CHAPTER

5 Tubular Sodium Transport

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Na^+, the primary extracellular cation, is of critical importance to the maintenance of extracellular fluid volume. The kidneys play the dominant role in regulating Na^+ excretion. Each day, the glomeruli filter roughly 25,000 mEq of Na^+. From this quantity, almost 10 times the total exchangeable Na^+ in the body, the kidneys typically absorb over 99%. A remarkable feature of the Na^+ absorptive process is the precision with which it is regulated. An individual consuming a typical diet containing 6 g of Na^+ will excrete 260 mEq of Na^+ per day. The same individual placed on a 2-g Na^+-restricted diet will promptly reduce Na^+ excretion to 87 mEq per day. Although the fraction of filtered Na^+ absorbed by the kidney changes from 99.0% on a standard diet to 99.6% on a Na^+-restricted diet, this small change represents the addition or removal of over 1 L per day to extracellular fluid volume. Thus, the kidneys absorb large amounts of filtered Na^+ with remarkably precise control.

The exquisitely sensitive regulation of Na^+ absorption by the kidneys relies on sequential actions of the various nephron segments, each with highly specialized transport capabilities. Figure 5.1 provides an overview of Na^+ transport along the nephron. In general, the absolute rates of Na^+ reabsorption are greatest in the proximal tubule and fall as the tubular fluid proceeds from proximal to distal segments. Conversely, the ability to transport Na^+ against steep tubular fluid to blood gradients and its physiologic control increase along the nephron. For example, the proximal tubule reabsorbs the bulk (60% to 70%) of the filtered Na^+ load, but as will be detailed later, does so against at most small electrochemical gradients. Moreover, the ability to alter Na^+ transport in the proximal tubule, in relative terms, is rather limited, usually varying by less than 25%. The collecting duct, in contrast, reabsorbs only a minor fraction ($\sim$2% to 4%) of the filtered Na^+ load. However, the collecting duct can transport Na^+ against a large electrochemical gradient to produce urine, which is almost Na^+ free ($<$10 mEq/L). In addition, the rate of Na^+ transport in the collecting duct can vary over a wide range (tenfold) in response to physiologic stimuli. The different nephron segments thus permit both high rates of Na^+ transport (proximal segments) and highly regulated Na^+ transport (distal segments).

Substantial progress has been made recently in identifying the proteins that mediate Na^+ transport in each nephron segment and in defining their interactions and regulation within each segment. Many Na^+ transport proteins have been linked to specific genetic disorders (Table 5.1). Given the primacy of renal Na^+ transport to the control of extracellular fluid volume, it is not surprising that the majority of these genetic disorders are characterized by either hypotension or hypertension. An updated and curated database of these genetic disorders is maintained at the Online Mendelian Inheritance in Man website (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM).

This chapter considers the transepithelial transport of Na^+ by the various nephron segments. The discussion of each nephron segment begins with a description of the general features of Na^+ transport in that segment along with pertinent structure–function relations. The mechanism of Na^+ transport is then considered on a cellular or subcellular level, with emphasis on recent electrophysiologic, biochemical, and molecular findings. Finally, each section includes a consideration of the factors that regulate Na^+ transport in the individual segments.

PRINCIPLES OF MEMBRANE TRANSPORT

This section describes physical principles that underlie the movement of ions across individual membranes and epithelia. However, it is not intended to be an extended treatment of the thermodynamic aspects of membrane transport processes.

Diffusion Processes

Solute transport across membranes may occur by diffusion or convection, or by a mediated process. Diffusion is the random Brownian motion of a molecule with respect to adjacent molecules and occurs as the consequence of thermal energy.[1] Because the diffusional movement of an

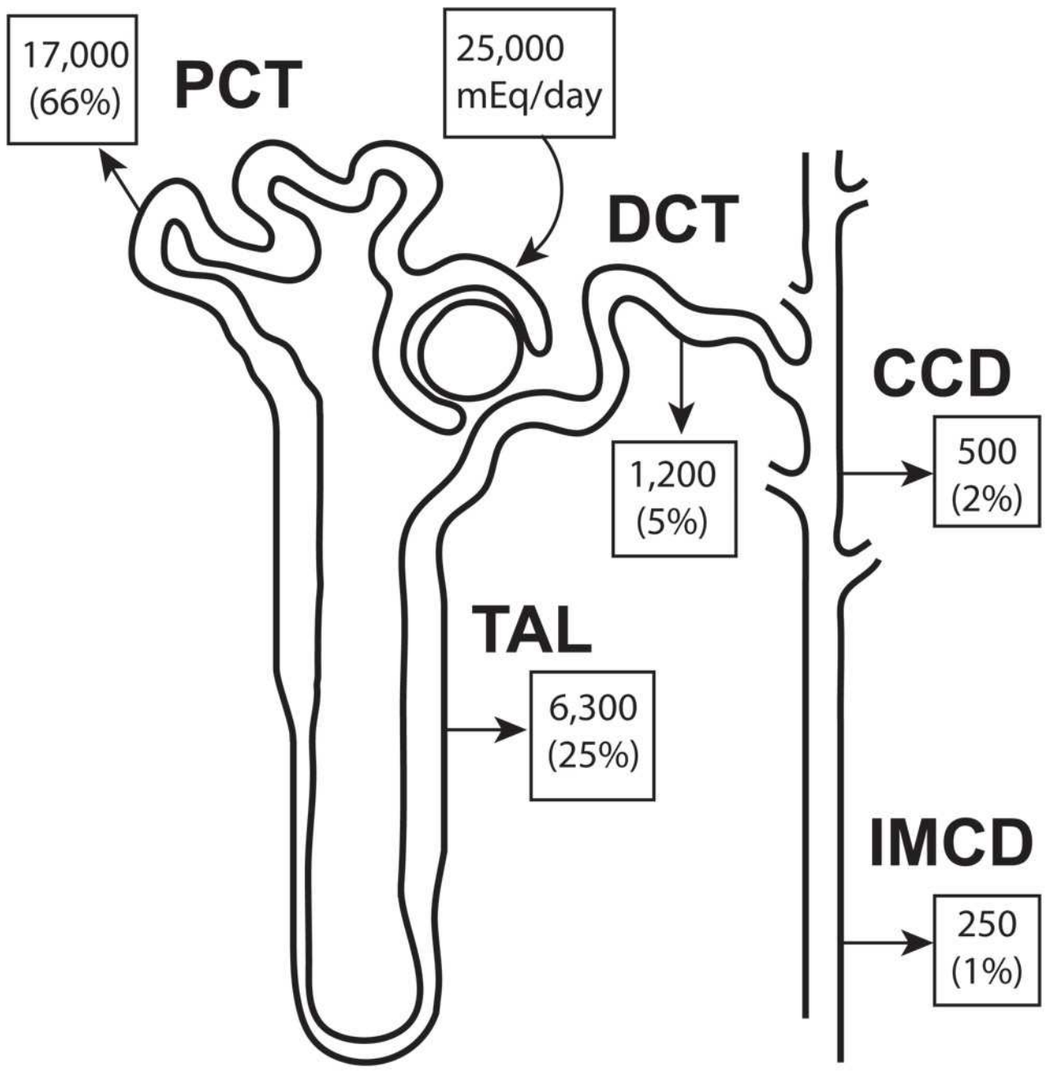


FIGURE 5.1 The contribution of various nephron segments to Na^+ transport. *PCT*, proximal convoluted tubule; *DCT*, distal convoluted tubule; *CCD*, cortical collecting duct; *TAL*, thick ascending limb; *IMCD*, inner medullary collecting duct.

individual molecule is random, a concentration gradient is required for any net transfer of molecules to occur across a membrane. Thus, the concentration gradient represents the driving force for net transport.

For charged solutes, the driving force for transport is the sum of the chemical and electrical potential gradients. The Nernst equation describes the equilibrium condition for a membrane permeable only to a single ionic species:

$$V_m = V_2 - V_1 = -(RT/ZF) \ln (C_2/C_1) \quad (1)$$

where R is the gas constant, T is the absolute temperature, Z is the valence of the solute, F is the Faraday constant, and C and V are concentration and electrical potential terms, respectively. At equilibrium, then, the voltage (V_m) across an ideally selective membrane is defined by the concentrations of the permeant ion on both sides of the membrane, C_2 and C_1, respectively. For systems containing more than one permeant ion, the equilibrium voltage can be described by the Goldman–Hodgkin–Katz (GHK) equation[2,3]:

$$V_m = -RT/F \ln [(P_{Na}C_{2Na} + P_KC_{2K} + P_{Cl}C_{1Cl})/(P_{Na}C_{1Na} + P_K C_{1K} + P_{Cl} C_{2Cl})] \quad (2)$$

where P_x is the permeability of the respective solutes, in this case, Na^+, K^+, and Cl^-. Thus, in a system containing multiple charged solutes, the transmembrane voltage is a function of the relative concentrations and permeabilities of each solute on the two sides of the membrane.

Convective Processes

Convection is the vectorial movement of an ensemble of molecules and is driven by an externally imposed force (e.g., hydrostatic pressure). Examples of convective transport include glomerular filtration and solvent drag, a process in which solute movement is coupled to water movement.

Bulk water flow may be driven by hydrostatic pressure and/or osmotic pressure. The familiar Starling equation:

$$J_v = K(\Delta P - \Delta\pi) \quad (3)$$

describes net volume flow (J_v) in response to hydrostatic (ΔP) and osmotic ($\Delta\pi$) pressure differences. The equivalence of osmotic and hydrostatic pressure is explicit in the Starling equation. The degree to which a solute exerts an osmotic pressure depends on the degree to which it permeates membranes. The ratio of the observed osmotic pressure to that predicted if a solute were excluded absolutely from a membrane is termed the reflection coefficient, σ:

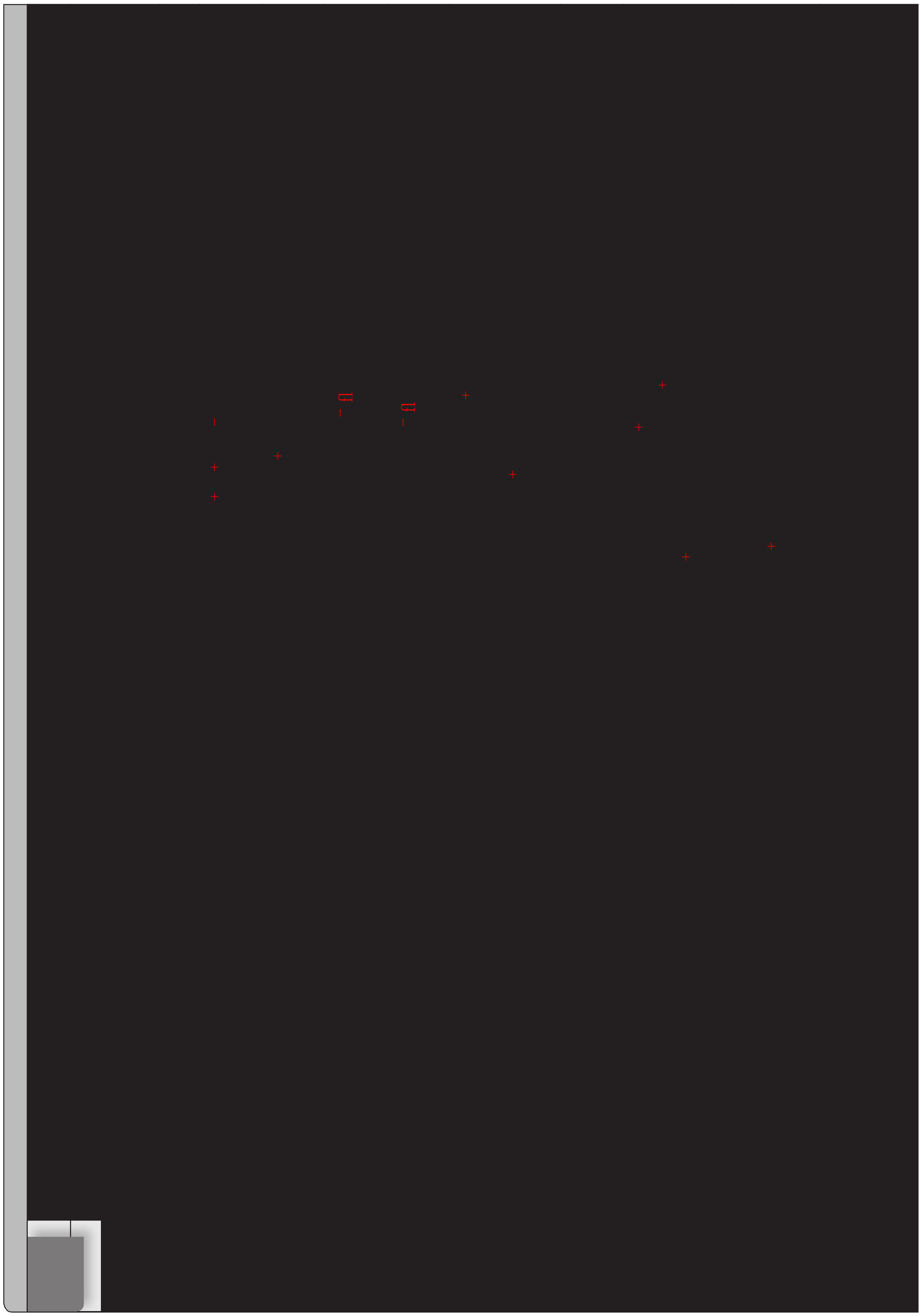
$$\sigma = \Delta\pi_{obs}/\Delta\pi_{theoretical} \quad (4)$$

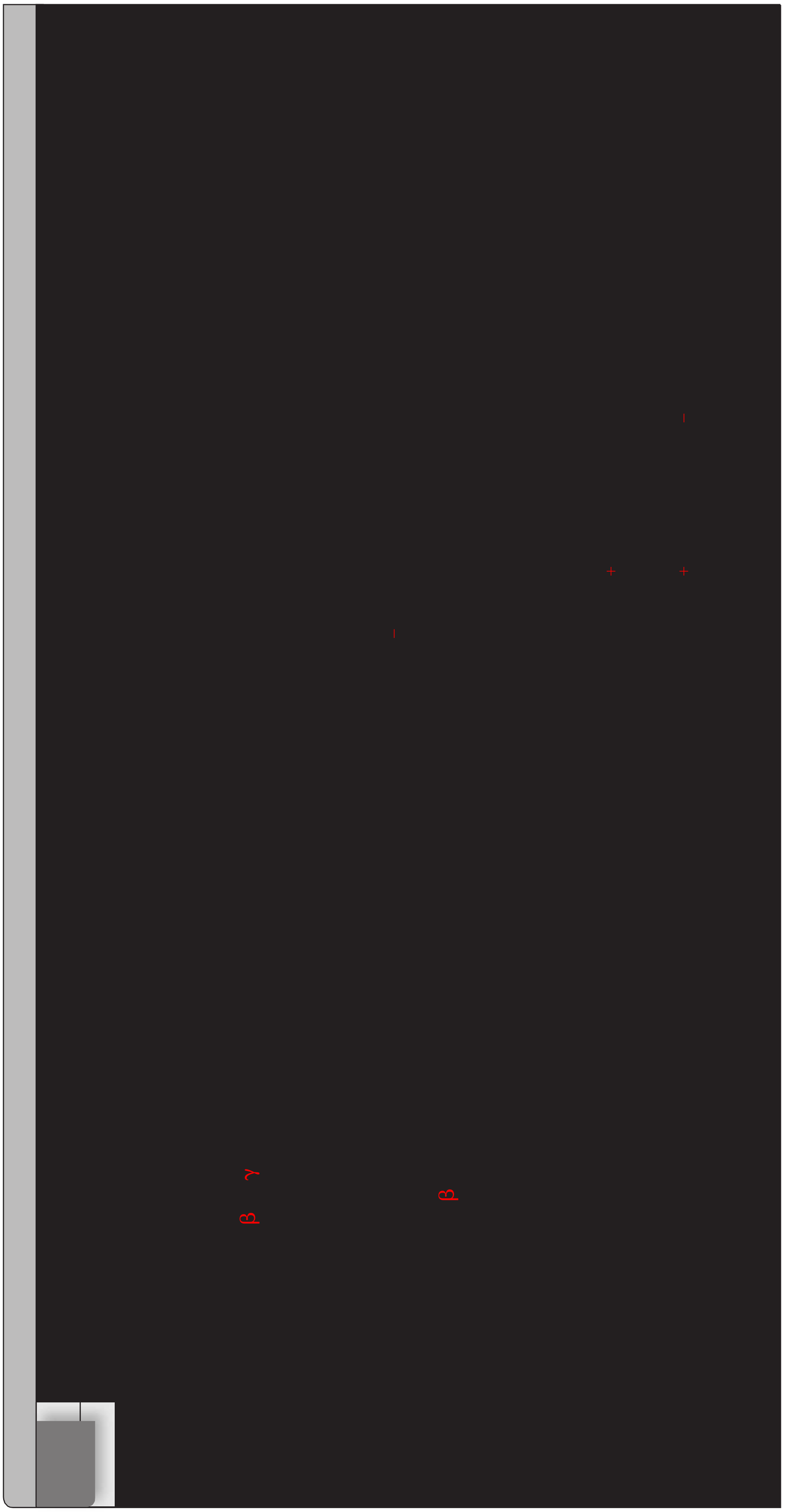
For impermeant solutes, $\sigma = 1$; for highly permeable solutes, σ approaches 0.

For solutes with $\sigma < 1$, transmembrane solute flux will be accelerated in the direction of volume flow. This acceleration is known as solvent drag.[4] Thus, the net passive flux of a permeable solute across a membrane may be driven by both diffusion and entrainment with solvent flow (i.e., solvent drag).

Facilitated Diffusion

Biologic membranes are composed primarily of lipid bilayers. Because the permeability of many hydrophilic solutes through lipid membranes is low, membranes contain proteins that facilitate the transport of certain solutes. Transport proteins, often termed carriers or transporters, have a high degree of specificity for the transported solute. Flux through the limited number of transporters saturates as the solute concentration is increased. An example of carrier-mediated facilitated diffusion is the entry of glucose into renal tubular cells mediated by the hexose transporter, GLUT-1.[5] The movement of ions through ion channels represents another form of facilitated diffusion. In this case, integral membrane proteins containing several membrane-spanning domains form pores in cell membranes through which ions permeate. Ion channels generally have a high degree of specificity for the ions being transported and very high transport rates. Facilitated diffusion mechanisms, like enzymes, serve only to accelerate the rate of transport, but do not affect the equilibrium distribution of solutes. In other words, facilitated diffusion, like simple diffusion and convection, is a passive process that tends to dissipate transmembrane gradients.

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Active Transport Processes

Active transport is a special case of facilitated transport in which chemical bond energy is supplied to the transport process so that the final distribution of the solute is remote from equilibrium. The coupling of solute transport to the energy source can take two forms. In primary active transport, solute transport is coupled directly to an energy-yielding reaction.

The most widely recognized example of primary active transport is the transport of Na^+ and K^+ by the Na^+,K^+-ATPase. This enzyme, often referred to as the sodium pump, couples the extrusion of cellular Na^+ to cellular K^+ uptake.[6] In renal tubules, this enzyme is localized to the basolateral membrane. In general, segments with high rates of active Na^+ transport have high Na^+,K^+–ATPase activity.[7] The hydrolysis of each ATP molecule ordinarily pumps three sodium ions out of the cell coupled to two potassium ions moving inward.[8] Therefore, the pump is electrogenic. The Na^+,K^+–ATPase is responsible for maintaining the cell Na^+ activity at a low level, which provides the energy for the Na^+-coupled transport of many other solutes. Thus, the inhibition of Na^+,K^+–ATPase (e.g., by peritubular ouabain addition) causes a significant rise in the cell Na^+ activity.[9] The affinity constant (K_m) of the pump for intracellular sodium, about 15 to 30 mM,[10] is similar to the intracellular sodium activity measured in proximal tubule cells.[9] Therefore, the pump is unsaturated with respect to sodium, and pump activity is very sensitive to changes in the intracellular sodium concentration activity.

In secondary active transport, solute movement against its electrochemical gradient is energized by the movement of another solute down its own gradient.[11] Na^+, because of its steep inward electrochemical gradient maintained by the sodium pump, often participates in the transport of other solutes, either in the same (cotransport or symport) or opposite (exchange or antiport) direction. Thus, by coupling solute transport with sodium movement into cells, cellular metabolic energy generated by the Na^+,K^+–ATPase is stored in the form of a Na^+ concentration gradient, analogous to a battery, and then dissipated in the transport of a variety of different solutes. Some examples of symport processes include Na^+-glucose and Na^+-amino acid cotransport; Na^+-proton and Na^+-calcium exchange are two examples of antiport processes.

PROXIMAL TUBULE

General Features

The proximal tubule is the major site for Na^+ absorption within the kidney and serves two major purposes. First, the proximal tubule protects the extracellular fluid volume by reclaiming the bulk, approximately 60% to 80%, of the glomerular filtrate.[12,13] The proximal tubule with its well-developed brush border membrane is optimally designed to perform the reabsorption of such a large fraction of the filtrate.[14] Second, the absorption of sodium in the proximal tubule provides, by way of coupled processes, the driving force for the absorption of other solutes, such as bicarbonate, glucose, phosphate, and amino acids.

Under most circumstances, fluid at any given point along the proximal tubule has virtually the same Na^+ concentration and osmolality as plasma.[13] The isosmotic nature of proximal tubule fluid absorption derives from the high water permeability of this segment,[15] which effectively clamps the osmolality of the tubular fluid at that of plasma. Although Na^+ transport in the proximal tubule occurs in the absence of large electrical or chemical gradients, the bulk of Na^+ absorption in the proximal tubule involves active transport. For example, Na^+ can be reabsorbed against both concentration[13,16] and electrical[17] gradients. In addition, fluid absorption and sodium transport cease when Na^+,K^+–ATPase activity is inhibited or when cell metabolism is slowed.[18,19]

A significant amount of proximal Na^+ transport also occurs passively.[20] For example, in the late convoluted and straight tubules (S2 and S3 segments), Na^+ diffuses passively out of the tubule driven by the lumen-positive electrical potential difference in those segments. This potential difference derives from a Cl^- concentration gradient across the tubule wall. Even in this case, however, it is the active transport of Na^+ in upstream portions of the proximal nephron that ultimately accounts for these gradients and potentials.

Nephron Heterogeneity

Analyses of proximal tubular Na^+ transport are complicated by two factors: a nonhomogeneous nephron population and axial changes in fluid composition. There is considerable heterogeneity of both morphologic and functional characteristics along the proximal tubule. The S1 segment cells have extensive basal interdigitations, numerous mitochondria, and a well-developed luminal brush border.[21] S3 segment cells, in contrast, are flatter and have fewer mitochondria, lower brush borders, and much less extensive basolateral membranes than S1 cells.[21] The Na^+,K^+–ATPase activity of the S3 segment is only 25% of that for the S1 segments.[22] As might be expected on the basis of these observations, the net rates of the Na^+ and fluid transport in the S3 straight segment are, in general, lower than in the S1 convoluted tubule.[23]

Juxtamedullary proximal convoluted tubules have higher rates of volume and bicarbonate absorption than their superficial counterparts,[23,24] although this disparity has not been noted between juxtamedullary and superficial straight segments.[25]

Glomerular ultrafiltrate in the early proximal tubule undergoes axial composition changes. Figure 5.2 illustrates that the chloride concentration rises as a consequence of the preferential absorption of $NaHCO_3^-$ over NaCl in this segment.[26] Glucose, amino acids, and other organic compounds are also absorbed avidly in association with Na^+ so that their luminal concentrations in this

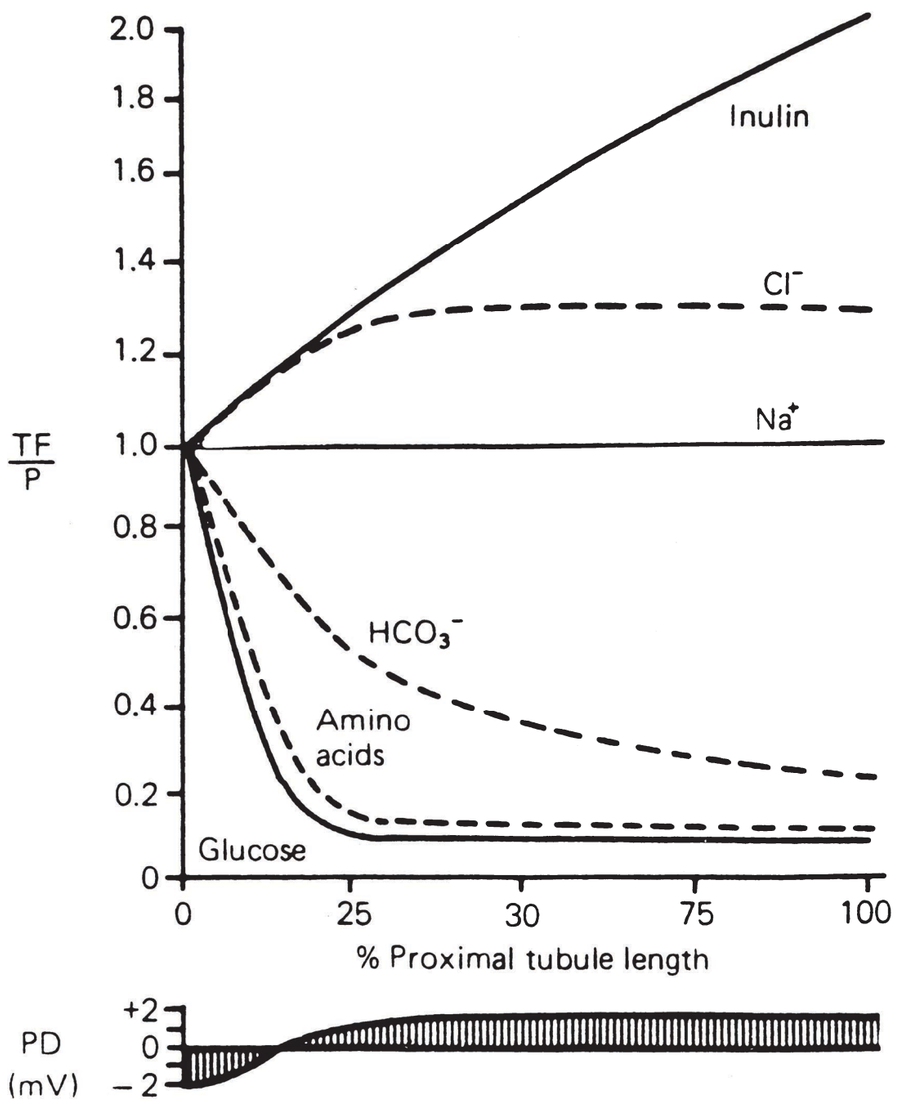

FIGURE 5.2 The profile of transepithelial voltage and solute concentrations along the mammalian proximal tubule. *TF/P*, tubular fluid/plasma concentration ratio; *PD*, potential difference. (From: Rector FC Jr. Sodium, bicarbonate, and chloride absorption by the proximal tubule. *Am J Physiol.* 1983;244:F461, with permission.)

segment approach zero.[26,27] The omission of glucose and amino acids from luminal fluids reduces both the potential difference and the volume absorptive rate.[24,28] In the S3 segment, on the other hand, the omission of glucose and alanine has no effect either on the potential difference or on the fluid absorptive rate,[25,29] although the deletion of all organic solutes does reduce volume absorption by 50%.[30] The rates of transport of glucose, amino acids, phosphate, and Na^+ in the early proximal convoluted tubule exceed those in the proximal straight tubule.[31–33] These rates correlate well with the relative basolateral membrane areas of the respective segments.[34]

Electrophysiology of the Proximal Tubule

Transepithelial Potential Difference

The electrogenic nature of the transport of Na^+ coupled to glucose and amino acids creates a lumen-negative transepithelial electrical potential difference in the early proximal convoluted tubule. The deletion of glucose and alanine from the luminal fluid reduces the potential difference from about −5.0 mV, lumen-negative, nearly to zero.[24,28] This transepithelial potential difference becomes lumen-positive (+2 to 4 mV) when the tubular fluid to plasma chloride concentration ratio is approximately 1.3.[35] This lumen-positive voltage is probably a diffusion potential arising from the Cl^- concentration gradient.

Electrical Resistance

The electrical resistance of the mammalian proximal tubule is remarkably low, making this tubule a classic example of a leaky epithelium, with resistances of 5 to 10 Ω-cm^2.[36,37] In the proximal tubule, the total cellular resistance (i.e., the sum of the apical and basolateral resistance) is 20- to 70-fold greater than the transepithelial resistance. This indicates that the paracellular resistance is low and that the predominant route for passive ion flows in the proximal tubule involves the paracellular pathway.

Ionic Selectivity

The initial convolution of the superficial proximal tubule is Na^+ selective; thereafter, the superficial convoluted and straight tubules are Cl^- selective.[29,38] In contrast, juxtamedullary proximal tubules are Na^+ selective throughout their course.[38] Because the Cl^- concentration rises as fluid flows along the convoluted tubule (Fig. 5.2), oppositely directed gradients for Cl^- and HCO_3^- in late convoluted and straight segments give rise to a lumen-positive transepithelial potential difference.[26] The latter indicates a higher permeability for Cl^- than HCO_3^-, both in superficial and juxtamedullary proximal tubules.[23,39]

MECHANISMS OF SODIUM REABSORPTION

Apical Membrane Sodium Entry

In the proximal tubule, Na^+ entry into the cell may be coupled to the movement of other solutes, such as glucose, chloride, or protons; or Na^+ may enter independently. In either case, the driving force for Na^+ entry is the steep electrochemical gradient favoring Na^+ influx.

The intracellular Na^+ activity ranges from 15 to 35 mM.[10,40] The entry of Na^+ into cells appears to be rate limiting for transepithelial Na^+ transport. Amphotericin B, a polyene antifungal, increases the permeability of the luminal membrane to Na^+ and causes a large rise in net sodium absorption.[41]

Na^+/H^+ Exchange

Directly coupled Na^+/H^+ exchange in the proximal tubular brush border is responsible for most proton secretion and for a large fraction of Na^+ reabsorption in the proximal tubule.[42] The mechanism whereby Na^+/H^+ exchange effects Na^+ reabsorption is presented in Figure 5.3. Briefly, entry of Na^+ is coupled to extrusion of a proton into the lumen. The proton titrates a filtered HCO_3^- molecule to form carbonic acid. Carbonic acid subsequently is dehydrated to CO_2 in a reaction catalyzed by carbonic anhydrase IV in the brush border membrane.[43] Within the cell, the reverse process occurs: carbonic acid formed by the hydration of CO_2 dissociates into H^+ and HCO_3^-. The H^+ is extruded into the lumen by Na^+/H^+ exchange or by the vacuolar H^+–ATPase to repeat another cycle while the HCO_3^- is transported into the blood via a $1Na^+$:$3HCO_3^-$ cotransport process (vide infra).

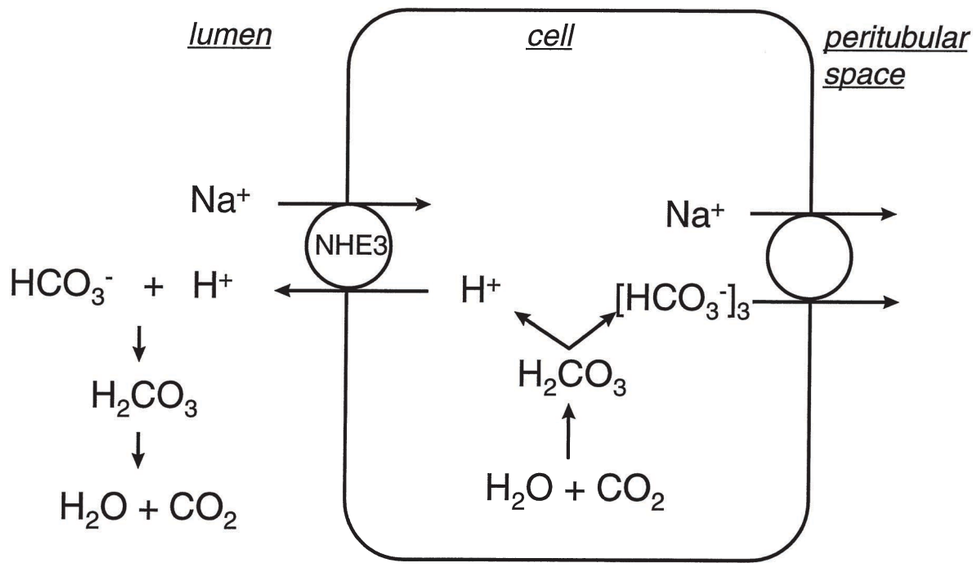

FIGURE 5.3 The scheme of $NaHCO_3$ transport mediated by Na^+/H^+ exchange. See text for explanation.

