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Molecular Regulation and Physiology of the H^+,K^+-ATPases in Kidney

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Two H^+, K^+-adenosine triphosphatase (ATPase) proteins participate in K^+ absorption and H^+ secretion in the renal medulla. Both the gastric ($HK\alpha_1$) and colonic ($HK\alpha_2$) H^+,K^+-ATPases have been localized and characterized by a number of techniques, and are known to be highly regulated in response to acid-base and electrolyte disturbances. Both ATPases are dimers of composition α/β that localize to the apical membrane and both interact with the tetraspanin protein CD63. Although CD63 interacts with the carboxy-terminus of the α-subunit of the colonic H^+,K^+-ATPase, it interacts with the β-subunit of the gastric H^+,K^+-ATPase. Pharmacologically, both ATPases are distinct; for example, the gastric H^+,K^+-ATPase is inhibited by Sch-28080, but the colonic H^+,K^+-ATPase is inhibited by ouabain (a classic inhibitor of the Na^+-pump) and is completely insensitive to Sch-28080. The α-subunit of the colonic H^+,K^+-ATPase is the only subunit of the X^+,K^+-ATPase superfamily that has 3 different splice variants that emerge by deletion or elongation of the amino-terminus. The messenger RNA and protein of one of these splice variants ($HK\alpha_{2C}$) is specifically up-regulated in newborn rats and becomes undetectable in adult rats. Therefore, $HK\alpha_2$, in addition to its role in potassium and acid-base homeostasis, appears to play a significant role in early growth and development. Finally, because chronic hypokalemia appears to be the most potent stimulus for upregulation of $HK\alpha_2$, we propose that the $HK\alpha_2$ participates importantly in the maintenance of chronic metabolic alkalosis.
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The intercalated cells (IC) of the collecting tubule are responsible for regulation of urinary acidification, and therefore play a central role in maintaining acid-base homeostasis. Two types of intercalated cells have been recognized: type A and type B intercalated cells. Type A intercalated cells secrete protons into the lumen of the tubule, thereby acidifying the urine. Stochiometrically, for each proton secreted, one bicarbonate ion is returned to systemic blood across the basolateral membrane on the Cl/HCO_3 exchanger. Type A cells and net H^+-secretory capacity are stimulated by acidemia. A schematic representation of a type A IC is displayed in Figure 1. In contrast, type B intercalated cells secrete $HCO3^-$ across the luminal membrane and protons across the basolateral membrane. Type B IC cells have the capacity to alkalinize tubule fluid, and therefore, final urine, but are recruited significantly for this purpose in metabolic alkalosis to excrete excess bicarbonate. Three adenosine triphosphate (ATP)-dependent proton transporters that mediate hydrogen excretion are expressed in the renal medulla: the colonic and gastric H^+,K^+-ATPases, and the H^+-ATPase. This review focuses on the mechanisms and molecular regulation of proton secretion by the H^+,K^+-ATPases.

Colonic H^+,K^+-ATPase

The colonic H^+,K^+-ATPase assembles and functions as an α/β heterodimer. The α-subunit, hereafter designated as $HK\alpha_2$, was cloned by Crowson and Shull[1] from a rat distal colon complementary DNA library. Therefore, it often is referred to as the colonic H^+,K^+-ATPase α-subunit. In addition to expression in distal colon, $HK\alpha_2$ also is expressed at low levels in other tissues, including kidney, where it is highly regulated.[1,2]

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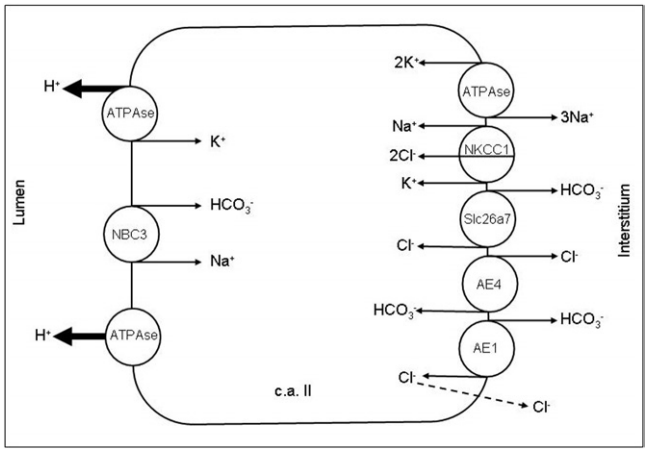

