Alterations in Renal and Hepatic Nitrogen Metabolism in Rats During HCl Ingestion

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The effect of prolonged metabolic acidosis on hepatic and renal enzymes associated with nitrogen metabolism was investigated. The rates of urinary ammonia and urea excretion were also determined. Administration of 9 mmol HCl daily for 8 days resulted in severe metabolic acidosis. The activity of the first two enzymes of the urea cycle, carbamoyl phosphate synthetase (CPS) and ornithine transcarbamoylase (OTC), was 30% greater in chronically acidotic rats than in pair-fed controls. There was also a fivefold increase in renal phosphate-dependent glutaminase (PDG) activity and an 18 to 24-fold increase in renal ammonia excretion. Urea excretion was not constant in the acidotic group, decreasing during the first 4 days and gradually returning to pair-fed control levels between the fourth and eighth day. The return to control levels of urinary urea excretion coincided with the plateau of urinary ammonia excretion that occurred by day 4 in the acidotic group. A similar pattern of urea nitrogen excretion has been observed in both NH_4Cl and HCl acidosis, ie, an initial decrease in urea excretion followed by a gradual increase with time. These results suggest that hepatic urea synthesis does not play a significant role in long-term regulation of the acid-base balance in rats during chronic metabolic acidosis.

UREA SYNTHESIS has traditionally been viewed as serving to dispose of excess ammonia resulting from the catabolism of dietary and endogenous protein. Two molecules of ammonia (one derived from aspartate) and one molecule of bicarbonate are consumed in the reaction, which is catalyzed by the sequential activity of the five urea cycle enzymes. The equivalent of two protons are also liberated during the first three steps of the cycle. Long-term flux through the urea cycle is primarily a function of the periportal amino acid load, ie, protein intake or mobilization of endogenous protein stores, and subsequent catabolism.[1-3]

Decreased urea excretion or diminished flux through the urea cycle has been a frequent finding during experimentally induced metabolic acidosis in vivo,[4-9] in vitro,[10-12] and during perfusion studies.[13-18] During such episodes, an increase in the renal extraction and metabolism of glutamine occurs, resulting in a significant increase in ammonium excretion in addition to increased production of bicarbonate during subsequent catabolism of the remaining α-ketoglutarate anion.[8,19] Because urea synthesis is a proto-generating process, the simultaneous decrease in urea synthesis and excretion is considered to result in a sparing of bicarbonate and a diminution of the acidosis.[20] Thus, a repartitioning of ammonia from urea to glutamine is viewed as a major homeostatic response in mammals by serving to increase the net systemic bicarbonate concentration. However, there is little information on the pattern of urea and ammonia excretion in whole organisms over extended periods, and in vitro and perfusion studies can only provide information on acute acidosis and do not take account of the interorgan cooperation and whole organism response to such perturbations. We have recently found a decrease in urea excretion during the first 2 days only of NH_4Cl ingestion in rats, followed by a significant increase in urea excretion relative to control levels by the seventh day.[21] Other groups have found no change in the endogenous rate of urea production following HCl, NH_4Cl, or NH_4HCO_3 infusion for short periods (1 to 3 hours) into the femoral vein of rats.[22] Similar rates of urea production were also found with either ammonium chloride or ammonium bicarbonate during short periods of infusion.[23] These findings were interpreted as suggestive of a primary role for ureogenesis in ammonia disposal rather than in acid-base homeostasis.

Investigations of the enzyme changes accompanying metabolic acidosis have focused mainly on the kidney.[24-27] Two studies have reported the effect of NH_4Cl acidosis on hepatic urea cycle enzymes.[9,21] However, the effect of chronic HCl acidosis on urea cycle enzymes has not been reported. In light of the recent finding that NH_4Cl results in only a temporary decrease in urea excretion during experimentally induced metabolic acidosis,[21] it was of interest to investigate the pattern of urea and ammonia excretion and the activity of the urea cycle enzymes during chronic HCl acidosis. In addition, information regarding the pattern of urea excretion during long-term ingestion of acid without the potentially complicating effects of the ammonium moiety might also provide further insight into the relative importance of hepatic urea production over longer periods.

