CHAPTER 8

The Physiology of the Loop of Henle

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OBJECTIVES

This chapter will:

1. Define the structural-function correlation of the loop of Henle.
2. Discuss sodium transport and the countercurrent system.
3. Analyze, at the molecular level, the mechanisms and factors regulating bicarbonate reabsorption.
4. Examine the role of the loop of Henle in the renal handling of ammonia/ammonium ion.
5. Discuss the reabsorption of divalent cations.

The loop of Henle is a very complex segment characterized by at least two peculiar properties, its extreme heterogeneity, and its particular anatomic configuration. This structure is defined anatomically as composing the pars recta of the proximal tubule (thick descending limb), the thin descending and ascending limbs, the thick ascending limb (TAL), and the macula densa. The loop of Henle is surrounded by tissue with increasing interstitial osmolality,[1] resulting from the noticeable addition of sodium, chloride, and urea contents,[2] which is accomplished through the countercurrent system. As shown by several investigators, medullary cells use cellular osmolytes to survive in this hypertonic environment.[2] In addition to its role in continuing the reabsorption of solutes, this part of the nephron is responsible for the kidney's ability to generate a concentrated or dilute urine.[1,3]

SODIUM TRANSPORT AND THE COUNTERCURRENT SYSTEM

A major function of the loop of Henle is the creation and maintenance of the interstitial osmotic gradient that increases from the renal cortex (approximately 290 mOsm/kg) to the tip of the medulla (approximately 1200 mOsm/kg). The loop of Henle reabsorbs approximately 40% of filtered sodium, mostly in the TAL, and approximately 25% of filtered water in the pars recta and thin descending limb. The thin descending limb is permeable to water but relatively impermeable to sodium, whereas the thin ascending limb and TAL are essentially impermeable to water.[4] Sodium is reabsorbed passively in the thin ascending limb but actively in the TAL. Active sodium reabsorption in the TAL is driven by the basolateral sodium pump (Na^+,K^+–adenosine triphosphatase [ATPase]), which maintains a low intracellular sodium concentration, allowing sodium entry from the lumen, mainly via the Na^+,K^+,$2Cl^-$ cotransporter.[5] This transporter, which is expressed exclusively along the loop of Henle, is the site of action of loop diuretics such as furosemide. Sodium exits the cell via the sodium pump, whereas chloride exits through the basolateral Cl^- channel ClCKa and ClCKb. Potassium ions may exit either via basolateral ion channels and a K^+,Cl^- cotransporter; in addition, potassium also recycles through apical membrane renal outer medullary potassium (ROMK) channels.[6] Potassium recycling is also partly responsible for generating the lumen-positive potential difference (transepithelial voltage [V_{te}]) found in this segment. This V_{te} drives additional sodium reabsorption through the paracellular pathway: For each sodium ion reabsorbed transcellularly, another one is reabsorbed paracellularly. Other cations (potassium, calcium, magnesium) also are reabsorbed by this route. These carriers act in synergy, and the reduced function of each one (NKCC2, ROMK, CLCKb, and its Barttin subunit) results in defective salt absorption, leading to a salt-losing tubulopathy named Bartter syndrome (BS). The description of several cases of BS patients carrying activating mutation of the calcium-sensing receptor (CaSR) raised the hypothesis that this protein is involved in the regulation of salt handling along the TAL. Further studies have demonstrated that the CaSR along the TAL is activated by extracellular calcium ions; once activated, CaSR inhibits NKCC2 and ROMK, plus Na,K–ATPase at basolateral level. This inhibition results in sodium and chloride wasting and into the reduction of the positive luminal potential that drives paracellular calcium and magnesium. Fig. 8.1 shows the schematic salt absorption along the TAL. A recent report describes several cases of X-linked polyhydramnios with prematurity and transient antenatal BS associated with mutation in MAGE-D2 gene. The latter encodes a protein that affects expression and function of both NKCC2 and Na-Cl cotransporters, along the distal tubule.

The U-shaped countercurrent arrangement of the loop of Henle, the differences in permeability of the descending and ascending limbs to sodium and water, and the active sodium reabsorption in the TAL are the basis of countercurrent multiplication and generation of the medullary osmotic gradient. Fluid entering the descending limb from the proximal tubule is isotonic (approximately 290 mOsm/kg). However, the raised medullary osmolarity drives water

