A tyrosine-based signal regulates H-K-ATPase-mediated potassium reabsorption in the kidney

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Wang, Tong, Nathalie Courtois-Coutry, Gerhard Giebisch, and Michael J. Caplan. A tyrosine-based signal regulates H-K-ATPase-mediated potassium reabsorption in the kidney. Am. J. Physiol. 275 (Renal Physiol. 44): F818–F826, 1998.—Isoforms of the H-K-ATPase participate in active K resorption in the renal collecting tubule. The cytoplasmic tail of the β-subunit of the gastric H-K-ATPase includes a 4 amino acid motif which is highly homologous to tyrosine-based endocytosis signals. We have generated transgenic mice expressing an H-K-ATPase β-subunit in which the tyrosine residue in this sequence has been mutated to alanine. Mice expressing the mutated protein manifest constitutive hypersecretion of gastric acid, demonstrating that the β-subunit tyrosine-based motif is required for the regulated endocytosis of the H-K pump and hence the cessation of gastric acid output. To test the possibility that the tyrosine-based sequence in the tail of the H-K-ATPase β-subunit plays a role in regulating the function of renal H-K-ATPases, we examined renal K clearance in normal and in transgenic mice. Blood pressure, urine volume, glomerular filtration rate (GFR), plasma Na, and Na excretion are similar in control and transgenic mice. However, plasma K concentrations are significantly higher in transgenic mice (4.76 ± 0.04 meq/l in control; n = 9, P < 0.05) and K excretion is lower in the transgenic animals (fractional excretion of K was 26.2 ± 3.62% in transgenic and 50.1 ± 4.78% in control; n = 9, P < 0.01). These data suggest that the tyrosine-based signal in the cytoplasmic tail of the H-K-ATPase β-subunit functions in the kidney as it does in the stomach to internalize H-K pump and thus inactivate pump function. Its elimination may result in the constitutive presence of the pump at the cell surface and lead to excessive urinary K reabsorption.

The P-type ATPases comprise a large and growing collection of ion pumps that share extensive structural and mechanistic homology (38). An important subset of this group, which includes the Na-K-ATPase and the H-K-ATPases, drive the import of potassium ions in exchange for the export of sodium ions or protons, respectively. All of these enzymes are heterodimers, composed of polytopic α-subunits predicted to span the membrane 10 times and heavily glycosylated β-subunits that cross the bilayer once in a type II orientation. The structural determinants involved in catalysis, including binding sites for ATP, transported cations, and specific inhibitors, all appear to map to the α-subunit polypeptides. Assembly of α- and β-subunits to form holoenzymes is required for the functional maturation of these pumps and appears to modulate their biochemical and cell biological properties (7, 19).

At least two H-K-ATPase isoforms are produced in the kidney. The best studied of these is the gastric H-K pump, which also mediates acid secretion in the stomach (25). In addition, an H-K-ATPase originally identified in the colon and cloned from a rat colonic cDNA library is also expressed in renal tubule epithelial cells (1, 4, 13, 17, 26, 31). An activity similar to that of the gastric H-K pump α-subunit (HKo1) is present in cells of the collecting tubule, whereas a pump with the pharmacological profile of the colonic H-K-ATPase α-subunit (HKα2) is detected in cells of the proximal tubule and Henle’s loop (4, 30). The protein sequences of both the α-subunits of HKo1 and HKα2 are ~65% identical to that of the corresponding Na-K-ATPase α-polypeptide and ~65% identical to each other (13, 30). It has been clearly demonstrated that the gastric H-K-ATPase β-subunit is expressed in renal epithelial cells (5, 6). Furthermore, both of these H-K-ATPase α-polypeptides can assemble productively with the gastric H-K-ATPase β-subunit when these proteins are coproduced in heterologous expression systems (11, 22, 23). No additional “nongastric” H-K-ATPase β-subunits have yet been isolated from mammalian cDNA libraries. It is likely, therefore, that in mammalian renal epithelial cells, it is the gastric β-subunit isoform which assembles with the “nongastric” pumps.

