Energy turnover and the production of ammonium by the kidney: effect of hypernatremia

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The purpose of this study was to explore further the relation between the rates of oxygen consumption and ammonium (NH_4^+) production in the kidney during chronic metabolic acidosis. The experimental model was the dog with chronic metabolic acidosis because of the extensive background literature in this species. Chronic metabolic acidosis was produced by the ingestion of 10 mmol $\text{NH}_4\text{Cl/kg}$ body weight for 5 days. There was a significant increase in the rate of oxygen extraction when hypernatremia was present. Despite this rise in the rate of oxygen consumption, there was no increase in the rate of NH_4^+ production nor in the rate of glutamine extraction. These data suggest that hypernatremia might prevent a further augmentation in glutamine extraction when the rate of oxygen consumption rises. In addition, a larger proportion of the NH_4^+ produced was excreted in the urine during hypernatremia. This increase was associated with a rise in the urine flow rate, but not with a fall in urine pH.

Key words: Acid–base, ATP, glutamine, energy metabolism, metabolic acidosis, metabolic regulation, net acid excretion, oxygen consumption.

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L'objectif de cette étude a été d'examiner davantage la relation entre les taux de consommation d'oxygène et la production d'ammonium (NH_4^+) dans le rein durant une acidose métabolique chronique. On a utilisé le chien en acidose métabolique chronique comme modèle expérimental en raison de l'abondante littérature sur cette espèce. L'acidose métabolique chronique a été produite par l'ingestion de 10 mmol de $\text{NH}_4\text{Cl/kg}$ de poids corporel pendant 5 jours. Le taux d'extraction d'oxygène a augmenté significativement en présence d'hypernatrémie. Malgré l'élévation du taux de consommation d'oxygène, les taux de production de NH_4^+ et d'extraction de glutamine n'ont pas augmenté. Ces résultats suggèrent qu'une hypernatrémie pourrait prévenir une autre augmentation de l'extraction de glutamine lorsque le taux de consommation d'oxygène augmente. En outre, une plus grande proportion du NH_4^+ produit a été excrétée dans l'urine durant l'hypernatrémie. Cette augmentation a été associée à l'élévation du taux de débit urinaire, mais non pas à une chute de pH urinaire.

Mots clés : acide–base, ATP, glutamine, métabolisme énergétique, acidose métabolique, régulation métabolique, excrétion nette d'acide, consommation d'oxygène.

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During chronic metabolic acidosis, the expected renal response is to generate new bicarbonate; this is accomplished mainly by the production and excretion of ammonium (NH_4^+). Two major steps are involved: first, glutamine is metabolized in cells of the proximal tubule, yielding NH_4^+ and bicarbonate as products (Halperin et al. 1991); second, the cation, NH_4^+ , must be transferred to the urine (Knepper et al. 1989). During the metabolism of glutamine to either glucose or CO_2 , ATP is produced (Halperin et al. 1982). Hence the rate of turnover of energy (ATP) could limit the synthesis of NH_4^+ in these cells because no organ or organelle can synthesize ATP at a rate that exceeds its rate of utilization even for 1 min, owing to the very small quantity of ADP in cells (Flatt 1972; Halperin et al. 1985b; Halperin et al. 1982; Jungas et al. 1992). Since at least 90% of the ATP turnover in the kidney appears to be related to sodium reabsorption (Mandel and Balaban 1981), one might anticipate that a direct relationship exists between the rates of oxygen consumption and glutamine oxidation (Halperin et al. 1985). Two exceptions to this rule have been observed in dogs with chronic metabolic acidosis. First, when fuels other than glutamine (ketoacids (Lemieux et al. 1980; Lemieux et al. 1971), fatty acids (Vinay et al. 1976)) were infused and oxidized, ATP was produced without the synthesis of NH_4^+ . Now there was a substantial (and pre-

dictable) decline in the rate of NH_4^+ production. Second, during acute hyponatremia, the rate of oxygen consumption rose, but no extra glutamine was extracted. In this setting, there was complete oxidation and no transamination of glutamine (Halperin and Chen 1990), presumably reflecting increased oxidation of alanine.

