

Ammonia production by isolated mouse proximal tubules perfused in vitro. Effect of metabolic acidosis.

G T Nagami, C M Sonu, K Kurokawa

J Clin Invest. 1986;**78**(1):124-129. <https://doi.org/10.1172/JCI112540>.

Research Article

We examined the effects of metabolic acidosis in vivo and reduced bath and luminal pH in vitro on total NH₃ (NH₃ + NH₄) production rates by isolated mouse proximal tubule segments. Midproximal tubule segments were obtained from mice with NH₄Cl-induced metabolic acidosis and from nonacidotic controls. The segments were perfused with modified Krebs-Ringer bicarbonate (KRB) buffer, incubated in KRB buffer containing 0.5 mM L-glutamine and 1.0 mM sodium acetate, and gassed with 95% O₂ and 5% CO₂. Isolated unperfused and perfused proximal tubules from acidotic mice produced total NH₃ at higher rates than corresponding tubules from nonacidotic mice. Perfusion of the tubular lumen stimulated total NH₃ production by tubules from both acidotic and nonacidotic mice. In contrast, lowering the bath pH to 7.0 by lowering the HCO₃⁻ concentration increased total NH₃ production rates by tubules from nonacidotic mice but not by tubules from acidotic mice. Reducing the HCO₃⁻ concentration of the bath buffer to 10 mM while maintaining a pH of 7.4 had no significant effect on total NH₃ production by tubules from nonacidotic mice. Lowering the luminal fluid pH by reducing the perfusate HCO₃⁻ from 25 mM to 10, 5, or 1.2 mM while maintaining a bath pH of 7.4 lowered collected luminal fluid pH but had no effect on total NH₃ production by proximal tubules from nonacidotic mice. [...]

Find the latest version:

<https://jci.me/112540/pdf>



Ammonia Production by Isolated Mouse Proximal Tubules Perfused In Vitro

Effect of Metabolic Acidosis

Glenn T. Nagami,*‡ Charles M. Sonu,*‡ and Kiyoshi Kurokawa§

*Nephrology Section, Research and Medical Services, Veterans Administration Medical Center, West Los Angeles, Los Angeles, California 90073; ‡Department of Medicine, School of Medicine, University of California at Los Angeles, Los Angeles, California 90024; and §Department of Medicine, University of Tokyo, Tokyo, Japan

Abstract

We examined the effects of metabolic acidosis *in vivo* and reduced bath and luminal pH *in vitro* on total NH_3 ($\text{NH}_3 + \text{NH}_4^+$) production rates by isolated mouse proximal tubule segments. Midproximal tubule segments were obtained from mice with NH_4Cl -induced metabolic acidosis and from nonacidotic controls. The segments were perfused with modified Krebs-Ringer bicarbonate (KRB) buffer, incubated in KRB buffer containing 0.5 mM L-glutamine and 1.0 mM sodium acetate, and gassed with 95% O_2 and 5% CO_2 . Isolated unperfused and perfused proximal tubules from acidotic mice produced total NH_3 at higher rates than corresponding tubules from nonacidotic mice. Perfusion of the tubular lumen stimulated total NH_3 production by tubules from both acidotic and nonacidotic mice. In contrast, lowering the bath pH to 7.0 by lowering the HCO_3^- concentration increased total NH_3 production rates by tubules from nonacidotic mice but not by tubules from acidotic mice. Reducing the HCO_3^- concentration of the bath buffer to 10 mM while maintaining a pH of 7.4 had no significant effect on total NH_3 production by tubules from nonacidotic mice. Lowering the luminal fluid pH by reducing the perfusate HCO_3^- from 25 mM to 10, 5, or 1.2 mM while maintaining a bath pH of 7.4 lowered collected luminal fluid pH but had no effect on total NH_3 production by proximal tubules from nonacidotic mice. These observations demonstrated that metabolic acidosis *in vivo* stimulated total NH_3 production in isolated mouse proximal tubule segments and that low peritubular pH and HCO_3^- stimulated total NH_3 production by proximal tubule segments from nonacidotic mice *in vitro*.

Introduction

Enhanced rates of ammonia production and excretion by the kidney play key roles in the defense against acid challenges (1, 2). The proximal tubule appears to be the main source of the ammonia that enters the final urine (3–7). Furthermore, the proximal tubule is the major site of enhanced addition of ammonia to the luminal fluid with metabolic acidosis (5, 6).

