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LABORATORY STUDY

Effect of Chronic Metabolic Acidosis on Renal Growth and Renal Sodium Handling in Uninephrectomized Rats

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ABSTRACT

Paucity studies have indicated that a systemic metabolic acidosis cause a decrease in salt and water reabsorption in the kidney. The following study was undertaken on male Wistar-Hannover rats (200–250 g) to investigate the effects of a chronic, NH_4Cl-induced metabolic acidosis on the renal handling of Na^+ in sham-operated and uninephrectomized rats, by lithium clearance. The present study shows that chronic acidosis (blood pH, 7.16 ± 0.13) caused a sustained increase in renal fractional Na^+ excretion (267.9 ± 36.4%), accompanied by a rise in the fractional proximal (113.3 ± 3.6%) and post-proximal (179.7 ± 20.2%) Na^+ and fractional K^+ (163.4 ± 5.6%) excretions when compared to pair-fed rats. These differences occurred in spite of an unchanged creatinine clearance and Na^+ filtered load. On the other hand, a body growth impairment was observed in the acidotic (control, 258 ± 3.7 g versus acidotic, 232 ± 4.6 g) and pair-fed rats (225 ± 3.6 g), whereas there was significant enhance in the kidney weights in acidotic rats (1.73 ± 0.05 g) com-

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pared to other experimental groups (control, 1.46 ± 0.05 g; pair-fed, 1.4 ± 0.05 g). The renal growth indexes after metabolic acidosis NH_4Cl-induced did not shown statistical difference at 1.5, 3.0 and 12 hours after uninephrectomy when were compared with pair-fed groups. However, from the fifth to tenth day after unilateral nephrectomy the renal growth index of acidotic group was significantly greater than pair-fed groups. Unilateral nephrectomy in acidotic animals caused a striking additional but transient increase in fractional renal sodium ($FENa^+$) and potassium (FEK^+) excretion from 1.5 to 3 hours post-surgery meanly associated with an enhanced post-proximal sodium excretion when compared to pair-fed uninephrectomize rats. By the fifth postoperative day the all functional values returned to baseline levels. This altered renal Na^+ handling and K^+ excretion may result from a reciprocal relationship between tubular metabolic pathway stimuli and ion transport. Further studies are required to investigate the acidosis involvement on functional kidney response.

Key words: Renal function; Renal sodium excretion; Lithium clearance; Metabolic acidosis.

INTRODUCTION

There is a surprising lack of experimental data on the actual mechanisms of the metabolic acidosis-induced disturbance in sodium handling in the renal tubules. Paucity studies have indicated that a systemic metabolic acidosis causes a decrease in salt and water reabsorption in the kidney (1,2). Ammonium chloride-induced chronic metabolic acidosis has been shown to cause a decrease in hydro-saline reabsorption in dog kidney (3,4). Using free water clearance technique (5) as an index of proximal tubular function, the effect of ammonium chloride acidosis on renal ion balance appeared to be localized to the proximal tubule segments in the dog. On the other hand, during sustained metabolic acidosis in man and other animals show a progressive increase in capacity to excrete ammonia in the urine (1,4,6). This process may continue until practically the entire load of acid is excreted as the ammonium salt.

Chronic ammonium chloride induced metabolic acidosis has been found to be an inducing factor for renal growth in rats. The hypertrophic mechanism involved in this process is not entirely known and could be due to increased proteic synthesis or due reduced proteic catabolism in the tubule renal cells (7, 13). Nephronal hypertrophy, characterize by an increase in cell protein content and cell size, is predominantly accounted for by an elevated renal tubule mass (7). This hypertrophy occurs in several disorders such as diabetic nephropathy, the remnant kidney, and renal insufficiency (7).

After a reduction in the renal mass, the remaining kidney undergoes hypertrophy with an associated increment in functional capacity (7). In the remaining kidney, there is an increased glomerular filtration rate; enhanced reabsorption of sodium, bicarbonate and tubular fluid; and cell hypertrophy. The increase in transport capacity is seen in various nephron segments but primarily occurs in the proximal tubule (7,8).

Renal lithium clearance has been used as a noninvasive method to estimate the output of sodium and fluid by the proximal renal tubules, thus allowing the study of various factors which influence sodium handling in different tubule segments (9, 10).

