Renal expression of the ammonia transporters, Rhbg and Rhcg, in response to chronic metabolic acidosis

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Renal expression of the ammonia transporters, Rhbg and Rhcg, in response to chronic metabolic acidosis. Am J Physiol Renal Physiol 290: F397–F408, 2006. First published September 6, 2005; doi:10.1152/ajprenal.00162.2005.—Chronic metabolic acidosis induces dramatic increases in net acid excretion that are predominantly due to increases in urinary ammonia excretion. The current study examines whether this increase is associated with changes in the expression of the renal ammonia transporter family members, Rh B glycoprotein (Rhbg) and Rh C glycoprotein (Rhcg). Chronic metabolic acidosis was induced in Sprague-Dawley rats by HCl ingestion for 1 wk; control animals were pair-fed. After 1 wk, metabolic acidosis had developed, and urinary ammonia excretion increased significantly. Rhcg protein expression was increased in both the outer medulla and the base of the inner medulla. Intercalated cells in the outer medullary collecting duct (OMCD) and in the inner medullary collecting duct (IMCD) in acid-loaded animals protruded into the tubule lumen and had a sharp, discrete band of apical Rhcg immunoreactivity, compared with a flatter cell profile and a broad band of apical immunolabel in control kidneys. In addition, basolateral Rhcg immunoreactivity was observed in both control and acidic kidneys. Cortical Rhcg protein expression and immunoreactivity were not detectably altered. Rhcg mRNA expression was not significantly altered in the cortex, outer medulla, or inner medulla by chronic metabolic acidosis. Rhbg protein and mRNA expression were unchanged in the cortex, outer and inner medulla, and no changes in Rhbg immunolabel were evident in these regions. We conclude that chronic metabolic acidosis increases Rhcg protein expression in intercalated cells in the OMCD and in the IMCD, where it is likely to mediate an important role in the increased urinary ammonia excretion.

Connecting Segment; Collecting Duct

Ammonia is the principal component of net acid excretion, a critical function of the kidneys, both under basal conditions and in response to metabolic acidosis (13, 20).1 The proximal tubules produce ammonia as ammonium, NH4+, in a process that results in equimolar bicarbonate formation (27, 40). Proximal tubule segments secrete ammonium into the luminal fluid; some (38, 39), but not all (50), studies suggest this occurs via the apical Na+/H+ exchanger, NHE-3. The thick ascending limb of the loop of Henle reabsorbs luminal ammonia, predominantly by transport of NH4+ by the apical Na+–K+–2Cl– cotransporter, NKCC2/BSC1 (13, 20, 27). This coordinated process results in renal interstitial ammonia accumulation (13, 20). Finally, the collecting duct secretes ammonia from the renal interstitium into the luminal fluid. Quantitatively, 70–80% of urinary ammonia is secreted by the collecting duct, emphasizing the critical importance of collecting duct ammonia transport (20, 48).

Increasing evidence suggests that a newly recognized ammonia transporter family, the Rh glycoprotein family, may play a critical role in collecting duct renal ammonia transport. Rh A glycoprotein (Rhag) is expressed by erythrocytes and erythroid-precursor cells (32), complements yeast deficient in endogenous ammonia transporters (37), and, as shown in seminal studies, mediates NH4+/H+ exchange (59, 60). Rh B glycoprotein (Rhbg) and Rh C glycoprotein (Rhcg) are nonerythroid Rh glycoproteins (31, 33), are homologous to Rhag and to bacterial and yeast ammonia transporters (31, 33), and, at least for Rhbg, have been shown to also complement yeast deficient in endogenous ammonia transporters (37). Heterologous expression studies demonstrate that both Rhbg and Rhcg transport ammonia (2, 34, 36, 41, 42, 44). Moreover, Rhbg and Rhcg are expressed in the distal convoluted tubule (DCT), connecting segment (CNT), initial collecting tubule (ICT), cortical collecting duct (CCD), outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD) (14, 31, 45, 55), sites where 70–80% of urinary ammonia is secreted (6, 7, 20, 48, 49). Immunohistochemical studies demonstrate basolateral Rhbg immunoreactivity in these sites (45, 55). Rhcg protein is expressed in the same cellular distribution, but has been reported to exhibit apical immunoreactivity (14, 55). Finally, transport studies using cultured mouse collecting duct cells show that carrier-mediated mechanisms that appear to involve Rhbg and Rhcg are critical components of basolateral and apical plasma membrane ammonia transport, respectively (21, 22). Thus Rhbg and Rhcg appear to be likely candidates to mediate important roles in renal ammonia metabolism.

