The renal H^+-K^+-ATPases: physiology, regulation, and structure

Michelle L. Gumz, I. Jeanette Lynch, Megan M. Greenlee, Brian D. Cain, and Charles S. Wingo

Abstract

The H^+-K^+-ATPases use the energy of ATP hydrolysis to pump hydrogen (H^+) and potassium (K^+) ions against their concentration gradients. Because they form a high-energy phosphorylated intermediate during the catalytic cycle, these enzymes are classified as P-type ATPases. They consist of two subunits. The large, ~100-kDa α-subunit contains 10 transmembrane helices and houses the catalytic site and the ion translocating pathways. A second, smaller glycosylated β-subunit is present in the kidney, the gastric or HKα1 isoform and the colonic or HKα2 isoform. In this review we discuss new information on the physiological function, regulation, and structure of the renal H^+-K^+-ATPases.

Keywords: ATPases; physiology; regulation; structure

Introduction

The renal H^+-K^+-ATPases use the energy of ATP hydrolysis to pump hydrogen (H^+) and potassium (K^+) ions against their concentration gradients. Because they form a high-energy phosphorylated intermediate during the catalytic cycle, these enzymes are classified as P-type ATPases. They consist of two subunits. The large, ~100-kDa α-subunit contains 10 transmembrane helices and houses the catalytic site and the ion translocating pathways. A second, smaller glycosylated β-subunit is present in the kidney, the gastric or HKα1 isoform and the colonic or HKα2 isoform. In this review we discuss new information on the physiological function, regulation, and structure of the renal H^+-K^+-ATPases.

Methods

The large, ~100-kDa α-subunit contains 10 transmembrane helices and houses the catalytic site and the ion translocating pathways. A second, smaller glycosylated β-subunit is present in the kidney, the gastric or HKα1 isoform and the colonic or HKα2 isoform. In this review we discuss new information on the physiological function, regulation, and structure of the renal H^+-K^+-ATPases.

Results

The large, ~100-kDa α-subunit contains 10 transmembrane helices and houses the catalytic site and the ion translocating pathways. A second, smaller glycosylated β-subunit is present in the kidney, the gastric or HKα1 isoform and the colonic or HKα2 isoform. In this review we discuss new information on the physiological function, regulation, and structure of the renal H^+-K^+-ATPases.

Discussion

The renal H^+-K^+-ATPases are ion pumps that use the energy of ATP hydrolysis to transport protons (H^+) in exchange for potassium ions (K^+). These enzymes consist of a catalytic α-subunit and a regulatory β-subunit. There are two catalytic subunits present in the kidney, the gastric or HKα1 isoform and the colonic or HKα2 isoform. In this review we discuss new information on the physiological function, regulation, and structure of the renal H^+-K^+-ATPases.

Conclusions

The H^+-K^+-ATPases are ion pumps that use the energy of ATP hydrolysis to transport protons (H^+) in exchange for potassium ions (K^+). These enzymes consist of a catalytic α-subunit and a regulatory β-subunit. There are two catalytic subunits present in the kidney, the gastric or HKα1 isoform and the colonic or HKα2 isoform. In this review we discuss new information on the physiological function, regulation, and structure of the renal H^+-K^+-ATPases.
localization of HKα1 and HKα2 expression in kidney

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Role of H+-K+-ATPases in K+ reabsorption and hypokalemia. The majority of reports indicate that HKα2 H+-K+-ATPase mRNA and protein expression are increased with dietary K+ depletion as shown in Table 2. This effect is well established in studies from rat and has been confirmed at the whole kidney level in mouse. Only one study (3) reported increased HKα1 mRNA expression with dietary K+ depletion. HKα2 mRNA and protein have been shown to be increased particularly in the medulla of rats fed a K+-depleted diet.

In each of the dietary K+ depletion studies, a Sch-28080-sensitive component, indicative of HKα1 H+-K+-ATPase, is present (see Tables 3 and 4). Additionally, luminal ouabain sensitivity, indicative of HKα2 H+-K+-ATPase, was reported in the rat OMCD and inner medullary collecting duct (IMCD) but was not additive with the effect of Sch-28080 (53, 56). In the terminal IMCD (tIMCD) of K+-restricted rats, ouabain inhibited steady-state acid secretion both in the presence and absence of Sch-28080 (85). Overall, the data suggest that both HKα1 and HKα2 H+-K+-ATPases participate in K+ reabsorption during K+ restriction, and the relative roles of these two H+-K+-ATPases in hypokalemia are discussed further below.