The mammalian Na^+/H^+ exchanger (NHE) is electroneutral with a stoichiometry of one proton for one sodium.[44] The exchanger is reversibly inhibited by high concentrations of amiloride.[45] Intracellular protons, via an internal activator site,[46] increase Na^+/H^+ exchange in response to intracellular acidosis.[47]

The apical and basolateral membranes of kidney cells contain different forms of NHEs with different affinities for amiloride.[48] The apical Na^+/H^+ exchanger is involved in urinary acidification and has a low amiloride affinity, whereas the basolateral exchanger has a high affinity for amiloride. The sensitivity of NHE1 to amiloride[49] and its basolateral localization[50] suggest that it represents the "housekeeping" NHE. In contrast, NHE3 is in the brush border membrane of proximal tubule cells.[51] NHE3 knockout mice have significantly reduced rates of Na^+ and HCO_3^- transport in proximal tubules.[52] In addition, pharmacologic inhibitors of NHE3 reduce proximal tubule Na^+ reabsorption by about one third.[53] However, a significant rate of amiloride-sensitive HCO_3^- transport still persists in the proximal tubules of NHE3 knockout mice,[54] indicating that the NHE3 is responsible for much, but not all, proximal tubular Na^+-coupled luminal acidification (vide infra). NHE8 is also expressed in the apical membranes of cortical tubules and may contribute to these processes.[55]

Sodium–Glucose Cotransport

Electrophysiologic studies in kidney proximal tubules show that apical membranes depolarize with addition of glucose to luminal fluids.[56] The depolarization occurs because the Na^+-glucose transporter is electrogenic. The Na^+-glucose transporter is specific for the D-stereoisomers of glucose, galactose, and α-methyl-D-glucoside.[57] The Na^+-glucose cotransporter has little affinity for cations other than Na^+.[58] Phlorizin inhibits Na^+-glucose cotransport by competing with glucose for its binding site.[59]

The rate of glucose transport by the early proximal tubule is greater than in late proximal segments.[60] In the proximal straight tubule, the K_m for D-glucose is 5 to 20 times lower than in the proximal convoluted tubule. Moreover, the transporter in the early cortical proximal tubule has a 1:1 Na^+ to glucose stoichiometry,[61] whereas the transporter in the straight medullary segment has a 2:1 stoichiometry.[62] By coupling the energy from two Na^+ ions moving down their electrochemical gradient to the transport of each glucose molecule, the medullary transporter is able to establish a much greater cellular to extracellular glucose concentration ratio than a 1:1 Na^+:glucose transporter.[27] The 2 Na^+:1 glucose transporter is, therefore, well suited to the straight segment, where tubular fluid glucose concentrations have already been reduced by glucose absorption in the more proximal segments.

A Na^+-glucose cotransporter (SGLT-1), which belongs to the SLC5 gene family,[63] mediates high affinity Na^+-glucose cotransport with a sodium-to-glucose coupling ratio of 2:1, whereas SGLT-2[64] shares 59% homology to SGLT-1 and mediates low-affinity Na^+-glucose cotransport with a sodium-to-glucose coupling ratio of 1:1.[65] In situ hybridization revealed high levels of a SGLT-2 message in the S1 segment of the proximal tubule.[66] Recently, better antibodies have confirmed that, in rat kidney, SGLT-1 immunolocalizes to the brush border membrane of all three segments of the proximal tubule.[5] By immunohistochemistry, SGLT-2 was detected at the brush border of the early proximal tubule in mice, which was absent in SGLT-2 knockout animals.[67] Thus, it appears that SGLT-2 may represent the low-affinity, high-capacity sodium–glucose cotransporter in the early proximal tubule, whereas SGLT-1 may represent the high-affinity, low-capacity transporter of the proximal straight tubule.[65]

Mutations in SGLT-2 form the basis for renal glycosuria (Table 5.1), an inherited condition characterized by a lowered threshold for tubular reabsorption of glucose.[68] In contrast, the dominant clinical manifestations of inactivating mutations of SGLT-1[69] relate to the failure to absorb sugars in the intestinal tract (glucose–galactose malabsorption). These findings suggest that SGLT-2 plays a much more significant role, quantitatively, than SGLT-1 in proximal tubule glucose reabsorption. SGLT-2 inhibitors are currently under investigation as potential therapeutic agents for the treatment of diabetes.[70]

Sodium–Amino Acid Cotransport

The proximal tubule reabsorbs amino acids from the tubular fluid via an active transport step at the luminal membrane.[31] Samarzija and Frömter,[71] using double-perfusion micropuncture techniques, observed a depolarization of the luminal membrane during amino acid transport, and they were able to identify five classes of amino acid transporters in the luminal membrane. Over the last decade, many of the transport proteins that mediate the different amino acid transport systems have been identified in kidney and intestine. This topic has been recently reviewed.[72]

Both Na^+-dependent and Na^+-independent amino acid uptake pathways have been characterized in the kidney.[72] Neutral amino acid transport appears to involve at least

three separate transport systems,[73] one that transports all neutral amino acids, one specific for imino acids, and one for the β-amino acids. Glycine may also have a specific transporter.[74] In the kidney, neutral amino acid transport is driven by a Na^+ gradient, as supported by experiments in slices, perfused tubules, and brush border membranes. The neutral amino acid transporter B^0AT1 (SLC6A19) cotransports one Na^+ per amino acid.[75] The K_m of the substrate decreases with an increasing cosubstrate concentration and vice versa. The initial step for transport involves the binding of the amino acid to B^0AT1, and this binding affinity increases under hyperpolarizing conditions.[76]

The acidic and basic amino acid groups each have their own transport systems.[77,78] At least one amino acid transporter, a Na^+-independent transporter for neutral and dibasic amino acids, has been cloned from the kidney.[79,80]

NaCl Transport

Two basic mechanisms account for NaCl reabsorption in the proximal tubule. In simple electrogenic Na^+ entry, sodium is transported actively through the cell, thereby creating a lumen-negative potential difference. Cl^- reabsorption then proceeds through the paracellular pathway driven by the lumen-negative potential difference. In electrically neutral NaCl transport, both Na^+ and Cl^- move through the cell at equal rates, such that no transepithelial potential and, hence, no driving force for paracellular Cl^- movement is generated.

Neutral NaCl Transport

Several lines of evidence indicate that a sizable fraction of proximal NaCl transport is transcellular and electroneutral.[81] First, by virtue of the coupling of Cl^- entry to apical Na^+ entry, the intracellular Cl^- activity of proximal tubule cells is greater than predicted from an equilibrium distribution.[82] Second, Cl^- absorption persists even when the driving force for passive, paracellular movement is abolished.[83] Conversely, Cl^- reabsorption is inhibited by cyanide in the absence of any change in the passive driving forces for Cl^- movement.[83] Finally, the luminal application of SITS,[84] an anion-exchange inhibitor, or removal of chloride from the tubule perfusate,[85] reduces net Na^+ reabsorption.

In principle, electroneutral NaCl transport across the apical membrane of proximal tubule cells could occur as directly coupled NaCl cotransport or as parallel Na^+/H^+ and Cl^-/base exchangers. There is no good evidence for the former process in the mammalian proximal tubule.[81] However, considerable evidence supports the view that electroneutral NaCl transport in the proximal tubule involves parallel exchangers. The coupling of Na^+ absorption to Cl^- absorption in this case occurs because of the relation between cell pH and concentration of base within the cell. With reference to Figure 5.4, the extrusion of H^+ in exchange for Na^+ results in the liberation of the base for participation in Cl^-/base exchange. The uphill entry of Cl^-, then, is indirectly coupled to the downhill entry of Na^+, because both are coupled to the transport of an acid–base pair. The model illustrated in Figure 5.4 uses formate/Cl^- exchange as the anionic component of electroneutral NaCl transport.

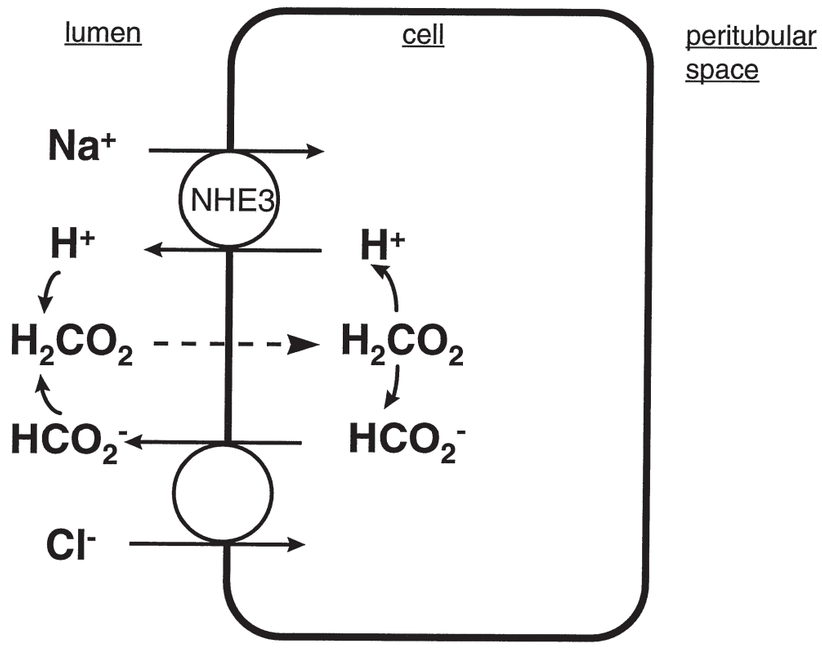

FIGURE 5.4 The scheme of neutral NaCl transport mediated by the parallel action of $Na^+ - H^+$ exchange and formate/Cl^- exchange. Formate (HCO_2^-) combines with H^+ in the tubular lumen to form formic acid (H_2CO_2), which reenters the cell by nonionic diffusion. A similar scheme applies for oxalate–Cl^- exchange.

As indicated previously, there is abundant evidence for a Na^+/H^+ exchanger in proximal tubule brush border membranes. With respect to NaCl transport, the inhibition of Na^+/H^+ exchange by high concentrations of amiloride[86] or more specific inhibitors of NHE3[53] results in a dramatic fall in transcellular NaCl transport. Likewise, knockout of NHE3 also reduces NaCl and fluid reabsorption in the proximal tubule.[52] Several Cl^-/base exchangers have been implicated in NaCl transport. Recent interest has focused on the role of Cl^-/formate (HCO_2^-) and Cl^-/oxalate ($C_2O_4^{2-}$) exchange in NaCl transport.

A Cl^-/formate exchanger is present in brush border membrane vesicles.[87] A role for Cl^-/formate exchange in neutral NaCl transport is suggested by the finding that the addition of formate to the luminal perfusate increases the rate of NaCl reabsorption in rabbit proximal tubules.[88] As depicted in Figure 5.4, formate is presumed to leave the cell in exchange for Cl^-. The secreted formate then combines with a proton, which was transported by the Na^+/H^+ exchanger to form formic acid. The formic acid then reenters the cell by nonionic diffusion and dissociates to supply substrate for the continuation of both exchange processes. CFEX (SLC26A6), a homolog of pendrin, is a protein capable of mediating Cl^-/formate exchange and is present in apical membranes of the proximal tubule.[89]

Cl^-/oxalate ($C_2O_4^{2-}$) exchange has also been demonstrated in brush border membrane vesicles.[90] It has been suggested that Cl^-/oxalate exchange may mediate neutral NaCl transport in a manner analogous to that described for Cl^-/formate exchange. It has also been suggested that NaCl absorption proceeds via the operation of three parallel transporters: the Na^+-sulfate cotransport, the sulfate/oxalate exchange, and the Cl^-/oxalate exchange.[91] Indeed, the CFEX protein, in addition to Cl^-/formate exchange, is also able to mediate Cl^-/oxalate, oxalate/formate, oxalate/oxalate, and oxalate/sulfate exchange.[92]

Other Na^+-dependent transport processes have been described in the apical membrane of the proximal tubule. However, they do not contribute significantly to Na^+ reabsorption because of the low concentrations of substrate present.

Simple Electrogenic Na^+ Entry

The classic Ussing model for salt reabsorption involves passive entry of Na^+ across apical membranes and extrusion across the basolateral membrane by Na^+,K^+–ATPase. A problem in assessing the contributions of electrogenic processes to Na^+ transport in the proximal tubule is the presence of other mechanisms of Na^+ entry. However, when the contributions of Na^+/H^+ exchange and Na^+ cotransport to net Na^+ absorption are minimized by deleting glucose, amino acids, and bicarbonate from the perfusate, a fraction of fluid absorption in isolated perfused straight segments persists and the transepithelial potential is −1.0 mV.[30] These results indicate that in the proximal straight tubule simple electrogenic Na^+ transport constitutes a mechanism for Na^+ absorption. A conductive Na^+ pathway has been demonstrated in brush border membrane vesicles.[93] Unlike the Na^+ channel found in the distal nephron segments, the Na^+ channel in the proximal tubule is not blocked by amiloride.[93]

In the proximal convoluted tubule, however, the deletion of glucose, bicarbonate, and amino acids completely abolishes fluid absorption.[23] Consequently, simple electrogenic proximal Na^+ transport may be limited to straight segments.

Passive NaCl Absorption

The rise in tubular fluid Cl^- concentration, and the attendant lumen-positive voltage (Fig. 5.2), provides a mechanism for passive NaCl absorption in late regions of the proximal nephron. In the Cl-selective superficial pars recta, approximately one-third of net NaCl absorption can be accounted for by this mechanism.[20]

Basolateral Membrane

The proximal tubule, particularly the S1 segment, possesses high Na^+,K^+–ATPase activity in the basolateral membrane. The Na^+,K^+–ATPase pumps Na^+, which entered cells apically, across basolateral membranes. In other words, the pump keeps the cell Na^+ activity low and maintains the electrochemical gradient for Na^+ entry across the apical membrane. Consequently, the inhibition of Na^+,K^+–ATPase activity with ouabain decreases transepithelial Na^+ reabsorption and increases the intracellular Na^+ activity in the proximal tubule.[94]

Na^+ also exits across the basolateral membrane in concert with HCO_3^-. Studies in intact tubules and in membrane vesicles have demonstrated an electrogenic, stilbene-sensitive Na^+-HCO_3^- cotransporter in the basolateral membrane of rat and rabbit proximal tubules.[95,96] The cotransporter transfers two net negative charges across the basolateral membrane. The stoichiometry of this process is 1 Na^+: 1 HCO_3^-: 1 CO_3^{2-} (or SO_3^{2-}).[97] Thus, this transport moiety for Na^+ extrusion, $[Na^+(HCO_3^-)_3]^{2-}$ is electronegative, with the lumen-negative cell interior providing a major driving for Na^+ extrusion. A number of Na^+-HCO_3^- cotransporters have been cloned, along with different splice variants.[98] One of these, NBC1 (encoded by SLC4A4 and subsequently renamed NBCe1-A) is localized to the basolateral membrane of the S1 and S2 segments of the proximal tubule.[99] As illustrated in Figure 5.3, the net result of these steps is the reabsorption of Na^+ and HCO_3^-, accounting for the bulk of HCO_3^- reabsorption and about 20% of Na^+ reabsorption in the proximal tubule. It is believed that this cotransport process accounts for the basolateral transport of most of the bicarbonate reclaimed from the luminal fluid.[97] Mutations in NBCe1-A cause proximal (type II) renal tubular acidosis and other defects in the eye, teeth, and mental development (Table 5.1). The regulation and role of NBC in acid–base transport in the proximal tubule is an area of active research that has been reviewed recently.[100]

The pathways for Cl^- exit across the basolateral membrane are less well defined. Several pathways for Cl^- exit across the basolateral membrane have been proposed: conductive Cl^- channels, KCl cotransport, and $Na^+(HCO_3^-)_2/Cl^-$ exchange (Fig. 5.5). Studies of rat proximal convoluted tubules[101] and rabbit convoluted and straight proximal tubule segments[102,103] using intracellular microelectrodes have indicated that the proximal tubule cell has a very low Cl^- conductance. Thus, in normal proximal convoluted tubule and proximal straight tubule segments, conductive Cl^- efflux across basolateral membranes appears to play a minor role in NaCl absorption. Under hypotonic conditions, however, cell swelling dramatically increases the basolateral membrane Cl^- conductance.[104]

Because the chemical gradient for K^+ to leave cells exceeds that for Cl^- entry, KCl cotransport can mediate basolateral Cl^- exit from proximal tubule cells. Ion-selective microelectrode studies have demonstrated KCl cotransport in basolateral membranes of rabbit proximal tubule cells.[103,105]

Stilbene-sensitive, Na^+-dependent Cl^-/HCO_3^- exchange has been demonstrated in rat[106] and rabbit[107,108] proximal tubules. In this case, the entry of 1 Na^+ and 2 HCO_3^- across the basolateral membrane is coupled to the efflux of Cl^-. The Na^+

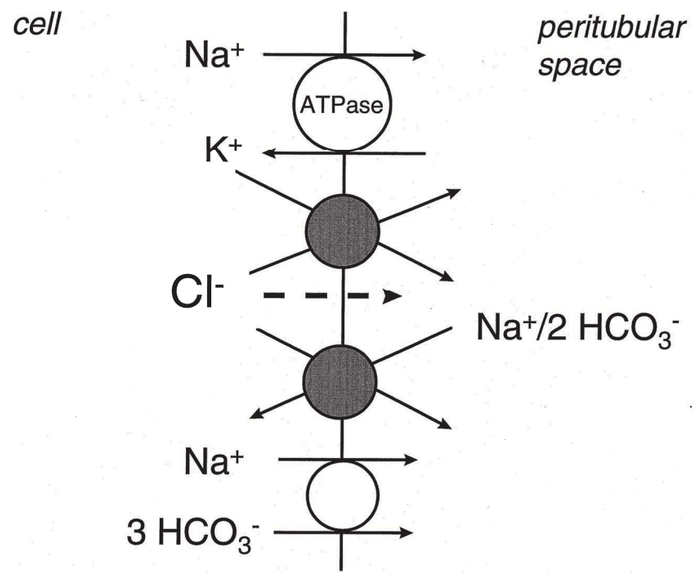

FIGURE 5.5 The transport pathways for Na^+ and Cl^- absorption across the basolateral membrane of proximal tubular cells. Cl^- can leave the cell via KCl cotransport, Na^+–$2HCO_3^-/Cl^-$ exchange, and Cl^- channels (minor). Na^+ exits via the Na^+,K^+–ATPase and Na^+–$3(HCO_3^-)$ cotransport.

and HCO_3^- that enter the cell are thought to be recycled through the $[Na^+(HCO^-_3)_3]^{2-}$ cotransporter (see previous). Indeed, $Na^+(HCO^-_3)_2/Cl^-$ exchange may account for much more Cl^- movement than KCl transport.[108] Na^+-independent Cl^-/HCO_3^- exchange is also present in the basement membrane of proximal tubules.[106–108] However, under physiologic conditions, this process mediates net Cl^- influx and does not contribute to net NaCl absorption.

CONTROL OF PROXIMAL TUBULAR SODIUM REABSORPTION

Glomerulotubular Balance (GTB)

The proximal tubule responds to an increase in glomerular filtration with an increase in the absolute rate of fluid absorption (APR) to minimize variations in the fractional proximal fluid absorption. This phenomenon is termed glomerulotubular balance (GTB). The efficiency of GTB—that is, the extent to which APR/GFR remains constant—is subject to physiologic and pathologic control. The prime factor modulating GTB in vivo is the effective circulating volume. Thus, at a constant or near constant GFR, volume expansion and volume contraction decrease and increase, respectively, the absolute rate of proximal Na^+ absorption. In other words, volume expansion and volume contraction reset GTB upward and downward, respectively.[109,110] This section considers some of the factors that modulate proximal Na^+ absorption.

Peritubular capillary oncotic pressure is one of the factors regulating the rate of salt and water absorption from the proximal tubule.[111] The oncotic pressure of the peritubular proteins favors the movement of fluid across the basement membrane, whereas capillary hydrostatic pressure retards this movement. Thus, at a given renal blood flow, an increase in the glomerular filtration rate and, hence, the filtration fraction, will cause an increase in the oncotic pressure in the postglomerular peritubular capillaries. At constant single nephron glomerular filtration rates (SNGFR), the perfusion of efferent capillaries with hypo-oncotic fluids decreases the absolute rate of proximal fluid absorption, whereas perfusion of the capillaries with hyperoncotic fluids increases proximal absorption.[112] The effects of the peritubular protein concentration can also be demonstrated in isolated perfused tubules.[113]

It is not precisely clear how the peritubular protein concentration modulates proximal fluid absorption. The effect is not simply because of the oncotic pressure exerted by the proteins, because changes in absorption do not occur when active transport is inhibited or from comparable changes in the transtubular hydrostatic pressure.[114] The prevailing views are that the peritubular protein may directly affect transcellular Na^+ transport[115] or the back leak of Na^+ through the paracellular pathway.[113,116]

Luminal factors also contribute to glomerulotubular balance. The flow dependence of proximal absorption has been investigated in the convoluted[15] and straight segments[30] of isolated, perfused rabbit nephrons. The key observations are (1) the volume absorptive rate is clearly dependent on the perfusion rate, (2) the flow dependence persists in the absence of active transport when anion gradients are present, and (3) the flow dependence is abolished in the absence of active transport and anion gradients.

An explanation for these results lies in a consideration of axial versus radial changes in fluid composition along the tubule.[117] The rate of passive Na^+ absorption in the late proximal tubule is dependent on the magnitude of the chloride gradient between the lumen and the bath. At low axial perfusion rates, the radial Cl^- gradient tends to dissipate as a function of distance along the tubule, but this dissipation is minimized at higher axial perfusion rates. Hence, the integrated driving force for passive NaCl absorption increases with the rate of tubule perfusion. In addition, the availability of solutes, such as glucose, amino acids, and bicarbonate, is also partly responsible for the flow dependence of proximal fluid absorption.[118]

The flow dependence of reabsorption in the proximal tubule has also been tested in the mouse proximal tubule. In this experimental model, which included the use of the NHE3 knockout mouse, the data supported the hypothesis that the "brush border" microvilli act as mechanosensors that transmit fluid dynamic torque to the actin cytoskeleton and thus modulate Na^+ absorption.[119]

Catecholamines

Renal denervation reduces proximal tubule Na^+ and fluid absorption.[120] Both α- and β-adrenergic receptors exist in the proximal tubule.[121,122] The rate of salt and water reabsorption in the proximal tubule is stimulated by α- and β-adrenergic agonists.[123] α-Adrenergic agonists increase apical Na^+ entry via the stimulation of Na^+/H^+ exchange[122] and also increase basolateral Na^+ efflux via Na^+,K^+–ATPase activity in rat proximal tubules by a pathway that involves the activation of calcineurin.[124] The effects of β-adrenergic agonists on Na^+,K^+–ATPase activity are less clear, with one study showing that they increase Na^+,K^+–ATPase activity via protein kinase C (PKC).[125] However, others have found that β-adrenergic agonists inhibit Na^+,K^+–ATPase activity via PKA.[126]

Dopamine, which is produced by proximal tubular cells,[127] inhibits Na^+ reabsorption. Dopamine inhibits Na^+,K^+–ATPase activity via its receptors DA-1 and DA-2.[128]

Parathyroid Hormone

Parathyroid hormone (PTH) causes a 30% to 50% reduction in proximal tubular Na^+ and phosphate absorption.[129] PTH stimulates adenylyl cyclase, cAMP production, and PKA, which in turn inhibits Na^+/H^+ exchange in several proximal tubule systems.[130] Weinman et al.[131] demonstrated that phosphorylation by PKA inhibits Na^+/H^+ exchange activity via a PDZ domain-dependent interaction with the NHE regulatory factor (NHERF). In the absence of NHERF, cAMP

does not inhibit the exchange activity of NHE3.[132] The current model for this inhibition is that NHE3 associates with PKA indirectly via NHERF and the cytoskeletal protein ezrin. PKA, when active, phosphorylates NHE3 at serines 552 and 605, which mediates the inhibition of the exchanger.[133] NHE3 is directly phosphorylated by other protein kinases, including calmodulin-dependent protein kinase II, which inhibits Na^+/H^+ exchange activity and PKC, which stimulates the exchanger.[134]

Angiotensin II

The systemic administration of low doses of angiotensin II (Ang II) inhibits the excretion of Na^+,[135] whereas inhibitors of Ang II increase Na^+ excretion.[136] Systemic Ang II causes changes in renal blood flow, aldosterone secretion, filtration fraction, and catecholamine release from renal sympathetic nerve endings.[137] Low concentrations of Ang II ($<10^{-9}$ M) cause an increase in proximal tubule fluid and bicarbonate reabsorption, effects that are mediated by the AT1 subtype of Ang II receptors present in both the brush border and basolateral membranes of the proximal tubule.[138] Higher concentrations ($>10^{-8}$ M) depress fluid and bicarbonate absorption, presumably via counterbalancing effects mediated by the lower affinity AT2 receptor.[138] Studies have demonstrated that the stimulatory effect of Ang II on fluid and bicarbonate reabsorption occurs via enhanced apical Na^+/H^+ exchange via NHE3 and basolateral Na^+-$(HCO_3^-)_3$ cotransport via NBC-1 in the proximal tubule.[139] The physiologic effects of Ang II may involve the coupling of these receptors to both phospholipase A_2 and inhibitory G proteins.[140,141]

Thyroid Hormone

On a clinical level, hypothyroidism is associated with a decreased cardiac output, renal blood flow, and glomerular filtration rate (GFR). Clearance studies in hypothyroid rats have documented decreases in GFR, renal Na^+ reabsorption, and renal Na^+,K^+–ATPase activity.[142] These changes are reversible after thyroid hormone replacement.[143] The thyroid hormone may exert direct effects to stimulate proximal tubular salt and fluid reabsorption via increased basolateral K^+ permeability[144] and/or direct stimulation of Na^+/H^+ exchange through an increase in NHE3 transcription.[145]

Corticosteroids

Although mineralocorticoids do not have an effect on proximal tubular sodium reabsorption,[146] there is evidence for glucocorticoid receptors in the proximal tubule.[147] Dexamethasone inhibits apical membrane Na^+-phosphate cotransport in cultured proximal tubular cells via PKC activation.[148] Dexamethasone also enhances the activity of apical NHE3 and the mRNA expression and functional activity of basolateral NBC-1.[149] The resulting increase in proximal tubule HCO_3^- reabsorption could contribute to the maintenance of the metabolic alkalosis that is associated with increased glucocorticoid production in vivo.

Nitric Oxide

Various forms of nitric oxide synthase (NOS) are expressed in the proximal tubule.[150] Low basal production of nitric oxide (NO) by the proximal tubule is boosted dramatically by lipopolysaccharide (LPS) and cytokines. Even under basal conditions, the proximal tubule may be affected by NO produced by adjacent cells, such as endothelium or other nephron segments. The overall effect of NO on proximal tubule Na^+ transport is controversial and may be biphasic, with acute inhibition and chronic stimulation of Na^+ reabsorption as assessed by pharmacologic agents and genetic knockouts of NOS, respectively.[151] In vitro, NO decreased Na^+/H^+ exchange and Na^+,K^+–ATPase activity in cultured proximal tubule cells.[152]

MECHANISM OF ISOTONIC FLUID ABSORPTION

Proximal tubule water absorption is coupled tightly to solute absorption, because the measured osmolality of the tubular fluid is generally identical to plasma. Three general mechanisms of solute–solvent coupling have been suggested to account for this isotonic absorption: lateral interspace hypertonicity, effective osmotic gradients because of different reflection coefficients for solutes in the tubular and peritubular fluids, and luminal fluid hypotonicity.

The standing gradient theory argues that active transport of salt into the lateral intercellular space raises the osmolality of the space, thus providing an osmotic gradient for fluid transport from the lumen to the interspace.[153] The tight junctions in this model are presumed to be impermeable to water, so that the osmotic flow of water from the cell into the hypertonic interspace raises the hydrostatic pressure in this compartment and forces fluid across the basement membrane.

An alternative explanation[117] proposes that an effective osmotic driving force for fluid absorption can exist between solutions of identical osmolalities if the reflection coefficients of the membrane for the solutes in the solutions differ. Specifically, the elevated tubular fluid-to-plasma Cl^- concentration found in the late proximal convolution and in the pars recta provides an effective osmotic driving force for fluid absorption because σHCO_3^- exceeds σCl^-. That is, the bicarbonate in the peritubular fluid is a more "effective" osmotic agent than is the chloride in the tubular fluid, and, thus, net water flows out of the tubule. Although this mechanism may be applicable to the proximal straight tubule, the reflection coefficients for NaCl and $NaHCO_3$ measured across the rabbit proximal convoluted tubule are virtually identical,[154] so oppositely directed Cl^- and HCO_3^- gradients may make only a negligible contribution to fluid absorption in convoluted segments.

Finally, because of the high osmotic water permeability of the proximal tubule, only small degrees of absolute luminal hypotonicity are needed to provide a sufficient driving

force to account for the observed rates of fluid reabsorption.[20] Experimental evidence supports the view that absolute luminal hypotonicity is a significant driving force for fluid reabsorption in the proximal tubule. Thus, when proximal tubules are perfused and bathed by symmetric NaCl solutions, the luminal fluid becomes slightly hypotonic.[155] The development of luminal hypotonicity can be amplified by maneuvers that decrease the water permeability of the proximal tubule. The aquaporin 1 (AQP1) water channel is abundantly expressed in the proximal tubule. In AQP1 knockout mice, the osmolality of tubular fluid at the end of the proximal tubule is significantly lower than in normal mice.[156] As the luminal fluid becomes more hypotonic, the resorbate becomes more hypertonic, and the degree of resorbate hypertonicity correlates with the rate of volume reabsorption by the tubules.[157]

THE LOOP OF HENLE

The dissociation of salt and water absorption by the loop of Henle is ultimately responsible for the capacity of the kidney, either to concentrate or to dilute the urine. The active absorption of NaCl in the water-impermeable thick ascending limb of Henle (TALH) serves both to dilute the urine and to supply the energy for the "single effect" of countercurrent multiplication. A functionally similar segment, known as the diluting segment, is found in amphibians and teleosts.[158]

The mammalian loop of Henle contains the descending thin limb (DTL), the ascending thin limb (ATL), and the thick ascending limb (TAL). The loop of Henle absorbs about 25% to 40% of the filtered Na^+ load.[159] Furthermore, the fluid leaving the loop is dilute, indicating that more NaCl is absorbed in the loop than water.

SALT TRANSPORT BY THE THIN DESCENDING AND THIN ASCENDING SEGMENTS

As the tubular fluid enters the descending thin limb and flows toward the tip of the renal papilla, it becomes more concentrated.[160] Passive models for urinary concentration indicate that this increase in osmotic pressure is attributable to water extraction rather than solute entry.[161] Current observations indicate that the aquaporin water channel AQP1 mediates water movement across the luminal surface of the DTL.[162] According to some reports, however, AQP1 expression is significantly lower in short loop nephrons than long loop nephrons,[163] suggesting that (1) not all DTLs in the kidney extract water to the same degree, and/or (2) water movement in short loop DTLs is facilitated by alternative water channels or via the paracellular route. In contrast to the DTL, in vitro microperfusion studies demonstrate that the ATL is relatively impermeable to water.[164]

The study of NaCl transport by the DTL and ATL has been complicated by the fact that the transport characteristics of these two nephron segments exhibit significant interspecies heterogeneity.[165] Although the Na^+ and Cl^- permeabilities appear to vary widely among rabbits, hamsters, and rats, one consistent finding was that the DTL is relatively less permeable to NaCl than the ATL. Coupled with the DTL's high permeability to water, the relative lack of solute permeability ensures that the osmotic pressure of fluid entering the renal papilla is greater than that of the fluid leaving it.[166] Thus, the formation of dilute urine by the loop of Henle begins in the ATL.