In the parietal cells of the stomach, the gastric H-K-ATPase is concentrated in the membranes of the intracellular tubulovesicular element compartment (TVE) (25). Secretagogue stimulation initiates the fusion of the TVEs with the apical plasmalemma, thus permitting their cargo of H-K pumps to secrete acid directly into the gastric luminal space. Inactivation of

The kidney employs numerous transport mechanisms in the maintenance of systemic potassium balance. The nature and direction of these transport processes varies over the length of the nephron, with individual segments initiating reabsorptive or secretory transepithelial potassium fluxes. Segment-specific potassium transport properties are determined both by the inventory of transport proteins expressed in particular epithelial cell types and by the subcellular destinations at which each of these proteins accumulates. Recently, progress has been made in characterizing the molecular and functional attributes of the diverse array of polypeptides involved in renal potassium transport. Through this effort, it has become apparent that several H-K-ATPases belonging to the P-type family of ion-transporting ATPases participate in potassium reabsorption from distinct localizations along the nephron (14, 30, 43). Little is known, however, of the mechanisms through which the activities of these ion pumps are regulated.
METHODS

Acid secretion is associated with the reinternalization of the H-K-ATPase and the regeneration of the TVEs. We have identified a tyrosine-based sequence motif in the cytoplasmic tail of the gastric H-K-ATPase β subunit that plays a critical role in this regulated endocytic process (12, 21). This motif is highly homologous to tyrosine-based coated pit localization signals, which will not function if their tyrosine residues are removed or replaced (3, 10, 20). We prepared a mutated form of the gastric H-K-ATPase β-subunit in which the tyrosine residue is replaced by an alanine (H-Y20A). The modified protein was then expressed in transgenic mice under the control of the cytomegalovirus (CMV) promoter. Analysis of gastric acid production revealed that these mice manifest a dominant hyper-acid-secreting phenotype, as would be expected if retrieval of the H-K pump from the plasma membrane is impeded (12).

Immuno localization studies further demonstrated that the H-K pump subunit polypeptides are retained at the parietal cell apical surfaces even in the absence of secretagogue stimulation. It would appear, therefore, that the tyrosine-based signal in the cytoplasmic tail of the gastric H-K-ATPase β-subunit is required to target the pump to a regulated storage compartment and to thus terminate acid secretion.

The CMV promoter drives expression of exogenous proteins in numerous cell types, including those of the renal tubule (39). We have previously shown that the H-Y20A protein is similarly capable of productively assembling to form holoenzyme (12). If the mechanisms that govern the downregulation of H-K-ATPase function in the kidney operate as they do in the stomach, then they are dependent upon the putative tyrosine-based endocytosis sequence in the cytoplasmic tail of the H-K-ATPase β-subunit. Were this the case, then expression of H-Y20A should result in the formation of pump complexes that are not substrates for internalization-inactivated activation. The resultant constitutive activation of H-K-ATPase activity might be expected to alter renal excretion of K and H and hence perturb systemic K or acid-base balance. We find that mice expressing the mutated protein tend to be hyperkalemic and to exhibit markedly reduced fractional and absolute K clearance. It would appear, therefore, that some or all of the H-K pumps expressed in the kidney are governed by cycles of regulated exo- and endocytosis.

Animal preparation and surgical procedures. Male control (C57BL/6) and H-Y20A transgenic mice weighing 39.9 ± 1.41 g were maintained on a regular laboratory diet and tap water until the day of the experiment. Ages of both control and H-Y20A transgenic animals were matched with nontransgenic littermates. The mice were anesthetized by intraperitoneal injection of 100 mg/kg body wt of Inactin [5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid; BYK-Gulden, Constanze, Germany] and placed on a thermostatically controlled surgical table to maintain body temperature at 37°C. After tracheotomy, the left jugular vein was exposed and cannulated with a PE-10 catheter for intravenous infusion. A carotid artery was also catheterized with PE-10 tubing for arterial blood collection and mean arterial pressure (MAP) measurement. The bladder was exposed and catheterized via a suprapubic incision with a 10-cm piece of PE-10 tubing for timed urine collections.