K+-stimulated ATPase activity has been reported in the CD of both rabbit and rat (74). As shown in Table 3, the most extensive studies of these separate types of K+-ATPase activities come from the Doucet laboratory (22), examining the distinct activities of the nephron segments and the CD in animals adapted to a normal or a K+–depleted diet. K+-ATPase activity has been identified in isolated microdissected nephron segments of rat, and all of the activities are sensitive to Sch-28080, which suggests that they arise from HKα1 H+-K+-ATPase. Three distinct enzymatic subtypes of K+-ATPase activity were described, which exhibit different characteristics (Table 3). In brief, K+-dependent ATPase activity in the CD was classified as either type I or type III. Type I was Sch-28080 sensitive and ouabain insensitive and was present in the cortical collecting duct (CCD) and OMCD of rats maintained on a normal-K+ diet (9). This activity exhibited a pharmacological profile that was virtually identical to the classic K+-ATPase obtained from microsomes from the gastric mucosa. Type III activity, defined as partially Sch-28080- and ouabain sensitive, replaced type I activity in rats fed a low-K+-diet (9). A recent study reported the existence of type I and type III activities in mouse and further characterized both activities in HKα1 or HKα2 H+-K+-ATPase-null mice (22). Dherbecourt et al. (22) reported that type I activity was no longer detectable in the HKα1-null mice, whereas type III activity was preserved. These data suggest that type I activity requires the expression of the HKα1 subunit. In contrast, type III activity was no longer detectable in the HKα2-null mice, whereas type I activity was preserved, which suggests that type III activity requires the expression of the HKα2 subunit. It should be noted that the

### Table 2. Effect of K+ restriction or depletion on HKα1 and HKα2 expression in kidney

<table>
<thead>
<tr>
<th>HKα1</th>
<th>Segment</th>
<th>Species</th>
<th>Reference</th>
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<td>Increased</td>
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<tr>
<td>Decreased</td>
<td>IMCD</td>
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<td>Protein</td>
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</tr>
<tr>
<td>Increased</td>
<td>Whole kidney</td>
<td>Rat</td>
<td>23</td>
</tr>
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<td>Medulla</td>
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<td>CD, MCD</td>
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<td>Ctx, OM, IM</td>
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<td>OMCD</td>
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<td></td>
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<td>IMCD</td>
<td>53</td>
<td></td>
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</tbody>
</table>

MCD, medullary collecting duct; Ctx, cortex.

### Table 3. K+-ATPase activities in kidney

<table>
<thead>
<tr>
<th>Type 1 K+-ATPase</th>
<th>Type 2 K+-ATPase</th>
<th>Type 3 K+-ATPase</th>
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</thead>
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<tr>
<td>IC50</td>
<td>Sch-28080</td>
<td>0.25 μM</td>
</tr>
<tr>
<td>IC50</td>
<td>ouabain</td>
<td>Insensitive</td>
</tr>
<tr>
<td>Cation activation</td>
<td>K+</td>
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<td>Effect of K+ depletion</td>
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<td>Decreases</td>
</tr>
<tr>
<td>Localization</td>
<td>CD</td>
<td>PT, TAL</td>
</tr>
</tbody>
</table>

Data derived from Ref. 22. PT, proximal tubule.
pharmacological maneuvers used in these studies could result in a nonspecific pharmacological effect of Sch-28080 as has been shown previously (12). Codina et al. (12) demonstrated a decline in intracellular ATP after Sch-28080 incubation in mMCD-3 cells. Thus the common Sch-28080 sensitivity reported for each of the K^+-ATPase activities described in Table 3 may require further clarification.

Another pharmacologically distinct K^+-ATPase activity (type II) was discovered in the early proximal tubule (PT) and TAL of rat (97). This unique activity was Sch-28080- and ouabain sensitive and was markedly reduced during K^+ depletion (9). Type II activity was similar to type III in pharmacology, yet different because 1) it was almost completely absent during K^+ depletion and 2) it was not stimulated by Na^+.

Beltowski and Wójcicka (8) reported the existence of type II activity in the cortex and medulla by an independent method; however, it was not possible to conclude in which nephron segment(s) the activity existed. The presence of type II activity has not been described in HK_2 or HK_2-null mice.

Role of H^+-K^+-ATPases in H^+ secretion and acid-base disturbances. The CD exhibits a significant Sch-28080-sensitive or K^+-dependent acid secretion mechanism in response to acute acid loading (10, 49, 57, 71, 90, 91, 96). Apical localization of H^+-K^+-ATPase in immunohistochemical studies supports the concept that the enzyme is important in both K^+ reabsorption and H^+ secretion (24, 65, 83). Data from K^+ tracer and HCO_3^- flux experiments clearly demonstrate that H^+-K^+-ATPase plays an important role in transportation of both cations in all segments of the CD (4, 85, 94, 102, 103).

