, an inverse relationship between FGF23 and the degree of hypophosphataemia has been found. It has been proposed that individual genetic and environmental background could help to find alternative pathways to bypass the otherwise increased FGF23 activity. In ADHR, for example, adjustment of the expression and secretion of the more stable mutated FGF23 from osteoblasts could in some cases compensate for the extended half-life of the hormone, thereby maintaining Pi homeostasis and resulting in patients with asymptomatic or milder clinical presentation.

XLH, as the most common familial hypophosphataemia, has the best established medical management, but similar treatment strategies could be adopted in the other hypophosphataemic conditions associated with elevated FGF23 levels such as ADHR and AHRH. Treatment is started in the first year of life with high doses of elemental phosphorus daily and $1,25(OH)_2D$ supplementation to avoid development of secondary hyperparathyroidism. $1,25(OH)_2D$ has to be increased later in childhood and needs to be adjusted to avoid hypercalcaemia and hypercalciuria. However, a balanced treatment is difficult to achieve and often patients develop nephrocalcinosis, and in later stages tertiary hyperparathyroidism with hypercalcaemia, hypertension, and kidney damage (Verge et al., 1991; Alon et al., 2003; Tournis et al., 2011). A more detailed discussion about the classical treatment and dosages can be found elsewhere (Bastepe and Juppner, 2008; Carpenter et al., 2011). Future treatments may also include administration of FGF23 antibody (Yamazaki et al., 2008; Aono et al., 2009), although a recent study identified complications related to this approach (Shalhoub et al., 2012).

Table 39.3 Familial hypophosphataemic disorders classified by pathophysiological cause

Disease		
Defective renal Na/Pi transporter activity		
	Hereditary hypophosphataemic rickets with hypercalciuria	HHRH
	Autosomal recessive Fanconi syndrome and hypophosphataemic rickets	ARFS
	Nephrolithiasis/osteoporosis, hypophosphataemic 2	
Increased FGF23 signalling pathway		
	X-linked hypophosphataemic rickets	XLH
	Autosomal-dominant hypophosphataemic rickets	ADHR
	Autosomal-recessive hypophosphataemic rickets	ARHR1
		ARHR2
	Hypophosphataemic rickets and hyperparathyroidism	
Activated PTH signalling pathway		
	Jansen-type metaphyseal chondrodysplasia	JMC
	Multiple endocrine neoplasia type 1	MEN1
	Hyperparathyroidism-jaw tumour syndrome	HPTJT
	Familial hypocalciuric hypercalcaemia	FHH
	Neonatal severe primary hyperparathyroidism	NSHPT
Decreased vitamin D signalling pathway		
	Vitamin D-dependent rickets type 1	VDDR1
	Vitamin D-dependent rickets type 1	VDDR2

Familial hypophosphataemias caused by impaired PTH or vitamin D signalling pathway

Several other hypophosphataemic inherited disorders have been associated with the two classical hormones controlling Pi and calcium homeostasis, PTH and vitamin D. Several syndromes listed in Table 39.3 are associated with hyperparathyroidism from disturbances of calcium homeostasis inducing subsequent hypophosphataemia.

Although the patients affected by these syndromes usually present with hypophosphataemia, the clinical features are associated with important abnormalities of calcium metabolism. The diseases associated with these pathways are discussed more extensively in Chapters 37 and 38.

Post-transplant hypophosphataemia

Post-transplant hypophosphataemia, a common disorder, is well described in the literature. Although described mainly in patients following renal transplantation (Gyory et al., 1969; Moorhead et al., 1974; Ward et al., 1977; Graf et al., 1979; Better, 1980; Garabedian et al., 1980; Olgaard et al., 1980; Rosenbaum et al., 1981; Sakhaee et al., 1985; Parfitt et al., 1986; Pabico and McKenna, 1988; Steiner et al., 1993; Levi, 2001), post-transplant hypophosphataemia also occurs in patients undergoing bone marrow transplantation (Raanani et al., 1995; Crook et al., 1996). In all reports, the decrease in serum Pi concentration was associated with an increase in urinary Pi excretion and a significant decrease in the measured or derived TmPi/GFR (Walton and Bijvoet, 1975). In addition to the impairment in renal tubular Pi reabsorption, evidence indicates that intestinal Pi absorption is impaired in transplant patients (Farrington et al., 1979; Rosental et al., 1982; Massari, 1997; Levi, 2001). The mechanism for post-transplant hypophosphataemia has not been fully elucidated, but it is linked to disordered regulation of renal tubular reabsorption of Pi. Although a role for PTH in mediating this defect is still uncertain, recent studies suggest a role for FGF23 (Sanchez Fructuoso et al., 2012a, 2012b).

Decrease in gastrointestinal absorption of phosphorus

Abnormalities of vitamin D metabolism

Vitamin D and its metabolites play an important role in Pi homeostasis (Gray et al., 1977). Vitamin D promotes the intestinal absorption of calcium and Pi, and is necessary to maintain the normal mineralization of bone.

Vitamin D deficient rickets

Diets deficient in vitamin D lead to the metabolic disorder known as rickets when it occurs in children and osteomalacia when it appears in adults (Nemere and Norman, 1987). Vitamin D deficiency in childhood results in severe deformities of bone, because of rapid growth.

Vitamin D-dependent (-resistant) rickets

These are recessively inherited forms of vitamin D refractory rickets. The conditions are characterized by hypophosphataemia, hypocalcaemia, elevated levels of serum alkaline phosphatase, and, in

Table 39.4 Serum FGF23, PTH, vitamin D, and calcium levels in hypophosphataemic disorders

Disease	Gene	FGF23	PTH	$1,25(OH)_2D$	Calcium
Defective renal Na/Pi transporter activity					
HHRH	*SLC34A3*	Low	Low	High	High
ARFS	*SLC34A1*	Low/normal	n.d.	Low	Normal
	NHERF1	Normal	Normal	High	Normal
Increased FGF23 signalling pathway					
XLH	*PHEX*	High/normal[a]	Normal	Low/normal[a]	Normal
ADHR	*FGF23*	High/normal	Normal	Low	Normal
ARHR1	*DMP1*	High	Normal	Low/normal	Normal
ARHR2	*ENPP1*	High/normal[a]	Normal	Low/normal[a]	Normal
	KL	High	High	High	High

[a] Normal refers here to values in normal range but inappropriately high or low in the context of hypophosphataemia.

some instances, generalized aminoaciduria and severe bone lesions. Currently, two main forms of vitamin D-dependent rickets have been characterized. The serum concentrations of $1,25(OH)_2D$ serves to differentiate the two types of vitamin D-dependent rickets Table 39.4.

Type I vitamin D-dependent rickets is associated with reduced calcitriol levels. It is caused by a mutation in the gene converting 25(OH)D to $1,25(OH)_2D_1$, the renal 1α -hydroxylase enzyme (Fu et al., 1997). This condition responds to very large doses of vitamin D_2 and D_3 (× 100–300 the normal requirement of physiological doses), or to 0.5 to 1.0 micrograms per day of $1,25(OH)_2D$.

Type II vitamin D-dependent rickets is characterized by end-organ resistance to$1,25(OH)_2D$. Plasma levels of $1,25(OH)_2D$ are elevated. This finding, in association with radiographic and biochemical signs of rickets, and implies resistance to $1,25(OH)_2D$ in its target tissues.

Clinical and biochemical manifestations of hypophosphataemia

The manifestations of hypophosphataemia are presented in Table 39.5. The clinical manifestations of hypophosphatemia and severe Pi depletion are related to disturbances in cellular energy and metabolism. Patients with mild degrees of hypophosphataemia are usually asymptomatic. However, if hypophosphataemia is severe, that is, if serum Pi levels are < 0.48 mmol/L (< 1.5 mg per dL), a series of haematological, neurological, and metabolic disorders may develop. In general, the patients become anorectic and weak, and mild bone pain may be present if the hypophosphataemia persists for several months (Table 39.5).

Treatment of hypophosphataemia

There are several general principles that apply to the treatment of hypophosphataemic patients. As with any predominantly intracellular ion (e.g. potassium), the state of total body phosphorus stores, as well as the magnitude of phosphorus losses, cannot be readily assessed by measurement of the concentrations in serum. In fact, under conditions in which a rapid shift of Pi has resulted from glucose infusion or hyperalimentation, total body stores of phosphorus may be normal, although with diminished intake and renal losses, there may be severe Pi depletion. Furthermore, the volume of distribution of Pi may vary widely, reflecting in part the intensity and duration of the underlying cause.

In clinical situations in which hypophosphataemia is to be expected (e.g. glucose infusion or hyperalimentation in the alcoholic or nutritionally compromised patient, and during treatment of diabetic ketoacidosis), careful monitoring of the concentration of serum Pi is crucial. In these situations, addition of Pi supplementation to prevent the development of severe hypophosphataemia may prove very helpful. It is now generally recommended that hyperalimentation solutions contain a Pi concentration of 12–15 mmol/L or 37–46.5 mg/dL, to provide an appropriate amount of phosphorus in patients where renal impairment is absent (Lentz et al., 1978). Phosphorus supplementation during glucose infusion or during the treatment of diabetic ketoacidosis is usually withheld until the serum Pi levels decrease to < 0.32 mmol/L (1 mg/dL). Phosphorus may be given orally to these patients and others with mild asymptomatic hypophosphataemia in the form of skimmed milk, which contains 0.9 mg/mL, Neutra-Phos (3.3 mg/mL), or phosphorus soda (129 mg/mL). However, intestinal absorption is quite variable, and diarrhoea often complicates the oral administration of phosphate-containing compounds. For these reasons, parenteral administration is usually recommended in the hospitalized patient. If oral therapy is permissible, Fleet Phospho-Soda may be given at a dosage of 60 mmol daily in three doses (21 mmol/5 mL or 643 mg/5 mL).

In patients with severe Pi depletion, it is difficult to determine the magnitude of the total deficit in Pi and to calculate a precise initial dose. It is usually prudent to proceed with caution and repair the deficit slowly. The most frequently recommended regimen is 0.08 mmol/kg body weight (2.5 mg/kg body weight) given over 6 hours for severe, but uncomplicated, hypophosphataemia and 0.16 mmol/kg body weight (5 mg/kg of body weight) in symptomatic patients (Lentz et al., 1978). Parenteral administration should be discontinued when the serum Pi concentration is > 0.65 mmol/L (> 2 mg/dL).

Hyperphosphataemia

In adults, hyperphosphataemia occurs at serum Pi levels > 1.6 mmol/L, whereas in children and adolescents, serum phosphorus levels > 2.24 mmol/L are considered abnormal. Hyperphosphataemia is a common clinical problem, particularly

Table 39.5 Clinical and biochemical manifestations of severe hypophosphataemia

Cardiovascular and skeletal muscle	Carbohydrate metabolism	Haematological alterations	Neurological manifestations	Skeletal abnormalities	Biochemical manifestations	Renal manifestations
A. Decreased cardiac output	A. Hyperinsulinaemia	A. Red blood cells 1. Decreased adenosine triphosphate (ATP) content 2. Decreased 2,3-DPG 3. Decreased P 50 4. Increased oxygen affinity 5. Decreased lifespan 6. Haemolysis 7. Spherocytosis	A. Anorexia	A. Bone pain	A. Low parathyroid hormone levels	A. Hypercalciuria
B. Muscle weakness	B. Decreased glucose metabolism	B. Leucocytes 1. Decreased phagocytosis 2. Decreased chemotaxis 3. Decreased bactericidal activity	B. Irritability C. Confusion	B. Radiolucent areas (X-ray)	B. Increased $1,25(OH)_2D_3$	B. Hypomagnesaemia
C. Decreased transmembrane resting potential		C. Platelets 1. Impaired clot retraction 2. Thrombocytopenia 3. Decreased ATP content 4. Megakaryocytosis 5. Decreased lifespan	D. Paraesthesias	C. Pseudofractures	C. Increased creatinine phosphokinase	C. Hypermagnesuria D. Hypophosphaturia
D. Rhabdomyolysis			E. Dysarthria	D. Rickets or osteomalacia	D. Increased aldolase	E. Decreased glomerular filtration rate
			F. Ataxia			F. Decreased Tm for bicarbonate
			G. Seizures			G. Decreased renal gluconeogenesis
			H. Coma			H. Decreased titratable acid excretion

in those with significantly reduced renal function, because the kidneys play a key role in eliminating phosphorus from the body.

A number of mechanisms result in hyperphosphataemia (Table 39.6), which are discussed in more detail below.

Impaired renal excretion

Since the kidney is the major organ via which phosphorus is removed from the body, elevated serum Pi occurs in patients with acute or chronic renal failure. Serum Pi levels are almost always elevated in patients with an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2 (see 'Epidemiology').

Increased intestinal absorption

Increased absorption occurs in those who have ingested large amounts of phosphate-containing foods or beverages such as dark colas, dairy products, meat, and beans (for a complete list see the National Kidney Foundation website at <http://www.kidney.org/atoz/content/phosphorus.cfm>) or those who have used a phosphate-containing enema in preparation for colonoscopy (Markowitz and Perazella, 2009). Patients with impaired colonic motility are also at risk of hyperphosphataemia, because decreased transit time of food through the colon allows for increased absorption of Pi from food.

Release from intracellular stores

Phosphorus is released from intracellular stores in rhabdomyolysis, where muscle breakdown releases large amounts of Pi and potassium into plasma (Chatzizisis et al., 2008). In rhabdomyolysis, the release of myoglobin is also often toxic to the kidney, which causes renal failure that further exacerbates the hyperphosphataemia.

Table 39.6 Causes of hyperphosphataemia

Impaired excretion	Increased absorption	Release from stores	Shift	Endocrinopathies
Acute or chronic renal failure	Ingestion of high phosphate foods	Rhabdomyolysis	Metabolic acidosis	Hypoparathyroidism
	Phosphate containing enemas	Tumour lysis syndrome	Lactic acidosis	Pseudohypoparathyroidism
	Impaired colonic motility	Tissue death	Diabetic ketoacidosis	Familial tumoural calcinosis

The tumour lysis syndrome is characterized by spontaneous or drug-induced death of predominantly haematological malignancies, which results in hyperuricaemia, hyperkalaemia and hyperphosphataemia (Abu-Alfa and Younes, 2010). These metabolic derangements can also lead to acute kidney injury (AKI), which further elevates serum Pi. Malignant hyperthermia occurs in susceptible individuals after exposure to inhalational anaesthetics, which cause release of calcium from intracellular stores within myocytes; this leads to unregulated myocyte contraction, which can result in rhabdomyolysis and hyperphosphataemia (Stowell, 2008). Finally, any cause of massive tissue ischaemia or cell death will result in hyperphosphataemia, because intracellular Pi leaks into plasma.

Shift from intracellular to extracellular pools

Shift of Pi from intracellular stores can cause a significant increase in serum Pi. Phosphorus shifts out of cells in patients with metabolic or lactic acidosis (O'Connor et al., 1977), and those with diabetic ketoacidosis (Kebler et al., 1985).

Endocrinopathies

PTH and FGF23 play central roles in the maintenance of Pi homeostasis. Both hormones are phosphaturic and so lack of the active form of either hormone results in hyperphosphataemia.

Hypoparathyroidism

Hypoparathyroidism may be surgically induced after removal of the parathyroid glands to treat hyperparathyroidism (Wen et al., 2010). More rarely the parathyroid glands are targets of autoimmune disease, as is the case in autoimmune polyglandular syndrome type 1 (Brown, 2009). Whether surgically induced or as a result of autoimmune disease, hypoparathyroidism results in hyperphosphataemia and hypocalcaemia (see Chapters 38).

Pseudohypoparathyroidism

In pseudohypoparathyroidism (PHP), patients may have isolated resistance to PTH or may also be resistant to other hormones (Mantovani, 2011). There are a variety of PHP syndromes, which are outlined in Table 39.7. In some syndromes a constellation of features known as Albright's hereditary osteodystrophy (AHO) are present. Features of AHO include short stature, subcutaneous ossifications, brachydactyly and varying severity of mental retardation. Some PHP syndromes are caused by inactivating mutations in the α-subunit of the G protein that transduces the signal from the PTH receptor to downstream effectors.