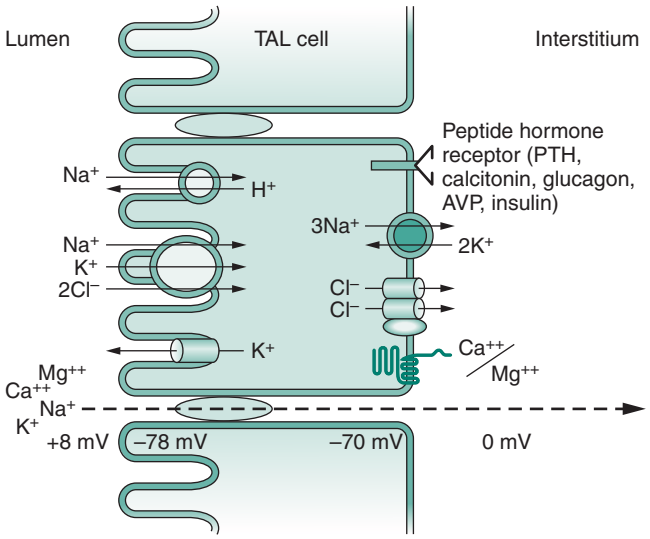

FIGURE 8.1 Schema of the thick ascending limb (TAL) cells with the main ion transport proteins localized on the luminal and basolateral membranes. Note the lumen's positive transepithelial potential difference. *AVP,* Arginine vasopressin; *Ca^{++},* calcium ion; *Cl^-,* chloride ion; *H^+,* hydrogen ion; *K^+,* potassium ion; *Mg^{++},* magnesium ion; *mV,* millivolt(s); *Na^+,* sodium ion; *PTH,* parathyroid hormone.

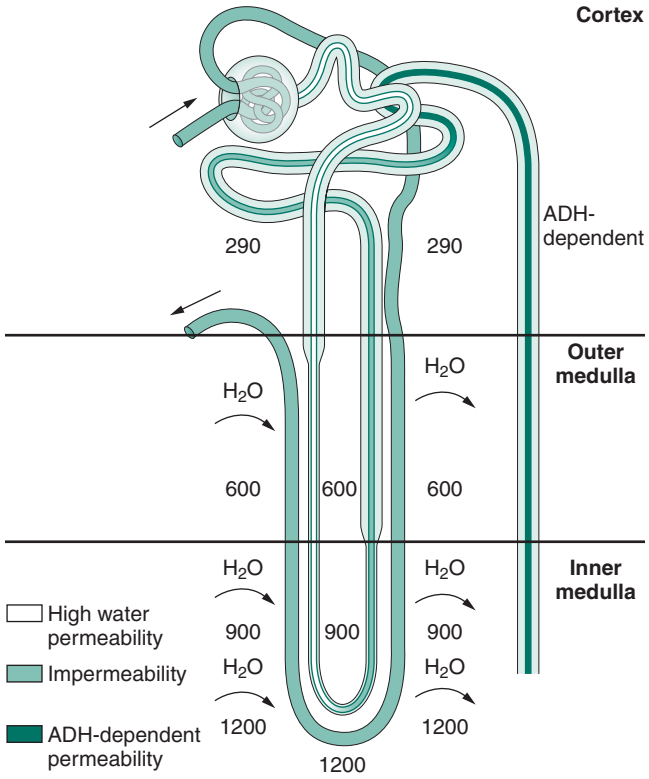

FIGURE 8.2 The U-shaped arrangements of the loop of Henle and vasa recta. The interstitial osmolality increases from the cortex (290 mOsm/kg H_2O) to the medulla (up to 1200 mOsm/kg H_2O). *ADH,* Antidiuretic hormone; *H_2O,* water molecule.

reabsorption from the thin descending limb, thereby raising the osmolarity and sodium chloride concentration of the fluid delivered to the ascending limb. Conversely, the active sodium chloride reabsorption in the ascending limb results in the generation of hypotonic (approximately 100 mOsm/kg) fluid delivered to the distal tubule (Fig. 8.2).

As already indicated, the ability to concentrate the urine requires an hypertonic medulla; this goal is achieved thanks to the active sodium reabsorption along the TAL. In addition, a significant role in this process involves kidney urea handling, which leads to urea accumulation in the medulla contributing to increased medulla tonicity.[2] Several urea transporters have been cloned. They belong to two different families encoded by two genes. UT-As proteins are expressed in several nephron segments (mainly thin descending limb and in the collecting ducts but not in the vasculature). UT-B transporters are expressed along the arterial vasa recta through the renal medulla and also are expressed in other cells and organs as erythrocytes, brain, heart, testis, and bladder. These proteins are crucial in the urine concentrating mechanism. In an animal model, UT-B deletion leads to the reduced ability to maximally concentrate the urine because of a defective ability to accumulate urea in the inner medulla, demonstrating the importance of urea recycling along the vasa recta. Similarly, UT-A2 KO mice and UT-A1/UTA3 deficient mice show an impaired ability to concentrate the urine. As the loop of Henle, the capillaries that supply the medulla have a special anatomic arrangement. Descending vasa recta lose water and gain solutes while ascending vasa recta gain water and loose solutes. This countercurrent exchange is highly efficient to produce concentrated urine. Conditions that decrease medullary flow, such as dehydration, improve urine concentrating ability by allowing more time for blood into vasa recta to achieve osmotic equilibration with the hypertonic interstitium. Conversely, an increased medullary flow, as in osmotic diuresis, decreases urine concentrating ability. The role of the collecting duct in this process is explained elsewhere.

