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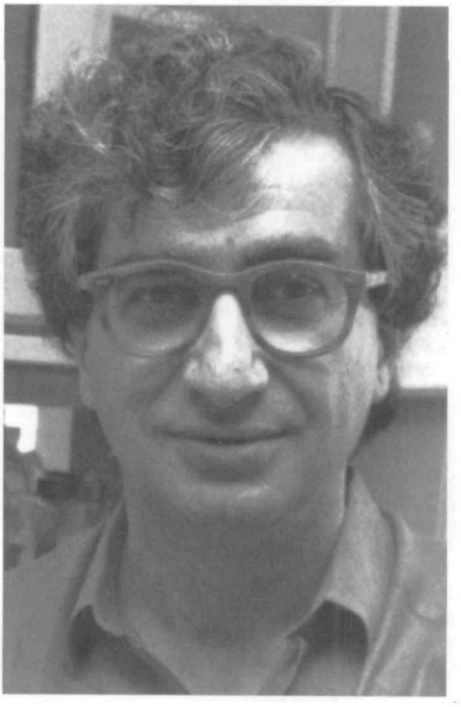

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# THE ROLE OF THE V-ATPase IN RENAL EPITHELIAL $H^+$ TRANSPORT

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## Summary

This article provides a brief review of the history of the identification and characterization of the renal V-ATPase, and a review of the physiology of renal bicarbonate reabsorption and $H^+$ transport.

## Introduction

To maintain acid–base homeostasis, the kidney must reabsorb the filtered bicarbonate and produce new bicarbonate to match daily non-volatile acid production. Over the last 60 years, the understanding of this process has progressed from descriptions of the response of the intact kidney through physiological manipulations down to the beginnings of an understanding of the control of renal acid excretion at the molecular level. The progressive investigation of the nature of renal $H^+$ transport ultimately resulted in a collision of classical physiology with the biochemistry and molecular biology of the V-ATPases. The concepts that emerged from the physiological studies of $H^+$ transport by urinary epithelia have provided a framework for modeling the regulation of vacuolar acidification.

## A brief summary of renal anatomy and function

The principal function of the kidney is to control the volume and composition of the extracellular fluid. One of the most important aspects of kidney function is the maintenance of the extracellular fluid bicarbonate concentration. Each day, 4000 mmol of bicarbonate are filtered by the glomerulus and reclaimed by the kidney. In addition, 50–100 mmol of hydrogen ion are produced daily by the catabolism of protein. This acid is titrated by the extracellular fluid bicarbonate and subsequently regenerated in the kidney. The kidney accomplishes both the reabsorption of filtered bicarbonate and the regeneration of bicarbonate by hydrogen ion secretion, a process in which V-ATPases have a pivotal role.

Key words: kidney; acid–base; hydrogen ion transport.

The human kidney is composed of 1 million individual functional units called nephrons (Fig. 1). Each nephron consists of a glomerulus, which forms a nearly protein-free ultrafiltrate of plasma from a capillary tuft, and the renal tubular epithelium, which reabsorbs and secretes solutes along its length, ultimately producing urine out of the filtrate. Tubular processing begins in the proximal tubule, a 'leaky' epithelium that is unable to sustain large electrical concentration or osmotic gradients. The proximal tubule has an apical brush border with dense microvilli providing a large surface area and reabsorbs 60–70% of the glomerular filtrate. The proximal tubule has three morphologically and functionally distinct segments, designated as the S1, S2 and S3 segments. Approximately 80% of the filtered bicarbonate is reabsorbed in the proximal tubule (Fig. 2). After leaving the proximal tubule, the filtrate enters the loop of Henle. The loop consists of the thin descending limb, the thin ascending limb and the thick ascending limb. The thin limbs do not transport solutes actively, but regulate solute and water flux out of the lumen by differential permeabilities to water and solutes. The thick ascending limb of the loop dilutes the urine by transporting solute out in excess of water and creates a hypertonic interstitium that is required for generating a concentrated urine. About 10% of the filtered bicarbonate is reabsorbed by the thick ascending limb. Filtrate leaving the loop enters the distal convoluted tubule, where additional reabsorption of sodium and 1–3% of the filtered bicarbonate takes place. The filtrate then enters the collecting tubule, which is a tight epithelium capable of generating and sustaining an electrical potential difference across the luminal membrane and large concentration and osmotic gradients. Unlike all of the other segments described above, which each consist of a relatively uniform cell type, the collecting duct is composed of two major types of cells, the principal cells (about 60% of the cells) and the intercalated cells. The principal cells reabsorb sodium, secrete potassium and absorb water and all of these transport functions are under the control of regulatory hormones. The intercalated cells secrete acid