Additional measurements of H^+-K^+-ATPase-mediated HCO_3^- flux in isolated CDs revealed that the enzyme’s activity could be controlled by the application of barium, a K^+ channel blocker (101, 105). Barium was shown to block HCO_3^- reabsorption in the CCD when applied either to the luminal perfusate, under K^+-replete conditions, or to the peritubular fluid, under K^+-restricted conditions. K_0 barium reabsorption was also inhibited by peritubular barium under K^+-restricted conditions. From these data, a model was proposed coordinating the H^+-K^+-ATPase with apical and basolateral channels, suggesting coordinated H^+ secretion with apical membrane recycling of K^+ during K^+ repletion and K^+ reabsorption via basolateral K^+ channels during K^+ depletion (Fig. 1).

Indeed, the mRNA expression of H^+-K^+-ATPases has been studied in states of metabolic acidosis as well. A recent study of CD genes regulated by chronic metabolic acidosis in mice showed that, after 3 days of 0.7 M NH_4Cl loading, HKo_1 and HKo_2 expression, determined by real-time PCR, in isolated OMCD increased ~15 fold and ~2-fold, respectively, compared with control levels (11). After 14 days of acid loading, OMCD expression of HKo_1 was not different from control, whereas HKo_2 gene expression was approximately threefold greater than control levels. Analysis of intracellular pH (pH_i) recovery in split-open CCD from rabbits subjected to chronic metabolic acidosis (0.075 M NH_4Cl for 10–14 days) demonstrated a threefold stimulation of Sch-28080-inhibitable and K^+-dependent H^+ secretion, suggesting that H^+-K^+-ATPases may play a role in the kidney’s response to chronic metabolic acidosis (72, 73).

Ammonia^3 increases HCO_3^- reabsorption in the rat CCD and IMCD (35, 84). The effects of ammonia on acid/base transport was investigated in the rabbit CCD (26–28). Ammonia stimulates a luminal Sch-28080- and ouabain-sensitive net HCO_3^- flux, suggesting that both isoforms of the H^+-K^+-ATPase are involved in mediating this response. Ammonia also stimulates the CCD H^+-K^+-ATPase through an intracellular calcium-dependent, microtubule-dependent and a Golgi vesicle transport-dependent process indicative of membrane vesicle insertion into the apical plasma membrane (27). These observations are consistent with studies on the stimulation of K^+ reabsorption by 10% CO_2 to mimic respiratory acidosis in the CCD of K^+-deplete rabbits that was blocked by calmodulin inhibition or attenuating changes in intracellular Ca_2^+ concentration ([Ca_2^+]_i) (102). Combined, these findings suggest that both ammonia and respiratory acidosis stimulate a calcium-dependent mechanism of H^+ extrusion via an H^+-K^+-ATPase.

Additionally, HKo_2 mRNA expression in the rabbit CCD has been shown to be significantly increased during acute metabolic alkalosis induced by dietary HCO_3^- loading (25).

Sch-28080 also significantly inhibited the increased HCO_3^- reabsorption observed in distal tubules (DT) and CCD from

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^3 The term “ammonia” refers to the total of (NH_4^+ + NH_3).
alkalotic rats, indicating that the HKβ1 H+-K+-ATPase may be stimulated as well (30, 92).

Table 4 provides a summary of the effects of both hypokalemia and acid-base disturbances on H+-K+-ATPase activity in the kidney.

**Na**^+^ and **NH**^+_4^ transport and **H**^+^-**K**^+-ATPase activity.** Substantial clinical evidence supports the assertion that diets with little K^+^ content can either worsen hypertension in humans with preexisting hypertension (34, 42) or predispose normotensive individuals to NaCl-sensitive hypertension (43). Moreover, mild K^+^ depletion appears to worsen hypertension in part by promoting renal Na^+^ retention (41). Given the effect of chronic hypokalemia to reduce plasma aldosterone concentrations, such observations suggest that an as yet not fully understood renal adaptive mechanism exists that exerts greater control of Na^+^ excretion than plasma aldosterone. Conversely, diets with a large K^+^ content have been associated with improvement in systemic hypertension (50, 64). At least for HKα1, there appears to be a significant interaction of Na^+^ and K^+^ for reabsorption by an apparent common mechanism. This observation is supported by work performed in native renal (104) and gastric (77) tissues. Interestingly, additional evidence from heterologous expression studies by Swarts et al. (78) suggests that HKα2 may have a greater affinity for NH^+_4^ than K^+^.