ACID-BASE TRANSPORT

The maintenance of a correct acid-base balance is essential for normal cell function. The kidney plays a central role in this process through several mechanisms, including the almost complete tubular reabsorption of filtered bicarbonate. Various nephron segments participate in this task; there is general agreement about the importance of the proximal tubule in bicarbonate reabsorption. However, other downstream segments participate in this process as well.[7] Thus the loop of Henle is potentially an important site of acid-base regulation because it reabsorbs, under physiologic conditions, a significant fraction (about 15%) of the filtered bicarbonate.

Studies on bicarbonate transport along the loop of Henle in the rat in vivo indicate that the descending limb of Henle's loop has low bicarbonate permeability.[8] Accordingly, it is unlikely to play a major role in the overall process of bicarbonate reabsorption. In contrast, perfusion studies of the S3 segment of the proximal tubule have demonstrated its ability to reabsorb bicarbonate. On the basis of these observations, two portions of the loop of Henle, the S3 segment of the proximal tubule and the TAL, participate significantly in the overall reabsorption of bicarbonate. However, it is possible that under physiologic conditions, the contribution of the S3 segment to bicarbonate reabsorption is only modest because the concentration of bicarbonate of the fluid entering this nephron segment is low (about 5 mM) as a consequence of avid bicarbonate reabsorption in the early segments (S1 and S2) of the proximal tubule.

The situation is different in the TAL. Micropuncture studies have shown that, by the time fluid has reached the tip of Henle's loop, as general effect of water reabsorption, the concentration of bicarbonate rises significantly.[9] As a consequence, bicarbonate reabsorption in the TAL is facilitated. The reabsorption of water in excess of bicarbonate in the descending limb of Henle and the transfer of bicarbonate in a concentration-dependent manner in the TAL constitute a potent system of bicarbonate retrieval along the TAL. It may be concluded that under physiologic conditions, bicarbonate reabsorption along the loop of Henle is largely a function of the TAL.[10]

At the molecular level, in vivo perfusion studies of the LOH have identified the Na^+,H^+ exchanger as the major proton-secreting mechanism responsible for bicarbonate reabsorption, thus confirming previous experiments performed in vitro on isolated TALs.[11,12] This antiporter is a ubiquitous membrane protein that pumps protons against an electrochemical gradient by using a downhill sodium gradient. Starting from the seminal work of Sardet and associates,[13] at least eight membrane isoforms (NHEs) have been cloned. Along the TAL, NHE3 has been localized to the luminal membrane and has been identified as the major proton-secreting NHE along this segment.[14] In addition, NHE2 also has been found along the TAL.[15] The role of NHE2 is unknown, but it has been postulated to offset the loss of function of NHE3. The transport process of bicarbonate is active in nature because its concentration in the fluid emerging from the loop is much lower than that measured at the tip of Henle's loop.[8] The TAL can lower luminal bicarbonate concentration to a limiting value of about 5 mM. Moreover, as demonstrated by both loop perfusions in vivo and perfusion studies of TAL ex vivo, the transport of bicarbonate is concentration dependent and sharply decreases after administration of a carbonic anhydrase inhibitor such as acetazolamide or methazolamide.[11] Maneuvers that interfere with the activity of basolateral Na^+,K^+–ATPase, such as the removal of either sodium from the basolateral and lumen, or of potassium from the basolateral, lead to almost complete cessation of bicarbonate reabsorption in perfused TAL in vitro. Furosemide and bumetanide, inhibitors of Na^+,K^+,$2Cl^-$ cotransport in the apical membrane of cells lining the TAL, stimulate bicarbonate reabsorption.[11] This effect is explained best by the fall in cell sodium concentration after exposure to inhibitors of Na^+,K^+,$2Cl^-$ transport and the rise of the sodium gradient across the apical membrane, which would be responsible for increasing the rate of Na^+-H^+ exchange.[12] Results from perfusion studies of the loop of Henle in vivo are consistent with the conclusion that NHE3 is the predominant isoform of the sodium-hydrogen exchanger involved in bicarbonate reabsorption, because such studies show that bicarbonate transport is reduced sharply by a specific NHE3 inhibitor but not by HOE 694, an agent known to block NHE2 exclusively.[16,17]