Familial tumoural calcinosis

Familial tumoural calcinosis (FTC) is another group of genetic syndromes that can cause hyperphosphataemia (Chefetz and Sprecher, 2009). There are two types of familial tumoural calcinosis: hyperphosphataemic and normophosphataemic FTC. Both types are inherited in an autosomal recessive fashion and in both renal function is normal. However, in hyperphosphataemic FTC, deposition of calcium phosphate in cutaneous, subcutaneous and perivascular tissues is much more pronounced than in normophosphataemic FTC. Hyperphosphataemic FTC can be caused by inactivating mutations in one of three genes: *FGF23, GALNT3*, or *KL*. The *FGF23* gene encodes FGF23, a powerful phosphaturic hormone, whereas the *GALNT3* gene encodes an O-glycosyltransferase that protects FGF23 from degradation. The *KL* gene encodes Klotho, a protein that among other things acts as a cofactor in FGF23 activation. In all of these cases lack of active FGF23 leads to profound hyperphosphataemia.

Epidemiology

Except in cases of massive Pi ingestion or release of Pi from intracellular stores, serum Pi levels are usually maintained in the normal range in those with normal kidney function and normal FGF23 and PTH levels. Chronic renal failure is the most common cause of hyperphosphataemia in the general population. Kestenbaum et al. showed in a study of 6730 patients with chronic kidney disease, 3490 of whom had a serum Pi level measured in the previous 18 months,

Table 39.7 Types of pseudohypoparathyroidism syndromes

Syndrome	Hormone resistance	AHO	Gα-subunit defect
PHP-Ia	Multiple: PTH, TSH, GHRH, Gn	Yes	Maternal inactivating mutations
PHP-Ib	PTH, TSH	No	Paternal inactivating mutations
PHP-Ic	Multiple: PTH, TSH, Gn	Yes	Some inactivating mutations reported.

AHO = Albright's hereditary osteodystrophy; GHRH = growth hormone releasing hormone; Gn = gonadotropins; PHP = pseudohypoparathyroidism; PTH = parathyroid hormone, TSH = thyroid stimulating hormone.

Table modified from Mantovani (2011).

that serum Pi levels begin to rise above normal when the eGFR falls below 30 mL/min (Kestenbaum et al., 2005). Elevated serum Pi levels are common in dialysis patients, with 50% of those receiving haemodialysis estimated to have serum Pi above the normal range (Lynch et al., 2011).

Clinical features

Mild hyperphosphataemia (serum Pi < 1.76 mmol/L) is asymptomatic; whereas severe hyperphosphataemia (serum Pi > 2.56 mmol/L) causes symptoms by inducing hypocalcaemia, which results from calcium precipitating with phosphorus. Hypocalcaemia often causes muscle cramps, but can also result in altered mental status, seizures, arrhythmias, and hypotension (Shiber and Mattu, 2002). Other manifestations of hyperphosphataemia include anorexia, nausea, and vomiting.

Calcium phosphate deposition in the kidney can cause renal failure. Acute Pi nephropathy may occur after ingestion of oral sodium phosphate purgatives, which are used in preparation for colonoscopy (Markowitz and Perazella, 2009). Criteria for diagnosis of acute Pi nephropathy are: (1) AKI after exposure to oral sodium phosphate purgatives, (2) renal biopsy showing widespread tubular and interstitial deposition of calcium phosphate crystals, (3) no prior evidence of hypercalcaemia or conditions known to cause hypercalcaemia, and (4) no evidence of any other renal disease. Those at greatest risk for acute Pi nephropathy are patients who are > 60 years of age, those with pre-existing renal disease, those with hypertension, concomitant use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers or diuretics, and patients who are female. The reported incidence of acute Pi nephropathy after ingestion of oral sodium phosphate varies widely among studies (from 0.5% to 6.3% of patients). This variability is due in part to the variation in the definition of AKI used.

An elevated calcium × phosphorus product (product > 60) is one of the risk factors for development of calcific uraemic arteriolopathy (CUA), although CUA may be seen in patients with relatively normal levels of serum calcium and Pi. CUA is most often seen in patients with CKD, particularly those requiring renal replacement therapy (Hayden et al., 2008). It is characterized by arteriolar calcification in the artery media and thrombosis within subcutaneous fat tissue. Clinically CUA leads to painful skin lesions and non-healing skin ulcerations. The prevalence of CUA is estimated to be between 1% and 5% of dialysis patients. Treatment is aimed at controlling mineral metabolism parameters and meticulous wound care. Sodium thiosulfate is also sometimes used as an antioxidant and anti-inflammatory agent. CUA can be a life-threatening disease, since many patients succumb to infection and sepsis.

Laboratory findings

Laboratory work-up of hyperphosphataemia includes creatinine, electrolytes (including calcium and Pi), albumin, PTH, 25(OH)D and $1,25(OH)_2D$, and a complete (full) blood count (CBC). The creatinine and electrolytes give an indication of renal function. The calcium (corrected for albumin), Pi and PTH allow assessment of whether there is hypoparathyroidism or resistance to PTH, or whether secondary hyperparathyroidism is present; 25(OH)D and $1,25(OH)_2D$ give an indication of whether vitamin D intoxication is present. This is important, because $1,25(OH)_2D$ increases Pi absorption in the gut, which can lead to hyperphosphataemia. The CBC gives an indication of whether a haematological malignancy is present. Renal biopsy is generally reserved only for suspected cases of acute Pi nephropathy. Imaging is not indicated unless it is part of the workup of AKI or CKD.

Treatment and outcomes

Shift or release

Hyperphosphataemia due to a shift from intracellular to extracellular pools or due to release from intracellular stores is treated by treating the underlying condition. It is also important to preserve renal function to ensure adequate excretion of Pi.

Impaired renal function

Dietary restriction of Pi is a key component of treatment in patients with impaired renal function, especially in those with CKD and an eGFR < 30 mL/min/1.73 m^2. In most cases of CKD with an eGFR < 30 mL/min/1.73 m^2 dietary restriction alone does not suffice and oral Pi binders must be used. There are several different formulations of these binders, which are discussed in more detail below.

Phosphate binders

Calcium-based phosphate binders

Calcium carbonate has been used for decades in dialysis patients whereas calcium acetate is a newer formulation. There have been a few trials comparing the efficacy of calcium carbonate with that of calcium acetate, but these are limited by small numbers of participants and relatively high dropout rates. Pflanz et al. conducted a randomized crossover trial in dialysis patients in which participants received equimolar doses of calcium carbonate or calcium acetate for 6 weeks each (Pflanz et al., 1994). Of the 31 patients originally enrolled, only 23 completed both arms of the study. Patients in the acetate group had significantly lower serum Pi, calcium × phosphorus product, and intact PTH (iPTH) after completion of treatment than those in the carbonate group, but they also had a significantly higher serum calcium level. Another trial by Janssen et al. compared the efficacy of aluminium hydroxide (discussed below), calcium carbonate, and calcium acetate in 53 dialysis patients (Janssen et al., 1996). In this trial the per gram dose of elemental calcium administered was the same in the carbonate and acetate groups, but the number of capsules of calcium acetate taken daily was less than the number of tablets of calcium carbonate. Serum Pi levels were equivalent in the carbonate and acetate groups, but significantly lower in the aluminium hydroxide group. Interestingly, in this trial the incidence of hypercalcaemia was significantly less in the acetate than the carbonate group.

Sevelamer

Sevelamer is a resin that binds phosphate. It is currently marketed as sevelamer carbonate, but most of the trials conducted used the original formulation of sevelamer hydrochloride. The most well-known trial comparing the efficacy of sevelamer with that of calcium-based Pi binders is the Dialysis Clinical Outcomes Revisited (DCOR) trial (Suki et al., 2007). There were 2103 dialysis patients enrolled in the study, of which 1068 patients completed treatment. Patients were randomized to the sevelamer arm or the calcium arm (70% received calcium acetate and 30% received calcium carbonate). There was no significant difference between the groups in all cause or cause-specific mortality. However, a subgroup analysis showed a significantly lower mortality rate in

the sevelamer group in patients over the age of 65. Two recent meta-analyses of trials comparing calcium-based Pi binders and sevelamer have also shown no statistically significant differences in cardiovascular mortality between patients taking either type of binder (Tonelli et al., 2007; Jamal et al., 2009). Tonelli et al. examined 14 publications of randomized trials involving 3193 dialysis patients. In comparison with calcium-containing Pi binders, sevelamer use was associated with slightly lower serum calcium levels, similar to slightly higher serum Pi levels, and a similar calcium × phosphorus product. There was no significant difference in all-cause mortality, cardiovascular mortality or the frequency of symptomatic bone disease. There was a trend towards decreased all-cause mortality in subjects taking non-calcium based versus calcium-based binders, but this was not statistically significant. Five trials involving 469 patients reported coronary artery calcification scores. There was no difference in coronary artery calcification between patients taking calcium versus non-calcium containing binders.

In summary, there is little hard evidence that sevelamer is superior to calcium-based Pi binders in decreasing the occurrence of clinically relevant endpoints such as cardiovascular mortality or bone disease.

Lanthanum

Lanthanum carbonate is a Pi binder that does not contain calcium or aluminium. Studies to date with this binder have not been adequately powered to show differences in mortality (Tonelli et al., 2010). Results of trials performed so far show no difference in cardiovascular complications in subjects taking lanthanum *versus* calcium-containing Pi binders. In the largest study, dialysis patients were randomized to receive lanthanum (N = 682) or their usual Pi binder (N = 677). Over a 2-year follow-up period serum Pi levels were similar between the two groups and the lanthanum group had better serum calcium levels and iPTH values than the usual Pi binder group. It should be noted, however, that the dropout rate among subjects in the lanthanum group was high (71%) over the study period.

Aluminium hydroxide

While aluminium hydroxide is highly effective in lowering serum Pi levels, its use is complicated by aluminium retention and aluminium-induced encephalopathy in patients with renal failure (McDermott et al., 1978; Salusky et al., 1991). Thus, aluminium-containing Pi binders should never be used for more than a few days. In our practice, aluminium hydroxide is only used for very short periods (< 1 week) in patients with very high serum Pi levels that are refractory to other Pi binders; repeat courses of aluminium hydroxide are never given.

Guidelines

Kidney Disease: Improving Global Outcomes (KDIGO) is a global non-profit organization whose mission is to improve the treatment and outcomes of CKD. KDIGO recommendations for monitoring calcium, Pi, and intact PTH are given in Table 39.8 (see <http://www.kdigo.org/pdf/KDIGO%20CKD-MBD%20GL%20KI%20Suppl%20113.pdf>)

For renal transplant patients, KDIGO guidelines recommend measuring serum calcium and Pi at least once weekly in the immediate post-transplant period until values stabilize.

Table 39.8 Frequency of measurement of calcium, phosphorus, and iPTH for different stages of CKD

CKD stage	Parameters	Frequency of measurement
3	Ca, Phos	6–12 months
3	iPTH	Depends on disease progression
4	Ca, Phos	3–6 months
4	iPTH	6–12 months
5 (including dialysis)	Ca, Phos	1–3 months
5 (including dialysis)	iPTH	3–6 months

Ca = calcium; iPTH = intact PTH; Phos = phosphorus.

In terms of treatment, KDIGO guidelines suggest maintaining serum Pi in the normal range for patients with CKD stages 3–5 and lowering serum Pi towards the normal range for patients on dialysis. Recommendations also state that calcium-based Pi binder use should be restricted in patients with persistent or recurrent hypercalcaemia, those with a low iPTH, adynamic bone disease, or evidence of vascular calcification. (A full set of KDIGO guidelines can be found at <http://www.kdigo.org/clinical_practice_guidelines/kdigo_guideline_for_ckd-mbd.php>)

National Kidney Foundation Kidney Disease Outcomes Quality Initiative

In the United States, the National Kidney Foundation (NKF) has issued its own set of guidelines that were last published in 2003. A new set of guidelines is to be published soon. In general, the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines offer similar recommendations to those of KDIGO. (A full set of the NKF KDOQI guidelines can be found at <http://www.kidney.org/professionals/kdoqi/guidelines_commentaries.cfm#guidelines>.)

Outcomes

Acute transient hyperphosphataemia due to AKI generally has no long-term sequelae. In patients with CKD, chronic hyperphosphataemia is believed to contribute to cardiovascular morbidity and mortality (Block et al., 2004; Tuttle and Short, 2009). Patients that develop acute Pi nephropathy usually do not recover their baseline renal function (Markowitz and Perazella, 2009). In one study in Iceland, of 15 patients diagnosed with acute Pi nephropathy after a mean follow up of 26.6 months, one patient reached end-stage renal disease, one patient died with progressive renal failure, and the remaining patients had CKD with a mean serum creatinine of 184.4 μmol/L (Pálmadóttir et al., 2010).

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CHAPTER 40

Approach to the patient with hypomagnesaemia

Martin Konrad and Karl P. Schlingmann

Introduction

Magnesium plays an important role in many different cellular processes. Magnesium homeostasis depends on the balanced regulation of intestinal absorption and renal excretion. Renal magnesium handling is a pure filtration-reabsorption process, as there is little evidence of tubular secretion (Fig. 40.1). About 80% of the total plasma magnesium (0.65–1.2 mmol/L) is filtered through the glomeruli; of this amount, 15–20% is reabsorbed by the proximal tubule. The thick ascending limb of Henle's loop (TALH) plays a major role in reclaiming filtered magnesium (55–70%), whereas only 5–10% is reabsorbed in the distal convoluted tubule (DCT). The DCT mediates the selective regulation of magnesium reabsorption and plays an important role in determining the final urinary excretion (Quamme, 1997). Only 3–5% of the filtered load normally appears in the urine.

Physiology of renal tubular magnesium reabsorption

Proximal tubule

In adults, the proximal tubular magnesium reabsorption rate (15–20%) is considerably less than the fractional reabsorption of sodium and calcium (de Rouffignac and Quamme, 1994). In contrast, at neonatal age, the proximal tubule reabsorbs about 70% of the filtered magnesium, which is the same as the fractional reabsorption of sodium and calcium (Lelievre-Pegorier et al., 1983). This difference clearly indicates that the permeability of the proximal tubule changes during development, so that more magnesium is delivered to the loop of Henle in the adult. This maturation in segmental handling of magnesium should be taken into consideration when renal magnesium handling in very young children is assessed.

Different hormones affect magnesium reabsorption in the proximal tubule by influencing salt and water transport (de Rouffignac, 1995). Reabsorption is load-dependent, that is, transport is greater with elevated luminal magnesium concentrations. Extracellular volume expansion or anything that retards salt and water transport, results in diminished fluid absorption and greater magnesium delivery to the loop of Henle and the DCT. The increase in distal delivery is normally reclaimed in these nephron segments, but may be large enough to cause an increase in urinary magnesium excretion and hypermagnesiuria (Quamme, 1989).

Loop of Henle

The cortical thick ascending limb of the loop of Henle (cTALH) reabsorbs the predominant portion of the filtered magnesium, amounting up to 70%. In this nephron segment, transepithelial magnesium reabsorption is passive moving from lumen to the interstitial space through the paracellular pathway (Fig. 40.2). The driving force for magnesium reabsorption is the positive luminal transepithelial voltage generated by K^+ recycling across the apical membrane (Fig. 40.2). Any influence that alters transepithelial voltage or the permeability of the paracellular pathway will alter magnesium reabsorption in the cTALH (de Rouffignac, 1995). The voltage in the TALH depends on apical ROMK potassium channel activity and Na^+-K^+-$2Cl^-$ cotransport that control current flow across the apical membrane. Sodium exits by the basolateral Na^+,K^+-ATPase and chloride through basolateral ClC-Ka and ClC-Kb members of the chloride channel family (ClC). For proper function, ClC-Ka and ClC-Kb channels require the co-expression of barttin, an essential βsubunit of these channels (Estévez et al., 2001). Changes in their transport rates will affect the transepithelial voltage and thus magnesium absorption. The permeability of the paracellular pathway also plays an important role in determining transepithelial magnesium transport. Paracellular magnesium movement is influenced by electrostatic charges of proteins comprising this route (de Rouffignac and Quamme, 1994). Moreover, there appears to be selectivity of the pathway to divalent cations (Quamme, 1989). Members of the claudin family of tight junction proteins, claudin-16 and claudin-19, have been identified in the TALH and are involved in controlling magnesium and calcium permeability of the paracellular pathway (Simon et al., 1999; Konrad et al., 2006). This notion is supported by the phenotype resulting from mutations in the encoding genes *CLDN16* and *CLDN19*. Affected individuals present with massive calcium and magnesium wasting due to defective reabsorption in the cTALH (Simon et al., 1999; Konrad et al., 2006).