Specifically regarding HKα1, studies under different physiological conditions supported a significant interaction of Na^+^ and K^+^ with a Sch-28080-sensitive absorptive mechanism and concluded that K^+^ and Na^+^ compete for the same reabsorptive pathway (103, 104). Competitive binding between Na^+^ and K^+^ on the H+-K^+-ATPase is also supported by the observation that two structurally different H+-K^+-ATPase inhibitors, Sch-28080 and A80915A, can inhibit Na^+^ reabsorption in the rabbit CCD under conditions of dietary Na^+^ depletion (106). This observation suggests that the HKα1 isoform can mediate Na^+^ absorption since Sch-28080 is the classic gastric-type inhibitor. Dherbecourt et al. (22) reported K^+^-ATPase activity (type III) in HKα1-null mice that is sensitive to both Sch-28080 and ouabain. Direct evidence distinguished type III activity from type I activity by several characteristics, but of particular note, type III activity can be stimulated by Na^+^, which suggests direct binding of Na^+^ on this enzyme (9).

If the HKα1 H+-K^+-ATPase does transport Na^+^ under physiological conditions, then the disruption of the apical Na^+^/H^+^ exchanger 3 (NHE-3) would lead to enhanced distal luminal Na^+^ delivery and plausibly an upregulation of HKα1 as observed by Nakamura et al. (54). Swarts et al. (77) concluded that Na^+^ as well as K^+^ could support the dephosphorylation of the HKα1-containing H+-K^+-ATPase by comparing the steady-state amount of the phosphorylated enzyme in tight and leaky gastric membrane vesicles.

Additionally, physiological evidence shows that one or more H+-K^+-ATPase isoforms are stimulated by dietary Na^+^ depletion, consistent with a role for H+-K^+-ATPase in Na^+^ reabsorption. The intercalated cells (IC) from the split-open CCD of rats on a low-Na^+^ diet had twofold higher K^+^ dependent pH^+_i^ recovery from an acid pulse compared with control (70). The control K^+^-dependent pH^+_i^ recovery was blocked by Sch-28080, but abolishment of pH^+_i^ recovery in the IC from the low-Na^+^ animals required the application of both Sch-28080 and ouabain. These results suggested that NaCl depletion induces an additional H+-K^+-ATPase isoform. However, other studies did not observe that dietary Na^+^ depletion upregulated kidney HKα2 mRNA (65, 75) or protein (75). These findings suggest at least two explanations, either that yet a third isoform (possibly an α/β pair that has not been characterized) is induced under these conditions or that the increase in pump activity occurred as a posttranslational event. Supporting the former, Petrovic et al. (61) proposed that the kidneys of HKα1-null mice possess a novel H+-K^+-ATPase that is neither HKα1 or HKα2.

The H+-K^+-ATPase has been implicated as a compensatory mechanism to other renal perturbations. HKα2 H+-K^+-ATPase has been demonstrated to be upregulated by ischemia-reperfusion injury or acetazolamide treatment. Rats subjected to ischemia-reperfusion injury substantially upregulate cortical HKα2 H^+-K^+-ATPase, and not HKα1, perhaps to compensate for the (~75%) downregulation of the cortical and medullary NHE-3 (89). NHE-3 is an apical Na^+^/H^+^ exchanger that facilitates

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**Table 4. Effect of pathophysiologival conditions on H^+-K^+-ATPase Activity**

<table>
<thead>
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<th>Effect on H^+-K^+-ATPase Activity</th>
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<tr>
<td></td>
<td></td>
<td>CDF</td>
<td>Rabbit</td>
<td>102, 103</td>
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<tr>
<td>Acute NH4Cl loading</td>
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<td>A-type IC</td>
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<td>CDF</td>
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<tr>
<td>Metabolic acidosis</td>
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<td>PC and IC</td>
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H^+-K^+-ATPase activity is often defined as K^+-dependent H^+^ transport and pharmacologically defined as omeprazole-, Sch-28080-, and/or ouabain-sensitive H^+^ or K^+^ flux. The reader should be aware that inhibitor sensitivities do not strictly define the activity as mediated by HKα1 or HKα2. DT, distal tubule; tIMCD, terminal IMCD; OMCDF or OMCDF, OMCD of the inner stripe.
over half of the acid secretion that occurs in the PT. Rats treated with acetazolamide, which causes renal HCO$_3^-$ wasting, also exhibited upregulated cortical HK$_{\alpha_2}$ (89). Of particular interest, NHE-3-null mice have profoundly reduced HCO$_3^-$ reabsorption in the PT, with only slightly decreased serum HCO$_3^-$ levels compared with wild type (WT) (66). In an attempt to resolve the compensatory acid secretion mechanism limiting the development of substantial metabolic acidosis in NHE-3-null mice, Nakamura et al. (54) studied net HCO$_3^-$ absorption in the OMCD and concluded that the HK$_{\alpha_1}$ H$^+$/K$^+$-ATPase is involved in the adaptive changes in these mice. Specifically, there was a greater Sch-28080-sensitive acid secretion and upregulation of HK$_{\alpha_1}$ mRNA in the OMCD from NHE-3-null compared with control mice. Nakamura et al. reported that renal HK$_{\alpha_2}$ H$^+$/K$^+$-ATPase does not play a compensatory role in the NHE-3-null mice since the renal HK$_{\alpha_2}$ mRNA was undetectable in kidney and there was no effect of luminal 1.0 mM ouabain. However, the axonal heterogeneity of each CD segment and the small mass of CD in the whole kidney or cortical sections limits the interpretation of these data.

