Enhanced ammonia secretion by proximal tubules from mice receiving NH₄Cl: role of angiotensin II

GLENN T. NAGAMI
(With the Technical Assistance of Evelyn M. Warech)
Nephrology Section, Medical and Research Services, Veterans Affairs Greater Los Angeles Healthcare System at West Los Angeles, Los Angeles 90073; and School of Medicine, University of California, Los Angeles, California 90095

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We previously demonstrated (21) that giving mice NH₄Cl for 7 days induced adaptive increases in the rates of tNH₃ production and secretion by mouse S2 proximal tubule segments. We and others also demonstrated (2, 15, 17) that angiotensin II (ANG II) has important effects on tNH₃ production and secretion by the proximal tubule. Thus in addition to the known effects of ANG II on acid secretion, bicarbonate reclamation, and fluid reabsorption by the proximal tubule (5–8, 12, 13), ANG II enhances new bicarbonate generation via the production and secretion of tNH₃.

Studies by Seikaly and colleagues (28) and Braam and co-workers (1, 22) demonstrated that ANG II is produced by the proximal tubule in such a manner that the concentrations of ANG II in the luminal and peritubular fluid compartments may be 100–1,000 times higher than in the systemic circulation. Concentrations of ANG II in the 10⁻⁹ M range may be present in the tubule fluid.

The purposes of the present studies were to 1) examine the effects of short-term (18-h) NH₄Cl administration on renal tNH₃ excretion and tNH₃ production and secretion by mouse S2 proximal tubule segments, 2) determine the effects of blocking the type 1 angiotensin (AT₁) receptor in vivo on the response of the kidney and proximal tubule to the short-term acid challenge, and 3) determine the direct effects of ANG II in vitro on tNH₃ production and secretion by S2 segments from control and NH₄Cl-treated mice. These studies demonstrated that 1) short-term acid administration stimulates the rates of renal tNH₃ excretion and luminal tNH₃ secretion by S2 proximal tubule segments without affecting tNH₃ production rates; 2) the enhanced renal tNH₃ excretion and tNH₃ secretion rates by proximal tubules with short-term acid treatment are blocked when the AT₁-receptor blocker losartan is coadministered with NH₄Cl treatment; and 3) the stimulatory effect of in vitro ANG II on tNH₃ production and secretion rates is greater in S2 segments.
derived from NH4Cl-treated mice compared with controls, and the effects of ANG II are inhibited by losartan in both controls and acid-treated mice.

METHODS

Animals. The studies performed were approved by the Animal Research Committee at the Veterans Affairs 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 100 mg/l losartan, 2% sucrose with losartan, or 2% sucrose alone (control) for 18 h (short term). At the end of 18 h, the mice were anesthetized with intramuscular injections of ketamine (0.09 mg/g body wt) and xylazine (0.01 mg/g body wt) and blood was obtained from the aorta for measurement of plasma total CO2 (tCO2) and potassium concentration. Urine was obtained from the bladder for determination of tNH3 and creatinine.

Microperfusion of mouse proximal tubule segments. S2 segments of the mouse proximal tubule comprising the late convoluted and early straight portions (0.9 μl) were dissected from the outer cortical nephrons under direct microscopic visualization. Each S2 segment was placed in a solution that was gassed with a gas jet of 95% O2-5% CO2 at pH 7.4 and 37°C.

Measurement of tNH3 production rates. In studies to determine tNH3 production rates by isolated perfused S2 segments, the bath solution was covered with pregassed mineral oil and continuously bubbled with a gas jet of 95% O2-5% CO2. The distal end of the perfused segment remained open to the bath medium so that tNH3 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 tNH3 using a microenzymatic method that coupled the conversion of 2-oxoglutarate, NADH, and tNH3 to NAD+ and glutamate (18). The volume of the bath solution was determined from the degree of dilution of trypan blue dye added in known amounts to the bath solution at the completion of the study.

Measurement of luminal tNH3 secretion rates. In studies to examine luminal tNH3 secretion rates, the fluid leaving the distal end of the perfused segment was collected with a pipette (18, 20). Luminal tNH3 secretion rates equaled the rate at which tNH3 left the distal end of the perfused segment in timed luminal fluid collections.