Figure 1 Schematic representation of a type A intercalated cell. The ATP-dependent H^+-transporters (H^+,K^+-ATPase and H^+-ATPase) are located on the apical membrane (thick arrows) and are critically important for urinary acidification. Model compiled from Wall,[52] Batlle and Flores,[53] and Giebisch et al.[54]

$HK\alpha_2$ has a molecular weight of approximately 100 kDa, and in common with the other members of the X^+,K^+-ATPase family, is predicted to span the plasma membrane 10 times and has intracellular carboxy and amino termini (http://www.ch.embnet.org/software/TMPRED_form.html). $HK\alpha_2$ also contains binding domains for ouabain,[3] is responsible for potassium/hydrogen exchange, and requires association with a β-subunit for functionality (ATPase enzymatic activity and transport function).[4-7]

Pharmacologic and Transport Properties of Colonic H^+,K^+-ATPase

Our laboratory has shown that rat $HK\alpha_2$ supports $^{86}Rb^+$-uptake when expressed in *Xenopus laevis* oocytes in a β-subunit–dependent manner. $^{86}Rb^+$-uptake was insensitive to Sch-28080 (10 μmol/L) but partially sensitive to ouabain (IC_{50}~250 μmol/L).[4] Cougnon et al[5] showed, using a similar approach, that $HK\alpha_2$ functions as an H^+/K^+ and Na^+/K^+ exchanger, and is sensitive to ouabain. A similar observation was made by Grishin et al[8] by cotransfecting HEK-293 cells with human ATP1AL1 (assumed to be the homolog of rat $HK\alpha_2$) and the β-subunit of the rabbit gastric H^+,K^+-ATPase. The transport activity of the expressed pump was more efficient in transporting sodium than protons at a coupling ratio of approximately 10 to 1. To show that $HK\alpha_2$ can function as a Na^+-pump it would be necessary to stably transfect HEK-293 cells with rat $HK\alpha_2$ and to select transfected cells in the presence of low concentrations of ouabain because the endogenous Na^+-pump of HEK-293 is very sensitive to low concentrations of ouabain.[9,10] However, an experiment of this type has not been reported to date.

Splice Variants of $HK\alpha_2$

Alignment of the amino acid sequences of the α_1-, α_2-, and α_3-subunits of Na^+,K^+-ATPase, the gastric H^+,K^+-ATPase α-subunit ($HK\alpha_1$), and $HK\alpha_2$ (PileUp program from SeqWeb (Accelrys,® San Diego, CA) reveals a high degree of sequence similarity. Although splice variants have not been identified for any of the -subunits of the Na^+-pump or for $HK\alpha_1$, splice variants for $HK\alpha_2$ have been reported. Kone and Higham[11] were the first to identify a splice variant of $HK\alpha_2$ that was truncated by 108 amino acids at the amino-terminus. This variant, $HK\alpha_{2B}$, was expressed in HEK-293 cells and displayed pharmacologic properties identical to $HK\alpha_2$. A second splice variant of $HK\alpha_2$ was identified by Campbell et al[12] in rabbit renal medulla. $HK\alpha_{2C}$ encodes a 61-residue amino-terminal extension to rabbit $HK\alpha_2$ and has not been expressed in heterologous systems thus far.

Immunolocalization of $HK\alpha_2$

$HK\alpha_2$ messenger RNA (mRNA) and protein are expressed at low levels in the renal medulla but abundantly in the distal colon.[1,2,13] A rabbit polyclonal antibody increased against the sequence of rat $HK\alpha_2$ that extends from amino acid 686 to 698 was developed by our laboratory.[13] Gallardo et al[14] and our laboratory[15] used this antibody to show by immunolocalization that $HK\alpha_2$ protein is expressed in the apical membrane of the distal colon (Fig 2).

