Role of angiotensin II in the enhancement of ammonia production and secretion by the proximal tubule in metabolic acidosis

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A MAJOR WAY that the kidneys defend the body against the introduction of excess acid is through the production and excretion of total ammonia ($tNH_3 = NH_3 + NH_4^+$) (15, 21, 29, 33, 36). The proximal tubule is the major site of this response to acid challenges, increasing its rates of $tNH_3$ production and secretion and generating new bicarbonate formation (8, 26). We previously demonstrated that chronic metabolic acidosis in vivo stimulates $tNH_3$ production and secretion by mouse S2 proximal tubule segments (24). The increased rates of ammoniagenesis persist even after the tubule is removed from the acidic animal and assayed in vitro under normal pH conditions. Such adaptive changes result from the induction of key ammoniagenic enzymes (5, 37).

In our previous study, short-term (18-h) acid challenges induced an adaptive enhancement in the rate of $tNH_3$ secretion, but not production in an angiotensin II (ANG II)-dependent manner (22). Giving the acid challenge concurrently with a type 1 ANG II (AT1) receptor blocker, losartan, blocked the acid-induced increase in $tNH_3$ secretion that was observed with the short-term acid challenge. It is well-known that during metabolic acidosis, the systemic renin-angiotensin system is stimulated (9, 28, 31), but the role of this system in the renal response to chronic acid challenges is unknown. Studies by others also demonstrated that local concentrations of ANG II in the kidney are substantial (3, 25, 34) and may act in a paracrine fashion (30). As ANG II directly stimulates $tNH_3$ production and secretion by the proximal tubule (14, 20), it may mediate a portion of the adaptive enhancement of $tNH_3$ production and secretion that is observed with metabolic acidosis.

The purposes of the present studies were to test whether AT1 receptor blockade alters the acidosis-induced adaptive increase in urinary $tNH_3$ excretion and S2 proximal tubule segment rates of $tNH_3$ production and secretion, to examine the effects of AT1 receptor blockade on the adaptive upregulation of key proteins involved in ammonia production and transport, and to determine the direct effects of ANG II in vitro on $tNH_3$ production and secretion by S2 segments from control and NH4Cl-treated mice. We found that NH4Cl-induced metabolic acidosis enhanced $tNH_3$ excretion by the kidney and $tNH_3$ production and secretion rates by mouse S2 proximal tubule segments in a losartan-inhibitable manner. In addition, metabolic acidosis enhanced the expression of renal cortical phosphate-dependent glutaminase (PDG) and phosphoenolpyruvate carboxykinase 1 (PCK1) and cortical brush-border membrane (BBM) NHE3 protein expression but only the enhancement of NHE3 expression was prevented by the provision of losartan with the acid load. The addition of ANG II in vitro to the lumen of microperfused S2 segments further stimulated rates of $tNH_3$ production and secretion in a losartan-inhibitable manner.

METHODS

Animals. The studies performed were approved by the Animal Research Committee (IACUC) at the VA Greater Los Angeles Healthcare System. Male Swiss-Webster mice (Hilltop, Scottsdale, PA) weighing 25–30 g were maintained on Purina Rodent Chow. Mice were provided 0.3 M NH4Cl in 2% sucrose, 0.3 M NH4Cl in 2% sucrose with losartan (100 mg/l), 2% sucrose with losartan, or 2% sucrose alone (control) for 7 days. At the end of 7 days, the mice were anesthetized with isoflurane and blood was obtained from the aorta for...
measurement of serum total CO\(_2\) and potassium concentration. Urine was obtained from the bladder for determination of tNH\(_3\) and creatinine, and urine pH was immediately estimated using pH paper in conjunction with standard pH solutions.