To explore the relationship between the consumption of oxygen and the metabolism of glutamine in the kidney further, the effect of hypernatremia on NH_4^+ production during chronic metabolic acidosis was explored. The rationale for selecting hypernatremia as a model was to provide a means to increase the oxygen consumption by the kidney (reabsorb more sodium) without increasing the GFR or renal blood flow. In this way, the relative importance of an upper limit on ammoniogenesis could be explored. In addition, specific effects of hypernatremia could also be evaluated. Results to be reported indicate that the rate of oxygen consumption rose as expected during hypernatremia, but that this was not accompanied by a rise in the rates of glutamine extraction or NH_4^+ production. Possible mechanisms to explain this lack of correlation are discussed.

Methods

Chemicals

Enzymes, cofactors, and metabolic intermediates were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest purity available.

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Animals

Twelve mongrel dogs of either sex (15–25 kg) received 5 mmol $\text{NH}_4\text{Cl/kg}$ body weight b.i.d. in Dr. Ballard's dog food for 5 days. Vomiting was not detected in any dogs. If the bicarbonate concentration in plasma was not <17 mmol/L on the morning of the experiment, an infusion of isotonic HCl at 4.4 mL/min was given to ensure that the degree of acidosis was comparable during the control period of all dogs. HCl was infused in six dogs; since the rates of oxygen consumption, glutamine extraction, and NH_4^+ production were similar to the remainder of the population, the data were combined for statistical analyses.

Experimental procedure

Dogs were anaesthetized with phenobarbital (30 mg/kg i.v. with additional doses as needed to maintain a constant depth of anaesthesia throughout the experiment); dogs were permitted to breathe spontaneously throughout the experiment. The abdomen was opened by midline incision and catheters were inserted into both renal veins and ureters. The left gonadal vein was ligated. Catheters were placed in the jugular vein for infusion and the femoral artery for sampling.

After obtaining baseline samples, each dog received a bolus of creatinine (400 $\mu\text{mol/kg}$) followed by a continuous intravenous infusion containing 150 mmol/L NaCl, 10 mmol/L KCl, 137 mmol/L mannitol, and 4.4 mmol/L creatinine at a rate of $0.3 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ body weight for 60–90 min before taking samples for the control period. This ensured that the rate of urine flow was high enough to obtain complete urine collections. Urine was collected for three 20-min periods with bracketing blood samples to calculate the rates of production and extraction of metabolites as previously described (Halperin et al. 1985b). This control period was similar for all dogs. Two groups of animals were assigned: the isotonic saline group and the hypertonic saline group. The rates of infusion of mannitol, creatinine, and KCl were constant throughout the experiment in both groups.

Isotonic saline group

These dogs received isotonic saline at $0.4 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ for 180 min after the control period. Samples of urine and blood were collected as described above.

Hypertonic saline group

Animals in this series received hypertonic saline (850 mmol/L) at $0.2 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ body weight for 60 min. The concentration of saline was decreased to 150 mmol/L for the next 45 min. Blood was drawn to ensure that hypernatremia was present (a rise in the plasma sodium concentration of 10–20%). Samples of blood and urine were collected as described above.

Analytical methods

The pH, PCO_2 , and PO_2 of blood and the urine pH were measured anaerobically at 37°C with a digital acid–base analyzer (Corning 178 blood pH analyzer). Analytical methods for hemoglobin, metabolites, oxygen, sodium, potassium, and creatinine were as previously described (Halperin et al. 1985b). The bicarbonate concentration in plasma was calculated using a pK' of 6.1 and a solubility for CO_2 of 0.03 (Hastings and Sendroy 1925; Van Slyke et al. 1928). The extraction of oxygen, glutamine, lactate, and citrate as well as the production of NH_4^+ , alanine, glucose, and glutamate by the kidney were measured by arteriovenous measurements in plasma (glutamine, glutamate) or blood (NH_4^+ , oxygen, lactate, alanine, citrate, glucose); these differences were multiplied by the rate of flow of plasma or blood as indicated. The concentrations of all metabolites in the renal venous blood were corrected for the rate of urine flow (Wolfe 1941).