MATERIALS AND METHODS

In Vivo Procedures

Two groups of 11 female Sprague-Dawley rats weighing approximately 225 g were transferred to metabolic cages 7 days before commencement of the experiment. During this period of acclimatization, they were given powdered pellets (Redmills, Kilkenny, Ireland; 17% protein), allowed free access to tap water, and exposed to a 12-hour light-dark cycle. After the acclimatization period, metabolic acidosis was induced in the experimental group in a modification of the method of Almond et al[28] by giving a mixture of equal amounts of 0.8 mol/L HCl and powdered pellets daily for 8 days. During a preliminary study, it was noted that the consumption of powdered pellets by the acidotic group was, on average, only 50% that of the control group. The control group was therefore pair-fed the same quantity of powdered pellets as the acidotic group, mixed with an equal volume of tap water. Urine was collected daily in flasks containing thymol preservative, and daily recordings were made of food and water intake. At the end of the experimental period, rats were anesthetized with ether and two separate

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blood samples were obtained by cardiac puncture. The first of these was obtained anaerobically and heparinized for analysis of blood acid-base measurements. The second sample was centrifuged at 3,000 × *g* for 15 minutes and subsequently subjected to serum urea analysis. Following ether overdose, the liver and kidneys were removed and stored at −70°C and −20°C for assay of the urea cycle enzymes and kidney glutaminase, respectively. Urea cycle enzyme activities were determined within 7 days of death.

Metabolite Determinations

Serum urea analysis was performed using a Technicon multiple analyzer, and blood acid-base analyses were made with a radiometer ABL_3 blood gas analyzer. Urinary ammonia was determined by the reductive amination of oxoglutarate to glutamate by NADH and sample ammonia using glutamate dehydrogenase.[29] Urinary urea was determined by this method following pretreatment of the sample with urease.[29]

Enzyme Activities

Hepatic carbamoyl phosphate synthetase ([CPS] EC 6.3.4.16) and ornithine transcarbamoylase ([OTC] EC 2.1.3.3) activities were assayed by measuring the amount of citrulline formed according to the method of Nuzum and Snodgrass,[30] except that no supplementary OTC was used in the determination of CPS. Hepatic arginase (EC 3.5.3.1) activity was assayed by measuring the amount of urea according to the method of Nuzum and Snodgrass.[30] A third group of rats fed laboratory pellets and chow ad libitum for the duration of the experimental period served as positive controls for the urea cycle enzymes. Renal phosphate-dependent glutaminase ([PDG] EC 3.5.1.2) and phosphate-independent glutaminase (PIG) levels were measured in whole homogenates of kidney by measuring the amount of ammonia in the supernatant released from added glutamine with and without addition of phosphate, according to the method of O'Donovan.[31]

Statistical Methods

Results are given as the mean ± SEM. Differences between groups were tested with the two-tailed Student *t* test. *P* values less than .05 are considered statistically significant.

RESULTS

Physiological changes in the rats associated with incorporating HCl into the diet are listed in Table 1. Rats given powdered pellets and water ad libitum normally consume approximately 25 g food daily (Lardner AL and O'Donovan DJ, unpublished observations, September 1996). When 0.8 mol/L HCl was mixed with the food, food consumption decreased to 12 g/d (Table 1). Fluid intake was similar in pair-fed and acid-fed

Table 1. Physiological Effects of HCl Ingestion on the Rat

Parameter	Control (pair-fed)	Acidotic
Food intake (g/d)	12.6 ± 0.5	11.8 ± 0.5
Fluid intake (mL/d)*	23.0 ± 1.0	25.7 ± 1.5
Final body weight (g)†	204 ± 6.4	191 ± 5.5
Kidney weight (g)	1.86 ± 0.05	1.90 ± 0.04
HCl (mmol/d)	—	9.4 ± 0.04
Urine flow rate (mL/d)	13.7 ± 0.7	17.0 ± 1.4
Serum urea (mmol/L)	9.04 ± 1.35	9.55 ± 1.28
Blood pH	7.37 ± 0.02	7.00 ± 0.06

NOTE. Values are the mean ± SEM for 11 rats in each group. Blood pH, serum urea, and kidney weight were measured on the final day (day 8) ($P < .0001$).

