Respective Roles of H-ATPase and H-K-ATPase in Ion Transport in the Kidney

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ABSTRACT

Two types of proton-translocating ATPases, H-ATPase and H-K-ATPase, are found in the renal tubular cells. H-ATPase is present in both endocytic vesicles and apical membranes in almost all nephron segments. On the other hand, H-K-ATPase is present only in the connecting tubule and collecting duct. There is evidence to suggest that H-ATPase may be involved in H secretion in almost all nephron segments. H-K-ATPase is involved not only in H secretion but also in K absorption in the collecting duct segments. Aldosterone administration and metabolic acidosis stimulate the activity of H-ATPase in all collecting duct segments, whereas hypokalemia has only a limited effect on H-ATPase activity. On the other hand, hypokalemia, as well as metabolic acidosis, stimulates H-K-ATPase activity in the collecting duct segments, whereas aldosterone administration alone plays a minor role in the regulation of this enzyme. The synthesis and transport of ammonia, which also play an important role in maintaining the acid-base balance, will not be discussed here. Several excellent and recent reviews are available on this topic (1,2).

GENERAL PROPERTIES OF DIFFERENT TYPES OF H-ATPases

There are three types of H-ATPases. The FO-F1 type of H-ATPase is present in the mitochondria where it acts as ATP synthase. This type of H-ATPase is also present in bacterial membranes and in chloroplasts (3). It is composed of 8 to 14 polypeptides. The mammalian enzyme is inhibited by oligomycin, azide, and low concentrations of dicyclohexylcarbodiimide (DCCD), but not by ouabain (inhibitor of Na-K-ATPase) or vanadate (inhibitor of E1-E2 type ATPases, including Na-K-ATPase).

The second type of H-ATPase is present in the membranes of intracellular organelles, such as endosomes, lysosomes, and clathrin-coated vesicles (4), and it acidifies the interior of these organelles. Because this H-ATPase is present in the intracellular vacuoles, it has been called vacuolar (or V) H-ATPase (5). The vacuolar H-ATPase is also present in the

monia and titrable acid. Both of these functions are achieved by the acidification of the tubule fluid. Most of the filtered bicarbonate is reabsorbed in the proximal tubule. The acidification of the luminal fluid in the distal tubule plays an important role in the transport of total ammonia and in the titration of phosphate and other buffers. During the last several years, it has been established that acidification of the tubule fluid is mediated, at least in part, by an electrogenic proton pump (H-ATPase) that is located in the luminal membrane of renal tubule cells in both proximal and distal nephron segments, including the collecting duct. Recent evidence suggests that an electroneutral H-K-ATPase present in the collecting duct may play a role in the acidification of tubule fluid. In this article, we will review the biochemical, immunohistological, and physiological evidence for the respective roles of H-ATPase and H-K-ATPase in ion transport in various nephron segments. We will also consider here the effects of certain physiological and pharmacological factors on the two enzymes.

To maintain acid-base balance, the human kidney not only reabsorbs the filtered bicarbonate but also generates bicarbonate by excretion of am-

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plasma membranes of subpopulations of cells in the mammalian collecting duct and turtle urinary bladder where it is responsible for urinary acidification (6,7). In addition, this type of H-ATPase is located in synaptic vesicles and chromaffin granules (8). The V type H-ATPases are composed of nine or more different polypeptides. They contain more cysteine residues than mitochondrial H-ATPase and are inhibited by low concentrations of N-ethylmaleimide (NEM), a sulphydryl alkylating agent (9). In addition, V type H-ATPases are inhibited by higher concentrations of DCCD than is the mitochondrial H-ATPase (ATP synthase) (5). They are not inhibited by azide, ouabain, or vanadate.

The functions of V type H-ATPases in various species and in various organs in mammals have been reviewed recently (3,5,8). In brief, the major function of V type H-ATPases is to acidify the interior of intracellular organelles. This acidification leads to activation of several secondary processes. For example, it has been demonstrated in several tissues that the acidification of endosomes is important for the dissociation of ligands from receptors after receptor-mediated endocytosis and the subsequent recycling of the receptors (5). Under normal conditions, endocytosed ligands such as low-density lipoproteins, insulin, and epidermal growth factor dissociate from their receptors in the acidic pH of the endosomes. However, treatment of cells with weak bases, such as NH4Cl or chloroquine, that neutralize acidic compartments in the cells results in accumulation of ligand-receptor complexes in the endosomes and a depletion of cell surface receptors. Similarly, the maintenance of an acidic pH within lysosomes is necessary for optimum activity of lysosomal hydrolases (10).