Renal clearance. Renal clearance techniques in mice were carried out as described by others (41) and modified from methods used in our laboratory (42). Experiments were performed simultaneously in one control and one H-Y20A transgenic mouse. Upon completion of surgery, 0.05 ml of isotonic saline was given intravenously to replace surgical fluid loss. Subsequently, a priming dose of 10µCi of [methoxy-3H]inulin (New England Nuclear, Boston, MA) was administered in 0.3-ml isotonic saline and a maintenance infusion of 0.9% NaCl and 4 mM of KCl, containing 10 µCi/ml of inulin, followed at a rate of 0.41 ml/h (~1/10 of the infusion rate previously used in rats). The KCl was added to the infusion solution to avoid dilution of the plasma K concentration.

Membrane preparation and gastric H-K-ATPase β-subunit abundance. Tissues were removed from either normal or H-Y20A mice and rinsed in cold PBS and homogenized. Microsomal membranes were obtained by centrifuging the homogenates as previously described (12). Membrane proteins were separated on a 8.5% polyacrylamide gel using SDS-PAGE. The proteins were transferred to polyvinylidene difluoride paper. The blot was blocked with BLOTTO (5% nonfat milk, 0.1% Tween 20, and phosphate-buffered saline, pH 7.4) for 2 h, then incubated with H-K-ATPase β-subunit primary antibody at 1:500 dilution followed by secondary antibody conjugated to horseradish peroxidase at 1:1,000 dilution (12, 21). Labeled proteins were visualized by the enhanced chemiluminescence detection method. The expression of the H-K-ATPase β-subunit protein in kidney was normalized to that of the Na-K-ATPase α-subunit. Preparation and characterization of the Na-K-ATPase α-subunit monoclonal antibody has been previously described (21, 22).

Statistics. Control and experimental groups were performed under identical experimental conditions. Data are presented as means ± SE. Student's t-test was used to compare control and experimental groups. The one-way ANOVA test was used for comparison of several experimental groups with a control group. The difference between the mean values of an experimental group and a control group are considered significant if P < 0.05.

Materials. [methoxy-3H]inulin was obtained from New England Nuclear Research Products.
brane fractions derived from the nongastric tissues of H-K-ATPase transgenic animals. As can be seen in Fig. 1, the gastric H-Y20A protein is expressed in the kidneys of the and D. Chow) was performed to determine whether the synthetic antibody directed against the H-K-ATPase b-subunit is not detected in crude membrane fractions prepared from the stomachs of control and H-Y20A animals, as revealed by the broad smear extending from 60–80 kDa. A band of ~60 kDa is detected in nongastric tissues only in those mice transgenic for H-Y20A.

Fig. 1. Expression of the H-Y20A protein in tissues of transgenic animals. Crude membrane fractions were prepared from the stomachs, colons, brains, kidneys, and livers of control and H-Y20A transgenic mice. Expression of the H-Y20A protein was detected by Western blotting using a monoclonal antibody directed against the gastric H-K-ATPase b-subunit. Gastric H-K-ATPase b-subunit is endogenously expressed in stomachs of control and H-Y20A animals, as indicated by the broad smear extending from ~60–80 kDa. A band of ~60 kDa is detected in nongastric tissues only in those mice transgenic for H-Y20A.

due at position 20 in the cytoplasmic tail of the H-K-ATPase b-subunit polypeptide is referred to as H-Y20A. The cDNA encoding H-Y20A was expressed in transgenic mice under the control of the CMV promoter. As previously described, three lines of transgenic mice were generated that transmit the exogenous H-Y20A transgenes. These data indicate that the CMV promoter drives strong renal expression of the exogenous H-Y20A mutated H-K-ATPase b-subunit and renal excretion of K, suggesting that the elevated plasma K noted in the transgenic animals is attributable to reduced renal K excretion.

Since H-K-ATPases catalyze both K absorption and H secretion, we also examined the urine pH and blood acid-base status in normal and H-Y20A mice. As indicated in Table 3, urine pH in H-Y20A mice was not significantly different from that in control mice. We also detected no significant differences in blood pH, Pco₂ and HCO₃ concentrations between normal and H-Y20A mice.