Measurement of tCO2 and potassium concentrations. The tCO2 was enzymatically determined from serum samples using the phosphoenolpyruvate carboxykinase reaction (Sigma). The measurements were linear over the range of concentrations observed. Potassium measurements were made by ion-sensitive electrode (16).

Solutions. KRB buffer solution contained the following electrolytes (in mM): 125 NaCl, 25 NaHCO3, 5 KCl, 1 MgCl2, 1 Na2HPO4, and 1 CaCl2. ANG II and losartan (Merck) were used in concentrations as specified in RESULTS. The low-sodium perfusion solution substituted N-methyl glutamine chloride for NaCl in the KRB buffer solution. All glutamine-containing solutions were freshly prepared using the purest form of L-glutamine available (Sigma).

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 (26). All data are presented as means ± SE.

RESULTS

Effects of losartan and short-term NH4Cl loading on serum tCO2, potassium concentration, and urinary tNH3 excretion. Groups of mice (n = 5/group) received the following in their drinking water for 18 h: 2% sucrose in water, 100 mg/l losartan in 2% sucrose, 0.3 M NH4Cl in 2% sucrose, or 0.3 M NH4Cl + 100 mg/l losartan in 2% sucrose (Table 1). Mice receiving losartan and sucrose in the drinking solution displayed no significant differences in serum tCO2 concentrations, potassium concentrations, or urinary tNH3 excretion per milligram of creatinine compared with control mice receiving sucrose alone. Mice receiving NH4Cl in the drinking solution displayed similar serum tCO2 and potassium concentrations as the controls but had higher urinary tNH3 excretion per milligram of creatinine. The lack of a decrease in the tCO2 concentration in mice receiving NH4Cl may have resulted from the short duration of the administration of NH4Cl and from the short-term compensatory effects of increased excretion of tNH3 that was observed. In contrast, mice receiving NH4Cl and losartan in the drinking solution displayed lower serum tCO2 concentrations and had lower rates of tNH3 excretion than mice receiving NH4Cl without losartan. The serum potassium concentrations did not significantly differ among the study groups. Thus although losartan by itself had no effect on renal tNH3 excretion, the enhanced renal tNH3 excretion that was observed with an 18-h NH4Cl challenge was blocked when losartan was coadministered with the NH4Cl treatment.

In vitro microperfusion of mouse S2 proximal tubule segments. Under the in vitro microperfusion conditions employed, the tNH3 production and secretion rates were stable for >45 min after the onset of tube incubation in vitro. All studies were completed within that time period.

Table 1. Effects of losartan and short-term NH4Cl challenge on ammonia excretion

<table>
<thead>
<tr>
<th>Total CO2 Concentration, mM</th>
<th>Serum Potassium Concentration, mM</th>
<th>Urine tNH3/Creatinine, μmol/mg creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% sucrose water)</td>
<td>23.2 ± 0.5</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>Losartan in 2% sucrose</td>
<td>23.1 ± 0.7</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>NH4Cl in 2% sucrose</td>
<td>23.9 ± 0.6</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>NH4Cl + losartan in 2% sucrose</td>
<td>20.1 ± 0.8*</td>
<td>4.5 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. tNH3, total NH3. *P < 0.05 vs. other groups; n = 5 mice per group.
Effects of NH₄Cl administration in vivo on tNH₃ production and net luminal tNH₃ secretion by S2 proximal tubule segments perfused in vitro. In previous studies (21), we demonstrated that tNH₃ production and secretion rates were enhanced in S2 proximal tubule segments derived from mice given NH₄Cl for 7 days. In the present study, we examined the effect of an 18-h exposure to NH₄Cl on the rates of tNH₃ production and transport by isolated proximal tubule segments microperfused under normal bicarbonate and pH concentrations in vitro. The tNH₃ production rates observed in S2 segments derived from mice receiving NH₄Cl for 18 h were not significantly different from the rates observed in S2 segments derived from control mice not receiving NH₄Cl. As depicted in Fig. 1A, the tNH₃ production rates were not significantly different in S2 segments derived from NH₄Cl-challenged mice vs. those from control mice (23.1 ± 1.3 vs. 20.7 ± 1.0 pmol·min⁻¹·mm⁻²; n = 5). By contrast, as shown in Fig. 1B, the rates of net luminal tNH₃ secretion were higher in five S2 segments derived from NH₄Cl-treated mice compared with five segments from controls (14.8 ± 0.3 vs. 10.1 ± 0.9 pmol·min⁻¹·mm⁻²; *P < 0.05). When losartan was given with NH₄Cl, the enhancement in luminal tNH₃ excretion that was observed with NH₄Cl loading alone was not seen (11.4 ± 0.4 pmol·min⁻¹·mm⁻²; n = 5). When losartan was given without NH₄Cl, the rate of luminal tNH₃ secretion by S2 proximal tubules (10.2 ± 1.0 pmol·min⁻¹·mm⁻²; n = 5) did not significantly differ from the rate observed in S2 segments from control mice. Unlike the results obtained with a chronic (7-day) NH₄Cl acid-challenge protocol (21), treatment with NH₄Cl for 18 h failed to significantly increase tNH₃ production rates in S2 segments. Nevertheless, the 18-h exposure to NH₄Cl did result in an adaptive increase in luminal tNH₃ secretion rates by S2 proximal tubule segments, and the administration of the AT₁-receptor blocker losartan with the acid challenge blocked the adaptive increase in net luminal tNH₃ secretion.