Previous studies examining the response of the various segments of the nephron to acidosis have employed micropuncture and microcatheterization techniques to assess luminal ammonia

entry (3–6) and have examined isolated unperfused dissected nephron segments to measure ammonia production rates (7). Although these approaches have provided important insights into ammonia handling by specific nephron segments, each of them has certain limitations. The micropuncture and microcatheterization techniques may only be used to examine certain accessible portions of the nephron and, since only luminal fluid entry of ammonia is measured, the micropuncture technique cannot be used to directly determine ammonia production rates. Metabolic studies on unperfused nephron segments may not accurately reflect what occurs in the more physiologic perfused state. We have demonstrated previously that perfusion of the tubular lumen markedly enhanced the rates of total ammonia production by isolated proximal tubule segments from nonacidotic mice (8). No studies have directly examined total ammonia production by isolated perfused proximal tubule segments from acidotic animals.

Acute lowering of pH *in vitro* has been shown to increase ammonia production by cortical tubule suspensions from nonacidotic rats but not by those from acidotic rats (9). The tubule fragments used in those studies have open lumens and purportedly transport fluid and solutes. Although most of these tubules appear to be portions of proximal tubules, the precise origin of these tubule fragments and the rates at which these segments transport fluid and solutes from lumen to surrounding bath fluid are unknown. No study has examined the effect of acute *in vitro* metabolic acidosis on defined perfused proximal segments or the effects of peritubular and luminal pH on total ammonia production.

The aim of the present study was to examine the regulation of total ammonia ($\text{NH}_3 + \text{NH}_4^+$) production by isolated mouse proximal segments from acidotic mice. We also examined the effect of reduced bath and luminal fluid pH *in vitro* on total ammonia production by perfused and unperfused proximal segments from acidotic and nonacidotic mice. We studied the mouse because we have shown previously that mice excrete ammonia into the urine under normal dietary conditions and that proximal tubules may be dissected without collagenase so that they are suitable for microperfusion work (8). In the present studies we demonstrated that mice with ammonium chloride-induced metabolic acidosis excrete higher amounts of ammonia into the urine compared with nonacidotic controls, that proximal tubules from acidotic mice produce total ammonia at higher rates than corresponding tubule segments from nonacidotic mice, and that acidic pH of the bathing medium, but not acidic intraluminal pH, stimulates total ammonia production by proximal tubules segments from nonacidotic mice.

Methods

Animals. Male Swiss-Webster mice weighing 25–35 g were given free access to a rodent chow diet (Ralston Purina Co., St. Louis, MO), and

Portions of this study were presented at the 17th and 18th Annual Meetings of the American Society of Nephrology, Washington, DC, December 1984 and December 1985.

Address correspondence to Dr. Nagami, Nephrology Section 691/W111L, Veterans Administration Medical Center, West Los Angeles, Los Angeles, CA 90073.

Received for publication 22 July 1985.

The Journal of Clinical Investigation, Inc.
Volume 78, July 1986, 124–129

0.3 M ammonium chloride in 2% sucrose or 2% sucrose for 7 d. At the end of the 7 d, the mice were anesthetized with intramuscular injections of ketamine (0.05 mg/g body weight) and xylazine (0.05 mg/g body weight), and blood was obtained from the aorta for measurement of plasma bicarbonate. Urine was obtained from the bladder for measurement of total ammonia and creatinine.

Isolation and perfusion of proximal segments. As described previously, mouse nephron segments consisting of the late convoluted and early straight portions of the proximal tubule (S_2) were dissected from the outer cortex of an acidotic or a nonacidotic control mouse kidney (8). The lengths of the segments ranged from 0.6 to 1.2 mm. Using the technique of Burg and colleagues (10), we cannulated and perfused the dissected proximal segment with Krebs-Ringer bicarbonate (KRB)¹ buffer (composition: NaCl, 125 mM; NaHCO_3 , 25 mM; KCl, 5 mM; MgSO_4 , 1.0 mM; Na_2PO_4 , 1.2 mM; and CaCl_2 , 1.0 mM) in a perfusion chamber modified to hold a volume of 200 μl . The tubules were incubated in KRB buffer containing 0.5 mM L-glutamine and 1.0 mM sodium acetate equilibrated at 37°C with a gas mixture of 95% O_2 and 5% CO_2 . The bath medium was covered with mineral oil pregassed with 95% O_2 and 5% CO_2 , and the oil above the perfused segment was continuously bubbled with a jet of 95% O_2 and 5% CO_2 gas so that the perfused tubule vigorously vibrated below the jet. At the end of an incubation period of 20–30 min, an aliquot of the bath medium was removed for assay of total ammonia. The final bath volume was determined from the dilution of known amounts of trypan blue dye added to the perfusion chamber.

In experiments examining the effect of lowered bath pH on total ammonia production, the pH of the incubation bath medium was lowered to 7.0 by reducing the sodium bicarbonate concentration to 10 mM and by raising the sodium chloride concentration, thus maintaining a constant osmolality. The partial pressure of carbon dioxide in the gas mixture remained constant. The effect of reduced intraluminal pH on total ammonia production was examined by lowering the perfusate pH in a graded fashion from 25 mM to 10, to 5, or to 1.2 mM. The pH of collected luminal fluid was measured using a pH selective liquid ion exchanger electrode (World Precision Instruments, Inc., New Haven, CT) in conjunction with a KCl reference electrode. In experiments examining the effect of low bicarbonate concentration at a nonacidotic pH, the bicarbonate concentration of the bath buffer was lowered to 10 mM and the carbon dioxide content of the gassing mixture was reduced from 5 to 2%, thus maintaining a pH of 7.4.