Chronic metabolic acidosis is a common clinical condition. Kidneys respond to chronic metabolic acidosis by increasing net acid excretion, predominantly by increasing urinary ammonia excretion (28, 43, 51). Furthermore, the increased renal

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ammonia excretion is associated with substantial increases in collecting duct ammonia secretion (6, 7, 20, 48, 49). The current study examines the hypothesis that changes in collecting duct ammonia secretion involve, at least in part, changes in the expression and/or cellular distribution of the mammalian ammonia transporter family members, Rhbg and Rhcg. We induced chronic metabolic acidosis in normal Sprague-Dawley rats by adding HCl to their diet; control rats were pair-fed to avoid changes due to differences in dietary intake. After 7 days, we examined expression of Rhbg and Rhcg using steady-state protein and mRNA quantification and immunohistochemistry. Our results indicate that metabolic acidosis increases Rhcg expression in the OMCD and in the IMCD.

METHODS

Animals. Normal Sprague-Dawley rats, weighing ~250 g, were obtained from Charles River Laboratories. Metabolic acidosis was induced using methods described previously (25). Briefly, acidosis rat chow was prepared by adding 1 liter of 0.8 M HCl solution to 1 kg rat chow. Control rat diet was made by mixing 1 liter of H2O to 1 kg of rat chow. The weight of food eaten by chronic metabolic acidosis rats was recorded every 24 h, and control rats were provided food on a pair-fed basis. On day 7, animals were placed in metabolic cages and urine was collected under mineral oil. The urine volume was recorded and an aliquot was frozen for later analysis. On the day of experiment, animals were euthanized using pentobarbital sodium. Blood was taken from the aortic root and placed in a heparinized tube for pH and PCO2 measurement. Animals were euthanized using pentobarbital sodium. Blood was taken from the aortic root and placed in a heparinized tube for pH and PCO2 measurement. Blood-gas measurements were performed on freshly obtained, heparinized arterial blood using an AVL Opti 1 blood-gas analyzer. Serum bicarbonate concentration was calculated from the pH and the PCO2 measurement.

Tissue processing for immunohistochemistry. On the day of fixation, animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). The kidneys were preserved by in vivo perfusion fixation through the abdominal aorta. The kidneys were first perfused briefly with PBS (pH 7.4) to rinse away all blood and, subsequently, with 3% paraformaldehyde-0.12% picric acid-PBS for 5 min. The kidneys were removed, cut into 2-mm-thick slices, and postfixed in the same fixative overnight. Samples of kidney from each animal were embedded in polyester wax (polyethylene glycol 400 disperseate, Polysciences, Warrington, PA), and 5-µm-thick sections were cut and mounted on gelatin-coated glass slides.

Rhbg and Rhcg RNA quantification. Total RNA was extracted using RNAlater (Qiagen, Valencia, CA) and stored at −70°C freezer until used. Rhbg and Rhcg mRNA were quantified using real-time RT-PCR using standard techniques reported previously (21, 57). Briefly, we designed rat Rhbg and Rhcg mRNA-specific primers and fluorescent probes using Primer Express software, version 1.5 (Perkin-Elmer Applied Biosystems, Foster City, CA). The forward primer for Rhbg was 5′-CCTGCCGCTGTCGTGTC-3′, the reverse primer was 5′-AGCGGACAAAGATCGCAAAG-3′, and the fluorescent probe was 6FAM-CTTCCTTCAAGCCGACCTCCTCCT-TAMRA. For Rhcg, the forward primer was 5′-TGGGATATACTGGCCTAGTCT-CA-3′, the reverse primer was 5′-GAGGGCTGCTCGGTGGTTG-3′, and the fluorescent probe was 6FAM-CTCAGCGATGTCCCTTCACCGAGA-CATMARA. 18S RNA expression was quantified using commercially available primers and probes (Applied Biosystems, Foster City, CA). RNA was reverse transcribed using the SuperScript First Strand Synthesis System for RT-PCR (Invitrogen) and random hexamers. Real-time RT-PCR was then performed on an ABI Prism GeneAmp 5700 Sequence Detection System (Applied Biosystems). Amplification was performed using a 25 µL of total fluid volume and TaqMan RT-PCR Master Mix Reagents (Applied Biosystems). We used a two-step cycle protocol including an initial 95°C denaturation step for 15 s and then 60°C for 1 min and 40 cycles of these alternating temperatures. Results were analyzed using GeneAmp 5700 SDS software, version 1.3 (PerkinElmer, Applied Biosystems). Expression was quantified using the ΔΔCT technique using 18S RNA as an internal standard.