The decrease in osmolality in the ATL is due primarily to a fall in the NaCl content of the luminal fluid. The electroneutral transport of NaCl appears to occur through two key mechanisms. The transepithelial movement of sodium from the ATL lumen occurs via the paracellular pathway,[167] whereas Cl^- diffusion occurs through a transcellular route. Yoshitomi et al.[168] detected conductive pathways for Cl^- in both the apical and basolateral membranes of ATL cells. The transcellular movement of Cl^- in the ATL is regulated, because the basolateral Cl^- conductance is inhibited at low pH[168] and by low intracellular Ca^{2+} concentrations.[169] Uchida et al.[170] cloned a Cl^- channel in 1993 from the rat renal medulla, ClC-K1 (termed ClC-Ka in humans), which represents the major mediator of transcellular Cl^- movement in the thin ascending limb. This channel, which belongs to the ClC family of Cl^- channels, is expressed exclusively within the kidney and has been localized by immunohistochemistry to both the apical and basolateral membranes of the thin ascending limb of Henle.[171] Its activity is dependent on the coexpression of barttin, an accessory protein that forms a complex with ClC-K1, increases its abundance at plasma membranes, and modifies channel gating.[172–175] The expression of ClC-K1 is increased by dehydration.[170] Genetic knockout of the ClC-K1 gene in mice produced a urinary-concentrating defect, confirming the role of passive NaCl transport in the thin ascending limb in the urinary concentrating mechanism.[176]

NaCl ABSORPTION IN THE THICK ASCENDING LIMB

General Features

The studies of Rocha and Kokko[177] and Burg and Green[178] were the first to investigate salt absorption in the thick ascending limb, and their work defined several key features of this unique epithelium. First, salt absorption in the medullary and cortical TAL generates a lumen-positive transepithelial voltage, which is sensitive to furosemide. Second, the transport of Cl^- occurs against both electrical and chemical gradients and involves an active transport process that is dependent on intact basolateral Na^+,K^+–ATPase activity.[179] A final important feature of the TAL is that this segment consists of a tight epithelium, which despite its high ionic conductance, possesses a very low permeability to water. The apical membrane of the TAL constitutes the major barrier to transcellular and paracellular water flow.[180] The high

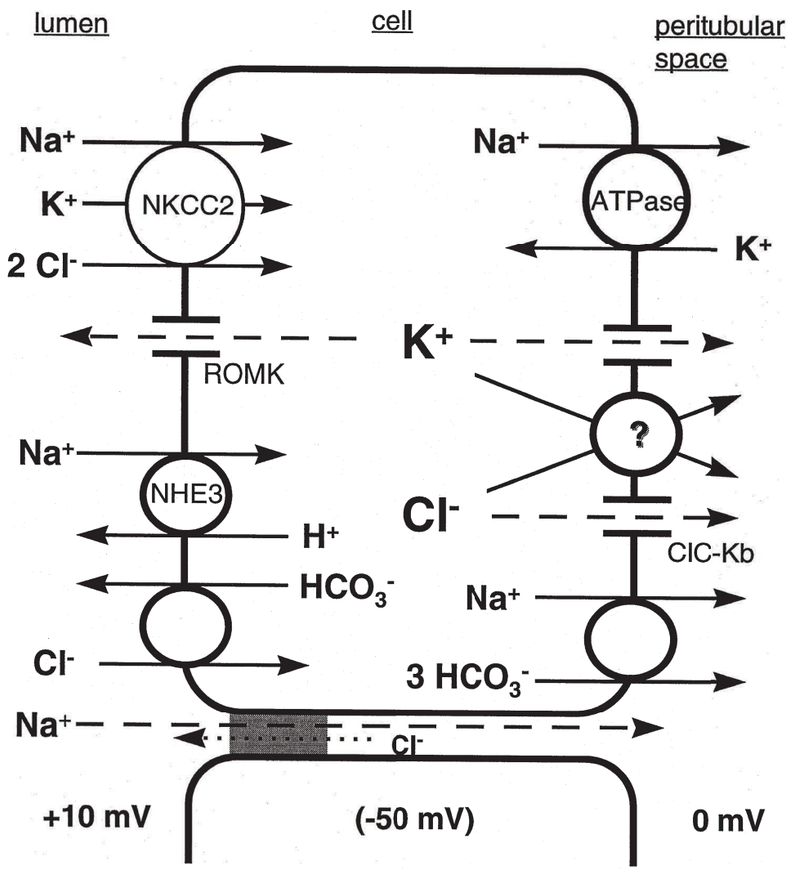

FIGURE 5.6 A model depicting the major elements of the mechanism of NaCl absorption by the thick ascending limb. *Dashed lines* indicate passive ion movements down electrochemical gradients. *ROMK*, renal outer medullary K^+ channel; *ClC-Kb*, chloride channel Kb.

ionic conductance and low water permeability effectively further dilutes fluid entering the TAL from the ascending thin limb.

Figure 5.6 integrates the results of several electrophysiologic and biochemical studies to provide a model of salt reabsorption in the thick ascending limb. According to this model, net Cl^- absorption by the TAL is a secondary active transport process. Luminal Cl^- entry into the cell is mediated by an electroneutral Na^+-K^+-$2Cl^-$ cotransport process driven predominantly by the favorable electrochemical gradient for Na^+ entry.[181] Because the Na^+ gradient is maintained by the continuous operation of the basolateral membrane Na^+,K^+–ATPase pump, the apical entry of Cl^- via the cotransporter ultimately depends on the operation of the basolateral Na^+,K^+–ATPase.

In contrast to the electroneutral entry of Cl^- across the apical membrane, the majority of Cl^- efflux across the basolateral membrane proceeds through conductive pathways.[182,183] A favorable electrochemical gradient for Cl^- efflux through dissipative pathways has been demonstrated by Greger et al.[184] in the rabbit cTAL. Intracellular Cl^- is maintained at concentrations above electrochemical equilibrium by the continued entry of Cl^- via the apical Na^+-K^+-$2Cl^-$ cotransporter.[185]

According to the model in Figure 5.6, K^+ that enters TAL cells via the Na^+-K^+-$2Cl^-$ cotransporter recycles, to a large extent, across the apical membrane via a K^+-conductive pathway. This apical K^+ recycling serves several purposes. First, it ensures a continued supply of luminal K^+ to sustain Na^+-K^+-$2Cl^-$ cotransport. Without recycling, the luminal K^+ concentration would fall rapidly as a consequence of K^+ entry via Na^+-K^+-$2Cl^-$ cotransport and would limit net NaCl absorption. Second, the apical membrane K^+ current provides a pathway for net K^+ secretion by the TAL. In mouse TAL, for example, the rate of K^+ secretion amounts to about 10% of the rate of net Cl^- absorption.[182] K^+ secretion in this segment is an active process, ultimately driven by the Na^+,K^+–ATPase, proceeding in the face of a lumen-positive transepithelial potential. Third, under open circuit conditions, the transcellular and paracellular pathways form a current loop in which the currents traversing the two pathways are of equal size, but which traverse in the opposite direction. The potassium current from cell to lumen polarizes the lumen and causes an equivalent current to flow from the lumen to the bath through the paracellular pathway.[186] Because the paracellular pathway is cation selective ($P_{Na}/P_{Cl} = 2$ to 6), the majority of the current through the paracellular pathway is carried by Na^+ moving from the lumen to the interstitium. This paracellular absorption of Na^+ increases the efficiency of Na^+ transport by the TAL.[187] With reference to Figure 5.6, for each Na^+ transported through the cell and requiring the use of ATP, one Na^+ is transported through the paracellular pathway without any additional energy expenditure. Finally, the apical K^+ current satisfies the continuity requirement imposed by a high degree of conductive Cl^- efflux across basolateral membranes.[182]

A small component of Na^+ transport by the TAL is accounted for by $NaHCO_3$ absorption.[188] In the rat TAL, the rate of $NaHCO_3$ absorption is roughly 5% to 10% of that for NaCl absorption. $NaHCO_3$ absorption appears to be mediated by an apical membrane amiloride-sensitive Na^+/H^+ exchanger and a basolateral membrane electrogenic Na^+-$3(HCO_3^-)$ cotransporter.[188]

The following sections will describe the individual components of the mechanism for TAL salt transport (Fig. 5.6) in greater detail.

Apical Na^+-K^+-$2Cl^-$ Cotransport

Studies of Cl^- transport across apical membranes of intact TAL segments[189] and in isolated membrane vesicle preparations[190] established that the predominant mode for Cl^- entry into the TAL cell is via a Na^+-K^+-$2Cl^-$ cotransporter. A characteristic feature of this transporter is its sensitivity to inhibition by furosemide, bumetanide, and other 5-sulfamoylbenzoic acid derivatives.[191] The measurement of isotope flux into TAL cells or membrane vesicles prepared from the inner stripe of the outer medulla yielded a stoichiometry of 1 Na^+:1 K^+:2 Cl^- cotransport.[190] K^+-independent NaCl cotransport has also been described under certain conditions.[192]

The proteins that mediate the Na^+-K^+-$2Cl^-$ cotransport have been cloned. An absorptive form of the Na^+-K^+-$2Cl^-$ cotransporter, referred to as NKCC2 or BSC1, was initially cloned by Gamba et al.[193] based on sequence homology to the thiazide-sensitive Na^+-Cl^- cotransporter (see the following). A second Na^+-K^+-$2Cl^-$ cotransporter, NKCC1, was cloned by Payne et al.[194] NKCC2 (BSC1) is the primary mediator of apical salt entry in the thick ascending limb. In situ hybridization and single-nephron reverse transcriptase polymerase

chain reaction (RT-PCR) studies demonstrated the expression of NKCC2 in the MTAL and CTAL,[195] and immunohistochemical studies indicate that NKCC2 is localized to the apical membrane of these nephron segments.[196] The importance of NKCC2 in mediating salt reabsorption in the TAL is illustrated by the fact that loss-of-function mutations of NKCC2 cause Bartter syndrome (Table 5.1),[197] a Mendelian salt-wasting disorder characterized by hypokalemia, metabolic alkalosis, hyperaldosteronism, and normal-to-low blood pressure, results from a defect in salt absorption by the thick ascending limb.

The NKCC2 cDNA encodes a glycoprotein containing ~1100 amino acids and having a predicted molecular weight of 115 to 120 kDa.[193] The full-length protein contains 12 transmembrane domains containing a sizable extracellular loop with N-glycosylation sites positioned between transmembrane segments 7 and 8, and large intracellular amino and carboxy termini flank the transmembrane regions. NKCC2 belongs to the SLC12A family of cation chloride cotransporters, which is part of the amino acid polyamine organocation cotransporter (APC) superfamily.[198] Based on the homology to other crystallized APC family members, the cotransporter structure probably consists of two clustered groups of five transmembrane helices that are positioned in a symmetric, inverted orientation.[199] The details regarding how this fold facilitates the three-ion cotransport remain obscure but surely will provide an initial framework for more detailed structure-function studies in the coming years.

The two cytoplasmic domains of NKCC2 mediate specific regulatory functions. The amino terminus is believed to be unstructured and contains several cytosol-exposed serines and threonines, which are phosphoacceptor sites. These residues are phosphorylated by at least three protein kinases that stimulate NKCC2 activity and/or plasma membrane expression (discussed in detail below).[200] The carboxy terminus is large and comprises ~40% of the total NKCC2 sequence. It may also contain phosphorylation sites, although to date this has not been explored in detail. It is clear, however, that the NKCC2 C-terminus serves as a hub for interactions with proteins that regulate its trafficking, including the glycolytic enzyme aldolase[201] and secretory membrane carrier protein 2 (SCAMP2).[202] It also serves as the interface for the formation of NKCC2 homodimers,[203] which was confirmed recently when the crystal structure of the C-terminus of the related prokaryotic cation chloride cotransporter MaCCC was solved.[199]

At least six isoforms of NKCC2 have been identified.[200] These isoforms are the result of alternative splicing of two regions of the NKCC2 gene: the first region is a 96 base pair region that encodes part of the second transmembrane domain, whereas the second region encompasses the extreme C-terminus. Three variants of the 96 base pair region are encoded by different versions of exon 4 of the NKCC2 gene. These exons are differentially spliced into NKCC2 pre-mRNAs to generate three distinct isoforms (A, B, and F), which alter the amino acid composition of the second transmembrane domain. Each of the A, B, and F isoforms can have either a long C-terminus, or a truncated C-terminus; although to date, the short isoforms have only been described in the murine TAL.[204] In addition, several "tandem" transcripts have been described in the human kidney; these contain combinations of exons 4A, 4B, and 4F spliced alongside one another into the NKCC2 pre-mRNA.[205] Transcripts containing exons 4A/4F, 4B/4A, and 4B/4A/4F have been reported. Because these tandem transcripts contain redundant sequences encoding for the second transmembrane domain, they probably cause the misfolding of NKCC2, resulting in the formation of nonfunctional isoforms. Because these isoforms may still form oligomers with NKCC2, they likely exert a dominant-negative effect on NKCC2 function and inhibit its activity.[206]

The A, B, and F isoforms show differential expression within the thick ascending limb. In the rat nephron, the A isoform was found in both the cortical and medullary TAL, the B isoform is restricted to the cortical TAL, whereas the F isoform is present in the medullary, but not the cortical, the TAL, and to a lesser extent, in the outer medullary collecting duct. Although some interspecies discrepancies have been noted, similar findings have generally been observed in the embryonic mouse and human kidney.[205,207,208]

When the 4A, 4B, and 4F exons are spliced into transcripts containing a long C-terminus, all three products are capable of mediating Na^+-K^+-$2Cl^-$ cotransport. However, the A, B, and F isoforms have different transport properties that may have physiologic relevance. Isoforms A and B have higher affinities for Na^+, K^+, and Cl^- than the F isoform. The A isoform possesses the highest transport capacity of all three isoforms. Based on the known distribution of the A, B, and F isoforms in the TAL, it is currently thought that the A isoform accounts for the high transport capacity of the medullary TAL, whereas the presence of the more active A and B isoforms in the cortical TAL allows for the continued reabsorption of salt to take place, even though the tubular fluid in this segment is more dilute than plasma. Supporting this is experimental evidence demonstrating that the NKCC2 A and B isoforms can both be strongly activated by Na^+, K^+, and Cl^- at concentrations that are much more dilute than the composition of tubular fluid in the cortical TAL.[208]

Apical K^+ Conductance

An important feature of the luminal membrane of the TAL is a barium-inhibitable potassium conductance.[185] This apical membrane K^+ conductance allows K^+ to be recycled from the cell back into the luminal fluid to support further NaCl absorption via the NaCl cotransporter. Using measured values of intracellular K^+ activity (rabbit cTAL),[209] apical membrane conductance, and intracellular voltage, it can be shown that the measured apical membrane conductance is sufficient to provide for the recycling of all of the potassium uptake via the Na^+-K^+-$2Cl^-$ cotransporter.

Three types of K^+ conductances have been characterized in the apical membrane of the thick ascending limb: a high-conductance (150 pS) calcium-activated K^+ channel, which

does not contribute to net K^+ transport,[210] an intermediate-conductance (70 pS) K^+ channel,[211] and a low-conductance (30 to 35 pS) K^+ channel.[211,212] The latter two conductances account for the majority of the apical K^+ secretion in the TAL.[213] Both the intermediate- and the low-conductance channels are inhibited by intracellular ATP and are increased by high dietary potassium.[214]

A major breakthrough in elucidating the molecular mechanism of K^+ secretion in the TAL was made when Ho et al.[215] cloned the ATP-dependent K^+ channel ROMK from rat kidney. This channel is the prototype for a large family of inward-rectifying K channels (Kir channels), and hence, is also called Kir 1.1. Based on its biophysical properties and regulation, investigators had long suspected that ROMK was the sole mediator of the 30-pS K^+ conductance in the TAL. This hypothesis was confirmed when studies of the thick ascending limb in the ROMK knockout mouse revealed the absence of a 30-pS channel.[216] Subsequent studies of the ROMK knockout mouse demonstrated that these mice also lack a 70pS conductance.[214] Thus, it is likely that ROMK also comprises at least a portion of the intermediate K^+ secretory conductance in this nephron segment. It has been proposed that the intermediate-conductance channel may be a heteromeric protein containing ROMK and other, as yet, unidentified subunits.

The notion that ROMK is the predominant channel responsible for apical K^+ recycling in the thick ascending limb is also supported by the finding of mutations in the ROMK gene in families with type II Bartter syndrome.[217,218] These mutations have generally been confirmed to result in defective K^+-channel function.[219] As noted, Bartter syndrome results from a defect in thick ascending limb salt transport. Thus, the presence of ROMK mutations as a cause of Bartter syndrome indicates the important role of ROMK in net salt absorption by the thick ascending limb (Table 5.1).

Basolateral Membrane Cl^- Transport

Cl^- exit across the basolateral membrane of TAL cells is largely conductive, proceeding down its electrochemical gradient through Cl^--selective channels in the basolateral membrane.[220] The primary mediator of basolateral chloride transport in the TAL in humans is thought to be the chloride channel ClC-Kb, the sequence of which is closely related to the aforementioned human ClC-Ka channel expressed in the ATL (see previously). The name for the corresponding rodent ortholog of ClC-Kb is ClC-K2. In contrast to the relatively narrow expression of ClC-K1, ClC-K2 is expressed broadly throughout the basolateral membranes of multiple segments in the rodent nephron, including the TAL, the distal convoluted tubule, the connecting tubule, and the collecting duct.[171,221,222] Inherited mutations of the ClC-Kb cause type III Bartter syndrome, which can manifest as a mixed Bartter/Gitelman phenotype, possibly owing to the expression of ClC-K2/ClC-Kb in the distal convoluted tubule (DCT) and TAL.[223]

Similar to ClC-K1/ClC-Ka channels in the ATL, ClC-K2/ClC-Kb channels require barttin accessory subunits to be fully functional. Barttin was originally identified by positional cloning of the BSND gene, which is responsible for type IV Bartter syndrome (Table 5.1), a severe form of hereditary salt wasting that is accompanied by sensorineural deafness.[224] Barttin is located in the basolateral membranes of the thin and thick ascending limb, distal nephron, and also in the stria vascularis of the inner ear, where it is believed to play a role in K^+ secretion into the endolymph. The regulation of ClC-K channels by barttin appears to be multifaceted, because the accessory subunit influences channel protein stability, subcellular localization, and gating.[172,173,175] The mutations of barttin identified in Bartter syndrome patients generally impair the ability of barttin to produce a Cl^- conductance when expressed with ClC-Kb.[172,173]

Although less completely studied, there do appear to be additional transport pathways that mediate basolateral Cl^- flux in the TAL. For example, a barium-sensitive transcellular K^+-Cl^- cotransport mechanism has been proposed (Fig. 5.6).[225] The expression of two KCl cotransporters that belong to the same family of SLC12 electroneutral cotransporters as NKCC2, KCC1,[226] and KCC4[227] have been observed. Thus, both of these cotransporters may participate in the extrusion of K^+ and Cl^- from cells in the cortical and medullary TAL. Studies in knockout animals, however, indicate that KCC4 is not the primary mediator of basolateral Cl^- flux in the TAL, because these mice do not develop a salt-wasting phenotype. Rather, the KCC4 knockout mouse develops metabolic acidosis and sensorineural deafness, suggesting that KCC4 plays important physiologic functions in the acid-secreting intercalated cells of the collecting duct and in the cells of the inner ear.[228]

Synchronous Na^+/H^+:Cl^-/HCO_3^- Exchange

Friedman and Andreoli[229] found that net Cl^- absorption and the transepithelial voltage were doubled when CO_2 and HCO_3^- were added to the external solutions bathing cortical TAL segments. Because the (CO_2 + HCO_3^-)-stimulated rate of NaCl absorption did not result in net CO_2 transport and could be abolished by the lipophilic carbonic anhydrase inhibitor ethoxyzolamide or by the luminal addition of the anion-exchange inhibitor SITS or DIDS, it was proposed that the apical membrane of the mouse cortical TAL contains parallel, near synchronous Na^+/H^+:Cl^-/HCO_3^- exchangers in addition to a Na^+-K^+-$2Cl^-$ cotransporter. The addition of CO_2 and HCO_3^- to the bathing solutions had no effect on net NaCl transport in either the rabbit cTAL or the mouse mTAL. Both the rat and mouse medullary TAL do contain Na^+/H^+ exchangers in their apical membranes. However, in these segments, Na^+/H^+ exchange plays a role in net HCO_3^- transport and cell pH regulation rather than transcellular NaCl absorption.[230] The NHE3 isoform of the Na^+/H^+ exchanger is the major isoform expressed in the apical membrane of the thick ascending limb.[231,232]

REGULATION OF SALT ABSORPTION IN THE TAL

Vasopressin

The peptide hormone arginine vasopressin (AVP, also known as antidiuretic hormone, ADH) remains the most extensively characterized stimulatory hormone for NaCl reabsorption in the TAL. The reabsorption of NaCl in the TAL is crucial for efficient urinary concentration, because it is this process that plays a key role in maintaining a hypertonic medullary interstitial solute gradient for water reabsorption in more distal portions of the nephron.[233] Thus, from a teleologic perspective, vasopressin ought to be an important regulator of this process. The cognate receptor for vasopressin, the V2 receptor (V2R), is expressed in both the cortical and medullary TAL, where it participates in signaling cascades that stimulate NKCC2 activity.[234]

Binding of AVP to V2R results in increased intracellular levels of cAMP in the TAL. The increase in cAMP levels ultimately drives the translocation of NKCC2 from intracellular subapical vesicles to the luminal plasma membrane. In addition, cAMP serves as a signal that increases the phosphorylation of residues in the NKCC2 amino terminus that stimulate cotransporter activity in vitro.[235,236] Although this process may be mediated upstream by the cAMP-dependent protein kinase A (PKA), it is unclear whether PKA directly phosphorylates the N-terminal residues that stimulate its activity. Rather, it appears that two other kinases in particular are important downstream mediators of this process. These kinases, the Ste20 SPS1-related proline alanine-rich kinase (SPAK) and oxidative stress responsive kinase 1 (OSR1), are structurally homologous, activated by vasopressin, bind to a defined docking site harbored within the NKCC2 N-terminus, and directly phosphorylate these previously identified stimulatory residues (Fig. 5.7).[237–239]

The importance of SPAK and OSR1 in NKCC2 function has recently been established in transgenic and knockout models.[237,240,241] A complete knockout of SPAK mRNA and protein expression yields a mild salt-wasting phenotype that approaches Gitelman syndrome, likely owing to the elimination of its activity in the DCT (see the following). However, the absence of a Bartter-like phenotype in these animals does not refute the importance of SPAK and OSR1 in the TAL. Rather, the Gitelman-like salt-wasting phenotype in these animals appears to be due to two factors. First, knockout of the SPAK gene not only ablates full-length kinase-active SPAK, but also eliminates the expression of a truncated kidney-specific kinase-defective SPAK isoform (KS-SPAK) that suppresses baseline SPAK and OSR1 activity in the TAL.[237] Second, in the SPAK knockout mouse, TAL-expressed OSR1 appears to compensate for the absence of SPAK activity. Both of these effects ultimately result in increased rather than decreased NKCC2 abundance and phosphorylation, which probably compensates for the lack of DCT salt reabsorption and gives rise to a relatively mild salt-wasting phenotype. The dominance of OSR1 over SPAK in the TAL was recently verified in studies of the OSR1 knockout mouse, which, in contrast to the SPAK knockout animal, exhibits a Bartter-like phenotype.[241] On the other hand, knock-in mice bearing a mutation that ablates a key catalytic activation site in SPAK do exhibit decreased NKCC2 phosphorylation; presumably, this inactivating mutation exerts a dominant-negative effect on NKCC2 phosphorylation by preventing the binding of stimulatory kinases such as OSR1 to the NKCC2 N-terminal docking site.[242] Although it is clear that SPAK and OSR1 are important kinases that are linked to tubular salt reabsorption in the TAL, it is unclear how they directly connect to the well-established upstream cAMP-dependent signaling cascades, which are mediated by vasopressin. Nevertheless, given the fact that vasopressin activates SPAK and OSR1 and stimulates NKCC2 phosphorylation at key SPAK/OSR1 phosphorylation sites, it would appear that these kinases are important downstream intermediaries of the vasopressin signaling pathway.

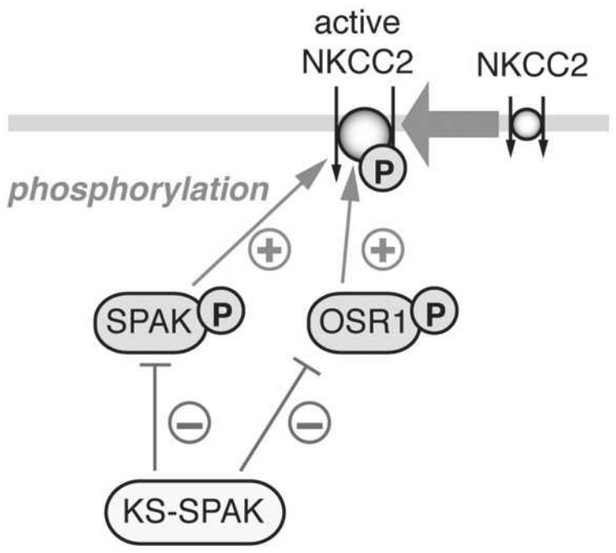

FIGURE 5.7 The current model for SPS1-related proline alanine-rich kinase /oxidative stress responsive kinase 1 (SPAK/OSR1) and kidney-specific (KS)-SPAK regulation of NKCC2 via phosphorylation in the thick ascending limb of the loop of Henle.

In addition to its effects on apical Na^+-K^+-$2Cl^-$ cotransport, vasopressin increases the transcellular electrical conductance of the mouse medullary TAL, and this conductance increase is a major element in the mechanism for the hormone-dependent increase in the rate of net salt absorption.[243,244] The available evidence suggests that both the apical and basolateral membrane conductances are increased by vasopressin. In apical membranes, AVP increases conductance by increasing the functional number of K^+ channels[182]; this increase occurs even when net salt absorption is abolished by furosemide. Ecelbarger at al.[245] showed that this increase in apical K^+ conductance was at least in part due to a dramatic upregulation in ROMK abundance and apical localization. Thus, the machinery for apical recycling of K^+ is increased in the TAL, which would aid in augmenting NKCC2-mediated NaCl reabsorption.

The predominant portion of the ADH-induced increase in cellular conductance is accounted for by an increase in the basolateral membrane Cl^- conductance.[182] Two mechanisms have been suggested for the hormone-

dependent increase in basolateral Cl^- conductance. Schlatter and Greger[183] have proposed that the ADH-induced increase in intracellular cAMP results in a direct increase in Cl^- channel activity. Such a mechanism has been amply demonstrated in Cl^--secreting epithelia, such as the trachea and intestine. Alternatively, ADH might enhance Cl^- conductance indirectly by increasing apical membrane Cl^- entry.[182] According to this proposal, an ADH-dependent activation of apical membrane NKCC2 and ROMK leads to an increase in intracellular Cl^- concentration. Because the activity of basolateral Cl^- channels is exquisitely sensitive to changes in intracellular Cl^- concentration,[246] this increase will translate into an increase in the basolateral membrane Cl^- conductance.

Prostaglandins

Prostaglandin 1 (PGE_2), the major product of prostaglandin synthesis in the renal medulla, participates in a local negative-feedback system that modulates the rate of NaCl absorption by the TAL. PGE_2 resulted in a 50% reduction in ADH-stimulated net Cl^- absorption in isolated perfused mammalian mTAL segments, but had no effect in the absence of ADH.[247] It is likely that PGE_2 inhibits ADH-stimulated generation of cAMP in the mTAL by activating an inhibitory G protein, G_i.[247] Although interstitial cells are a major source of PGE_2 production in the renal medulla, thick ascending limb cells can metabolize arachidonic acid through at least two pathways. Escalante et al.[248] demonstrated that purified medullary thick ascending limb cells produce 20-hydroxyeicosatetraenoic acid (20-HETE) via the cytochrome P450 enzyme, ω-hydroxylase. 20-HETE was subsequently shown to inhibit NaCl transport in the thick ascending limb at steps, which include the apical K^+ channel[249] and the Na^+-K^+-$2Cl^-$ cotransporter.[248] Thick ascending limb cells, particularly in the macula densa (MD), also express COX-2.[250] COX-2 expression in these cells may be coupled to renin secretion. Thus, COX-2 expression is increased by salt restriction, diuretics, and, in Bartter syndrome, all conditions characterized by hyperreninemia.[251] Moreover, COX-2 inhibitors reduce renin secretion in these settings.[252]

Osmolality

Peritubular osmolality is a third factor regulating mTAL salt absorption. In isolated mouse and rabbit TAL segments, increases in peritubular osmolality rapidly and reversibly inhibit the ADH-stimulated rate of net Cl^- absorption.[243] Molony and Andreoli[253] determined that hypertonicity inhibits the basolateral membrane Cl^- conductance. This inhibition of transcellular salt absorption occurs at a locus beyond the generation of cAMP, because supramaximal concentrations of either ADH or cAMP are unable to reverse the hypertonicity-mediated effect.[254] Thus, increasing the absolute magnitude of interstitial osmolality provides a negative feedback signal, which can reduce ADH-dependent salt absorption by the medullary TAL.

Hypercalcemia

Hypercalcemia often results in an ADH-resistant urinary concentrating defect, that is, nephrogenic diabetes insipidus.[255] At least part of this concentrating defect results from the inhibition of ADH-stimulated cAMP production in the TAL by calcium.[256] Preincubation of tubule segments with pertussis toxin abolishes the effect of hypercalcemia on cAMP generation, indicating that the inhibition of cAMP generation is mediated through the activation of G_i.[257] The effects of hypercalcemia are mediated by a G protein–coupled calcium-sensing receptor (CaSR) present on the basolateral membrane of TAL cells.[258] Activation of this receptor also inhibits the activity of the 70-pS apical K channel (ROMK, see previous) via the production of 20-HETE, a P450 metabolite of arachidonic acid.[259,260] Even changes in serum calcium within the physiologic range can alter NaCl absorption via the CaSR.[261] This may help to explain the hypotensive effect of high calcium intake in salt-sensitive hypertensive individuals.[262]

Modulation of NaCl Absorption by Other Peptide Hormones

In addition to ADH, a number of other peptide hormones stimulate adenylate cyclase activity in the TAL. In the mouse and rat, glucagon stimulates NaCl reabsorption and increases the transepithelial potential in isolated microperfused mTAL segments. Calcitonin and PTH stimulate sodium transport in the cortical, but not the medullary, portions of the TAL.[263]

Ang II receptors are present in the thick ascending limb.[264] Ang II has been reported to both stimulate and inhibit sodium transport in the thick ascending limb.[265,266] Chronic infusion of Ang II increased the abundance of NKCC2 in the rat outer medulla by 87%.[267] In a model of heart failure, in which Ang II levels are elevated, NKCC2 expression was also increased and this increase could be prevented by treatment with an angiotensin receptor antagonist.[268]

Adrenergic Agents

β-Agonist-sensitive adenylate cyclase activity is present in the rat, but not the rabbit TAL.[269] Likewise, β-adrenoceptors have been detected along the rat TAL by autoradiographic localization.[270] The physiologic effects of adrenergic agents have been tested in micropuncture and in in vitro microperfusion studies. DiBona and Sawin[271] demonstrated an enhancement of loop NaCl absorption during low-frequency renal nerve stimulation. Acute renal denervation, on the other hand, depressed NaCl absorption by the loop of Henle.[272]

Micromolar concentrations of isoproterenol stimulate net Cl^- absorption by in vitro perfused mouse medullary and cortical TAL.[273] The effects of isoproterenol on NaCl absorption in these segments can be blocked by propranolol.

Nitric Oxide

Acute administration of nitric oxide donor or L-arginine, the substrate for NOS, decreases NaCl absorption in isolated perfused thick ascending limb segments.[274] The effect of

L-arginine on NaCl transport can be blocked by L-NAME, an inhibitor of NOS, indicating that endogenous production of NO mediates the effect of L-arginine. The inhibitory effect of NO on net NaCl absorption appears to involve, at least in part, the inhibition of NKCC2 activity.[275] The thick ascending limb expresses all three isoforms of NOS.[276] Plato et al.[277] used mice deficient in the various NOS isoforms to determine that the effect of L-arginine is mediated by eNOS rather than iNOS or nNOS. In contrast to its effect on NaCl absorption, NO stimulates $NaHCO_3^-$ absorption in the thick ascending limb.[266] Finally, although short-term exposure to NO inhibits NaCl absorption, chronic exposure increases NKCC2 expression,[278] which could translate into increased NaCl absorption.

Sodium Balance

Dietary Na^+ restriction in rats results in a transient decrease in NKCC2 expression,[279] whereas high Na^+ intake has no major effect on NKCC2 expression.[280] Chronic treatment with furosemide in conjunction with a high sodium diet increases NKCC2 expression.[281] The latter phenomenon may account for the development of diuretic resistance and the interdose rebound in sodium absorption in patients chronically treated with loop diuretics.