Effect of amiloride on luminal tNH₃ secretion after 18-h acid challenge. We previously demonstrated (18) that microperfusing normal mouse S2 proximal tubule segments with the low-sodium perfusion solution containing 0.1 mM amiloride inhibits luminal fluid acidification by 100% and net luminal tNH₃ secretion by 90%. These results were consistent with transport of NH₄⁺ on the Na⁺/H⁺ exchanger (10). In the present study, we examined the effect of luminal perfusion with a low-sodium perfusate containing amiloride on net luminal tNH₃ secretion by S2 proximal tubule segments derived from control mice and mice given NH₄Cl for 18 h (Fig. 2). As with control S2 segments derived from non-acid-loaded mice, net tNH₃ secretion was inhibited by >90% by perfusion with the low-sodium amiloride-containing solutions. Net luminal tNH₃ secretion fell from 10.4 ± 0.8 to 1.0 ± 0.2 pmol·min⁻¹·mm⁻² in S2 segments from control mice and from 15.1 ± 0.1 to 1.4 ± 0.4 pmol·min⁻¹·mm⁻² in segments from acid-challenged mice (*P < 0.01; n = 5/group). Thus the enhanced tNH₃ transport mechanism observed in mice exposed to short-term acid loading appeared to be similar in nature to the mechanism observed in controls with both being inhibited by conditions that would inhibit Na⁺/H⁺ (NH₄⁺) exchange (10, 18).

Effects of luminal ANG II on tNH₃ production and net luminal secretion rates in S2 segments derived from NH₄Cl-loaded mice. In our previous studies (17), we demonstrated that ANG II added to the luminal perfusion alone was not seen (10.1 ± 0.9 pmol·min⁻¹·mm⁻²; n = 5) did not significantly differ from the rate observed in S2 segments from control mice. Unlike the results obtained with a chronic (7-day) NH₄Cl acid-challenge protocol (21), treatment with NH₄Cl for 18 h failed to significantly increase tNH₃ production rates in S2 segments. Nevertheless, the 18-h exposure to NH₄Cl did result in an adaptive increase in luminal tNH₃ secretion rates by S2 proximal tubule segments, and the administration of the AT₁-receptor blocker losartan with the acid challenge blocked the adaptive increase in net luminal tNH₃ secretion.