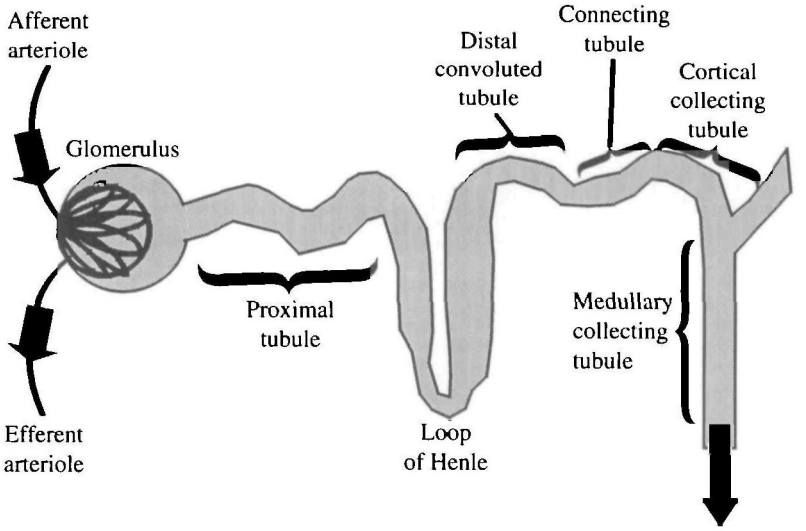


Fig. 1. Diagram of the nephron showing the different segments of the tubule discussed in the text.

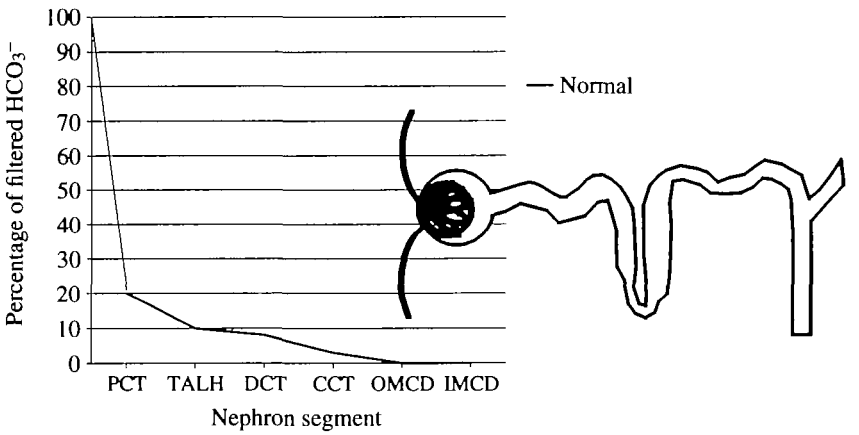


Fig. 2. Sites for bicarbonate reabsorption along the nephron. PCT, proximal convoluted tubule; TALH, thick ascending limb of the loop of Henle; DCT, distal convoluted tubule; CCT, cortical collecting tubule; OMCD, outer medullary collecting duct; IMCD, inner medullary collecting duct.

and actively reabsorb potassium; a subpopulation of intercalated cells also secretes bicarbonate and reabsorbs chloride.

## A historical perspective on urinary acidification

Studies in the 1930s demonstrated that the kidney could reabsorb all of the filtered bicarbonate ion and could excrete quantities of acid exceeding the amount filtered by the glomerulus and therefore must be secreting acid into the urine (Steinmetz, 1974). The next level in understanding the process of renal bicarbonate reabsorption came with the advent of methods to analyze the contributions of different parts of the nephron. The micropuncture method developed in the 1950s allows one only to sample tubular fluid by micropipette from the portions of the nephron that appear on the kidney surface. This method limited the analysis of the segmental regulation of bicarbonate reabsorption to the proximal tubule, the distal convoluted tubule and the cortical collecting tubule. Studies with the micropuncture method revealed that, although most of the bicarbonate is reabsorbed in the proximal tubule, the final regulation of bicarbonate reabsorption occurred in the distal parts of the nephron beyond the loop of Henle. For example, when animals were given a high-acid diet, they showed very little change in proximal tubular bicarbonate reabsorption, but greatly increased their excretion of acid in the distal nephron. After an initial understanding of the sites of bicarbonate reabsorption had developed, investigations focused on the cellular mechanism of bicarbonate reabsorption. Was bicarbonate ion itself transported, or was bicarbonate reabsorbed as a result of hydrogen ion secretion titrating the bicarbonate to $CO_2$, which then diffused across the epithelium? It had been known for some time that the kidney was rich in the enzyme carbonic anhydrase, which catalyzed the dehydration of carbonic acid to $CO_2$ and water and several inhibitors of the enzyme had been identified. It was demonstrated that