Microperfusion of mouse proximal tubule segments. S2 segments of the mouse proximal tubule comprising the late convoluted and early straight portions (0.9 ± 0.2 mm) were dissected from outer cortical nephrons under direct microscopic visualization. Each S2 segment was placed in a temperature-regulated chamber mounted over the objective of an inverted microscope and was microperfused using concentric pipettes so that the luminal aspect of the S2 segment was cannulated and perfused with Krebs-Ringer bicarbonate (KRB) buffer (16, 17). The flow rates (20.1 ± 0.3 nl/min) did not differ among the groups studied. The S2 segment was bathed in ~300 μl KRB buffer containing 0.5 mM l-glutamine pregressed with 95% O\(_2\)-5% CO\(_2\), pH 7.4 at 37°C.

Measurement of tNH\(_3\) production rates. In studies measuring tNH\(_3\) production rates by isolated perfused S2 segments, the bath solution was covered with a gas jet of 95% O\(_2\)-5% CO\(_2\). The distal end of the perfused segment remained open to the bath medium so that tNH\(_3\) entered the bath solution via the fluid leaving the distal end of the perfused segment and via direct release into the bath medium through the basolateral aspect of the S2 segment. At the end of a 20- to 30-min incubation period, an aliquot of the bath solution was taken for analysis of tNH\(_3\) using a microenzymatic method coupling the conversion of 2-oxoglutarate, NADH and tNH\(_3\) to NAD and glutamate

Measurement of luminal tNH\(_3\) secretion rates. In studies examining luminal tNH\(_3\) secretion rates, the fluid leaving the distal end of the perfused segment was collected with a pipette (16, 23). Luminal tNH\(_3\) secretion rates equaled the rate at which tNH\(_3\) left the bath solution via the fluid leaving the distal end of the perfused segment and via direct release into the bath medium through the basolateral aspect of the S2 segment. At the end of a 20- to 30-min incubation period, an aliquot of the bath solution was taken for analysis of tNH\(_3\) using a microenzymatic method coupling the conversion of 2-oxoglutarate, NADH and tNH\(_3\) to NAD\(^+\) and glutamate

Preparation of proteins from renal cortical tissue. Mice were given control diets with or without losartan or 7-day NH\(_4\)Cl loads as noted above. At the end of 7 days, mice were euthanized, kidneys were removed, and renal cortical tissue was dissected. Isolated tissue was placed in a mannitol buffer [150 mM mannitol, 80 mM HEPEs, pH 7.5, 0.2 mM EDTA with antiprotease cocktail (Roche)], homogenized with a Polytron homogenizer, and the homogenate was centrifuged at 200 g for 10 min (4°C). An aliquot of the supernatant was taken for total protein determination in the crude homogenate, placed in lysis buffer [330 mM sucrose, 20 mM Tris, pH 7.5, 0.2 mM EDTA, 1% Triton X-100, 0.5% NP-40 and antiprotease cocktail (Roche)], and kept on ice for 20 min. The lysed homogenate was centrifuged at 14,000 g at 4°C for 20 min, the protein concentration of the supernatant was determined, and the samples were stored at −80°C for subsequent immunoblot analysis.

Preparation of BBM proteins. After the aliquot for total proteins was taken, the remainder of the supernatant of the crude homogenate was used to prepare BBMs, as described by Booth and Kenny (2) and modified by Karniski et al. (12, 13). The supernatant of the homogenate was treated with magnesium sulfate to bring the final concentration of magnesium to 11 mM. The preparation was mixed at 4°C for 20 min and then processed through a series of low-speed centrifugations (2,300 g × 8 min, 3,800 g × 8 min, 4,400 g × 8 min), each time saving the supernatant until a high-speed centrifugation step (14,000 g for 12 min) was performed to harvest the BBM vesicles. The pelleted vesicles were resuspended in lysis buffer and kept on ice with intermittent mixing for 20 min before protein assay and storage.