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fusional solution significantly stimulated tNH₃ production in S2 segments derived from normal mice. In the present study, we examined the effects of 10⁻⁹ M ANG II in the tubule lumen on tNH₃ production and net luminal secretion rates in control mice and mice given NH₄Cl for 18 h before the study (n = 5/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 (21.0 ± 0.6 pmol·min⁻¹·mm⁻¹ without ANG II and 28.1 ± 1.0 pmol·min⁻¹·mm⁻¹ with ANG II; P < 0.05) and acid-loaded mice (22.6 ± 0.9 vs. 39.4 ± 1.5 pmol·min⁻¹·mm⁻¹; P < 0.01). ANG II had a significantly greater stimulatory effect on tNH₃ production rates by S2 segments from NH₄Cl-treated mice compared with its effects on S2 segments from nonacidotic controls (P < 0.05). In previous studies (17), we demonstrated that the stimulatory effect of ANG II on total ammonia production was blocked by concurrent provision of the angiotensin-receptor blocker saralasin to the luminal fluid. The stimulatory effect of luminal ANG II on the tNH₃ production rate by S2 segments from acid-loaded mice was also inhibited by the addition of losartan (10⁻⁶ M) to the luminal fluid (21.4 ± 0.9 pmol·min⁻¹·mm⁻¹). These studies indicate that acid loading increases the ammoniagenic response of the proximal tubule to luminal ANG II that is mediated through interactions with an AT₁ receptor.

We also examined the effect of luminal 10⁻⁹ M ANG II on net luminal tNH₃ secretion in S2 proximal tubule segments from control and acid-loaded mice. ANG II increased luminal tNH₃ secretion by S2 proximal tubule segments from control mice (no ANG II, 11.1 ± 1.0 pmol·min⁻¹·mm⁻¹ vs. ANG II, 20.0 ± 1.5 pmol·min⁻¹·mm⁻¹; n = 5; P < 0.05; Fig. 4). The net luminal tNH₃ secretion rate by S2 segments from mice receiving NH₄Cl for 18 h was also markedly stimulated (no ANG II, 14.8 ± 0.5 pmol·min⁻¹·mm⁻¹ vs. ANG II, 30.4 ± 1.6 pmol·min⁻¹·mm⁻¹; n = 4; P < 0.01). The effect of ANG II on net luminal tNH₃ secretion in S2 proximal tubule segments from NH₄Cl-treated mice was greater than the effect observed in tubules from control mice (P < 0.05) and was blocked by 10⁻⁶ M losartan (15.3 ± 0.8 pmol·min⁻¹·mm⁻¹; n = 5). Thus ANG II stimulates net luminal tNH₃ secretion rates to a significantly higher extent in tubules from acid-loaded mice compared with those observed in non-acid-loaded controls. Furthermore, the effect of ANG II on luminal tNH₃ secretion rates in NH₄Cl-treated mice is abolished by the addition of losartan to the luminal perfusion solution.

DISCUSSION

In response to the introduction of excess acid to the body, the kidney increases urinary tNH₃ excretion before the induction of enhanced amounts of enzymes involved in tNH₃ production (4, 29). Thus the early response of the kidney to acid loading is to facilitate transport of tNH₃ into the urine. Enhanced rates of transport occur in the proximal tubule in response to acute acid loading (29). We previously demonstrated that with prolonged (7-day) treatment with NH₄Cl, both tNH₃ production and secretion rates underwent adaptive enhancement (20). The results of the present study demonstrated that short-term (18-h) NH₄Cl treatment induced an adaptive enhancement of luminal tNH₃ secretion without inducing a significant adaptive increase in the tNH₃ production rate. Thus the early response to acid challenges by the mouse proximal tubule may be to increase tNH₃ transport mechanisms before inducing increased amounts of ammoniagenic enzymes. The secretion of tNH₃ by the proximal tubule with its subsequent excretion into the urine would increase net acid excretion from the body.

ANG II has important effects on tNH₃ production and transport in the proximal tubule (2, 15, 17). Lumi-
nal ANG II not only stimulates tNH₃ production but also stimulates ammonia secretion into the luminal fluid (17). With acidosis in vivo the renin-angiotensin system has been shown to be upregulated (3, 9, 25). The results of the present study demonstrate that providing an inhibitor of AT₁ receptors, losartan, with the NH₄Cl challenge blocked both the increase in urinary tNH₃ excretion and the adaptive increase in the net luminal tNH₃ secretion rate by the proximal tubule that were observed with 18-h NH₄Cl administration without losartan. The administration of losartan had no effect on the basal rates of urinary tNH₃ excretion or the rates of tNH₃ production or secretion by proximal tubule segments from control mice not treated with NH₄Cl. These results suggest that ANG II plays an important role in modulating renal tNH₃ excretion and tNH₃ transport by the proximal tubule in response to acid challenges.