Immunohistochemistry. Immunolocalization of Rhbg and Rhcg was accomplished using immunoperoxidase procedures and a commercially available kit (Dako, DakoCytomation). The sections were dewaxed in ethanol and rehydrated, rinsed in PBS, treated for 15 min with 5% normal goat serum (Vector Laboratories) in PBS, and then incubated at 4°C overnight with affinity-purified anti-Rhbg and anti-Rhcg antibodies. The sections were then washed in PBS, and endogenous peroxidase activity was blocked by incubating the sections in 0.3% H2O2 for 30 min. The sections were washed in PBS and incubated for 30 min with biotinylated goat anti-rabbit IgG secondary antibody (Vector Laboratories) diluted 1:200 in PBS and again washed with PBS. The sections were treated for 30 min with the avidin-biotin complex reagent, rinsed with PBS, and then exposed to diaminobenzidine. The sections were washed in distilled water, and in some experiments the sections were counterstained with hematoxylin. The sections were then dehydrated with xylene and mounted with Permount (Fisher Scientific, Fair Lawn, NJ). Sections were examined on a Nikon E600 microscope equipped with DIC optics and photographed using a DXM1200F digital camera and ACT-1 software (Nikon USA).

Double-labeling for Rhcg and AE1. Colocalization of Rhcg with AE1 was performed using sequential immunoperoxidase procedures and a commercially available kit (Dako, DakoCytomation). The tissue

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sections were dewaxed, had endogenous peroxidase quenched using DAKO peroxidase solution for 30 min, then were washed, incubated with DAKO serum-free blocking solution, washed, and incubated with anti-AE1 primary antibody diluted 1:500 overnight at 4°C in a humidified chamber. The sections were then washed, incubated with biotinylated anti-mouse and anti-rabbit secondary antibody (DAKO LSAB2 kit) for 30 min, washed, incubated with streptavidin for 30 min, washed, exposed to diaminobenzidine for 5 min, and then washed. To detect Rhcg immunoreactivity, the above procedure was repeated with the substitution of anti-Rhcg primary antibody (1:2,000) and the substitution of Vector SG for dianimobenzidine. The sections were dehydrated with graded increases in ethanol, treated with xylene, and mounted using Permount. Sections were examined on a Nikon E600 microscope equipped with DIC optics and photographed using a DXM1200F digital camera and ACT-1 software (Nikon).

Statistics. Statistical analyses were performed using paired t-test calculations. P < 0.05 was used for statistical significance. In each analysis, n refers to the number of animals in each group.

RESULTS

Physiological data. Serum electrolyte measurements are summarized in Table 1. HCl ingestion resulted in moderate hyperchloremic metabolic acidosis associated with a mild hypernatremia compared with control, pair-fed rats. Importantly, the serum potassium concentration did not change significantly. These results are consistent with chronic metabolic acidosis.

Urinary electrolyte measurements are summarized in Table 2. HCl-induced metabolic acidosis increased urinary ammonia excretion significantly but did not alter urinary urea nitrogen, sodium, or potassium excretion significantly. The marked increase in urinary ammonia excretion despite only moderate chronic metabolic acidosis is consistent with previous reports (58). There was modest polyuria, as reported previously (18, 25). These observations confirm that this is a model of chronic metabolic acidosis associated with increased renal ammonia metabolism.

Antibody characterization in rat kidney tissue. The antibodies used in these studies have been characterized previously in mouse tissue (23, 55, 57). To confirm their specificity in rat tissues, we performed an initial set of immunoblot analyses. As shown in Fig. 1, anti-Rhbg antibodies recognized a protein of ~59 kDa. Immunoreactivity was blocked by preincubating the antibodies with the immunizing peptide for Rhbg. Antibodies to Rhcg recognized a protein of ~54 kDa; immunoreactivity was blocked by preincubating the antibodies with the immunizing peptide. These apparent molecular weights for both Rhbg and Rhcg are similar to that previously observed in the mouse and rat kidney (14, 45, 55).