Luminal ammonia output was measured in some experiments by using a second pipette to collect the luminal fluid leaving the distal end of the perfused segment.

Ammonia assay. We developed a method for measuring the small amounts of ammonia produced by an individual proximal segment (8). The method is based upon a series of enzymatic reactions which are coupled to a sensitive NAD-specific bioluminescence reaction. In the presence of excess NADH, α -ketoglutarate, and glutamic dehydrogenase, total ammonia in the sample or blank quantitatively reacts with NADH to form NAD^+ . The excess NADH is selectively destroyed by acid hydrolysis and the remaining NAD^+ is measured by a modification of the bioluminescence method of Brolin et al. (11). The relationship between plateau light emission and total ammonia concentration is linear and the coefficient of variation in the range of ammonia concentrations observed in our samples was 0.05–0.10.

Calculations and data analysis. As described previously (8), we measured total ammonia production by tubules in which the distal end of the tubule remained open to the bath. The rate of accumulation of total ammonia in the bathing medium reflected ammonia entering the bath from the basolateral and luminal portions of the tubule. Luminal ammonia output was defined as the rate at which total ammonia left the distal end of the perfused segment in timed luminal fluid samples collected with a collecting pipette.

Fluid reabsorption was measured by adding extensively dialyzed [^3H]methoxyinulin to the perfusion solution as previously described (12).

Perfusion flow rate was determined by the rate that [^3H]methoxyinulin left the distal end of the perfused segment.

Data are presented as mean \pm SE and were analyzed by analysis of variance.

Results

20 mice receiving 300 mM ammonium chloride in 2% sucrose for 7 d had a plasma bicarbonate concentration of 16.8 ± 0.6 mM, which was significantly lower than 20 control mice receiving 2% sucrose in water only (20.4 ± 0.4 mM) ($P < 0.01$). In addition, urinary excretion of total ammonia per milligram creatinine was higher in ammonium chloride-treated mice than that observed in nonacidotic control mice, 943 ± 93 and 46.0 ± 6.4 $\mu\text{mol}/\text{mg}$, respectively ($P < 0.001$). Thus, mice receiving ammonium chloride in 2% sucrose developed metabolic acidosis and had enhanced rates of total ammonia excretion compared with control mice receiving 2% sucrose without ammonium chloride.

Total ammonia production and fluid reabsorption rates for perfused proximal segments from control mice were stable for 90 min under the experimental conditions examined (8). In the present study, fluid reabsorption rates were measured in four proximal tubule segments from nonacidotic mice; the segments were perfused with KRB buffer and bathed in KRB buffer with 0.5 mM L-glutamine and 1.0 mM sodium acetate as substrates. The mean rate of fluid reabsorption was 0.94 ± 0.16 nl/min per mm. Total ammonia production rates by perfused segments from acidotic mice were stable for 60 min. Fluid reabsorption rates were measured in four proximal tubule segments from NH_4Cl -treated mice. The mean fluid reabsorption rate was 0.83 ± 0.11 nl/min per mm. All experiments were completed within 60 min of dissection of the proximal segments. All tubules excluded trypan blue added to the bath medium at the end of the experimental period.

Total ammonia production rates were measured in unperfused and perfused proximal segments from acidotic and control mice (Fig. 1). The total ammonia production rate of five unperfused proximal tubules from acidotic mice was 21.0 ± 2.0 pmol/min per mm which was significantly higher than the rate of seven unperfused proximal tubules from nonacidotic controls, 5.9 ± 0.3 pmol/min per mm ($P < 0.01$). The total ammonia production rate by six proximal segments from acidotic mice perfused at a flow rate of 18.7 ± 1.3 nl/min was 41.9 ± 3.2 pmol/min per mm, which was significantly higher than the rate observed in seven proximal tubules from control mice perfused at a flow

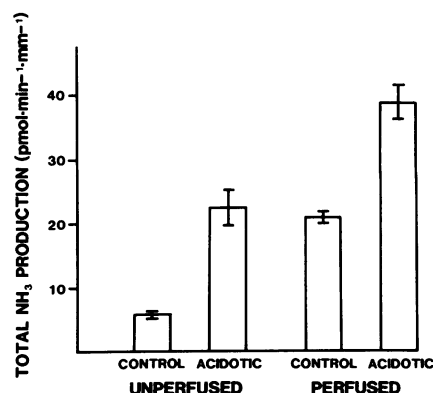


Figure 1. Total ammonia production by unperfused and perfused isolated proximal tubules from control and acidotic mice.