Verlander et al[16] used a chicken $HK\alpha_{2C}$–specific polyclonal antibody in immunolocalization experiments to show that

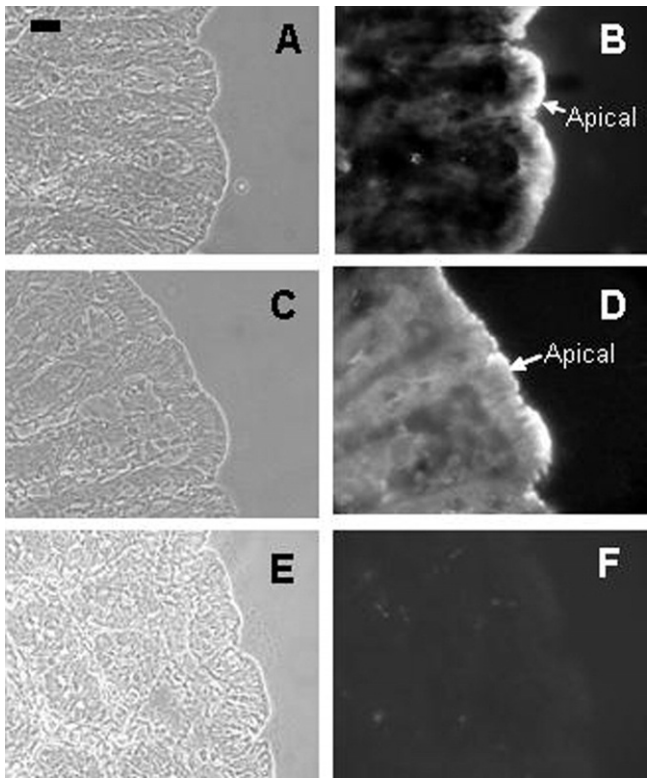

Figure 2 $HK\alpha_2$ and $NK\beta_1$ localize to the apical membrane of the distal colon. Immunolocalization experiments were performed as described by our laboratory.[15] (A, C, and E) Bright field images of the same distal colon sections used in immunolocalization experiments. (B) Labeling with anti-$HK\alpha_2$ antibody. (D) Labeling with anti $NK\beta_1$. (F) Labeling without primary antibody. The bar indicates 20 μm. Note that strong basolateral staining with anti-$NK\beta_1$ was observed if the sample was treated briefly with CHAPS (1%).[15]

HKα_{2c} is expressed in the apical membranes of type A IC, type B IC, and principal cells. These findings are compatible with the observation[17] that HKα_2 also was immunolocalized to rat principal cells. The physiologic significance of localization in principal cells has not been elucidated completely, however, because these cells have not been viewed as participants in urinary acidification.

In addition to expression in kidney and distal colon, HKα_2 also is expressed abundantly in prostate. Pestov et al[18] showed HKα_2 protein in the apical membrane of rat anterior prostate, where it colocalizes with β_1-Na^+,K^+-ATPase (NKβ_1).[19]

β_1-Na^+,K^+-ATPase is the Physiologic β-Subunit for HKα_2

The different α-subunits of Na^+,K^+-ATPase and HKα_1 require assembly with a specific β-subunit for protection from degradation, trafficking out of the endoplasmic reticulum (ER) to the plasma membrane, and for function. Although a unique β-subunit has not been identified for HKα_2, immunoprecipitation experiments performed by Kraut et al[20] and by our laboratory[21] have revealed that HKα_2 co-immunoprecipitates with NKβ_1. These data are consistent with the observation that NKβ_1 is expressed in the apical membrane of the distal colon[15] and rat anterior prostate.[18] These results also are consistent with the observation that NKβ_1 is more efficient than NKβ_3 (also expressed in kidney and distal colon) in supporting $^{86}Rb^+$-uptake when cotransfected with HKα_2 in HEK-293 cells (HKα_2 plus NKβ_1 versus HKα_2 plus NKβ_3).[15] By addition of enhanced green fluorescent protein to the amino terminus of HKα_2, we also showed that NKβ_1 is more efficient than NKβ_3 in the translocation of HKα_2 to the plasma membrane.[15] Therefore, abundant evidence supports the view that β_1-Na^+,K^+-ATPase is the physiologic β-subunit for HKα_2 in kidney, colon, and prostate.