inhibition of carbonic anhydrase produced an 'acid disequilibrium pH' in the lumen of the kidney tubule, defined as a pH in the fluid of the tubular lumen that was lower than the pH of the same fluid allowed to come to equilibrium. This result suggested that the kidney secreted hydrogen ion as a mechanism for bicarbonate reabsorption. Since estimates of intracellular pH from the distribution of weak acids showed that the cytoplasmic pH of the renal tubular cell was very close to the pH of extracellular fluid, it was clear from thermodynamic considerations that the secretion of hydrogen ion must be due to an active transporter, probably present in the luminal membrane (Steinmetz, 1974).

The next advances in elucidating the cellular mechanisms responsible for active hydrogen ion secretion in epithelia were made in the amphibian and reptilian bladder. The bladder of these lower vertebrates performs many of the functions carried out by the mammalian collecting duct. The bladder could be mounted as a flat sheet separating two chambers and the fluxes of ions and current could be easily measured. The turtle bladder reabsorbed sodium and acidified its lumen; treatment with ouabain on the serosal side eliminated sodium transport and allowed the characteristics of hydrogen ion secretion to be examined. The turtle bladder could be shown to acidify its lumen in the virtual absence of $CO_2$, again supporting the model of hydrogen ion secretion as a means for bicarbonate reabsorption. After its transport of sodium had been eliminated with ouabain, the bladder generated a lumen-positive potential difference. When acidification of the lumen was studied in bladders under short-circuited conditions, which allows the net charge transferred across the bladder to be measured simultaneously with ion fluxes, it was found that the rate of hydrogen ion secretion (measured with an automatic titration device) exactly matched the addition of positive charge to the lumen. This result suggested that the pump was electrogenic. The imposition of an adverse pH gradient or a lumen-positive potential difference across the lumen slowed the rate of proton transport equally if the magnitude of the pH gradient were converted to an equivalent Nernst potential. These findings also supported the model that the pump was electrogenic. The luminal acidification was dependent on metabolism and was nullified under conditions where bladder ATP production was inhibited. These findings and the electrogenic nature of the transporter suggested that an active proton pump was present. Studies with inhibitors showed that acidification was eliminated by the addition of dicyclohexylcarbodiimide (DCCD) to the lumen, suggesting that the pump might resemble the F-ATPases, but further characterization of the enzyme became difficult in the intact cell (Al-Awqati, 1978; Steinmetz, 1986).

During the time that these advances in understanding the cellular basis of proton transport in epithelia were emerging from studies in the turtle urinary bladder, physiological methods for studying the transport function of individual segments of the nephron had improved greatly. With the isolated perfused tubule technique, individual segments of almost any part of the nephron could be isolated by microdissection and perfused, allowing their transport properties to be determined. This approach revealed that the collecting duct of the kidney had an electrogenic proton pump and an assortment of other ion transporters nearly identical to those identified earlier in the turtle urinary bladder (Steinmetz, 1986).

The next stride in identification of the nature of the renal epithelial proton pump came

with the development of methods to examine proton transport in isolated membrane vesicles. Studies in mitochondrial inner membrane vesicles, in membrane vesicles from the stomach, chromaffin granules and other tissues had shown that addition of ATP to the vesicles caused the membranes to take up a weak base upon acidification of the vesicle interior. Membranes isolated from the turtle urinary bladder were found to have an electrogenic ATP-initiated acidification that was not inhibited by the mitochondrial F-ATPase inhibitors azide and oligomycin, distinguishing the proton pump in these vesicles from contaminating submitochondrial vesicles. The kidney membrane proton pump was also resistant to vanadate, distinguishing it from the P-ATPases, but was sensitive to DCCD and sulfhydryl reagents. The proton pump in turtle bladder membranes therefore had the properties that subsequently would be found in all V-ATPases (Gluck *et al.* 1982). A proton pump with properties identical to those in the turtle bladder was found in the mammalian kidney (Gluck and Al-Awqati, 1984). Simultaneously, reports were emerging from numerous laboratories that proton pumps acidifying a variety of different vacuolar compartments also had the same properties (Forgac, 1989). Was the proton pump studied in the kidney the same enzyme responsible for urinary acidification, or were the proton-transporting membranes in the bladder and kidney merely contaminants from vacuolar compartments? If this enzyme was responsible for urinary acidification, what were the properties of this new type of proton pump?