Distal convoluted tubule

Despite a net reabsorption of only 5–10% of the filtered magnesium, the DCT plays an essential role in determining the final urinary magnesium excretion, since little or no magnesium is reabsorbed beyond this segment (Quamme, 1997). Magnesium transport within the DCT is transcellular and active in nature (Fig. 40.3). Magnesium enters the cell through selective ion channels (TRPM6) across the apical membrane, driven by the transmembrane negative

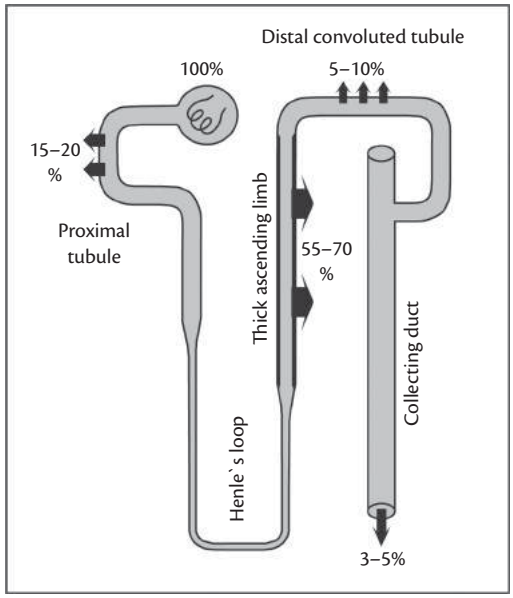

Fig. 40.1 Segmental magnesium reabsorption along the nephron.

electrical potential (Dai et al., 2001). Apical magnesium entry is the rate-limiting step in reabsorption and many of the hormonal and non-hormonal controls act at this site. Cellular magnesium is actively extruded at the basolateral membrane, possibly by a sodium-dependent exchange mechanism, which is still unresolved at the molecular level (de Rouffignac and Quamme, 1994). Magnesium transport in the DCT is dependent on luminal magnesium concentration and apical transmembrane voltage (Dai et al., 2001). (For more details, see Chapter 27.)

Clinical assessment of magnesium deficiency

Although magnesium is an abundant cation in the whole body, > 99% is located either intracellularly or in the skeleton. The < 1% of total magnesium present in the body fluids is the most assessable for clinical testing, and the total serum magnesium concentration is the most widely used measure of magnesium status; although its limitations in reflecting magnesium deficiency are well recognized (Elin, 1994). The reference range for normal total serum magnesium concentration is a subject of ongoing debate, but concentrations of 0.7–1.1 mmol/L are widely accepted. Because the measurement of serum magnesium concentration does not necessarily reflect the true total body magnesium content, it has been suggested that measurement of ionized serum magnesium or intracellular magnesium concentrations might provide more precise information on magnesium status. However, the relevance of such measurements to body magnesium stores has been questioned, because the ionized serum magnesium and intracellular magnesium did not correlate with tissue magnesium and the correlation with the results of magnesium retention tests was contradictory (Arnold et al., 1995; Hébert et al., 1997; Hashimoto et al., 2000). The use of stable magnesium isotopes and muscle ^{31}P-nuclear magnetic resonance spectroscopy represent promising new methods for non-invasive estimation of body and/or tissue magnesium pools. However, they are not particularly suitable for routine measurements.

Hypomagnesaemia develops late in the course of magnesium deficiency and intracellular magnesium depletion may be present despite normal serum magnesium levels. Due to the kidney's ability to sensitively adapt its magnesium transport rate to imminent deficiency, the urinary magnesium excretion rate is important in the assessment of the magnesium status. In hypomagnesaemic patients, urinary magnesium excretion rates help to distinguish renal magnesium wasting from extra-renal losses. In the presence of hypomagnesaemia, the 24-hour magnesium excretion is expected to decrease below 1 mmol (Sutton and Domrongkitchaiporn, 1993). Magnesium/creatinine ratios and fractional magnesium excretion rates have also been advocated as indicators of evolving magnesium deficiency (Elisaf et al., 1997; Tang et al., 2000). However, the interpretation of these results seems to be limited due to intra- and inter-individual variability (Nicoll et al., 1991; Djurhuus et al., 1995).

In patients at risk for magnesium deficiency, but with normal serum magnesium levels, the magnesium status can be further evaluated by determining the amount of magnesium excreted in the urine following an intravenous infusion of magnesium. This procedure has been described as 'parenteral magnesium loading test' and is still the gold standard for the evaluation of the body magnesium status (Elin, 1994; Hébert et al., 1997). Normal subjects excrete at least 80% of an intravenous magnesium load within 24 hours, whereas patients with magnesium deficiency excrete much less. The magnesium loading test, however, requires normal renal

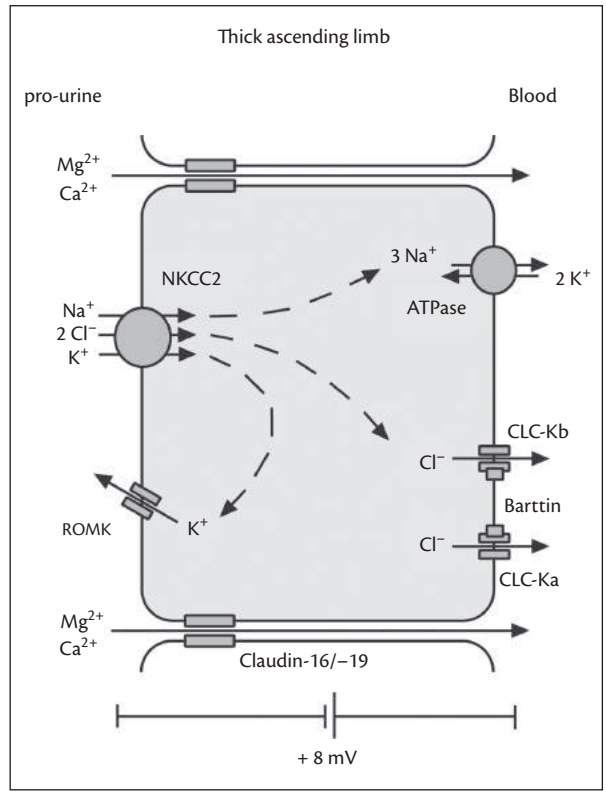

Fig. 40.2 Magnesium reabsorption in the cortical thick ascending limb of Henle's loop (TALH). The membrane proteins influencing magnesium reabsorption are indicated. Magnesium reabsorption is passive and occurs through the paracellular pathway. The driving force is the lumen-positive transcellular voltage which is generated by the transcellular reabsorption of NaCl and the potassium recycling back to the tubular fluid via ROMK.

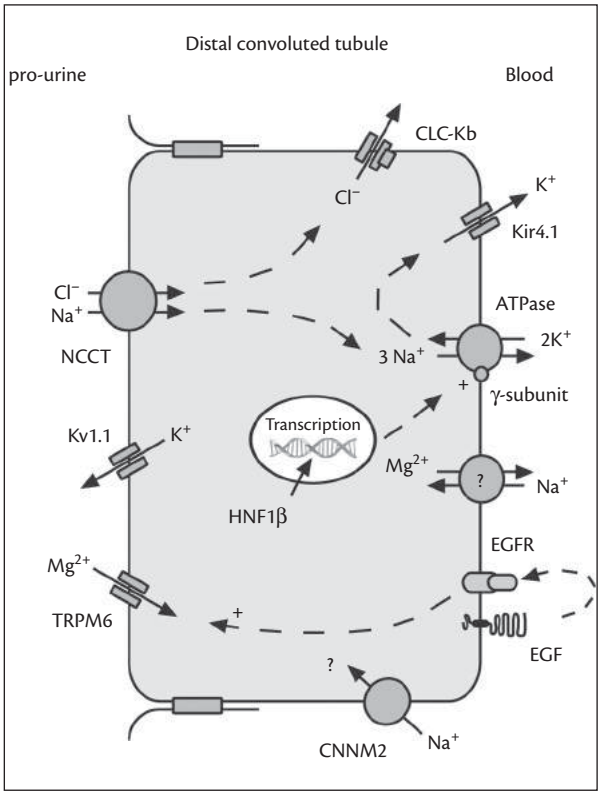

Fig. 40.3 Magnesium reabsorption in the distal convoluted tubule. The key proteins influencing magnesium reabsorption are indicated. Magnesium transport through DCT cells is active and mainly depends on the negative plasma membrane potential because the chemical gradient is very low. The Kv1.1 potassium channel determines the transmembrane voltage that allows magnesium entry through TRPM6. The molecular identity of the basolateral extrusion mechanism is still unknown but this mechanism seems to depend on a sodium gradient, which results from the coordinated action of NCCT, Na^+-K^+-ATPase and Kir4.1. The paracrine action of EGF regulates apical magnesium transport via TRPM6. HNF1B increases the transcription of the γ subunit of the Na^+-K^+-ATPase. The exact role of CNNM2 on magnesium reabsorption is still unknown.

handling of magnesium. If excess magnesium is being excreted by the kidneys due to diuresis, the magnesium load test may yield an inappropriate negative result. Conversely, if renal function is impaired and less blood is being filtered, this test could give a false-positive result.

Pathophysiology of renal magnesium handling

Inherited magnesium-wasting disorders

Hereditary hypomagnesaemia comprises a still growing number of rare genetically determined disorders primarily or secondarily affecting renal magnesium handling. In recent years, numerous genetic defects in genes encoding components of the renal tubular salt and electrolyte transport machinery or regulating factors have been described (Table 40.1). The spectrum ranges from the most frequent variant, the Gitelman syndrome with a primary defect in salt reabsorption in the DCT to extremely rare disorders discovered in single patients or families only. In the clinical setting, the assessment of additional biochemical parameters in serum and urine together with extrarenal finding and the mode of inheritance may help to confine the possibly underlying genetic defects (Table 40.2).

Hypomagnesaemia associated with abnormal renal salt handling

Tubular salt reabsorption affects the membrane potential of tubular epithelial cells and is involved in the generation of the transepithelial potential, both of which are a prerequisite for the processes of magnesium reabsorption along the different segments of the nephron. Salt-wasting disorders with hypokalaemia and metabolic alkalosis, also known as Bartter-like syndromes, impair tubular reabsorption of sodium chloride in different parts of the distal nephron. The renal conservation of magnesium is secondarily affected to a varying extent according to the nephron segment affected in each of the Bartter-like syndromes or EAST syndrome (for details see Chapter 31).

Table 40.1 Inherited disorders of magnesium handling

Disorder	OMIM #	Inheritance	Gene	Protein
Primary salt-wasting disorders				
Classic Bartter syndrome	607364	AR	*CLCNKB*	ClC-Kb, chloride channel
Gitelman syndrome	263800	AR	*SLC12A3*	NCCT, NaCl cotransporter
EAST/SeSAME syndrome	612780	AR	*KCNJ10*	Kir4.1, potassium channel
Familial hypomagnesaemia with hypercalciuria/ nephrocalcinosis	248250	AR	*CLDN16*	Claudin-16, tight junction
	248190	AR	*CLDN19*	Claudin-19, tight junction
Hypomagnesaemia/secondary hypocalcaemia	602014	AR	*TRPM6*	TRPM6, cation channel
Isolated dominant hypomagnesaemia	154020	AD	*FXYD2*	Gamma subunit Na/K/ATPase
	176260	AD	*KCNA1*	Kv1.1, potassium channel
	613882	AD	*CNNM2*	Cyclin M2
HNF1B nephropathy	137920	AD	*HNF1B*	HNF1beta, transcription factor
Isolated recessive hypomagnesaemia	611718	AR	*EGF*	Pro-EGF, epidermal growth factor
Hypomagnesaemia/metabolic syndrome	50005	maternal	*MTTI*	Mitochondrial tRNA

Table 40.2 Clinical and biochemical characteristics in inherited magnesium disorders

Disorder	Serum Mg^{2+}	Serum Ca^{2+}	Serum K^+	Blood pH	Urine Mg^{2+}	Urine Ca^{2+}	Other findings
Primary salt-wasting disorders							
Classic Bartter syndrome	↔↘	↓↑	↓↓	↑	↔↗	↔	Failure to thrive, polyuria
Gitelman syndrome	↓	↔	↓	↑	↑	↓	Chondrocalcinosis
EAST/SeSAME syndrome	↓	↔	↓	↑	↑	↓	Epilepsy, ataxia, deafness
Familial hypomagnesaemia with hypercalciuria/ nephrocalcinosis	↓	↔	↔	↔	↑↑	↑↑	Nephrocalcinosis, renal failure Ocular abnormalities
Hypomagnesaemia/secondary hypocalcaemia	↓↓	↓	↔	↔	↔↗	↔↗	Mental retardation?
Isolated dominant hypomagnesaemia							
Related to FXYD2 defects	↓↓	↔	↔	↔	↔↗	↓	None
Related to KCNA1 defects	↓↓	↔	↔	?	↔↗	↔	Episodic ataxia, myokymia
Related to CNNM2 defects	↓↓	↔	↔	↔	↔↗	?	Unknown
HNF1B nephropathy	↓	↔	?	?	↔↗	↔↘	Cystic kidneys, diabetes (MODY5)
Isolated recessive hypomagnesaemia	↓↓	↔	↔	↔	↔↗	↔	Mental retardation

Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis

Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis (FHHNC) is an autosomal recessive disorder caused by mutations in two different members of the claudin family of tight junction proteins, namely claudin-16 and claudin-19 (Simon et al., 1999; Konrad et al., 2006). Since its first description, > 100 different patients have been reported, allowing a comprehensive characterization of the clinical spectrum of this disorder and discrimination from other magnesium-losing tubular diseases (Praga et al., 1995; Benigno et al., 2000; Weber et al., 2001; Wolf et al., 2002; Godron et al., 2012). Due to excessive renal magnesium and calcium wasting, affected individuals develop the characteristic triad of hypomagnesaemia, hypercalciuria, and nephrocalcinosis that gave the disease its name. The majority of patients present during early childhood with recurrent urinary tract infections, polyuria/polydipsia, nephrolithiasis, and/or failure to thrive. Clinical signs of severe hypomagnesaemia such as seizures and muscular tetany are less common. Additional biochemical abnormalities include elevated serum intact parathyroid hormone (iPTH levels) *before* the onset of chronic renal failure, incomplete distal tubule acidosis, hypocitraturia, and hyperuricaemia present in most patients (Weber et al., 2000). The prognosis of FHHNC patients is rather poor with a high risk of progressive renal failure early during adolescence. A considerable number of patients already exhibit a markedly reduced GFR (< 60 mL/min/1.73 m^2) at the time of diagnosis. Hypomagnesaemia may completely disappear with the decline of GFR due to reduction in filtered magnesium limiting urinary magnesium excretion.

Whereas the renal phenotype is almost identical in carriers of *CLDN16* and *CLDN19* mutations, ocular involvement, including severe myopia, nystagmus, or macular coloboma, is observed only in patients with *CLDN19* mutations (Nicholson et al., 1995; Praga et al., 1995; Konrad et al., 2006; Haisch et al., 2011; Godron et al., 2012).

In addition to oral magnesium supplementation, therapy aims at the reduction of calcium excretion to prevent the progression of nephrocalcinosis and stone formation, because the degree of renal calcification has been correlated with progression of chronic renal failure (Praga et al., 1995). However, therapeutic strategies do not seem to significantly influence the progression of renal failure. Supportive therapy is important for the protection of kidney function and should include provision of sufficient fluids and effective treatment of stone formation and bacterial colonization. As expected, renal transplantation is performed without evidence of recurrence because the primary defect resides in the kidney.

Based on clinical observations and clearance studies, it had been postulated that the primary defect in FHHNC was related to disturbed magnesium and calcium reabsorption in the TALH (Rodríguez-Soriano and Vallo, 1994). In 1999, Simon et al. identified a new gene (*CLDN16*, formerly *PCLN1*), which is mutated in patients with FHHNC (Simon et al., 1999) *CLDN16* codes for claudin-16, a member of the claudin family, which is important for the formation and function of tight junctions. The individual composition of tight junction strands with different claudins confers the characteristic properties of different epithelia regarding paracellular permeability and/or transepithelial resistance. In this context, a crucial role has been attributed to the first extracellular domain of the claudin proteins, which is extremely variable in number and position of charged amino acid residues (Colegio et al., 2003). Individual charges have been shown to influence paracellular ion selectivity, suggesting that claudins positioned on opposing cells forming the paracellular pathway provide charge-selective pores within the tight junction complex.