Table 2. 24-h urine content

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Control (n = 6)</th>
<th>HCl (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺, mmol/l</td>
<td>134±2</td>
<td>138±0.7*</td>
</tr>
<tr>
<td>K⁺, mmol/l</td>
<td>6.4±0.4</td>
<td>5.7±0.7</td>
</tr>
<tr>
<td>Cl⁻, mmol/l</td>
<td>105±1</td>
<td>119±1†</td>
</tr>
<tr>
<td>pH (from ABG)</td>
<td>7.37±0.02</td>
<td>7.21±0.06*</td>
</tr>
<tr>
<td>HCO₃⁻, mmol/l</td>
<td>26.0±7</td>
<td>19±1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. control. †P < 0.001 vs. control. ‡P < 0.0025 vs. control.

Rhcg protein expression. To begin examining the mechanisms by which urinary ammonia excretion increases in response to chronic metabolic acidosis, we quantified renal Rhbg protein expression using immunoblot analysis of the renal cortex, outer medulla, and the base of inner medulla. Figure 2 summarizes these results. Chronic metabolic acidosis did not result in significant increases in steady-state Rhbg protein expression in the cortex, outer medulla, or inner medulla (P = not significant for each region, n = 6 in each group). The level of Rhbg expression in samples from the tip of the inner medulla was too low for accurate quantification, consistent with the relative lack of intercalated cells in the tip of the inner medulla (8, 9).

Rhcg protein expression. Similar studies examined Rhcg protein expression. Significant increases in Rhcg immunoreactivity were observed in proteins isolated from the outer medulla (P < 0.05) and the base of the inner medulla (P < 0.001), but not the renal cortex. Figure 3 summarizes these results. Rhcg expression in samples from the tip of the inner medulla was too
low for accurate quantification, consistent with the relative lack of intercalated cells in the tip of the inner medulla (8, 9).

Rhcg mRNA quantification. Because chronic metabolic acidosis increased Rhcg protein expression in the outer and inner medulla, we next quantified Rhcg mRNA expression. Figure 4 summarizes these results. No significant differences in Rhcg mRNA expression were observed in any of these regions ($P$ not significant in cortex, outer medulla, and inner medulla, $n = 6$). Increased Rhcg protein expression in the outer and inner medulla appears to independent of changes in steady-state mRNA expression.

Rhbg immunolocalization. Examination of Rhbg immunoreactivity in kidneys from control animals revealed basolateral immunoreactivity in the DCT, CNT, ICT, CCD, OMCD in the outer and inner stripes, and in a subset of cells in the IMCD. These results are similar to those reported previously in rat and mouse kidneys (45, 55). In kidneys from acidic rats examined using light microscopy, there were no detectable differences in Rhbg immunoreactivity in the cortex, outer medulla, or the inner medulla. Figures 5 and 6 show representative results.

Rhcg immunolocalization. In the cortex of control rats, Rhcg immunoreactivity was present in the DCT, CNT, ICT, and CCD in a cellular distribution similar to that reported previously (14, 55). However, in contrast to previous reports, both apical and basolateral Rhcg immunoreactivity was observed (Fig. 7). In general, basolateral Rhcg immunoreactivity was...
less intense than apical immunoreactivity. Chronic metabolic acidosis did not detectably alter Rhcg immunoreactivity compared with control conditions (Fig. 7).

In both the inner and outer stripe of the OMCD, all cells expressed Rhcg immunoreactivity (Fig. 8). In general, intercalated cells in control rat kidneys were cuboidal and exhibited a broad band of apical Rhcg immunoreactivity (Fig. 8B, black arrows), with a lesser intensity of basolateral immunoreactivity (Fig. 8B, black arrowheads). Apical Rhcg immunoreactivity in OMCD principal cells was less intense than in intercalated cells (Fig. 8B, white arrows). The greater intensity of apical Rhcg immunoreactivity in OMCD intercalated cells compared with principal cells is similar to previous reports (14, 55). Weak basolateral Rhcg immunoreactivity was also observed in
OMCD principal cells under control conditions (Fig. 8B, white arrowheads).