The predominant role in sodium reabsorption and H^+ secretion through the NHE3 promoted by blocking the NKCC2 with furosemide has been proposed recently as the urine acidification mechanism induced by furosemide. Furosemide plus fludrocortisone administration has been validated as an alternative method to ammonium chloride administration to maximize acid secretion along the collecting duct and diagnose a distal renal tubular acidosis. Conventionally, furosemide induces an increase in sodium delivery to the collecting duct that will stimulate the ENaC-mediated proton secretion in the A-type intercalated cells. Investigators have proposed that the furosemide-inducing urine acidification could be dependent on a direct increased secretion of protons in the TAL through the increased activity of the NHE3, highlighting the role of the TAL in the renal acid-base homeostasis.[18]

The role of additional transporters participating in bicarbonate transport along the loop of Henle is uncertain. Conceivably, H^+ transport by H^+-ATPase could mediate some bicarbonate reabsorption in the S3 segment of the proximal tubule and the TAL.[11] Microperfusion studies performed on LOH with the use of bafilomycin, an inhibitor of electrogenic H^+-ATPase, have demonstrated the presence of a modest but functionally significant active H^+-ATPase. Moreover, immunohistochemical evidence for the presence of proton ATPase along the TAL has been reported.[19] Recently we found critical the role of the intracellular H^+-ATPase for the cells of the medullary TAL. Suppression of the subunit Atp6ap2 of the H^+-ATPase determines lysosomal dysfunction and impairment of the autophagy flux coupling a phenotype of distal renal tubular acidosis to urinary concentrating defect.[20]

Basolateral Membrane

Epithelial polarity plays the main role in the solute reabsorption by generating diffusion gradient cell-to-lumen and cell-to-interstitium. Transcellular bicarbonate reabsorption depends also on effective mechanisms of base exit across the basolateral membranes of bicarbonate-transporting tubule cells. The main exit pathway for bicarbonate along the TAL seems to be the anion exchanger 2 (AE2). By exchanging Cl^- with HCO_3^-, this protein guarantees net bicarbonate absorption in the interstitium of the TAL at cortical and medullary level.[21] However, experimental evidence, obtained in perfused TAL and in fused cells of the frog's diluting segment, have demonstrated the presence of sodium bicarbonate cotransporter that shares many properties with a cotransporter in the basolateral membrane of proximal tubule cells.[22,23] This sodium coupled bicarbonate transporter, identified as NBCn1, works by importing bicarbonate in the cells coupled with sodium. NBCn1 function is devoted primarily to the ammonia reabsorption along the medullary TAL.[24]

Some properties of the basolateral acid-base transporters deserve mention. First, basolateral sodium-hydrogen exchange, maintained by the NHE1 isoform of the Na^+-H^+ transporter family, has been identified in the TAL,[25] and its activity has been shown to alter apical Na^+-H^+ exchange and thus net bicarbonate reabsorption. Perfusion experiments in which net transport of bicarbonate and cell pH were monitored showed that basolateral Na^+-H^+ exchange enhances transepithelial bicarbonate reabsorption. These results are unexpected because stimulation of basolateral Na^+-H^+ exchange should increase cell pH and thus lower apical Na^+-H^+ exchange. The mechanism of such "cross-talk" between basolateral and apical membrane Na^+-H^+ exchanges, and their coordination, is incompletely understood. However, the identification of basolateral Na^+-H^+ exchange as a potential site of physiologic regulation of luminal acidification and of bicarbonate transport across the TAL is of great interest.

In addition to NHE1, NHE4 has been localized on the basolateral membrane of TAL. Functional experiments have led to the hypothesis that this particular isoform may be involved specifically in ammonium transport across the basolateral membrane of TAL.[26]

Cell pH and Bicarbonate Transport

The regulation of transepithelial bicarbonate reabsorption by intracellular pH (pH_i) and hyperosmolarity is also a subject