The majority of mutations reported in FHHNC are simple missense mutations affecting the transmembrane domains and the extracellular loops, with a particular clustering in the first extracellular loop that contains the ion selectivity filter. Within this domain, patients originating from Germany or Eastern European countries exhibit a common mutation (L151F) due to a founder

effect (Weber et al., 2001). Defects in *CLDN16* have also been shown to underlie the development of a chronic interstitial nephritis in Japanese cattle that rapidly develop chronic renal failure shortly after birth (Ohba et al., 2000). Interestingly, affected animals typically show *hypocalcaemia* but no hypomagnesaemia, which might be explained by advanced renal failure present at the time of examination. The fact that, in contrast to the point mutations identified in human FHHNC, large deletions of *CLDN16* are responsible for the disease in cattle, might explain the more severe phenotype with early-onset renal failure. However, *Cldn16* knockout mice do not display renal failure during the first months of life (Will et al., 2010). In FHHNC patients, progressive renal failure is generally thought to more likely be a consequence of massive urinary calcium wasting and nephrocalcinosis. A study of a large cohort of FHHNC patients showed that the presence of *CLDN16* mutations leading to a complete loss of function on both alleles display a younger age at manifestation, as well as a more rapid decline in renal function compared with patients with at least one allele with residual claudin-16 function (Konrad et al., 2008). These findings support the theory that a complete lack of claudin-16 is associated with a more severe phenotype, whereas some residual function delays the progression of renal failure.

There is evidence from family analyses that carriers of heterozygous *CLDN16* mutations may also present with clinical symptoms. Two independent studies reported a high incidence of hypercalciuria, nephrolithiasis, and nephrocalcinosis in first-degree relatives of FHHNC patients (Praga et al., 1995; Weber et al., 2001). A subsequent study also found a tendency towards mild hypomagnesaemia in family members with heterozygous *CLDN16* mutations (Blanchard et al., 2001). Thus, one might speculate that *CLDN16* mutations are involved in idiopathic hypercalciuric stone formation.

In addition to mutations causing FHHNC, a homozygous *CLDN16* mutation (T303G) affecting the C-terminal PDZ domain has been identified in two families with isolated hypercalciuria and nephrocalcinosis without disturbances in renal magnesium handling (Müller et al., 2003). Interestingly, the hypercalciuria disappeared during follow-up and urinary calcium levels reached normal values beyond puberty. Transient transfection of MDCK cells with the CLDN16 (T303G) mutant revealed mistargeting of the mutant claudin-16 to the apical membrane. It still remains to be determined why this type of targeting defect is associated with transient isolated hypercalciuria without increased magnesium excretion.

Molecular genetic studies FHHNC patients with severe ocular involvement resulted in identification of mutations in a second member of the claudin family, claudin-19 (encoded by *CLDN19*) (Konrad et al., 2008). Claudin-19 is expressed together with claudin-16, predominantly in the TALH. Tight-junction strands in this part of the renal tubule also express claudin-10 and claudin-18 (Hou et al., 2009). These other claudins are able to maintain the barrier function of the tight junction complex in the absence of claudin-16 and -19; however, claudin-16 and -19-depleted tight junctions displayed a loss in cation permselectivity (Hou et al., 2009). Unfortunately, it remains an unanswered question whether claudin-16 and -19 directly take part in the formation of a paracellular pore structure selective for magnesium and calcium, or if they are simply involved in generating the cation selectivity of the tight junction complex required for maintaining the lumen-positive potential difference in the TALH (Hou and Goodenough, 2010). In this context, it is interesting to note that claudin-16 and claudin-19 deficient mice also display increased renal losses of sodium, as well as of potassium, in addition to the disturbance in renal magnesium and calcium handling (Hou et al., 2009).

Patients with claudin-19 defects exhibit a renal phenotype indistinguishable from patients with defective claudin-16 function (Konrad et al., 2008). However, the ocular phenotype observed in patients with claudin-19 defects is much more severe. The molecular basis for this phenomenon is the expression of claudin-19 in different layers of the retina (Konrad et al., 2008). Patients show abnormal development of the optic disc, leading to severe visual impairment and the development of horizontal nystagmus.

Hypomagnesaemia with secondary hypocalcaemia

Hypomagnesaemia with secondary hypocalcaemia (HSH) is an autosomal recessive disorder caused by mutations in the *TRPM6* gene coding for TRPM6, a member of the transient receptor potential cation channel family (Schlingmann et al., 2002; Walder et al., 2002). Since its first description in 1968 (Paunier et al., 1968), at least 50 HSH kindreds have been described (Walder et al., 1997, 2002; Schlingmann et al., 2005; Jalkanen et al., 2006). Almost all patients present in early infancy with generalized seizures refractory to anticonvulsant treatment or other symptoms of increased neuromuscular excitability such as muscle spasms or tetany. Laboratory evaluation at initial presentation reveals dramatically reduced serum magnesium levels of around 0.2 mmol/L. Hypomagnesaemia is accompanied by hypoparathyroidism with barely detectable iPTH levels, and consecutive hypocalcaemia with total serum calcium levels around 1.6 mmol/L. The unexpected finding of hypoparathyroidism is thought to result from an inhibition of iPTH synthesis and secretion in the presence of extreme hypomagnesaemia (Anast et al., 1972; Cole and Quamme, 2000; Vetter and Lohse, 2002). In addition, iPTH-induced release of calcium from bone is substantially impaired in hypomagnesaemia, because magnesium depletion interferes with the generation of cAMP in response to iPTH (Cole and Quamme, 2000). The hypocalcaemia is resistant to treatment with calcium or vitamin D.

Treatment of HSH consists of immediate administration of magnesium (equivalent to 25–50 mg magnesium sulfate) per kilogram body weight, up to a maximum of 8 mmol magnesium (equivalent to 2 g magnesium sulfate) (Schlingmann et al., 2004, 2005). Administration of magnesium alone rapidly leads to relief of clinical symptoms, normocalcaemia, and normalization of iPTH levels. Acute parenteral therapy is followed by lifelong high-dose oral magnesium supplementation (Shalev et al., 1998). In the majority of patients, organic magnesium salts such as aspartate or citrate are used. Daily requirements of up to 4 mmol/kg of body weight per day (16 times the recommended daily allowance) have been described (Cole et al., 2000), although an average daily requirement of around 1 mmol/kg of body weight per day seems to be sufficient in most HSH patients. Adolescent patients usually tolerate oral magnesium to a lesser extent than infants and younger children, who on average receive larger amounts per kilogram of body weight. While gastrointestinal complaints are clearly aggravated with increasing amounts of oral magnesium in the same patient, the susceptibility to diarrhoea shows marked inter-individual variability.

The laxative effect of magnesium salts can result in pronounced gastrointestinal side effects that occasionally necessitate considering alternative routes of administration such as intravenous or subcutaneous injections. Splitting of oral doses can reduce fluctuations of serum magnesium levels and peak urinary excretion and also alleviate diarrhoea. Additional intramuscular magnesium injections might be necessary to reduce oral intake. A regimen consisting of daily intramuscular injections given over a 20-year period has been described (Cole et al., 2000). Ultimately, the authors used continuous nocturnal administration via nasogastric tube as a therapeutic alternative to improve quality of life. In another HSH patient, hypomagnesaemic seizures only ceased after implantation of a subcutaneous pump system that provided continuous magnesium infusion (Aries et al., 2000).

Delay in diagnosis may lead to neurological deficits or may even be fatal, since seizures are refractory to anticonvulsive treatment. Several HSH patients with severe mental retardation after long-lasting seizures have been reported.

In contrast to all other known forms of hereditary hypomagnesaemia, pathophysiologic studies in affected patients using radioactive magnesium isotopes pointed to a primary defect in intestinal magnesium absorption (Lombeck et al., 1975). The presence of an additional renal magnesium loss in HSH was controversial until magnesium-loading studies clearly demonstrated a renal magnesium leak (Matzkin et al., 1989). With rising serum magnesium levels during substitution, renal magnesium loss, which is barely detectable at initial presentation, becomes evident, demonstrating a decreased renal threshold for magnesium (Walder et al., 2002). Despite remaining hypomagnesaemic with serum levels around 0.6 mmol/L, HSH patients display inappropriately high fractional excretion of magnesium in the range of 2–4% (Schlingmann et al., 2002); assuming intact renal magnesium conservation, fractional excretions rate would be expected to drop below 1% in the presence of hypomagnesaemia (Ahmad and Swaminathan, 2000).

A positional candidate gene approach allowed the identification of mutations in the *TRPM6* gene as the underlying defect in HSH (Schlingmann et al., 2002; Walder et al., 2002). *TRPM6* codes for a member of the transient receptor potential (TRP) family of cation channels. All TRP channels share the common feature of six transmembrane domains with a putative pore-forming region between the fifth and sixth transmembrane domain and intracellular N- and C-termini. Four TRP protein subunits assemble to form a functional channel complex.

The TRPM subfamily comprises eight members that exhibit a significant diversity in domain structure, as well as cation selectivity and activation mechanisms. Three members—TRPM2, TRPM6, and TRPM7—are set apart from all other known ion channels because they harbour enzyme domains in their respective C termini and represent prototypes of an intriguing new family of enzyme-coupled ion channels. TRPM6 and TRPM7 contain protein kinase domains with sequence similarity to elongation factor 2 (eEF-2) serine/threonine kinases (Dorovkov and Ryazanov, 2004; Ryazanova et al., 2004).

The functional characterization of TRPM7 demonstrated permeability for various cations, including calcium and magnesium (Monteilh-Zoller et al., 2003). TRPM7 gating was shown to be regulated by intracellular magnesium and magnesium-nucleotide complexes (Nadler et al., 2001). Targeted deletion of TRPM7 in cell lines results in intracellular magnesium depletion and growth arrest (Schmitz et al., 2003). These data point to the essential role of ubiquitously expressed TRPM7 for cellular magnesium homeostasis.

TRPM6 is highly homologous to the TRPM7 ion channel. To date, only one group has succeeded in the functional expression of TRPM6 in a mammalian cell line (Voets et al., 2004). The authors were able to show channel properties similar to those observed for TRPM7. In contrast, another group demonstrated that heteromultimerization with TRPM7 is essential for correct membrane targeting of TRPM6 (Chubanov et al., 2004). In this study, TRPM7-induced currents were significantly increased by co-expression of TRPM6. Heteromultimerization has been described previously for other members of the TRP family (Hofmann et al., 2002). The detection of TRPM6 expression in the DCT, together with functional studies in HSH patients who clearly demonstrated a renal magnesium leak, points to an important role of TRPM6 for active transcellular magnesium reabsorption in the DCT (Schlingmann et al., 2002; Voets et al., 2004). Whether TRPM6 alone or in cooperation with TRPM7 constitutes the apical magnesium channel in DCT cells remains to be clarified in future studies.

TRPM6 mutations in HSH patients comprise the following classes of mutations: stop mutations and frame shift mutations, both leading to premature stops of translation, as well as splice site mutations, which impede proper mRNA synthesis and presumably lead to absence of the corresponding exon and large exon deletions (Schlingmann et al., 2002; Walder et al., 2002). Thus far most mutations described in HSH result in truncated TRPM6 proteins. Only a few missense mutations have been identified (Schlingmann et al., 2002; Jalkanen et al., 2005; Chubanov et al., 2007). Functional characterization of these mutations revealed a complete loss of function of the TRPM6 protein (Chubanov et al., 2004, 2007). Obviously, complete lack of TRPM6 ion channel function is required for the development of the typical HSH phenotype. It is intriguing to speculate whether minor changes in TRPM6 function by single point mutations might result in a less severe clinical picture or even in subclinical magnesium deficiency.

How does an impairment of TRPM6 protein function impede epithelial magnesium transport in the intestine and kidney? The observation that in HSH patients the administration of high oral doses of magnesium are successful in achieving at least subnormal serum magnesium levels supports the existing evidence of two independent transport systems for magnesium in the gastrointestinal tract (Schweigel and Martens, 2000). TRPM6 probably represents a molecular component of active transcellular magnesium transport. An increased intraluminal magnesium concentration achieved by increased oral intake would allow compensation for the defect of the active transcellular pathway by increasing absorption via the passive paracellular route (Kerstan and Quamme, 2002; Schlingmann et al., 2002).

Isolated dominant hypomagnesaemia

FXYD2 (γ subunit)

Isolated dominant hypomagnesaemia (IDH) was first linked to a mutation in the *FXYD2* gene on chromosome 11q23 which codes for a γ subunit of the Na^+,K^+-ATPase (Meij et al., 2000). Only two related families with a *FXYD2* mutation have been described so far (Geven et al., 1987b; Meij et al., 2000). The index patients of both families presented with generalized seizures at ages 7 and 13 years, respectively. Serum magnesium levels in the two patients at that time were around 0.4 mmol/L. One index patient was treated for

seizures of unknown origin with antiepileptic drugs until serum magnesium levels were evaluated adolescence. At that time severe mental retardation was evident. Serum magnesium measurements performed in members of both families revealed low serum magnesium levels around 0.5 mmol/L in several apparently healthy individuals. Detailed haplotype analyses identified a common haplotype segregating in the two families, which suggested a common ancestor. Indeed, the mutational screening of the *FXYD2* gene demonstrated the identical mutation G41R in all affected individuals of both family branches (Meij et al., 2000).

A ^{28}Mg-retention study in one index patient pointed to a primary renal defect (Geven et al., 1987b). The intestinal absorption of magnesium was preserved and even stimulated in compensation for the increased renal losses. Urinary magnesium measurements in affected family members revealed daily magnesium excretions of around 5 mmol per day despite profound hypomagnesaemia (Geven et al., 1987b). In addition, urinary calcium excretions were found to be low in all hypomagnesaemic family members, a finding reminiscent of patients presenting with Gitelman syndrome; however, in contrast to Gitelman syndrome, no other associated biochemical findings were reported.

The γ subunit encoded by *FXYD2* is a member of a family of small single transmembrane proteins that share the common amino acid motif F-X-Y-D. It comprises two isoforms (γ-a and γ-b) that are differentially expressed in the kidney. The γ-a isoform is present predominantly in the proximal tubule, whereas expression of the γ-b isoform predominates in the distal nephron, especially in the DCT and connecting tubule (Arystarkhova et al., 2002b). The ubiquitous Na^+,K^+-ATPase is a dimeric enzyme invariably consisting of one α and one β subunit. FXYD proteins constitute a third or γ subunit that represents a tissue-specific regulator of Na^+,K^+-ATPase and produces distinct effects on the affinity of the Na^+,K^+-ATPase for sodium, potassium, and ATP. The FXYD2 γ subunit increases the apparent affinity of Na^+,K^+-ATPase for ATP, while decreasing its sodium affinity (Arystarkhova et al., 2002a). Thus, it might provide a mechanism for balancing energy utilization and maintaining appropriate Na and K gradients across the cell membrane.

Expression studies of the mutant G41R-γ subunit in mammalian renal tubule cells revealed a dominant negative effect of the mutation leading to retention of the γ subunit within the cell. Whereas initial data pointed to retention of the entire Na^+,K^+-ATPase complex in intracellular compartments, subsequent data suggest an isolated trafficking defect of the mutant γ subunit (Blostein et al., 2003). The mutant γ subunit is retarded in the Golgi complex, which points to disturbed post-translational processing. The assumption of a dominant negative effect was first substantiated by the observation that individuals with a large heterozygous deletion of chromosome 11q that includes the *FXYD2* gene exhibit normal serum magnesium levels (Meij et al., 2003). Meanwhile, it could be shown that wild type γ subunits oligomerize within the cell before trafficking to the plasma membrane. The G41R-mutant was shown to also oligomerize with itself and the wild type γ subunit and thus prevent proper routing of wild type γ subunits to the plasma membrane and incorporation into functional ATPase complexes (Cairo et al., 2008).