1. Abbreviation used in this paper: KRB, Krebs-Ringer bicarbonate buffer.

rate of 21.1 ± 1.1 nl/min, 21.5 ± 2.4 pmol/min per mm ($P < 0.01$). In addition, perfusion of proximal tubule segments increased the rate of ammonia production by tubules from acidotic mice or from nonacidotic controls ($P < 0.01$). Thus, chronic metabolic acidosis in vivo altered midproximal tubule segments so that even when removed from the kidney and incubated at pH 7.4 in vitro they produced ammonia at higher rates than segments from nonacidotic control mice.

We examined the effect of lowering the pH of the bath medium on total ammonia production by proximal tubule segments from control and acidotic mice (Figs. 2 and 3). Reducing the sodium bicarbonate concentration to 10 mM while maintaining a constant partial pressure of carbon dioxide resulted in a measured bath pH of 7.04 ± 0.02 , which was significantly lower than the pH observed when the bath bicarbonate concentration was 25 mM, 7.44 ± 0.04 ($P < 0.001$). Total ammonia production was measured in unperfused and perfused proximal tubule segments from control mice (Fig. 2). Lowering the bath pH from 7.4 to 7.0 increased the rate of total ammonia production by unperfused proximal tubule segments from control mice, 21.0 ± 0.9 pmol/min per mm at pH 7.0 ($n = 5$) vs. 5.9 ± 0.3 at pH 7.4 ($n = 7$) ($P < 0.01$). Nine proximal tubule segments from control mice perfused at a flow rate of 21.1 ± 0.8 nl/min and bathed at pH 7.0 produced total ammonia at a rate of 31.1 ± 1.4 pmol/min per mm, which was significantly higher than the rate observed in seven segments from control mice perfused at a similar flow rate but bathed at pH 7.4, 21.5 ± 1.1 pmol/min per mm ($P < 0.01$). Total ammonia production rates by unperfused and perfused proximal segments from acidotic mice were measured while they were bathed at pH 7.0 or 7.4 (Fig. 3). Unperfused proximal tubule segments from acidotic mice produced ammonia at rates of 22.8 ± 0.9 when incubated at pH 7.0 ($n = 5$) and 21.0 ± 2.0 when incubated at pH 7.4 ($n = 5$) ($P > 0.1$). Perfused segments from acidotic mice produced total ammonia at rates of 37.6 ± 1.6 pmol/min per mm when incubated at pH 7.0 ($n = 5$) and 41.9 ± 3.2 when incubated at pH 7.4 ($n = 6$) ($P > 0.1$). Thus, lowering the bath pH did not significantly alter the rates of ammonia production by perfused or unperfused proximal segments from acidotic mice.

To determine the reversibility of the effects of low pH in vitro on total ammonia production by perfused proximal tubules,

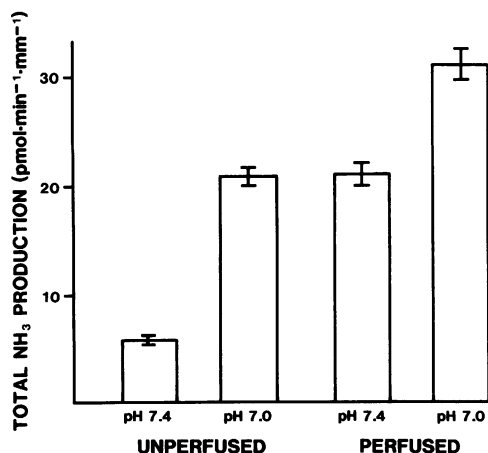


Figure 2. Total ammonia production by unperfused and perfused isolated proximal tubules from control mice incubated in bath media at pH 7.4 and 7.0.

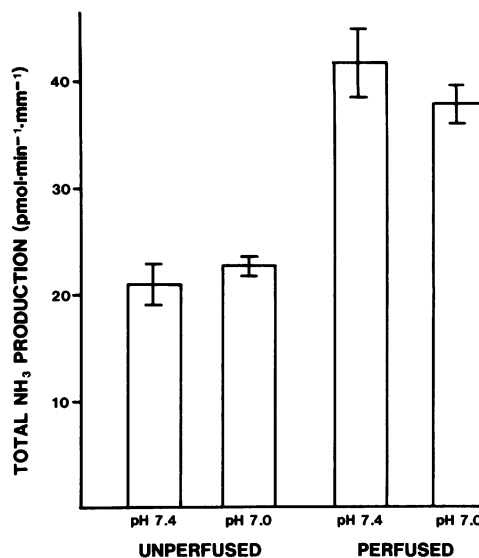


Figure 3. Total ammonia production by unperfused and perfused isolated proximal tubules from acidotic mice incubated in bath medium at pH 7.4 or 7.0.