The major transport mechanism responsible for the increase in transport of tNH₃ with NH₄Cl treatment appeared to be fundamentally the same as the mechanism present under basal dietary conditions. As occurs in proximal tubules from control mice (18), luminal ammonia secretion in proximal tubules from NH₄Cl-treated mice was 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 the Na⁺/H⁺ exchanger (18), and the enhanced transport rate observed in S2 segments from NH₄Cl-treated mice may be due to the induction of additional amiloride-sensitive transporters or to the enhanced efficiency of existing tNH₃ transporters.

Luminal ANG II markedly stimulated tNH₃ production and secretion rates in proximal tubule segments dissected from non-acid-treated control mice and mice receiving NH₄Cl treatment for 18 h. The increase in the tNH₃ production rate in response to ANG II occurred in the absence of a significant rise in basal tNH₃ production rates with NH₄Cl treatment. A significantly greater increment in the luminal tNH₃ secretion rate in response to the addition of ANG II to the luminal perfusion solution was observed in S2 segments from mice that received short-term acid loading in vivo compared with that observed in segments from controls. In other words, acid loading with NH₄Cl in vivo greatly enhances the stimulatory effect of luminal ANG II in vitro on tNH₃ production and secretory rates. The results of the present study differ from the results of Quan and Baum (24), who demonstrated that AT₁ receptor and angiotensin-converting enzyme inhibition had a significant effect on fluid reabsorption in the absence of exogenous ANG II and that exogenous ANG II had no effect on fluid reabsorption rates. By contrast, our results demonstrated that the stimulation of tNH₃ production and transport rates by exogenous ANG II was blocked by losartan, but the rates did not fall below basal values. The differences observed may be due to differences in species, experimental protocols, or the functions measured. The concentration of ANG II that was present in the fluid of the tubule lumen with in vitro microperfusion in our studies was unknown. It is likely that the luminal concentrations were much lower than concentrations reported with in vivo microperfusion sampling (1, 22, 28) due to dilution and the lack of upstream cells that could contribute to the final ANG II concentration. As a result, our studies do not rule out a possible paracrine role of ANG II in vivo.

The ANG II that is present in the lumen of the proximal tubule in vivo could play a key role in enabling the proximal tubule to optimally generate and secrete more tNH₃ to maintain acid-base balance in the face of acid challenges.

In a recent study in human subjects, Henger and colleagues (9) demonstrated the importance of ANG II in maintaining high renal tNH₃ excretion rates in normal subjects with experimentally induced metabolic acidosis. They showed that in individuals with established NH₄Cl-induced acidosis and high rates of tNH₃ excretion, angiotensin receptor blockade led to net acid accumulation over a 4-day period that largely resulted from a reduction in urinary tNH₃ excretion and led to a worsening of acidosis. Our experiments differed from those of Henger and co-workers in that the mice used in our studies were not initially acidic when exposed to losartan and were not given an agent that blocks the action of aldosterone. In our study, the adaptive increases in tNH₃ excretion rates by the kidney and tNH₃ secretion rates by the proximal tubule in response to a short-term exposure to NH₄Cl were blocked by concurrent administration of the AT₁ receptor blocker losartan. Although the experimental design of the study by Henger and co-workers involved human subjects and pretreatment with NH₄Cl and spironolactone, their results were complementary to ours: their results demonstrated the importance of ANG II in allowing the kidneys to maintain optimal tNH₃ excretion with established acidosis, whereas our results demonstrated the role of ANG II in the enhancement of tNH₃ excretion in response to a short-term acid challenge.

In summary, the present studies demonstrated that 1) short-term acid loading enhances urinary tNH₃ excretion by mice and net luminal tNH₃ secretion by isolated perfused mouse S2 segments derived from NH₄Cl-treated mice, 2) ANG II plays an important role in the adaptive increase in urinary tNH₃ excretion and S2 proximal tubule tNH₃ secretion, and 3) short-term acid challenges increase the response of the proximal tubule to the stimulatory effects of ANG II on tNH₃ production and luminal secretion. Taken together, these results indicate the important role of ANG II in the regulation of the adaptation and response of the proximal tubule to acid challenges.

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REFERENCES