The proton-motive force generated by V type H-ATPases in synaptic vesicles and chromaffin granules is used for the uptake and storage of neurotransmitters (8). The mechanism of neurotransmitter uptake may vary with the transporter of the neurotransmitter. Thus, like Na-K-ATPase, V type H-ATPases provide energy for several secondary processes. In fact, it has been suggested that the nervous system cannot function without V type H-ATPases because of a lack of accumulation of neurotransmitters in the absence of H-ATPase (8).

V type H-ATPases isolated from different kidney membrane compartments have been shown to differ in their enzymatic and structural properties. For example, two H-ATPases isolated from brush borders and microsomes of the bovine kidney differ in their 56K subunit, substrate specificity, pH optimum, and sensitivity to inhibition by Cu2+ (11). It has been suggested that the activity of V type H-ATPase in vivo may be regulated by two cytosolic proteins, a stimulatory protein and an inhibitory protein (12). Some differences in the enzyme activity of V type H-ATPases in different organelles may also be because of secondary factors present in the organelles. These include the presence or absence of a Cl- channel and the lipid composition of the organelle membrane (8). The acidification by V type H-ATPase in various organelles may be controlled by the primary and secondary factors mentioned above. In contrast, the acidification of urine by V type H-ATPase is probably controlled by the rates of enzyme biogenesis and by enzyme insertion into and retrieval from the plasma membrane (7). There is evidence that the activity of the H-ATPase and its insertion into and retrieval from the plasma membrane in the collecting duct are influenced by adrenal hormones and changes in acid-base balance (discussed below).

The third type of H-ATPase is characteristic of the mammalian gastric mucosa (13). It belongs to the E1-E2 type of ATPases that form phosphorylated intermediates and are vanadate sensitive. It is composed of two subunits and is inhibited by omeprazole and an imidazopyridine compound (SCH 28080). It resembles Na-K-ATPase but is not inhibited by ouabain. This type of H-ATPase is also present in certain fungi. Recently, ouabain-insensitive K-ATPase activity has been demonstrated in the mammalian collecting duct (14,15). The renal K-ATPase is pharmacologically similar to gastric H-K-ATPase (15).

The gastric H-K-ATPase is responsible for transport of H into the lumen in exchange for K. This transport process is accompanied by a back diffusion of K into the lumen via a K channel and transport of Cl from cell to the lumen via an apical Cl channel. These processes lead to net secretion of HCl into the lumen (16). The recognition of the role of H-K-ATPase in gastric acid secretion led to the development of omeprazole (PRILoseC®; Merck Sharp & Dohme, West Point, PA), which is used in pathological hypersecretory conditions such as Zollinger-Ellison syndrome and in gastroesophageal reflux diseases (13). The physiological role of renal H-K-ATPase in H and K transport is discussed in a later section.

**DISTRIBUTION OF H-ATPase ALONG THE NEPHRON**

The distribution of H-ATPase in the various nephron segments has been studied by both biochemical and immunohistochemical techniques. Because NEM has been used as an inhibitor of the V type H-ATPase, the enzyme is also called NEM-sensitive ATPase. We will use the term NEM-sensitive ATPase and H-ATPase interchangeably. Figure 1 illustrates the locations of the major nephron segments in which enzyme activities have been measured in both the rat and rabbit. The distribution of NEM-sensitive ATPase activity along the rabbit nephron is given in Figure 2. Enzyme activity is high in the connecting
Figure 1. A diagramatic sketch of the nephron to show the location of segments microdissected for assay of ATPases. PCT (S1), PCT, initial portion; PST (S2), proximal straight tubule from cortex; PST (S3), proximal straight portion from medulla; OMCD (O.S.), OMCD from outer stripe; OMCD (I.S.), OMCD from inner stripe.