As noted above, dietary K$^+$ depletion has been shown to produce a volume- and Na$^+$-dependent form of hypertension despite reduction of plasma aldosterone concentration. Such evidence suggests that certain H$^+$/K$^+$-ATPase isoforms could participate in NaCl uptake, and experimental evidence supports the functional coupling of an H$^+$/K$^+$ ATPase and an apical Cl$^-$/HCO$_3^-$ exchanger in the CCD, possibly in the B-type IC (106). Thus the precise cation specificity of the luminal and cytosolic binding sites of H$^+$/K$^+$-ATPase isoforms may have profound importance if the binding affinities differ between species. These observations could help to explain not only the well-known interaction of K$^+$ intake on Na$^+$ intake but also the difference in the adaptive responses to K$^+$ depletion of humans versus commonly studied laboratory animals.

Knockout studies. HK$_{\alpha_1}$-null mice have been generated (76) and have survived, although they exhibited achlorhydria, hypergastrinemia, and metaplasia of the gastric epithelium. The secretory membranes and gastric mucosa of the HK$_{\alpha_1}$-null mice were also abnormal. Although no overt renal phenotype was observed in the HK$_{\alpha_1}$-null mice, no detailed study has been published on the renal function of these mice. Subsequent studies indicate that such investigations are essential to fully understand the role of these two H$^+$ pumps in solute transport (46). Petrovic et al. (61) reported the possible existence of a novel and compensatory H$^+$/K$^+$-ATPase activity. Measurement of K$^+$-dependent H$^+$ secretion in the CCD of HK$_{\alpha_1}$-null animals revealed the presence of an apparently unique Sch-28080- and ouabain-insensitive transporter. Review of the human genome for a third P-type ATPase $\alpha$-subunit gene did not reveal a likely candidate gene (http://biobase.dk/~axe/patbase.html). However, such an activity could be produced via alternative splicing of existing genes or posttranslational modification of an ion pump subunit. Since the H$^+$/K$^+$-ATPases require an additional $\beta$-subunit to function, the activity detected by Petrovic et al. may be due to a previously unrecognized HK$\alpha_2/\beta$ pair that confers these unique pharmacological properties. P-type ATPase $\alpha$-subunits can exhibit modified profiles depending on the identity of the coassembled $\beta$-subunit (29). Specifically, the work of Geering and coworkers (19) has clearly demonstrated that different $\beta$-subunits confer different protease sensitivities to HK$_{\alpha_2}$ and that these structural changes can be observed in response to K$^+$ and ouabain.

The ability of HK$_{\alpha_2}$-null mice to retain fecal and urinary K$^+$ has been studied under control and K$^+$-depleted conditions (48). Under control conditions, no dramatic differences were observed between WT and HK$_{\alpha_2}$-null mice. However, when fed a K$^+$-free diet for 18 days, the null mice lost four times more fecal K$^+$ than the WT animals. However, these knockouts did not display a significant renal phenotype. The HK$_{\alpha_2}$-null mice also lost twice as much body weight as WT and had smaller plasma and muscle K$^+$ concentration (K$^+$) than WT mice. These studies clearly demonstrated that the HK$_{\alpha_2}$ H$^+$/K$^+$-ATPase has a pivotal role in maintaining K$^+$ balance, at least in the colon.

A previous report suggested that the HK$_{\alpha_2}$ H$^+$/K$^+$-ATPase could function as a Na$^+$/K$^+$-ATPase in the colon (62). A subsequent study involving the HK$_{\alpha_2}$-null animals examined the effect of dietary Na$^+$ depletion in such mice versus WT control animals from the same strain (75). The HK$_{\alpha_2}$-null mice had a greater rate of fecal K$^+$ excretion than the WT animals when fed a Na$^+$-restricted diet. In addition, regardless of diet, the null mice exhibited smaller values of epithelial Na$^+$ channel (ENaC)-mediated short-circuit current in the distal colon. The authors postulated that HK$_{\alpha_2}$ plays an important role not only in K$^+$ conservation but also in Na$^+$ absorption by the colon, which suggests that HK$_{\alpha_2}$ H$^+$/K$^+$-ATPase may also mediate Na$^+$ reabsorption by the kidney.