Immunoblotting of protein preparations. Sixty micrograms of isolated proteins from the homogenate or 100 μg from the BBM preparation were denatured under reducing conditions and separated by SDS-PAGE on 4–12% gradient gels (Invitrogen). The separated proteins were electroblotted (Owl semidy semidy semidy semidy electroblotter) to nitrocellulose membranes for immune staining for glutaminase, PCK1, or NHE3 using an anti-glutaminase antibody (kind gift of Dr. N. Cuthoys), anti-PCK1 antibody (Cayman Chemical), or anti-NHE3 antibody (kind gift of Dr. O. Moe), respectively. Secondary antibody, chemiluminescent detection kit (Pierce), and scanning densitometry were used to determine relative expression after exposing the membranes to the primary antibodies. Immunoblotting was performed on three sets of mice exposed to sucrose alone, sucrose + losartan, sucrose + NH\(_4\)Cl, and sucrose + NH\(_4\)Cl and losartan.

Measurements of total CO\(_2\) and potassium concentrations. Total CO\(_2\) (tCO\(_2\) = HCO\(_3\)\(^−\) + dissolved CO\(_2\)) was determined on serum samples enzymatically using the phosphoeneopryuvate carboxykinase reaction (Sigma Chemical). The measurements were linear over the range of concentrations observed. Potassium measurements were made by ion-sensitive electrode (18).

Solutions. KRB buffer solution contained the following electrolytes (in mM) 125 NaCl, 25 NaHCO\(_3\), 5 KCl, 1 MgCl\(_2\), 1 NaH\(_2\)PO\(_4\), and 1 CaCl\(_2\). ANG II and losartan (Merck) were used in the concentrations specified in the results. The low (25 mM) sodium perfusion solution substituted N-methylglucamine chloride for NaCl in the KRB buffer solution. All glutamine-containing solutions were freshly prepared using the purest form of l-glutamine available (Sigma Chemical).

Statistical analysis. Comparisons between two groups of data were done using Student’s t-test, whereas comparisons among multiple groups were made using ANOVA with multiple comparisons by the method of Scheffé (32) and by single group t-test (GraphPad InStat). All data are presented as means ± SE.

### RESULTS

**Effects of losartan and long-term NH\(_4\)Cl loading on serum tCO\(_2\) and potassium concentrations, and urinary tNH\(_3\) excretion.** Groups of mice (n = 5 in each) received the following in their drinking water for 7 days: 2% sucrose in water, losartan (100 mg/l) in 2% sucrose, 0.3 M NH\(_4\)Cl in 2% sucrose, or 0.3 M NH\(_4\)Cl + losartan (100 mg/l) in 2% sucrose (Table 1). Mice receiving losartan and sucrose in the drinking solution displayed no significant differences in serum tCO\(_2\) concentrations or in urinary tNH\(_3\) excretion per milligram of creatinine compared with control mice receiving sucrose alone. However, mice receiving NH\(_4\)Cl displayed lower serum tCO\(_2\) concentra-
tions and higher urinary tNH₃ excretion per milligram of creatinine, relative to mice receiving sucrose and sucrose + losartan. Mice concurrently receiving NH₄Cl and losartan displayed an even lower serum tCO₂ concentration (P < 0.01 vs. other groups) and had reduced tNH₃ excretion per milligram of creatinine relative to mice receiving NH₄Cl without losartan (P < 0.01). Serum potassium concentrations did not significantly differ among the study groups. Urine pH from acid-loaded mice, as measured by pH paper, was low (pH 5.5 to 6.0) in both losartan-treated and -untreated mice. Thus, although losartan by itself had no effect on renal tNH₃ excretion, coadministration of losartan with the NH₄Cl treatment significantly attenuated the acidosis-induced enhancement in renal tNH₃ excretion without affecting urinary pH. Losartan treatment also led to a greater reduction in the serum tCO₂ concentration in NH₄Cl-treated mice.