Figure 2. NEM-sensitive ATPase activity in individual segments along the rabbit nephron. The abbreviations used for each segment are the same as those described in the legend to Figure 1. The PST represents S2 segment; MTAL and OMCD represent MTAL (I.S.) and OMCD (I.S.), respectively; IMCD represents IMCD1. Each bar represents mean ± SE of three to five animals (data taken from references 17 and 42).

Figure 3. NEM-sensitive ATPase activity in individual segments along the rat nephron. The abbreviations used for each segment are the same as those described in the legend to Figure 1. MTAL and OMCD represent MTAL (I.S.) and OMCD (I.S.), respectively; IMCD represents IMCD1. Each bar represents mean ± SE of 7 to 15 animals (compiled from references 18, 20, and 37).

Figure 4. Distribution of H-K-ATPase along the nephron

Recently, we and others have reported the presence of ouabain-insensitive K-stimulated ATPase activity in collecting duct segments of both rat and rabbit (14,15). In both species, H-ATPase activity is present in the CNT, CCD, OMCD, and inner medullary collecting duct (IMCD), but not in other nephron segments (Figure 4). There are no quantitative differ-

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There are significant species differences in NEM-sensitive ATPase activity along the nephron. The enzyme activities in proximal as well distal tubule segments are higher in the rat than in the rabbit (Figure 3). However, enzyme activities in the collecting duct segments are similar in rat and rabbit (17,19–23). Na-K-ATPase activity in the proximal convoluted tubule (PCT) and DCT is also higher in rat than in rabbit (24). The higher activity of NEM-sensitive ATPase and Na-K-ATPase may be related to higher single nephron GFR in the rat.

Immunohistochemical studies with antibodies raised against three subunits of bovine kidney H-ATPase have demonstrated labeling of apical vesicles and the apical plasma membrane of the PCT, thick ascending limbs, DCT, and intercalated cells in the cortical collecting duct (CCD) and outer medullary collecting duct (OMCD) (25).
ences in H-ATPase activity in the CCD and OMCD between rabbit and rat, and the enzyme activity is inhibited by vanadate, omeprazole, and SCH 28080. The pharmacological characterization of the enzyme suggests that ouabain-insensitive K-ATPase is similar to the well-characterized gastric H-K-ATPase (13). Immunohistochemical studies have demonstrated that a monoclonal antibody against gastric H-K-ATPase labels intercalated cells in the CCD and OMCD in both rabbit and rat (26).

ROLE OF H-ATPASE IN THE PROXIMAL TUBULE

Unlike ouabain, which is a specific inhibitor of Na-K-ATPase, there is no specific inhibitor of the V type H-ATPase that would unequivocally prove its physiological role in the kidney. Therefore, we rely on a combination of biochemical, immunohistochemical, and electrophysiological experiments to explain the role of H-ATPase in the various nephron segments.

Approximately 80% of the filtered bicarbonate is reabsorbed in the proximal tubule. A major mechanism for bicarbonate absorption includes H secretion by a Na:H exchanger in the apical membrane (Figure 5). This process is energized by the sodium gradient created by the Na-K-ATPase in the basolateral membrane. In the luminal fluid, secreted H combines with filtered bicarbonate to form carbonic acid, which is dehydrated by luminal carbonic anhydrase to CO₂. Carbon dioxide diffuses into the cell where H is regenerated by a reverse process. Bicarbonate is transported across the basolateral membrane via a Na(HCO₃)₃ transporter (27,28).

There are several pieces of evidence to suggest that a part of the bicarbonate reabsorption in the PCT occurs via a H-ATPase. Bicarbonate reabsorption in the rat PCT is not completely inhibited by luminal amiloride and t-butyl amiloride, two inhibitors of the Na:H exchanger (29,30), and it has been estimated that approximately 35% of bicarbonate absorption occurs by a sodium-independent process (independent of the Na:H exchanger) (30). Furthermore, luminal DCCD, an inhibitor of H-ATPase, was reported to decrease bicarbonate absorption in the PCT by 21% (31). Biochemical studies have demonstrated the presence of an ATP-driven proton pump in brush-border membranes isolated from the rat renal cortex (32,33). In agreement with these findings, H-ATPase immunolabeling has been demonstrated on the apical membrane of the rat PCT. Taken together, these results suggest that H-ATPase is responsible for part of the proton secretion in the PCT (Figure 5).