To investigate the relationship between the level of expression of the exogenous H-Y20A mutated H-K-ATPase b-subunit and renal excretion of K, we performed Western blots using the H-K-ATPase b-subunit antibody on kidney membranes derived from H-Y20A animals in which the clearance measurements were done. As can be seen in Fig. 4A, a broad band of molecular mass ~50–60 kDa corresponding to the heavily glycosylated H-K-ATPase b-subunit protein is present in each of the lanes derived from H-Y20A mice.
Expression levels were quantitated by subjecting the blots to scanning densitometry. These data are plotted in Fig. 4B, which depicts the amount of the H-K-ATPase β-subunit polypeptide present in the kidneys of one control and 5 H-Y20A mice. Densitometry reveals that H-Y20A mouse 4 expressed the highest level of HKβ, whereas mouse 2 expressed the second highest amount, and mouse 1 (control mouse) expressed no detectable HKβ protein. We have also reprobed the blot shown in Fig. 4A with an antibody directed against the α-subunit of the Na-K-ATPase to ensure that renal epithelial membrane proteins were evenly loaded in each lane. We find no differences in the levels of detectable sodium pump α-subunit (not shown), demonstrating that the differences in H-K-ATPase β-subunit signal plotted in Fig. 4B directly reflect this protein’s actual abundance in the kidney.

The level of renal H-K-ATPase β-subunit expression was then correlated with the plasma K concentrations and renal K excretion values obtained for the individual H-Y20A mice. Figure 5 illustrates the plasma K

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<th>Table 2. Plasma Na and K and urine excretion of Na and K in the normal and H-Y20A transgenic mice</th>
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Values are means ± SE from 9 animals of each group (normal control or H-Y20A transgenic mouse). PNa, plasma Na; PK, plasma K; ENa, absolute excretion of Na; EK, absolute excretion of K; FENa, fractional excretion of Na; FEK, fractional excretion of K. *Significant difference from normal control mouse (P < 0.05). †Significant difference from normal control mouse (P < 0.01).

Fig. 2. Plasma K (PK, A) and Na (PNa, B) concentrations in control and H-Y20A transgenic mice. Data are means ± SE (n = 9). Plasma Na and K concentrations were measured at the end of each 30-min experimental period. *H-Y20A cation concentrations that are significantly different from the corresponding control values (P < 0.05).

Fig. 3. K excretion in normal and H-Y20A transgenic mice. Absolute (EK, A) and fractional (FEK, B) excretion of K are presented for both control and H-Y20A mice (n = 9). *H-Y20A K excretion measurements that are significantly different from the corresponding control values (P < 0.05).
and K excretion values obtained from the animals analyzed in Fig. 4. Comparison of Fig. 5 and Fig. 4B suggests that a close relationship exists between H-Y20A expression and renal K retention. Mouse 4 manifests the highest plasma K and the lowest EK and FEK, whereas similar but somewhat less extreme behavior is detected in mouse 2. The control animal (mouse 1) had normal plasma K and K excretion rates. The inverse relationship which exists between expression of the H-Y20A transgene and renal K excretion strongly suggests that the physiological phenotype observed in the H-Y20A mice can be ascribed to the presence of the altered β-subunit protein.

**DISCUSSION**

The regulation of renal potassium excretion is accomplished through complex interactions between several pathways for secretion and absorption along the nephron. A growing body of evidence indicates that potassium absorption in the initial and cortical collecting tubule is mediated by several members of the H-K-ATPase family of ion pumps (14, 30, 43). Control of H-K-ATPase function in the stomach is accomplished through cycles of regulated exo- and endocytosis, in which intracellular pools of gastric H-K pump are delivered to apical cell surface in response to secretory

### Table 3. Acid-base status in normal and H-Y20A transgenic mice

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<th>Blood pH</th>
<th>PCO2, mmHg</th>
<th>HCO3, mM</th>
<th>Urine pH</th>
<th>Hct, %</th>
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<tr>
<td>Control</td>
<td>7.34 ± 0.03</td>
<td>44.44 ± 2.47</td>
<td>24.86 ± 1.39</td>
<td>5.91 ± 0.06</td>
<td>46.08 ± 0.43</td>
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<tr>
<td>H-Y20A</td>
<td>7.36 ± 0.02</td>
<td>40.65 ± 2.71</td>
<td>22.76 ± 0.96</td>
<td>5.87 ± 0.10</td>
<td>45.62 ± 0.55</td>
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Values are means ± SE from 9 animals of each group. Hct, hematocrit.