Calculations

Results are reported as the mean $\pm$ SEM. For each dog, the means of three values in each period were treated as a single value and the results from the two kidneys were averaged. The GFR, rates of renal plasma or blood flow and the extraction or production of metabolites were calculated as described previously (Halperin et al. 1985b).

TABLE 1. Effect of isotonic saline on the extraction of metabolites in dogs with chronic metabolic acidosis

	Control	Isotonic saline
Plasma lactate ($\mu\text{mol/L}$)	541 ± 70	519 ± 95
Plasma alanine ($\mu\text{mol/L}$)	513 ± 38	455 ± 40
Plasma citrate ($\mu\text{mol/L}$)	123 ± 5	110 ± 6
Plasma glutamine ($\mu\text{mol/L}$)	706 ± 106	568 ± 86
Plasma glutamate ($\mu\text{mol/L}$)	45 ± 7	44 ± 10
Plasma GFR (mL/min)	28 ± 4	27 ± 3
Extraction ($\mu\text{mol/min}$)		
Oxygen	209 ± 26	207 ± 27
Glutamine	29 ± 4	22 ± 3
Lactate	13 ± 3	10 ± 3
Citrate	2.8 ± 0.5	3.4 ± 1.1
Production ($\mu\text{mol/min}$)		
NH_4^+	45 ± 4	43 ± 4
Alanine	5.9 ± 1.6	3.2 ± 1.0
Glutamate	1.1 ± 0.4	0.8 ± 0.3

NOTE: Results are from five dogs prepared as described in the Methods section. Data were obtained during the control period and following the prolonged infusion of isotonic saline. The results are the mean $\pm$ SEM.

Statistical comparisons were performed by paired analyses on data from individual dogs; this helped to minimize inter-animal variations.

Results

To ensure that the changes reported in this manuscript reflected the infusion of hypertonic saline, data were obtained in five dogs infused with isotonic rather than hypertonic saline, but with a similar quantity of sodium. There were no significant changes observed in the level of metabolites in blood or plasma, nor in the extraction or production of metabolites in the dogs infused with isotonic saline (Table 1). The sodium concentration in plasma of these dogs was 137 ± 3 mmol/L, and the acid–base parameters in plasma were similar to the hypertonic saline group (pH 7.17 ± 0.03 , bicarbonate 13 ± 1 mmol/L; Table 2).

The acid–base, electrolyte, and metabolite levels in plasma in the dogs infused with hypertonic saline are reported in Table 2. During the control period, the dogs had chronic metabolic acidosis with acidemia (pH 7.16 ± 0.03) and a low concentration of bicarbonate in plasma (14 ± 1 mmol/L). The potassium concentration was somewhat lower than normal (3.6 ± 0.2 mmol/L), a consistent finding with this protocol (Halperin et al. 1985b). After the control period, hypertonic saline was infused to induce hypernatremia (158 ± 1 mmol/L). There were no significant changes in pH, bicarbonate, or potassium in plasma. The concentration of metabolites in blood was also not altered significantly following the infusion of hypertonic saline (Table 2).

The data on urinary excretion are presented in Table 3. The pH of the urine was 5.7 and the rate of NH_4^+ excretion was 26 ± 4 $\mu\text{mol/min}$. Of the NH_4^+ produced, two thirds was excreted in the urine. The infusion of hypertonic saline led to a significant rise in the rate of excretion of sodium and chloride as expected. Although there was a small rise in urine pH and the rate of urine flow, these changes were not statistically significant. Nevertheless, there was a significant rise in the percentage of NH_4^+ produced that was excreted in the urine (78 vs. 66%, Table 3).