H-ATPase is not only present in the apical membrane but also in the endocytic vesicles in the PCT (25). In addition, immunostaining with H-ATPase antibodies has been demonstrated in invaginations at the base of the microvilli (25), which may be areas of active endocytosis. Endosomal H-ATPase has characteristics similar to that of the brush-border membrane (34). Therefore, endosomes may serve as an additional source of H-ATPase for proximal tubular acidification by fusing with the luminal membrane and inserting the H pump into this membrane. The vacuolar H-ATPase is responsible for the acidic interior of the endosomes, which is important for the receptor-ligand dissociation that occurs after receptor-mediated endocytosis. The low pH of the lysosomes is also dependent on a V type H-ATPase. The acidification of endocytic vesicles and lysosomes plays an important role in the reabsorption and degradation of filtered proteins in this segment. Thus,
H-ATPase in the PCT may be involved not only in H secretion into the luminal fluid but also in several other processes.

It has been reported that metabolic acidosis decreases NEM-sensitive ATPase in the PCT from superficial nephrons, but not in the PCT from juxta-medullary nephrons, of the rat (23). The physiological significance of this observation is not known at the present time.

ROLE OF H-ATPase IN THE THICK ASCENDING LIMB OF HENLE'S LOOP

Both medullary and cortical thick ascending limbs (MTAL and CTAL) are impermeable to water. Basolateral Na-K-ATPase provides the driving force for the Na:K:2Cl transporter in the luminal membrane (35). Chloride exits the cell passively across the basolateral membranes via a Cl channel and a KCl cotransport system. K is recycled back to the tubular lumen through luminal K channels and to the blood through the basolateral KCl cotransport system. The sum of these processes results in reabsorption of NaCl and dilution of luminal fluid.

The thick ascending limbs have been thought to play a minor role in the acidification of urine. Recently, however, it has been demonstrated that the rat CTAL and MTAL absorb bicarbonate at rates comparable with those in the collecting ducts (36,37). Bicarbonate absorption in the thick ascending limbs is an active process that occurs by a combination of H secretion across the apical membrane and base efflux across the basolateral membrane. The H secretion occurs mainly via a Na:H exchanger, which is inhibited by amiloride (37), as in the PCT (Figure 5). In addition, preliminary observations have provided evidence for the presence of a sodium-independent acid extrusion mechanism, presumably mediated by a H-ATPase (38).

NEM-sensitive ATPase activity in the MTAL and CTAL is influenced by changes in acid-base balance (20,22,33), and recent studies have revealed an inverse linear relationship between NEM-sensitive ATPase activity in MTAL and CTAL and total CO2 in the plasma (39). Taken together, these results suggest that H-ATPase may also be involved in bicarbonate reabsorption in the rat MTAL and CTAL.

ROLE OF H-ATPase IN THE DISTAL CONVOLUTED TUBULE AND THE CONNECTING TUBULE

In the past, the term "distal tubule" has been used to include the nephron segments from the macula densa to the first junction of two nephrons forming a CCD in a medullary ray. Later, it was shown that this long distal tubule is a heterogenous structure that is comprised of three parts. The early part is the "true" DCT; the middle part is the CNT; and the last part is the initial cortical collecting duct (ICCD) (40). These three segments are easily distinguishable in the rabbit but not in the rat. The DCT contains DCT cells, whereas the CNT has two cell types: CNT cells and intercalated cells. The ICCD and the CCD (from the medullary ray) also have two main cell types, the principal cell and the intercalated cell. The intercalated cells in the CNT are similar to those in the CCD.

Bicarbonate absorption has not been studied in the rabbit DCT because this is a very small segment (<0.5 mm in length). In a micropuncture study of the rat DCT, it has been shown that this segment probably does not secrete H except in very severe acidosis (41). Although NEM-sensitive ATPase activity in the DCT is greater than in the CTAL or CCD in the rat, the enzyme activity in the DCT is not influenced by changes in acid-base balance (20,22).