*Combined intake from water bottle and food mixture.

†Rats were of similar weight at the start of the experiment.

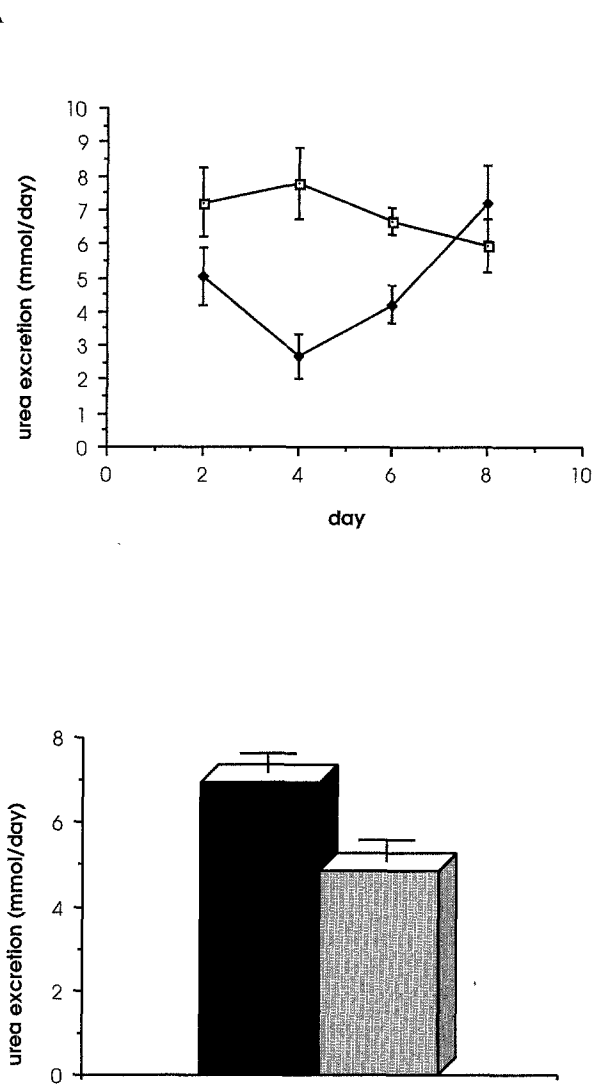

Fig 1. (A) Effect of chronic HCl ingestion on the rate of urinary urea excretion in (□) control and (◆) acidotic rats. (B) Mean urea excretion for the 8-day period in (▨) chronically acidotic and (■) control rats. Values are the mean ± SEM (n = 10 rats per group).

groups when account was taken of the volume of HCl in the acidotic group. Thus, with this experimental design, rats ingested greater than 9 mmol HCl daily. This volume of acidifying substance is slightly greater than the amount rats will consume when allowed to drink a 0.28-mol/L NH_4Cl solution ad libitum. The urine flow rate was 60% and 66% of fluid intake for the control and acidotic groups, respectively. Ingestion of HCl resulted in a small but nonsignificant decrease in body weight, while the kidney weight was not affected (Table 1). Blood pH was significantly decreased by 0.37 units in the acidotic group (Table 1), as were blood bicarbonate and acid-base excess (results not shown). These results indicate the presence of systemic acidosis in rats given HCl.

Ingestion of HCl did not significantly alter the serum urea concentration on the final day (Table 1). Urinary urea output during the 8 days is shown in Fig 1A and B. Urea excretion in the pair-fed control group (Fig 1A) was approximately 7 mmol/d. Urinary urea in the acidotic group decreased to less

than 3 mmol/d during the first 4 days of HCl ingestion. However, between days 4 and 8, daily urea excretion gradually increased such that by day 8 there was no significant difference in urea excretion between the control and acidotic groups. The mean urinary urea excretion for the 8-day period is shown in Fig 1B. Acidotic animals excreted a mean of 30% less urea than pair-fed controls over the 8-day period. Serum urea levels normally reflect urea output by the liver, provided urea clearance by the kidney is not altered. Although renal urea clearance was not measured in these experiments, the lack of difference between control and acidotic animals in both serum and urinary urea on day 8 would suggest that renal urea clearance was not affected by HCl acidosis in the present study.