![Image of Western blot analysis](image-url)  
**Fig. 4.** H-Y20A protein abundance in individual transgenic mice. A: Western blot analysis employing an antibody directed against the gastric H-K-ATPase β-subunit was performed on membranes derived from kidneys of control (lane 1) and transgenic animals (lanes 2–6). A ~60-kDa band is detected only in membranes derived from the H-Y20A transgenic mice. B: densitometry was performed to quantify the level of H-Y20A protein expression detected in blot presented in A.

![Image of bar charts](image-url)  
**Fig. 5.** Comparison of plasma K concentrations and K excretion measurements in 6 individual mice. Plasma K (A), absolute K excretion (B), and fractional K excretion (C) measurements were obtained from the same 6 animals whose kidneys were subsequently examined in the Western blot analysis depicted in Fig. 5. Data are presented in the same order as in Fig. 5 (mouse 1 is a control, mice 2–6 are H-Y20A transgenics). All values are means ± SE from 4 measurements.
gogue stimulation and are subsequently reinternalized to discontinue acid secretion (25). This endocytosis step appears to be directed by a tyrosine-based signal present in the cytoplasmic tail of the H-K-ATPase β-subunit. Disruption of this signal results in the constitutive presence of the H-K pump at the plasma membrane and continuous hypersecretion of gastric acid (12). We have now provided evidence that a similar mechanism plays a role in governing the function of H-K-ATPase in the kidney.

Expression of a mutated form of the gastric H-K-ATPase β-subunit, in which the critical tyrosine residue of the putative internalization sequence has been substituted with an alanine (H-Y20A) in transgenic mice, produces a phenotype consistent with the decreased secretion or hyper-reabsorption of potassium from the renal tubule fluid. Serum potassium is significantly elevated in the H-Y20A mice, whereas absolute and fractional renal potassium excretion are markedly reduced. It is highly unlikely that reduced potassium excretion resulted from decreased potassium secretion by principal cells, since the observed elevation of serum potassium would be expected to increase, not decrease, urinary excretion of potassium. Thus these observations suggest that the presence of an H-K-ATPase β-subunit that had been deprived of its capacity to participate in regulated endocytosis results in significant perturbations of the mechanisms which normally govern renal potassium transport. It is logical to conclude, therefore, that under normal circumstances endocytosis, driven by the H-K-ATPase β-subunit's tyrosine-based signal, is a pivotal step in the regulation of renal H-K pump function.

The CMV promoter used in the present studies to drive H-Y20A expression has been shown to be effective in a broad array of cell types (39). In the kidney, H-Y20A protein is produced by the glomerulus as well as by epithelial cells along the entire length of the renal tubule (data not shown). It must be noted, however, that expression of the β-subunit of the H-K-ATPase alone produces no enzymatic or ion pumping activity (7, 19). It is only in combination with a catalytic α-subunit that the β-subunit can participate in cation transport. Although the Na-K-ATPase α-subunit is present at high levels throughout the nephron, we have previously demonstrated that the gastric H-K-ATPase β-subunit does not complex efficiently with the sodium pump α-polypeptide when the two are coproduced in mammalian cells (22). The influence of the H-Y20A β-subunit on renal potassium excretion is likely, therefore, to reflect the assembly of H-Y20A with H-K-ATPase α-subunits endogenously expressed in one or more renal epithelial cell types. We hypothesize that these same cells endogenously express H-K-ATPase β-subunits that normally associate with these α-subunits and whose endocytosis signals normally regulate holoenzyme function. Expression of these endogenous β-subunits probably persists in the H-Y20A transgenic animals. Furthermore, we would expect those α-subunits that assemble with the endogenous wild-type β-subunits to be properly regulated by endocytosis. In contrast, the pool of α-subunits that interact with the exogenous H-Y20A polypeptides will not be accessible to the cellular internalization machinery and thus not be subject to inactivation. Consequently, the H-Y20A amino acid substitution behaves as a genetically dominant mutation, producing a phenotype despite the persistent expression of the unaltered protein. It is worth noting that the magnitude of this phenotype appears to correlate with the level of H-Y20A protein expression, consistent with the concept that the endogenous wild-type and H-Y20A β-subunits compete with one another for assembly with the population of endogenous H-K-ATPase α-subunits.