Since the production of NH_4^+ from glutamine is accompanied by the production of ATP (Halperin et al. 1982), results

TABLE 2. Values in blood in dogs with chronic metabolic acidosis before and after the induction of hypernatremia

	Control	Hypernatremia
Plasma		
Na (mmol/L)	142 ± 1	158 ± 1 ^a
K (mmol/L)	3.6 ± 0.2	3.8 ± 0.1
HCO ₃ (mmol/L)	14 ± 1	13 ± 1
pH	7.16 ± 0.03	7.17 ± 0.03
P _{CO} ₂ (mmHg)	40 ± 2	35 ± 2
Glutamine (μmol/L)	598 ± 67	625 ± 58
Glutamate (μmol/L)	42 ± 5	42 ± 6
Blood		
Lactate (μmol/L)	539 ± 59	706 ± 238
Alanine (μmol/L)	504 ± 35	436 ± 46
Citrate (μmol/L)	100 ± 11	80 ± 5.1
Hematocrit (%)	37 ± 1	36 ± 1

NOTE: For details, see Methods. Results are presented as the mean ± SEM. The values in each period are the mean of triplicate determinations and the number of dogs was 7. ^a*p* < 0.05 in paired values.

of the extraction and production of compounds of interest are presented in three ways in Table 4. First, the absolute values are shown. In addition, results are expressed per 100 μmol of oxygen extracted or per millimole of sodium reabsorbed (the latter two methods reflect energy considerations). There was a small decline in the rates of glutamine extraction and NH₄⁺ production/100 μmol of O₂ extracted during the periods with hypernatremia, but these changes were not statistically significant.

Oxygen extraction was 208 μmol/min in the control period. Since each mole of oxygen yields 6 moles of ATP, this will provide 1248 μmol of ATP/min. The rate of sodium reabsorption was 3747 μmol/min (Table 3), or close to 3 mmol sodium reabsorbed/mmol ATP expended. During the infusion of hypertonic saline, the rate of oxygen consumption rose significantly as expected (to 241 ± 30 μmol/min, Table 4), while the rate of sodium reabsorption was also higher (4306 μmol/min, Table 3), maintaining the same ratio of close to 3 mmol sodium reabsorbed/mmol ATP expended.

Discussion

To excrete a large quantity of NH₄⁺ in the urine, this cation must be produced in the proximal tubule (Halperin et al. 1991). To synthesize NH₄⁺, glutamine is extracted from the glomerular filtrate and the peritubular blood (Lemieux et al. 1974; Pilkington et al. 1970). Metabolism of glutamine to produce NH₄⁺ is a process that yields, ultimately, bicarbonate and ATP as products (Halperin et al. 1982). For net gain of bicarbonate for the body, NH₄⁺ must be made an end-product of metabolism; this occurs when the cation NH₄⁺ is excreted in the urine (Halperin and Jungas 1983). It is also apparent that the synthesis of NH₄⁺ has energy (turnover of ATP) considerations (Halperin et al. 1982). Since cells contain such a tiny quantity of the precursor of ATP, ADP, continued flux in pathways that produce ATP can only occur if ATP is reconverted to ADP (Flatt 1972; Halperin et al. 1982; Jungas et al. 1992). This occurs in the kidney primarily when sodium is reabsorbed (flux through Na–K ATPase) (Halperin et al. 1982). Hence one might expect that more NH₄⁺ could be synthesized if the rate of filtration (and reabsorption) of sodium were to rise. The purpose of these experiments was to explore this relationship in more detail. In addition, the importance of

TABLE 3. Effect of hypernatremia on values in urine in dogs with chronic metabolic acidosis