These issues were not resolved until the proton pump had been isolated and its structure and location in the kidney established. The studies of the proton pump in kidney membrane vesicles had provided an assay for the enzyme: vanadate- and azide-resistant *N*-ethylmaleimide (NEM)-sensitive ATPase activity (Gluck and Al-Awqati, 1984). With the use of this assay, the V-ATPase from the kidney was isolated by a multiple step chromatographic procedure (Gluck and Caldwell, 1987, 1988). Its structure was strikingly similar to that of the V-ATPase from yeast (Anraku; Stevens; Kane; this volume), *Neurospora* (B. Bowman and E. Bowman, this volume), plant (Sze; Taiz; this volume) and coated vesicle (Forgac; Stone; this volume). The multi-subunit structure of these enzymes and their biochemical properties are discussed in detail in this volume.

Monoclonal and polyclonal antibodies were prepared against the isolated kidney V-ATPase, allowing its location in the kidney to be established by immunocytochemistry (Brown *et al.* 1988*a*,*b*). These experiments confirmed that the kidney V-ATPase resides on the plasma membrane of the renal tubular epithelial cells in the same segments of the nephron in which proton pumps had been identified by physiological studies.

## Cellular mechanisms of proton and bicarbonate transport in the nephron

### *Proximal tubule*

Fig. 3 shows a schematic drawing of transporters engaged in proton and bicarbonate transport in the proximal tubule. (Note that this drawing excludes many of the other transport proteins of the proximal tubule, such as the sodium cotransporters for glucose, phosphate, amino acid and organic anion transport.) The proximal tubule is a leaky epithelium responsible for reabsorption of over 60% of the glomerular filtrate and has a dense apical brush border, composed of microvilli, enhancing its capacity for bulk

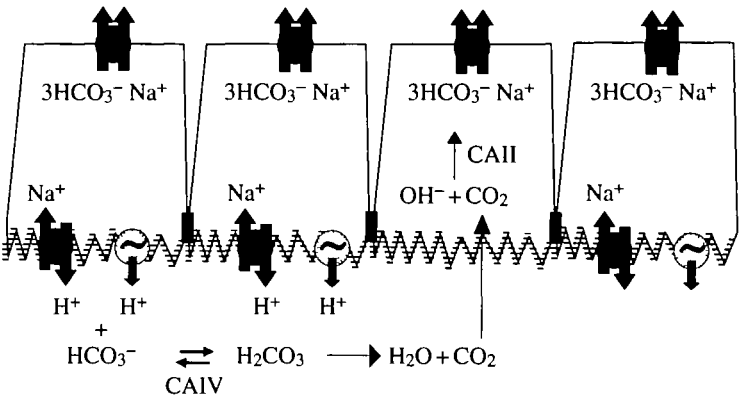


Fig. 3. Diagram of proximal tubule bicarbonate transport. CA, carbonic anhydrase.

transport. Each of the three segments of the proximal tubule, S1, S2 and S3, is composed of a uniform population of cells whose morphology and transport properties vary from those of the other proximal segments. The proximal tubule reabsorbs approximately 80% of the bicarbonate entering the lumen in the glomerular filtrate. The secretion of $H^+$ results in the formation of luminal carbonic acid, which is then dehydrated efficiently to $CO_2$ and water by type IV carbonic anhydrase, a glycophosphatidylinositol-linked ectoenzyme residing in the brush border. Two-thirds of apical $H^+$ secretion originates from a brush-border $Na^+/H^+$ antiporter; the remainder arises from a V-ATPase present in the microvilli only in the initial part of the proximal tubule and in the apical invaginations at the base of the microvilli in the entire proximal tubule (Brown *et al.* 1988*a*,*b*). The brush-border V-ATPase has enzymatic and structural differences from the V-ATPase in microsomes (Wang and Gluck, 1990; Simon and Burckhardt, 1990), much of which may originate from intercalated cells and lysosomes (Gluck, this volume). Hydroxyl ions generated intracellularly by the $Na^+/H^+$ antiporter and V-ATPase in the brush border are converted into bicarbonate ion by cytosolic type II carbonic anhydrase. Bicarbonate exits the basolateral membrane mostly through an electrogenic $Na^+/HCO_3^-$ symporter that probably transports $1Na^+$, $1HCO_3^-$ and $1CO_3^{2-}$ together, resulting in net exit of two negative charges, driven by the inside-negative potential. An electroneutral $Cl^-/HCO_3^-$ antiporter accounts for less than 10% of basolateral bicarbonate exit (Alpern, 1990).