A variety of changes was observed in the OMCD in response to chronic metabolic acidosis. Some cells in OMCD in the outer and inner stripe had a rounded apical region, extended into the tubule lumen and exhibited a sharp, discrete band of apical Rhcg immunoreactivity (Fig. 8, C and D, arrows). The relative number of these cells, their morphological appearance, and their intense apical Rhcg immunoreactivity suggested they were intercalated cells. To confirm this, we colocalized Rhcg with AE1, the basolateral anion exchanger present in OMCD intercalated cells (11), in tissue sections from control and chronic metabolic acidosis rats. Cells with intense apical Rhcg immunoreactivity in OMCD in both the outer and inner stripe
in both control and chronic metabolic acidosis kidneys exhibited basolateral AE1 immunoreactivity, identifying these cells as intercalated cells (Fig. 9, A and B). In addition to apical immunoreactivity, basolateral Rhcg immunoreactivity was observed in both intercalated and principal cells (Fig. 8D, black and white arrowheads, respectively). Rhcg immunoreactivity was not observed in cells other than the OMCD in the outer medulla, either under control conditions or in response to chronic metabolic acidosis. Although the intensity of immunolabel was not demonstrably different between control and acidicotic kidneys, immunoblot analysis (see Fig. 3) is a more quantitative method for assessing changes in protein expression. Thus these results indicate that chronic metabolic acidosis increases Rhcg protein expression in the OMCD.
In the IMCD of control kidneys, intercalated cells were cuboidal and exhibited a broad band of apical Rhcg immunoreactivity (Fig. 8, E and F). Detectable Rhcg immunoreactivity was not observed in principal cells or IMCD cells (Fig. 8, E and F). These findings are consistent with previous reports in the mouse and rat kidney (14, 55). However, in contrast to previous reports, we observed both apical and basolateral Rhcg immunoreactivity in IMCD intercalated cells (Fig. 8, E and F).

Chronic metabolic acidosis resulted in changes in the IMCD. Similar to the OMCD, the apical region of some cells was rounded, extended into the tubule lumen and exhibited a sharp, discrete band of apical Rhcg immunoreactivity (black arrows). Basolateral Rhcg immunoreactivity (black arrowhead) is present in a narrow band and is less intense than the apical immunoreactivity. Principal cells have a cuboidal appearance and exhibit both apical (white arrows) and basolateral (white arrowheads) Rhcg immunoreactivity that is less intense than in intercalated cells. E and F: representative findings from the IMCD of a control kidney. Rhcg immunoreactivity is present in the apical (arrows) and basolateral region (arrowhead) of intercalated cells and not in adjacent principal cells or IMCD cells. G and H: findings from the IMCD from chronic metabolic acidosis kidneys. The apical region of intercalated cells projects into the tubule lumen and exhibits discrete apical Rhcg immunoreactivity (black arrows). Intercalated cells in the IMCD of chronic metabolic acidosis kidneys also exhibit weak basolateral immunoreactivity (black arrowhead). A–H: *denotes the collecting duct lumen. Rhcg immunoreactivity was not detectable in other medullary structures either under control conditions or in response to chronic metabolic acidosis. A–H: representative of findings in 6 control and 6 chronic metabolic acidosis rats using Rhcg antibody at 1:2,000 dilution.