of study. Compared with the behavior of Na^+-H^+ exchange in most epithelia, the NHE3-mediated Na^+-H^+ exchange in the apical membrane of TAL has a much higher apparent affinity for intracellular H^+.[27] Thus exchange activity is relatively insensitive to changes in cell pH over the physiologic range, and the turnover rate of the transporter is already near maximum at normal cell pH and does not respond to pH changes (pH_i between 6.5 and 7.2). This situation contrasts with the characteristics of Na^+-H^+ exchanges in other epithelia, in which transport activity drops sharply when pH is altered in the range of 6.5 to 7.1.[28] It has been suggested that the insensitivity of apical Na^+-H^+ exchange in the TAL reflects an adaptation to prevent fluctuations of transepithelial bicarbonate transport during changes in pH_i that may be related to ammonium (NH_4^+) transport. NH_4^+ entry into cells of the TAL has been shown to occur by carrier-mediated electroneutral NH_4^+,$2Cl^-$ transport (NH_4^+ replacing Na^+ and K^+ on the Na^+,K^+,$2Cl^-$ transporter; see later) and may lead to fluctuations of pH_i. Insensitivity of the apical Na^+-H^+ exchanger to pH_i changes thus would uncouple bicarbonate reabsorption from NH_4^+ excretion. Changes in external osmolality also modulate apical Na^+-H^+ exchange and bicarbonate absorption in TAL.[29] External hyperosmolarity lowers transepithelial bicarbonate transport, whereas hypotonicity stimulates transport.[30] It appears that the mechanism by which hyperosmolarity reduces apical Na^+-H^+ exchange involves an acid shift in the pH_i dependence of bicarbonate transport and a decrease in the transporter's sensitivity to the stimulating effect of pH_i. The opposite effect, stimulation of Na^+-H^+ exchange and bicarbonate reabsorption during decrease in medullary osmolarity, may play a role in greater urinary acidification and diminished bicarbonate excretion when loop diuretics affect medullary washout of solutes.[31]

Other Regulating Factors

Systemic acid-base disturbances also modulate bicarbonate transport: acidosis increases bicarbonate reabsorption, whereas metabolic alkalosis has the opposite effect.[32] The loop of Henle can participate in the tubular adaptation to an increase in filtered load of bicarbonate by increasing net loop of Henle bicarbonate transport. In this setting, at the molecular level, NHE3 RNA and protein abundance also were stimulated and, accordingly, NHE3 activity increased.[33] Finally NHE3 expression and abundance were highly stimulated in the early phase of diabetes, which is characterized by increased glomerular filtration rate.[34]

AMMONIA AND AMMONIUM ION TRANSPORT

Ammonium ion (NH_4^+) is generated by proximal tubular cells and is secreted partly within the tubular fluid. NH_4^+ then reaches the TAL of Henle's loop, where it is largely reabsorbed. Absorption of NH_4^+ and ammonia (NH_3) by the medullary TAL (MTAL) in absence of water transport provides the energy for total ammonia accumulation (the sum of NH_4^+ and NH_3) in the medullary interstitium, which favors ammonia secretion into the tubular fluid of adjacent medullary collecting ducts. Thus a major part of the NH_4^+ excreted in urine derives from the NH_4^+ synthesized by proximal tubular cells and absorbed by the MTAL. The diffusion of NH_3 coupled to H^+ transport and trapping as NH_4^+ in the acidic lumen of the collecting duct make up an important mechanism of transepithelial ammonium transport (Fig. 8.3).[35]

As stated earlier, a major fraction of the NH_4^+ delivered by the proximal tubule must be reabsorbed by the MTAL to accumulate in the medullary interstitium and to be secreted directly in contiguous collecting ducts. Total ammonia is absorbed by the MTAL primarily as NH_4^+ by means of secondary active transporters. Diffusion of NH_4^+ from lumen to peritubular space also takes place through the paracellular pathway as a consequence of the lumen-positive transepithelial voltage of the MTAL. NH_4^+ absorption