The activities of CPS, OTC, and arginase on day 8 are listed in Table 2. These enzymes were assayed in rats fed ad libitum, as well as the acidotic and pair-fed control groups. A reduction in food intake from 25 to 12 g/d resulted in 34% lower CPS activity relative to the level in rats fed ad libitum, while incorporation of HCl in the diet resulted in similar values as obtained with the ad libitum diet. Thus, CPS activity following ingestion of HCl for 8 days was 52% greater than in the pair-fed control group. A similar trend occurred with hepatic OTC activity, with the value in the acidotic group exceeding that of the pair-fed controls by 28% ($P < .025$). Similar to CPS activity, OTC activity was 25% lower in the pair-fed group versus the ad libitum control group. Acidosis did not significantly influence hepatic arginase activity.

Renal PDG activity is shown in Fig 2. PDG was increased nearly fivefold in acidotic animals ($P < .0001$), whereas there was no difference in PIG activity between the groups (results not shown).

Daily urinary ammonia excretion is shown in Fig 3. Ammonia excretion in the pair-fed control group was, on average, 0.19 ± 0.02 mmol/d. On all days examined, HCl acidosis induced large (18- to 26-fold) increases in urinary ammonia excretion ($P < .001$), reaching a plateau by day 4.

DISCUSSION

The present study indicates that the induction of chronic metabolic acidosis by HCl ingestion results in higher activities of both CPS and OTC in the rat liver relative to the levels in pair-fed controls. The decreased activity in the pair-fed controls relative to the ad libitum controls is consistent with a reduction in dietary protein intake of the former.[2-3] Enzyme activities in the acidotic group are the same as those of the rats fed ad libitum, suggesting that HCl acidosis prevented the decrease in activity associated with reduced protein intake. However, it is possible that a temporary decrease in CPS and OTC activity occurred at earlier time points coincident with the maximum decrease in urea excretion. Further studies will establish if this is so. Such specific acid-induced changes in hepatic mitochondrial enzymes have not previously been reported. It is noteworthy that there was no effect of HCl on arginase, which is located in the cytosol, suggesting that HCl exerted its effects exclusively within the mitochondrial matrix. Previous groups have reported little or no change in the activity of the urea cycle

Table 2. Hepatic Urea Cycle Enzyme Activity (IU)

Group	CPS	OTC	Arginase
Control (ad libitum)	21.0 ± 1.6	265.0 ± 13.4	ND
Control (pair-fed)	14.0 ± 1.5	197.8 ± 15.4	1,150 ± 118
HCl acidotic	21.2 ± 1.7*	253.4 ± 13.4*	1,030 ± 90

NOTE. Data show the effect of chronic HCl ingestion on hepatic urea cycle enzyme activity on day 8. Assays were made in triplicate in whole-liver homogenates. One unit is defined as the amount of enzyme catalyzing the formation of 1 μmol citrulline (CPS and OTC) or urea (arginase)/g wet liver weight/min. Values are the mean ± SEM from 8 rats per group.

Abbreviation: ND, not determined.

*$P < .05$, acidotic *v* pair-fed.