Effects of in vivo NH₄Cl administration on tNH₃ production and net luminal tNH₃ secretion by S2 proximal tubule segments perfused in vitro. In previous studies, we demonstrated that tNH₃ production and secretion rates were enhanced in S2 proximal tubule segments derived from mice given NH₄Cl for 7 days (24). In the present study, we examined the effect of a 7-day exposure to NH₄Cl with or without concurrent administration of losartan on the rates of tNH₃ production and transport by isolated S2 segments perfused in vitro under normal bicarbonate and pH concentrations. Changes in tNH₃ production and secretion that persisted after dissecting the tubule from the in vivo environment represented chronic adaptive changes. The tNH₃ production and luminal secretion rates observed in S2 segments derived from mice that received NH₄Cl for 7 days were higher than those observed in segments from control mice not receiving NH₄Cl. However, losartan given with the acid load blocked this increase (Fig. 1). As depicted in Fig. 1A, the tNH₃ production rates were higher in segments from mice receiving NH₄Cl (31.1 ± 1.2 pmol·min⁻¹·mm⁻¹) compared with mice receiving NH₄Cl with losartan (22.2 ± 0.8 pmol·min⁻¹·mm⁻¹), mice given losartan alone (19.8 ± 0.8 pmol·min⁻¹·mm⁻¹), or control mice (20.2 ± 1.0 pmol·min⁻¹·mm⁻¹; n = 5 in each group, P < 0.01). As shown in Fig. 1B, the rates of net luminal tNH₃ secretion were higher in S2 segments derived from NH₄Cl-treated mice (21.6 ± 0.3 pmol·min⁻¹·mm⁻¹) compared with segments from NH₄Cl + losartan-treated mice (12.1 ± 1.0 pmol·min⁻¹·mm⁻¹), losartan-treated mice (9.8 ± 1.1 pmol·min⁻¹·mm⁻¹), and controls (10.0 ± 1.0 pmol·min⁻¹·mm⁻¹; n = 5 for each group, P < 0.01). Thus, NH₄Cl-induced metabolic acidosis was associated with adaptive increases in both tNH₃ production and luminal tNH₃ secretion rates by S2 proximal tubule segments and the administration of losartan with the NH₄Cl blocked these adaptive increases.

Effect of 7-day acid challenge and losartan on ammoniagenic enzymes. To further explore the mechanism by which losartan prevented the adaptive enhancement of tNH₃ production, we examined the impact of losartan administration on the protein expression of two key ammoniagenic enzymes in the renal cortex: PDG and PCK1. As has been described in rats (11, 27), PDG and PCK1 protein expression levels were enhanced with metabolic acidosis induced by 7-day NH₄Cl acid loading [3.6 ± 0.3- and 2.6 ± 0.3-fold increases, respectively (n = 3 for each condition, P < 0.05 vs controls)]; however, the administration of losartan had no effect on the PDG or PCK1 protein levels under acid- or nonacid-loaded conditions (Fig. 2).