The CNT is believed to behave like the CCD with respect to the regulation of H-ATPase (27,42). The CNT is also involved in K secretion (43,44). Morphological studies have demonstrated that chronic potassium loading increases the basolateral membrane area of CNT cells (45,46).
ROLE OF H-ATPase IN THE CCD

The principal cells are the predominant cell type (>60%) in the CCD, and they are responsible for Na absorption and K secretion. These cells have Na and K conductive pathways in the luminal membrane and Na-K-ATPase in the basolateral membrane (Figure 6). The Na channel in the luminal membrane is sensitive to low concentrations (10^-7 M) of amiloride.

The CCD is also involved in H secretion, which occurs via a sodium-independent mechanism (47,48). In addition, the CCD of bicarbonate-loaded animals is capable of bicarbonate secretion (49). The intercalated cells are thought to be responsible for H transport in this segment. It has been shown morphologically that the CCD contains two subtypes of intercalated cells called A cells and B cells (40). There are several pieces of evidence to suggest that type A intercalated cells are responsible for H secretion. A model for bicarbonate reabsorption (or H secretion) in type A intercalated cells includes an apical H-pump and a basolateral Cl-HCO3 exchanger (Figure 6). Immunohistochemical studies have provided support for this model (Figure 7). The H pump is a V type H-ATPase (4), and the Cl-HCO3 exchanger is similar to erythrocyte band 3 (50–52). It has been shown that respiratory acidosis increases the surface area of the luminal membrane and decreases the apical tubular structures in type A intercalated cells in the CCD, supporting a role of these cells in acid secretion (53).

There is evidence that type B intercalated cells are involved in bicarbonate secretion, at least in the rabbit. The biochemical mechanism of HCO3 secretion in the CCD is not well understood. It has been suggested that the type B intercalated cells is similar to the type A cell but has a reversed polarity with a H-ATPase in the basolateral membrane and a Cl-HCO3 exchanger in the apical membrane. Immunohistochemical studies have demonstrated that the basolateral membrane of type B intercalated cells in the rat kidney is stained with H-ATPase antibodies (25). However, the apical membrane of type B intercalated cell does not label with antibodies against band 3 protein (50–52). In addition, it has been demonstrated that the Cl-HCO3 exchanger in the B cell is not regulated by acute changes in peritubular Pco2 or intracellular pH (54). Furthermore, it has been suggested that the Cl-HCO3 exchanger in the B cell may act as a Cl self-exchanger (55). All of these observations indicate that the Cl-HCO3 exchanger in these

Figure 7. Light micrograph of rat kidney cortex illustrating H⁺-ATPase immunostaining by a horseradish peroxidase technique using a rabbit polyclonal antibody against H⁺-ATPase from bovine brain (kindly provided by Dr. Dennis Stone). Immunostaining is observed in the S segment of the proximal tubule where it is located in the apical region beneath the brush border corresponding to coated pits and endocytic vesicles (arrowheads). In the CCD, there is heavy immunostaining of the apical region of type A intercalated cells (closed arrow), whereas type B intercalated cells exhibit diffuse cytoplasmic and basal staining (open arrow). Magnification, x1,000. Courtesy of Dr. Jin Kim.
cells is different from that present in the basolateral membrane of the type A cell.

McKinney and Burg (49) first showed that bicarbonate reabsorption in the CCD is influenced by the acid-base status of the animal. These observations have been confirmed by other investigators both in rabbits and in rat (27). NEM-sensitive ATPase activity in the rat CCD is increased during metabolic acidosis (20,23) and decreased during metabolic alkalosis (22). The enzyme activity in the CCD is inversely proportional to plasma total CO2 (39). These results suggest that the CCD is an important site for renal adaptation to chronic acidosis and alkalosis and that changes in the activity of H-ATPase is at least partially responsible for this adaptation. Acid respiratory acidosis is associated with a migration of membrane vesicles to the apical plasma membrane of the CCD, indicating exocytotic insertion of the H-ATPase into the luminal membrane (53). The opposite may happen during a decrease in bicarbonate absorption when peritubular bicarbonate is increased acutely (56).

NEM-sensitive ATPase activity in the CCD is not only stimulated by metabolic acidosis but is also influenced by adrenal steroids. Aldosterone administration increases and adrenalectomy decreases NEM-sensitive ATPase activity in the CCD (17,57). The demonstration that changes in acid-base balance can produce a change in NEM-sensitive ATPase activity in adrenalectomized rabbits (42) suggests that acidosis (or alkalosis) and aldosterone act independently of each other. Chronic treatment of the rabbit with deoxycorticosterone, a mineralocorticoid, increases bicarbonate secretion in the CCD, indicating exocytotic insertion of the H-ATPase into the apical membrane of type A cell (54). The opposite may happen during a decrease in bicarbonate absorption when peritubular bicarbonate is increased acutely (56).