Molecular Regulation of HKα_2 mRNA and Protein Expression

Studies performed in several laboratories consistently have shown that HKα_2 mRNA and protein expression increases in the renal medulla during chronic potassium depletion.[13,22] This finding supports the view that the colonic H^+,K^+-ATPase plays a central role in potassium conservation. However, this interpretation has been challenged by the absence of the expected phenotype in the HKα_2–deficient mouse model.[23] That is to say that HK$\alpha_{2-/-}$ mice developed normally and did not display easily discernable abnormalities in potassium homeostasis. Nevertheless, with dietary potassium deprivation HK$\alpha_{2-/-}$ mice developed a lower plasma K^+ and displayed inappropriate and persistent fecal and urinary K^+ wasting. Based on knowledge of the means by which the HKα_2–deficient mouse model was generated,[23] it would be predicted that if a mutated HKα_2 protein were synthesized in HK$\alpha_{2-/-}$, the last 84 amino acids at the carboxy-terminus should be truncated. Because these mice did not display the anticipated phenotype, we considered the possibility that the carboxy-terminus of HKα_2, although absent in the HK$\alpha_{2-/-}$ mouse, might not be required for functionality. To determine whether the terminal 84 amino acids were critical for functionality, we created a deletion mutation of HKα_2 that truncated these terminal amino acids (ΔHKα_2). This mutation, which was expressed in HEK-293 cells, assembled poorly with NKβ_1, and degraded more rapidly. Moreover, in keeping with these findings, the addition of enhanced green fluorescent protein to the amino-terminus of HKα_2 showed that the protein failed to migrate to the plasma membrane efficiently. Therefore, we concluded that the Meneton mutation was indeed nonfunctional. These arguments opened the possibility that alternative potassium transporters might compensate for the absence of HKα_2 in the HKα_2-deficient mouse model, or that other potassium-retaining transporters might be upregulated.

Association of CD63 With the Carboxy-Terminus of HKα_2

In an attempt to identify potential modifier proteins that might interact with the HKα_2 carboxy-terminus, we subsequently used this 84 amino acid sequence to screen a mouse kidney complementary DNA library, using the yeast 2-hybrid method.[24] A tetraspanin protein, CD63, was identified and shown by co-immunoprecipitation to assemble with the HKα_2 carboxy-terminus. A current model of HKα_2/NKβ_1/CD63 interaction is displayed in Figure 3. By specifically suppressing the expression of endogenous CD63 by small interfering RNA (siRNA) in transiently transfected HEK-293 cells, we showed in CD63 knockdown cells that HKα_2/NKβ_1 migrated more efficiently to the plasma membrane, and $^{86}Rb^+$ uptake was significantly greater than in cells expressing CD63 protein.[24] From this finding it appears reasonable to conclude that CD63 interacts with HKα_2/NKβ_1 to regulate endocytosis from the cell surface. This view is consistent with the findings of Duffield et al.[25] In these studies it was shown that CD63 assembles with the β-subunit of the rabbit gastric H^+,K^+-ATPase. These investigators concluded that CD63 participates importantly in the regulation of the trafficking of the gastric H^+,K^+-ATPase. Therefore, these 2 studies, when taken together, suggest a previously unappreciated protein-trafficking role for the tetraspanin protein, CD63. In our studies CD63 appears to function additionally as a suppressor protein.