*Regulation of bicarbonate reabsorption in the proximal tubule*

In the proximal tubule, proton secretion varies with the plasma bicarbonate concentration, the plasma $P_{CO_2}$, the plasma potassium concentration, plasma angiotensin II levels and several other factors. All of these factors affect the rate of luminal $Na^+/H^+$ antiporter activity; it has not been established whether they also affect the luminal V-ATPase activity. The brush-border $Na^+/H^+$ antiporter has a regulatory site in the cytoplasmic domain that increases the rate of antiport greatly at pH values below 7.2. An increase in the $P_{CO_2}$ or a decrease in the plasma bicarbonate concentration acidifies the cytosol and stimulates the antiporter; the opposite changes in $P_{CO_2}$ and plasma bicarbonate concentration suppress antiporter activity. Elevations in the plasma

potassium concentration depolarize the proximal tubule cell, reducing the voltage-driven basolateral exit of bicarbonate; the resulting elevation of intracellular pH suppresses the luminal $Na^+/H^+$ antiporter. Lowering the plasma potassium concentration hyperpolarizes the cell, increases bicarbonate exit and consequently stimulates the luminal $Na^+/H^+$ antiporter. Angiotensin stimulates the luminal antiporter directly through an undetermined mechanism, by signalling mechanisms involving a change in intracellular calcium concentration.

Since the proximal tubule absorbs 80% of the filtered bicarbonate, suppression of proximal $H^+$ secretion by only 10% can increase bicarbonate delivery to the loop of Henle by 30%, resulting in up to a two fold increase in bicarbonate delivery to the collecting tubule; the extra bicarbonate is excreted unless there are stimuli for increased collecting tubule $H^+$ secretion (discussed below). This capacity of the proximal tubule to excrete bicarbonate efficiently allows the kidney to avoid elevations in the plasma bicarbonate concentration. In several clinical conditions, for example extracellular fluid volume depletion and vomiting, factors that stimulate $H^+$ secretion, such as angiotensin II and potassium depletion, may impair the capacity to excrete bicarbonate and produce an elevated serum bicarbonate concentration (alkalosis).

### *Thick ascending limb*

The apical membrane of the thick ascending limb has a $Na^+/H^+$ antiporter that is responsible for 80% of the hydrogen ion secretion in this segment; a V-ATPase in the apical membrane secretes the remaining 20% (Good, 1990; Borensztein *et al.* 1991). Bicarbonate generated intracellularly exits the basolateral membrane, probably through a $Na^+/HCO_3^-$ cotransporter similar to the one in the proximal tubule.

### *Regulation of bicarbonate transport in the thick ascending limb*

In the thick ascending limb, proton secretion is affected by vasopressin, glucagon and the non-volatile acid intake or production of the animal. Vasopressin, glucagon and any agent that raises cell cyclic AMP levels suppress $H^+$ secretion; much of this effect is probably on the apical $Na^+/H^+$ antiporter, although an effect on the plasma membrane V-ATPase has not been excluded. Acidosis (acidification of the extracellular fluid of the animal) stimulates thick ascending limb $H^+$ secretion, possibly by lowering cell pH and stimulating the apical $Na^+/H^+$ antiporter through an internal pH-sensitive modifier site.

### *Distal convoluted tubule*

The distal convoluted tubule consists of a uniform cell type whose primary functions are sodium reabsorption and potassium secretion. In normal animals, bicarbonate reabsorption rates are low in the distal convoluted tubule, but increase fivefold in animals subjected to a sustained increase in acid intake, or to potassium depletion (Lucci *et al.* 1982; Capasso *et al.* 1986). The mechanism responsible for this increase is unknown. An apical plasma membrane V-ATPase appears to be the sole transporter engendering luminal hydrogen ion secretion in this segment and the mechanism for basolateral bicarbonate exit has not been established.

*Collecting tubule*

Fig. 4 shows a schematic drawing of transporters engaged in proton and bicarbonate transport in the cortical collecting tubule. The collecting tubule has two major cell types, the principal cells and the intercalated cells and is made up of several morphologically and functionally distinct segments: the connecting tubule, the cortical collecting tubule and the outer medullary collecting tubule, consisting of the outer and inner stripe. The inner medullary collecting tubule (or papillary collecting tubule) has intercalated cells only in the first third; the last two-thirds of the segment consists of a uniform cell type called principal cells.