Urinary magnesium wasting together with the expression pattern of the *FXYD2* gene indicate a defect of active transcellular magnesium reabsorption in the DCT in affected individuals. Meij et al. have suggested that diminished intracellular potassium might depolarize the apical membrane, resulting in a decrease in magnesium uptake (Meij et al., 2000). Alternatively, an increase in intracellular sodium could impair basolateral magnesium transport, which is presumably achieved by a sodium-coupled exchange mechanism. Another explanation could be that the γ subunit is involved not only in Na^+,K^+-ATPase function, but also is an essential component of a yet unidentified ATP-dependent transport system specific for magnesium.

An interesting finding in IDH linked to the *FXYD2*-G41R mutation is hypocalciuria. In patients with the FXYD2 mutant, there is no evidence for renal salt wasting and no stimulation of the renin–angiotensin–aldosterone system (RAAS). Therefore, the mechanism leading to hypocalciuria in IDH related to *FXYD2* mutations remains unclear. One could speculate that, similar to Gitelman syndrome, a defect in Na^+,K^+-ATPase function and energy metabolism might lead to a cell apoptosis in the early DCT responsible for magnesium reabsorption, while later parts of the distal nephron remain intact.

KCNA1

Genetic heterogeneity in isolated dominant hypomagnesaemia was demonstrated by the identification of a dominant-negative missense mutation in *KCNA1* encoding the voltage-gated potassium channel Kv1.1 (Glaudemans et al., 2009). The phenotype of affected individuals originating from a large Brazilian family included recurrent episodes of muscle cramps, tetanic episodes and tremor, and muscle weakness, starting in infancy. Laboratory analyses revealed isolated hypomagnesaemia < 0.4 mmol/L, while urinary magnesium excretion was found to be inappropriately elevated, pointing to a renal magnesium leak. No alterations in renal calcium handling were observed.

Interestingly, *KCNA1* mutations had been identified before in patients with episodic ataxia with myokymia (OMIM 160120), a neurologic disorder characterized by periodical appearance of incoordination and imbalance, as well as myokymia, an involuntary, spontaneous, and localized trembling of muscles (Browne et al., 1994). In addition to muscle cramps and tetany attributed to magnesium deficiency, these symptoms were also present in members of the above mentioned Brazilian kindred with hypomagnesaemia.

By using a genome-wide single nucleotide polymorphism-based linkage strategy followed by subsequent conventional sequencing of candidate genes identified a heterozygous mutation in the *KCNA1* gene co-segregating with the disease (Glaudemans et al., 2009). The mutation leads to a non-conservative amino acid exchange (N255D) in the encoded Kv1.1 potassium channel. Functional voltage-gated potassium channels of the KCNA family are composed of homo- or heterotetramers in assembly with other Kv channel subunits. Co-expression of the mutant N255D-Kv1.1 with wild type channel subunits in HEK293 cells resulted in a significant reduction in current amplitudes, compatible with a dominant-negative effect of the mutant. The dominant-negative effect seems to be the result of an impaired gating of the potassium channel tetramer, since trafficking to the plasma membrane is preserved (van der Wijst et al., 2010).

Kv1.1 expression was demonstrated in the kidney to be co-localized with TRPM6 at the apical membrane of the DCT. Glaudemans et al. propose a model in which Kv1.1 permits hyperpolarization of the DCT apical cell membrane potential as a prerequisite for TRPM6-mediated magnesium entry (Fig. 40.3). Thus these authors, for the first time, linked magnesium reabsorption in the DCT to potassium secretion, and identified a new dependency

between renal magnesium and potassium handling at the molecular level (Glaudemans et al., 2009).

CNNM2

Another form of isolated dominant hypomagnesaemia was recently described in which mutations in *CNNM2* were discovered (Stuiver et al., 2011). *CNNM2* (or ACDP2) had been identified before by differential gene expression microarray analysis of genes upregulated in face of hypomagnesaemia in mice (Goytain and Quamme, 2005). The authors had demonstrated that the CNNM2 protein is able to induce the transport of different divalent cations, including magnesium (Goytain and Quamme, 2005). Furthermore, the *CNNM2* gene locus had been linked to serum magnesium levels in a genome-wide association study (Meyer et al., 2010).

Stuiver and colleagues screened *CNNM2* as a candidate gene in patients with unresolved magnesium wasting disorders and identified heterozygous mutations in two unrelated families with isolated dominant hypomagnesaemia (Stuiver et al., 2011). In affected individuals, clinical symptoms and age at manifestation seem to be highly variable with symptoms ranging from convulsive episodes in early childhood to muscle weakness, vertigo and headaches during adolescence. Other heterozygous carriers from both families remained asymptomatic. Serum magnesium levels in affected individuals were in the range of 0.4–0.5 mmol/L and failed to normalize under oral magnesium supplementation. All other serum electrolytes were found to be normal. Since data on urinary calcium excretions vary between the described index patients, it remains unclear if the finding of hypocalciuria seen in a number of other inherited magnesium wasting disorders (see above) is also a concomitant feature in patients with *CNNM2* mutations.

The *CNNM2* gene codes for CNNM2 or Cyclin M2, a transmembrane protein that is expressed in kidney at the basolateral membrane of TALH and DCT, but also in other organs especially the brain (Stuiver et al., 2011). CNNM2 is one of four members of a protein family with a conserved domain structure (for which this family was previously named ancient conserved domain proteins or ACDP family) with sequence similarities to the bacterial CorC protein involved in bacterial magnesium transport (Gibson et al., 1991).

Whereas a truncating frame-shift mutation was identified in one of the described families, affected individuals of the second family carry a missense mutation, leading to a non-conservative amino acid exchange of the CNNM2 protein (T568I) (Stuiver et al., 2011). Functional characterization of the mutant T568I-CNNM2 demonstrated that protein trafficking was preserved in HEK293 cells; however, patch clamp analyses revealed a significant reduction in magnesium-sensitive, inwardly-rectifying sodium currents. In contrast to previous experiments in *Xenopus* oocytes by Goytain and Quamme (2005), the authors did not observe significant magnesium currents with overexpression of CNNM2 in HEK293 cells (Stuiver et al., 2011). The localization data, together with the functional studies, lead to the assumption that CNNM2 might represent a basolateral magnesium sensing mechanism in renal tubular cells, rather than being a molecular component of the yet uncharacterized basolateral magnesium extrusion machinery itself. Since Stuiver and colleagues identified a truncating mutation, as well as a missense mutation in their families they only speculate on a reduced amount of functional protein as a putative mechanism for the dominant mode of inheritance (Stuiver et al., 2011). Therefore, it remains a subject of further study as to how exactly CNNM2 is involved in basolateral magnesium transport processes.

Hepatocyte nuclear factor 1B nephropathy

Hepatocyte nuclear factor 1B (HNF1B) is a transcription factor critical for the development of kidney and pancreas. Heterozygous mutations in *HNF1B* were first been implicated in a subtype of maturity-onset diabetes of the young (MODY5) before an association with developmental renal disease was reported. The renal phenotype is highly variable, including enlarged hyperechogenic kidneys, multicystic kidney disease, renal agenesis, renal hypoplasia, cystic dysplasia, as well as hyperuricaemic nephropathy. The association of diabetes with renal cysts leads to the term 'renal cysts and diabetes (RCAD) syndrome'. *HNF1B* mutations are present in heterozygous state, either inherited or *de novo*, and comprise point mutations, as well as whole-gene deletions (Heidet et al., 2010). Interestingly, around 50% of affected individuals present with hypomagnesaemia due to renal magnesium wasting (Heidet et al., 2010; Adalat et al., 2009). The degree of hypomagnesaemia is usually mild to moderate (~ 0.65 mmol/L) and the defect in renal magnesium conservation is accompanied by the occurrence of hypocalciuria. The *HNF1B* gene encodes a transcription factor regulating the expression of numerous renal genes, including the *FXYD2* gene which contains several HNF1B -binding sites in its promoter region (Adalat et al., 2009). In accordance with the phenotype and *in silico* data, Adalat and colleagues could show that HNF1B was able to stimulate the expression of *FXYD2 in vitro*. Therefore, defective FXYD2 transcription represents a putative mechanism explaining renal magnesium wasting in patients with *HNF1B* mutations.

Isolated recessive hypomagnesaemia

Geven and colleagues initially reported a form of isolated hypomagnesaemia in a consanguineous family, indicating autosomal recessive inheritance (Geven et al., 1987a). Two affected girls presented with generalized convulsions during infancy. Unfortunately, late diagnosis resulted in neurodevelopmental deficits in both patients. A thorough clinical and laboratory workup at 4 and 8 years of age, respectively, revealed serum magnesium levels of 0.5–0.6 mmol/L with no other associated electrolyte abnormalities. A ^{28}Mg-retention study in one patient pointed to a primary renal defect, while intestinal magnesium uptake was preserved (Geven et al., 1987a). Both patients exhibited renal magnesium excretions of 3–6 mmol per day, despite hypomagnesaemia, which confirmed renal magnesium wasting, whereas calcium excretion rates were in the normal range.

Using a homozygosity mapping strategy, Groenestege and colleagues identified a candidate interval on chromosome 4q (Groenestege et al., 2007). Subsequent screening of candidate genes within this region resulted in the identification of a homozygous missense mutation in the *EGF* gene, leading to a non-conservative amino acid exchange in the encoded pro-EGF protein (pro-epidermal growth factor) in the two sisters (Groenestege et al., 2007). Pro-EGF is a small peptide hormone expressed in various tissues, including the kidney (predominantly in the DCT). Pro-EGF is a membrane protein that is inserted in both the luminal and basolateral membrane of polarized epithelia. After membrane insertion, it is processed by unknown proteases into active EGF peptide. EGF activates specialized EGF receptors (EGFRs),

which are expressed in the basolateral membrane. This activation leads to an increase in TRPM6 trafficking to the luminal membrane and increased magnesium reabsorption (Thebault et al., 2009). The mutation described in IRH (P1070L) disrupts the basolateral sorting motif in pro-EGF (Groenestege et al., 2007). Therefore, the activation of EGFRs in the basolateral membrane is compromised, which finally leads to reduced magnesium reabsorption. Despite acting in a paracrine fashion in the DCT, the authors speculate on a role for EGF as a selectively acting magnesiotropic hormone (Groenestege et al., 2007).

Mitochondrial hypomagnesaemia

In 2004, a mutation in the mitochondrial isoleucine tRNA gene, $tRNA^{Ile}$, or *MTTI*, was discovered in a large Caucasian kindred (Wilson et al., 2004). Extensive clinical evaluation of this family was initiated after the discovery of hypomagnesaemia in the index patient. Pedigree analysis was compatible with mitochondrial inheritance as the phenotype was exclusively transmitted by affected females. The phenotype includes hypomagnesaemia, hypercholesterolaemia, and hypertension. Among the adults on the maternal lineage, the majority of offspring exhibit at least one of the mentioned symptoms; approximately half of the individuals show a combination of two or more symptoms, and around one-sixth had all three features. Serum magnesium levels of family members in the maternal lineage greatly vary, ranging from 0.3 to 1.0 mmol/L, with approximately 50% of individuals being hypomagnesaemic (< 0.75 mmol/L).

Hypomagnesaemic individuals showed higher fractional excretions rates (median around 7.5%) than their normomagnesaemic relatives in the maternal lineage (median around 3%), which clearly pointed to renal magnesium wasting. Hypomagnesaemia was accompanied by decreased urinary calcium excretion, a finding pointing to the DCT as the affected tubular segment.

The mitochondrial mutation observed in the examined family affects the $tRNA^{Ile}$ gene *MTTI* (Wilson et al., 2004). The observed nucleotide exchange occurs at the T nucleotide directly adjacent to the anticodon triplet. This position is highly conserved among species and critical for codon-anticodon recognition. The functional consequences of the tRNA defect for mitochondrial function remain to be elucidated in detail. As ATP consumption along the tubule is highest in the DCT, the authors speculate on an impaired energy metabolism of DCT cells as a consequence of the mitochondrial defect, which in turn could lead to disturbed transcellular magnesium reabsorption.

Acquired renal magnesium wasting disorders

Cisplatin and carboplatin

The cytostatic agent cisplatin and the newer antineoplastic drug, carboplatin, are widely used in various protocols for the therapy of solid tumours (Lajer and Daugaard, 1999). Among diverse side effects, nephrotoxicity receives most attention as the major dose-limiting factor (Yao et al., 2007). Carboplatin has been reported to have less severe side effects than cisplatin (English et al., 1999; Boulikas and Vougiouka, 2004; Carrick et al., 2004). Hypomagnesaemia following renal magnesium wasting is regularly observed in patients treated with cisplatin (Lajer and Daugaard, 1999; Goren, 2003; Hodgkinson et al., 2006). The incidence of magnesium deficiency is > 30%, but increases to > 70% have been observed with longer cisplatin usage and greater accumulated doses (Hodgkinson et al., 2006).

Cisplatin exerts direct damage renal tubular cells and causes proximal, as well as distal, tubular dysfunction (Yao et al., 2007). Acute cisplatin toxicity involves defective mitochondrial function, decreased ATPase activity, altered cell cation content, and altered solute transport. In the distal tubule, the reabsorption of salt and particularly of magnesium are compromised. In rodents, cisplatin leads to an impairment of proximal tubular function, whereas in dogs and humans morphological changes appear predominantly in the DCT and the collecting duct (Mavichak et al., 1985; Swainson et al., 1985; Magil et al., 1986). Thus, for cisplatin toxicity the interpolation from animal studies may be misleading, but the evidence from both clinical and experimental studies indicate that the drug acts on distal tubular magnesium transport. Since there is no magnesium reabsorption within the cortical collecting ducts, it is likely that actions within the DCT are responsible for the renal magnesium leak. Using micropuncture, Mavichak et al. showed that magnesium reabsorption was diminished in the distal tubule of rats receiving cisplatin (Mavichak et al., 1985). The molecular mechanisms for the acute, apparently selective effects on magnesium remain undefined. It would be of interest to determine if amiloride retains its magnesium-conserving actions in these patients (Quamme, 1997). The effects of cisplatin may persist for months or years later—long after the inorganic platinum has disappeared from the renal tissue (Bianchetti et al., 1991; Markmann et al., 1991). Whatever cellular mechanisms are involved they must include genetic alterations of magnesium transport. The fact that cisplatin exerts its cytotoxicity binding cellular DNA may be relevant (Lajer and Daugaard, 1999; Boulikas and Vougiouka, 2004). The chronic alterations in distal tubular ion transport comprise a clinical picture reminiscent of Gitelman syndrome (Panichpisal et al., 2006). Morphological studies in humans with cisplatin nephrotoxicity could demonstrate focal tubular necrosis predominantly in the DCT, a feature also observed in NCCT-deficient mice, suggestive of DCT cell apoptosis (Arany and Safirstein, 2003; Loffing et al., 2004). Therefore, fluid repletion, and especially magnesium supplementation with sufficient doses, should be promoted routinely to prevent acute and chronic cisplatin nephrotoxicity, and the risk of adverse effects from hypomagnesaemia.

Aminoglycosides

Aminoglycosides, such as gentamicin, induce renal impairment in up to 35% of patients, dependent on the dose and duration of administration. In addition, aminoglycosides cause hypermagnesiuria and hypomagnesaemia (Shah and Kirschenbaum, 1991). As many as 25% of patients receiving gentamicin will exhibit hypomagnesaemia (Shah and Kirschenbaum, 1991). The hypermagnesiuric response occurs soon after the onset of therapy; it is dose dependent and readily reversible on withdrawal. As with adults, neonates also display an immediate increase of calcium and magnesium excretion after gentamicin infusion (Elliott et al., 2000; Giapros et al., 2004). Magnesium wasting is associated with hypokalaemia and hypercalciuria that may also lead to diminished plasma calcium concentrations (Keating et al., 1977). This would suggest that aminoglycosides affect renal magnesium and calcium transport in the tubular segments where both are reabsorbed. Experimental studies with animals support this notion (Garland et al., 1994). The

cellular mechanisms are not completely understood, but hypermagnesiuria and hypercalciuria are observed in the absence of histopathological changes (Weinberg et al., 1983). Because gentamicin is a polyvalent cation, it was postulated that it may have effects on the CaSR (Kang et al., 2000; Dai et al., 2001; McLarnon et al., 2002; Ward et al., 2005). Activation of this receptor by polyvalent cations inhibits the passive reabsorption of magnesium and calcium in the TALH and active hormone-mediated transport in the DCT, leading to renal magnesium and calcium wasting.