THE DISTAL NEPHRON

Anatomic Considerations

The distal nephron may be divided into three segments: the DCT, the connecting tubule (CNT), and the collecting duct. Perhaps owing to the nature of the original micropuncture studies characterizing the distal nephron, the DCT was initially thought to be a segment consisting of a homogeneous population of epithelial cells. However, more recent work clearly indicates that the DCT can be further divided into two functionally distinct subsegments, referred to as the "early" and "late" DCT (DCT1 and DCT2, respectively).[282] One of the primary features that distinguishes between the early and late portions of the DCT is the differential sensitivity of these segments to the mineralocorticoid hormone aldosterone. More specifically, the late DCT expresses the enzyme 11beta-hydroxysteroid dehydrogenase 2 (11β-HSD2), which metabolizes cortisol, thereby rendering mineralocorticoid receptors sensitive to aldosterone.[283] For this reason, the late DCT, CNT, and the cortical collecting duct (CCD) are collectively termed the aldosterone-sensitive distal nephron (ASDN).

Na^+ Transport in the Distal Convoluted Tubule and the Connecting Segment

General Characteristics

The DCT absorbs roughly 5% to 10% of the filtered Na^+ load.[284] Fluid enters the DCT with a Na^+ concentration of 25 to 30 mM, but salt is added along the initial 20% of the DCT, so that the Na^+ concentration averages 50 mM at a point 200 to 300 μm from the macula densa.[285] From there, tubular Na^+ concentration decreases along the DCT to a value of approximately 30 mM at the end.[286] Tubular fluid to plasma Na^+ ratios as low as 0.10 have been observed during stationary microperfusion.[287] This finding, together with the presence of the lumen-negative potential difference (see the following), clearly establishes the active nature of Na^+ absorption in this segment.

Na^+ absorption by the DCT is load dependent. That is, over a wide range of delivery rates, the proportion of Na^+ absorbed by the DCT remains constant at 80%.[286] At high tubular fluid flow rates, the fall in luminal Na^+ concentration along the tubule is attenuated; thus, more Na^+ is available to distal Na^+ absorptive sites at high rather than at low flow rates.

Electrophysiologic Considerations

Depending on the type of electrodes that were used, measurements of the transepithelial voltage in the earliest loops of the DCT vary from slightly lumen negative to slightly lumen positive.[288–291] Consistent among all measurements, however, is the observation that the transepithelial voltage becomes progressively more lumen negative as tubular fluid passes to the end of the DCT and into the CNT and CCD.

The progressively lumen-negative transepithelial electrical potential is primarily due to a change in Na^+ transport pathways from the early DCT to more downstream nephron segments. As shown previously by Ellison et al.,[292] Na^+ reabsorption in the early DCT is largely mediated by an electroneutral, thiazide-sensitive NaCl cotransporter, whereas Na^+ absorption in the late DCT also involves an amiloride-sensitive electrogenic pathway.[282] These and other mechanisms of Na^+ absorption are discussed in detail in the following sections.

The Mechanism of Na^+ Absorption

A general model for these mechanism by which Na^+ is reabsorbed in the early and late DCT is presented in Figure 5.8.

The Apical NaCl Cotransport. The absorption of Na^+ and Cl^- in the early DCT are coupled.[293] The coupling of Cl^- to Na^+ entry provides a mechanism to maintain the intracellular Cl^- activity above its electrochemical equilibrium.[294] The early distal tubule is the site of action of thiazide diuretics.[295] Autoradiographic studies[296] and immunocytochemical studies[297] have demonstrated thiazide-binding sites in the apical membranes of DCT and connecting tubule cells.

The thiazide-sensitive NaCl cotransporter (NCC, TSC, SLC12A3) mediates electroneutral NaCl reabsorption in the early and late DCT (Fig. 5.8).[298] The cotransporter shares considerable sequence homology to the bumetanide-sensitive Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) present in the TAL,[193] yet exhibits markedly different inhibitor sensitivity and ionic requirements. NCC transports NaCl with a

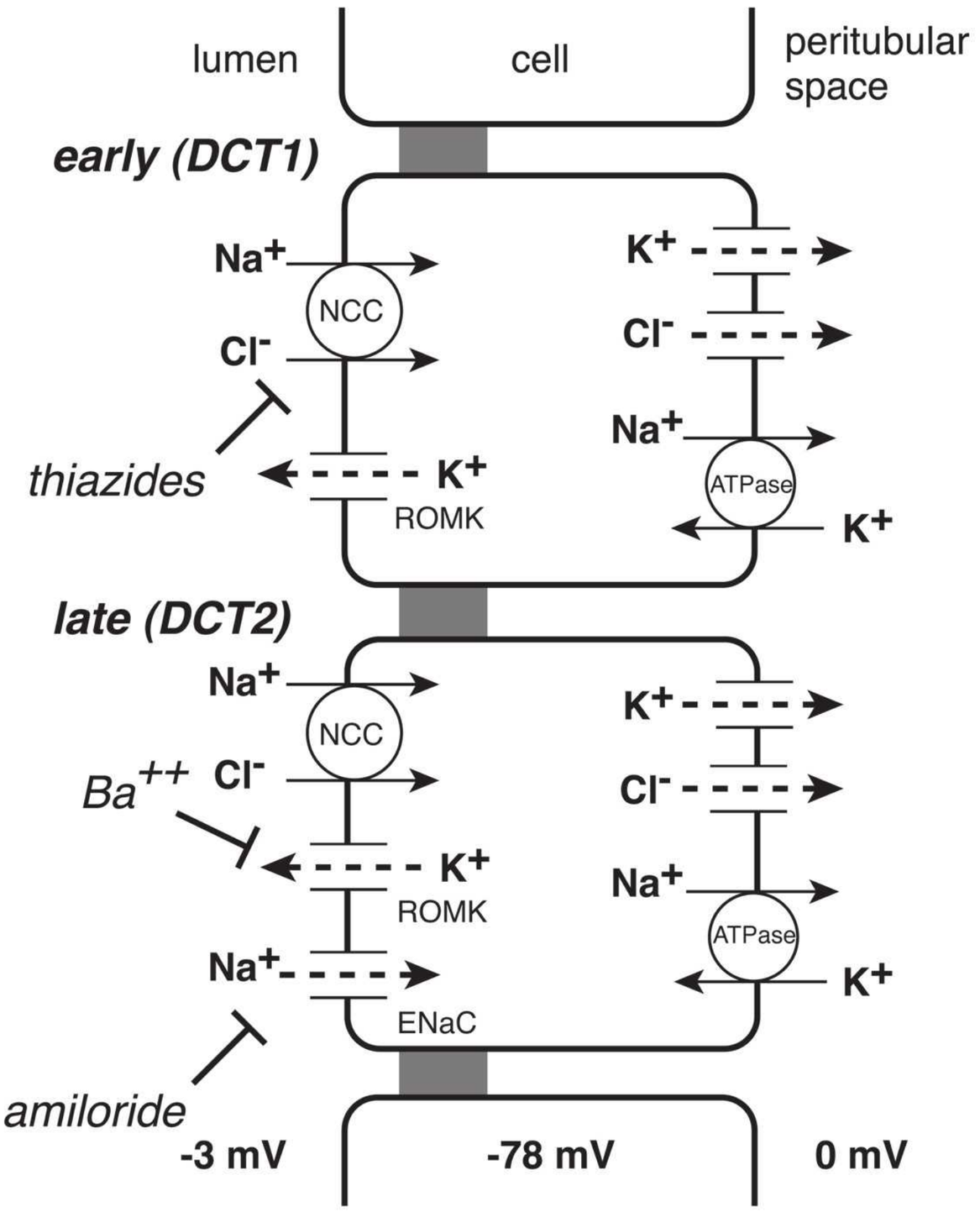

FIGURE 5.8 A model of NaCl absorption by cells of the early and late distal convoluted tubule (DCT). The early and late DCT express NCC, a thiazide-sensitive, electroneutral NaCl cotransporter. The late DCT also expresses the amiloride-sensitive Na^+ channel ENaC. Basolateral K^+ channels include Kir4.1, mutated in EAST/SeSAME (epilepsy, ataxia, sensorineural deafness, and tubulopathy/seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) syndrome, a hereditary salt-wasting disorder. *ROMK*, renal outer medullary K channel; *NCC*, thiazide-sensitive NaCl cotransporter.

1:1 stoichiometry, is K^+-independent, and is inhibited by thiazide diuretics.[299] NCC is expressed in the apical membrane of DCT cells and extends, in most species, into the connecting segment.[300]

Gitelman Syndrome. Loss-of-function mutations in the NCC gene have been linked to the pathogenesis of Gitelman syndrome.[218] This syndrome resembles Bartter syndrome (hypokalemia, alkalosis, sodium wasting), except that urinary calcium excretion is reduced in Gitelman syndrome and is elevated in most cases of Bartter syndrome. Thus, physiologically, Gitelman syndrome mimics the effects of thiazide diuretics. Several studies have evaluated the consequences of mutations causing Gitelman syndrome on NCC function, and in most cases, these mutations alter the NCC coding sequence in such a way that they reduce NCC expression and trafficking to the apical plasma membrane of the DCT.[301,302] In this regard, most of these disease-causing mutations result in the conformational misfolding of NCC, resulting in the recognition of mutant NCC by chaperone-dependent endoplasmic reticulum (ER) quality control machinery that targets the cotransporter for proteasomal degradation.[301,303]

Familial Hyperkalemic Hypertension. Studies of another inherited disorder of distal Na^+ transport, familial hyperkalemic hypertension ([FHHt] type 2 pseudohypoaldosteronism, Gordon syndrome), have yielded additional insights into the regulation of NCC function. FHHt is the phenotypic opposite of Gitelman syndrome and is characterized by hypertension, hyperkalemia, and metabolic acidosis (Table 5.1). The disorder is largely corrected by the infusion of sodium with poorly reabsorbable anions, or by treatment with thiazide diuretics.[304] These features suggested that the hypertension in FHHt is chloride dependent, and that an increase in NCC activity may be involved in the pathogenesis of PHA II. Positional cloning demonstrated that FHHt is caused by mutations in either of two with-no-lysine (WNK) serine-threonine kinases, WNK1 or WNK4.[305] These kinases have been intensely studied since their linkage to FHHt in 2001, and are well-established regulators of NCC, but the mechanisms by which they affect NCC function are complex. Both WNK1 and WNK4 have been shown to regulate NCC plasma membrane trafficking and activity (Fig. 5.9). In the current model it is believed that WNK4 acts as an inhibitor of NCC trafficking at the baseline state because it prevents the cotransporter from trafficking from the biosynthetic pathway to the cell surface.[306–308] In contrast to this inhibitory effect on NCC traffic, it has been hypothesized that under certain physiologic states that favor NaCl reabsorption, WNK4 stimulates NCC phosphorylation. Because NCC is structurally similar to NKCC2, it is activated by the serine–threonine kinases SPAK and OSR1 (see previous section). WNK4 and WNK1 activate SPAK and OSR1, which then can

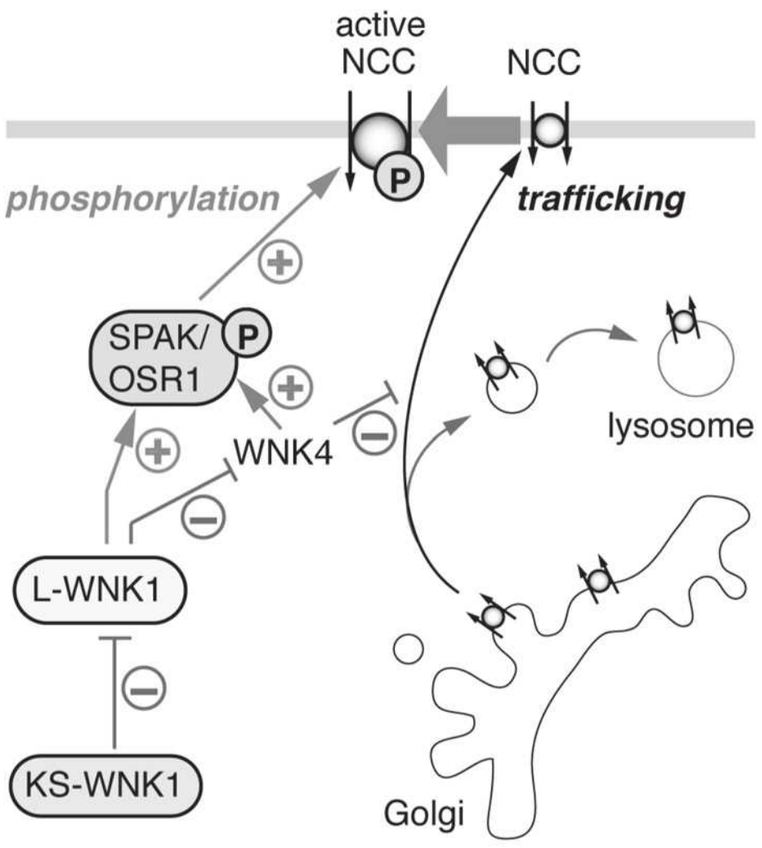

FIGURE 5.9 A model of thiazide-sensitive cotransporter (NCC) regulation by the with-no-lysine SPS1-related proline alanine-rich kinase/oxidative stress responsive kinase 1 (WNK-SPAK/OSR1) signaling pathway. With-No-Lysine (WNK) kinases regulate NCC trafficking and phosphorylation. *L-WNK1*, full-length kinase-active ("long") WNK1; *KS-WNK1*, short kinase-defective "kidney-specific" WNK1.

bind to NCC and stimulate its activity by phosphorylating a cluster of serines and threonines in its NH_2-terminus.[307] In contrast to the TAL, only the full-length kinase active SPAK is expressed in the DCT (i.e., no inhibitory KS-SPAK isoforms are expressed).[237] Due to the observations that WNK4 inhibits NCC under some experimental conditions and stimulates it under others, several have proposed that the WNK signaling pathway acts as a "switch" that converts the DCT from a salt-wasting to a salt-reabsorptive segment, although more work is needed to confirm this hypothesis.[309–311] In FHHt, missense mutations of WNK4 stimulate NCC activity through two mechanisms. First, mutant WNK4 is incapable of reversing the inhibitory effect on NCC at the baseline; this results in increased NCC surface expression.[308,312] Second, the FHHt-associated mutant increases NCC phosphorylation, presumably through enhanced SPAK/OSR1 activity. Thus, through incompletely defined mechanisms, mutant WNK4 appears to "lock" the DCT into a high Na^+ reabsorptive state by increasing the total number of active NCC molecules at the cell surface, resulting in thiazide-sensitive hypertension.[313]

In contrast, WNK1 appears to influence NCC traffic indirectly by suppressing the inhibitory effect of WNK4 (Fig. 5.9). This effect requires intact WNK1 kinase activity and releases NCC from intracellular retention, thereby facilitating NCC delivery to the cell surface.[307,308] Unlike WNK4, WNK1 mutations that cause FHHt do not alter the kinase's coding sequence. Rather, WNK1 mutations are large intronic deletions that increase the mRNA abundance of WNK1 isoforms.[305] Kinase-active WNK1 is capable of phosphorylating and activating SPAK and OSR1.[314] Thus, the WNK1 gene mutations that cause FHHt presumably do so by reversing the inhibitory effect of WNK4 on NCC traffic and by stimulating NCC phosphorylation.[315] As with SPAK gene expression in the TAL (see previous), long and short WNK1 isoforms are expressed in the DCT. The long form of WNK1 (L-WNK1) is kinase active and stimulates NCC activity and trafficking through the mechanisms described previously. A short kidney-specific kinase-defective product (KS-WNK1), in contrast, suppresses L-WNK1 activity (Fig. 5.9).[316] The balance of these two isoforms has been postulated to act as a "switch" that controls overall L-WNK1 kinase activity in the DCT, which should in turn regulate NCC activity.[310] Consistent with this idea, selective knockout of KS-WNK1 expression increases NCC activity, whereas overexpression of the kidney-specific isoform in KS-WNK1 transgenic mice causes salt-wasting through NCC inhibition.[317]

The Basolateral Electrogenic Na^+ Pump. In both subsegments, the basolateral extrusion of Na^+ from the cytosol into the peritubular space (and eventually, the plasma) occurs via the electrogenic Na^+,K^+–ATPase. The activity of this pump results in the generation of a constant transepithelial voltage across the basolateral membrane of −60 to −90 mV.[318,319] A reduction in the luminal sodium concentration causes V_{bl} to depolarize, whereas increases in the sodium concentration hyperpolarizes V_{bl}. In addition, V_{bl} depolarizes after ouabain treatment.[320] These observations are consistent with the notion that apical Na^+ entry stimulates the electrogenic Na^+,K^+–ATPase system in the basolateral membrane.

Basolateral K^+ Efflux. Recent insights from rare Mendelian diseases highlight the importance of basolateral K^+ transport in tubular sodium transport in the distal nephron. In 2008, two groups identified that patients with mutations in the K^+ channel gene KCNJ10 (Kir4.1) develop hereditary salt wasting.[321,322] Patients with these mutations develop a complex constellation of neurologic defects in addition to the renal salt wasting. The disease has been named EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) by some investigators and SeSAME syndrome (seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance) by others (Table 5.1). The salt-wasting phenotype in these patients is reminiscent of Gitelman syndrome, suggesting that the disorder results in an impairment of salt transport in the DCT. Indeed, Kir4.1 is expressed on the basolateral membrane of DCT cells, and patients with EAST/SeSAME syndrome mutations develop markedly reduced infoldings of the DCT basolateral membrane. This deficiency in total basolateral membrane content results in a decrease in the number of surface Na^+,K^+–ATPase molecules, resulting in decreased sodium pump activity and impaired salt reabsorptive capacity.[323] Additionally, it is thought that loss-of-function mutations of Kir4.1 impair K^+ recycling across the basolateral membrane, which reduces the efficacy of Na^+,K^+–ATPase (Fig. 5.8).[321]

Basolateral Cl^- Transport. As mentioned previously, ClC-Kb channels and barttin are both expressed in the DCT, where they mediate basolateral Cl^- reabsorption.[172] Because the apical transport mechanism in the DCT via NCC occurs through the coupled reabsorption of Na^+ and Cl^-, basolateral Cl^- transport is essential to the development of a gradient for Na^+ entry and to reduce the intracellular Cl^- concentration, which stimulates SPAK/OSR1–mediated NCC phosphorylation.[324] Mutations in either ClC-Kb or the accessory subunit result in impaired distal salt reabsorption. Because barttin is required for adequate basolateral Cl^- reabsorption in the loop of Henle and DCT (see previous), patients with barttin mutations develop a more severe form of Bartter syndrome.[172]

Apical Conductive Na^+ Channels. The entry of sodium into the Amphiuma distal tubular cell[325] and late rat DCT cell[326] is inhibited by amiloride, a Na^+ channel blocker. A Na^+ channel in the apical membrane would serve to depolarize the membrane and create the observed lumen-negative transepithelial potential. This transepithelial voltage, in turn, is a driving force for passive Cl^- reabsorption. Na^+ channel subunits have been found by immunolocalization in the late DCT in mouse and rat kidney,[327,328] but not the human kidney.[329]

The Regulation of NaCl transport in the Distal Convoluted Tubule

Na^+ Delivery. NaCl reabsorption in the DCT is dependent on the delivered load of NaCl.[286] The DCT responds to chronic increases in the delivery of NaCl with an increase in the capacity for NaCl transport,[330] as well as marked ultrastructural changes in the DCT cell. These morphologic changes include an increase in the size of the DCT cell, an increase in the basolateral membrane surface area, and an increase in the size of mitochondria.[330] Accompanying the functional and morphologic changes are an increase in Na^+,K^+–ATPase activity and an increase in thiazide-binding sites.[331] These effects appear to result from an increase in Na^+ entry into the DCT cell rather than the increase in distal NaCl delivery or changes in plasma aldosterone or ADH levels that occur with chronic furosemide treatment. Inhibition of NaCl entry into DCT cells with chronic thiazide treatment resulted in a loss of cell height, loss of normal polarity, and apoptosis of the DCT cells.[332] The cellular mechanisms whereby NaCl entry affects transport function and morphology are not known.

Dietary Na^+. Studies in rats and rabbits have yielded conflicting results regarding the effects of increased dietary Na^+ on DCT morphology and Na^+ transport. In rats, no consistent effect of a high Na^+ diet on either cell morphology, transport rates, or thiazide-receptor density could be demonstrated.[330,331,333] Rats fed a low Na^+ diet[279] or treated with thiazide diuretics,[281] however, demonstrated a significant increase in NCC expression, which may have been because of the stimulation of mineralocorticoid secretion.[334] In contrast, rabbits fed a high Na^+ diet developed an increased rate of DCT Na^+ reabsorption and an increase in Na^+,K^+–ATPase activity.[335]

Steroid Hormones. Currently available evidence suggests that adrenal steroid hormones regulate Na^+ transport in the DCT. The presence of both mineralocorticoid and glucocorticoid receptors in the DCT has been demonstrated by immunohistochemistry and by hormone binding.[283,336,337] In addition, an adrenalectomy resulted in a decrease in Na^+,K^+–ATPase activity in the DCT.[338] The Na^+,K^+–ATPase activity could be restored by replacement doses of glucocorticoids, but not by mineralocorticoids.[338,339] Microperfusion studies of superficial distal tubules (containing both DCT and CNT), however, demonstrated an increase in Na^+ transport in animals receiving aldosterone infusions.[340,341] Both the thiazide-sensitive and the thiazide-insensitive components of Na^+ transport were increased by aldosterone.[341] The former may reflect neutral NaCl cotransport in the DCT, whereas the latter reflects electrogenic Na^+ absorption in the late DCT or CNT. Aldosterone infusion also resulted in an increase in thiazide-binding sites in the renal cortex, as determined by [^{3}H]metolazone binding, an increase in the natriuretic response to thiazide diuretics, and a large increase in NCC protein.[341–343] These findings establish NCC as an aldosterone-regulated transporter. By combining immunohistochemical and in situ hybridization techniques, Bostanjoglo et al.[283] determined that DCT cells coexpress NCC, mineralocorticoid receptors, and 11beta-hydroxysteroid dehydrogenase type 2, an enzyme typically found in mineralocorticoid target sites. Thus, DCT cells, particularly those in the late portions of the DCT, express the key elements required for selective mineralocorticoid actions.

In 1998, Kim et al.[343] showed that aldosterone increases the total protein abundance of NCC. This effect did not correlate with changes in NCC mRNA abundance, suggesting that aldosterone regulates NCC abundance by posttranslational mechanisms. Recent work indicates that at least two molecular mechanisms account for this effect. Aldosterone rapidly induces and activates the serine–threonine kinase serum and glucocorticoid-regulated kinase 1 (SGK1), which directly phosphorylates and inactivates two inhibitors of NCC trafficking, WNK4 (described previously), and neural precursor cell-derived, developmentally downregulated 4-2 (Nedd4-2), an E3 ubiquitin ligase.[344,345] SGK1-mediated phosphorylation of two residues located at the C-terminus of WNK4 releases NCC from inhibition. This results in WNK4 inactivation and diverts the cotransporter away from intracellular degradation pathways, allowing NCC to traffic directly to the plasma membrane from the biosynthetic pathway.[344] By phosphorylating Nedd4-2, SGK1 suppresses the ability of the E3 ligase to attach ubiquitin molecules to NCC; this

reduces NCC degradation and increases its plasma membrane expression.[345] Both of these effects provide an explanation for the aldosterone-induced increase in NCC total protein abundance that was initially seen by Kim et al[343] Aldosterone also appears to stimulate NCC transport activity by promoting NCC phosphorylation, an effect that occurs independently of the effects of mineralocorticoids on NCC traffic.[346,347] The mechanism by which this effect occurs remains unclear but appears to correlate directly with increased SPAK/OSR1 activity, suggesting that additional connections between aldosterone and the WNK/SPAK/OSR1 signaling pathway may exist.

As mentioned previously, glucocorticoids increase Na^+,K^+–ATPase activity following an adrenalectomy in the DCT.[338,339] This effect was not blocked by spironolactone, a mineralocorticoid receptor antagonist, suggesting that glucocorticoids were acting via glucocorticoid receptors rather than mineralocorticoid receptors.[339] In addition, dexamethasone infusions increased thiazide-sensitive NaCl transport and [^{3}H]metolazone binding sites in adrenalectomized rats.[341,342] Nevertheless, the role of glucocorticoids in the physiologic regulation of Na^+ transport in the DCT remains unclear.

Gonadal steroid hormones may also influence NaCl transport in the DCT. Chen et al.[348] reported gender differences in the density of thiazide receptors and in the natriuretic response to thiazides in rats. Female rats had higher levels of thiazide-binding sites in the renal cortex than males. The levels in females fell following ovariectomy, whereas levels rose in males following orchiectomy. Moreover, the increase in urinary Na^+ excretion caused by thiazides was greater in females than in males, suggesting that the differences in thiazide-binding sites were reflective of differences in thiazide-sensitive salt transport in vivo. Likewise, using antibodies against NCC, Verlander et al.[349] found that estrogen treatment increased NCC expression in the DCT. These results are consistent with the view that male sex hormones (e.g., testosterone) may downregulate NCC expression and salt transport, whereas estrogens increase NCC expression and salt transport in the DCT. The authors are not aware of gender differences in the response of humans to thiazide diuretics.

Vasopressin. Recent work indicates that vasopressin is a potent activator of the thiazide-sensitive cotransporter.[350,351] Two groups have shown that the vasopressin analog deamino-Cys-1, d-Arg-8 vasopressin (dDAVP) stimulates increases in NCC abundance, trafficking to the plasma membrane, and activation through SPAK/OSR1-mediated phosphorylation of its amino terminus. This effect is probably dependent on cyclic AMP and protein kinase A (PKA), well-established intermediaries of vasopressin-dependent signaling.[352] The effect on NCC abundance may be a Nedd4-2-dependent process, because PKA can phosphorylate and inactivate Nedd4-2 through mechanisms similar to SGK1[353]; however, this hypothesis is yet to be tested.

Na^+ TRANSPORT IN THE CORTICAL COLLECTING DUCT

General Considerations

The transport processes in the collecting duct mediate final adjustments in urinary composition. The collecting duct is a major locus of action of mineralocorticoid hormones and plays a major role in K^+ homeostasis and acid–base balance. Quantitatively, it is a minor site of Na^+ absorption, reclaiming only about 2% to 4% of the filtered Na^+ load.[354]

Electrophysiologic Aspects

The transepithelial voltage in the CCD varies widely from +10 to −100 mV,[295,355,356] which largely results from differences in mineralocorticoid levels at the time of measurement in the animals. The reported values for transepithelial resistance also vary widely depending on apical Na^+ and K^+ channel activities, as shown by the effects of luminal amiloride and barium, respectively, on transepithelial resistance.[356,357] The basolateral membrane is conductive to K^+ and, at least in rabbits, Cl^-.[357]

Mechanisms of Salt Absorption in Collecting Ducts

A proposed model for Na^+ absorption and K^+ secretion in the CCD is presented in Figure 5.10. As indicated previously, apical membranes of CCD principal cells possess conductive pathways for Na^+ and K^+.[358,359] Na^+ enters principal cells through Na^+ channels in the apical membrane down its electrochemical gradient. Na^+ is then pumped across the basolateral membrane by the Na^+,K^+–ATPase in exchange for K^+. The Na^+ current across the apical membrane depolarizes

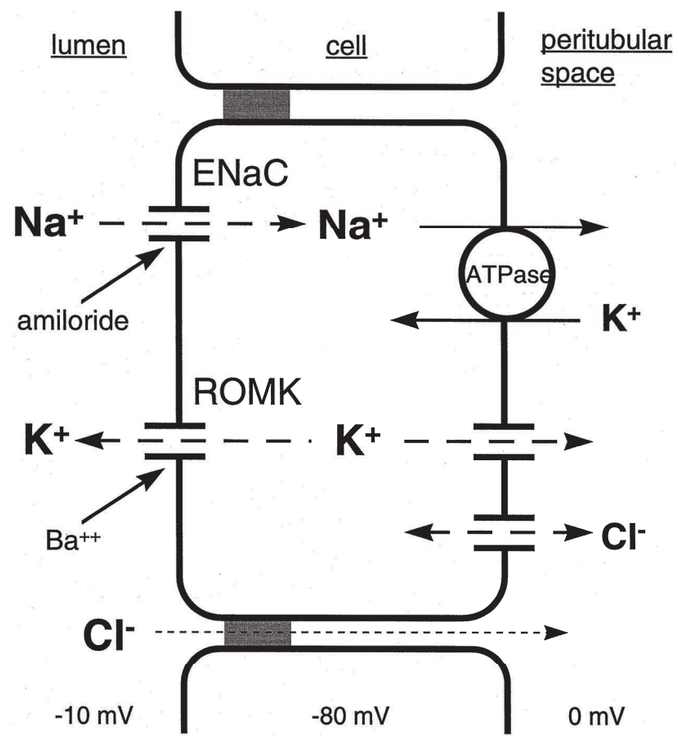

FIGURE 5.10 A model of salt transport by the principal cell of the cortical collecting duct. Apical Na^+ entry proceeds via amiloride-sensitive Na^+ channels (ENaC). Apical K^+ channels (ROMK) mediate K^+ secretion by this segment. Cl^- absorption is driven by the lumen-negative voltage through the paracellular pathway.

the cell, so that the cellular K^+ is above its equilibrium concentration, and thus leaves the cell through conductive pathways in the apical or basolateral membrane.[360]

Apical Na^+ Channels

Apical Na^+ entry depolarizes the apical membrane relative to the basolateral membrane, causing a lumen-negative transepithelial voltage, which in turn provides the driving force for Cl^- reabsorption through the paracellular pathway. Patch-clamp studies of collecting duct cells and urinary bladder cells provided some details regarding the electrophysiologic properties and regulation of apical membrane Na^+ channels.[361] The amiloride-sensitive epithelial Na^+ channel (ENaC) has been cloned,[362,363] has a single-channel conductance to Na^+ of 4 to 5 pS, and is highly selective for Na^+ over K^+ ($P_{Na}/P_K > 20$). ENaC exhibits slow gating, with openings and closings lasting several seconds. Amiloride blocks ENaC at submicromolar concentrations.[364]

ENaC consists of three homologous subunits (α, β, and γ).[363] The subunits share a common structure consisting of two transmembrane domains, intracellular N- and C-termini, and a large extracellular loop.[365,366] Although the α subunit of ENaC can form Na^+ channels on its own,[362] coexpression of all three subunits dramatically increases the membrane Na^+ conductance.[363] Based on homology to the related acid-sensing ion channel (ASIC1), the crystal structure of which was recently solved,[367] native ENaC likely exists as an α, β, and γ heterotrimer. The single channel properties of the expressed channel closely resemble those of the 4 to 5 pS highly selective channel studied in native tissues.[363] The large extracellular domains of the α and γ subunits can get proteolytically cleaved by furin in the biosynthetic pathway.[368,369] Other proteases like prostasin and plasmin may cleave the γ subunit.[370] These cleavage events release small inhibitory fragments of the α and γ subunits, causing an increase in the open probability of ENaC.[370] ENaC activity is also enhanced by increasing luminal flow rates, as the extracellular domain of ENaC subunits respond to laminar shear stress.[371,372] Thus, collecting duct Na^+ absorption is enhanced with increased distal fluid delivery via ENaC, a mechanosensitive channel.[373]

The key importance of ENaC for Na^+ reabsorption in the collecting duct is highlighted by naturally occurring mutations in ENaC that are responsible for Liddle syndrome, an autosomal dominant form of hypertension, and pseudohypoaldosteronism type 1, an autosomal recessive form of salt wasting (see Table 5.1).[374] In Liddle syndrome, mutations in the ENaC subunits result in an increase in amiloride-sensitive Na^+ channel activity with a consequent increase in sodium reabsorption and volume-mediated hypertension.[375–377] Most Liddle mutations occur in the cytoplasmic C-termini of the γ (SCNN1G) and β (SCNN1B) subunits.[375] These mutations affect a conserved PY motif in the C-terminus, which is necessary for interaction with the ubiquitin ligase Nedd4-2 and subsequent internalization and degradation of ENaC.[378] Liddle syndrome mutations cause excessive ENaC accumulation at the apical plasma membrane, increasing Na^+ absorption. Pseudohypoaldosteronism type 1 (PHA-1), the clinical opposite of Liddle syndrome, is caused by homozygous inactivating mutations in the ENaC channel, resulting in a syndrome of Na^+-wasting, hypotension, and hyperkalemia.[379] The majority of mutations causing PHA-1 are frameshift or nonsense mutations that result in truncated, nonfunctional ENaC proteins.[374]

Apical Electroneutral Na^+ Transport

It was first observed in rats that a portion of Na^+ entry across the apical membrane in perfused CCD segments was sensitive to luminal hydrochlorothiazide, which inhibited Na^+ and Cl^- absorption without changing the transepithelial voltage.[380] In addition, amiloride inhibited Na^+ transport in this segment by only 50%, and the effects of amiloride and hydrochlorothiazide were additive. It was thus concluded that the CCD may possess two parallel transport pathways for Na^+: an electrogenic pathway involving amiloride-sensitive Na^+ channels and a thiazide-sensitive neutral NaCl cotransport pathway.[380] More recently, a Na^+-dependent Cl^-/HCO_3^- exchanger (NDCBE/SLC4A8) was identified in intercalated cells and found to mediate the amiloride-resistant, thiazide-sensitive electroneutral Na^+ reabsorption in the CCDs of mice.[381] That NCC knockout mice also exhibited significant natriuresis following treatment with thiazide diuretics suggests the importance of this pathway. The mechanism for net NaCl reabsorption in this setting involves the parallel activity of the NDCBE and Na^+-independent Cl^-/HCO_3^- exchange via pendrin/SLC26A4 at the apical membrane of type B intercalated cells. The basolateral reabsorption pathways for the Na^+ and Cl^- that enter at the apical membrane in this model are the Na^+,K^+–ATPase and ClC-K Cl^- channels. Thus, a novel target of thiazide diuretics distinct from NCC has been identified in the collecting duct with the implication that Cl^- transport by intercalated cells plays an important role in blood pressure regulation.[382] Because pendrin is a key mediator of bicarbonate secretion, and thus acid–base regulation, these findings also suggest a strong link between acid–base and volume/blood pressure regulation.