The connecting tubule principal cells resemble the cells of the distal convoluted tubule in having low levels of a V-ATPase on the apical plasma membrane and resemble principal cells of the cortical collecting tubule in having an apical amiloride-sensitive sodium channel and an apical potassium channel. The mechanism for basolateral bicarbonate exit has not been established. The $Na^+/K^+$-ATPase in the basolateral membrane maintains a low cell sodium concentration and an inside-negative potential that provides a driving force for luminal sodium entry down its electrochemical gradient. The connecting tubule normally has a lumen-negative voltage of $-5$ to $-30$ mV as a result of the diffusion potential created by sodium entering through conductive apical channels. This lumen-negative potential is an important driving force for potassium secretion through apical potassium channels in the principal cell and for electrogenic $H^+$ secretion by the A-type intercalated cell.

In both the connecting tubule and cortical collecting tubule, there are two major types of intercalated cells, the acid-secreting 'A-type' (or $\alpha$) intercalated cells and the bicarbonate-secreting 'B-type' (or $\beta$) intercalated cells (Brown *et al.* 1988*a*,*b*; Steinmetz, 1986; Schuster, 1990*a*,*b*). The A-type intercalated cell is designed for electrogenic transepithelial $H^+$ secretion. A-type intercalated cells have a V-ATPase in the luminal (apical) pole of the cell that is present both in the plasma membrane and in a specialized

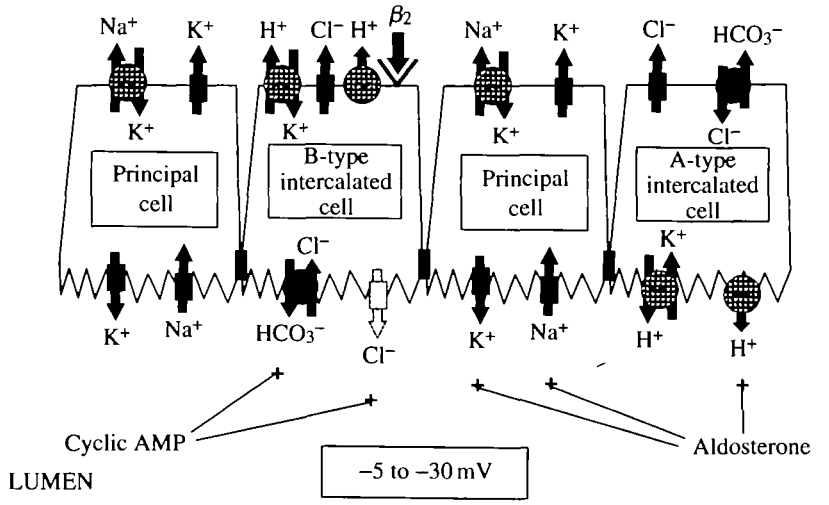


Fig. 4 . Diagram of cortical collecting tubule bicarbonate and proton transport.

intracellular tubulovesicular system (Brown *et al.* 1988*a,b*, 1989) from which additional V-ATPase-containing membrane may be recruited to the plasmalemma in response to an appropriate physiological stimulus (Schwartz and Al-Awqati, 1985; Bastani *et al.* 1991). Cytosolic hydroxyl ions generated by the plasma membrane V-ATPase are converted to $HCO_3^-$ by type II carbonic anhydrase (Brown, 1989; Kim *et al.* 1990; Brechue *et al.* 1991) and the bicarbonate exits the basolateral membrane through a $Cl^-/HCO_3^-$antiporter that is an exon splice variant of the red cell anion transporter (Brosius *et al.* 1989), known commonly as band 3 protein; antibodies to red cell band 3 stain the basolateral membrane of the A-type intercalated cell (Drenckhahn *et al.* 1985; Schuster *et al.* 1986; Verlander *et al.* 1988; Alper *et al.* 1989). The chloride taken up by $Cl^-/HCO_3^-$antiport exits the cell through a basolateral chloride channel (Dietl *et al.* 1991). The location of these transporters in the A-type intercalated cell preserves the electroneutrality of cellular ion fluxes, but maintains electrogenic transepithelial hydrogen ion secretion. The A-type intercalated cells also have an electroneutral $H^+/K^+$-ATPase, similar to the enzyme in the stomach, in the apical membrane (Wingo *et al.* 1990); the major physiological role of this transporter is probably not proton secretion, but active potassium reabsorption during potassium depletion. The B-type intercalated cell is designed for electroneutral chloride reabsorption and bicarbonate secretion. B-type intercalated cells have a V-ATPase on the *basolateral* membrane (Brown *et al.* 1988*a,b*). Bicarbonate generated intracellularly exits on the luminal side through an electroneutral $Cl^-/HCO_3^-$ antiporter (Schuster, 1991) that does not cross-react with antibodies to band 3 (Schuster *et al.* 1986; Verlander *et al.* 1988; Alper *et al.* 1989) and whose structure is unknown. This system of transporters provides a mechanism for active transepithelial chloride reabsorption. Chloride enters from the lumen through the electroneutral transporter down a concentration gradient; cell chloride concentration is low because of the inside-negative potential of the cell. Chloride normally exits, driven by the inside-negative potential, through a chloride channel in the basolateral membrane. As discussed below, $\beta$-adrenergic agents open a chloride channel in the luminal membrane (Schuster and Stokes, 1987).