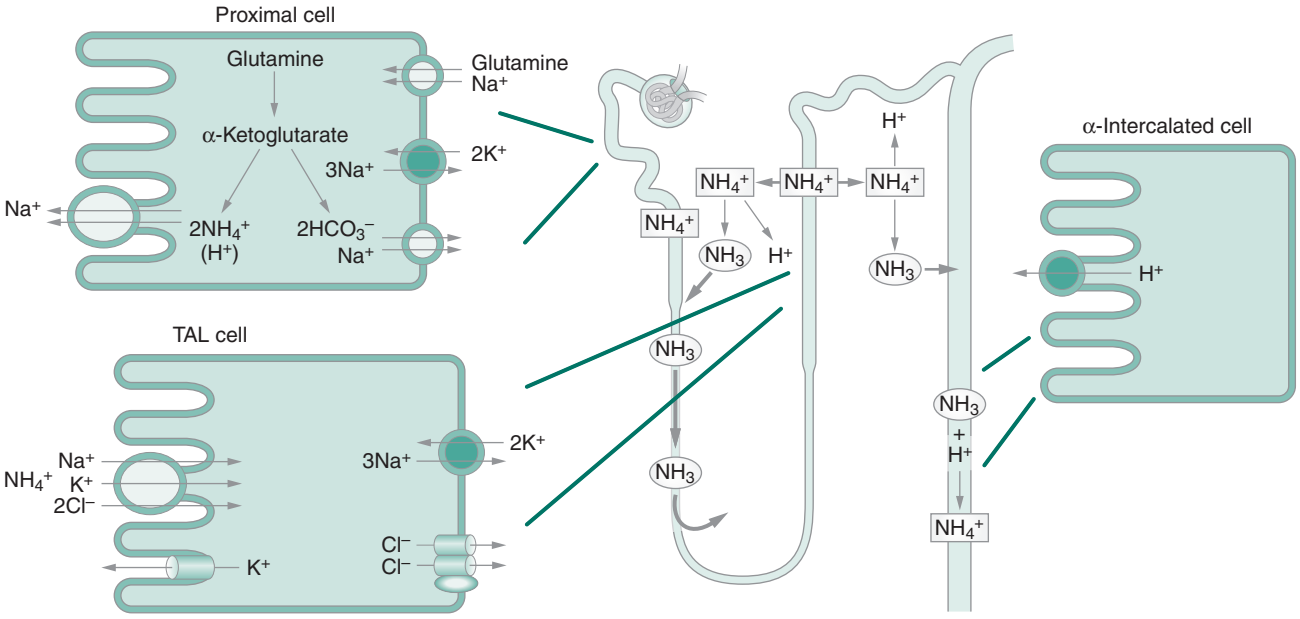

FIGURE 8.3 Renal handling of ammonia (NH_3) and ammonium ion (NH_4^+). For details see text. *Cl^-,* chloride ion; *H^+,* hydrogen ion; *HCO_3^-,* bicarbonate ion; *K^+,* potassium ion; *Na^+,* sodium ion.

is regulated by the acid-base status. Indeed, the ability of the MTAL that has been isolated and perfused in vitro to absorb total ammonia is increased during chronic metabolic acidosis (CMA).[36] The $Na^+,K^+(NH_4^+),2Cl^-$ cotransporter is the main apical NH_4^+ carrier and is responsible for 50% to 65% of NH_4^+ luminal uptake. An electroneutral barium- and verapamil-sensitive $K^+,NH_4^+(H^+)$ antiport mechanism is responsible for the rest of the MTAL NH_4^+ luminal uptake. On the basolateral side of TAL cells, the Na^+-H^+ exchanger NHE1 of the MTAL significantly contributes to the cell-to-peritubular space NH_4^+ transport. As previously stated, the capacity of the MTAL to absorb NH_4^+ increases during chronic metabolic acidosis, and this adaptation favors the renal elimination of an acid load.[37] The mechanism explaining this MTAL adaptation is the increased expression and activity of $Na^+,K^+(NH_4^+),2Cl^-$ by metabolic acidosis.[38]

THE FUNCTION OF THE MACULA DENSA

The *macula densa* is a region of specialized epithelial cells of the TAL where there is close anatomic contact between the TAL and the vascular pole of its own glomerulus.[39] Macula densa cells differ from the other cells of the TAL; they have large nuclei and are closely packed, thus looking like a plaque (leading to the term macula densa). They are part of the juxtaglomerular apparatus, which consists of the extraglomerular matrix (secreted by the mesangial cells of the glomerulus) and the granular cells of the afferent arterioles, which are the site of production, storage, and release of renin (Fig. 8.4). The juxtaglomerular apparatus is a part of a complex feedback mechanism that regulates renal blood flow, glomerular filtration rate, and sodium balance. The complex mechanism matches the amount of sodium that escapes the proximal tubule and thus is delivered to the TAL, with the capacity of more distal nephron segments to reabsorb sodium; it does so by altering the glomerular filtration rate and the filtered load of sodium, a process known as *tubuloglomerular feedback*.

Macula densa cells detect changes in luminal sodium chloride concentration through a complicated series of ion transport–related intracellular events. Sodium chloride entry via an $Na^+,K^+,2Cl^-$ cotransporter and exit of chloride ions through a basolateral channel lead to cell depolarization and elevations in cytosolic calcium.[40] Communication from macula densa cells to the glomerular vascular elements involves the release of ATP across the macula densa basolateral membrane through an axion channel with high conductance. Increased sodium and fluid delivery to the TAL and macula densa region signals the cells of the juxtaglomerular apparatus to release renin and produce angiotensin II locally as well as other vasoconstrictors, which act on the afferent arteriole to decrease filtration and thereby sodium and fluid delivery to the TAL and beyond.[41]