we examined the rate of total ammonia production in tubules exposed to pH 7.0 (bicarbonate concentration, 10 mM) KRB buffer containing 0.5 mM L-glutamine and 1.0 mM sodium acetate for 4 or 10 min, washed with pH 7.4 (bicarbonate concentration, 25 mM) KRB buffer containing 0.5 mM L-glutamine and 1.0 mM sodium acetate, preincubated for 5 min in the pH 7.4 KRB buffer, washed with the pH 7.4 KRB buffer, and then incubated for 30 min in the pH 7.4 KRB buffer containing L-glutamine and sodium acetate (Fig. 4). Washing was accomplished by carefully exchanging the bath medium several times. Seven proximal tubule segments perfused at a flow rate of 21.5 ± 0.9 nl/min, preincubated at pH 7.0 for 4 min, and then incubated at pH 7.4 produced total ammonia at a rate of 19.2 ± 0.9 pmol/min per mm for at least 30 min, which was not significantly different from the rate of total ammonia production

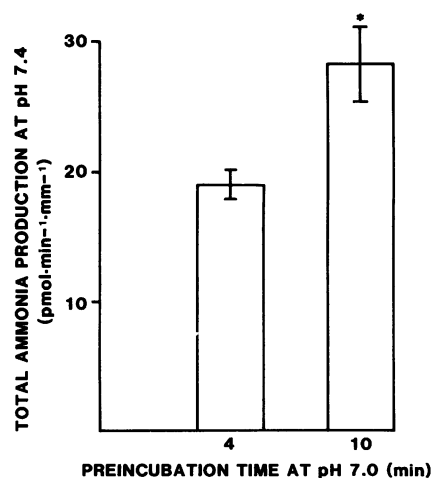


Figure 4. Total ammonia production by isolated perfused mouse proximal tubules preincubated for 4 or 10 min at pH 7.0 and then incubated at pH 7.4 (* $P < 0.01$ compared with tubules preincubated for 4 min).

by proximal tubules incubated solely at pH 7.4. Thus, 4-min exposure of tubules to the acidic bath pH induced no persistent effects in the tubules. On the other hand, proximal tubule segments perfused at a flow rate of 21.2 ± 0.7 nl/min, preincubated at pH 7.0 for 10 min, and then incubated for 30 min at pH 7.4 produced total ammonia at a rate of 28.6 ± 2.2 pmol/min per mm, which was significantly higher than the rates observed in tubule segments perfused at similar flow rates but preincubated for only 4 min at pH 7.0 or incubated solely at pH 7.4 ($P < 0.01$). Thus, incubation of these proximal tubule segments at low pH and bicarbonate concentration in vitro for 10 min induced changes in the proximal tubule which persisted for at least 30 min after the pH of the bath medium was increased from 7.0 to 7.4.

To assess the relative role of pH versus bicarbonate concentration on the regulation of total ammonia production by isolated mouse proximal tubules, we lowered the bicarbonate concentration of the incubation buffer to 10 mM and lowered the carbon dioxide content of the gassing mixture from 5 to 2% so that the pH remained at 7.4. Proximal tubule segments ($n = 9$) incubated in 10 mM bicarbonate KRB buffer at a pH 7.4 and perfused at 21.2 ± 0.5 nl/min produced total ammonia at a rate of 20.7 ± 1.4 pmol/min per mm, which did not differ from proximal tubule segments incubated in a buffer containing 25 mM bicarbonate at the same pH. Thus, low bicarbonate in the absence of low pH had no measurable effect on the rate of total ammonia production.

We examined the effects of lowering the perfusate pH on total ammonia production rate by perfused proximal tubules to determine the effects of low luminal versus low peritubular pH on the regulation of total ammonia production (Table I). We lowered the perfusate pH in graded fashion from 7.42 ± 0.03 to 7.04 ± 0.02 , 6.75 ± 0.02 , or 6.15 ± 0.03 by lowering perfusate bicarbonate concentrations from 25 mM to 10, 5, or 1.2 mM. The bath pH was maintained at 7.4. To verify that we actually lowered the luminal fluid pH, we measured the pH of collected luminal fluid samples using a pH selective ion exchanger with a reference electrode. The measured collected luminal fluid pH fell when the perfusate pH fell (Table I). The rates of total ammonia production by proximal tubules perfused with the low pH buffers did not differ significantly from each other or from tubules perfused with pH 7.4 buffer.