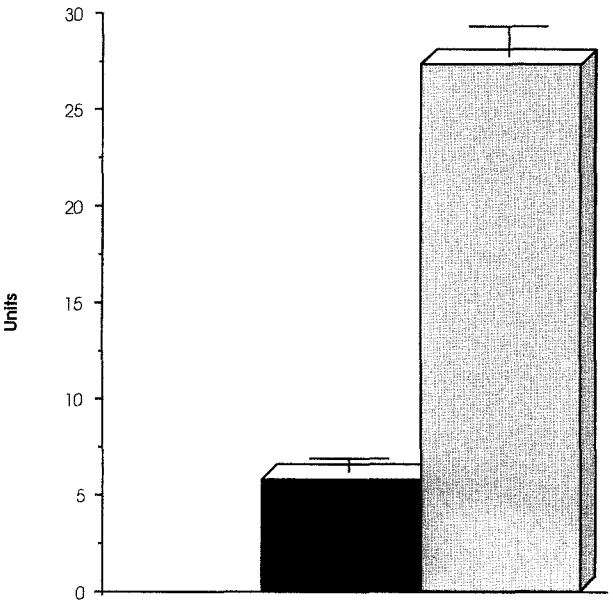

Fig 2. Effect of chronic HCl ingestion on (■) control and (▨) acidotic rat renal PDG activity on day 8. Assays were made in whole-kidney homogenates. One unit of activity is the amount of enzyme catalyzing the formation of 1 μmol NH_3 from glutamine/min. All estimations were made in duplicate. Values are the mean ± SEM (n = 11 rats per group).

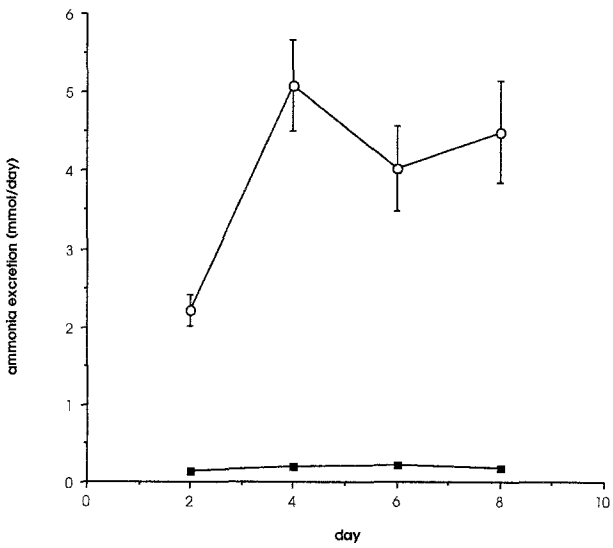

Fig 3. Effect of chronic HCl ingestion on urinary ammonia excretion in (■) control and (○) acidotic rats. Values are the mean ± SEM (n = 6 rats per group).

enzymes following NH_4Cl administration to rats for 4 to 7 days.[9,21] However, food intake does not decrease in animals consuming NH_4Cl in the drinking water.[21] It is therefore possible that the lack of reduction in food consumption by rats during NH_4Cl acidosis may mask the stabilizing effect of metabolic acidosis and reduced food intake on mitochondrial CPS and OTC activities. Whether the increases in mitochondrial enzyme activity serve an adaptive role in chronic HCl acidosis is not known and remains to be established.

The fivefold increase in renal PDG activity is consistent with the development of chronic metabolic acidosis and is in general agreement with findings from other in vivo studies.[4,5,7-9,19] The increased ammoniagenesis resulting from hydrolysis of glutamine extracted from the renal artery during chronic acidosis provides an expendable cation that can be easily excreted in the urine. Urinary ammonia output in our experiment showed a marked increase in the acidotic group on all days, reaching a plateau by day 4. Rector et al[19] found that ammonia excretion peaked at day 6 following administration of 5 mmol NH_4Cl daily. Oliver and Bourke[7] reported a ninefold increase in ammonia excretion following ingestion of 6 mmol HCl daily. The greater acid load consumed in the present study may account for the earlier plateau and greater excretion of ammonia.

The overall rate of urinary urea excretion was decreased by 30% in acidotic rats and is consistent with the results from Oliver and Bourke.[7] Phromphetcharat et al[8] reported a 42% decrease in urea excretion in male rats given 8 to 11.5 mmol NH_4Cl daily for 1 week. Both groups pooled their urea estimations and presented them as a single result. However, it is clear that when urinary urea excretion is reported in this manner, the pattern of urea excretion is masked. When examined over the 8-day period, urinary urea excretion in the present study decreased over the first 4 days and then gradually returned to normal over the following 4 days. This increase coincided with the maximum urinary ammonia excretion and indicates that during the first 4 days of metabolic acidosis, an overall shift in nitrogen partitioning from urea formation to ammonia production occurs. This is consistent with the accepted role for coordinated liver and kidney function in minimizing bicarbonate consumption via decreased ureogenesis and maximizing its production via renal catabolism of glutamine carbon skeleton.