A recent report on the HK$_{\alpha_2}$-null mice provided in vivo evidence for the importance of the HK$_{\alpha_2}$ H$^+$/K$^+$-ATPase as a H$^+$ pump (60). Previously, Pestov et al. (58) had shown that the HK$_{\alpha_2}$ H$^+$/K$^+$-ATPase was expressed in the apical membrane of the anterior prostate epithelium. In a convincing study, this group evaluated the acidification of anterior prostate fluid in the HK$_{\alpha_2}$-null mice (60). They found that the pH of prostate fluid in the HK$_{\alpha_2}$-null animals was 6.96 compared with 6.38 in WT mice. This loss of acidification strongly suggested that the HK$_{\alpha_2}$ H$^+$/K$^+$-ATPase functions as a H$^+$ pump in vivo and is required for acidification of luminal prostate fluids. Additionally, mice with disruption for $Atp12a$ (encoding for HK$_{\alpha_2}$) have loss of apical immunohistochemical localization for NaK$_{\beta_1}$, supporting the role of this $\beta$-subunit as the authentic in vivo subunit in the mouse anterior prostate for the apically localized HK$_{\alpha_2}$/NaK$_{\beta_1}$ H$^+$/K$^+$-ATPase (59) similar to the work of Codina et al. (14) in rat medulla. Indeed, we recently investigated (46) rates of pH recovery and acid extrusion in response to acute acid loading in two types of IC from the CCD of WT mice and in these null mice. The results confirm a significant and separate contribution of both HK$_{\alpha_1}$ and HK$_{\alpha_2}$ to acid extrusion in both A-type and B-type IC in mice fed a normal diet. Moreover, it is now clear that both HK$_{\alpha_1}$ and HK$_{\alpha_2}$ independently contribute to H$^+$ secretion in mice fed normal mouse chow and that combined disruption of both catalytic subunits (HK$_{\alpha_1,2}$) reveals independent effects of both gene products at least in A-type IC (46). Such observations suggest that these two pumps independently secrete H$^+$ in exchange for cellular K$^+/cation reabsorption, which supports the role of both isoforms in renal solute transport.

In summary, HK$_{\alpha_1}$- and HK$_{\alpha_2}$-null mice have been generated, but the renal phenotype of these mouse models has not been fully characterized. Examining the HK$_{\alpha_1,2}$-null mice would be of particular interest.
null mice during K⁺ and Na⁺ depletion, as well as acid and alkali loading, will provide valuable insight into the physiological function of renal H⁺-K⁺-ATPases in pathophysiological states.

**Regulation of Renal H⁺-K⁺-ATPases**

**Signaling molecules.** Regulation of the Na⁺-K⁺-ATPase by accessory subunits and signaling molecules is considerably better understood compared with that of the H⁺-K⁺-ATPases. In an effort to characterize more fully these mechanisms of regulation for the HKα₁ H⁺-K⁺-ATPase, Cornelius and Mahmoud (18) undertook a profiling study to explore the acute regulation mechanisms of the gastric H⁺-K⁺-ATPase. It is well established that the activity of the gastric enzyme depends on trafficking of the enzyme from intracellular vesicles to the apical membrane of gastric parietal cells. The signals regulating this trafficking event are not fully understood but most likely involve second messenger molecules such as cAMP, Ca²⁺, and diacylglycerol. The protein kinases involved in these signaling pathways include protein kinases A and C (PKA, PKC). Consensus sequence sites for these kinases are present in the HKα₁ protein. Using a combination of proteolytic fingerprinting and 3²P-kinase assays, these investigators demonstrated the presence of a PKC phosphorylation site at the NH₂ terminus of HKα₁ and a PKA phosphorylation site at the COOH terminus. However, the latter site was only phosphorylated in the presence of detergent, indicating that it may or may not be physiologically relevant. The PKC phosphorylation site is likely Ser 27, a highly conserved PKC site. The catalytic activity of the gastric H⁺-K⁺-ATPase was significantly increased by NH₂-terminal PKC (Ca²⁺-dependent isoforms) phosphorylation. These investigators concluded that, since the activation of catalytic activity occurred at saturating ATP concentrations, the most likely reaction step affected by PKC phosphorylation was the E₁→P to E₂-P transition. Phosphorylation of HKα₁ at the NH₂-terminal PKC site may therefore serve as a "conformational switching signal," with implications for regulation of H⁺ secretion.