Effect of 7-day acid challenge on expression of BBM-associated NHE3. As Na⁺-H⁺ (NH₄⁺) exchange may be involved in tNH₃ secretion by the proximal tubule (14, 16), we examined the effect of acid loading on the BBM expression of an important NHE isoform, NHE3. Renal cortical BBMs from mice receiving 2% sucrose + NH₄Cl had higher levels of NHE3 expression (2.1 ± 0.1-fold higher, n = 3, P < 0.05) compared with those from mice receiving 2% sucrose alone. BBM NHE3 abundance was similar to levels observed in control (2% sucrose) mice than in the mice receiving 2% sucrose with losartan (relative level 1.1 ± 0.1 compared with control, n = 3) and in mice receiving 2% sucrose with NH₄Cl and losartan (relative expression level 1.3 ± 0.1 compared with control, n = 3). These results demonstrated that BBM NHE3 expression was upregulated by acid loading as has been described by others in rats (1) and that losartan inhibited this upregulation, raising the possibility that one of the mechanisms by which losartan prevented an adaptive increase in tNH₃.
Effect of amiloride and luminal sodium reduction on luminal tNH₃ secretion after 7-day acid challenge. We previously demonstrated that microperfusing normal mouse S2 proximal tubule segments with the low sodium perfusion solution containing 0.1 mM amiloride inhibited net luminal tNH₃ secretion by 90% (16). These results were consistent with transport of NH₄⁺ by a Na⁺-H⁺ exchanger (14). In the present study, we examined the effect of luminal perfusion with low (25 mM) sodium solution containing 0.1 mM amiloride (low Na⁺/amiloride) on tNH₃ production and net luminal secretion rates in S2 proximal tubule segments derived from control mice and mice given NH₄Cl. These perfusion conditions had no effect on NHE3 expression in the absence of NH₄Cl treatment compared with levels observed in nonacidic, losartan-treated group with nonacidic, nonlosartan-treated controls, but had no effect on NHE3 expression in the absence of NH₄Cl treatment (relative abundance = 1.1 ± 0.1 for nonacidotic, losartan-treated group compared with control mice (20). In the present study, we examined the effects of 10⁻⁹ M ANG II into the tubule lumen on tNH₃ production and net luminal secretion rates in control mice and mice given a 7-day NH₄Cl challenge (n = 5 in each group). As depicted in Fig. 3, addition of ANG II to the luminal perfusion solution stimulated tNH₃ production rates by S2 segments from both control mice (20.2 ± 1.0 without ANG II and 32.0 ± 1.6 pmol·min⁻¹·mm⁻¹ with ANG II, P < 0.01) and acidic mice (39.8 ± 1.2 pmol·min⁻¹·mm⁻¹ without ANG II vs. 50.1 ± 1.1 with ANG II, P < 0.01). Thus, ANG II stimulated tNH₃ production rates by S2 proximal tubule segments from both acidic and nonacidotic mice (P < 0.01). In previous studies, we demonstrated that the stimulatory effect of ANG II on tNH₃ production was blocked by concurrent provision of losartan in the luminal fluid (22). The stimulatory effect of acute addition of ANG II to the luminal perfusion solution on tNH₃ production rate by S2 segments from acid-loaded mice observed here was also inhibited by the addition of losartan (10⁻⁶ M) to the ANG II-containing luminal perfusate (40.1 ± 1.5 pmol·min⁻¹·mm⁻¹).
ANG II has important effects on tNH₃ production and transport in the proximal tubule (4, 19, 20). Acidosis in vivo is associated with upregulation of the renin-angiotensin system (6, 10, 31). Luminal ANG II not only stimulates tNH₃ production but also stimulates tNH₃ secretion into the luminal fluid (20). Our previous studies demonstrated that a short-term (18 h) NH₄Cl load resulted in an adaptive increase in luminal tNH₃ secretion rates in the absence of a measurable increase in production rates in S2 proximal tubule segments (22). The adaptive increase in tNH₃ secretion in response to NH₄Cl loading was blocked by concurrent administration of the AT1 receptor blocker losartan.