Control of Na^+ Absorption in the Cortical Collecting Duct

Aldosterone

Aldosterone is one of the key regulators of Na^+ transport in the collecting duct, where it increases the rates of Na^+ absorption and K^+ secretion.[383,384] The major target site for mineralocorticoid effects is the principal cell of the CCD, although actions in the DCT have also been documented (see previous). Mineralocorticoid effects are produced by the binding of either mineralocorticoids or glucocorticoids[385] to mineralocorticoid receptors found predominantly in the CCD.[336,337] In addition, the binding of glucocorticoids to glucocorticoid receptors can also produce mineralocorticoid responses.[385] This lack of specificity results from two

factors: first, mineralocorticoid receptors do not discriminate between aldosterone and glucocorticoids,[386] so that either class of steroids can bind to and activate the receptor. Second, the DNA binding domains of mineralocorticoid and glucocorticoid receptors are highly conserved such that both receptors can activate many of the same genes.[387] The specificity for mineralocorticoids in vivo is provided by the selective degradation of glucocorticoids, but not mineralocorticoids, by the enzyme 11 β-hydroxysteroid dehydrogenase.[386] 11β-Hydroxysteroid dehydrogenase activity is high in CCD segments.[388,389] Illustrating the important role of this enzyme in regulating access of hormones to the mineralocorticoid receptor, genetic deficiency of this enzyme produces a syndrome, apparent mineralocorticoid excess, resembling hyperaldosteronism (hypertension, hypokalemia, metabolic alkalosis), except that aldosterone levels are low.[390] The clinical manifestations result from the stimulation of mineralocorticoid receptors by circulating glucocorticoids.

In isolated perfused CCDs from mineralocorticoid-treated rabbits, there is an increase in both the Na^+ and K^+ conductance of the apical membrane and an increase in basolateral Na^+,K^+–ATPase activity.[356] The functional changes after aldosterone treatment are accompanied by morphologic changes in principal cells. The basolateral membrane length of principal cells falls by 35% after an adrenalectomy, and the administration of aldosterone, but not dexamethasone, restores the membrane length to control levels.[391]

An early effect of aldosterone, occurring within a few hours of exposure, is an increase in the sodium permeability of the apical membrane of the CCD principal cell. Results from electrophysiologic and immunologic studies support the view that the early aldosterone-induced increase in apical sodium permeability is because of the activation of quiescent Na^+ channels rather than the synthesis and/or the insertion of new Na^+ channels into the membrane.[392–394] Several pathways have been implicated in the aldosterone-mediated increase in Na^+ channel activity. An important downstream mediator of aldosterone is the serum and glucocorticoid-regulated kinase SGK1.[395] SGK1 is expressed in the thick ascending limb, DCT, connecting segment, and cortical collecting tubules.[396] Aldosterone increases the transcription of SGK1 in vitro,[395] although the effects of aldosterone on SGK1 protein expression in vivo appear to be minor.[396] Coexpression of SGK1 with ENaC results in markedly greater sodium currents than seen with ENaC alone.[395,397] SGK1 is required for the stimulation of sodium transport by aldosterone both in vitro[397] and in vivo.[398] SGK1 increases sodium currents by increasing cell surface expression of ENaC[399] and also increasing the open probability of individual ENaC channels.[400] The effect of SGK1 on cell surface expression of ENaC is largely mediated by Nedd4-2. Specifically, Nedd4-2 is a substrate for SGK1. Upon phosphorylation by SGK1, the affinity of Nedd4-2 for the PY domains of ENaC is diminished,[401] whereas Nedd4-2 binding to 14-3-3 scaffolding proteins is increased.[402,403] Thus, 14-3-3 proteins act to sequester Nedd4-2 and prevent it from binding to ENaC. As discussed earlier, the binding of Nedd4-2 to ENaC induces internalization and degradation of the channel.

Recently, additional aldosterone-induced proteins have been discovered, including the glucocorticoid-induced leucine zipper protein (GILZ1), the transcription of which is rapidly induced by aldosterone in collecting duct principal cells. GILZ1 stimulates ENaC-mediated Na^+ transport and ENaC surface expression by inhibiting Raf-1 in the extracellular signal-regulated kinase (ERK) signaling pathway.[404,405] Soundararajan and colleagues[406] have also identified another aldosterone-induced protein, CNK3 (connector enhancer of kinase suppressor of Ras 3), which also stimulates ENaC and appears to be a scaffolding protein. The emerging story is that a multiprotein complex exists in CCD cells that promotes context-specific aldosterone signal transduction to induce ENaC activity and apical Na^+ conductance. Specifically, aldosterone stimulates ENaC activity through synergistic aldosterone-dependent activation by SGK1 via Nedd4-2 and by GILZ1 via Raf-1 and CNK3.

Nongenomic effects of aldosterone (rapid effects that are mineralocorticoid receptor-independent) have also been described in many tissues, including the kidney.[407] Elucidating the mechanisms of such effects is an area of active investigation and appears to include the mitogen-activated protein kinase (MAPK) pathway and the methylation of proteins and lipids. Aldosterone also increases the activity of a number of cellular methyltransferase enzymes.[408] Moreover, the β subunit of ENaC itself is a substrate for methylation, and, when methylated, exhibits increased Na^+ transport activity.[409] The effects of aldosterone on Na^+ channel activity may also involve small GTP-binding proteins, for example, Ras,[410] and phosphatidylinositol 3-kinase.[411] SGK1 is a downstream mediator of phosphoinositide-3-kinase.[412]

Sometime after the increase in apical membrane Na^+ conductance occurs, the basolateral membrane Na^+,K^+–ATPase activity and pump current increase.[413] This increase is due initially to the effect of increased cell sodium activity on existing pump units.[414] Later, aldosterone induces the synthesis of additional Na^+,K^+–ATPase pump subunits.[415] Increased apical membrane Na^+ entry may promote the late synthesis of Na^+,K^+–ATPase, because the inhibition of sodium entry by amiloride markedly reduced the aldosterone-induced increase in Na^+,K^+–ATPase activity.[416]

Aldosterone also exerts an additional, late effect on the amiloride-sensitive Na^+ conductance. Patch-clamp studies in rats exposed to high levels of aldosterone for several days demonstrated a large increase in the amiloride-sensitive whole cell Na^+ conductance,[417] which correlated with an increase in the number of active Na^+ channels in the apical membrane.[418] Increases in ENaC mRNA[419] and protein[334] levels in aldosterone-treated tissues suggest that the synthesis of new ENaC channels may contribute to the late aldosterone-induced increase in Na^+ conductance. SGK1, in addition to increasing the cell surface expression of ENaC, also regulates the transcription of ENaC subunits, principally α.[420]

Antidiuretic Hormone (ADH)

Exposure of rat CCD segments in vitro to ADH results in a sustained stimulation of Na^+ absorption.[421] ADH increases the transepithelial potential, depolarizes the apical membrane, and increases the conductance of the apical membrane of principal cells.[422] These changes are entirely reversed by luminal amiloride, indicating that ADH increases the apical membrane sodium conductance of the principal cell. These effects of ADH are mediated intracellularly by cAMP.[423] Moreover, the effects of ADH on Na^+ transport in the rat CCD are enhanced by prior treatment of the animals with mineralocorticoids.[421]

ADH and aldosterone increase apical membrane ENaC activity through both overlapping and distinct mechanisms of action. Like aldosterone via SGK1, ADH induces enhanced PKA-dependent phosphorylation of Nedd4-2 at phosphorylation sites that overlap those of SGK1.[353] As described previously, these phosphorylation events promote sequestration of Nedd4-2 through enhanced binding to 14-3-3 proteins. In contrast to aldosterone, which can activate quiescent channels, ADH, via cAMP, promotes the insertion of additional Na^+ channels into the apical membrane.[424] In addition, PKA-mediated phosphorylation may also directly stimulate the activity of ENaC channels already present in the apical membrane, potentially both directly[425] and indirectly via Nedd4-2 inhibition, causing enhanced channel residency time at the membrane and thus proteolytic cleavage.[370] Long-term exposure to ADH may also increase the capacity of the collecting duct for Na^+ transport by increasing the expression of both Na^+,K^+–ATPase and ENaC.[426]

Other Agents

Bradykinin is produced in the connecting duct[427] and binds to specific receptors in the CCD.[428] An intrarenal infusion of bradykinin produces a diuresis. Bradykinin has been reported to reduce Na^+ absorption in rat CCD segments.[429] More recently, bradykinin was shown to acutely and reversibly decrease the open probability of ENaC in patch-clamp studies performed ex vivo on split open CCDs isolated from rats. This effect appears to be mediated specifically through the bradykinin B_2 receptor.[430]

As will be discussed in the following section, the major site of action of atrial natriuretic peptide (ANP) is the inner medullary collecting duct. ANP stimulates cGMP production in the CCD,[431] and inhibits the hydro-osmotic actions of ADH in the CCD.[432] Biphasic effects of ANP on conscious, sedated rats have been observed with acute water and sodium excretion within minutes, followed by retention after 90 minutes, which was associated with increased apical membrane expression of α- and γ-ENaC. These delayed effects may represent a compensatory response to increase sodium and water reabsorption and prevent volume depletion in response to prolonged ANP infusion.[433]

α2-Adrenergic agonists inhibit sodium reabsorption in the rat CCD.[434] This inhibition is associated with an increase in the apical membrane resistance, reflecting decreased Na^+ entry through Na^+ channels. These changes appear to result from an inhibition of adenylate cyclase and antagonism of ADH by α2 agonists.[435]

Prostaglandin E_2 exerts diuretic and natriuretic effects on the kidney. Part of this action is mediated by an inhibition of Na^+ absorption in the CCD.[436] The application of PGE_2 to the basolateral surface of perfused rabbit CCD segments reversibly inhibits the negative transepithelial voltage and net sodium absorption.[436] The effect of PGE_2 on sodium transport is coupled to a rise in intracellular [Ca^{2+}] and is dependent on the activation of PKC.[437] Four PGE_2 receptor subtypes, designated EP1, EP2, EP3, and EP4, have been characterized. Studies using receptor subtype–specific agonists and antagonists suggest that the EP1 receptor mediates PGE_2-dependent inhibition of Na^+ transport in the CCD.[438] This inhibition of Na^+ transport is associated with depolarization of the apical membrane voltage, consistent with inhibition of the basolateral Na^+,K^+-ATPase.[439] In contrast to the inhibitory effect of basolateral PGE_2 on Na^+ transport, luminal PGE_2 increases transepithelial voltage and presumably Na^+ absorption via the EP4 receptor.[440]

Epidermal growth factor (EGF) reduces Na^+ absorption in rabbit CCD by about 50% via the inhibition of apical electrogenic Na^+ entry via ENaC.[441,442] More recent studies performed on cultured mouse CCD cells suggest that the effects are mediated by the ErbB2 EGF receptor and that there is actually a biphasic effect of EGF on ENaC activity.[443] Acutely (<4 h), EGF treatment increases the ENaC current, an effect that appears to be mediated via the PI-3-kinase pathway. Chronically (>8 h), the ENaC current was inhibited via effects through the MEK/ERK pathway.[443]

Endothelin-1 (ET-1) is heavily secreted from the basolateral aspect of CD epithelial cells where it binds to basolateral ET_B receptors and may thus act in an autocrine fashion to inhibit both Na^+ and water reabsorption in this segment, as shown in vitro.[444] The physiologic relevance of these findings has been confirmed in vivo, because mice with CD-specific knockout of ET-1 or ET receptors are hypertensive on a normal Na^+ diet and have exacerbated Na^+ retention and hypertension when placed on a high-Na^+ diet. ET-1 inhibits ENaC activity through Src- and MAPK-dependent pathways.[444]

Nitric oxide is also a downstream mediator of ET-1,[444] where it decreases Na^+ transport in rat CCD segments by 40% to 80%.[445] The addition of nitric oxide to tubules decreased the intracellular [Na^+], but did not affect the activity of basolateral Na^+,K^+–ATPase, suggesting that the primary effect of nitric oxide is to inhibit apical Na^+ entry via ENaC. The inhibitory mechanism involving NO remains to be determined.[444]

Dopamine inhibits ADH-dependent Na^+ transport and transepithelial voltage in rat and rabbit CCD segments, although the particular basolateral dopamine receptor subtype may be species specific.[446,447]

Ang II stimulates sodium channel activity in rabbit and mouse CCDs.[448] Chronic Ang II infusion also increases the abundance of the α subunit of ENaC, the rate-limiting subunit for ENaC assembly.[449] Both the acute effects of Ang II on ENaC activity and the chronic effects on ENaC abundance are mediated by the AT1 receptor.[448,449] A more recent study performed on rats suggests that the Ang II-induced ENaC stimulation downstream of the AT1 receptor involves a Ca^{2+}-independent PKC pathway that induces superoxide generation. The blocking of arachidonic acid–induced inhibition of ENaC may also play a role.[450]

Bicarbonate (HCO_3^-) changes in the CCD lumen, which may reflect changes in total-body acid–base status or local HCO_3^- secretion from neighboring intercalated cells via the apical Na^+-independent Cl^-/HCO_3^- exchanger, pendrin, have been shown recently to modulate Na^+ reabsorption in the CCD.[451,452] Specifically, the bicarbonate-stimulated soluble adenylyl cyclase (sAC) appears to stimulate both basal and agonist-stimulated Na^+ reabsorption in the kidney collecting duct, acting to enhance Na^+,K^+–ATPase catalytic activity.[451] Another study suggests that pendrin increases ENaC abundance and activity, at least in part by increasing luminal [HCO_3^-].[452]

The elucidation of other cellular signaling pathways and kinases that are important in the regulation of ENaC, and Na^+ transport in the CCD is a very active area of research and involves signaling pathways downstream of diverse stimuli, including inflammation (IkappaB kinase/NF-kappaB/NF-κB pathway)[453] and metabolic stress (AMP-activated protein kinase).[454] A full treatment of these signaling pathways is beyond the scope of this chapter, but the interested reader is referred to a recent review.[455]

NA^+ TRANSPORT IN THE OUTER MEDULLARY COLLECTING DUCT

The transport properties of the outer medullary collecting duct (OMCD) have been studied by in vitro perfusion of isolated tubule segments. The functional properties of the OMCD differ depending on the location of the segment within the outer medulla. Segments within the outer stripe of the outer medulla ($OMCD_o$) exhibit electrophysiologic properties resembling the cortical collecting duct; that is, a lumen negative transepithelial voltage and electrogenic apical Na^+ entry.[456] Compared to the CCD, the $OMCD_o$ displays a less negative transepithelial voltage, much lower ionic permeabilities, and a lower rate of active reabsorption of Na^+.[457] As the collecting duct descends into the medulla, principal cells, which mediate Na^+ and K^+ transport in the CCD (see previous), are replaced by cells the electrical properties of which are similar to intercalated cells of the CCD (i.e., the apical membrane lacks a demonstrable Na^+ or K^+ conductance).[458] Within the inner stripe of outer medulla ($OMCD_i$), principal cells are virtually absent and no net Na^+ absorption occurs.[457]

NA^+ TRANSPORT IN THE INNER MEDULLARY COLLECTING DUCT

Mechanism of Na^+ Transport

The analysis of salt transport by the inner medullary collecting duct (IMCD) has been confounded by problems of axial tubule heterogeneity, species variability, and differences in experimental approach. Based on morphologic factors, the IMCD has been divided into three subsegments: $IMCD_1$, $IMCD_2$, and $IMCD_3$.[459] This morphologic heterogeneity is paralleled, to some extent, by functional heterogeneity. For example, the urea permeability and its responsiveness to ADH increases from $IMCD_1$ to $IMCD_3$ with increasing medullary depth.[460] Further complicating the analysis is the observation that similar subsegments from different species exhibit different properties relating to salt transport.[461,462] Finally, for unclear reasons, studies examining IMCD function in vivo (e.g., by microcatheterization)[463] have yielded markedly different results than have in vitro studies of isolated perfused tubules.[461,464]

Microelectrode impalement studies of IMCD segments from rats demonstrated that the apical membrane constituted the major cellular resistance, and the luminal application of amiloride increased the apical membrane voltage and resistance and decreased the transepithelial voltage.[461] These results, and others,[465] are consistent with the presence of an amiloride-sensitive sodium conductance in the apical membrane of IMCD cells. Patch-clamp studies of cultured rat IMCD cells indicate that Na^+ entry is mediated by a 20 to 30 pS amiloride-sensitive, nonselective, cGMP-gated cation channel in the apical membrane.[465,466] The basolateral membrane of IMCD cells contains the Na^+,K^+–ATPase, a K^+ conductance and a HCO_3^- conductance.[461]

The results discussed previously can be combined into a model for sodium transport in the IMCD (Fig. 5.11). Na^+

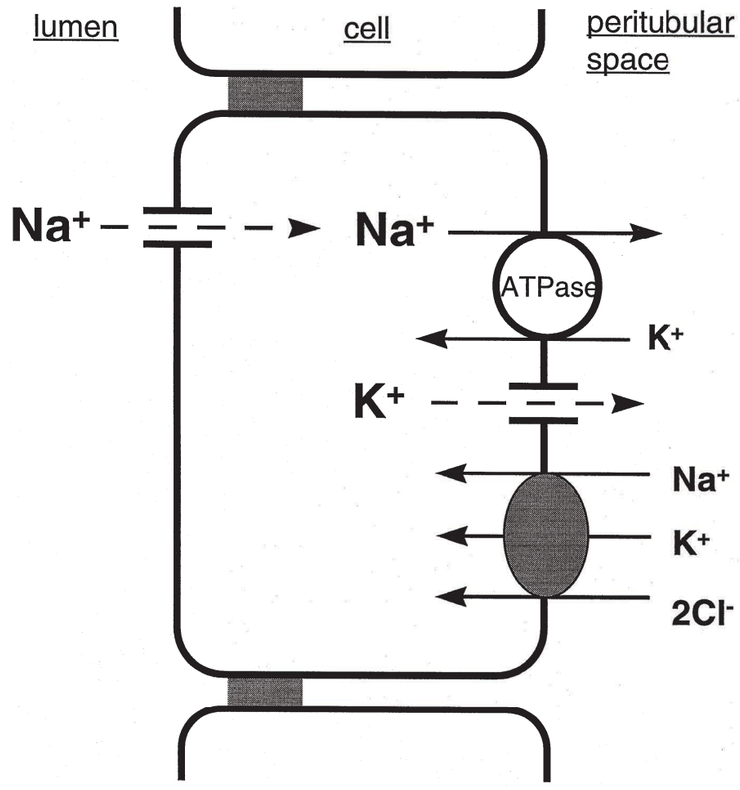

FIGURE 5.11 A model of Na^+ transport in the inner medullary collecting duct. Apical Na^+ entry proceeds via a nonselective amiloride-sensitive cation channel. Basolateral Na^+-K^+-$2Cl^-$ cotransport may be involved in Na^+ secretion.

entry across the apical membrane occurs down its steep electrochemical gradient through amiloride-sensitive Na^+ channels. Na^+ is extruded across the basolateral membrane by the Na^+,K^+–ATPase. The basolateral membrane K^+ conductance serves to recycle the K^+ that enters via the Na^+,K^+–ATPase. The K^+ conductance also hyperpolarizes the cell, thereby favoring Na^+ entry across the apical membrane.

There is some evidence for additional electroneutral Na^+ entry pathways in rat IMCD cells, although their importance in net Na^+ absorption by the IMCD in other species remains unclear. Furosemide and thiazide diuretics both inhibit a portion of Na^+ absorption by rat IMCD segments in vivo.[467,468] However, Zeidel and colleagues[469] failed to demonstrate sensitivity of rabbit IMCDs to loop diuretics and found that conductive Na^+ entry at the apical membrane predominated. A Na^+-K^+-$2Cl^-$ cotransporter appears to be present in the basolateral membrane of terminal IMCD segments.[470] The cloned Na^+-K^+-$2Cl^-$ cotransporter from cultured IMCD cells represents the "secretory" isoform of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) rather than the absorptive isoform present in the apical membrane of the thick ascending limb.[471] Finally, the Na^+-HCO_3^- cotransporter NBCn1 has been found in the basolateral membrane of rat IMCDs, which may contribute to cellular defense against both acidification and volume changes in this segment.[472]

Regulation of Na^+ Transport

The IMCD appears to be the major target site for the potent diuretic hormone ANP.[473] This hormone, working through cGMP,[474] inhibits Na^+ entry via apical Na^+ channels. In cell-attached and excised patches of cultured inner medullary cells, both ANP and dibutyryl cGMP inhibited the activity of the cation channel.[475]

In addition to inhibiting Na^+ reabsorption, ANP may stimulate Na^+ secretion in the IMCD.[470] In isolated perfused IMCD segments, ANP increased the bath to lumen flux rate of Na^+ and Cl^-, an effect that was inhibited by peritubular furosemide and by omission of either counterion, thus suggesting the role of a basolateral membrane Na^+-K^+-$2Cl^-$ cotransporter.[476] Sands et al.,[464] however, found no effect of ANP on the Na^+ permeability of the rat IMCD.

In one study,[477] ADH stimulated amiloride-sensitive Na^+ absorption by terminal IMCD segments perfused in vitro. Other studies, however, failed to find an effect of ADH or cAMP on the amiloride-sensitive cation channel in apical membranes of cultured IMCD cells.[464,465]

Micropuncture studies by Ullrich and Papavassiliou[478] demonstrated that mineralocorticoids increased net Na^+ absorption in the terminal IMCD, which was attributed to a decrease in the passive Na^+ permeability of the tubule leading to a decrease in back leak of NaCl into the lumen. Mineralocorticoids may also increase active Na^+ reabsorption in the IMCD. For example, aldosterone produced a three- to seven-fold stimulation of electrogenic Na^+ transport in cultured rat IMCD cells,[479] and chronic mineralocorticoid exposure in vivo increased the activity of Na^+,K^+ ATPase in the IMCD.[480]

REFERENCES

1. Einstein A. The elementary theory of the Brownian motion. Z. Elecktrochem. 1908;14:235.
2. Goldman D. Potential, impedance, and rectification in membranes. J Gen Physiol. 1944;27:37. http://www.ncbi.nlm.nih.gov/pubmed/19873371
3. Hodgkin AL, Katz B. The effect of sodium ions on the electrical activity of giant axon of the squid. J Physiol. 1949;108:37. http://www.ncbi.nlm.nih.gov/pubmed/18128147
4. Kedem O, Katchalsky A. Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. Biochim Biophys Acta. 1958;27:229. http://www.ncbi.nlm.nih.gov/pubmed/13522722
5. Takata K, Kasahara T, Kasahara M, et al. Localization of Na(+)-dependent active type and erythrocyte/HepG2-type glucose transporters in rat kidney: immunofuorescence and immunogold study. J Histochem Cytochem. 1991;39:287. http://www.ncbi.nlm.nih.gov/pubmed/1993828
6. Reuss L, Lewis SA, Wills NK, et al. Ion transport processes in basolateral membranes of epithelia. Fed Proc. 1984;43:2488.
7. Schmidt U, Dubach UC. Na K ATPase in the rat nephron related to sodium transport; results with quantitative histochemistry. Part II. Curr Probl Clin Biochem. 1971;3:320. http://www.ncbi.nlm.nih.gov/pubmed/4280963
8. Kirk KL, Halm DR, Dawson DC. Active sodium transport by turtle colon via an electrogenic Na-K exchange pump. Nature. 1980;287:237. http://www.ncbi.nlm.nih.gov/pubmed/6253798
9. Wang W, Messner G, Oberleithner H, et al. The effect of ouabain on intracellular activities of K+, Na+, Cl-, H+ and Ca2+ in proximal tubules of frog kidneys. Pfugers Arch. 1984;401:6.
10. Soltoff SP, Mandel LJ. Active ion transport in the renal proximal tubule. II. Ionic dependence of the Na pump. J Gen Physiol. 1984;84:623. http://www.ncbi.nlm.nih.gov/pubmed/6094705
11. Schultz SG, Curran PF. Coupled transport of sodium and organic solutes. Physiol Rev. 1970;50:637. http://www.ncbi.nlm.nih.gov/pubmed/4919599
12. Lassiter WE, Gottschalk CW, Mylle M. Micropuncture study of net transtubular movement of water and urea in nondiuretic mammalian kidney. Am J Physiol. 1961; 200:1139. http://www.ncbi.nlm.nih.gov/pubmed/13759324
13. Ullrich KJ, Schmidt-Nielson B, O'Dell R, et al. Micropuncture study of composition of proximal and distal tubular fuid in rat kidney. Am J Physiol. 1963;204:527. http://www.ncbi.nlm.nih.gov/pubmed/13995307
14. Welling LW, Welling DJ. Surface areas of brush border and lateral cell walls in the rabbit proximal nephron. Kidney Int. 1975;8:343. http://www.ncbi.nlm.nih.gov/pubmed/1206850
15. Andreoli TE, Schafer JA, Troutman SL. Perfusion rate-dependence of transepithelial osmosis in isolated proximal convoluted tubules: estimation of the hydraulic conductance. Kidney Int. 1978;14:263. http://www.ncbi.nlm.nih.gov/pubmed/723152
16. Giebisch G, Klose RM, Malnic G, et al. Sodium movement across single perfused proximal tubules of rat kidneys. J Gen Physiol. 1964;47:1175. http://www.ncbi.nlm.nih.gov/pubmed/14192552
17. Barratt LJ, Rector FC Jr, Kokko JP, et al. Factors governing the transepithelial potential difference across the proximal tubule of the rat kidney. J Clin Invest. 1974;53:454. http://www.ncbi.nlm.nih.gov/pubmed/11344559
18. Andreoli TE, Schafer JA, Troutman SL, et al. Solvent drag component of Cl- fux in superficial proximal straight tubules: evidence for a paracellular component of isotonic fuid absorption. Am J Physiol. 1979;237:F455.
19. Burg MB, Orloff J. Electrical potential difference across proximal convoluted tubules. Am J Physiol. 1970;219:1714.
20. Schafer JA, Patlak CS, Andreoli TE. A component of fuid absorption linked to passive ion fows in the superficial pars recta. J Gen Physiol. 1975;66:445. http://www.ncbi.nlm.nih.gov/pubmed/1181377
21. Maunsbach AB. Observations on the segmentation of the proximal tubule in the rat kidney. Comparison of results from phase contrast, fuorescence and electron microscopy. J Ultrastruct Res. 1966;16:239. http://www.ncbi.nlm.nih.gov/pubmed/5333381
22. Doucet A, Katz AI, Morel F. Determination of Na-K-ATPase activity in single segments of the mammalian nephron. Am J Physiol. 1979;237:F105.
23. Jacobson HR. Transport characteristics of in vitro perfused proximal convoluted tubules. Kidney Int. 1982;22:425. http://www.ncbi.nlm.nih.gov/pubmed/6759752

24. Jacobson HR. Characteristics of volume reabsorption in rabbit superficial and juxtamedullary proximal convoluted tubules. J Clin Invest. 1979;63:410. http://www.ncbi.nlm.nih.gov/pubmed/429562

25. Kawamura S, Imai M, Seldin DW, et al. Characteristics of salt and water transport in superficial and juxtamedullary straight segments of proximal tubules. J Clin Invest. 1975;55:1269. http://www.ncbi.nlm.nih.gov/pubmed/1133172

26. Rector FC Jr. Sodium, bicarbonate, and chloride absorption by the proximal tubule. Am J Physiol. 1983;244:F461.

27. Schafer JA, Williams JC Jr. Transport of metabolic substrates by the proximal nephron. Annu Rev Physiol. 1985;47:103. http://www.ncbi.nlm.nih.gov/pubmed/2581501

28. Kokko JP. Proximal tubule potential difference. Dependence on glucose, HCO3, and amino acids. J Clin Invest. 1973;52:1362.

29. Schafer JA, Troutman SL, Andreoli TE. Volume reabsorption, transepithelial potential differences, and ionic permeability properties in mammalian superficial proximal straight tubules. J Gen Physiol. 1974;64:582. http://www.ncbi.nlm.nih.gov/pubmed/4443793

30. Schafer JA, Troutman SL, Watkins ML, et al. Volume absorption in the pars recta. I. "Simple" active Na+ transport. Am J Physiol. 1978;234:F332.

31. Barfuss DW, Schafer JA. Active amino acid absorption by proximal convoluted and proximal straight tubules. Am J Physiol. 1979;236:F149.

32. McKeown JW Brazy PC, Dennis VW Intrarenal heterogeneity for fuid, phosphate, and glucose absorption in the rabbit. Am J Physiol. 1979;237:F312.

33. Schafer JA, Barfuss DW. The study of pars recta function by the perfusion of isolated tubule segments. Kidney Int. 1982;22:434. http://www.ncbi.nlm.nih.gov/pubmed/6759753

34. Welling LW, Welling DJ. Relationship between structure and function in renal proximal tubule. J Electron Microsc Tech. 1988;9:171. http://www.ncbi.nlm.nih.gov/pubmed/3199236

35. Seely JF, Chirito E. Studies of the electrical potential difference in rat proximal tubule. Am J Physiol. 1975;229:72. http://www.ncbi.nlm.nih.gov/pubmed/1147059

36. Lutz MD, Cardinal J, Burg MB. Electrical resistance of renal proximal tubule perfused in vitro. Am J Physiol. 1973;225:729. http://www.ncbi.nlm.nih.gov/pubmed/4726511

37. Seely JF. Variation in electrical resistance along length of rat proximal convoluted tubule. Am J Physiol. 1973;225:48. http://www.ncbi.nlm.nih.gov/pubmed/4714410

38. Jacobson HR, Kokko JP. Intrinsic differences in various segments of the proximal convoluted tubule. J Clin Invest. 1976;57:818.

39. Berry CA, Warnock DG, Rector FC Jr. Ion selectivity and proximal salt reabsorption. Am J Physiol. 1978;235:F234.

40. Gullans SR, Avison MJ, Ogino T, et al. NMR measurements of intracellular sodium in the rabbit proximal tubule. Am J Physiol. 1985;249:F160.

41. Stroup RF, Weinman E, Hayslett JP, et al. Effect of luminal permeability on net transport across the amphibian proximal tubule. Am J Physiol. 1974;226:1110.

42. Warnock DG, Rector FC Jr. Proton secretion by the kidney. Annu Rev Physiol. 1979; 41:197. http://www.ncbi.nlm.nih.gov/pubmed/35088

43. Wistrand PJ, Knuuttila KG. Renal membrane-bound carbonic anhydrase. Purification and properties. Kidney Int. 1989;35:851. http://www.ncbi.nlm.nih.gov/pubmed/2496258

44. Kinsella JL, Aronson PS. Properties of the Na+ -H+ exchanger in renal microvillus membrane vesicles. Am J Physiol. 1980;238:F461.