Another group of investigators used rat CCK to study the mechanism behind the PKA-dependent activation of the HKα₁ H⁺-K⁺-ATPase by isoproterenol (45). Using a [³²P]ATPase assay in combination with various inhibitors, Laroche-Joubert et al. (45) investigated a β-adrenergic receptor pathway specifically in β-IC of the CCK. They found that isoproterenol activated a G protein-coupled receptor that in turn led to the release of cAMP and activation of PKA. This was followed by stimulation of a pathway involving a tyrosine kinase-Ras-Raf-1-MEK-ERK cascade, ultimately resulting in activation of the HKα₁ H⁺-K⁺-ATPase as characterized by its sensitivity to Sch-28080.

**HKα₂: PKA.** Consensus sites for PKA are present in the amino acid sequence of HKα₂. In an effort to determine the effects of PKA signaling on the ouabain-sensitive H⁺-K⁺-ATPase in the kidney, Beltowski et al. (7) treated male Wistar rats with a nonhydrolyzable analog of cAMP. Analysis of ouabain-sensitive H⁺-K⁺-ATPase activity in the microsomal fractions from either renal cortex or medulla revealed 30% or greater increases in activity. This effect was sensitive to brefeldin A, a compound that prevents trans-Golgi processing of plasma membrane proteins, suggesting that the PKA-mediated activation of the HKα₂ H⁺-K⁺-ATPase was dependent on insertion of a protein into the plasma membrane. Whether this protein is the enzyme itself or another regulatory protein required for H⁺-K⁺-ATPase activity remains to be determined.

A recent report from the Codina and DuBose laboratories may shed some light on the above question. Codina et al. (17) mutated the COOH-terminal PKA consensus site, Ser 955, to either an alanine or an aspartic acid. A thorough analysis of these mutants was performed by assaying HKα₂ protein expression and functionality (by ⁸⁶Rb uptake assay) at the plasma membrane, total HKα₂ levels by immunoblot, and ³⁵S-met labeling to determine the rate of HKα₂ protein synthesis and degradation. These studies were conducted in HEK293 cells and revealed that phosphorylation of HKα₂ at Ser 955 promoted maturation of the HKα₂ protein. Inhibition of PKA activity by expression of the PKA inhibitor resulted in decreased expression of HKα₂ and a subsequent decrease in ⁸⁶Rb uptake. These results indicate that phosphorylation of HKα₂ by PKA may contribute to the enzyme being properly trafficked to the plasma membrane.

**Structure of H⁺-K⁺-ATPases**

To date, no high-resolution structure of the HKα₁ or the HKα₂ H⁺-K⁺-ATPase has been published. However, several structures of a related P-type ATPase, the single-subunit Ca²⁺-ATPase, have been published (79–82). The Ca²⁺-ATPase has been crystallized with bound analogs, representing various stages of the catalytic cycle, thereby allowing the pumping mechanism of a P-type ATPase to be visualized (see supplemental video in Ref. 80). Resolution of the Ca²⁺-ATPase structures has led to several modeling exercises in order to apply this knowledge to related enzymes, including the HKα₁ and HKα₂ H⁺-K⁺-ATPases.

Our laboratory (31) published the first such study, using the first high-resolution structure of the Ca²⁺-ATPase (Protein Data Bank ID: 1eul) as template to build a homology model of HKα₂. The overall shape of the HKα₂ H⁺-K⁺-ATPase model appeared to be conserved compared with the Ca²⁺-ATPase. Important features shared between the HKα₂ model and the Ca²⁺-ATPase included the A (actuator), P (phosphorylation), N (nucleotide binding), and M (transmembrane) domains of the subunit. Extensive modeling of the HKα₁ H⁺-K⁺-ATPase has also been performed based on several of the Ca²⁺-ATPase structures. HKα₁ H⁺-K⁺-ATPase models in both the E₁ and E₂ conformations have been used together with biochemical experiments to evaluate the docking of K⁺-competitive inhibitors in the pump (5) as well as to investigate a salt bridge required for K⁺ binding (36). The most comprehensive modeling studies on HKα₁ came from the laboratory of Sachs and were based on the E₁-Ca²⁺, E₂-thapsigargin (51), and E₂-magnesium fluoride (52) structures. In these two elegant studies, Munson et al. presented homology models of the HKα₁ H⁺-K⁺-ATPase and investigated them based on a large body of biochemical data concerning structure/function relationships in this ion pump.