In response to prolonged acid challenges, the kidney increases urinary tNH₃ excretion by increasing synthesis of proteins involved in tNH₃ production (7, 11, 27, 35). In the present study, we demonstrated that prolonged (7 days) NH₄Cl loading resulted in an increase in urinary tNH₃ excretion and in adaptive increases in both tNH₃ production and secretion rates by isolated S2 segments which were attenuated by concurrent administration of losartan with the acid load. The 7-day acid load enhanced tNH₃ production rates by S2 proximal tubule segments by 54% and secretion rates by 116% compared with nonacid-loaded controls. The enhanced secretion rates with acid loading appeared to result from a combination of the increased tNH₃ production rates as well as by an increased rate of secretion as a percentage of the total production rate, such that 69% of the tNH₃ produced by segments from acidic mice was secreted into the lumen compared with 50% in segments from nonacidotic control mice. When losartan was given with the acid load, the tNH₃ production and secretion rates returned to levels observed in tubules from nonacidotic controls such that the proportion of secreted tNH₃ made by the proximal tubule segment was 54% compared with 50% observed in tubules from nonacidotic controls and 69% in tubules from acidic mice. The prevention of the enhanced S2 proximal tubular tNH₃ production and secretion rates by losartan given with acid loading was associated with a reduction in urinary tNH₃ excretion and in a greater degree of NH₄Cl-induced acidosis (lower bicarbonate concentration). The reduction in urinary tNH₃ excretion appeared to occur without a loss of the ability to acidify the urine, suggesting that distal acidification processes remained intact. In the absence of an acid load, the proportion of secreted tNH₃ was 40% compared with 50% observed in segments from nonacidotic control mice. Thus, as is the case with briefer acid loading, the response of the proximal tubule to prolonged acid challenges depends on intact AT1 receptor function.

The mechanism by which losartan prevented acid-induced adaptive increases in urinary tNH₃ excretion and in proximal tubular rates of tNH₃ production and secretion depended, in part, on the prevention in the adaptive increase in NHE3 expression that normally occurs with acidosis (1). In our mouse model of in vivo acidosis, we noted a losartan-inhibitable

**DISCUSSION**

**Fig. 4.** ANG II (10⁻⁹ M) added to the lumen perfusion solution increased total ammonia production rates in S2 proximal tubule segments derived from control and acidic mice. The increment in rate was similar in both groups. Losartan blocked the stimulatory effect of ANG II on production rates. *P < 0.05 vs. other groups, n = 5 for each group.

P < 0.05 vs. perfusion with ANG II but without losartan. Therefore, ANG II increased tNH₃ production rates through its action on the AT1 receptor in proximal tubule segments from both acidic and control mice.

We also tested the effect of luminal ANG II (10⁻⁹ M) on net luminal tNH₃ secretion in S2 proximal tubule segments from control and acid-loaded mice. ANG II increased luminal tNH₃ secretion rates by S2 proximal tubule segments from control mice (–ANG II: 11.1 ± 1.0 pmol·min⁻¹·mm⁻¹, n = 5, P < 0.01; Fig. 5). ANG II also stimulated net luminal tNH₃ secretion by S2 segments from mice receiving NH₄Cl (–ANG II: 21.6 ± 0.5 pmol·min⁻¹·mm⁻¹, n = 5, P < 0.01). This stimulation by ANG II of net luminal tNH₃ secretion rates in S2 segments was significantly reduced by the addition of 10⁻⁶ M losartan with ANG II to the luminal perfusion solution in both acidic and nonacidotic groups (12.3 ± 0.8 or 20.2 ± 0.9 pmol·min⁻¹·mm⁻¹, respectively, n = 5, P < 0.01 vs. +ANG II without losartan). Thus, ANG II acts through its type 1 (AT1) receptor to stimulate net luminal tNH₃ secretion in proximal tubule segments from acidic and control mice.

![Fig. 5.](http://www.ajprenal.org)
increase in BBM-associated NHE3 protein. As we described previously, and as was again demonstrated in this study, blockade of NHE (luminal perfusion with a 25 mM sodium perfusate and 0.1 mM amiloride) largely blocked tNH3 entry into the lumen. However, the acute inhibition of NHE activity did not acutely affect tNH3 production rates. Therefore, although a chronic reduction of NHE3 expression on the BBM by losartan could reduce luminal transport activity of tNH3, it is unclear whether the prevention of the acid-induced increase in NHE3 also fully explains the ability of losartan to inhibit the adaptive increase in tNH3 production. Nevertheless, it is possible that enhancing tNH3 transport out of the cells and into the lumen kinetically favors the forward reactions for generating tNH3 due to rapid product removal and that reducing this efflux pathway in a sustained manner could dampen production rates.