To characterize the release into the bath medium of total ammonia by perfused proximal tubule segments from acidotic and control mice, we examined, in some experiments, luminal ammonia output, defined as the rate that total ammonia left the distal end of the perfused segments in timed luminal fluid collections (Fig. 5). The perfusion and bath solutions were at pH

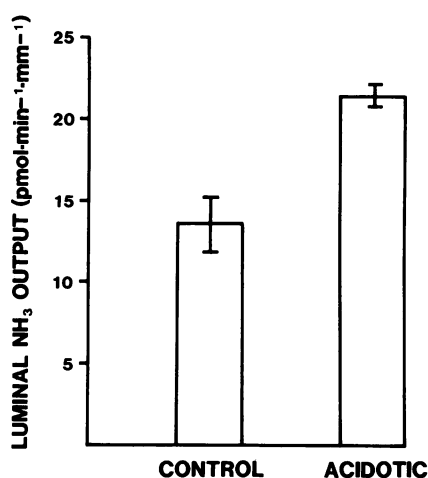


Figure 5. Luminal ammonia output by proximal segments from acidotic and control mice incubated in and perfused with KRB buffer at pH 7.4.

7.4. The luminal ammonia output observed in five perfused proximal segments from acidotic mice was 20.6 ± 0.8 pmol/min per mm, which was significantly higher than luminal ammonia output by eight perfused segments from control mice, 12.3 ± 0.5 pmol/min per mm ($P < 0.001$). Perfusion flow rates were not significantly different in the two groups (22.2 ± 2.5 vs. 20.9 ± 1.1 nl/min). Thus, the ammonia leaving the distal end of perfused proximal segments from acidotic mice was higher than that observed in proximal tubule segments from controls.

The relatively large contribution of luminal ammonia output to total ammonia production was explained by high luminal ammonia concentrations in collected luminal fluid samples. The luminal ammonia concentration was 0.96 ± 0.09 mM in perfused tubules from acidotic mice and 0.59 ± 0.02 mM in perfused tubules from controls. In contrast, bath ammonia concentrations were much lower, ranging from 1 to 3 μ M in perfused segments from control mice and from 4 to 6 μ M in perfused segments from acidotic mice. As a result, luminal ammonia concentrations were over 200 times higher than bath ammonia concentrations observed with incubation and perfusion of proximal segments from acidotic and control mice.

Discussion

The present studies employed our recently developed technique for measuring rates of total ammonia production by perfused proximal tubule segments (8). The advantages of this technique are that total ammonia production rates may be measured directly in defined and functionally active proximal tubule segments and that luminal and peritubular conditions may be altered in a controlled fashion.

The results of the present studies indicated that NH_4Cl -induced metabolic acidosis in vivo increased total ammonia production by segments of the midportion of the proximal tubule incubated in vitro. Metabolic acidosis in vivo induces adaptive changes in these segments, which persisted even after they were removed from the mouse and incubated at a nonacidotic pH of 7.4. Unperfused proximal segments from mice with metabolic acidosis produced ammonia at higher rates than segments from nonacidotic controls incubated at pH 7.4. These findings were

Table I. Effect of Perfusate pH on Total Ammonia Production Rates by Proximal Tubules

| Perfusate pH | n | Collected fluid pH | Flow rates | Total NH ₃ production rates |
|--------------|---|--------------------|------------|--|
| | | | nl/min | pmol/min per mm |
| 7.42±0.03 | 7 | 7.36±0.03 | 21.1±1.1 | 21.5±2.4 |
| 7.04±0.02 | 5 | 7.19±0.04 | 21.9±1.0 | 20.3±1.0 |
| 6.75±0.02 | 5 | 6.97±0.02 | 21.3±0.3 | 19.2±0.7 |
| 6.15±0.03 | 5 | 6.75±0.04 | 21.9±0.9 | 22.0±2.0 |

consistent with observations made in unperfused midproximal tubule segments (S₂) from rats with metabolic acidosis (7), although the magnitude of the increase in the rate of ammonia production with acidosis was higher in proximal tubule segments from our acidotic mice than was reported in corresponding segments from the acidotic rat.

Perfusion of the lumen of midproximal tubule segments from acidotic and nonacidotic mice resulted in measurable rates of active fluid reabsorption. The rates of fluid transport in unperfused segments could not be directly measured but must have been very low, since the lumen appeared to collapse during the incubation period. Luminal perfusion markedly increased the rate of total ammonia production by segments from acidotic and nonacidotic mice, so that the total ammonia production rate in perfused proximal segments from acidotic mice remained higher than the rate observed in perfused segments from nonacidotic control mice. The increased rate of ammonia production observed in proximal tubule segments from acidotic mice indicated that the rate of ammonia production may be stimulated further by perfusion of the lumen.