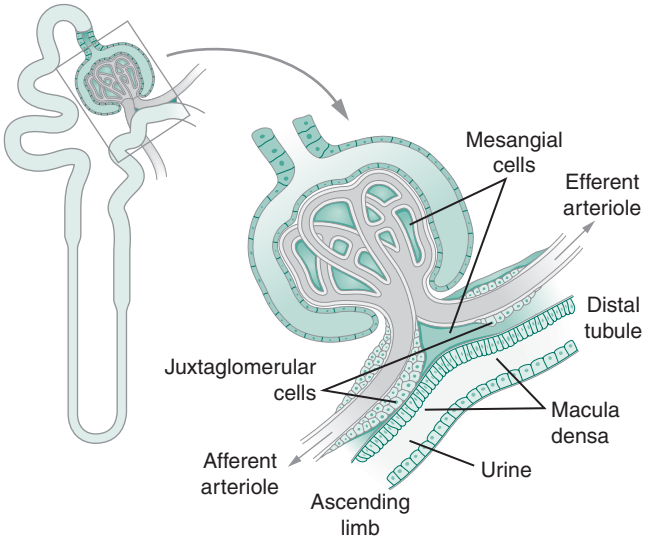

FIGURE 8.4 Schematic view of the juxtaglomerular apparatus.

TRANSPORT OF DIVALENT CATIONS

Calcium Transport

Plasma calcium is approximately 50% protein bound; only the remaining 50% is filterable. The proximal tubule is the major site of calcium ion (Ca^{2+}) transport, reabsorbing around 65% of the filtered load. Along the loop of Henle, the thin limbs have a minor role, whereas the TAL transports calcium mainly paracellularly, driven by a substantial lumen-positive V_{te}. However, a significant component of calcium reabsorption along the TAL is transcellular, as indicated by the finding that the loss of V_{te} does not suppress completely calcium transport and by the presence of Ca^{2+}-ATPase in the basolateral membrane.[42] Calcium transport is affected by parathyroid hormone, which stimulates calcium transport along the TAL and distal tubule through accumulation of cyclic adenosine monophosphate. However, when parathyroid hormone is present in excess, its anticalciuretic effect is offset by the increased filtered load of calcium because of enhanced gastrointestinal absorption of calcium and its release from bone. The action and importance of calcitonin as a regulator of calcium excretion are uncertain, but it is probably also anticalciuretic, acting (like parathyroid hormone) at the TAL and distal tubule. In contrast to thiazides, which induce hypocalciuria, loop diuretics such as furosemide increase calcium excretion, presumably because of their predominant action on the TAL's V_{te}-dependent paracellular calcium reabsorption. Clinical correlates of the differing effects of thiazide and loop diuretics on calcium excretion are Gitelman and Bartter syndromes, characterized by hypocalciuria and hypercalciuria, respectively.[43]

Changes in acid-base balance also affect calcium excretion. Metabolic acidosis is associated with an increase in calcium excretion, whereas metabolic alkalosis has the opposite effect. Although there is evidence that the calcium channels in the distal tubule are pH sensitive, much of the effect on calcium excretion occurs through alterations in filtered load. The buffering of hydrogen ions by the skeleton leaches calcium from bone, and in addition, a fall in plasma pH reduces calcium binding by proteins and thereby increases free calcium ions; these effects increase the filtered load of calcium.

Magnesium Reabsorption

Magnesium is the fourth most abundant cation in the body and the second most common cation in the intracellular fluid. The kidney provides the most sensitive control for magnesium balance. About 80% of the total serum magnesium is ultrafilterable through the glomerular membrane. The proximal tubule of the adult animal reabsorbs only a small fraction (10%–15%) of the filtered magnesium.

Micropuncture experiments indicate that approximately 60% of the filtered magnesium is reabsorbed in the loop of Henle.[44] Magnesium reabsorption in the loop occurs within the cortical thick ascending limb (CTAL) by passive means. The driving force for paracellular magnesium reabsorption is the lumen-positive voltage of the TAL.[45] Along this segment, a specific tight junction protein called claudin 16 (paracellin 1) is necessary for paracellular magnesium reabsorption.[46] Support for the importance of such protein comes from finding that mutations of the claudin 16 gene are associated with severe renal magnesium wasting.[47]

Many hormones (parathyroid hormone, calcitonin, glucagons, arginine vasopressin) and nonhormonal factors (magnesium restriction, acid-base balance, potassium depletion) influence renal reabsorption of magnesium to variable extents in the CTAL. Dietary magnesium restriction leads to renal magnesium conservation with diminished urinary magnesium excretion. Adaptation of magnesium transport with dietary magnesium restriction occurs in the CTAL and distal tubule. Elevation of plasma magnesium and calcium concentrations inhibits magnesium and calcium reabsorption, leading to hypermagnesuria and hypercalciuria. The identification of a calcium-magnesium–sensing receptor located on the peritubular sides of TAL and distal tubule cells explains this phenomenon (see later). Loop diuretics, such as furosemide and bumetanide, diminish salt absorption in the CTAL. Finally, metabolic acidosis, potassium depletion, or phosphate restriction can diminish magnesium reabsorption within the loop and distal tubule.[48]