The Sachs model of HKα₁ bound to ADP-Mg²⁺ is pictured in Fig. 2. The model is colored from dark blue at the NH₂ terminus to red at the COOH terminus. The A, N, P, and transmembrane domains are labeled. In their two reports, Munson et al. used the HKα₁ models to consider the mecha-
10 transmembrane helices in the membrane domain are labeled M1–M10. For termination. The A or actuator domain is shown in light blue, the N or nucleotide E2-K inhibitors. Docking of the inhibitor prevented access of K\(^+\) into the E1 HK. Shin and Sachs (69) for the HK\(1_{-H9251}\) model. To address this issue, Munson et al. replaced the Ca\(^2+\) with K\(^+\) access from the lumen depends on K\(^+\) conductance through the KCNQ1/KCNE2 complex, whereas CLIC6 is proposed to conduct Cl\(^-\) (67). These theoretical models are consistent with site-directed mutagenesis studies in which the WT and mutant (E820A) HK\(1_{-H9251}\) subunit were expressed with the HK\(\beta\) subunit in Sf9 cells. The WT and mutant enzymes both showed a biphasic activation by K\(^+\) on ATP hydrolysis, but with the mutant having a significantly reduced K\(^+\) affinity (33), and showed that Glu 795 is essential for HK\(\alpha_{1}\) H\(^+-\)K\(^+\)-ATPase activity (32).

The application of these modeling exercises to the kidney could potentially answer important questions about the ion affinities and transport properties of HK\(\alpha_{1}\) and HK\(\alpha_{2}\). For example, given the extensive data that suggest a role for either or both of the HK\(\alpha_{1}\) and HK\(\alpha_{2}\) H\(^+-\)K\(^+\)-ATPases in Na\(^+-\)K\(^+\)-ATPases is likely to be focused on delineating their role in the transport of ions other than H\(^+\) and K\(^+\).

**Conclusions and the Future**

Despite the well-established catalytic cycle of the H\(^+-\)K\(^+\)-ATPase family of ion-motive pumps, much further investigation into the function and regulation of H\(^+-\)K\(^+\)-ATPases is needed to ascertain the specific physiological roles of these ion pumps. The available evidence, albeit imperfect, supports the assertion that progress on the functional importance of these pumps during normal and physiologically stressed conditions will be imperative to assess the specific role of the enzyme in both electrolyte and acid-base homeostasis. A recent report demon-
strates the impairment of $H^+$ secretion in either or both $H^+\cdot K^+\cdot$ATPase $\alpha$-subunit-null mice on a normal diet. Lynch et al. (46) found that both HK$\alpha_1$ and HK$\alpha_2$ play a crucial role in acid secretion in the CCD. The regulation of $H^+\cdot K^+\cdot$ATPases by ammonia and the possible transport of NH$_4^+$ in place of $K^+$ bear implications for acid-base handling. The response of the HK$\alpha_1$, HK$\alpha_2$, and HK$\alpha_1/2$-null mice to acid and base loading will elucidate the enzyme’s role in acid-base balance.

The importance of HK$\alpha_2$ in $K^+$ homeostasis was previously studied. HK$\alpha_2$-null mice displayed a reduced conservation of fecal $K^+$ during $K^+$ depletion, leading to lower plasma $[K^+]$ than $K^+$-depleted WT mice. Both WT and HK$\alpha_2$-null mice conserved urinary $K^+$, and only a trend toward increased urinary $K^+$ excretion in the null mice was observed. This retention of urinary $K^+$ may be due to the presence and activation of the HK$\alpha_1$ subunit or a considerable reduction of $K^+$ secretion. Investigation of the HK$\alpha_1$-null and HK$\alpha_1/2$-null mice on a $K^+$-depleted diet will provide insight into the functional importance of $H^+\cdot K^+$-ATPases in $K^+$-depleted conditions.

Evidence implicates $H^+\cdot K^+$ ATPases in $Na^+$ transport. HK$\alpha_2$-null mice excrete 2.8 times more fecal $K^+$ and slightly more fecal $Na^+$ on a $Na^+$-depleted diet than $Na^+$-depleted WT mice (75). The lack of an overt renal phenotype in HK$\alpha_2$-null mice during $Na^+$ depletion may be a result of compensation by the HK$\alpha_1$ subunit or differences in specific tissues. The upregulation of $H^+\cdot K^+$-ATPases in the kidneys of NHE-3-null mice also suggests that this enzyme may have a physiological role in both $H^+$ and $Na^+$ transport. To fully investigate the role of $H^+\cdot K^+$-ATPases in $Na^+$ transport, both the HK$\alpha_1$-null and HK$\alpha_1/2$-null mice need to be examined similarly under $Na^+$-depleted conditions. HK$\alpha_1/2$-null mice provide an invaluable tool that can be used to assess the physiological significance of $H^+\cdot K^+$-ATPases, especially within pathological dietary conditions.

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DISCLOSURES

No conflicts of interest are declared by the author.

REFERENCES


REVIEW