	Control	Hypernatremia
pH	5.7 ± 0.3	5.9 ± 0.2
NH ₄ (μmol/min)	26 ± 4	32 ± 5 ^a
NH ₄ excretion as a % of production	66 ± 2	78 ± 3 ^a
Flow rate (mL/min)	1.19 ± 0.25	1.79 ± 0.23
GFR (mL/min)	27 ± 2.5	29 ± 2.7
RBF (mL/min)	170 ± 20	134 ± 18
RPF (mL/min)	105 ± 11	85 ± 10
Na (μmol/min)	69 ± 11	154 ± 18 ^a
K (μmol/min)	31 ± 6	28 ± 4
Cl (μmol/min)	127 ± 14	195 ± 20 ^a
Reabsorption of Na ⁺ (mmol/100 mL GFR)	13.8 ± 0.14	14.8 ± 0.20

NOTE: For details, see Table 1. Results are reported as the mean ± SEM for average values from the two kidneys. RBF, renal blood flow; RPF, renal plasma flow. ^a*p* < 0.05 in paired observations.

factors influencing the transfer of NH₄⁺ from proximal cells to the urine was assessed.

Production of NH₄⁺

There are several ways to analyze the rate of NH₄⁺ production in the kidney during chronic metabolic acidosis. We have previously emphasized that it should be evaluated per unit GFR if there is no variation in the sodium concentration in the plasma, as this should reflect a constant rate of ATP turnover. Since the filtered load of sodium no longer bears a constant relation to the GRF during hypernatremia, it is not valid to express the rate of NH₄⁺ production per unit GFR in this case. Rather it is more reasonable to calculate the rates of production and extraction of metabolites in the kidney per millimole of oxygen consumed or per millimole of sodium reabsorbed. An assumption implicit in this approach is that the proportion of oxygen consumed or sodium reabsorbed in the proximal convoluted tubule does not change to a major extent after the induction of hypernatremia. This latter point cannot be verified in vivo. Nevertheless, in dogs with extracellular fluid volume expansion, the proportion of sodium that is reabsorbed in the proximal tubule should not vary to a large extent.

One might anticipate that the rate of NH₄⁺ production might rise in dogs with chronic metabolic acidosis and hypernatremia, as there should be an increased rate of turnover of ATP (consumption of oxygen) in the kidneys of these dogs. Indeed, the rate of oxygen consumption by the kidney did rise (Table 4); of note, the GFR did not change during the period with hypernatremia (Table 3). This rise seemed to be related to the increase in Na reabsorption (Table 3) because there was no effect of hypernatremia on the rate of oxygen consumption if the latter is expressed per millimole of Na reabsorbed (Table 4).

Since the production of NH₄⁺ is linked to the extraction of glutamine and the production of ATP (Halperin et al. 1982), we were surprised not to find an increased rate of NH₄⁺ production during hypernatremia (Table 4). In fact, the rates of NH₄⁺ production and glutamine extraction (per sodium reabsorbed) actually declined (Table 4). Further, there did not appear to be a difference in the rate of transamination (production of alanine) as distinct from the effects of hyponatremia (Halperin and Chen 1990). Therefore, a process that was not

TABLE 4. Effect of hypernatremia on the production and extraction of metabolites by the kidney

Parameter	Units	Control	Hypernatremia
Production			
NH ₄ ⁺	μmol/min	39±5	41±7
	μmol/100 μmol O ₂	19±2.1	17±2.4
	μmol/mmol Na	10.5±1.4	9.7±1.3 ^a
Alanine	μmol/min	3.0±1.2	5.5±1.0 ^a
	μmol/100 μmol O ₂	1.4±0.6	2.3±0.4
	μmol/mmol Na	0.8±0.3	1.3±0.2
Glutamate	μmol/min	0.6±0.2	0.5±0.2
	μmol/100 μmol O ₂	0.3±0.1	0.2±0.1
	μmol/mmol Na	0.2±0.1	0.1±0.1
Extraction			
Oxygen	μmol/min	208±17	241±30 ^a
	μmol/mmol Na	56±2	56±3
Glutamine	μmol/min	24.7±5.1	23.4±6.2
	μmol/100 μmol O ₂	11.7±2.1	9.3±1.9
	μmol/mmol Na	6.6±1.3	5.4±1.3 ^a
Lactate	μmol/min	13.0±2.6	15.6±4.2
	μmol/100 μmol O ₂	6.6±1.6	6.9±2.1
	μmol/mmol Na	3.5±0.7	3.6±0.9
Citrate	μmol/min	2.7±0.6	2.1±0.2
	μmol/100 μmol O ₂	1.2±0.2	0.9±0.1
	μmol/mmol Na	0.7±0.12	0.5±0.04