45. Aronson PS. Mechanisms of active H+ secretion in the proximal tubule. Am J Physiol. 1983;245:F647.

46. Aronson PS, Nee J, Suhm MA. Modifier role of internal H+ in activating the Na+ -H+ exchanger in renal microvillus membrane vesicles. Nature. 1982;299:161. http://www.ncbi.nlm.nih.gov/pubmed/7110335

47. Boron WF, Boulpaep EL. Intracellular pH regulation in the renal proximal tubule of the salamander. Na-H exchange. J Gen Physiol. 1983;81:29. http://www.ncbi.nlm.nih.gov/pubmed/6833996

48. Haggerty JG, Agarwal N, Reilly RF, et al. Pharmacologically different Na/H antiporters on the apical and basolateral surfaces of cultured porcine kidney cells (LLC-PK1). Proc Natl Acad Sci USA. 1988;85:6797. http://www.ncbi.nlm.nih.gov/pubmed/2901105

49. Sardet C, Franchi A, Pouyssegur J. Molecular cloning, primary structure, and expression of the human growth factor-activatable Na+/H+ antiporter. Cell. 1989;56:271. http://www.ncbi.nlm.nih.gov/pubmed/2536298

50. Biemesderfer D, Reilly RF, Exner M, et al. Immunocytochemical characterization of Na(+)-H+ exchanger isoform NHE-1 in rabbit kidney. Am J Physiol. 1992;263:F833.

51. Biemesderfer D, Pizzonia J, Abu-Alfa A, et al. NHE3: a Na+/H+ exchanger isoform of renal brush border. Am J Physiol. 1993;265:F736. http://www.ncbi.nlm.nih.gov/pubmed/8238556

52. Schultheis PJ, Clarke LL, Meneton P, et al. Renal and intestinal absorptive defects in mice lacking the NHE3 Na+/H+ exchanger. Nat Genet. 1998;19:282. http://www.ncbi.nlm.nih.gov/pubmed/9662405

53. Vallon V Schwark JR, Richter K, et al. Role of Na(+)/H(+) exchanger NHE3 in nephron function: micropuncture studies with S3226, an inhibitor of NHE3. Am J Physiol Renal Physiol. 2000;278:F375.

54. Choi JY, Shah M, Lee MG, et al. Novel amiloride-sensitive sodium-dependent proton secretion in the mouse proximal convoluted tubule. J Clin Invest. 2000;105:1141.

55. Goyal S, Vanden Heuvel G, Aronson PS. Renal expression of novel Na+/H+ exchanger isoform NHE8. Am J Physiol Renal Physiol. 2003;284:F467.

56. Biagi B, Kubota T, Sohtell M, et al. Intracellular potentials in rabbit proximal tubules perfused in vitro. Am J Physiol. 1981;240:F200.

57. Sacktor B. Sodium-coupled hexose transport. Kidney Int. 1989;36:342. http://www.ncbi.nlm.nih.gov/pubmed/2687560

58. Aronson PS, Sacktor B. The Na+ gradient-dependent transport of D-glucose in renal brush border membranes. J Biol Chem. 1975;250:6032. http://www.ncbi.nlm.nih.gov/pubmed/1150669

59. Aronson PS. Energy-dependence of phlorizin binding to isolated renal microvillus membranes. Evidence concerning the mechanism of coupling between the electrochemical Na+ gradient and the sugar transport. J Membr Biol. 1978;42:81. http://www.ncbi.nlm.nih.gov/pubmed/671529

60. Barfuss DW, Schafer JA. Differences in active and passive glucose transport along the proximal nephron. Am J Physiol. 1981;241:F322.

61. Turner RJ, Moran A. Stoichiometric studies of the renal outer cortical brush border membrane D-glucose transporter. J Membr Biol. 1982;67:73. http://www.ncbi.nlm.nih.gov/pubmed/7201526

62. Turner RJ, Moran A. Further studies of proximal tubular brush border membrane D-glucose transport heterogeneity. J Membr Biol. 1982;70:37. http://www.ncbi.nlm.nih.gov/pubmed/7142902

63. Hediger MA, Coady MJ, Ikeda TS, et al. Expression cloning and cDNA sequencing of the Na+/glucose co-transporter. Nature. 1987;330:379. http://www.ncbi.nlm.nih.gov/pubmed/2446136

64. Wells RG, Pajor AM, Kanai Y, et al. Cloning of a human kidney cDNA with similarity to the sodium-glucose cotransporter. Am J Physiol. 1992;263:F459.

65. Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. Physiol Rev. 2011;91:733. http://www.ncbi.nlm.nih.gov/pubmed/21527736

66. Kanai Y, Lee WS, You G, et al. The human kidney low affinity Na+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. J Clin Invest. 1994;93:397. http://www.ncbi.nlm.nih.gov/pubmed/8282810

67. Vallon V, Platt KA, Cunard R, et al. SGLT2 mediates glucose reabsorption in the early proximal tubule. J Am Soc Nephrol. 2010;22:104. http://www.ncbi.nlm.nih.gov/pubmed/20616166

68. van den Heuvel LP, Assink K, Willemsen M, et al. Autosomal recessive renal glucosuria attributable to a mutation in the sodium glucose cotransporter (SGLT2). Hum Genet. 2002;111:544. http://www.ncbi.nlm.nih.gov/pubmed/12436245

69. Martin MG, Turk E, Lostao MP, et al. Defects in Na+/glucose cotransporter (SGLT1) trafficking and function cause glucose-galactose malabsorption. Nat Genet. 1996;12:216. http://www.ncbi.nlm.nih.gov/pubmed/8563765

70. Patel AK, Fonseca V Turning glucosuria into a therapy: Efficacy and safety with SGLT2 inhibitors. Curr Diab Rep. 2010;10:101. http://www.ncbi.nlm.nih.gov/pubmed/20425568

71. Samarzija I, Frömter E. Electrophysiological analysis of rat renal sugar and amino acid transport. I. Basic phenomena. Pfugers Arch. 1982;393:179. http://www.ncbi.nlm.nih.gov/pubmed/7099920

72. Broer S. Amino acid transport across mammalian intestinal and renal epithelia. Physiol Rev. 2008;88:249. http://www.ncbi.nlm.nih.gov/pubmed/18195088

73. Samarzija I, Fromter E. Electrophysiological analysis of rat renal sugar and amino acid transport. III. Neutral amino acids. Pfugers Arch. 1982;393:119. http://www.ncbi.nlm.nih.gov/pubmed/7099922

74. Hammerman MR, Sacktor B. Na+ -dependent transport of glycine in renal brush border membrane vesicles. Evidence for a single specific transport system. Biochim Biophys Acta. 1982;686:189. http://www.ncbi.nlm.nih.gov/pubmed/7082661

75. Bohmer C, Broer A, Munzinger M, et al. Characterization of mouse amino acid transporter B0AT1 (slc6a19). Biochem J. 2005;389:745. http://www.ncbi.nlm.nih.gov/pubmed/15804236

76. Camargo SM, Makrides V, Virkki LV, et al. Steady-state kinetic characterization of the mouse B(0)AT1 sodium-dependent neutral amino acid transporter. Pflugers Arch. 2005;451:338.
http://www.ncbi.nlm.nih.gov/pubmed/16133263
77. Samarzija I, Fromter E. Electrophysiological analysis of rat renal sugar and amino acid transport. IV Basic amino acids. Pflugers Arch. 1982;393:210.
http://www.ncbi.nlm.nih.gov/pubmed/6808460
78. Samarzija I, Fromter E. Electrophysiological analysis of rat renal sugar and amino acid transport. V. Acidic amino acids. Pflugers Arch. 1982;393:215.
http://www.ncbi.nlm.nih.gov/pubmed/6124929
79. Tate SS, Yan N, Udenfriend S. Expression cloning of a Na(+)-independent neutral amino acid transporter from rat kidney. Proc Natl Acad Sci USA. 1992;89:1.
http://www.ncbi.nlm.nih.gov/pubmed/1729674
80. Wells RG, Hediger MA. Cloning of a rat kidney cDNA that stimulates dibasic and neutral amino acid transport and has sequence similarity to glucosidases. Proc Natl Acad Sci USA. 1992;89:5596.
81. Berry CA, Rector FC Jr. Mechanism of proximal NaCl reabsorption in the proximal tubule of the mammalian kidney. Semin Nephrol. 1991;11:86.
http://www.ncbi.nlm.nih.gov/pubmed/2034928
82. Cassola AC, Mollenhauer M, Fromter E. The intracellular chloride activity of rat kidney proximal tubular cells. Pflugers Arch. 1983;399:259.
http://www.ncbi.nlm.nih.gov/pubmed/6664828
83. Alpern RJ, Howlin KJ, Preisig PA. Active and passive components of chloride transport in the rat proximal convoluted tubule. J Clin Invest. 1985;76:1360.
84. Lucci MS, Warnock DG. Effects of anion-transport inhibitors on NaCl reabsorption in the rat superficial proximal convoluted tubule. J Clin Invest. 1979;64:570.
http://www.ncbi.nlm.nih.gov/pubmed/457869
85. Green R, Bishop JH, Giebisch G. Ionic requirements of proximal tubular sodium transport. III. Selective luminal anion substitution. Am J Physiol. 1979;236:F268.
86. Baum M. Evidence that parallel Na+ -H+ and Cl(-)-HCO3-(OH-) antiporters transport NaCl in the proximal tubule. Am J Physiol. 1987;252:F338.
87. Karniski LP, Aronson PS. Chloride/formate exchange with formic acid recycling: a mechanism of active chloride transport across epithelial membranes. Proc Natl Acad Sci USA. 1985;82:6362.
88. Schild L, Giebisch G, Karniski LP, et al. Effect of formate on volume reabsorption in the rabbit proximal tubule. J Clin Invest. 1987;79:32.
http://www.ncbi.nlm.nih.gov/pubmed/3793930
89. Knauf F, Yang CL, Thomson RB, et al. Identification of a chloride-formate exchanger expressed on the brush border membrane of renal proximal tubule cells. Proc Natl Acad Sci USA. 2001;98:9425.
http://www.ncbi.nlm.nih.gov/pubmed/11459928
90. Karniski LP, Aronson PS. Anion exchange pathways for Cl- transport in rabbit renal microvillus membranes. Am J Physiol. 1987;253:F513.
91. Aronson PS. Ion exchangers mediating NaCl transport in the renal proximal tubule. Cell Biochem Biophys. 2002;36:147.
http://www.ncbi.nlm.nih.gov/pubmed/12139400
92. Jiang Z, Grichtchenko, II, Boron WF, et al. Specificity of anion exchange mediated by mouse Slc26a6. J Biol Chem. 2002;277:33963.
http://www.ncbi.nlm.nih.gov/pubmed/12119287
93. Warnock DG, Yee VJ. Sodium uptake mechanisms in brush-border membrane vesicles prepared from rabbit renal cortex. Biochim Biophys Acta. 1982;684:137.
http://www.ncbi.nlm.nih.gov/pubmed/7055550
94. Lewis SA, Wills NK. Apical membrane permeability and kinetic properties of the sodium pump in rabbit urinary bladder. J Physiol. 1983;341:169.
http://www.ncbi.nlm.nih.gov/pubmed/6312027
95. Alpern RJ. Mechanism of basolateral membrane H+/OH-/HCO-3 transport in the rat proximal convoluted tubule. A sodium-coupled electrogenic process. J Gen Physiol. 1985;86:613.
http://www.ncbi.nlm.nih.gov/pubmed/2999293
96. Akiba T, Alpern RJ, Eveloff J, et al. Electrogenic sodium/bicarbonate cotransport in rabbit renal cortical basolateral membrane vesicles. J Clin Invest. 1986;78:1472.
97. Aronson PS, Soleimani M, Grassl SM. Properties of the renal Na(+)-HCO3-cotransporter. Semin Nephrol. 1991;11:28.
http://www.ncbi.nlm.nih.gov/pubmed/1900129
98. Aalkjaer C, Frische S, Leipziger J, et al. Sodium coupled bicarbonate transporters in the kidney, an update. Acta Physiol Scand. 2004;181:505.
http://www.ncbi.nlm.nih.gov/pubmed/15283764
99. Maunsbach AB, Vorum H, Kwon TH, et al. Immunoelectron microscopic localization of the electrogenic Na/HCO(3) cotransporter in rat and ambystoma kidney. J Am Soc Nephrol. 2000;11:2179.
100. Boron WF. Acid-base transport by the renal proximal tubule. J Am Soc Nephrol. 2006; 17:2368.
http://www.ncbi.nlm.nih.gov/pubmed/16914536
101. Burckhardt BC, Cassola AC, Fromter E. Electrophysiological analysis of bicarbonate permeation across the peritubular cell membrane of rat kidney proximal tubule. II. Exclusion of HCO3(-)-effects on other ion permeabilities and of coupled electroneutral HCO3(-)-transport. Pflugers Arch. 1984;401:43.
http://www.ncbi.nlm.nih.gov/pubmed/6089091
102. Cardinal J, Lapointe JY, Laprade R. Luminal and peritubular ionic substitutions and intracellular potential of the rabbit proximal convoluted tubule. Am J Physiol. 1984; 247:F352.
103. Ishibashi K, Sasaki S, Yoshiyama N. Intracellular chloride activity of rabbit proximal straight tubule perfused in vitro. Am J Physiol. 1988;255:F49.
104. Welling PA, O'Neil RG. Cell swelling activates basolateral membrane Cl and K conductances in rabbit proximal tubule. Am J Physiol. 1990;258:F951.
105. Sasaki S, Ishibashi K, Yoshiyama N, et al. KCl co-transport across the basolateral membrane of rabbit renal proximal straight tubules. J Clin Invest. 1988;81:194.
http://www.ncbi.nlm.nih.gov/pubmed/3335635
106. Alpern RJ, Chambers M. Basolateral membrane Cl/HCO3 exchange in the rat proximal convoluted tubule. Na-dependent and -independent modes. J Gen Physiol. 1987;89:581.
http://www.ncbi.nlm.nih.gov/pubmed/2953859
107. Sasaki S, Yoshiyama N. Interaction of chloride and bicarbonate transport across the basolateral membrane of rabbit proximal straight tubule. Evidence for sodium coupled chloride/bicarbonate exchange. J Clin Invest. 1988;81:1004.
http://www.ncbi.nlm.nih.gov/pubmed/2450891
108. Ishibashi K, Rector FC Jr, Berry CA. Chloride transport across the basolateral membrane of rabbit proximal convoluted tubules. Am J Physiol. 1990;258:F1569.
109. Daugharty TM, Ueki IF, Nicholas DP, et al. Comparative renal effects of isoncotic and colloid-free volume expansion in the rat. Am J Physiol. 1972; 222:225.
http://www.ncbi.nlm.nih.gov/pubmed/5060222
110. Daugharty TM, Ueki IF, Nicholas DP, et al. Renal response to chronic intravenous salt loading in the rat. J Clin Invest. 1973;52:21.
http://www.ncbi.nlm.nih.gov/pubmed/4682387
111. Brenner BM, Troy JL, Daugharty TM, et al. Quantitative importance of changes in postglomerular colloid osmotic pressure in mediating glomerulotubular balance in the rat. J Clin Invest. 1973;52:190.
http://www.ncbi.nlm.nih.gov/pubmed/4682382
112. Brenner BM, Troy JL, Daugharty TM. On the mechanism of inhibition in fluid reabsorption by the renal proximal tubule of the volume-expanded rat. J Clin Invest. 1971;50:1596.
http://www.ncbi.nlm.nih.gov/pubmed/5125390
113. Imai M, Kokko JP. Effect of peritubular protein concentration on reabsorption of sodium and water in isolated perfused proximal tubules. J Clin Invest. 1972;51:314.
http://www.ncbi.nlm.nih.gov/pubmed/5009115
114. Grantham JJ, Qualizza PB, Welling LW Influence of serum proteins on net fluid reabsorption of isolated proximal tubules. Kidney Int. 1972;2:66.
http://www.ncbi.nlm.nih.gov/pubmed/4671530
115. Baum M, Berry CA. Peritubular protein modulates neutral active NaCl absorption in rabbit proximal convoluted tubule. Am J Physiol. 1985;248:F790.
116. Grandchamp A, Boulpaep EL. Pressure control of sodium reabsorption and intercellular backflux across proximal kidney tubule. J Clin Invest. 1974;54:69.
http://www.ncbi.nlm.nih.gov/pubmed/4834883
117. Schafer JA, Troutman SL, Watkins ML, et al. Flow dependence of fluid transport in the isolated superficial pars recta: evidence that osmotic disequilibrium between external solutions drives isotonic fluid absorption. Kidney Int. 1981;20:588.
http://www.ncbi.nlm.nih.gov/pubmed/7343709
118. Haberle DA, Shiigai TT, Maier G, et al. Dependency of proximal tubular fluid transport on the load of glomerular filtrate. Kidney Int. 1981;20:18.
http://www.ncbi.nlm.nih.gov/pubmed/7300109
119. Du Z, Duan Y, Yan Q, et al. Mechanosensory function of microvilli of the kidney proximal tubule. Proc Natl Acad Sci USA. 2004;101:13068.
http://www.ncbi.nlm.nih.gov/pubmed/15319475
120. Szenasi G, Bencsath P, Takacs L. Proximal tubular transport and urinary excretion of sodium after renal denervation in sodium depleted rats. Pflugers Arch. 1985;403:146.
http://www.ncbi.nlm.nih.gov/pubmed/3982965
121. Morgunov NS, You YD, Hirsch DJ. Response of mouse proximal straight tubule and medullary thick ascending limb to beta-agonist. J Am Soc Nephrol. 1993;4:1151.

122. Liu F, Nesbitt T, Drezner MK, et al. Proximal nephron Na+/H+ exchange is regulated by alpha 1A- and alpha 1B-adrenergic receptor subtypes. Mol Pharmacol. 1997;52:1010.
123. Bello-Reuss E. Effect of catecholamines on fuid reabsorption by the isolated proximal convoluted tubule. Am J Physiol. 1980;238:F347.
124. Aperia A, Ibarra F, Svensson LB, et al. Calcineurin mediates alpha-adrenergic stimulation of Na+ ,K(+)-ATPase activity in renal tubule cells. Proc Natl Acad Sci USA. 1992;89:7394.
125. Singh H, Linas SL. Role of protein kinase C in beta 2-adrenoceptor function in cultured rat proximal tubule epithelial cells. Am J Physiol. 1997;273:F193.
126. Cheng XJ, Fisone G, Aizman O, et al. PKA-mediated phosphorylation and inhibition of Na(+)-K(+)-ATPase in response to beta-adrenergic hormone. Am J Physiol. 1997;273:C893.
127. Seri I, Kone BC, Gullans SR, et al. Locally formed dopamine inhibits Na+ -K+ -ATPase activity in rat renal cortical tubule cells. Am J Physiol. 1988;255:F666.
128. Bertorello A, Aperia A. Inhibition of proximal tubule Na(+)-K(+)-ATPase activity requires simultaneous activation of DA1 and DA2 receptors. Am J Physiol. 1990;259:F924.
129. Baumann K, Chan YL, Bode F, et al. Effect of parathyroid hormone and cyclic adenosine 3 ‘ ,5’-monophosphate on isotonic fuid reabsorption: polarity of proximal tubular cells. Kidney Int. 1977;11:77.
http://www.ncbi.nlm.nih.gov/pubmed/191680
130. Gesek FA, Schoolwerth AC. Hormonal interactions with the proximal Na(+)-H+ exchanger. Am J Physiol. 1990;258:F514.
131. Weinman EJ, Steplock D, Wang Y, et al. Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane Na(+)-H+ exchanger. J Clin Invest. 1995;95:2143.
http://www.ncbi.nlm.nih.gov/pubmed/7738182
132. Yun CH, Oh S, Zizak M, et al. cAMP-mediated inhibition of the epithelial brush border Na+/H+ exchanger, NHE3, requires an associated regulatory protein. Proc Natl Acad Sci USA. 1997;94:3010.
133. Alexander RT, Grinstein S. Tethering, recycling and activation of the epithelial sodium-proton exchanger, NHE3. J Exp Biol. 2009;212:1630.
http://www.ncbi.nlm.nih.gov/pubmed/19448073
134. Weinman EJ, Dubinsky WP, Fisher K, et al. Regulation of reconstituted renal Na+/H+ exchanger by calcium-dependent protein kinases. J Membr Biol. 1988;103:237.
http://www.ncbi.nlm.nih.gov/pubmed/2846848
135. Waugh WH. Angiotensin II: local renal effects of physiological increments in concentration. Can J Physiol Pharmacol. 1972;50:711.
http://www.ncbi.nlm.nih.gov/pubmed/4340507
136. Hall JE, Guyton AC, Trippodo NC, et al. Intrarenal control of electrolyte excretion by angiotensin II. Am J Physiol. 1977;232:F538.
137. Seely JF, Levy M. Control of extracellular fuid volume. In: Brenner BM, Rector FC Jr, eds. The Kidney. Philadelphia, PA: W. B. Saunders; 1982: 371.
138. Valles P, Wysocki J, Batlle D. Angiotensin II and renal tubular ion transport. Sci World J. 2005;5:680.
http://www.ncbi.nlm.nih.gov/pubmed/16142301
139. Geibel J, Giebisch G, Boron WF. Angiotensin II stimulates both Na(+)-H+ exchange and Na+/$HCO3^-$ cotransport in the rabbit proximal tubule. Proc Natl Acad Sci USA. 1990;87:7917.
http://www.ncbi.nlm.nih.gov/pubmed/2172967
140. Li L, Wang YP, Capparelli AW, et al. Effect of luminal angiotensin II on proximal tubule fuid transport: role of apical phospholipase A2. Am J Physiol. 1994;266:F202.
141. Liu FY, Cogan MG. Angiotensin II stimulates early proximal bicarbonate absorption in the rat by decreasing cyclic adenosine monophosphate. J Clin Invest. 1989;84:83.
http://www.ncbi.nlm.nih.gov/pubmed/2544631
142. Katz AI, Lindheimer MD. Renal sodium- and potassium-activated adenosine triphosphatase and sodium reabsorption in the hypothyroid rat. J Clin Invest. 1973;52:796.
143. De Santo NG, Capasso G, Paduano C, et al. Tubular transport processes in proximal tubules of hypothyroid rats. Micropuncture studies on isotonic fuid, amino acid and buffer reabsorption. Pfugers Arch. 1980;384:117.
http://www.ncbi.nlm.nih.gov/pubmed/6446079
144. Capasso G, Lin JT, De Santo NG, et al. Short term effect of low doses of tri-iodothyronine on proximal tubular membrane Na-K-ATPase and potassium permeability in thyroidectomized rats. Pfugers Arch. 1985;403:90.
http://www.ncbi.nlm.nih.gov/pubmed/2984639
145. Cano A, Baum M, Moe OW. Thyroid hormone stimulates the renal Na/H exchanger NHE3 by transcriptional activation. Am J Physiol. 1999;276:C102.
146. Lynch RE, Schneider EG, Willis LR, et al. Absence of mineralocorticoid-dependent sodium reabsorption in dog proximal tubule. AmJ Physiol. 1972;223:40.
http://www.ncbi.nlm.nih.gov/pubmed/5039071
147. Garg LC, Narang N, Wingo CS. Glucocorticoid effects on Na-K-ATPase in rabbit nephron segments. Am J Physiol. 1985;248:F487.
148. Vrtovsnik F, Jourdain M, Cherqui G, et al. Glucocorticoid inhibition of Na-Pi cotransport in renal epithelial cells is mediated by protein kinase C. J Biol Chem. 1994;269:8872.
http://www.ncbi.nlm.nih.gov/pubmed/8132623
149. Ali R, Amlal H, Burnham CE, et al. Glucocorticoids enhance the expression of the basolateral Na+ :HCO3- cotransporter in renal proximal tubules. Kidney Int. 2000;57:1063.
150. Liang M, Knox FG. Production and functional roles of nitric oxide in the proximal tubule. Am J Physiol Regul Integr Comp Physiol. 2000;278:R1117.
151. Garvin JL, Herrera M, Ortiz PA. Regulation of renal NaCl transport by nitric oxide, endothelin, and ATP: clinical implications. Annu Rev Physiol. 2011;73:359.
http://www.ncbi.nlm.nih.gov/pubmed/20936940
152. Roczniak A, Burns KD. Nitric oxide stimulates guanylate cyclase and regulates sodium transport in rabbit proximal tubule. Am J Physiol. 1996;270:F106.
153. Diamond JM, Bossert WH. Standing-gradient osmotic fow. A mechanism for coupling of water and solute transport in epithelia. J Gen Physiol. 1967;50:2061.
http://www.ncbi.nlm.nih.gov/pubmed/6066064
154. Jacobson HR, Kokko JP, Seldin DW, et al. Lack of solvent drag of NaCl and NaHCO3 in rabbit proximal tubules. Am J Physiol. 1982;243:F342.
155. Gonzalez E, Carpi-Medina P, Whittembury G. Cell osmotic water permeability of isolated rabbit proximal straight tubules. Am J Physiol. 1982;242:F321.
156. Vallon V, Verkman AS, Schnermann J. Luminal hypotonicity in proximal tubules of aquaporin-1-knockout mice. Am J Physiol Renal Physiol. 2000;278:F1030.
157. Barfuss DW, Schafer JA. Rate of formation and composition of absorbate from proximal nephron segments. Am J Physiol. 1984;247:F117.
158. Dantzler WH. Studies on nonmammalian nephrons. Kidney Int. 1982; 22:560.
159. Bennett CM, Brenner BM, Berliner RW. Micropuncture study of nephron function in the rhesus monkey. J Clin Invest. 1968;47:203.
http://www.ncbi.nlm.nih.gov/pubmed/16695942
160. Kokko JP. Sodium chloride and water transport in the descending limb of Henle. J Clin Invest. 1970;49:1838.
http://www.ncbi.nlm.nih.gov/pubmed/5460286
161. Kokko JP, Rector FC Jr. Countercurrent multiplication system without active transport in inner medulla. Kidney Int. 1972;2:214.
http://www.ncbi.nlm.nih.gov/pubmed/4657922
162. Nielsen S, Pallone T, Smith BL, et al. Aquaporin-1 water channels in short and long loop descending thin limbs and in descending vasa recta in rat kidney. Am J Physiol. 1995;268:F1023.
163. Zhai XY, Fenton RA, Andreasen A, et al. Aquaporin-1 is not expressed in descending thin limbs of short-loop nephrons. J Am Soc Nephrol. 2007;18:2937.
http://www.ncbi.nlm.nih.gov/pubmed/17942963
164. Imai M, Kokko JP. Sodium chloride, urea, and water transport in the thin ascending limb of Henle. Generation of osmotic gradients by passive diffusion of **solutes.** J Clin Invest. 1974;53:393.
http://www.ncbi.nlm.nih.gov/pubmed/11344552
165. Imai M. Functional heterogeneity of the descending limbs of Henle’s loop. II. Interspecies differences among rabbits, rats, and hamsters. Pfugers Arch. 1984;402:393.
http://www.ncbi.nlm.nih.gov/pubmed/6522246
166. Jamison RL. Micropuncture study of segments of thin loop of Henle in the rat. Am J Physiol. 1968;215:236.
http://www.ncbi.nlm.nih.gov/pubmed/5659337
167. Imai M, Kokko JP. Mechanism of sodium and chloride transport in the thin ascending limb of Henle. J Clin Invest. 1976;58:1054.
http://www.ncbi.nlm.nih.gov/pubmed/993330
168. Yoshitomi K, Kondo Y, Imai M. Evidence for conductive Cl^- pathways across the cell membranes of the thin ascending limb of Henle’s loop. J Clin Invest. 1988;82:866.
http://www.ncbi.nlm.nih.gov/pubmed/2458388
169. Kondo Y, Yoshitomi K, Imai M. Effect of Ca2+ on Cl” transport in thin ascending limb of Henle’s loop. Am J Physiol. 1988;254:F232.
170. Uchida S, Sasaki S, Furukawa T, et al. Molecular cloning of a chloride channel that is regulated by dehydration and expressed predominantly in kidney medulla. J Biol Chem. 1993;268:3821.
http://www.ncbi.nlm.nih.gov/pubmed/7680033
171. Uchida S, Sasaki S, Nitta K, et al. Localization and functional characterization of rat kidney-specific chloride channel, ClC-K1. J Clin Invest. 1995;95:104.
http://www.ncbi.nlm.nih.gov/pubmed/7814604
172. Estevez R, Boettger T, Stein V, et al. Barttin is a Cl^- channel beta-subunit crucial for renal Cl” reabsorption and inner ear K+ secretion. Nature. 2001; 414:558.
http://www.ncbi.nlm.nih.gov/pubmed/11734858