Fig. 8. Rhcg immunoreactivity in the medulla in response to chronic metabolic acidosis. A and B: Rhcg immunoreactivity in the OMCD in the inner stripe in a control kidney. OMCD intercalated cells exhibit a broad apical band of intense immunoreactivity (black arrows); basolateral immunoreactivity (black arrowheads) a weaker, thinner band compared with the apical immunoreactivity. Adjacent principal cells exhibit faint apical (white arrows) and basolateral (white arrowheads) immunoreactivity. Both intercalated cells and principal cells have a cuboidal appearance. C and D: representative findings in the OMCD in the inner stripe of chronic metabolic acidosis kidneys. The apical region of intercalated cells is rounded and protrudes into the tubule lumen more prominently than in control conditions and exhibits intense apical Rhcg immunoreactivity (black arrows). Basolateral Rhcg immunoreactivity (black arrowhead) is present in a narrow band and is less intense than the apical immunoreactivity. Principal cells have a cuboidal appearance and exhibit both apical (white arrows) and basolateral (white arrowheads) Rhcg immunoreactivity that is less intense than in intercalated cells. E and F: representative findings from the IMCD of a control kidney. Rhcg immunoreactivity is present in the apical (arrows) and basolateral region (arrowhead) of intercalated cells and not in adjacent principal cells or IMCD cells. G and H: findings from the IMCD from chronic metabolic acidosis kidneys. The apical region of intercalated cells projects into the tubule lumen and exhibits discrete apical Rhcg immunoreactivity (black arrows). Intercalated cells in the IMCD of chronic metabolic acidosis kidneys also exhibit weak basolateral immunoreactivity (black arrowhead). A–H: *denotes the collecting duct lumen. Rhcg immunoreactivity was not detectable in other medullary structures either under control conditions or in response to chronic metabolic acidosis. A–H: representative of findings in 6 control and 6 chronic metabolic acidosis rats using Rhcg antibody at 1:2,000 dilution.
Also similar to the OMCD, the relative number of these cells, their morphological appearance, and their intense apical Rhcg immunoreactivity suggested they were intercalated cells, which we confirmed by colocalization of Rhcg with AE1 (Fig. 9, C and D). Although immunolabel intensity was not demonstrably different between control and acidotic kidneys, immunoblot analysis (see Fig. 3) is a more quantitative method for assessing changes in protein expression. Similarly to the outer medulla, chronic metabolic acidosis did not result in Rhcg immunoreactivity in cells other than in the collecting duct; thus
the increased Rhcg protein expression observed by immuno-
 blot analysis reflects increased expression in IMCD.

DISCUSSION

The current studies are the first examining the effect of chronic acid loading on the renal expression of the ammonia transporter family members, Rhbg and Rhcg. Chronic metabolic acidosis increased Rhcg protein expression in the outer medulla and the inner medulla; this increase was associated with more distinct apical Rhcg immunoreactivity and with protrusion into the tubule lumen by OMCD and IMCD intercalated cells. No change was observed in Rhcg protein expression or immunoreactivity in the cortex. Basolateral Rhcg immunoreactivity was observed for the first time in both control and acidic kidneys. There were no detectable changes in Rhbg protein expression or immunoreactivity in the cortex, outer medulla, or inner medulla. Taken together, these results suggest that the increased renal ammonia secretion that occurs in response to chronic metabolic acidosis may be due, at least in part, to enhanced apical expression of the ammonia transporter family member, Rhcg, in intercalated cells in the outer and inner medulla collecting duct.

Chronic metabolic acidosis increases total renal ammonia excretion (6, 28, 43, 51) through mechanisms that involve increased collecting duct ammonia secretion (5, 6, 18). Multiple factors can regulate collecting duct ammonia secretion. Importantly, recent studies have identified expression of the ammonia transporter family members, Rhbg and Rhcg, in the renal collecting duct (14, 45, 55), raising the possibility that important role in the regulation of transepithelial collecting duct ammonia secretion. This possibility is further supported by the recent demonstration that ammonia secretion by the cultured collecting duct, mIMCD-3, cell involves an apical, transporter-mediated process that appears to be attributable to Rhcg (22). Finally, seminal studies have shown that the related ammonia transporter family member, Rhag, mediates NH$_4^+$/$H^+$ exchange (59, 60); studies examining the nonerythroid ammonia transporters, Rhbg and Rhcg, have confirmed that they transport ammonia, although slight differences in the exact substrate specificity have been observed in different studies (2, 34, 36, 41, 42, 44). Thus increasing evidence supports an important role of Rhbg and Rhcg in collecting duct ammonia secretion. The current study, by demonstrating enhanced Rhcg expression in the OMCD and the IMCD, adds to these previous studies by demonstrating that increased apical ammonia transport mediated by increased Rhcg expression may be an important component of the renal collecting duct response to chronic metabolic acidosis.