Calcineurin inhibitors

The calcineurin inhibitors ciclosporin and tacrolimus (FK506) are widely prescribed as immunosuppressants to organ transplant recipients and in numerous immunological disorders. On this therapy patients are at high risk of developing renal injury and hypertension. Tubular dysfunction with subsequent disturbance of mineral metabolism is another common side effect. Both drugs commonly lead to renal magnesium wasting and hypomagnesaemia (Rob et al., 1996). These drugs also cause modest hypercalcaemia with hypercalciuria and hypokalaemia (Rob et al., 1996). The hypomagnesaemic effect is probably attenuated by the fall in GFR and reduction in filtered magnesium, but this defect appears to be specific for magnesium (Wong and Dirks, 1988). Calcineurin inhibitor therapy is associated with an inappropriately high fractional excretion rate of magnesium, suggesting impaired passive reabsorption in the TALH or active magnesium transport in the DCT (Lote et al., 2000).

Chang and colleagues have reported that ciclosporin reduces claudin-16 expression in the TALH (Chang et al., 2007). Furthermore, ciclosporin and tacrolimus have been shown to inhibit PTH-stimulated magnesium uptake in a mouse DCT (mDCT) cell line (Kim et al., 2006). Accordingly, Ledeganck and colleagues could show that ciclosporin decreases the renal expression of TRPM6, TRPM7, NCC, and EGF (Ledeganck et al., 2011). In contrast to the study by Chang et al. (2007), these authors did not find a decrease in claudin-16 or claudin-19 expression in their rat model (Ledeganck et al., 2011). In a previous animal study, Nijenhuis et al. had demonstrated that tacrolimus induces a decrease in TRPV5, calbindin-D28k, and TRPM6 at the mRNA level (Nijenhuis et al., 2004). They could also show a decrease in TRPV5 and calbindin-D28k at the protein level. These effects appeared to be specific, because no morphologic features of tubular toxicity were observed. Finally, Ledeganck and co-workers could demonstrate that the EGF-mediated increase in TRPM6 abundance was abrogated by ciclosporin (Ledeganck et al., 2011). As mentioned above, ciclosporin, beyond its effect on magnesium transport in the DCT, downregulates the sodium chloride cotransporter NCCT in the DCT, resulting in renal salt wasting (Ledeganck et al., 2011).

With respect to magnesium, it is interesting to note that TRPM6 expression in the intestine was not changed on tacrolimus administration. It is not known whether these drugs act through calcineurin, which is the intracellular receptor for these agents. It is speculated that FK506-binding proteins, which are known to bind and regulate the calcium-permeable transient receptor potential-like (TRPL) cation channels, might be involved because tacrolimus disrupts this binding (Mervaala et al., 1999). By analogy, consider that certain FKBPs might also regulate TRPV5 or TRPM6 expression or activity. Hypomagnesaemia has been implicated as a contributor to the nephrotoxicity and arterial hypertension associated with calcineurin inhibitors. Mervaala and colleagues could demonstrate that the adverse effects of ciclosporin in spontaneously hypertensive rats largely depend on dietary sodium and that these adverse effects can be prevented by magnesium supplementation (Mervaala et al., 1999). Magnesium supplementation also had a beneficial effect on ciclosporin nephrotoxicity in a rat model used by Miura and colleagues (Miura et al., 2002).

EGF receptor antibodies

The EGF hormone axis has been implicated in renal magnesium handling by the discovery of a homozygous mutation in the *EGF* gene in a family with isolated recessive hypomagnesaemia (see above) (Groenestege et al., 2007). The way for these findings was paved by the observation that anticancer treatments with monoclonal antibodies against the EGF receptor (EGFR) resulted in renal magnesium wasting and hypomagnesaemia (Tejpar et al., 2007). Of note, patients treated with EGFR targeting antibodies (cetuximab, panitumumab) for colorectal cancer usually receive combination therapy with platinum compounds, potentially aggravating the effects on serum magnesium levels. A significant number of patients receiving such a chemotherapeutic regimen show decreasing serum magnesium concentrations over time (Tejpar et al., 2007; Cao et al., 2010). Twenty-four-hour urine collections, as well as magnesium loading tests in single patients, demonstrated a defect in renal magnesium conservation. Together with the genetic findings in isolated recessive hypomagnesaemia due to a pro-EGF mutation, these findings imply a selective effect of EGFR targeting on transcellular magnesium transport in the DCT. There, TRPM6 mediated magnesium uptake into DCT cells is stimulated by basolaterally secreted EGF via its receptor (EGFR) (Groenestege et al., 2007). The initial report of Tejpar and colleagues describes oral as well as intravenous magnesium supplementation in patients with different degrees of hypomagnesaemia after cetuximab treatment, but with limited success in correcting serum magnesium levels (Tejpar et al., 2007). Subsequent studies investigated the development of hypomagnesaemia under EGFR targeting therapy in relation to the antitumour effect and outcome of patients (Vincenzi et al., 2008, 2011). They found that the development of hypomagnesaemia is correlated with the tumour response rate and outcome. Patients with a reduction of serum magnesium > 50% showed a better tumour response rate and a better overall survival (Vincenzi et al., 2011). The authors, therefore, consider serum magnesium as an easily measurable biomarker for efficacy in colorectal cancer patients treated with cetuximab.

Proton-pump inhibitors

Over the last 20 years, proton-pump inhibitors (PPIs) used for the reduction of gastric acidity have emerged as one of the most widely prescribed class of drug worldwide (Cundy and Mackay, 2011). Due to the large number of patients, even rare side effects could become apparent that remain undiscovered during initial clinical trials. Hypomagnesaemia, clinically apparent as muscle cramps, tetany, nausea, vomiting, but also cerebral convulsions, has been observed in a small, but significant number of patients receiving PPIs. It is conceivable that in a substantially larger number of patients, the relationship between low serum magnesium levels and the use of PPIs remains unrecognized. A recent review summarizing the data from previous publications reveals severely lowered serum magnesium levels < 0.4 mmol/L with concomitant hypocalcaemia, a

laboratory constellation reminiscent of HSH due to TRPM6 defects (Cundy and Mackay, 2011). A previous report on hypomagnesaemia following PPI treatment had already described suppressed parathyroid hormone levels during phases of severe hypomagnesaemia as the probable cause of hypocalcaemia (as in HSH) (Epstein et al., 2006). Although a number of patients also receive diuretics, this finding does not explain the extraordinary degree of magnesium deficiency observed in patients receiving PPIs.

What is the mechanism underlying hypomagnesaemia in PPI users? Data regarding urinary magnesium excretions in hypomagnesaemic PPI are contradictory. Fractional magnesium excretions were reported to be low in face of profound hypomagnesaemia, pointing to an intact tubular magnesium reabsorption. However, as observed in HSH patients, a renal magnesium leak might only become apparent if serum magnesium levels reach a certain threshold. An alternative explanation could involve a disturbed intestinal reabsorption of magnesium. Unfortunately, the molecular link between proton pump inhibition and hypomagnesaemia remains unclear so far. In relation to the severe degree of hypomagnesaemia, possible molecular mechanisms include an inhibition of TRPM6, leading to a combined intestinal and renal defect, but also a disturbance of ATPases or ATPase-subunits other than gastric H^+-K^+-ATPase involved in epithelial magnesium transport.

In any case, it is recommended to monitor serum magnesium levels in patients receiving PPIs, particularly those with concomitant cardiac disease and risk for arrhythmia. Moreover, attention should be drawn to the medication list of patients presenting with hypomagnesaemia and secondary hypocalcaemia due to apparent hypoparathyroidism.

Miscellaneous agents

A number of antibiotics, anti-tuberculosis therapies, and antiviral drugs may result in renal magnesium wasting (Shah and Kirschenbaum, 1991; Ahmad and Swaminathan, 2000). The cellular basis of this effect on magnesium reabsorption is still largely unknown. Many are associated with general cytotoxicity. Amphotericin B may lead to acquired distal renal tubular acidosis, which in turn can reduce renal magnesium reabsorption (Ahmad and Swaminathan, 2000). Pamidronate used in the treatment of tumour-associated hypercalcaemia has been reported to cause transient hypomagnesaemia (Elisaf et al., 1998). The cellular mechanisms are difficult to predict, since this drug is used in patients with hypercalcaemia, which may aggravate renal magnesium wasting.

Metabolic acidosis

It has long been known that systemic acidosis is associated with renal magnesium wasting. Acute metabolic acidosis produced by infusion of NH_4Cl or HCl leads to significant increases in urinary magnesium excretion (Quamme, 1997; Ahmad and Swaminathan, 2000). Chronic acidosis also leads to urinary magnesium wasting which, as with the acidosis itself, may be partially corrected by the administration of bicarbonate (Quamme, 1997). In contrast to metabolic acidosis, acute and chronic metabolic alkalosis consistently lead to a fall in urinary magnesium excretion (Quamme, 1997).

The cellular basis for the acid–base effects on magnesium transport appears to be diverse. Di Stefano et al. perfused isolated mouse cTAL segments harvested from mice maintained on alkali in the drinking water (20 mM sodium bicarbonate) for 3 days (Di Stefano et al., 1995). They showed that alkalosis doubled magnesium absorption from control levels without a change in transepithelial voltage. They interpreted these data to indicate that alkalosis changes the permeability of the paracellular pathway, so that magnesium moves passively through the pathway to a greater degree, resulting in more magnesium absorption. The effects of metabolic acidosis were not determined in this study (Di Stefano et al., 1995). Nevertheless, these data suggest that pH influences the paracellular pathway, perhaps through alteration of claudins. Dai et al. have used an mDCT cell line to determine the effects of extracellular pH changes on cellular magnesium uptake (Dai et al., 2001). The results of these experiments showed that acute alkalosis markedly enhances magnesium uptake, whereas acidosis diminishes transport.

Metabolic acidosis of any aetiology would be expected to lead to diminished magnesium reabsorption in the distal tubule. Diabetic subjects frequently present with hypomagnesaemia and cellular magnesium deficiency (Husmann et al., 1997; Khan et al., 1999; Takaya et al., 2003). This is, in part, due to urinary magnesium wasting (Tosiello, 1996). In combination, insulin deficiency and ketoacidosis of uncontrolled diabetes diminish magnesium transport in the TALH (Guerrero-Romero et al., 2002; Barbagallo et al., 2003) and DCT (Dai et al., 2001), resulting in exacerbation of renal magnesium wasting.

Phosphate restriction and phosphate depletion

One of the hallmarks of hypophosphataemia and cellular phosphate depletion is the striking increase in urinary calcium and magnesium excretion (Kelepouris and Agus, 1998; Ahmad and Swaminathan, 2000; Dai et al., 2001). The hypermagnesiuria may be sufficiently large to lead to overt hypomagnesaemia (Coburn and Massry, 1970). The increase in divalent ion excretion in both human disease and experimental animal models occurs within hours of dietary phosphate restriction. Three mechanisms have been proposed to account for the increased renal excretion: (1) mobilization of calcium and magnesium from bone, (2) suppression of PTH secretion, and (3) disturbed tubular transport (Dai et al., 2001).

It is evident from clearance experiments that the urinary excretion of divalent cations in phosphate-depleted human subjects is inappropriate for the plasma concentration, supporting the notion of defective tubular transport (Coburn and Massry, 1970). Dai et al. have shown that cellular phosphate depletion leads to diminished magnesium uptake in mDCT cells (Dai et al., 1997b). This observation supports the notion that the DCT may be involved, in part, in decreased magnesium absorption and increased magnesium excretion associated with hypophosphataemia. The reasons why cellular phosphate depletion leads to diminished magnesium reabsorption are not known.

Cellular potassium depletion

Hypokalaemia and cellular potassium depletion are associated with diminished magnesium absorption within TALH and DCT that may lead to increased magnesium excretion (Quamme, 1997). The increase in urinary excretion of divalent cations may be explained by the effect of potassium depletion on salt absorption in the TALH. Chloride conservation is impaired in potassium-depleted rats, which may be related to a change in basolateral Na^+,K^+-ATPase activity, resulting in impaired apical NKCC2-mediated sodium chloride co-transport.

To date, there is no direct evidence for changes in magnesium absorption in the TALH with potassium depletion. However, as magnesium and calcium are absorbed by passive mechanisms, it is probable that impaired salt transport can lead to diminished divalent cation absorption in this segment.

Studies using isolated mDCT cells suggest that potassium depletion may have additional effects on magnesium transport in the DCT (Dai et al., 1997a). Cellular potassium depletion results in the inhibition of magnesium uptake into mDCT cells, as determined by microfluorescence. The exact mechanism for the disturbed magnesium entry is not known, but might involve a diminished potassium excretion via Kv1.1 (KCNA1), as suggested by the discovery of mutant Kv1.1 in a dominantly inherited form of hypomagnesaemia (see above) (Glaudemans et al., 2009). The authors propose a model in which Kv1.1 activity is critical for the establishment and maintenance of the DCT cell membrane potential as a prerequisite for magnesium entry via TRPM6. Further studies are required to fully explain the defective magnesium transport in the DCT associated with cellular potassium depletion.

Experimental and clinical data suggest a close association of serum magnesium, potassium, and phosphate levels. Crook et al. reported a twofold increase in the prevalence of hypophosphataemia (plasma phosphate < 0.8 mmol/L) and a sixfold increase in hypokalaemia (plasma potassium < 3.5 mmol/L) in patients with hypomagnesaemia (plasma magnesium < 0.70 mmol/L) (Crook, 1994). A trilogy consisting of hypomagnesaemia, hypophosphataemia, and hypokalaemia was also found in 8% of patients with hypomagnesaemia and 17% of patients with severe hypomagnesaemia (plasma magnesium < 0.50 mmol/L). The evidence suggests that hypokalaemia and hypophosphataemia may have profound effects on tubular magnesium transport. Many of the syndromes associated with potassium depletion and phosphate depletion are complicated by concurrent alterations in acid–base balance (Crook, 1994). There is evidence that acid–base changes have different effects on magnesium transport relative to potassium or phosphate depletion, such that the three disturbances may act in an additive manner to compromise renal magnesium conservation (Dai et al., 2001).

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CHAPTER 41

Approach to the patient with renal Fanconi syndrome, glycosuria, or aminoaciduria

Detlef Bockenhauer and Robert Kleta

Introduction

The proximal tubule reabsorbs the bulk of the glomerular filtrate. Up to 80% of filtered salt and water is returned back into the circulation in this tubular segment (Gottschalk, 1961; Lassiter et al., 1961; Bockenhauer et al., 2008). Moreover, several solutes, such as phosphate, glucose, low-molecular weight proteins (LWMPs), and amino acids are exclusively reabsorbed in the proximal tubule. An entire orchestra of specialized apical and basolateral transporters, as well as paracellular molecules, mediate this reabsorption. Defects in proximal tubular function can be isolated (e.g. isolated renal glycosuria, aminoacidurias, or hypophosphataemic rickets) or generalized. In the latter case it is called the Fanconi–Debre–de Toni syndrome, based on the initial clinical descriptions (Fanconi, 1931, 1936, de Toni, 1933; Debre et al., 1934). However, in clinical practice it is usually referred to as just the 'renal Fanconi syndrome'. Severity of proximal tubular dysfunction can vary: a complete loss of function with intact glomerular filtration rate (GFR) and no compensation elsewhere is probably not compatible with life, since it would result in a urine output of > 100 L per day, assuming a daily filtrate volume of 150 L.

Clinical manifestations of renal Fanconi syndrome

The clinical presentation of renal Fanconi syndrome depends on the underlying cause, severity, and age at presentation. Onset of symptoms is usually insidious and non-specific. Polyuria/polydipsia is typically present. Not uncommonly the diagnosis is incidental, prompted by blood or urine tests obtained for another indication. Some patients may experience bone pain and metabolic bone disease. In children, rickets is a common presenting symptom, as originally described by Fanconi, Debre, and de Toni (Fanconi, 1931, 1936, de Toni, 1933; Debre et al., 1934). Several factors contribute to the development of rickets: (a) the renal phosphate losses with secondary hypophosphataemia and (b) renal calcium losses, and (c) deficiency of 1,25(OH) vitamin D. Vitamin D is fat-soluble and circulates in the plasma by means of a carrier protein, vitamin D binding protein (DBP). DBP is a LMWP and the complex of DBP and vitamin D is filtered and then reabsorbed in the proximal tubule (Negri, 2006). Following uptake in the epithelial cell of the proximal tubule, vitamin D can be converted into its most active form by 1α-hydroxylation. Consequently, there is a deficiency of 1α-hydroxylated vitamin D with impaired proximal tubular function.