Lowering the bath pH by reducing the bicarbonate concentration *in vitro* stimulated total ammonia production by renal cortical tubule suspensions from rats (9). Our results indicated that lowering the bath pH and bicarbonate concentration stimulated total ammonia production by unperfused and perfused midproximal segments from nonacidotic control mice. In contrast, low pH *in vitro* did not affect total ammonia production rates by unperfused or perfused segments from acidotic mice. The latter results were consistent with observations in cortical tubule suspensions from acidotic rats (9) in which no further enhancement of total ammonia production occurred with reduced pH *in vitro*. Thus, perfusion of the tubular lumen and low pH stimulated ammonia production by proximal segments from normal mice while perfusion of the tubular lumen, but not low pH, stimulated ammonia production by proximal segments from acidotic mice. The reason for the difference in response to low pH by tubules from nonacidotic and acidotic mice may be that the metabolic machinery for ammonia production which was sensitive to pH changes was already maximally stimulated in proximal tubules from chronically acidotic mice so that lowering the pH *in vitro* did not stimulate further ammonia production. On the other hand, luminal perfusion increased total ammonia production by proximal tubule segments from both acidotic and nonacidotic mice, indicating that perfusion of the tubular lumen and low peritubular pH *in vitro* stimulated total ammonia production by a different mechanism.

The mechanism for perfusion-induced increases in the rate of total ammonia production remains unclear. We have demonstrated previously that increased fluid reabsorption rates, which are associated with perfusion of the lumen and increased flow, do not play an important role in the enhanced rate of total ammonia production observed with perfusion (8). Other possible mechanisms by which perfusion stimulates total ammonia production include the removal of an inhibitor of ammoniogenesis, such as a prostaglandin (13), or increased delivery of the substrate, L-glutamine, to the sites of glutamine metabolism, which could occur with backflux of glutamine from bath to lumen and with subsequent luminal uptake and intracellular metabolism or with metabolism within the lumen (14). Further studies to delineate the mechanism of perfusion-induced stimulation of total ammonia production are required.

In the present studies, exposure of proximal tubules to low

bath pH and bicarbonate concentration induced changes within the proximal tubule that led to persistently high total ammonia production rates even after the bathing medium was returned to pH 7.4. The mechanism for the sustained response to low pH was independent of hormonal or neural influences because the response occurred *in vitro*. Whether induction of the synthesis of important ammoniagenic enzymes, such as phosphate-dependent glutaminase, occurred in 10 min of *in vitro* acidic pH could not be determined because of technical problems in the direct measurement of rates of enzyme synthesis in individual tubule segments over short periods of time.

Our data showing that low bath pH stimulated total ammonia production rates by proximal tubules while low luminal fluid pH had no effect on total ammonia production rates indicated that peritubular rather than intraluminal low pH was the important stimulus for ammonia production. This was the first direct demonstration that peritubular rather than intraluminal low pH stimulates total ammonia production by the proximal tubule. These data suggested that peritubular pH rather than intraluminal pH may affect factors, such as intracellular pH, that may regulate total ammonia production. Indeed, such an interpretation would be consistent with Alpern's data in the doubly microperfused rat proximal tubule, which indicated that lowering the pH of the peritubular fluid had a greater effect on lowering the intracellular pH than lowering the pH of the luminal fluid (15).

Our results indicating that low pH and bicarbonate concentration of the bath, but not low bicarbonate concentration alone, stimulated total ammonia production are consistent with the data of Baverel and Lund in rat cortical tubule suspensions (16). They also demonstrate that isohydric reduction of the bicarbonate concentration of the bath medium does not stimulate total ammonia production. In contrast, results of experiments by Scaduto and Schoolwerth suggest that bicarbonate ion, independent of pH, may play an important role in the regulation of ammonia production by isolated mitochondria (17). The apparent differences in results may have arisen from differences between the experimental models studied. In the intact tubule, ammonia production rates depend upon the interaction of many factors, such as substrate transport, cytosolic metabolism, and mitochondrial metabolism, while in the isolated mitochondrial preparation fewer factors may be involved. Furthermore, our study differed from the study of Scaduto and Schoolwerth in that we reduced bicarbonate concentration at a constant pH while Scaduto and Schoolwerth completely removed bicarbonate from the medium. Indeed, others have demonstrated that differences in the intracellular pH (18) and mitochondrial matrix pH (19) may be observed when the incubation medium buffer is changed at a constant pH from a bicarbonate-CO₂ buffer to a nonbicarbonate buffer.

In conclusion, total ammonia production by isolated proximal tubule segments is enhanced by *in vivo* acidosis. Perfusion of the tubule lumen increases total ammonia production rates in proximal tubule segments from acidotic and nonacidotic mice, whereas lowering the bath pH and bicarbonate concentration stimulates total ammonia production by proximal tubule segments from nonacidotic mice, but not by segments from acidotic mice. Thus, perfusion and low bath pH stimulate total ammonia production by different mechanisms. Preincubation of proximal tubule segments from nonacidotic mice at a bath pH of 7.0 for 10 min results in persistently elevated rates of total ammonia production, even after the segments are washed and then in-

cubated at pH 7.4. This result is in agreement with the persistently high rates of total ammonia production by isolated proximal tubule segments from mice with chronic metabolic acidosis. In addition, low peritubular bicarbonate concentration without low pH, and low intraluminal pH and bicarbonate concentration do not alter total ammonia production rates. These findings demonstrate the importance of the role of peritubular pH on the regulation of total ammonia production.