The following study was undertaken to further investigation the effects of a chronic, NH_4Cl-induced metabolic acidosis on the renal growth and the tubule sodium handling, by lithium clearance, in sham-operated and uninephrectomized rats.

MATERIAL AND METHODS

The experiments were performed on sixty, male Wistar-Hannover rats (200-250 g) allowed free access to water and normal rat chow. The general guidelines established by the Declaration of Helsinki (1964) for laboratory animals were followed throughout the study.

Experimental Design

Metabolic acidosis was produced by substituting the drinking water for 0.25 M solution of NH_4Cl (Merck). All animals drank *ad libitum* but records of volume were kept, and only those rats which had drunk nearly equivalent amount of water or NH_4Cl were select for use. NH_4Cl-treated rats were maintained on their respective regimens for 10 days. To simulate the hypophagia induced by NH_4Cl metabolic acidosis, the renal function was also performed in a subgroup of animals (pair-fed) submitted to food-restricted pattern similar to the average food intake observed in the experimental acidotic rats. On the 11th day the renal function of rats having both kidneys (sham-operated) and uninephrectomized rats was studied at intervals of 1,5 h, 3 h and 12 h after unilateral nephrectomy and then again after 5 and 10 days.

Fourteen hours before the renal test, 60mmol LiCl/100 g body weight was given by gavage. The rats were subsequently housed individually in metabolic cages with free access to tap water but no food. The experiment was performed at the same time in each group of control, pair-fed and acidotic animals. At 8:00 a.m., each animal received a load of tap water by gavage (5% of body weight), followed by a second load of the same volume one hour later. Twenty minutes after the second load, spontaneously voided urine was collected over a 2 h period. The voided urine passed through the funnel in the bottom of the cage into a graduated centrifuge tube. At the end of the experiment, blood samples were drawn by cardiac puncture and the kidney was immediately removed, decapsulated and weighed.

Unilateral Nephrectomy

Ethyl ether was used to anesthetize the animals and a 1 cm incision was made immediately below the last left rib. The kidney was carefully removed, drained and weighed on a precise balance. The adrenal was kept in place intact. After certifying the absence of excessive bleeding, the incision was sutured.

Biochemical Analysis

Plasma and urine sodium, potassium and lithium concentrations were measured by

flame photometry, while the creatinine concentrations were determined spectrophotometrically by the alkaline picrate method (11).

Statistics and Calculations

The results are reported as means ± SEM per 100 g body weight. Renal clearances (C) was calculated by a standard formula (C = UV/P) using the plasma creatinine and lithium levels for each period. Creatinine clearance was used to estimate glomerular filtration rate (GFR) and lithium clearance (CLi^+) was used to assess proximal tubule output. Fractional sodium ($FENa^+$) and potassium (FEK^+) excretion was calculated as CNa^+/CCr and CK^+/CCr respectively, where CNa+ and CK+ is ion clearance and CCr is creatinine clearance. The fractional proximal ($PFENa^+$) and post-proximal ($PPFENa^+$) sodium excretion were calculated as CLi/CCr x 100 and CNa^+/CLi^+x100, respectively (10). Changes in fractional excretion were estimated using the control and pair-fed values. The renal growth index was calculated as the percentage variant of renal weight of the same rat multiplied by 100. Statistical analysis of the data was performed using one-way analysis of variance for repeated measurements. When the results were significant, the Bonferroni's contrast test was used to determine the extent of the differences. A p value < 0.05 was considered to indicate significance.