Ammonia secretion is believed generally to involve $H^+$ secretion in parallel with ammonia secretion. Intercalated cells in the OMCD respond to chronic metabolic acidosis with increased apical plasma membrane surface area (35) and with increased targeting of $H^+$-ATPase to the apical plasma membrane (3, 4, 53). $H^+$-$K^+$-ATPase, which may contribute 40–60% of OMCD and IMCD $H^+$ secretion (1, 52, 56), is increased by chronic metabolic acidosis in some (17), but not all (12, 52), reports. The current study, by showing increased expression of the ammonia transporter family member, Rhcg, in the OMCD and IMCD suggests a coordinated response of both $H^+$ and ammonia transport mechanisms in chronic metabolic acidosis.

Chronic metabolic acidosis appears to regulate Rhcg protein expression in the outer and inner medulla through mechanisms independent of changes in steady-state Rhcg mRNA expression. This suggests that the increased protein expression is either due to an increased mRNA translation rate or to increased protein stability. We are unaware of any data supporting the possibility that chronic metabolic acidosis increases mRNA translation efficiency, but cannot exclude this possibility at present. However, extracellular ammonia, which increases in response to chronic metabolic acidosis, induces hypertrophy in renal mesangial cells and proximal tubule cells (10, 30, 47) through mechanisms that appear to involve increased protein stability (29, 30). Whether such a mechanism regulates Rhcg protein stability is unknown at present. An alternative possibility is that acidosis increases the number of intercalated cells in both the OMCD and the IMCD. However, previous studies have shown that chronic metabolic acidosis does not alter the number of collecting duct intercalated cells (24, 54).

Renal cortical Rhcg expression and immunoreactivity did not appreciably change in response to chronic metabolic acidosis. The lack of response in the DCT and CNT is consistent with only minimal changes in the rate of net ammonia secretion between the early and late distal tubule in response to chronic metabolic acidosis (49). Why Rhcg expression in the CCD did not detectably change is less clear. One possible explanation is that chronic metabolic acidosis may regulate Rhcg-mediated transport through posttranslational modifications, such as phosphorylation-dephosphorylation. Consistent with this possibility is that computer modeling identifies multiple consensus phosphorylation sites in Rhcg.

An unexpected observation was the detection of significant basolateral Rhcg immunoreactivity, in addition to the previously reported apical Rhcg immunoreactivity. The lack of basolateral Rhcg immunoreactivity in the mouse kidney (55) may reflect species-dependent differences. Previous reports examining the rat kidney did not identify basolateral Rhcg immunoreactivity (14, 45); the reason for the difference in the current study is not clear but may reflect the different sensitivities of immunofluorescence compared with immunohistochemical microscopy. More importantly, the physiological role of basolateral Rhcg is not yet apparent.

Chronic metabolic acidosis did not detectably alter Rhbg protein expression or immunoreactivity in the cortex, outer medulla, or inner medulla. This could indicate that mechanisms independent of protein expression regulate Rhbg-mediated ammonia transport. One possibility relates to the observation that chronic extracellular ammonia exposure, which occurs in chronic metabolic acidosis, acidifies CCD intercalated and principal cells (15, 16, 19). Because intracellular acidification stimulates basolateral collecting duct ammonia uptake (21), this may be an important regulatory mechanism. Another possible regulatory mechanism is through phosphorylation-dephosphorylation. Similar to Rhcg, computer modeling identifies multiple consensus phosphorylation sites in Rhbg. Which, if any, regulate Rhbg-mediated ammonia transport is unknown at present. It is also important to note that a preliminary report suggested that metabolic acidosis increases Rhbg
protein expression in the cortex but not the outer medulla (46). The reasons for the different observations in this preliminary report (46) and in the current study are unclear. Finally, it is possible that Rhbg-mediated ammonia transport is not regulated in response to chronic metabolic acidosis.

In summary, the current studies are the first to examine the effects of a chronic acid-base disturbance on expression of the recently identified ammonia transporter family members, Rhbg and Rhcg. Chronic metabolic acidosis results in increased urinary ammonia excretion associated with increased Rhcg protein expression in the outer medulla and the inner medulla, protrusion of OMCD and IMCD intercalated cells into the tubule lumen, and a sharper, more distinct band of apical Rhcg immunoreactivity in OMCD and IMCD intercalated cells. Thus changes in ammonia secretion by the kidney in response to chronic metabolic acidosis appear to involve, at least in part, specific changes in transcellular ammonia secretion mediated by Rhcg in intercalated cells in the OMCD and IMCD.

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