NOTE: For details, see Table 1. Results are expressed as means ± SEM. The values are as μmol/min or per 100 μmol of O₂ extracted or per mmol of Na reabsorbed.

^a*p* < 0.05 in paired observations versus the control period.

defined during hypernatremia was responsible for diminishing the rates of glutamine extraction and NH₄⁺ production.

We shall consider five possibilities to explain the failure to elevate the rate of NH₄⁺ production during hypernatremia in dogs with chronic metabolic acidosis. First, the process could already be at its maximum rate, given the amount of enzymes responsible for the metabolism of glutamine in the kidney. Second, the rise in oxygen consumption might have occurred in nephron segments other than the proximal tubule, the site where NH₄⁺ is produced (Halperin et al. 1991). Third, there may be a lack of available substrate (glutamine) in proximal cells. Although this is possible, the glutamine concentration did not decline during hypernatremia (Table 2). Fourth, hypernatremia may have led to the production of an inhibitor of ammoniogenesis. None of the usual factors such as a rise in blood pH (Pitts 1964), hyperkalemia (Tannen 1987), a lower glutamine concentration in plasma (Halperin and Chen 1987), a decline in oxygen extraction (Halperin et al. 1985b) or an increase in the rate of lactate extraction (Dies et al. 1981; Halperin et al. 1985a; Leal-Pinto et al. 1972; Lemieux et al. 1986; Lemieux et al. 1985; Levy 1962) could account for the failure to increase the quantity of NH₄⁺ produced (Tables 2 and 4). Fifth, it is possible that the pH of the intracellular fluid of proximal cells was higher during hypernatremia. This might occur if there is a volume regulatory increase with alkalization in those cells that produce NH₄⁺ (Reid et al. 1986), although most cells of the proximal tubule do not manifest a volume regulatory increase (Gagnon et al. 1982; Linshaw et al. 1977; Lohr and Grantham 1986).

Transfer of NH₄⁺ to the urine

Two major factors are associated with the transfer of NH₄⁺ to the urine, the pH of urine and the rate of urine flow (Knepper et al. 1989; Nagami 1988). During hypernatremia, more of the

NH₄⁺ that was produced was excreted in the urine (78 vs. 66%, *p* < 0.05, Table 3). The urine pH was not lower at this time (5.9 vs. 5.7, Table 3). Hence it is unlikely that a primary stimulus of enhanced secretion of H⁺ in the collecting duct could explain these results. This is in keeping with previous observations that the highest rates of NH₄⁺ excretion were associated with higher rather than lower values for urine pH (Madison and Seldin 1958; Richardson and Halperin 1987; Simpson 1971; Stinebaugh and Schloeder 1966). It is reasonable to presume that the higher proportion of NH₄⁺ excreted during hypernatremia was the result of a higher rate of urine flow (Wilcox et al. 1984) (Table 3); this is consistent with data obtained during the infusion of a large quantity of hypotonic saline (Halperin and Chen 1990).

In summary, the acute induction of hypernatremia led to the excretion of a higher proportion of the NH₄⁺ that was produced (78 vs. 66%). The rate of NH₄⁺ production was lower than expected when considering the energy constraints on the rate of glutamine metabolism in cells of the proximal tubule. None of the factors known to modulate this rate changed in the appropriate direction. This suggests that there may be other factors that regulate the rate of NH₄⁺ production.

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