The transport properties of the cortical collecting tubule have very few differences from those of the connecting tubule. The principal cells of the cortical collecting tubule have no detectable plasma membrane V-ATPase; they have an apical amiloride-sensitive sodium channel and an apical potassium channel. The cortical collecting tubule also maintains a lumen-negative voltage of $-5$ to $-30$ mV as a result of the sodium diffusion potential. The cortical collecting tubule has both A-type and B-type intercalated cells that have nearly the same transport properties as those in the connecting tubule. The A-type intercalated cell in the cortical collecting tubule has a $Na^+/H^+$ antiporter in the basolateral membrane that is the primary regulator of intracellular pH (Weiner and Hamm, 1990); it has not yet been determined whether the A-type intercalated cells of the connecting tubule also have this transporter.

Fig. 5 shows a schematic drawing of transporters engaged in proton and bicarbonate transport in the outer medullary collecting tubule. The outer medulla is the portion of the renal medulla that contains thick ascending limbs. It contains two anatomical regions: the outer stripe, which contains S3 segments of the proximal tubule (also called the *pars*

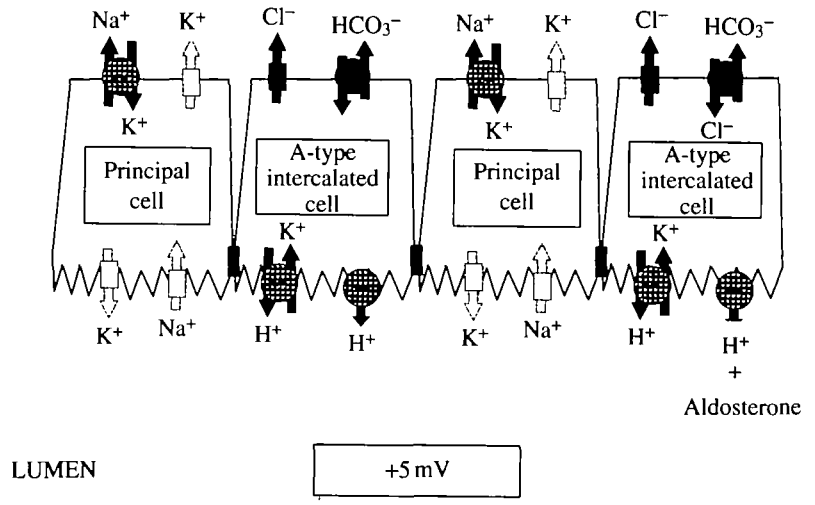


Fig. 5. Diagram of medullary collecting tubule bicarbonate and proton transport.

*recta*) and the inner stripe, which lacks S3 segments. The outer medullary collecting tubule in the outer and inner stripes contains principal cells, which do not have any demonstrable plasma membrane V-ATPase. No sodium reabsorption has been found in this segment. The transepithelial potential is normally at least +5 mV as a result of electrogenic proton secretion by the intercalated cells. All of the intercalated cells in the outer medulla are A-type cells, with a V-ATPase in the plasmalemma and a tubulovesicular system at the apical pole, an $H^+/K^+$-ATPase in the apical membrane, and a band-3-immunoreactive $Cl^-/HCO_3^-$antiporter, a chloride channel and a $Na^+/H^+$ antiporter (Breyer and Jacobson, 1989) in the basolateral membrane. Some evidence suggests that the electroneutrality of transepithelial $H^+$ transport in the outer medullary collecting tubule may be maintained by a paracellular chloride permeability (Stone *et al.* 1983).