The molecular identity of the H-K-ATPase β-subunit isoform (or isoforms) that normally mediates the endocytic regulation of H-K-ATPase function in the kidney remains to be established. It has been well documented, however, that the gastric H-K-ATPase β-subunit protein is expressed in the mouse and rat nephron (5, 6). A cDNA encoding a second H-K-ATPase β-subunit protein has been cloned from the toad bladder, but it has yet to be demonstrated that this protein, or any other molecular relative, is present in the mammalian kidney (28). In vitro expression studies reveal that all of the H-K-ATPase α-subunit isoforms identified to date can assemble productively with the gastric H-K-ATPase β-subunit polypeptide (9, 11, 22, 23). It is possible, therefore, that the gastric β-subunit is the only H-K-ATPase β-subunit expressed in the mammalian kidney and that it is a constituent of every H-K-ATPase holoenzyme complex which functions in the renal tubule. Were this the case, it would suggest that all of the different H-K-ATPase α-subunit isoforms identified in the nephron are subject to endocytic regulation. It is also possible, however, that mammalian nongastric α-subunits interact with one or more as yet to be identified β-polypeptide. If such novel β-subunits are identified in the mammalian kidney, then it will be interesting to determine whether tyrosine-based internalization signals are included within the amino acid sequences of their cytoplasmic tails. In this context, it is worth noting that the cytoplasmic tail of the toad bladder H-K-ATPase β-subunit does not include a sequence that closely resembles an endocytosis motif (28).

The identities of the α-subunit isoform or isoforms involved in endocytic regulation and the cell type in which it functions also remain to be established. Measurements of ATP hydrolysis performed on microdissected tubule segments suggest that K-ATPase activities distinct from that of the Na-K-ATPase are present at several sites along the nephron (4, 8, 14–16, 18, 29, 30, 43, 44). An enzyme catalyzing ouabain- and Sch-28080-inhibitable ATP hydrolysis is present in both the proximal convoluted tubule and the thick ascending limb. In contrast, the K-ATPase of the cortical and outer medullary collecting tubules is insensitive to ouabain and highly sensitive to Sch-28080, consistent with the pharmacological profile of the gastric H-K-ATPase. It is possible, therefore, that this particular activity is mediated by a population of gastric H-K-ATPase whose expression in the kidneys of at least
some mammalian species has been detected at both the protein and nucleic acid levels. Evidence has also been presented for expression in the collecting duct of yet another functional isotype of K-ATPase, inhibitable by both ouabain and by Sch-28080 with higher affinities than the proximal tubule activity. In contrast to the other two isoforms, this third ATPase appears also to be stimulated by Na (4). Each of the enzymatically identified K-ATPase activities in the kidney are affected differently by mineralocorticoid hormones and adaptation to physiological stresses such as K deprivation (2, 4, 16, 44). Potassium depletion appears not to alter or to reduce expression levels of the collecting duct’s highly Sch-28080-sensitive “gastric-like” ATPase. In contrast, this treatment reduces expression of the proximal and thick ascending limb enzymes while simultaneously boosting levels of the ouabain- and Sch-28080-inhibitable collecting duct activity. Whether a simple concordance can be established between these biochemically and pharmacologically defined enzymes and the cloned α-subunit sequences discussed above remains to be clarified. Future experiments employing pharmacological or molecular biological techniques to discriminate among these activities will be required to determine which of them are constitutively activated in the H-Y20A mouse.