173. Waldegger S, Jeck N, Barth P, et al. Barttin increases surface expression and changes current properties of ClC-K channels. Pfugers Arch. 2002;444:411.
http://www.ncbi.nlm.nih.gov/pubmed/12111250
174. Scholl U, Hebeisen S, Janssen AG, et al. Barttin modulates trafficking and function of ClC-K channels. Proc Natl Acad Sci USA. 2006;103:11411.
http://www.ncbi.nlm.nih.gov/pubmed/16849430
175. Fischer M, Janssen AG, Fahlke C. Barttin activates ClC-K channel function by modulating gating. J Am Soc Nephrol. 2010;21:1281.
176. Matsumura Y, Uchida S, Kondo Y, et al. Overt nephrogenic diabetes insipidus in mice lacking the CLC-K1 chloride channel. Nat Genet. 1999;21:95.
http://www.ncbi.nlm.nih.gov/pubmed/9916798
177. Rocha AS, Kokko JP. Sodium chloride and water transport in the medullary thick ascending limb of Henle. Evidence for active chloride transport. J Clin Invest. 1973;52:612.
http://www.ncbi.nlm.nih.gov/pubmed/4685086
178. Burg MB, Green N. Function of the thick ascending limb of Henle's loop. Am J Physiol. 1973;224:659.
http://www.ncbi.nlm.nih.gov/pubmed/4691283
179. Katz AI, Doucet A, Morel F. Na-K-ATPase activity along the rabbit, rat, and mouse nephron. Am J Physiol. 1979;237:F114.
180. Hebert SC. Hypertonic cell volume regulation in mouse thick limbs. I. ADH dependency and nephron heterogeneity. Am J Physiol. 1986;250:C907.
181. Oberleithner H, Guggino W, Giebisch G. Mechanism of distal tubular chloride transport in Amphiuma kidney. Am J Physiol. 1982;242:F331.
182. Hebert SC, Andreoli TE. Effects of antidiuretic hormone on cellular conductive pathways in mouse medullary thick ascending limbs of Henle: II. determinants of the ADH-mediated increases in transepithelial voltage and in net Cl-absorption. J Membr Biol. 1984;80:221.
http://www.ncbi.nlm.nih.gov/pubmed/6094817
183. Schlatter E, Greger R. cAMP increases the basolateral Cl^- conductance in the isolated perfused medullary thick ascending limb of Henle's loop of the mouse. Pfugers Arch. 1985;405:367.
http://www.ncbi.nlm.nih.gov/pubmed/3001635
184. Greger R, Oberleithner H, Schlatter E, et al. Chloride activity in cells of isolated perfused cortical thick ascending limbs of rabbit kidney. Pfugers Arch. 1983;399:29.
http://www.ncbi.nlm.nih.gov/pubmed/6646997
185. Greger R, Schlatter E. Properties of the lumen membrane of the cortical thick ascending limb of Henle's loop of rabbit kidney. Pfugers Arch. 1983;396:315.
http://www.ncbi.nlm.nih.gov/pubmed/6844136
186. Molony DA, Reeves WB, Andreoli TE. Na^+ :K^+ :$2Cl^-$ cotransport and the thick ascending limb. Kidney Int. 1989;36:418.
187. Hebert SC, Reeves WB, Molony DA, et al. The medullary thick limb: function and modulation of the single-effect multiplier. Kidney Int. 1987;31:580.
http://www.ncbi.nlm.nih.gov/pubmed/3550227
188. Good DW. Bicarbonate absorption by the thick ascending limb of Henle's loop. Semin Nephrol. 1990;10:132.
http://www.ncbi.nlm.nih.gov/pubmed/2180016
189. Greger R. Chloride reabsorption in the rabbit cortical thick ascending limb of the loop of Henle. A sodium dependent process. Pfugers Arch. 1981;390:38.
http://www.ncbi.nlm.nih.gov/pubmed/6264385
190. Koenig B, Ricapito S, Kinne R. Chloride transport in the thick ascending limb of Henle's loop: potassium dependence and stoichiometry of the NaCl cotransport system in plasma membrane vesicles. Pfugers Arch. 1983;399:173.
http://www.ncbi.nlm.nih.gov/pubmed/6657458
191. Reeves WB, Molony DA. The physiology of loop diuretic action. Semin Nephrol. 1988;8:225.
http://www.ncbi.nlm.nih.gov/pubmed/3059427
192. Eveloff J, Kinne R. Sodium-chloride transport in the medullary thick ascending limb of Henle's loop: evidence for a sodium-chloride cotransport system in plasma membrane vesicles. J Membr Biol. 1983;72:173.
http://www.ncbi.nlm.nih.gov/pubmed/6854622
193. Gamba G, Miyanoshita A, Lombardi M, et al. Molecular cloning, primary structure, and characterization of two members of the mammalian electroneutral sodium-(potassium)-chloride cotransporter family expressed in kidney. J Biol Chem. 1994;269:17713.
http://www.ncbi.nlm.nih.gov/pubmed/8021284
194. Payne JA, Xu JC, Haas M, et al. Primary structure, functional expression, and chromosomal localization of the bumetanide-sensitive Na-K-Cl cotrans-porter in human colon. J Biol Chem. 1995;270:17977.
195. Yang T, Huang YG, Singh I, et al. Localization of bumetanide- and thiazide-sensitive Na-K-Cl cotransporters along the rat nephron. Am J Physiol. 1996;271:F931.
196. Kaplan MR, Plotkin MD, Lee WS, et al. Apical localization of the Na-K-Cl cotransporter, rBSC1, on rat thick ascending limbs. Kidney Int. 1996;49:40.
http://www.ncbi.nlm.nih.gov/pubmed/8770947
197. Simon DB, Karet FE, Hamdan JM, et al. Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-2Cl cotransporter NKCC2. Nat Genet. 1996;13:183.
http://www.ncbi.nlm.nih.gov/pubmed/8640224
198. Monette MY, Rinehart J, Lifton RP, et al. Rare mutations in the human Na-K-Cl cotransporter (NKCC2) associated with lower blood pressure exhibit impaired processing and transport function. Am J Physiol Renal Physiol. 2011;300:F840.
199. Krishnamurthy H, Piscitelli CL, Gouaux E. Unlocking the molecular secrets of sodium-coupled transporters. Nature. 2009;459:347.
http://www.ncbi.nlm.nih.gov/pubmed/19458710
200. Ares GR, Caceres PS, Ortiz PA. Molecular regulation of NKCC2 in the thick ascending limb. Am J Physiol Renal Physiol. 2011;301:F1143.
201. Benziane B, Demaretz S, Defontaine N, et al. NKCC2 surface expression in mammalian cells: down-regulation by novel interaction with aldolase B. J Biol Chem. 2007;282:33817.
http://www.ncbi.nlm.nih.gov/pubmed/17848580
202. Zaarour N, Defontaine N, Demaretz S, et al. Secretory carrier membrane protein 2 regulates exocytic insertion of NKCC2 into the cell membrane. J Biol Chem. 2011;286:9489.
http://www.ncbi.nlm.nih.gov/pubmed/21205824
203. Parvin MN, Turner RJ. Identification of key residues involved in the dimerization of the secretory Na(+)-K(+)-2Cl(-) cotransporter NKCC1. Biochemistry 2011;50:9857.
http://www.ncbi.nlm.nih.gov/pubmed/21970294
204. Plata C, Mount DB, Rubio V, et al. Isoforms of the Na-K-2Cl cotransporter in murine TAL II. Functional characterization and activation by cAMP. Am J Physiol. 1999;276:F359.
205. Carota I, Theilig F, Oppermann M, et al. Localization and functional characterization of the human NKCC2 isoforms. Acta Physiol (Oxf). 2010;199:327.
http://www.ncbi.nlm.nih.gov/pubmed/20146722
206. Castrop H, Schnermann J. Isoforms of renal Na-K-2Cl cotransporter NKCC2: expression and functional significance. Am J Physiol Renal Physiol. 2008;295:F859.
207. Gimenez I, Isenring P, Forbush B. Spatially distributed alternative splice variants of the renal Na-K-Cl cotransporter exhibit dramatically different affinities for the transported ions. J Biol Chem. 2002;277:8767.
http://www.ncbi.nlm.nih.gov/pubmed/11815599
208. Plata C, Meade P, Vazquez N, et al. Functional properties of the apical Na+ -K+ -2Cl- cotransporter isoforms. J Biol Chem. 2002;277:11004.
http://www.ncbi.nlm.nih.gov/pubmed/11790783
209. Greger R, Weidtke C, Schlatter E, et al. Potassium activity in cells of isolated perfused cortical thick ascending limbs of rabbit kidney. Pfugers Arch. 1984;401:52.
http://www.ncbi.nlm.nih.gov/pubmed/6089092
210. Guggino SE, Guggino WB, Green N, et al. Ca2+-activated K+ channels in cultured medullary thick ascending limb cells. Am J Physiol. 1987;252:C121.
211. Wang WH. Two types of K+ channel in thick ascending limb of rat kidney. Am J Physiol. 1994;267:F599.
212. Wang WH, White S, Geibel J, et al. A potassium channel in the apical membrane of rabbit thick ascending limb of Henle's loop. Am J Physiol. 1990;258:F244.
213. Giebisch G, Hebert SC, Wang WH New aspects of renal potassium transport. Pfugers Arch. 2003;446:289.
http://www.ncbi.nlm.nih.gov/pubmed/12684792
214. Lu M, Wang T, Yan Q, et al. ROMK is required for expression of the 70-pS K channel in the thick ascending limb. Am J Physiol Renal Physiol. 2004;286:F490.
215. Ho K, Nichols CG, Lederer WJ, et al. Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. Nature. 1993;362:31.
http://www.ncbi.nlm.nih.gov/pubmed/7680431
216. Lu M, Wang T, Yan Q, et al. Absence of small conductance K+ channel (SK) activity in apical membranes of thick ascending limb and cortical collecting duct in ROMK (Bartter's) knockout mice. J Biol Chem. 2002;277:37881.
http://www.ncbi.nlm.nih.gov/pubmed/12130653
217. Simon DB, Karet FE, Rodriguez-Soriano J, et al. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K+ channel, ROMK. Nat Genet. 1996;14:152.
http://www.ncbi.nlm.nih.gov/pubmed/8841184
218. Simon DB, Lifton RP. Ion transporter mutations in Gitelman's and Bartter's syndromes. Curr Opin Nephrol Hypertens. 1998;7:43.
http://www.ncbi.nlm.nih.gov/pubmed/9442362
219. Schwalbe RA, Bianchi L, Accili EA, et al. Functional consequences of ROMK mutants linked to antenatal Bartter's syndrome and implications for treatment. Hum Mol Genet. 1998;7:975.
http://www.ncbi.nlm.nih.gov/pubmed/9580661

220. Wangemann P, Wittner M, Di Stefano A, et al. Cl(-)-channel blockers in the thick ascending limb of the loop of Henle. Structure activity relationship. Pf ugers Arch. 1986;407(Suppl 2):S128.
221. Yoshikawa M, Uchida S, Yamauchi A, et al. Localization of rat CLC-K2 chloride channel mRNA in the kidney. Am J Physiol. 1999;276:F552.
222. Kobayashi K, Uchida S, Mizutani S, et al. Intrarenal and cellular localization of CLC-K2 protein in the mouse kidney. J Am Soc Nephrol. 2001;12:1327.
223. Kramer BK, Bergler T, Stoelcker B, et al. Mechanisms of disease: the kidney-specific chloride channels ClCKA and ClCKB, the Barttin subunit, and their clinical relevance. Nat Clin Pract Nephrol. 2008;4:38.
http://www.ncbi.nlm.nih.gov/pubmed/18094726
224. Birkenhager R, Otto E, Schurmann MJ, et al. Mutation of BSND causes Bartter syndrome with sensorineural deafness and kidney failure. Nat Genet. 2001;29:310.
http://www.ncbi.nlm.nih.gov/pubmed/11687798
225. Di Stefano A, Greger R, Desf eurs E, et al. A Ba(2+)-insensitive K+ conductance in the basolateral membrane of rabbit cortical thick ascending limb cells. Cell Physiol Biochem. 1998;8:89.
http://www.ncbi.nlm.nih.gov/pubmed/9547022
226. Di Stefano A, Jounier S, Wittner M. Evidence supporting a role for KCl cotransporter in the thick ascending limb of Henle's loop. Kidney Int. 2001;60:1809.
227. Velazquez H, Silva T. Cloning and localization of KCC4 in rabbit kidney: expression in distal convoluted tubule. Am J Physiol Renal Physiol. 2003;285:F49.
228. Boettger T, Hubner CA, Maier H, et al. Deafness and renal tubular acidosis in mice lacking the K-Cl co-transporter Kcc4. Nature. 2002;416:874.
http://www.ncbi.nlm.nih.gov/pubmed/11976689
229. Friedman PA, Andreoli TE. CO2-stimulated NaCl absorption in the mouse renal cortical thick ascending limb of Henle. Evidence for synchronous Na +/H+ and Cl-/HCO3- exchange in apical plasma membranes. J Gen Physiol. 1982;80:683.
http://www.ncbi.nlm.nih.gov/pubmed/6816900
230. Kikeri D, Azar S, Sun A, et al. Na(+)-H+ antiporter and Na(+)-(HCO3-) n symporter regulate intracellular pH in mouse medullary thick limbs of Henle. Am J Physiol. 1990;258:F445.
231. Amemiya M, Loffing J, Lotscher M, et al. Expression of NHE-3 in the apical membrane of rat renal proximal tubule and thick ascending limb. Kidney Int. 1995;48:1206.
http://www.ncbi.nlm.nih.gov/pubmed/8569082
232. Laghmani K, Borensztein P, Ambuhl P, et al. Chronic metabolic acidosis enhances NHE-3 protein abundance and transport activity in the rat thick ascending limb by increasing NHE-3 mRNA. J Clin Invest. 1997;99:24.
http://www.ncbi.nlm.nih.gov/pubmed/9011571
233. Hebert SC, Andreoli TE. Control of NaCl transport in the thick ascending limb. Am J Physiol. 1984;246:F745.
234. Mutig K, Paliege A, Kahl T, et al. Vasopressin V2 receptor expression along rat, mouse, and human renal epithelia with focus on TAL. Am J Physiol Renal Physiol. 2007; 293:F1166.
235. Gimenez I, Forbush B. Short-term stimulation of the renal Na-K-Cl cotransporter (NKCC2) by vasopressin involves phosphorylation and membrane translocation of the protein. J Biol Chem. 2003;278:26946.
236. Gunaratne R, Braucht DW, Rinschen MM, et al. Quantitative phosphoproteomic analysis reveals cAMP/vasopressin-dependent signaling pathways in native renal thick ascending limb cells. Proc Natl Acad Sci USA. 2010;107:15653.
237. McCormick JA, Mutig K, Nelson JH, et al. A SPAK isoform switch modulates renal salt transport and blood pressure. Cell Metab. 2011;14:352.
http://www.ncbi.nlm.nih.gov/pubmed/21907141
238. Richardson C, Sakamoto K, de los Heros P, et al. Regulation of the NKCC2 ion cotransporter by SPAK-OSR1-dependent and -independent pathways. J Cell Sci. 2011;124:789.
http://www.ncbi.nlm.nih.gov/pubmed/21321328
239. Moriguchi T, Urushiyama S, Hisamoto N, et al. WNK1 regulates phosphorylation of cation-chloride-coupled cotransporters via the STE20-related kinases, SPAK and OSR1. J Biol Chem. 2005;280:42685.
http://www.ncbi.nlm.nih.gov/pubmed/16263722
240. Yang SS, Lo YF, Wu CC, et al. SPAK-knockout mice manifest Gitelman syndrome and impaired vasoconstriction. J Am Soc Nephrol. 2010;21:1868.
http://www.ncbi.nlm.nih.gov/pubmed/20813865
241. Lin SH, Yu IS, Jiang ST, et al. Impaired phosphorylation of Na(+)-K(+)-2Cl(-) cotransporter by oxidative stress-responsive kinase-1 deficiency manifests hypotension and Bartter-like syndrome. Proc Natl Acad Sci USA. 2011;108:17538.
http://www.ncbi.nlm.nih.gov/pubmed/21972418
242. Rafiqi FH, Zuber AM, Glover M, et al. Role of the WNK-activated SPAK kinase in regulating blood pressure. EMBO Mol Med. 2010;2:63.
http://www.ncbi.nlm.nih.gov/pubmed/20091762
243. Hebert SC, Culpepper RM, Andreoli TE. NaCl transport in mouse medullary thick ascending limbs. II. ADH enhancement of transcellular NaCl cotransport; origin of transepithelial voltage. Am J Physiol. 1981;241:F432.
244. Hebert SC, Friedman PA, Andreoli TE. Effects of antidiuretic hormone on cellular conductive pathways in mouse medullary thick ascending limbs of Henle: I. ADH increases transcellular conductance pathways. J Membr Biol. 1984;80:201.
http://www.ncbi.nlm.nih.gov/pubmed/6094816
245. Ecelbarger CA, Kim GH, Knepper MA, et al. Regulation of potassium channel Kir 1.1 (ROMK) abundance in the thick ascending limb of Henle's loop. J Am Soc Nephrol. 2001;12:10.
http://www.ncbi.nlm.nih.gov/pubmed/18265082
246. Reeves WB, Andreoli TE. Cl- transport in basolateral renal medullary vesicles: II. Cl- channels in planar lipid bilayers. J Membr Biol. 1990;113:57.
http://www.ncbi.nlm.nih.gov/pubmed/1689386
247. Culpepper RM, Andreoli TE. PGE2, forskolin, and cholera toxin interactions in modulating NaCl transport in mouse mTALH. Am J Physiol. 1984;247:F784.
248. Escalante B, Erlij D, Falck JR, et al. Effect of cytochrome P450 arachidonate metabolites on ion transport in rabbit kidney loop of Henle. Science. 1991;251:799.
http://www.ncbi.nlm.nih.gov/pubmed/1846705
249. Lu M, Zhu Y, Balazy M, et al. Effect of angiotensin II on the apical K+ channel in the thick ascending limb of the rat kidney. J Gen Physiol. 1996;108:537.
http://www.ncbi.nlm.nih.gov/pubmed/8972391
250. Harris RC, McKanna JA, Akai Y, et al. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. J Clin Invest. 1994;94:2504.
251. Cheng HF, Harris RC. Cyclooxygenases, the kidney, and hypertension. Hypertension. 2004;43:525.
http://www.ncbi.nlm.nih.gov/pubmed/14732722
252. Harding P, Sigmon DH, Alfie ME, et al. Cyclooxygenase-2 mediates increased renal renin content induced by low-sodium diet. Hypertension. 1997;29:297.
http://www.ncbi.nlm.nih.gov/pubmed/9039118
253. Molony DA, Andreoli TE. Diluting power of thick limbs of Henle. I. Peritubular hypertonicity blocks basolateral Cl^- channels. Am J Physiol. 1988; 255:F1128.
254. Hebert SC, Culpepper RM, Andreoli TE. NaCl transport in mouse medullary thick ascending limbs. III. Modulation of the ADH effect by peritubular osmolality. Am J Physiol. 1981;241:F443.
255. Khanna A. Acquired nephrogenic diabetes insipidus. Semin Nephrol. 2006;26:244.
http://www.ncbi.nlm.nih.gov/pubmed/16713497
256. Takaichi K, Kurokawa K. High Ca2+ inhibits peptide hormone-dependent cAMP production specifically in thick ascending limbs of Henle. Miner Electrolyte Metab. 1986;12:342.
http://www.ncbi.nlm.nih.gov/pubmed/3027519
257. Takaichi K, Kurokawa K. Inhibitory guanosine triphosphate-binding protein-mediated regulation of vasopressin action in isolated single medullary tubules of mouse kidney. J Clin Invest. 1988;82:1437.
258. Riccardi D, Lee WS, Lee K, et al. Localization of the extracellular Ca(2+)-sensing receptor and PTH/PTHrP receptor in rat kidney. Am J Physiol. 1996;271:F951.
259. Wang W, Lu M, Balazy M, et al. Phospholipase A2 is involved in mediating the effect of extracellular Ca2+ on apical K+ channels in rat TAL. Am J Physiol. 1997;273:F421.
260. Wang WH, Lu M, Hebert SC. Cytochrome P-450 metabolites mediate extracellular Ca(2+)-induced inhibition of apical K+ channels in the TAL. Am J Physiol. 1996;271:C103.
261. el-Hajj Fuleihan G, Seifter J, Scott J, et al. Calcium-regulated renal calcium handling in healthy men: relationship to sodium handling. J Clin Endocrinol Metab. 1998;83:2366.
http://www.ncbi.nlm.nih.gov/pubmed/9661610
262. McCarron DA. Diet and blood pressure—the paradigm shift. Science. 1998;281:933.
http://www.ncbi.nlm.nih.gov/pubmed/9722467
263. Wittner M, Di Stefano A. A hormonal mosaic modulating the function of the thick ascending limb of Henle's loop. Exp Nephrol. 1993;1:285.
http://www.ncbi.nlm.nih.gov/pubmed/8081979
264. Bouby N, Hus-Citharel A, Marchetti J, et al. Expression of type 1 angiotensin II receptor subtypes and angiotensin II-induced calcium mobilization along the rat nephron. J Am Soc Nephrol. 1997;8:1658.
http://www.ncbi.nlm.nih.gov/pubmed/9355068

265. Amlal H, LeGoff C, Vernimmen C, et al. ANG II controls Na(+)-K+ (NH4+)-2Cl- cotransport via 20-HETE and PKC in medullary thick ascending limb. Am J Physiol. 1998;274:C1047.
266. Good DW, George T, Wang DH. Angiotensin II inhibits HCO-3 absorption via a cytochrome P-450-dependent pathway in MTAL. Am J Physiol. 1999;276:F726.
267. Kwon TH, Nielsen J, Kim YH, et al. Regulation of sodium transporters in the thick ascending limb of rat kidney: response to angiotensin II. Am J Physiol Renal Physiol. 2003;285:F152.
268. Staahltoft D, Nielsen S, Janjua NR, et al. Losartan treatment normalizes renal sodium and water handling in rats with mild congestive heart failure. Am J Physiol Renal Physiol. 2002;282:F307.
269. Morel F. Sites of hormone action in the mammalian nephron. Am J Physiol. 1981;240:F159.
270. Summers RJ, Kuhar MJ. Autoradiographic localization of beta-adrenoceptors in rat kidney. Eur J Pharmacol. 1983;91:305.
http://www.ncbi.nlm.nih.gov/pubmed/6311583
271. DiBona GF, Sawin LL. Effect of renal nerve stimulation on NaCl and H2O transport in Henle's loop of the rat. Am J Physiol. 1982;243:F576.
272. Bencsath P, Szenasi G, Takacs L. Water and electrolyte transport in Henle's loop and distal tubule after renal sympathectomy in the rat. Am J Physiol. 1985;249:F308.
273. Bailly C, Imbert-Teboul M, Roinel N, et al. Isoproterenol increases Ca, Mg, and NaCl reabsorption in mouse thick ascending limb. Am J Physiol. 1990;258:F1224.
274. Ortiz PA, Garvin JL. Role of nitric oxide in the regulation of nephron transport. Am J Physiol Renal Physiol. 2002;282:F777.
275. Ortiz PA, Hong NJ, Garvin JL. NO decreases thick ascending limb chloride absorption by reducing Na(+)-K(+)-2Cl(-) cotransporter activity. Am J Physiol Renal Physiol. 2001;281:F819.
276. Ortiz PA, Garvin JL. Cardiovascular and renal control in NOS-deficient mouse models. Am J Physiol Regul Integr Comp Physiol. 2003;284:R628.
277. Plato CF, Shesely EG, Garvin JL. eNOS mediates L-arginine-induced inhibition of thick ascending limb chloride flux. Hypertension. 2000;35:319.
http://www.ncbi.nlm.nih.gov/pubmed/10642318
278. Turban S, Wang XY, Knepper MA. Regulation of NHE3, NKCC2, and NCC abundance in kidney during aldosterone escape phenomenon: role of NO. Am J Physiol Renal Physiol. 2003;285:F843.
279. Masilamani S, Wang X, Kim GH, et al. Time course of renal Na-K-ATPase, NHE3, NKCC2, NCC, and ENaC abundance changes with dietary NaCl restriction. Am J Physiol Renal Physiol. 2002;283:F648.
280. Song J, Hu X, Shi M, et al. Effects of dietary fat, NaCl, and fructose on renal sodium and water transporter abundances and systemic blood pressure. Am J Physiol Renal Physiol. 2004;287:F1204.
281. Na KY, Oh YK, Han JS, et al. Upregulation of Na+ transporter abundances in response to chronic thiazide or loop diuretic treatment in rats. Am J Physiol Renal Physiol. 2003;284:F133.
282. Reilly RF, Ellison DH. Mammalian distal tubule: physiology, pathophysiology, and molecular anatomy. Physiol Rev. 2000;80:277.
http://www.ncbi.nlm.nih.gov/pubmed/10617770
283. Bostanjoglo M, Reeves WB, Reilly RF, et al. 11Beta-hydroxysteroid dehydro-genase, mineralocorticoid receptor, and thiazide-sensitive Na-Cl cotransporter expression by distal tubules. J Am Soc Nephrol. 1998;9:1347.
http://www.ncbi.nlm.nih.gov/pubmed/9697656
284. Hierholzer K, Wiederholt M. Some aspects of distal tubular solute and water transport. Kidney Int. 1976;9:198.
http://www.ncbi.nlm.nih.gov/pubmed/940262
285. Schnermann J, Briggs J, Schubert G. In situ studies of the distal convoluted tubule in the rat. I. Evidence for NaCl secretion. Am J Physiol. 1982;243:F160.
286. Khuri RN, Strieder N, Wiederholt M, et al. Effects of graded solute diuresis on renal tubular sodium transport in the rat. Am J Physiol. 1975;228:1262.
287. Malnic G, Klose RM, Giebisch G. Micropuncture study of distal tubular potassium and sodium transport in rat nephron. Am J Physiol. 1966;211:529.
http://www.ncbi.nlm.nih.gov/pubmed/5927880
288. Hayslett JP, Boulpaep EL, Kashgarian M, et al. Electrical characteristics of the mammalian distal tubule: Comparison of Ling-Gerard and macroelectrodes. Kidney Int. 1977;12:324.
http://www.ncbi.nlm.nih.gov/pubmed/604621
289. Wright FS. Increasing magnitude of electrical potential along the renal distal tubule. Am J Physiol. 1971;220:624.
http://www.ncbi.nlm.nih.gov/pubmed/5545669
290. Allen GG, Barratt LJ. Origin of positive transepithelial potential difference in early distal segments of rat kidney. Kidney Int. 1985;27:622.
http://www.ncbi.nlm.nih.gov/pubmed/4010149
291. Barratt LJ, Rector FC Jr, Kokko JP, et al. Transepithelial potential difference profile of the distal tubule of the rat kidney. Kidney Int. 1975;8:368.
http://www.ncbi.nlm.nih.gov/pubmed/1206853
292. Ellison DH, Velazquez H, Wright FS. Thiazide-sensitive sodium chloride cotransport in early distal tubule. Am J Physiol. 1987;253:F546.
293. Velazquez H, Good DW, Wright FS. Mutual dependence of sodium and chloride absorption by renal distal tubule. Am J Physiol. 1984;247:F904.
294. Boron WF, Sackin H. Measurement of intracellular ionic composition and activities in renal tubules. Annu Rev Physiol. 1983;45:483.
http://www.ncbi.nlm.nih.gov/pubmed/6342522
295. Costanzo LS. Localization of diuretic action in microperfused rat distal tubules: Ca and Na transport. Am J Physiol. 1985;248:F527.
296. Beaumont K, Vaughn DA, Healy DP. Thiazide diuretic receptors: autoradiographic localization in rat kidney with [3H]metolazone. J Pharmacol Exp Ther. 1989;250:414.
http://www.ncbi.nlm.nih.gov/pubmed/2746509
297. Ellison DH, Biemesderfer D, Morrisey J, et al. Immunocytochemical characterization of the high-affinity thiazide diuretic receptor in rabbit renal cortex. Am J Physiol. 1993;264:F141.
298. Gamba G, Saltzberg SN, Lombardi M, et al. Primary structure and functional expression of a cDNA encoding the thiazide-sensitive, electroneutral sodium-chloride cotransporter. Proc Natl Acad Sci USA. 1993;90:2749.
299. Monroy A, Plata C, Hebert SC, et al. Characterization of the thiazide-sensitive Na(+)-Cl(-) cotransporter: a new model for ions and diuretics interaction. Am J Physiol Renal Physiol. 2000;279:F161.
300. Gamba G. The thiazide-sensitive Na+ -Cl- cotransporter: molecular biology, functional properties, and regulation by WNKs. Am J Physiol Renal Physiol. 2009;297:F838.
301. Kunchaparty S, Palcso M, Berkman J, et al. Defective processing and expression of thiazide-sensitive Na-Cl cotransporter as a cause of Gitelman's syndrome. Am J Physiol. 1999;277:F643.
302. De Jong JC, Van Der Vliet WA, Van Den Heuvel LP, et al. Functional expression of mutations in the human NaCl cotransporter: evidence for impaired routing mechanisms in Gitelman's syndrome. J Am Soc Nephrol. 2002;13:1442.
303. Needham PG, Mikoluk K, Dhakarwal P, et al. The thiazide-sensitive NaCl cotransporter is targeted for chaperone-dependent endoplasmic reticulum-associated degradation. J Biol Chem. 2011;286:43611.
http://www.ncbi.nlm.nih.gov/pubmed/22027832
304. Hadchouel J, Delaloy C, Faure S, et al. Familial hyperkalemic hypertension. J Am Soc Nephrol. 2006;17:208.
http://www.ncbi.nlm.nih.gov/pubmed/16221868
305. Wilson FH, Disse-Nicodeme S, Choate KA, et al. Human hypertension caused by mutations in WNK kinases. Science. 2001;293:1107.
http://www.ncbi.nlm.nih.gov/pubmed/11498583
306. Subramanya AR, Liu J, Ellison DH, et al. WNK4 diverts the thiazide-sensitive NaCl cotransporter to the lysosome and stimulates AP-3 interaction. J Biol Chem. 2009;284:18471.
http://www.ncbi.nlm.nih.gov/pubmed/19401467
307. Golbang AP, Cope G, Hamad A, et al. Regulation of the expression of the Na/Cl cotransporter by WNK4 and WNK1: evidence that accelerated dynamin-dependent endocytosis is not involved. Am J Physiol Renal Physiol. 2006;291:F1369.
308. Yang CL, Angell J, Mitchell R, et al. WNK kinases regulate thiazide-sensitive Na-Cl cotransport. J Clin Invest. 2003;111:1039.
http://www.ncbi.nlm.nih.gov/pubmed/12671053
309. San-Cristobal P, Pacheco-Alvarez D, Richardson C, et al. Angiotensin II signaling increases activity of the renal Na-Cl cotransporter through a WNK4-SPAK-dependent pathway. Proc Natl Acad Sci USA. 2009;106:4384.
http://www.ncbi.nlm.nih.gov/pubmed/19240212
310. Welling PA, Chang YP, Delpire E, et al. Multigene kinase network, kidney transport, and salt in essential hypertension. Kidney Int. 2010;77:1063.
311. Hoorn EJ, Nelson JH, McCormick JA, et al. The WNK kinase network regulating sodium, potassium, and blood pressure. J Am Soc Nephrol. 2011;22:605.
312. Wilson FH, Kahle KT, Sabath E, et al. Molecular pathogenesis of inherited hypertension with hyperkalemia: the Na-Cl cotransporter is inhibited by wild-type but not mutant WNK4. Proc Natl Acad Sci USA. 2003;100:680.
http://www.ncbi.nlm.nih.gov/pubmed/12515852
313. Yang SS, Morimoto T, Rai T, et al. Molecular pathogenesis of pseudo-hypoaldosteronism type II: generation and analysis of a Wnk4(D561A/+) knockin mouse model. Cell Metab. 2007;5:331.
http://www.ncbi.nlm.nih.gov/pubmed/17488636
314. Richardson C, Rafiqi FH, Karlsson HK, et al. Activation of the thiazide-sensitive Na+ -Cl^- cotransporter by the WNK-regulated kinases SPAK and OSR1. J Cell Sci. 2008;121:675.
http://www.ncbi.nlm.nih.gov/pubmed/18270262