Potential Role for HKα_2 in Growth and Development

Postnatal growth is associated with an increase in total body potassium.[26] Over the first 15 days of life a newborn rat will increase in body weight from approximately 1 to about 10 to 15 g. This means that during a short period the newborn rat must increase its total body potassium 10- to 15-fold. Potassium is derived from maternal milk; the kidneys and, to a lesser extent, the distal colon play a critical role in minimizing K^+ excretion. Therefore, growth requires a state of avid potassium conservation. Net positive potassium balance is achieved by 2 mechanisms: secretion is reduced,[27-29] and

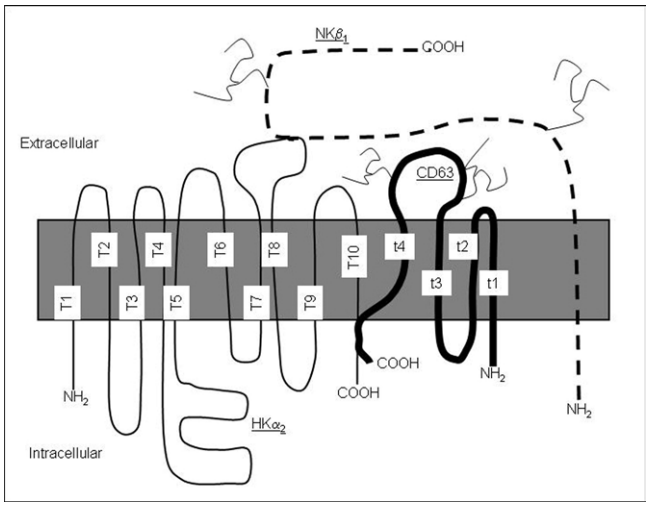

Figure 3 Proposed model for membrane localization of the HKα_2/NKβ_1/CD63 complex. HKα_2 (thin continuous line) is represented as a membrane-spanning protein (T1-T10) with cytosolic amino and carboxy termini. The β-subunit (discontinuous thick line) extends once across the cell surface. The amino-terminus is cytosolic and the carboxy-terminus is extracellular. The tetraspanin CD63 is represented as a thick continuous line (t1-t4); the amino and carboxy-termini are cytosolic. The β-subunit and CD63 are glycosylated (represented by branches). The model proposes that the β-subunit interacts with the extracellular domain of HKα_2 between T7 and T8 and the carboxy-terminus of CD63 interacts with the carboxy-terminus of HKα_2.

absorption is increased.[30,31] We studied the potential role of the colonic H^+,K^+-ATPase in potassium conservation during development. Although HKα_2 mRNA was not detected before birth, it was abundant in newborn rats (1 and 8 days of age) (Fig 4, left panel). Furthermore, this apparent upregulation subsided progressively into adulthood. Immunoblot analysis (Fig 4, middle panel) recognized a protein in newborn rats of a slightly higher molecular weight than HKα_2, suggesting the possibility that HKα_{2C} could function as the physiologic HKα_2 in newborn rats. However, HKα_{2C} protein was not detected in controls. This later observation is consistent with previous observations from our laboratory that HKα_2 is expressed at low levels in rats receiving a diet containing potassium.[13] The anti-HKα_2 antibody did not recognize HKα_{2C} if immune serum was preincubated with immunizing peptide (250 μmol/L) for 1 hour at 4°C (Fig 4, right panel).

Pathophysiologic Role of HKα_2

Distal Renal Tubular Acidosis (dRTA)

The colonic H^+,K^+-ATPase has been implicated in several pathophysiologic conditions. First, there was 1 infant reported with severe chronic hypokalemia and a hyperchloremic metabolic alkalosis as well as other typical clinical features of classic distal renal tubular acidosis.[32] The investigators suggested that abnormalities of the colonic H^+,K^+-ATPase might be the explanation for the severe hypokalemia and renal acidification defect. To sustain growth and development, large amounts of potassium and bicarbonate supplementation were required. Nevertheless, a specific defect in HKα_2 has not been proven to exist in this or other forms of classic distal RTA associated with hypokalemia. Whether an endemic form of classic distal RTA associated with striking hypokalemia, as reported in northeastern Thailand, represents failure of adaptation of HKα_2 is possible, but has not been proven unequivocally.