In light of the significant effects that the H-Y20A mutation exerts on potassium homeostasis, it is perhaps surprising that similar perturbations of acid-base metabolism are not observed. Since all of the H-K-ATPase isoforms examined to date are capable of mediating proton secretion (11, 23, 27, 34), one might expect the observed hyperkalemia in the transgenic mice to be accompanied by a metabolic alkalosis caused by inappropriate urinary acidification. The fact that both urine and plasma pH are unaltered by H-Y20A argues that other renal mechanisms compensate for any constitutive acid secretion that the H-Y20A mutation might induce. The magnitude of the contribution of H-K-ATPase activity to renal acid secretion in animals on a normal K diet has not been established but is probably modest. It is likely that the activity of the renal H-K-ATPases in acid-base balance is subordinate to that associated with the V-type H-ATPase expressed by intercalated cells (33). Two forms of intercalated cells can be identified in the mammalian kidney. Whereas a-type intercalated cells mediate acid secretion through apically disposed proton pumps, β-type intercalated cells employ basolateral proton pumps to drive bicarbonate secretion. Perturbations of acid-base balance induce changes in the relative populations of a- and β-type cells, indicating that the kidney can adapt to acid or base loads by specifically enhancing the functional capacities of acid- or base-extruding cells (40). It is possible, therefore, that H-Y20A mice adapt to constitutive H-K pump acid secretion by selectively increasing their census of β-type intercalated cells at the expense of the a variety. Future studies will be required to address this point.

It is also worth noting that at least one of the nongastric H-K-ATPase isoforms may catalyze very little proton secretion under normal circumstances. The protein encoded by the human ATPAL1 gene belongs to such a family of nongastric H-K-ATPases (24, 35). When expressed in HEK-293 cells in association with the gastric H-K-ATPase β-subunit, this pump mediates both potassium and proton transport (23). The magnitude of the proton efflux, however, is less than one-tenth that of the corresponding potassium influx. Recent experiments suggest that the ATPAL1 pump can also drive active sodium extrusion. It is possible, therefore, that some or all of the nongastric H-K-ATPase isoforms expressed in the kidney function primarily as sodium pumps. Were this the case, then their constitutive activation would not be expected to exert substantial effects on acid secretion. Studies are underway to further characterize nongastric H-K-ATPase function in vitro.

The fact that H-Y20A mice are able to maintain elevated but stable levels of serum potassium in the face of significant reductions in renal potassium excretion raises questions as to what mechanisms may operate to achieve potassium balance in these animals. It is unlikely that H-Y20A mice reduce their food intake and thereby limit the load of dietary potassium that needs to be excreted, since their average weights did not decline. Instead, it is possible that colonic absorption of potassium may be selectively reduced or secretion increased in H-Y20A mice as a consequence of the elevation of plasma potassium. It should be noted in this context that colonic expression of H-Y20A in the transgenic mice is much lower than that detected in the kidney (see Fig. 1). Thus any potential stimulatory influence of the H-Y20A mutation on the function of colonic H-K-ATPase may be minimal. Finally, the possibility must be considered that the present experimental setting did not represent a true steady state. This alternative seems probable because, although fairly constant renal function was maintained over a 2-h period, the animals were anesthetized, received 150 mM NaCl and 4 mM KCl intravenously, and were subject to several blood collections during the procedure. Each of these perturbations might accentuate potassium retention during the experimental period. Future potassium balance studies will be required to address these issues.

The activities of several renal transport systems appear to be governed through cycles of regulated membrane insertion and internalization (37). In the absence of antidiuretic hormone (ADH), the aquaporin-2 water channel resides in an intracellular membranous compartment of the collecting duct principal cells (32). Binding of ADH to its receptor elevates intracellular cAMP, which simultaneously triggers the fusion of the water channel vesicles with the apical plasma membrane and initiates their rapid endocytosis. Similarly, the binding of parathyroid hormone to its receptors on proximal tubule cells activates the uptake of Na-P, cotransporters, thus downregulating the phosphate reabsorptive capacity of the proximal tubule and increasing renal phosphate excretion (36). The data presented here strongly suggest that renal H-K-
ATPase activity is similarly modulated through exocytosis and endocytic membrane fusion events that modify the size of the pump population expressed at the cell surface. It remains to be determined, however, what physiological signals activate H-K-ATPase insertion and internalization in the kidney and what cellular mechanisms participate in these events. The identification of a tyrosine-based sequence motif that plays a critical role in this process provides a valuable tool which can now be used to identify the machinery that epithelial cells employ to effect this regulation.

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