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Both isoproterenol and 8-bromo-cAMP stimulate net bicarbonate secretion in the CCD (60). The effect is mediated by stimulation of the Cl: HCO3 exchange in the apical membrane of type B intercalated cell (61). Thus, acid-base secretion in the CCD is not only regulated by H-ATPase activity but also by the activity of the Cl: HCO3 exchange in the intercalated cells of the CCD.

ROLE OF H-ATPase IN THE OMCD

The OMCD has been subdivided into the OMCD1 (from the outer stripe) and OMCD (from the inner stripe) (Figure 1). The OMCD1 has been studied more extensively than the OMCD. The OMCD is an important site of sodium-independent H secretion (62). The intercalated cells of the OMCD are all similar to type A cells. Immunocytochemical studies show the presence of H-ATPase in the apical membrane and band 3 protein in the basolateral membrane of the intercalated cells of the OMCD (50–52). Thus, the cellular model for H secretion in the OMCD is the same as in the CCD (Figure 6). Both respiratory and metabolic acidosis increases the apical surface area of intercalated cells in the OMCD (63).

NEM-sensitive ATPase is present in the OMCD of both rabbit and rat (17,20). The enzyme activity in the OMCD is comparable to that in the CCD (Figure 2). There is an increase in NEM-sensitive ATPase activity in the OMCD during acidosis and a decrease in the enzyme activity during metabolic alkalosis (20,22). There is an inverse linear relationship between NEM-sensitive ATPase activity and plasma total CO2 content in the rat (39). These results support the described model in which a NEM-sensitive H pump is responsible for H secretion (Figure 6).

Aldosterone administration increases NEM-sensitive H-ATPase activity in the OMCD of both rabbit and rat (17,64) and stimulates electrogenic H secretion in the rabbit OMCD (65). These results suggest that H secretion in the OMCD is influenced by adrenal steroids via regulation of H-ATPase activity.

Hypokalemia also increases NEM-sensitive activity in the rat OMCD (59). The effect is limited to the OMCD and is not seen in the CCD or IMCD. The mechanism of this action of hypokalemia on NEM-sensitive ATPase in the OMCD is not known.

ROLE OF H-ATPase IN THE IMCD

The IMCD is a heterogenous structure. It has been subdivided into three subsegments: the initial one third (IMCD1, or IMCD1), the middle one third (IMCD2), and the last one third (IMCD3) (Figure 1). In spite of the morphological differences between the IMCD1 and IMCD2, the two subsegments show similar properties with respect to urea transport and response to hormones (66). Therefore, these subsegments together have been called the terminal IMCD (IMCD). The intercalated cells are absent in the rabbit IMCD and are present to a very small extent (10%) in the IMCD1 of the rat (67). Traditionally, nonintercalated cells in the IMCD of all species were considered to be principal cells. However, morphological reexamination of the IMCD showed that the cells in the IMCD have several characteristics different from the principal cells of the CCD, OMCD, and IMCD1. Therefore, the cells in the IMCD1 have been called IMCD cells (68).

There is some evidence to suggest that bicarbonate is absorbed independently of sodium in the rat IMCD (69). Metabolic acidosis increases and metabolic alkalosis decreases NEM-sensitive ATPase activity in the rat IMCD1 (20,22). Because intercalated cells (type A) are present in the rat IMCD1, it is likely that intercalated cells are sites of NEM-sensitive ATPase modulation by systemic acid-base changes. Therefore,
the mechanism of H secretion in the rat IMCD may to some extent be similar to that in the OMCD and CCD (Figure 6).