Metabolic Alkalosis

Because chronic hypokalemia is a frequent accompanying feature of chronic metabolic alkalosis, it is likely that upregulation of HKα_2 may be an important participant in the maintenance phase of metabolic alkalosis. In addition to enhanced urinary acidification, chronic hypokalemia also participates importantly in the maintenance of metabolic alkalosis through increased production and excretion of ammonium.[33] This combined effect of chronic hypokalemia, upregulation of HKα_2, and ammonium production and excretion, would greatly augment net acid excretion during the maintenance phase of chronic metabolic alkalosis, and persist until the hypokalemia were corrected.

Gastric H^+,K^+-ATPase

The gastric H^+,K^+-ATPase is abundant in gastric acinar cells and is responsible for gastric acid secretion.[34,35] The functional protein is a heterodimer composed of α/β subunits. The α-subunit (HKα_1) internalizes potassium in exchange for hydrogen and is inhibited by low concentrations of Sch-28080 and omeprazole,[34,36,37] but is insensitive to ouabain.[9,38,39] The unique β-subunit (HK βG) is heavily glycosylated and has a molecular weight of approximately 35 kd. Both HKα_1 and HKβG protein and mRNA are expressed in the kidney, but much less abundantly than in the stomach.[40]

Renal Regulation of HKα_1

Although expressed in renal cortex and medulla, HKα_1 protein, unlike HKα_2, is not upregulated during chronic hypokalemia.[2,13] Nevertheless, others have shown by in situ hy-

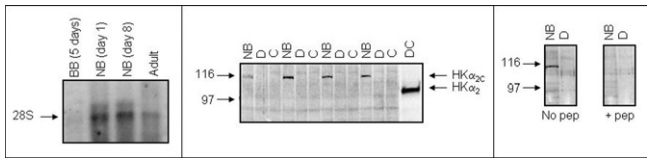

Figure 4 HKα_2 mRNA and HKα_{2C} protein are upregulated in postnatal growth and development in rats. Left panel: Northern analysis using a specific probe for HKα_2 was performed as described previously by our laboratory.[2] The results show upregulation of HKα_2 mRNA in newborn rats compared with adults. Middle panel: Immunoblot analysis using an antibody specific for HKα_2 suggested upregulation of HKα_{2C} protein in postnatal growth and development in rats. Right panel: The immunizing peptide (1 hour, 250 μmol/L) blocked detection of HKα_{2C} by the anti-HKα_2 antibody. BB, before birth; NB, newborn; D, dam; C, control; DC, distal colon; Pep, immunizing peptide. Markers for the mRNA and proteins gels are indicated on the left of each panel.

bridization that HKα_1 mRNA abundance in the renal cortex increases during chronic hypokalemia. Ahn et al[41,42] showed that chronic potassium restriction results in modestly enhanced renal cortical expression of HKα_1 and suggested that this isoform may participate in potassium conservation by the connecting tubule (CNT) and cortical collecting duct during potassium deprivation. This contrasts with HKα_2 mRNA, which is preferentially upregulated in the renal medulla, as is HKα_2 protein.[2] In addition, in isolated inner medullary collecting tubules, Wall et al[43] showed that J_{tCO2} increased in tubules harvested from chronically hypokalemic rats. A component of bicarbonate absorption was inhibited by low concentrations of Sch-28080, suggesting that HKα_1 or a HKα_1-type ATPase was upregulated during these experimental conditions. The residual component of bicarbonate absorption (~20%) was inhibited by high concentrations of ouabain, suggesting that the ouabain-sensitive fraction of J_{tCO2} could be attributable to HKα_2.[44,45]

Enzymatic Activities of HKα_1- and HKα_2-Deficient Mouse Models

For a number of years Doucet and Barlet,[46] Buffin-Meyer et al,[47] and Cheval et al[48] have studied the properties of different K^+-ATPase activities in isolated nephron segments. This group defined 3 distinct types of K^+-ATPase activities according to pharmacologic response and augmentation in response to chronic hypokalemia: types I, II, and III. According to the nomenclature adopted by these investigators, K^+-ATPase type I is sensitive to Sch-28080 and insensitive to ouabain, and is present in collecting ducts predominately; K^+-ATPase type II is ouabain sensitive and is localized to proximal tubules and thick ascending limbs; K^+-ATPase type III is observed only during chronic hypokalemia in the collecting duct and is sensitive to ouabain and Sch-28080. To determine whether the molecular entity responsible for rat K^+-ATPase type III is HKα_1, HKα_2, or an unknown new α-subunit, Dherbecourt et al[49] evaluated K^+-ATPase activity in the absence or presence of ouabain or Sch-28080 from tubules harvested from wild-type mice or HKα_1-[50] or HKα_2[23]-deficient mice.