We examined the effects of NH4Cl loading and losartan on expression of key ammoniagenic enzymes: PDG and PCK1 (11, 27). In the present studies, we found that the protein expression levels of these enzymes in renal cortical tissue were increased with acid loads, but losartan had no effect on their expression levels under control or acid-loaded conditions. Therefore, since we suspect that inhibition of tNH3 production may not be completely explained by a reduction in the transport of tNH3 (noting the lack of an acute effect of inhibition of luminal tNH3 secretion on total production rates), the existence of other undefined losartan-sensitive, acid-adaptive ammoniagenic pathways is likely and will be explored in future studies.

The major transport mechanism responsible for increased transport of tNH3 with NH4Cl treatment appeared to be fundamentally the same as the mechanism present under nonacid loading conditions. As occurred in proximal tubules from control mice (16), luminal tNH3 secretion rates observed in proximal tubule segments from NH4Cl-treated acidic mice were substantially inhibited by perfusion of the luminal fluid with a low sodium perfusate containing amiloride. Thus, net luminal tNH3 secretion by S2 segments from control and acid-treated mice may be mediated via a Na+/H+ exchanger. As mentioned previously, acid loading increased brush-border expression of NHE3 and this increase was prevented by providing losartan with the acid load. Therefore, the enhanced transport rate observed in S2 segments from acidic mice may be due to the induction of additional tNH3 transporters.

As in S2 segments from nonacidotic mice (20) and mice receiving an 18-h NH4Cl challenge, luminal ANG II markedly stimulated tNH3 production and secretion rates in proximal tubule segments from mice receiving NH4Cl for 7 days. One difference between the results observed in the present study compared with our previous observations was that the ANG II-induced increment in rates of tNH3 production and secretion rates after the 7-day acid load appeared to be quantitatively similar in tubules derived from acidic and nonacidotic mice, whereas a briefer acid challenge was associated with an enhanced stimulatory response to ANG II. In either case, however, luminal ANG II stimulated tNH3 production and secretion rates beyond the levels observed with acid challenge alone. When measured in vivo, ANG II has been found in the fluid of the tubule lumen at the concentrations used in the present experiments (25, 34). Therefore, our experimental addition of ANG II to the luminal perfusion solution may actually reflect the “native” state in the nephron in vivo and, with 18-h or 7-day acid challenges, ANG II would probably be present in sufficient quantities to enhance the renal response to either short-term or long-term acid challenges.

In human subjects, ANG II may be important in maintaining high renal tNH3 excretion rates in individuals with experimentally induced acidosis (10). Although Henger and co-workers (10) demonstrated that individuals given an acid load may display a reduction in urinary ammonia and net acid excretion in response to angiotensin receptor blockade, treatment with anti-angiotensin agents which are commonly used in patients with chronic kidney disease and hypertension are infrequent causes of metabolic acidosis. However, upon exposure to high acid loads, humans may develop overt acid-base problems with type 1 angiotensin receptor blocker similar to the mice used in this study.

In summary, we showed that 1) 7 days of NH4Cl loading results in acidosis in vivo, enhanced urinary tNH3 excretion, and enhanced tNH3 production and net luminal secretion rates by isolated perfused mouse S2 segments derived from acidic mice; 2) ANG II acting through its type 1 receptor is necessary for this adaptive increase in urinary tNH3 excretion and S2 proximal tubule (NH3 production and secretion rates; 3) ANG II added to the tubule lumen stimulated tNH3 production and secretion in S2 segments from acidic and nonacidotic control mice; and 4) acid loading also enhances protein expression levels of PDG, PCK1, and NHE3, but only upregulation of brush-border NHE3 expression is blocked by provision of losartan with the acid load. Taken together, these results indicate that ANG II plays a critical role in the adaptation and response of the proximal tubule to prolonged acid challenges.

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