Recently, we have determined the NEM-sensitive ATPase activity in all three subsegments of the rabbit. The enzyme activity in the IMCD2 and IMCD3 was equal to or greater than that measured in the IMCD1 (our unpublished observations). Because the subsegments of the rabbit IMCD do not have intercalated cells, it is likely that H-ATPase is present in the IMCD cells. Whether the enzyme is present in the apical membrane or in the intracellular organelles of the IMCD cell is not known. Recent studies in the isolated IMCD of the rat have provided evidence for H secretion, which was increased by chronic acid loading (70). Taken together, these results indicate that the IMCD is capable of H secretion despite the absence of intercalated cells. The mechanism of H secretion in the IMCD is not known at the present time.

**ROLE OF H-K-ATPase IN THE CNT, CCD, AND OMCD**

The rabbit OMCD has been shown to absorb K during K restriction (71,72). The demonstration that omeprazole (an inhibitor of gastric H-K-ATPase) inhibits K absorption and H secretion in the OMCD of rabbits maintained on a low-K diet (73) suggests that at least part of K absorption occurs via H-K-ATPase. Micropuncture and in vivo micropuncture experiments suggest that there is H secretion and K absorption in the rat distal tubule during K depletion (74). The reabsorption of potassium was reduced by SCH 28080, an inhibitor of H-K-ATPase. The late distal tubule in the rat probably represents the CNT and the ICCD. Taken together, these results suggest that renal H-K-ATPase is responsible for K absorption in the CNT, ICCD, and OMCD during K depletion.

H-K-ATPase activity is present in CNT and OMCD of both rat and rabbit (Figure 4) (14,15). Immunohistochemical studies indicate that H-K-ATPase is present in the intercalated cells of the CCD and OMCD of both rat and rabbit (26). Therefore, it is likely that intercalated cells are responsible for K secretion in exchange for H secretion in the CCD and OMCD (Figure 6). Recently, we examined H-K-ATPase activity in three subsegments of the IMCD (75) and found that H-K-ATPase activity was higher in the IMCD2 than in the IMCD1 and IMCD3. The rabbit IMCD does not have intercalated cells. Therefore, H-K-ATPase may also be present in the IMCD cells.

A low-potassium diet increases and a high-potassium diet suppresses the activity of H-K-ATPase in the CNT, CCD, and OMCD (14,76). These results suggest that the enzyme is regulated by body potassium. Potassium depletion increases bicarbonate reabsorption in the CDD (77). Because it has been demonstrated that potassium depletion does not change the activity of NEM-sensitive ATPase activity in the CCD (59), it is likely that the increased bicarbonate reabsorption may be mediated by H-K-ATPase. In addition, the H-K-ATPase activity is influenced by aldosterone. During hyperaldosteronism, the enzyme activity is increased in the CNT and CCD, but not in the OMCD and IMCD (78,79). Aldosterone stimulates the activity of Na-K-ATPase in CCD to a much greater extent than it does H-K-ATPase. Therefore, under normokalemic conditions, the overall effect of aldosterone will be to produce a net secretion of K. However, during K deficiency, there may be net reabsorption at least in the OMCD. The stimulatory effect of aldosterone on H-K-ATPase (79), along with its stimulatory effect on NEM-sensitive H-ATPase (20), will be additive for H secretion in the CNT.

Metabolic acidosis increases and metabolic alkalosis decreases the activity of H-K-ATPase in the rat OMCD (80). The stimulatory effect of metabolic acidosis is additive to the stimulation produced by hypokalemia, and there is an inverse linear relationship between H-K-ATPase activity in the OMCD and plasma bicarbonate concentration (80). On the basis of rubidium flux measurements, it has been suggested that there may be an interaction between Na absorption and H-K-pump activity in the CCD (81). The mechanism of this interaction is not known at this time.

The complete physiological and pathophysiological roles of renal H-K-ATPase have not been established at the present time. However, a recent study has reported that a decrease in H-K-ATPase activity in the CDD of rats treated with vanadate was associated with the development of distal tubular acidosis (82).

**H-ATPase and H-K-ATPase in Cultured Renal Tubular Cells**

In addition to microdissected nephron segments, renal epithelial cells in culture have proven to be useful in understanding the mechanism of electrolyte transport in the renal tubule (83). Both established cell lines and primary cultures have been used as a tool to study various transporters and to determine the mechanism of action of hormones. The sources, nephronal site of origin, hormone responsiveness, transport and metabolic functions, and electrical properties of established cell lines have been reviewed recently (83).