### *Regulation of ion transport in the collecting tubule*

The rate of hydrogen ion transport in the collecting duct is influenced by a number of factors. In the cortical and outer medullary collecting tubule, proton secretion by the A-type intercalated cells varies with the proton electrochemical gradient across the apical plasma membrane, plasma aldosterone levels, the plasma bicarbonate concentration, the plasma $P_{CO_2}$ and the non-volatile acid intake or production of the animal. pH and electrical gradients vary the rate of electrogenic $H^+$ secretion, presumably by direct kinetic effects of imposing an adverse electrochemical gradient across the V-ATPase. An increase in the lumen-negative potential stimulates proton secretion. A decrease in urinary pH suppresses $H^+$ secretion; net acid secretion stops at an apical pH of between 4.5 and 5.0. The quantity of hydrogen ion secreted depends on the concentration of urinary buffers. The principal buffers are phosphate and ammonia. As urine pH declines, the partition of ammonia into the collecting duct lumen increases, permitting more

protons to be excreted. Increased acid intake and several other factors that stimulate hydrogen ion secretion also increase the generation of ammonia by the proximal tubule. Aldosterone increases $H^+$ secretion by elevating the apical sodium conductance of principal cells (in the cortical collecting tubule), thus increasing the lumen-negative potential. It also stimulates $H^+$ secretion directly, by an undetermined mechanism, independently of changes in the transepithelial potential. An increase or decrease in the plasma bicarbonate concentration suppresses or stimulates, respectively, $H^+$ secretion. The mechanism of these effects is poorly understood. Decreasing the plasma bicarbonate level lowers the intracellular pH (pHi) of the intercalated cell; cytosolic acidification produces a transient and sustained elevation in intracellular calcium activity (Hays and Alpern, 1991). The effects of the elevated cell calcium activity are complex. The sustained elevation of calcium activity causes an immediate inhibition of V-ATPase activity; the transient elevation produces a delayed increase in V-ATPase that is inhibited by cytochalasin D, possibly indicating membrane insertion (Hays and Alpern, 1991). Elevations in the plasma $P_{CO_2}$ stimulate $H^+$ secretion; at least part of this effect entails inducing recruitment of V-ATPase to the apical membrane by exocytosis (Gluck *et al.* 1982; Schwartz and Al-Awqati, 1985; Steinmetz, 1986; Verlander *et al.* 1987; Brown, 1989*a*,*b*), but changes in the kinetics of the V-ATPase also appear to participate (McKinney and Davidson, 1987, 1988). Administration of HCl to rats produces a profound redistribution of V-ATPase from the intracellular tubulovesicular compartment to the apical plasma membrane in A-type intercalated cells (Madsen and Tisher, 1984; Bastani *et al.* 1991); the magnitude of the response does not appear to be controlled directly by the plasma bicarbonate concentration (Bastani *et al.* 1991).

Chloride reabsorption and bicarbonate secretion by the B-type intercalated cells, which are present only in the connecting tubule and cortical collecting tubule, vary with the luminal chloride concentration, $\beta$-adrenergic stimulation and the non-volatile acid intake or production of the animal. Apical chloride delivery increases $Cl^-/HCO_3^-$ antiport by concentration-dependent kinetic effects (Schuster and Stokes, 1987; Levine *et al.* 1990). $\beta$-Adrenergic stimulation and other agents that raise cyclic AMP levels, stimulates apical $Cl^-/HCO_3^-$ antiport by an undetermined mechanism. The same agents may also increase the apical chloride conductance of the B-type intercalated cell, allowing chloride to exit on the apical side, endowing a net effect of electrogenic bicarbonate secretion (Schuster, 1989, 1991). The physiological role of the response to $\beta$-adrenergic agents may be to facilitate potassium excretion by increasing the lumen-negative potential in the cortical collecting tubule. Administration of HCl to rats or rabbits suppresses cortical collecting tubule bicarbonate secretion by an unknown mechanism (McKinney and Burg, 1977; Levine *et al.* 1988). In chloride depletion, apical $Cl^-/HCO_3^-$ antiport is stimulated (Galla *et al.* 1991), accompanied by an increase in the amount of immunocytochemically detectable V-ATPase in the basolateral membrane of the B-type intercalated cells (Verlander *et al.* 1992).

The arrangement of transporters in the cortical and outer medullary collecting tubule provides a great deal of flexibility in regulating ion reabsorption and secretion. In response to extracellular fluid volume depletion, aldosterone stimulates conductive sodium reabsorption, electrogenic hydrogen ion secretion and electroneutral $Cl^-/HCO_3^-$

antiport, resulting in the net reabsorption of NaCl and $NaHCO_3$. Increased acid intake suppresses electroneutral $Cl^-/HCO_3^-$ antiport, giving an increased reabsorption and generation of $HCO_3^-$.

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