Collecting ducts isolated from wild-type mice (expressing HKα_1 and HKα_2 protein) with a normal plasma potassium level displayed K^+-ATPase activity that was inhibited by Sch-28080 but not by ouabain. However, the K^+-ATPase activity in collecting ducts harvested from mice maintained on a low-potassium diet was inhibited by both ouabain and Sch-28080.

In the second set of experiments, HKα_1–deficient mice (assumed to express HKα_2 protein) were maintained on a diet containing potassium and displayed no K^+-ATPase activity in collecting ducts. However, in chronically hypokalemic HKα_1–deficient mice, K^+-ATPase activity was inhibited by Sch-28080 and by ouabain. Therefore, although HKα_1 protein is not expressed, a Sch-28080–sensitive component was observed. This observation is in agreement with the findings of these investigators that HKα_2 is ouabain and Sch-28080 sensitive.

Finally, these investigators performed similar experiments using HKα_2–deficient mice (assumed to express HKα_1 protein). When consuming a diet containing potassium, K^+-ATPase activity was inhibited in HK$\alpha_{2-/-}$ animals by Sch-28080 but not by ouabain. This observation is consistent with expression of HKα_1 protein in HKα_2-deficient mice. However, during chronic hypokalemia no H^+,K^+-ATPase activity was observed, compatible with findings in our laboratory that HKα_1 does not show a regulatory response to chronic hypokalemia.

Taken together, the experiments performed with wild-type, HK$\alpha_{1-/-}$, and HK$\alpha_{2-/-}$ mice support the view that chronic hypokalemia upregulates expression of HKα_2 protein that in some conditions is sensitive to both ouabain and Sch-28080 (type III K^+-ATPase).

The findings of Doucet et al[23,46,50] also are compatible with previous studies from our laboratory[43] and from others[51] in the field who observed an increase in J_{tCO2} in the collecting duct during chronic potassium depletion. Furthermore, the increment in J_{tCO2} was sensitive to both Sch-28080 and ouabain.[43]

The observation that Sch-28080-sensitive K^+-ATPase was not present in HK$\alpha_{2-/-}$ mice during chronic hypokalemia suggests that chronic potassium depletion may downregulate expression of HKα_1 in HK$\alpha_{2-/-}$ mice. Quantification of HKα_1 mRNA and/or protein in potassium-deficient HK$\alpha_{2-/-}$ mice has not been reported.

Summary

HKα_2 plays a critical role in potassium and acid-base homeostasis through regulated H^+/K^+ exchange in intercalated cells of the mammalian collecting duct. HKα_2 assembles specifically with NKβ_1, and is inhibited by high concentrations of ouabain. Although insensitive to Sch-28080 in vitro, there is evidence that HKα_2 may be sensitive to Sch-28080 in vivo. These features are unique among members of the X^+,K^+-ATPase family. Furthermore, the molecular regulation of HKα_2 appears to depend on assembly with the tetraspanin CD63. In addition to participation in potassium and acid-base homeostasis, HKα_2 participates uniquely in growth and development in the early postnatal period. Although specific examples of genetic forms of distal renal tubular acidosis have not established an abnormality in H^+,K^+-ATPase expression or function, isolated case reports have suggested that an H^+,K^+-ATPase might be abnormal in certain forms of this disease. In addition, it appears likely that the colonic H^+,K^+-ATPase in the collecting duct may play a pivotal role in the maintenance of metabolic alkalosis when accompanied by chronic hypokalemia.

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