The increase in urea production between days 4 and 8 is unexpected and has not been previously reported. It suggests that during prolonged metabolic acidosis in vivo, hepatic ureogenesis does not play a significant role in acid-base homeostasis as has been traditionally assumed. This calls into question the importance of bicarbonate sparing by the liver during longer acidotic episodes. The mechanisms responsible for the return of urea excretion to control levels are unclear; this may have been at least partly due to an increase in skeletal muscle proteolysis, which has been shown to increase during metabolic acidosis in vivo[32,33] and is postulated by several investigators to constitute an important source of systemic bicarbonate during same.[17,20] An increase in skeletal muscle proteolysis might also provide ammonia substrate for ureogenesis, thus increasing net urea excretion. The first step of branched-chain amino acid degradation in muscle involves transamination with α-ketoglutarate, resulting in the formation of glutamate and the corresponding α-keto acid. The conversion of glutamate to alanine and subsequent release of the latter from skeletal muscle could theoretically provide ammonia substrate for urea synthesis. However, alanine output by skeletal muscle has been shown to decrease during metabolic acidosis,[34] thus making it unlikely that the increase in urea excretion derives from skeletal muscle protein degradation. Glutamine synthetase activity and glutamine release by muscle also increases during metabolic acidosis[35] and is presently considered to provide a substantial proportion of renal glutamine requirements and subsequent ammonia excretion.[36] On the basis of these considerations, degradation of skeletal muscle amino acids is likely to result in increased excretion of ammonia rather than urea by the kidneys during acidosis. However, further studies are required to establish unequivocally the ultimate fate of the ammonia moiety resulting from skeletal muscle proteolysis during chronic acidosis. Although an increase in hepatic amino acid degradation has been shown to occur in acute metabolic acidosis,[17] no alteration in the former was observed during chronic (7-day) metabolic acidosis.[18] The duration, means of induction, and total administered dose of HCl acidosis during the latter study were similar to ours. It is unlikely therefore that in the present study nitrogen substrate for ureogenesis was provided by an increase in hepatic protein breakdown. It is therefore tempting to speculate that between days 1 and 4 of the present study, renal glutamine requirements were met primarily by dietary protein, causing a decrease in urea formation and excretion. During this period, a gradual adaptation of skeletal muscle proteolysis also occurred, resulting in a switch from exogenous to endogenous glutamine provision for renal ammonia excretion and a corresponding return to control levels of urea synthesis over the following 4 days. Further studies are necessary to establish if this is so.

The pattern of nitrogen excretion during NH_4Cl[21] and HCl acidosis differs both qualitatively and quantitatively. Ammonia excretion was greater during HCl versus NH_4Cl acidosis, probably as a result of the milder degree of acidosis induced with the ammonium salt. Urea excretion with the latter decreased during the first 2 days and returned to control levels by day 4, increasing significantly relative to control levels by day 7.[21] During HCl acidosis, urea excretion decreased for 4 days and only returned to control levels by day 8. Given the slight nonsignificant increase in urea excretion in the HCl acidotic group versus the controls on day 8, it is possible that after this time a gradual increase in urea excretion relative to control levels might also occur, similar to that prevailing during NH_4Cl acidosis. Further studies are required to establish the pattern of urea and ammonia excretion from day 8 onward during HCl acidosis. However, it is noteworthy that a similar pattern constituting an initial decrease followed by a gradual increase in urea excretion occurred with both salts. This suggests that, irrespective of the type of metabolic acidosis, nitrogen partitioning between urea and glutamine constitutes only a temporary homeostatic mechanism during chronic acidotic episodes.

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