Very little effort has been made to examine H-ATPase or H-K-ATPase activity in cultured cells. A recent report suggests that Madin-Darby canine kidney (MDCK) cells express an apical H-K pump, which is stimulated by aldosterone (84). Proton secretion by MDCK cells is inhibited by omeprazole, an inhibitor of H-K-ATPase, and by barium, a K channel blocker that will prevent apical K recycling. Also, there is
preliminary evidence for H secretion mediated by H-ATPase and by H-K-ATPase in another cell line, RCCT-28A (85). However, neither H-ATPase nor H-K-ATPase has been characterized biochemically in these cells.

REGULATION OF URINARY ACIDIFICATION AND K EXCRETION

There is a complex relationship between the concentration of aldosterone, potassium, and total CO₂ in the plasma and transport of H and K in the collecting duct segments. The available data suggest that H secretion in the CCD and OMCD depends on H-ATPase and H-K-ATPase, both of which are stimulated by metabolic acidosis (Figure 6). In addition, both morphological and physiological studies indicate that a decrease in plasma pH is a major stimulus for urinary acidification. The intracellular signal(s) for the activation of the H pump and H-K pump during acidosis has (have) not been established. These intracellular signals may stimulate the insertion of the two pumps into the apical membrane or induce the synthesis of additional pumps, or both.

Chronic administration of DOCA to rabbits increases H secretion in the OMCD (65). In addition, H-ATPase activity in the CCD and OMCD of the rabbit is stimulated by aldosterone treatment (17). These results suggest that aldosterone receptors shown to be present in the CCD and OMCD (86) may not only be present in the principal cells but are also present in intercalated cells. Mineralocorticoids produce kaliuresis and antinatriuresis by increasing in Na-K-ATPase activity in the CCD (18,87). The increase in Na absorption will increase lumen negative voltage, which will stimulate H secretion indirectly in the CCD. Thus, aldosterone increases H secretion directly via stimulation of H-ATPase and indirectly by stimulation of Na-K-ATPase in the CCD.

Urinary K excretion is the net result of K secretion in the CCD and K absorption in the OMCD. K secretion in the CCD depends on Na-K-ATPase in the basolateral membrane of principal cells, and K absorption in the OMCD is mediated by the H-K-ATPase present in intercalated cells. Potassium loading increases plasma aldosterone, which in turn will stimulate K secretion. In addition, potassium loading itself stimulates Na-K-ATPase (88) and K secretion (89) in the CCD of adrenalectomized animals. In contrast, potassium loading decreases H-K-ATPase activity in the CCD and OMCD (76), suggesting a decrease in K absorption. The opposite is true for hypokalemia, which is associated with an increase in H-K-ATPase activity and in K reabsorption (43). Thus, it seems that plasma K is the principal regulator of urinary K excretion.

In spite of the stimulatory effect of aldosterone on H-K-ATPase activity in the CCD, administration of aldosterone is associated with potassium wastage and excess acid excretion, whereas aldosterone deficiency is associated with retention of both potassium and acid (90). Because aldosterone also increases the activity of Na-K-ATPase, which is responsible for K secretion in the CCD, and because the activity of Na-K-ATPase in the CCD is greater than that of H-K-ATPase, the effect of aldosterone on K secretion will be greater than on K absorption, resulting in K wasting.

SUMMARY AND CONCLUSIONS

In summary, there are at least two H-ATPases (an electrogenic H-ATPase and electroneutral H-K-ATPase) that mediate Na-independent H secretion in the distal nephron. Both of these ATPases are present in the intercalated cells of the collecting duct. The activity of both types of H-ATPases is regulated by plasma aldosterone, potassium intake, and changes in acid-base balance. H-ATPase is also present in other segments of the nephron, but less is known about its regulation in those segments.

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REFERENCES


"Study with me, then, a few things in the spirit of truth alone, so that we may establish the manner of Nature's operations . . . . For this essay which I plan will shed light upon the structure of the kidney. Do not stop to question whether these ideas are new or old, but ask, more properly, whether they harmonize with Nature. And be assured of this one thing, that I never reached my idea of the structure of the kidney by the aid of books, but by the long, patient and varied use of the microscope. I have gotten the rest by the deductions of reason, slowly, and with an open mind, as is my custom."