Biochemical abnormalities

Once suspected, the diagnosis of renal Fanconi syndrome is easily confirmed. Since many solutes, including LMWP, phosphate, amino acids, and glucose are exclusively reabsorbed in the proximal tubule, wasting all these substances in the urine clearly establishes the diagnosis. In addition, the biochemical profile includes a hypokalaemic, hyperchloraemic metabolic acidosis with hypophosphataemia and hypercalciuria. Markers of disturbed calcium/phosphate homeostasis, such as parathyroid hormone (PTH) and alkaline phosphatase are typically elevated. Moreover, plasma levels of vitamin D, especially 1,25-OH vitamin D are depressed (see above).

Proteinuria

Proteinuria consists mainly of LMWP, that is, those proteins that are physiologically filtered and need to be reabsorbed in the proximal tubule via the endocytic receptors megalin and cubilin (Christensen and Gburek, 2004). Under physiologic circumstances > 99% of filtered proteins are reabsorbed this way. Determination of LMWP, for example, by measurement of urinary α1-microglobulin, β2-microglobulin, or retinol-binding protein (RBP) in relation to urine creatinine, is an exquisitely sensitive marker of proximal tubular function. Median RBP to creatinine ratios were shown to be more than 1000-fold higher in patients with proximal tubular disorders, such as Dent disease compared with normal controls (Norden et al., 2000). Of note, LMWP proteinuria is typically missed or significantly underestimated by traditional urine dipstick testing. While albumin is usually considered a marker of glomerular function, it is important to remember that some albumin is also filtered and reabsorbed (Birn et al., 2000; Hryciw et al., 2006; Comper et al., 2008). The magnitude of physiologic albumin filtration is debated, but some animal experiments suggest it may actually be in the nephrotic range (Gekle, 2007; Pollock and Poronnik, 2007; Russo et al., 2007). Therefore, it is not surprising that albuminuria is also a typical feature of renal Fanconi syndrome, although plasma levels

of albumin are usually normal (Norden et al., 2001). In an inherited form of renal Fanconi syndrome without glomerular failure, total proteinuria was reported to be approximately 850 mg/day/m^2 (Tolaymat et al., 1992). Moreover, two siblings with a homozygous frameshift mutation in *CUBN*, encoding a key endocytic receptor in the proximal tubule, Cubulin, were recently described who had proteinuria up to 2 g per day (Ovunc et al., 2011).

Aminoaciduria

Amino acids, like LMWP, are exclusively reabsorbed in the proximal tubule. Approximately 50 g (450 mmol) of free amino acids are filtered daily into the primary urine by human kidney glomeruli and > 99% are reabsorbed under physiologic conditions (Camargo et al., 2012). Distinct transporters are expressed on the apical and basolateral side of the proximal tubular epithelium to facilitate transcellular amino acid transport (Camargo et al., 2008). Consequently, generalized aminoaciduria is a typical feature of renal Fanconi syndrome, although usually not as dramatically increased as the LMWP (Norden et al., 2004).

Organic aciduria

Organic acids are also exclusively reabsorbed in the proximal tubule and excretion is increased in renal Fanconi syndrome (Cogan, 1982); transport is mediated by specialized transport molecules, including the urate transporter SLC22A12 and SLC2A9, involved in renal hypouricaemia (Enomoto et al., 2002; Matsuo et al., 2008; Vitart et al., 2008). Consequently, patients with renal Fanconi syndrome typically have hyperuricosuria with hypouricaemia. Other organic acid transporters include the sodium dicarboxylate transporter NaDC1, involved in citrate reabsorption, leading to hypercitraturia with impaired proximal tubular function (Unwin et al., 2004). Several of these transporters are also involved in renal handling of drugs, such as probenecid, furosemide, or penicillin, potentially altering pharmacokinetics in renal Fanconi syndrome (Roch-Ramel, 1998). The increased excretion of lactate may have diagnostic implications in mitochondrial cytopathies: these are typically characterized by elevated plasma lactate levels. However, in those with associated renal Fanconi syndrome (see 'Pathogenesis'), the leakage of lactate in the urine can result in misleading normal plasma levels (Niaudet and Rotig, 1996).

Glycosuria

Under physiologic conditions, virtually all filtered glucose is reabsorbed in the proximal tubule. Thus, glycosuria is another marker of proximal tubular dysfunction. Glycosuria can be isolated (see 'Isolated renal glycosuria'), or seen in the context of renal Fanconi syndrome. Obviously, a concomitant blood glucose level is necessary to determine whether the glycosuria is secondary to an increased filtered load (hyperglycaemia) or impaired tubular reabsorption (normoglycaemia). It is important to remember, that dipsticks only pick up a urine glucose concentration > 5 mmol/L. Hence, glycosuria, especially in the context of polyuria with dilute urine, can be missed. A formal biochemical measurement is preferable and a 24-hour urine collection for glucose contents the gold standard. Normally, < 1.5 mmol (300 mg)/day/1.73 m^2 are excreted (Elsas and Rosenberg, 1969). Assuming a blood glucose level of 5 mmol/L and a GFR of 100 mL/min/1.73 m^2 means that under physiologic conditions > 99.5% of filtered glucose is reabsorbed.

Phosphaturia

Renal phosphate wasting with secondary hypophosphataemia is another hallmark of proximal tubule dysfunction with consequent clinical signs and symptoms, that is, rickets or bone disease. This can be isolated, as in hypophosphataemic rickets or in the context of generalized proximal tubule dysfunction, such as in renal Fanconi syndrome.

By convention and tradition, urine phosphate handling is usually assessed as the tubular reabsorption (TRP), which is the complement to the fractional excretion of phosphate (FEP). It is calculated as: TRP (%) = 100 – FEP (%). If 10% of filtered phosphate is excreted (FEP 10%), then 90% of filtered phosphate must have been reabsorbed (TRP 90%). By definition, a TRP > 70% is considered normal, however this can be misleading: after a large phosphate load, a TRP < 70% may be physiologic. Conversely, with severe hypophosphataemia and a decreased filtered load, a TRP of > 70% may be pathologic. To account for the filtered load, urinary phosphate excretion is best assessed using the tubular threshold concentration for phosphate excretion, corrected for glomerular filtration rate (TmP/GFR) (Walton and Bijvoet, 1975). It is calculated as follows:

$$\text{TmP/GFR} = \text{Phosphate plasma} - \text{Phosphate urine} / \text{Creatinine urine} \times \text{Creatinine plasma}$$

Normal values are age dependent and listed in Table 41.1.

Bicarbonaturia and metabolic acidosis

Under physiologic conditions the vast majority of filtered bicarbonate is reabsorbed in the proximal tubule, mainly facilitated via the sodium-hydrogen exchanger NHE3 and by carbonic anhydrase CAII (Aronson, 2002; Bobulescu and Moe, 2009). Consequently, bicarbonaturia, and proximal renal tubular acidosis (type 2 renal tubular acidosis), is another obligatory symptom in renal Fanconi syndrome (Rothstein et al., 1990). However, in clinical practice urinary bicarbonate is rarely measured and even if it is, in steady state it may be normal: the functionally impaired proximal tubule has a decreased bicarbonate threshold and the initial bicarbonate wasting leads to a reduction in plasma bicarbonate levels until the filtered load matches the bicarbonate threshold.

Table 41.1 Normal age-specific values for TmP/GFR

Age	mmol/L
< 1 month	1.48–3.43
1–3 months	1.48–3.30
4–6 months	1.15–2.60
7 months–2 years	1.10–2.70
2–4 years	1.04–2.79
4–6 years	1.05–2.60
6–8 years	1.26–2.35
8–10 years	1.10–2.31
10–12 years	1.15–2.58
12–15 years	1.18–2.09
> 15 years	0.80–1.35

Derived from Kruse et al. (1982), Bistarakis et al. (1986), and Shaw et al. (1990).

Hypercalciuria

Micropuncture studies have shown that approximately 70% of filtered calcium is reabsorbed in the proximal tubule (Hoenderop et al., 2005). The molecular pathway remains to be elucidated, but absorption is presumed to be passive and in parallel with sodium and water reabsorption (Suki, 1979). Consequently, impaired proximal tubular reabsorption of sodium and water also affects calcium transport, and hypercalciuria is another characteristic of renal Fanconi syndrome. Nephrocalcinosis and stone formation can ensue, but interestingly are rather uncommon. Presumably, the polyuria inherent in Fanconi syndrome is protective against these complications. Moreover, the impaired reabsorption and therefore increased luminal concentration of citrate (see 'Organic aciduria') is also protective (Pajor, 1999). Nephrocalcinosis/lithiasis is seen more commonly with Dent disease and Lowe syndrome (Thakker, 2000; Bockenhauer et al., 2008; Bokenkamp et al., 2009; Claverie-Martin et al., 2011). The reason for the preponderance to stone formation in these disorders is unclear, but argues for a special role of the underlying molecules, CLCN5 and OCRL, respectively, for calcium transport.

Hypokalaemia

Hypokalaemia due to renal potassium wasting is another typical feature of renal Fanconi syndrome. There are two potential mechanisms that contribute to potassium wasting: (a) decreased proximal tubular reabsorption. Like most ions, potassium is predominantly reabsorbed in the proximal tubule and impaired proximal tubular function will increase potassium delivery to the distal tubule (Wright and Giebisch, 1978). (b) Aldosterone–mediated distal potassium secretion. The volume depletion occurring as a result of impaired proximal sodium reabsorption can lead to activation of the renin–angiotensin–aldosterone system and potassium secretion in the collecting duct (Sebastian et al., 1971).

Pathogenesis

The aetiology of renal Fanconi syndrome varies with the age of onset, severity of symptoms, and exposure to certain toxins or medications (see Table 41.2 and Table 41.3). The variety of causes suggests that multiple pathways can disturb proximal tubular function, yet they can be grouped according to some common mechanisms. However, the mechanism of several causes of renal Fanconi syndrome remains to be elucidated, including those associated with valproic acid (Lande et al., 1993; Zaki and Springate, 2002) and aristolochic acid (Chinese herb nephropathy) (Yang et al., 2002; Kazama et al., 2004; Lee et al., 2004; Hong et al., 2006).

Disruption of cellular energy production

This appears to be the key mechanism leading to generalized proximal tubular dysfunction. Consistent with this notion, renal Fanconi syndrome is often associated with mitochondrial cytopathies, including defined phenotypes, such as Pearson, Kearns–Sayre, Leigh, and MELAS syndromes (Van Biervliet et al., 1977; Sperl et al., 1988; Superti-Furga et al., 1993; Wendel et al., 1995; Niaudet and Rotig, 1996; Mourmans et al., 1997; Kuwertz-Broking, 2000; Neiberger et al., 2002; Rotig, 2003). Several acquired forms probably also act by perturbing mitochondrial function. One of the most common causes of renal Fanconi syndrome in adults nowadays is exposure to antiretroviral medications, especially tenofovir (Verhelst et al., 2002; Earle et al., 2004; Malik et al., 2005; Hussain et al., 2006; Izzedine et al., 2006; Woodward et al., 2009; Hall et al., 2011). In one study, 1.5% of patients exposed to this drug developed renal Fanconi syndrome associated with marked ultrastructural changes of the mitochondria (Woodward et al., 2009). Potentially, genetic variations in genes encoding transporters such as MRP2 (ABCC2) affect intracellular concentrations of tenofovir, where it may affect mitochondrial DNA repair/synthesis (Izzedine et al., 2006). Aminoglycosides, especially gentamicin, have also been associated with renal Fanconi syndrome (Melnick et al., 1994; Gainza et al., 1997; Alexandridis et al., 2003; Ghiculescu and Kubler, 2006). Aminoglycosides bind to bacterial ribosomes, reducing fidelity of transcription, leading to errors in bacterial protein synthesis (Spahn and Prescott, 1996). The bacterial ancestry of mitochondria makes them particularly susceptible to aminoglycoside toxicity (Bockenhauer et al., 2009). Renal Fanconi syndrome associated with heavy metal intoxication (Thevenod, 2003; Barbier et al., 2005; Gonick, 2008; Johri et al., 2010; Sirac et al., 2011), paraquat (Gil et al., 2005) and suramin (Rago et al., 1974) is presumably also due to mitochondrial dysfunction.

An interesting aspect of the cellular energy production pathway as a cause of renal Fanconi syndrome was provided by the recent discovery of proximal tubular dysfunction associated with bi-allelic mutations in *SLC34A1*, encoding the renal phosphate transporter NaPi-IIa (Magen et al., 2010). It has been speculated that the intracellular deficiency of phosphate may impair proximal tubular ATP generation. However, further clinical details from more affected individuals are needed to convincingly demonstrate generalized proximal tubular dysfunction. Moreover, it is unclear, why renal Fanconi syndrome does not develop in other phosphaturic disorders, such as hypophosphataemic rickets.

Impaired endocytosis and intracellular trafficking

Impaired proximal tubular endocytosis is not only a key symptom of renal Fanconi syndrome, but may also contribute to its development. At least, this is suggested by two other genetic forms of partial proximal tubular dysfunction, Dent disease (Devuyst et al., 2005; Wang et al., 2005; Guggino, 2007) and Lowe syndrome (Erdmann et al., 2007; Ooms et al., 2009; Cui et al., 2010). Dent disease can be caused by mutations in either CLCN5, a chloride/proton antiporter (also called Dent 1 disease) or OCRL, an inositol polyphosphate-5-phosphatase (Dent 2 disease) (Lloyd et al., 1996; Hoopes et al., 2005). Interestingly, OCRL was initially identified as the molecular basis of the oculo-cerebral-renal syndrome of Lowe (Attree et al., 1992). It is still unclear why some patients with OCRL mutations appear to have an isolated renal problem (Dent 2), whereas others have the full-blown Lowe syndrome. Careful clinical studies, however, reveal a spectrum of severity with OCRL mutations without a clear distinction between the two diagnostic categories. There is evidence of extrarenal manifestations also in some Dent 2 patients, including elevated muscle enzymes, mild to moderate mental impairment, and stunted growth (Utsch et al., 2006; Bokenkamp et al., 2009).