315. McCormick JA, Yang CL, Ellison DH. WNK kinases and renal sodium transport in health and disease: an integrated view. Hypertension. 2008;51:588.
http://www.ncbi.nlm.nih.gov/pubmed/18212265
316. Subramanya AR, Yang CL, Zhu X, et al. Dominant-negative regulation of WNK1 by its kidney-specific kinase-defective isoform. Am J Physiol Renal Physiol. 2006;290:F619.
317. Hadchouel J, Soukaseum C, Busst C, et al. Decreased ENaC expression compensates the increased NCC activity following inactivation of the kidney-specific isoform of WNK1 and prevents hypertension. Proc Natl Acad Sci USA. 2010;107:18109.
318. Greger R, Velazquez H. The cortical thick ascending limb and early distal convoluted tubule in the urinary concentrating mechanism. Kidney Int. 1987;31:590.
http://www.ncbi.nlm.nih.gov/pubmed/3550228
319. Yoshitomi K, Shimizu T, Taniguchi J, et al. Electrophysiological characterization of rabbit distal convoluted tubule cell. Pfugers Arch. 1989;414:457.
http://www.ncbi.nlm.nih.gov/pubmed/2477793
320. Velazquez H, Greger R. Electrical properties of the early distal convoluted tubules of the rabbit kidney. Renal Physiol. 1986;9:55.
321. Bockenhauer D, Feather S, Stanescu HC, et al. Epilepsy, ataxia, sensorineural deafness, tubulopathy, and KCNJ10 mutations. N Engl J Med. 2009;360:1960.
322. Scholl UI, Choi M, Liu T, et al. Seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME syndrome) caused by mutations in KCNJ10. Proc Natl Acad Sci USA. 2009;106:5842.
http://www.ncbi.nlm.nih.gov/pubmed/19289823
323. Reichold M, Zdebik AA, Lieberer E, et al. KCNJ10 gene mutations causing EAST syndrome (epilepsy, ataxia, sensorineural deafness, and tubulopathy) disrupt channel function. Proc Natl Acad Sci USA. 2010;107:14490.
324. Pacheco-Alvarez D, Cristobal PS, Meade P, et al. The Na+ :Cl- cotransporter is activated and phosphorylated at the amino-terminal domain upon intracellular chloride depletion. J Biol Chem. 2006;281:28755.
http://www.ncbi.nlm.nih.gov/pubmed/16887815
325. Wiederholt M, Hansen L, Giebisch G. Dependence of distal peritubular membrane potential on luminal sodium and peritubular potassium. Pfugers Arch. 1975;355:R55.
326. Costanzo LS. Comparison of calcium and sodium transport in early and late rat distal tubules: effect of amiloride. Am J Physiol. 1984;246:F937.
327. Loffing J, Pietri L, Aregger F, et al. Differential subcellular localization of ENaC subunits in mouse kidney in response to high- and low-Na diets. Am J Physiol Renal Physiol. 2000;279:F252.
328. Schmitt R, Ellison DH, Farman N, et al. Developmental expression of sodium entry pathways in rat nephron. Am J Physiol. 1999;276:F367.
329. Biner HL, Arpin-Bott MP, Loffing J, et al. Human cortical distal nephron: distribution of electrolyte and water transport pathways. J Am Soc Nephrol. 2002;13:836.
http://www.ncbi.nlm.nih.gov/pubmed/11912242
330. Ellison DH, Velazquez H, Wright FS. Adaptation of the distal convoluted tubule of the rat. Structural and functional effects of dietary salt intake and chronic diuretic infusion. J Clin Invest. 1989;83:113.
http://www.ncbi.nlm.nih.gov/pubmed/2910903
331. Chen ZF, Vaughn DA, Beaumont K, et al. Effects of diuretic treatment and of dietary sodium on renal binding of 3H-metolazone. J Am Soc Nephrol. 1990;1:91.
http://www.ncbi.nlm.nih.gov/pubmed/1966553
332. Loffing J, Loffing-Cueni D, Hegyi I, et al. Thiazide treatment of rats provokes apoptosis in distal tubule cells. Kidney Int. 1996;50:1180.
http://www.ncbi.nlm.nih.gov/pubmed/8887276
333. Fanestil DD, Vaughn DA, Blakely P. Dietary NaCl and KCl do not regulate renal density of the thiazide diuretic receptor. Am J Physiol. 1997;273:R1241.
334. Knepper MA, Kim GH, Masilamani S. Renal tubule sodium transporter abundance profiling in rat kidney: response to aldosterone and variations in NaCl intake. Ann NY Acad Sci. 2003;986:562.
http://www.ncbi.nlm.nih.gov/pubmed/12763890
335. Shimizu T, Yoshitomi K, Taniguchi J, et al. Effect of high NaCl intake on Na+ and K+ transport in the rabbit distal convoluted tubule. Pfugers Arch. 1989;414:500.
http://www.ncbi.nlm.nih.gov/pubmed/2550887
336. Farman N, Bonvalet JP. Aldosterone binding in isolated tubules. III. Autoradiography along the rat nephron. Am J Physiol. 1983;245:F606.
337. Farman N, Oblin ME, Lombes M, et al. Immunolocalization of gluco- and mineralocorticoid receptors in rabbit kidney. Am J Physiol. 1991;260:C226.
338. El Mernissi G, Doucet A. Specific activity of Na-K-ATPase after adrenalectomy and hormone replacement along the rabbit nephron. Pfugers Arch. 1984;402:258.
http://www.ncbi.nlm.nih.gov/pubmed/6097867
339. El Mernissi G, Doucet A. Short-term effects of aldosterone and dexametha-sone on Na-K-ATPase along the rabbit nephron. Pfugers Arch. 1983;399:147.
http://www.ncbi.nlm.nih.gov/pubmed/6139788
340. Stanton BA. Regulation by adrenal corticosteroids of sodium and potassium transport in loop of Henle and distal tubule of rat kidney. J Clin Invest. 1986;78:1612.
341. Velazquez H, Bartiss A, Bernstein P, et al. Adrenal steroids stimulate thiazide-sensitive NaCl transport by rat renal distal tubules. Am J Physiol. 1996;270:F211.
342. Chen Z, Vaughn DA, Blakely P, et al. Adrenocortical steroids increase renal thiazide diuretic receptor density and response. J Am Soc Nephrol. 1994;5:1361.
343. Kim GH, Masilamani S, Turner R, et al. The thiazide-sensitive Na-Cl cotransporter is an aldosterone-induced protein. Proc Natl Acad Sci USA. 1998;95:14552.
http://www.ncbi.nlm.nih.gov/pubmed/9826738
344. Rozansky DJ, Cornwall T, Subramanya AR, et al. Aldosterone mediates activation of the thiazide-sensitive Na-Cl cotransporter through an SGK1 and WNK4 signaling pathway. J Clin Invest. 2009;119:2601.
http://www.ncbi.nlm.nih.gov/pubmed/19690383
345. Arroyo JP, Lagnaz D, Ronzaud C, et al. Nedd4-2 modulates renal Na+ -Cl- cotransporter via the aldosterone-SGK1-Nedd4-2 pathway. J Am Soc Nephrol. 2011;22:1707.
http://www.ncbi.nlm.nih.gov/pubmed/21852580
346. Chiga M, Rai T, Yang SS, et al. Dietary salt regulates the phosphorylation of OSR1/SPAK kinases and the sodium chloride cotransporter through aldosterone. Kidney Int. 2008;74:1403.
347. Vallon V, Schroth J, Lang F, et al. Expression and phosphorylation of the Na+ -Cl- cotransporter NCC in vivo is regulated by dietary salt, potassium, and SGK1. Am J Physiol Renal Physiol. 2009;297:F704.
348. Chen Z, Vaughn DA, Fanestil DD. Influence of gender on renal thiazide diuretic receptor density and response. J Am Soc Nephrol. 1994;5:1112.
http://www.ncbi.nlm.nih.gov/pubmed/7849251
349. Verlander JW, Tran TM, Zhang L, et al. Estradiol enhances thiazide-sensitive NaCl cotransporter density in the apical plasma membrane of the distal convoluted tubule in ovariectomized rats. J Clin Invest. 1998;101:1661.
http://www.ncbi.nlm.nih.gov/pubmed/9541496
350. Pedersen NB, Hofmeister MV, Rosenbaek LL, et al. Vasopressin induces phosphorylation of the thiazide-sensitive sodium chloride cotransporter in the distal convoluted tubule. Kidney Int. 2010;78:160.
http://www.ncbi.nlm.nih.gov/pubmed/20445498
351. Mutig K, Saritas T, Uchida S, et al. Short-term stimulation of the thiazide-sensitive Na+ -Cl- cotransporter by vasopressin involves phosphorylation and membrane translocation. Am J Physiol Renal Physiol. 2010;298:F502.
352. Nedvetsky PI, Tamma G, Beulshausen S, et al. Regulation of aquaporin-2 trafficking. Handb Exp Pharmacol. 2009:133.
353. Snyder PM, Olson DR, Kabra R, et al. cAMP and serum and glucocorticoid-inducible kinase (SGK) regulate the epithelial Na(+) channel through convergent phosphorylation of Nedd4-2. J Biol Chem. 2004;279:45753.
354. Windhager EE, Giebisch G. Micropuncture study of renal tubular transfer of sodium chloride in the rat. Am J Physiol. 1961;200:581.
http://www.ncbi.nlm.nih.gov/pubmed/13785775
355. Boulpaep EL. Recent advances in electrophysiology of the nephron. Annu Rev Physiol. 1976;38:21.
http://www.ncbi.nlm.nih.gov/pubmed/769655
356. Sansom SC, O'Neil RG Mineralocorticoid regulation of apical cell membrane Na+ and K+ transport of the cortical collecting duct. Am J Physiol. 1985;248:F858.
357. Sansom SC, Weinman EJ, O'Neil RG. Microelectrode assessment of chloride-conductive properties of cortical collecting duct. Am J Physiol. 1984;247:F291.
358. Stokes JB. Potassium secretion by cortical collecting tubule: relation to sodium absorption, luminal sodium concentration, and transepithelial voltage. Am J Physiol. 1981;241:F395.
359. O'Neil RG, Sansom SC. Characterization of apical cell membrane Na+ and K+ conductances of cortical collecting duct using microelectrode techniques. Am J Physiol. 1984;247:F14.
360. Sansom SC, Agulian S, Muto S, et al. K activity of CCD principal cells from normal and DOCA-treated rabbits. Am J Physiol. 1989;256:F136.
361. Garty H, Palmer LG. Epithelial sodium channels: function, structure, and regulation. Physiol Rev. 1997;77:359.
http://www.ncbi.nlm.nih.gov/pubmed/9114818
362. Canessa CM, Horisberger JD, Rossier BC. Epithelial sodium channel related to proteins involved in neurodegeneration. Nature. 1993;361:467.
http://www.ncbi.nlm.nih.gov/pubmed/8381523

363. Canessa CM, Schild L, Buell G, et al. Amiloride-sensitive epithelial Na+ channel is made of three homologous subunits. Nature. 1994;367:463.
http://www.ncbi.nlm.nih.gov/pubmed/8107805
364. Palmer LG, Frindt G. Amiloride-sensitive Na channels from the apical membrane of the rat cortical collecting tubule. Proc Natl Acad Sci USA. 1986;83:2767.
365. Canessa CM, Merillat AM, Rossier BC. Membrane topology of the epithelial sodium channel in intact cells. Am J Physiol. 1994;267:C1682.
366. Snyder PM, McDonald FJ, Stokes JB, et al. Membrane topology of the amiloride-sensitive epithelial sodium channel. J Biol Chem. 1994;269:24379.
http://www.ncbi.nlm.nih.gov/pubmed/7929098
367. Jasti J, Furukawa H, Gonzales EB, et al. Structure of acid-sensing ion channel 1 at 1.9 A resolution and low pH. Nature. 2007;449:316.
http://www.ncbi.nlm.nih.gov/pubmed/17882215
368. Hughey RP, Bruns JB, Kinlough CL, et al. Epithelial sodium channels are activated by furin-dependent proteolysis. J Biol Chem. 2004;279:18111.
369. Hughey RP, Mueller GM, Bruns JB, et al. Maturation of the epithelial Na+ channel involves proteolytic processing of the alpha- and gamma-subunits. J Biol Chem. 2003;278:37073.
http://www.ncbi.nlm.nih.gov/pubmed/12871941
370. Kleyman TR, Carattino MD, Hughey RP. ENaC at the cutting edge: regulation of epithelial sodium channels by proteases. J Biol Chem. 2009;284:20447.
http://www.ncbi.nlm.nih.gov/pubmed/19401469
371. Carattino MD, Sheng S, Kleyman TR. Epithelial Na+ channels are activated by laminar shear stress. J Biol Chem. 2004;279:4120.
http://www.ncbi.nlm.nih.gov/pubmed/14625286
372. Abi-Antoun T, Shi S, Tolino LA, et al. Second transmembrane domain modulates epithelial sodium channel gating in response to shear stress. Am J Physiol Renal Physiol. 2011;300:F1089.
373. Morimoto T, Liu W Woda C, et al. Mechanism underlying f ow stimulation of sodium absorption in the mammalian collecting duct. Am J Physiol Renal Physiol. 2006;291:F663.
374. Schild L. The epithelial sodium channel: from molecule to disease. Rev Physiol Biochem Pharmacol. 2004;151:93.
http://www.ncbi.nlm.nih.gov/pubmed/15146350
375. Shimkets RA, Warnock DG, Bositis CM, et al. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell. 1994;79:407.
http://www.ncbi.nlm.nih.gov/pubmed/7954808
376. Hansson JH, Nelson-Williams C, Suzuki H, et al. Hypertension caused by a truncated epithelial sodium channel gamma subunit: genetic heterogeneity of Liddle syndrome. Nat Genet. 1995;11:76.
http://www.ncbi.nlm.nih.gov/pubmed/7550319
377. Tamura H, Schild L, Enomoto N, et al. Liddle disease caused by a missense mutation of beta subunit of the epithelial sodium channel gene. J Clin Invest. 1996;97:1780.
http://www.ncbi.nlm.nih.gov/pubmed/8601645
378. Snyder PM, Steines JC, Olson DR. Relative contribution of Nedd4 and Nedd4-2 to ENaC regulation in epithelia determined by RNA interference. J Biol Chem. 2004;279:5042.
http://www.ncbi.nlm.nih.gov/pubmed/14645220
379. Chang SS, Grunder S, Hanukoglu A, et al. Mutations in subunits of the epithelial sodium channel cause salt wasting with hyperkalaemic acidosis, pseudohypoaldosteronism type 1. Nat Genet. 1996;12:248.
http://www.ncbi.nlm.nih.gov/pubmed/8589714
380. Terada Y, Knepper MA. Thiazide-sensitive NaCl absorption in rat cortical collecting duct. Am J Physiol. 1990;259:F519.
381. Leviel F, Hubner CA, Houillier P, et al. The Na +-dependent chloride-bicarbonate exchanger SLC4A8 mediates an electroneutral Na+ reabsorption process in the renal cortical collecting ducts of mice. J Clin Invest. 2010;120:1627.
382. Eladari D, Hubner CA. Novel mechanisms for NaCl reabsorption in the collecting duct. Curr Opin Nephrol Hypertens. 2011;20:506.
http://www.ncbi.nlm.nih.gov/pubmed/21610493
383. Tomaschitz A, Pilz S, Ritz E, et al. Aldosterone and arterial hypertension. Nat Rev Endocrinol. 2010;6:83.
http://www.ncbi.nlm.nih.gov/pubmed/20027193
384. Wang WH, Giebisch G. Regulation of potassium (K) handling in the renal collecting duct. Pf ugers Arch. 2009;458:157.
http://www.ncbi.nlm.nih.gov/pubmed/18839206
385. Naray-Fejes-Toth A, Fejes-Toth G. Glucocorticoid receptors mediate mineralocorticoid-like effects in cultured collecting duct cells. Am J Physiol. 1990;259:F672.
386. Funder JW, Pearce PT, Smith R, et al. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. Science. 1988;242:583.
http://www.ncbi.nlm.nih.gov/pubmed/2845584
387. Pearce D, Yamamoto KR. Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. Science. 1993;259:1161.
388. Bonvalet JP, Doignon I, Blot-Chabaud M, et al. Distribution of 11 beta-hydroxysteroid dehydrogenase along the rabbit nephron. J Clin Invest. 1990;86:832.
389. Naray-Fejes-Toth A, Watlington CO, Fejes-Toth G. 11 beta-Hydroxysteroid dehydrogenase activity in the renal target cells of aldosterone. Endocrinology. 1991;129:17.
http://www.ncbi.nlm.nih.gov/pubmed/2055180
390. Mune T, Rogerson FM, Nikkila H, et al. Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. Nat Genet. 1995;10:394.
http://www.ncbi.nlm.nih.gov/pubmed/7670488
391. Stanton B, Janzen A, Klein-Robbenhaar G, et al. Ultrastructure of rat initial collecting tubule. Effect of adrenal corticosteroid treatment. J Clin Invest. 1985;75:1327.
http://www.ncbi.nlm.nih.gov/pubmed/2985657
392. Palmer LG, Li JH, Lindemann B, et al. Aldosterone control of the density of sodium channels in the toad urinary bladder. J Membr Biol. 1982;64:91.
http://www.ncbi.nlm.nih.gov/pubmed/6276550
393. Kemendy AE, Kleyman TR, Eaton DC. Aldosterone alters the open probability of amiloride-blockable sodium channels in A6 epithelia. Am J Physiol. 1992;263:C825.
394. Kleyman TR, Coupaye-Gerard B, Ernst SA. Aldosterone does not alter apical cell-surface expression of epithelial Na+ channels in the amphibian cell line A6. J Biol Chem. 1992;267:9622.
http://www.ncbi.nlm.nih.gov/pubmed/1315763
395. Chen SY, Bhargava A, Mastroberardino L, et al. Epithelial sodium channel regulated by aldosterone-induced protein sgk. Proc Natl Acad Sci USA. 1999;96:2514.
http://www.ncbi.nlm.nih.gov/pubmed/10051674
396. Alvarez de la Rosa D, Coric T, Todorovic N, et al. Distribution and regulation of expression of serum- and glucocorticoid-induced kinase-1 in the rat kidney. J Physiol. 2003;551:455.
http://www.ncbi.nlm.nih.gov/pubmed/12816971
397. Helms MN, Fejes-Toth G, Naray-Fejes-Toth A. Hormone-regulated transepithelial Na+ transport in mammalian CCD cells requires SGK1 expression. Am J Physiol Renal Physiol. 2003;284:F480.
398. Wulff P, Vallon V Huang DY, et al. Impaired renal Na(+) retention in the sgk1-knockout mouse. J Clin Invest. 2002;110:1263.
399. Alvarez de la Rosa D, Zhang P, Naray-Fejes-Toth A, et al. The serum and glucocorticoid kinase sgk increases the abundance of epithelial sodium channels in the plasma membrane of Xenopus oocytes. J Biol Chem. 1999;274:37834.
http://www.ncbi.nlm.nih.gov/pubmed/10608847
400. Alvarez de la Rosa D, Paunescu TG, Els WJ, et al. Mechanisms of regulation of epithelial sodium channel by SGK1 in A6 cells. J Gen Physiol. 2004;124:395.
http://www.ncbi.nlm.nih.gov/pubmed/15452200
401. Snyder PM, Olson DR, Thomas BC. Serum and glucocorticoid-regulated kinase modulates Nedd4-2-mediated inhibition of the epithelial Na+ channel. J Biol Chem. 2002;277:5.
http://www.ncbi.nlm.nih.gov/pubmed/11696533
402. Bhalla V, Daidie D, Li H, et al. Serum- and glucocorticoid-regulated kinase 1 regulates ubiquitin ligase neural precursor cell-expressed, developmentally down-regulated protein 4-2 by inducing interaction with 14-3-3. Mol Endocrinol. 2005;19:3073.
403. Ichimura T, Yamamura H, Sasamoto K, et al. 14-3-3 proteins modulate the expression of epithelial Na+ channels by phosphorylation-dependent interaction with Nedd4-2 ubiquitin ligase. J Biol Chem. 2005;280:13187.
http://www.ncbi.nlm.nih.gov/pubmed/15677482
404. Soundararajan R, Zhang TT, Wang J, et al. A novel role for glucocorticoid-induced leucine zipper protein in epithelial sodium channel-mediated sodium transport. J Biol Chem. 2005;280:39970.
http://www.ncbi.nlm.nih.gov/pubmed/16216878
405. Soundararajan R, Wang J, Melters D, et al. Differential activities of glucocorticoid-induced leucine zipper protein isoforms. J Biol Chem. 2007;282:36303.
http://www.ncbi.nlm.nih.gov/pubmed/17956870
406. Soundararajan R, Pearce D, Ziera T. The role of the ENaC-regulatory complex in aldosterone-mediated sodium transport. Mol Cell Endocrinol. 2011;350:242.
407. Boldyreff B, Wehling M. Non-genomic actions of aldosterone: mechanisms and consequences in kidney cells. Nephrol Dial Transplant. 2003;18:1693.
http://www.ncbi.nlm.nih.gov/pubmed/12937210
408. Stockand JD, Edinger RS, Eaton DC, et al. Toward understanding the role of methylation in aldosterone-sensitive Na(+) transport. News Physiol Sci. 2000;15:161.
http://www.ncbi.nlm.nih.gov/pubmed/11390901

409. Rokaw MD, Wang JM, Edinger RS, et al. Carboxylmethylation of the beta subunit of xENaC regulates channel activity. J Biol Chem. 1998;273:28746.
410. Stockand JD, Spier BJ, Worrell RT, et al. Regulation of Na(+) reabsorption by the aldosterone-induced small G protein K-Ras2A. J Biol Chem. 1999;274:35449.
http://www.ncbi.nlm.nih.gov/pubmed/10585415
411. Blazer-Yost BL, Paunescu TG, Helman SI, et al. Phosphoinositide 3-kinase is required for aldosterone-regulated sodium reabsorption. Am J Physiol. 1999;277:C531.
412. Park J, Leong ML, Buse P, et al. Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. Embo J. 1999;18:3024.
413. Sansom SC, O'Neil RG. Effects of mineralocorticoids on transport properties of cortical collecting duct basolateral membrane. Am J Physiol. 1986;251:F743.
414. Barlet-Bas C, Khadouri C, Marsy S, et al. Enhanced intracellular sodium concentration in kidney cells recruits a latent pool of Na-K-ATPase whose size is modulated by corticosteroids. J Biol Chem. 1990;265:7799.
http://www.ncbi.nlm.nih.gov/pubmed/2159458
415. Verrey F, Kraehenbuhl JP, Rossier BC. Aldosterone induces a rapid increase in the rate of Na,K-ATPase gene transcription in cultured kidney cells. Mol Endocrinol. 1989;3:1369.
http://www.ncbi.nlm.nih.gov/pubmed/2558308
416. Palmer LG, Antonian L, Frindt G. Regulation of the Na-K pump of the rat cortical collecting tubule by aldosterone. J Gen Physiol. 1993;102:43.
http://www.ncbi.nlm.nih.gov/pubmed/8397277
417. Frindt G, Sackin H, Palmer LG. Whole-cell currents in rat cortical collecting tubule: low-Na diet increases amiloride-sensitive conductance. Am J Physiol. 1990;258:F562.
418. Pacha J, Frindt G, Antonian L, et al. Regulation of Na channels of the rat cortical collecting tubule by aldosterone. J Gen Physiol. 1993;102:25.
http://www.ncbi.nlm.nih.gov/pubmed/8397276
419. May A, Puoti A, Gaeggeler HP, et al. Early effect of aldosterone on the rate of synthesis of the epithelial sodium channel alpha subunit in A6 renal cells. J Am Soc Nephrol. 1997;8:1813.
420. Boyd C, Naray-Fejes-Toth A. Gene regulation of ENaC subunits by serum- and glucocorticoid-inducible kinase-1. Am J Physiol Renal Physiol. 2005;288:F505.
421. Reif MC, Troutman SL, Schafer JA. Sodium transport by rat cortical collecting tubule. Effects of vasopressin and desoxycorticosterone. J Clin Invest. 1986;77:1291.
http://www.ncbi.nlm.nih.gov/pubmed/2420830
422. Schlatter E, Schafer JA. Electrophysiological studies in principal cells of rat cortical collecting tubules. ADH increases the apical membrane Na+ -conductance. Pfugers Arch. 1987;409:81.
http://www.ncbi.nlm.nih.gov/pubmed/2441357
423. Schafer JA, Troutman SL. cAMP mediates the increase in apical membrane Na+ conductance produced in rat CCD by vasopressin. Am J Physiol. 1990;259:F823.
424. Marunaka Y, Eaton DC. Effects of vasopressin and cAMP on single amiloride-blockable Na channels. Am J Physiol. 1991;260:C1071.
425. Ismailov, II, McDuffe JH, Benos DJ. Protein kinase A phosphorylation and G protein regulation of purified renal Na+ channels in planar bilayer membranes. J Biol Chem. 1994;269:10235.
http://www.ncbi.nlm.nih.gov/pubmed/8144604
426. Djelidi S, Fay M, Cluzeaud F, et al. Transcriptional regulation of sodium transport by vasopressin in renal cells. J Biol Chem. 1997;272:32919.
427. Proud D, Nakamura S, Carone FA, et al. Kallikrein-kinin and renin-angiotensin systems in rat renal lymph. Kidney Int. 1984;25:880.
428. Figueroa CD, Gonzalez CB, Grigoriev S, et al. Probing for the bradykinin B2 receptor in rat kidney by anti-peptide and anti-ligand antibodies. J Histochem Cytochem. 1995;43:137.
http://www.ncbi.nlm.nih.gov/pubmed/7822771
429. Tomita K, Pisano JJ, Knepper MA. Control of sodium and potassium transport in the cortical collecting duct of the rat. Effects of bradykinin, vasopressin, and deoxycorticosterone. J Clin Invest. 1985;76:132.
http://www.ncbi.nlm.nih.gov/pubmed/4019771
430. Zaika O, Mamenko M, O'Neil RG, et al. Bradykinin acutely inhibits activity of the epithelial Na+ channel in mammalian aldosterone-sensitive distal nephron. Am J Physiol Renal Physiol. 2011;300:F1105.
431. Nonoguchi H, Knepper MA, Manganiello VC. Effects of atrial natriuretic factor on cyclic guanosine monophosphate and cyclic adenosine monophosphate accumulation in microdissected nephron segments from rats. J Clin Invest. 1987;79:500.
http://www.ncbi.nlm.nih.gov/pubmed/3027127
432. Dillingham MA, Anderson RJ. Inhibition of vasopressin action by atrial natriuretic factor. Science. 1986;231:1572.
http://www.ncbi.nlm.nih.gov/pubmed/3006248
433. Wang W, Li C, Nejsum LN, et al. Biphasic effects of ANP infusion in conscious, euvolumic rats: roles of AQP2 and ENaC trafficking. Am J Physiol Renal Physiol. 2006;290:F530.
434. Rouch AJ, Chen L, Troutman SL, et al. Na+ transport in isolated rat CCD: effects of bradykinin, ANP, clonidine, and hydrochlorothiazide. Am J Physiol. 1991;260:F86.
435. Gudme CN, Larsen AL, Nielsen R. Alpha2-adrenoceptors inhibit antidiuretic hormone-stimulated Na+ absorption across tight epithelia (Rana esculenta). Pfugers Arch. 2001;442:346.
http://www.ncbi.nlm.nih.gov/pubmed/11484764
436. Stokes JB, Kokko JP. Inhibition of sodium transport by prostaglandin E2 across the isolated, perfused rabbit collecting tubule. J Clin Invest. 1977;59:1099.
437. Hebert RL, Jacobson HR, Breyer MD. Prostaglandin E2 inhibits sodium transport in rabbit cortical collecting duct by increasing intracellular calcium. J Clin Invest. 1991;87:1992.
438. Guan Y, Zhang Y, Breyer RM, et al. Prostaglandin E2 inhibits renal collecting duct Na+ absorption by activating the EP1 receptor. J Clin Invest. 1998;102:194.
http://www.ncbi.nlm.nih.gov/pubmed/9649573
439. Warden DH, Stokes JB. EGF and PGE2 inhibit rabbit CCD Na+ transport by different mechanisms: PGE2 inhibits Na(+)-K+ pump. Am J Physiol. 1993;264:F670.
440. Sakairi Y, Jacobson HR, Noland TD, et al. Luminal prostaglandin E receptors regulate salt and water transport in rabbit cortical collecting duct. Am J Physiol. 1995;269:F257.
441. Breyer MD, Jacobson HR, Breyer JA. Epidermal growth factor inhibits the hydroosmotic effect of vasopressin in the isolated perfused rabbit cortical collecting tubule. J Clin Invest. 1988;82:1313.
http://www.ncbi.nlm.nih.gov/pubmed/2844852
442. Muto S, Furuya H, Tabei K, et al. Site and mechanism of action of epidermal growth factor in rabbit cortical collecting duct. Am J Physiol. 1991;260:F163.
443. Levchenko V, Zheleznova NN, Pavlov TS, et al. EGF and its related growth factors mediate sodium transport in mpkCCDc14 cells via ErbB2 (neu/HER-2) receptor. J Cell Physiol. 2010;223:252.
http://www.ncbi.nlm.nih.gov/pubmed/20049896
444. Kohan DE. Endothelin and collecting duct sodium and water transport. Contrib Nephrol. 2011;172:94.
http://www.ncbi.nlm.nih.gov/pubmed/21893992
445. Stoos BA, Garcia NH, Garvin JL. Nitric oxide inhibits sodium reabsorption in the isolated perfused cortical collecting duct. J Am Soc Nephrol. 1995;6:89.
http://www.ncbi.nlm.nih.gov/pubmed/7579075
446. Sun D, Schafer JA. Dopamine inhibits AVP-dependent Na+ transport and water permeability in rat CCD via a D4-like receptor. Am J Physiol. 1996;271:F391.
447. Saito O, Ando Y, Kusano E, et al. Functional characterization of basolateral and luminal dopamine receptors in rabbit CCD. Am J Physiol Renal Physiol. 2001;281:F114.
448. Peti-Peterdi J, Warnock DG, Bell PD. Angiotensin II directly stimulates ENaC activity in the cortical collecting duct via AT(1) receptors. J Am Soc Nephrol. 2002;13:1131.
449. Beutler KT, Masilamani S, Turban S, et al. Long-term regulation of ENaC expression in kidney by angiotensin II. Hypertension. 2003;41:1143.
http://www.ncbi.nlm.nih.gov/pubmed/12682079
450. Sun P, Yue P, Wang WH. Angiotensin II stimulates epithelial sodium channels (ENaC) in the cortical collecting duct of the rat kidney. Am J Physiol Renal Physiol. 2011 Dec 14 [Epub ahead of print].
451. Hallows KR, Wang H, Edinger RS, et al. Regulation of epithelial Na+ transport by soluble adenylyl cyclase in kidney collecting duct cells. J Biol Chem. 2009;284:5774.
http://www.ncbi.nlm.nih.gov/pubmed/19126549
452. Pech V, Pham TD, Hong S, et al. Pendrin modulates ENaC function by changing luminal HCO3. J Am Soc Nephrol. 2010;21:1928.
453. Edinger RS, Lebowitz J, Li H, et al. Functional regulation of the epithelial Na+ channel by IkappaB kinase-beta occurs via phosphorylation of the ubiquitin ligase Nedd4-2. J Biol Chem. 2009;284:150.
http://www.ncbi.nlm.nih.gov/pubmed/18981174
454. Carattino MD, Edinger RS, Grieser HJ, et al. Epithelial sodium channel inhibition by AMP-activated protein kinase in oocytes and polarized renal epithelial cells. J Biol Chem. 2005;280:17608.
http://www.ncbi.nlm.nih.gov/pubmed/15753079
455. Bhalla V, Hallows KR. Mechanisms of ENaC regulation and clinical implications. J Am Soc Nephrol. 2008;19:1845.
http://www.ncbi.nlm.nih.gov/pubmed/18753254

456. Koeppen BM. Conductive properties of the rabbit outer medullary collecting duct: outer stripe. Am J Physiol. 1986;250:F70.
457. Stokes JB. Na and K transport across the cortical and outer medullary collecting tubule of the rabbit: evidence for diffusion across the outer medullary portion. Am J Physiol. 1982;242:F514.
458. Koeppen BM. Conductive properties of the rabbit outer medullary collecting duct: inner stripe. Am J Physiol. 1985;248:F500.
459. Clapp WL, Madsen KM, Verlander JW, et al. Morphologic heterogeneity along the rat inner medullary collecting duct. Lab Invest. 1989;60:219.
http://www.ncbi.nlm.nih.gov/pubmed/2915516
460. Sands JM, Nonoguchi H, Knepper MA. Vasopressin effects on urea and H2O transport in inner medullary collecting duct subsegments. Am J Physiol. 1987;253:F823.
461. Stanton BA. Characterization of apical and basolateral membrane conductances of rat inner medullary collecting duct. Am J Physiol. 1989;256:F862.
462. Imai M, Yoshitomi K. Electrophysiological study of inner medullary collecting duct of hamsters. Pfugers Arch. 1990;416:180.
http://www.ncbi.nlm.nih.gov/pubmed/2162031
463. Sonnenberg H, Honrath U, Wilson DR. Effects of amiloride in the medullary collecting duct of rat kidney. Kidney Int. 1987;31:1121.
464. Sands JM, Nonoguchi H, Knepper MA. Hormone effects on NaCl permeability of rat inner medullary collecting duct. Am J Physiol. 1988;255:F421.
465. Light DB, McCann FV, Keller TM, et al. Amiloride-sensitive cation channel in apical membrane of inner medullary collecting duct. Am J Physiol. 1988;255:F278.
466. Vandorpe DH, Ciampolillo F, Green RB, et al. Cyclic nucleotide-gated cation channels mediate sodium absorption by IMCD (mIMCD-K2) cells. Am J Physiol. 1997;272:C901.
467. Wilson DR, Honrath U, Sonnenberg H. Furosemide action on collecting ducts: effect of prostaglandin synthesis inhibition. Am J Physiol. 1983; 244:F666.
468. Wilson DR, Honrath U, Sonnenberg H. Thiazide diuretic effect on medullary collecting duct function in the rat. Kidney Int. 1983;23:711.
http://www.ncbi.nlm.nih.gov/pubmed/6876566
469. Zeidel ML, Kikeri D, Silva P, et al. Atrial natriuretic peptides inhibit conductive sodium uptake by rabbit inner medullary collecting duct cells. J Clin Invest. 1988;82:1067.
470. Rocha AS, Kudo LH. Factors governing sodium and chloride transport across the inner medullary collecting duct. Kidney Int. 1990;38:654.
http://www.ncbi.nlm.nih.gov/pubmed/2146432
471. Delpire E, Rauchman MI, Beier DR, et al. Molecular cloning and chromosome localization of a putative basolateral Na(+)-K(+)-2Cl- cotransporter from mouse inner medullary collecting duct (mIMCD-3) cells. J Biol Chem. 1994;269:25677.
http://www.ncbi.nlm.nih.gov/pubmed/7929272
472. Praetorius J, Kim YH, Bouzinova EV, et al. NBCn1 is a basolateral Na+-HCO3- cotransporter in rat kidney inner medullary collecting ducts. Am J Physiol Renal Physiol. 2004;286:F903.
473. Zeidel ML. Renal actions of atrial natriuretic peptide: regulation of collecting duct sodium and water transport. Annu Rev Physiol. 1990;52:747.
http://www.ncbi.nlm.nih.gov/pubmed/2139559
474. Zeidel ML, Silva P, Brenner BM, et al. cGMP mediates effects of atrial peptides on medullary collecting duct cells. Am J Physiol. 1987;252:F551.
475. Light DB, Schwiebert EM, Karlson KH, et al. Atrial natriuretic peptide inhibits a cation channel in renal inner medullary collecting duct cells. Science. 1989;243:383.
http://www.ncbi.nlm.nih.gov/pubmed/2463673
476. Rocha AS, Kudo LH. Atrial peptide and cGMP effects on NaCl transport in inner medullary collecting duct. Am J Physiol. 1990;259:F258.
477. Kudo LH, van Baak AA, Rocha AS. Effect of vasopressin on sodium transport across inner medullary collecting duct. Am J Physiol. 1990;258:F1438.
478. Ullrich KJ, Papavassiliou F. Sodium reabsorption in the papillary collecting duct of rats. Effect of adrenalectomy, low Na+ diet, acetazolamide, HCO-3-free solutions and of amiloride. Pfugers Arch. 1979;379:49.
http://www.ncbi.nlm.nih.gov/pubmed/571103
479. Husted RF, Stokes JB. Separate regulation of Na+ and anion transport by IMCD: location, aldosterone, hypertonicity, TGF-beta 1, and cAMP. Am J Physiol. 1996;271:F433.
480. Terada Y, Knepper MA. Na +-K +-ATPase activities in renal tubule segments of rat inner medulla. Am J Physiol. 1989;256:F218.