The Calcium-Magnesium–Sensing Receptor

Calcium and magnesium transport in the TAL are influenced by the calcium-magnesium–sensing receptor, which has been localized to the basolateral membrane. When activated by a rise in plasma calcium/magnesium concentration, it causes reductions in sodium chloride reabsorption and V_{te}, thereby inhibiting reabsorption of calcium and magnesium.[49]

The signal transduction pathway includes stimulation of arachidonic acid (AA) production through direct or indirect activation of phospholipase A_2 (PLA_2), which is metabolized via the cytochrome P450 pathway to an active metabolite that inhibits the apical potassium channel and, perhaps, the Na^+,K^+,$2Cl^-$ cotransporter (Fig. 8.5). Both actions lower overall cotransporter activity, thereby reducing the lumen-positive voltage and paracellular transport of divalent cations. The calcium-magnesium–sensing receptor probably also directly or indirectly (by raising intracellular Ca^{2+}) inhibits adenylate cyclase and causes decrease of hormone-stimulated divalent cation transport.[50]

CONCLUSION

The loop of Henle is an important segment for fluid and ion transport. It participates in the generation of concentrated urine and is involved in the reabsorption of sodium, potassium, and chloride. The major and unique transport system is the Na^+,K^+,$2Cl^-$ transporter, the site of action of the loop diuretics. Along this segment, about 15% of the filtered bicarbonate is reabsorbed mainly through Na^+-H^+ exchange; in addition, the TAL actively reabsorbs NH_4^+, an important step for the diffusion of NH_3 in the thin descending limb and for its trapping along the collecting ducts. Finally, the TAL is responsible for a significant fraction of calcium and magnesium reabsorption.

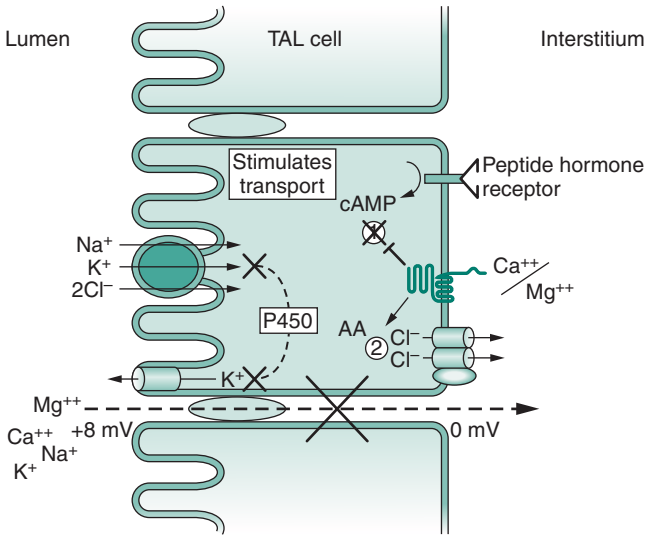

FIGURE 8.5 Cell signaling of the calcium/magnesium sensing receptor. For details see text. *1*, Adenylate cyclase; *2*, phospholipase A_2; *AA,* arachidonic acid; *cAMP,* cyclic adenosine monophosphate; *Ca^{2+},* calcium ion; *Cl^-,* chloride ion; *H^+,* hydrogen ion; *K^+,* potassium ion; *Mg^{++},* magnesium ion; *mV,* millivolt(s); *Na^+,* sodium ion; *P450,* cytochrome P450; *TAL,* thick ascending limb.

Key Points

1. The loop of Henle is an important site for fluid and solute reabsorption. Moreover, it is the segment that actively participates in the concentration of the urine through the countercurrent system.
2. Ions transport is localized mainly along the thick ascending limb, where a complex system links basolateral Na^+,K^+-ATPase to the luminal Na^+,K^+,$2Cl^-$ transporter, whereas potassium recycles through the apical membrane, and chloride exits through specific chloride channels.
3. Bicarbonate is reabsorbed along the loop mainly at the level of the S3 segment and the thick ascending limb. The major transport system is the Na^+/H^+ antiporter (NHE3), with a small contribution from H^+-ATPase. Bicarbonate transport is regulated by several factors, including medullary osmolality, systemic pH, and various hormones.
4. $NH4^+$ is reabsorbed actively along the thick ascending limb through the Na^+,K^+,$2Cl^-$ cotransport system, a fundamental step for NH_3 diffusion in the thin descending limb and trapping along the collecting duct.
5. Divalent cations (Ca^{2+} and Mg^{2+}) are reabsorbed along the thick ascending limb mainly through the paracellular pathway, which is driven by the electronegative potential difference. The calcium-magnesium–sensing receptor is intricately involved in the regulation of the divalent ions transport.

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