Both of these proteins are involved in proximal tubular endocytosis and recently it was shown that CLCN5 is involved not only in endocytosis, but also in exocytic trafficking of proximal tubular transporters (Lin et al., 2011). Similarly, OCRL appears to play an important role in the regulation of membrane trafficking (Erdmann et al., 2007; Mao et al., 2009; Cui et al., 2010). However,

Table 41.2 Aetiology of renal Fanconi syndromes and associated clinical features by age of onset

Age at onset	Disorder (OMIM #)	Gene (inheritance)	Associated features	Biochemical/diagnostic test
Neonatal	Galactosaemia (230400)	*GALT* (AR)	Liver dysfunction, jaundice, sepsis, encephalopathy	Red cell galactose 1-phosphate; enzyme tests
	Mitochondrial disorders	Multiple (AR or mitochondrial)	Usually multisystem dysfunction (brain, muscle, liver, heart, kidney)	Plasma/cerebrospinal fluid: lactate/pyruvate (may be normal due to urinary losses), muscle enzymology
	Tyrosinaemia type 1 (276700)	*FAH* (AR)	FTT, hepatic enlargement and dysfunction	Plasma amino acids, urine organic acids (succinyl acetone)
	ARC syndrome (208085)	*VPS33B* (AR)	Arthrogryposis, cholestasis, giant platelets, FTT	Clinical picture, platelet morphology
Infancy	Fructosaemia (229600)	*ALDOB* (AR)	Rapid onset of vomiting after fructose ingestion, hypoglycaemia, hepatomegaly	Hepatic fructose-1-phosphate aldolase B activity (liver biopsy)
	Cystinosis (219800)	*CTNS* (AR)	FTT, vomiting, corneal cystine crystals (may be absent, if age < 18 months)	Leucocyte cystine concentration
	Fanconi–Bickel syndrome (227810)	*SLC2A2* (AR)	FTT, hepatomegaly, hypoglycaemia, glycosuria, galactosuria	Molecular, glycogen storage (liver biopsy)
	Lowe syndrome (309000)	*OCRL* (X-linked)	Cataracts, hypotonia, developmental delay	Molecular
Childhood	Dent disease (300009)	*CLCN5* (X-linked)	Nephrocalcinosis, Phosphate and bicarbonate wasting often absent	Molecular
	Wilson disease (277900)	*ATP7B* (AR)	Hepatic and neurological disease, Kayser–Fleischer rings	Plasma copper, coeruloplasmin, liver biopsy
	Isolated incomplete renal Fanconi syndrome (613388)	*SLC34A1* (AR)		
	Isolated renal Fanconi syndrome with kidney failure (134600)	? (AD)	Rickets, glomerular kidney failure during adolescence	
	Isolated renal Fanconi syndrome without kidney failure (615605)	*EHHADH* (AD)	Rickets	

AR = autosomal recessive; AD = autosomal dominant; FTT = failure to thrive.

even though Dent disease and Lowe syndrome are typically cited as causes of renal Fanconi syndrome, it is debatable whether they always do cause a generalized proximal tubular dysfunction (Kleta, 2008). In a detailed clinical assessment of 16 patients with Lowe syndrome, we showed that there was a spectrum of severity with respect to renal involvement: while all had hypercalciuria and LMWP, and almost all aminoaciduria, the majority had no clear evidence of phosphate or bicarbonate wasting and none had appreciable glycosuria (Bockenhauer et al., 2008). The clinical features in Dent disease and proven CLCN5 mutations can be even more restricted: while they all have tubular proteinuria, some may have no other recognisable tubular abnormality, not even hypercalciuria, which was considered an invariant feature of this disease (Ludwig et al., 2006). Therefore, if renal Fanconi syndrome is defined exclusively as a generalized proximal tubular dysfunction affecting *all* transport pathways, Dent disease and Lowe syndrome do not strictly or consistently fulfil this diagnostic criterion.

Unspecified renal Fanconi syndromes

Renal Fanconi syndrome can also occur with non-specific damage to the proximal tubule, for instance from ischaemia (Ashworth and Molitoris, 1999). The high-transport activity in this tubular segment makes it particularly susceptible to damage from deprivation of metabolic fuel and oxygen. Renal Fanconi syndrome can also occur in monoclonal gammopathies (Kleta et al., 2004), thought to be a result of tubular obstruction from aggregated light chains (myeloma casts) and/or intracellular crystal formation due to incomplete lysosomal digestion of specific light chains (Lajoie et al., 2000; Messiaen et al., 2000; Kobayashi et al., 2006; Vanmassenhove et al., 2010). Similarly, the development of renal Fanconi syndrome in Fanconi–Bickel syndrome (systemic glycogen storage disorder due to autosomal recessive GLUT2—a passive basolateral glucose transporter—deficiency) is not understood.

Table 41.3 Acquired causes of the renal Fanconi syndrome

Anti-cancer drugs
Ifosfamide (Burk et al., 1990; Pratt et al., 1991; Rossi et al., 1992, 1999a, 1999b; Rossi and Ehrich, 1993; Negro et al., 1998; Ciarimboli et al., 2011)
Streptozocin (Sadoff, 1970; Kintzel, 2001)
Antibiotics
Aminoglycoside (Melnick et al., 1994; Gainza et al., 1997; Alexandridis et al., 2003; Ghiculescu and Kubler, 2006; Zietse et al., 2009)
Expired tetracyclines (Cleveland et al., 1965; Guggenbichler and Schabel, 1979; Montoliu et al., 1981; Zietse et al., 2009)
Antiretrovirals
Adefovir/cidofovir/tenofovir (Vittecoq et al., 1997; Verhelst et al., 2002; Malik et al., 2005; Hussain et al., 2006; Izzedine et al., 2006; Woodward et al., 2009; Jhaveri et al., 2010; Girgis et al., 2011; Hall et al., 2011)
Dideoxyinosine (Crowther et al., 1993; Izzedine et al., 2005)
Heavy metals
Lead poisoning (Chisolm, 1968; Barbier et al., 2005)
Cadmium (Kazantzis et al., 1963; Thevenod, 2003; Barbier et al., 2005; Rago et al., 2010)
Others
Sodium valproate (Lande et al., 1993; Zaki and Springate, 2002)
Aristolochic acid (Chinese herb nephropathy) (Yang et al., 2002; Lee et al., 2004; Hong et al., 2006)
Toluene/glue sniffing (Streicher et al., 1981)
Fumaric acid (Fliegner and Spiegel, 1992; Raschka and Koch, 1999; Haring et al., 2011)
Suramin (Rago et al., 1994)
Paraquat (Vaziri et al., 1979; Gil et al., 2005)
L-Lysine (Lo et al., 1996)

Treatment

Treatment of the underlying cause

Treatment of renal Fanconi syndrome should be aimed primarily at the underlying problem. Potentially causative toxins, such as heavy metals or aristolochic acid need to be identified and stopped. Potentially causative medications, such as aminoglycosides, valproate or tenofovir should be weaned off in close collaboration with the prescribing specialities and replaced by alternative drugs. However, for most of the genetic forms of renal Fanconi syndrome, there is no specific treatment available, with the notable exception of cystinosis (specific treatment with cysteamine) (Kleta and Gahl, 2004; Kleta et al., 2005), and potentially those mitochondrial cytopathies that respond to treatment with ubiquinone (Montini et al., 2008).

Supportive treatment

In those patients awaiting normalization of proximal tubular function after removal of the underlying cause and in those forms of renal Fanconi syndrome without specific treatment, supportive treatment is needed. This includes treatment of the metabolic acidosis with sodium and/or potassium bicarbonate. Often this is prescribed in the form of citrate: each Mol of citrate is converted by the liver into 2 Mol of bicarbonate and it is thought that in addition to correcting the acidosis it may decrease the risk of nephrolithiasis by increasing urinary citrate (Nicar et al., 1984). Dosage is titrated to normalize plasma bicarbonate levels. Supplementation with potassium chloride in addition to potassium bicarbonate or citrate may be necessary to normalize plasma potassium levels. Sodium chloride may be needed in those patients with evidence of volume depletion. Phosphate supplementation is given to increase plasma phosphate levels, although normalization may not always be achievable, as dosage is limited by gastrointestinal side effects, especially diarrhoea, which may actually worsen the salt and fluid losses.

A key complication of renal Fanconi syndrome is rickets (see above) and besides phosphate supplementation, the primary treatment is 1α-hydroxylated vitamin D. This can be in the form of 1,25(calcitriol) or 1-hydroxylated (1α-calcidol) vitamin D, although the latter is often preferred due to the longer half-life. Dosage should be titrated to achieve normalization of plasma calcium, PTH, and alkaline phosphatase.

Constipation is a common complication, especially in younger children and a consequence of the renal fluid losses and should be treated by adequate fluid supplementation and laxatives, if needed.

Isolated glycosuria

Isolated renal glycosuria is defined as renal glucose excretion above the normal range, that is, > 1.5 mmol/1.73 m^2 per day (Elsas and Rosenberg, 1969), in the absence of other proximal tubular defects, pregnancy, or hyperglycaemia. Therefore, isolated renal glycosuria is caused by a specific defect in renal glucose transport. Glucose reabsorption in the proximal tubule is mediated by three key transporters: on the apical side are expressed at least two secondary active sodium-glucose cotransporters: the high-affinity, low-capacity transporter SGLT1 (SLC5A1) and the low-affinity, high-capacity transporter SGLT2 (SLC5A2). Transport on the basolateral side is facilitated by GLUT2 (SLC2A2) (Brown, 2003). GLUT2 is also expressed in the liver and recessive mutations in GLUT2 cause a glycogen storage disorder called Fanconi–Bickel syndrome (Santer et al., 1997). SGLT1 is also expressed in enterocytes and recessive mutations in this transporter cause glucose/galactose malabsorption (Turk et al., 1991). In contrast, SGLT2 is functionally expressed exclusively in the proximal tubule and mutations in this transporter are the basis of isolated renal glycosuria (van den Heuvel et al., 2002; Santer et al., 2003; Calado et al., 2004, 2006, 2008; Francis et al., 2004; Kleta et al., 2004). Inheritance can be dominant or recessive and there is some genotype/phenotype correlation: patients with only one mutant allele typically have milder (< 10 g/day/1.73 m^2) or absent glycosuria, whereas those with two mutant alleles usually excrete more (Santer and Calado, 2010). Despite the definition of isolated renal glycosuria, other proximal tubular abnormalities can sometimes be seen in patients with defined SGLT2 mutations: some have accompanying hypercalciuria (Schneider et al., 1992; Scholl-Burgi et al., 2004) and others aminoaciduria (Gotzsche, 1977; Sankarasubbaiyan et al., 2001). The reasons for this are not clear. However, since calcium reabsorption in the proximal tubule is passive and parallels sodium reabsorption, it can be speculated that the decreased sodium-glucose transport also impairs calcium re-uptake. Regarding the aminoaciduria,

it has been hypothesized that glycosuria causes dissipation of the electric gradient of sodium-dependent amino acid transporters (Santer and Calado, 2010). While there may be some complications of isolated glycosuria, such as an increased incidence of urinary tract infections (De Marchi et al., 1983; De Paoli et al., 1984), episodes of dehydration/volume depletion (Calado et al., 2008), and ketosis during starvation (Oemar et al., 1987), it is usually considered a benign condition and not a disease and so no treatment is needed. Indeed, currently, with obesity and hypertension being some of the most serious threats to public health, renal sodium and glucose losses may be beneficial (109). Pharmacological blockade of SGLT2 is now a treatment option for type 2 diabetes (Oku et al., 1999; Santer and Calado, 2010). The role of another gene linked to glucose transport in the kidney, *SLC16A12*, has not been investigated yet (Kloeckener-Gruissem et al., 2008).

Aminoacidurias

Virtually all filtered amino acids are reabsorbed in the proximal tubule by specialized transporters. The modern classification (solute carriers SLC) is based on the molecular identity of these transporters; prior to this a complicated system based on functional properties was used with letters and symbols indicating amino acid specificity, chemical properties of transported amino acids (acidic, neutral, basic) and whether transport was sodium-dependent or not (Camargo et al., 2012). For instance, the transporter involved in Hartnup disorder, now called SLC6A19, was previously called B(0) AT1, with the first letter indicating broad amino acid specificity the capital B indicating that transport was sodium-dependent, and the 0 indicating that it was selective for neutral amino acids (Kleta et al., 2004). The functional information contained in these names actually gives diagnostic clues, since aminoacidurias are characterized by the loss of specific amino acids in the urine (see below). Some transporters consist of two subunits, so-called heterodimeric amino acid transporters. For instance, the transporter involved in cystinuria consists of two subunits, now called SLC7A9 and SLC3A1 (Chillaron et al., 2010). SLC7A9, previously called b(0,+) AT, constitutes the actual transporter subunit with broad amino acid specificity (b), sodium-independence (lower case b) and selectivity for neutral (0) and basic (+) amino acids. SLC3A1 encodes a subunit necessary for proper trafficking of the transporter and is called rBAT for 'related to BAT' (Camargo et al., 2008).

Cystinuria (OMIM #220100)

Cystinuria is characterized by an excessive excretion of the dibasic amino acids cystine, lysine, and ornithine. The key manifestation of cystinuria is urolithiasis and specific clinical and molecular features of cystinuria are described in Chapter 203.

Lysinuric protein intolerance (OMIM #222700)

Lysinuric protein intolerance is a rare disorder with severe though non-specific clinical manifestations. The diagnosis is prompted by the recognition of excess urinary excretion of lysine, arginine and ornithine and the urinary amino acid findings are similar to cystinuria. However, in contrast to cystinuria, urinary cystine is only mildly elevated and urolithiasis is not a recognized feature of lysinuric protein intolerance (Sebastio et al., 2011). Whereas the transporter underlying cystinuria mediates apical uptake of dibasic amino acids, it is the basolateral export of these amino acids that is impaired in lysinuric protein intolerance. The underlying basolateral transporter is also heteromeric and consists of two subunits, SLC7A7 (y+-LAT1), the actual catalytic unit, and SLC3A2, as the associated heavy chain (Verrey et al., 2000). So far, only mutations in SLC7A7 have been identified in Lysinuric protein intolerance (Borsani et al., 1999; Torrents et al., 1999; Sebastio et al., 2011). This transporter is also expressed in enterocytes, leading to not only renal losses of dibasic amino acids, but also to impaired intestinal uptake (Sebastio et al., 2011). It is also expressed in the lungs, potentially explaining the pulmonary complications of the disease (see below) (Rotoli et al., 2005). Affected patients typically come to medical attention during infancy with non-specific symptoms, such as failure to thrive and developmental delay. Breastfeeding and infant formulas delay the onset, probably due to their low protein content, and so symptoms become apparent with the introduction of solids. Patients usually experience vomiting and diarrhoea after ingesting protein (hence the name). Later, symptoms as diverse as hepato-splenomegaly, interstitial lung disease with alveolar proteinosis, osteopenia, and chronic kidney disease and bone marrow abnormalities can develop (Parenti et al., 1995). The pathogenesis of these complications is thought to relate to unbalanced metabolism of specific amino acids, especially arginine (Palacin et al., 2004). This amino acid is involved in the urea cycle, potentially explaining the hyperammonaemia. However, ammonia is only raised directly after protein-rich meals, so that timing of diagnostic blood draws is critical. Arginine is also a precursor for nitric oxide (NO) and the manifold functions of NO may explain some of the divergent symptoms of Lysinuric protein intolerance (Sebastio et al., 2011).

Hartnup disorder (OMIM #234500)

Hartnup disorder is named after the family in which this defect of neutral amino acid transport was first described in London, United Kingdom (Baron et al., 1956). Interestingly, affected patients nowadays are typically asymptomatic and it was only the protein-restricted diet after World War II that precipitated the pellagra-like skin rash, cerebellar ataxia and psychosis-like symptoms that characterize this disorder and prompted its recognition. Hartnup disorder is caused by mutations in the transporter for neutral amino acids SLC6A19 (see above), which is expressed on the apical side of proximal tubular epithelial cells (Kleta et al., 2004). Biochemically, the disorder is characterized by excess excretion of neutral amino acids. Proline and glycine excretions are not increased. Plasma amino acid levels are usually in the normal range. Symptoms are thought to relate to a deficiency in tryptophan, which only manifests with a protein-restricted diet. Tryptophan is a precursor for niacin (pellagra-like rash) and serotonin (neurological symptoms). Consequently, treatment consists of a protein-rich diet. The skin rash can also be alleviated by niacin supplementation.

Iminoglycinuria (OMIM #242600)

Iminoglycinuria is not linked to clinical manifestations, apart from an excess urinary excretion of glycine and the imino acids proline and hydroxyproline. Iminoglycinuria had previously been linked to mental retardation, deafness and visual impairment, but this probably just reflected an ascertainment bias, as the respective patient populations were screened by urinary amino acid determinations (Fraser et al., 1968; Rosenberg et al., 1968). The incidence of iminoglycinuria is estimated to be 1:10,000. Clinical heterogeneity of this disorder was suggested by the additional presence of an

intestinal uptake defect for imino acids in some patients (Rosenberg et al., 1968).

The molecular basis of iminoglycinuria remains to be definitively resolved. Multiallelic mutations and polymorphisms in four genes, *SLC36A2* (*PAT-2*), *SLC6A20* (*SIT-1*), *SLC6A19* (*B0AT1*), and *SLC6A18* (*B0AT3*) have been suggested (Broer et al., 2008), but this remains controversial (Broer et al., 2012).

Dicarboxylic aminoaciduria (OMIM 222730)

Dicarboxylic aminoaciduria refers to the excess excretion of the dicarboxylic amino acids aspartate and glutamate in the urine. It is an autosomal recessive condition caused by mutations in SLC1A1 (EAAC1/EAAT3) (Bailey et al., 2011). It has an estimated incidence of 1:35,000 (Auray-Blais et al., 2007). Like iminoglycinuria, it has been associated with intellectual impairment, but this may again reflect an association bias (Swarna et al., 1989, 2004), as dicarboxylic aminoaciduria has also been described in otherwise asymptomatic individuals (Melancon et al., 1977).

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