RESULTS

Groups of ten animals (acidotic control (sham-operated); acidotic uninephrectomized and pair-fed groups) were studied. Metabolic acidosis was confirmed by a blood pH of 7.16 ± 0.13. All rats survived and were clinically healthy up to tenth day of the study. As show in Table 1, body growth impairment was observed in the acidotic sham-operated rats after ten days of NH_4Cl or pair-fed intake. In contrast, the renal growth index was significantly less for the pair-fed compared to acidotic sham-operated group. There was also a significant difference between serum potassium, but not in sodium and lithium levels in acidotic sham-operated compared to pair-fed rats (Table 1) The renal growth indexes after metabolic acidosis NH_4Cl-induced did not shown statistical difference at 1.5, 3.0 and 12 hours after uninephrectomy when were compared with pair-fed groups. However, from the fifth to tenth day after unilateral nephrectomy the renal growth index of acidotic group was significantly greater than pair-fed groups (Figure 1). The data from the renal function studies in the experimental groups ten days after metabolic acidosis NH_4Cl-induced is shown in Figure 1. Metabolic acidosis caused a sustained increase in basal renal fractional sodium and potassium excretion, accompanied by a rise in the fractional proximal and post-proximal sodium excretion when sham-operated were compared to pair-fed rats (Figure 1). These differences occurred in spite of an unchanged creatinine clearance (Figure 1) and sodium filtered load. The urinary flow rates throughout the renal tubule sodium handling studies were not significantly different between unilateral nephrectomized rats and sham-operated animals. Unilateral nephrectomy in acidotic animals caused a striking additional but transient increase in fractional renal sodium ($FENa^+$) and potassium (FEK^+) excretion from 1.5 to 3 hours post-surgery. By the 1.5 to 3 hours post-uninephrectomy the fractional urinary sodium and potassium excretion in acidotic rats was associated with an enhanced post-proximal

Table 1

Effect of Metabolic Acidosis Induced by NH_4Cl Administration (NH_4) in Body Weight, Renal Weight and Serum Sodium, Potassium and Lithium Levels Compared to Sham-Operated (Sham) and Pair-Fed (PF) Rats. Data Are Reported as Means ± SEM for 10 Rats Per Group.

	Body Weight (g)	Renal Weight (g)	Liquid Intake (mL)	Na^+ (mM)	K^+ (mM)	Li^+ (mM)
Groups						
Sham	258 ± 3.7	1.46 ± 0.05	34.9 ± 0.9	143 ± 0.9	3.7 ± 0.1	100 ± 5.0
PF	225 ± 3.6	1.40 ± 0.05	34.8 ± 0.5	142 ± 1.2	3.5 ± 0.1	113 ± 6.0
NH_4	232 ± 4.3 [a]	1.73 ± 0.05 [b]	35.7 ± 0.7	143 ± 0.4	4.4 ± 0.1 [a,b]	109 ± 8.0

[a,b] $p < 0.01$ compared to Co or PF respectively (ANOVA and Bonferroni' contract test).