Acknowledgments

This research was supported by the Veterans Administration, by a research grant from the National Kidney Foundation of Southern California, and, in part, by a grant-in-aid from the American Heart Association with funds contributed in part by its Florida affiliate. Dr. Nagami is a Research Associate and was an Associate Investigator of the Veterans Administration.

References

1. Pitts, R. F. 1973. Production and excretion of ammonia in relation to acid-base regulation. In *Handbook of Physiology, Section 8: Renal Physiology*. J. Orloff and R. W. Berliner, editors. American Physiological Society, Washington, DC. 455–496.
2. Tannen, R. L. 1978. Ammonia metabolism. *Am. J. Physiol.* 235: F265–F277.
3. Glabman, S., R. M. Klose, and G. Giebisch. 1963. Micropuncture study of ammonia excretion in the rat. *Am. J. Physiol.* 205:127–132.
4. Hayes, C. P., Jr., J. S. Mayson, E. E. Owen, and R. R. Robinson. 1964. A micropuncture evaluation of renal ammonia excretion in the rat. *Am. J. Physiol.* 207:77–83.
5. Sajo, I. M., M. B. Goldstein, H. Sonnenberg, B. J. Stinebaugh, D. R. Wilson, and M. L. Halperin. 1981. Sites of ammonia addition to tubular fluid in rats with chronic metabolic acidosis. *Kidney Int.* 20:353–358.
6. Buerkert, J., D. Martin, and D. Trigg. 1982. Ammonia handling by superficial and juxtamedullary nephrons in the rat. *J. Clin. Invest.* 70:1–12.
7. Good, D. W., and M. B. Burg. 1984. Ammonia production by individual segments of the rat nephron. *J. Clin. Invest.* 73:602–610.
8. Nagami, G. T., and K. Kurokawa. 1985. Regulation of ammonia production by mouse proximal tubules perfused in vitro. Effect of luminal perfusion. *J. Clin. Invest.* 75:844–849.
9. Vinay, P., G. Lemieux, A. Gougoux, and C. Lemieux. 1980. Response of the rat and dog kidney to H^+ concentration in vitro: a comparative study with slices and tubules. *Int. J. Biochem.* 12:89–98.
10. Burg, M. B., J. J. Grantham, M. Abramow, and J. Orloff. 1966. Preparation and study of fragments of single rabbit nephrons. *Am. J. Physiol.* 210:1293–1298.
11. Brolin, S. E., A. Agren, J. P. Wersall, and S. Hjerren. 1978. Simplified bioluminescence analysis by continuous enzymatic reduction of $NADP^+$ using bacterial luciferase. In *Proceedings of the International Symposium on Analytical Applications of Bioluminescence and Chemiluminescence*. E. Schram and P. Stanley, editors. State Printing and Publishing, Inc., Westlake Village, CA. 109–121.
12. Yanagawa, N., G. T. Nagami, O. Jo, J. Uemasu, and K. Kurokawa. 1984. Dissociation of gluconeogenesis from fluid and phosphate reabsorption in isolated rabbit proximal tubules. *Kidney Int.* 25:869–873.
13. Jones, E. R., T. R. Beck, S. Kapoor, R. Shay, and R. G. Narins. 1984. Prostaglandins inhibited renal ammoniogenesis in the rat. *J. Clin. Invest.* 74:992–1002.
14. Silbernagl, S. 1985. Ammoniogenesis in the lumen of the proximal tubule or not? *Contrib. Nephrol.* 47:157–164.
15. Alpern, R. J. 1986. Control of cell pH (pH_i) in the rat proximal convoluted tubule (PCT) by apical and basolateral membrane (BLM) transporters. *Kidney Int.* 29:362. (Abstr.)
16. Baverel, G., and P. Lund. 1979. A role for bicarbonate in the regulation of mammalian glutamine. *Biochem. J.* 184:599–606.
17. Scaduto, R. C., Jr., and A. C. Schoolwerth. 1985. Effect of bicarbonate on glutamine and glutamate metabolism by rat kidney cortex mitochondria. *Am. J. Physiol.* 249:F573–F581.
18. Kleinman, J. G., R. A. Ware, and J. H. Schwartz. 1981. Anion transport regulates intracellular pH in renal cortical tissue. *Biochim. Biophys. Acta.* 648:87–92.
19. Simpson, D. P., and S. R. Hager. 1984. Bicarbonate-carbon dioxide buffer system: a determinant of the mitochondrial pH gradient. *Am. J. Physiol.* 247:F440–F446.