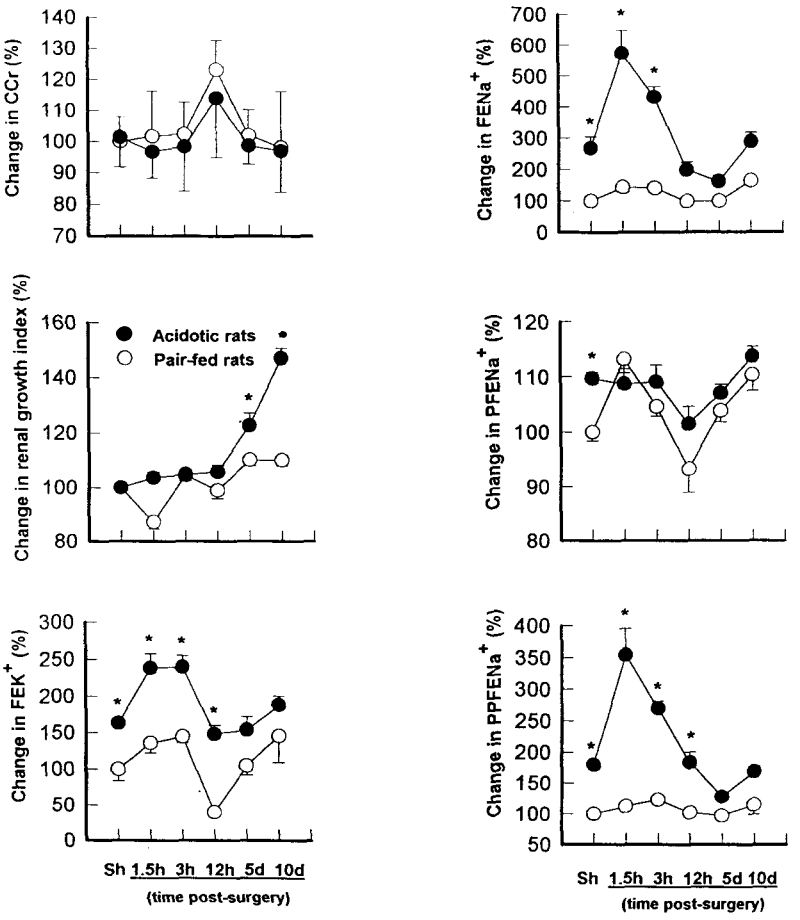

Figure 1. Effect of metabolic acidosis in uninephrectomized rats on renal creatinine clearance (CCr), renal growth index, fractional sodium (FENa) and potassium (FEK) excretion, and fractional proximal (PFENa) and post-proximal (PPFENa) tubule sodium excretion compared to acidotic sham-operated (Sh) and uninephrectomized pair-fed (PF) rats. The data are reported as percent of sham-operated or pair-fed values and represented as the mean f SEM for 10 rats per group. * $P < 0.05$ compared to the control or pair-fed animals (ANOVA and Bonferroni contrast test).

sodium excretion when compared to pair-fed uninephrectomize rats (Figure 1). At this time, the increased urinary excretion of ions occurred in spite of an unchanged creatinine clearance. However, twelve hours after uninephrectomy there was a statistically significant increase of creatinine clearance to a similar extent in acidotic and pair-fed rats. By the fifth postoperative day the all-functional values returned to baseline levels. The high urinary sodium excretion in acidotic sham-operated animals was not enhanced by unilateral nephrectomy. On the other hand, at 1.5 to 3 postoperative hours the fractional proximal sodium reabsorption in uninephrectomized pair-fed group was significantly depressed (Figure 1).

DISCUSSION

These findings are in agreement with previous studies (2,3,5), which have demonstrated decreased sodium transport in the presence of reduced blood pH. Absolute sodium excretion and fractional excretion of sodium were increased in sham-operated acidotic animals, despite similarities in the filtered load of sodium with pair-fed rats. Sartorius, Roemmoelt and Pitts (12), showed the humans, administered an oral ammonium chloride load, demonstrate a natriuresis in the early phases of the induced acidosis. Studies in rats and dogs undergoing a metabolic acidosis indicate that there is a decrease in renal tubular reabsorption of salt and water (2–4), and micropuncture studies localize the site of depression of sodium reabsorption to the proximal tubule (5,6). The relationship between sodium reabsorption and blood bicarbonate levels has been the subject of study (13). This study demonstrated that isotonic sodium and water reabsorption in the rat proximal tubules was correlated to the bicarbonate concentration in the peritubular capillaries. A depression of pericapillary bicarbonate level was correlated to a reduction in the proximal tubular reabsorption of salt and fluid. This observation may explain at least in part, our lithium clearance data in sham-operated rats, undergoing a metabolic acidosis, indicating that there is a decrease in tubular sodium reabsorption in the proximal tubule segments (2,5). The present data shows also a proximal sodium rejection followed by an enhanced distal fractional sodium excretion in the absence of change in creatinine clearance. In our study, the metabolic acidosis in sham-operated rats induced by NH_4Cl feeding caused an absolute increase in kidney weight. The data are consistent with *in vitro* studies of proximal tubule cells (14), where cells hypertrophy were accompanied by a marked decline in protein breakdown but not in cells protein synthesis after application of NH_4Cl. Also, the Na^+/H^+ exchanger, H^-/K^- ATPase and H^+-ATPase stimulation has been implicated as an important initiating signal for subsequent events leading to cell proliferation (21–24). Fine et al. (20), demonstrated that in renal proximal tubular cells *in vitro*, activation of the Na^+/H^+ antiporter correlated better with cell hypertrophy than with proliferation. In contrast with our observations, acidification *in vitro* has failed to produce cells hypertrophy when not produced by ammonium chloride (15,16). It has become apparent only when metabolic acidosis cause an effect of increased ammonia production rather than of the acidosis per se (17).

In agreement with several previous studies (8,18,19), we found that fractional proximal reabsorption was reduced acutely after uninephrectomy in pair-fed rats. The cause of this glomerulo-tubular imbalance remains unknown, but it appears to be short-lived, within 5 days fractional proximal reabsorption had increased so that fractional reabsorption had returned to a value similar to that in basal sham-operated animals. It seems likely that this increased rate of ion reabsorption is the *in vivo* parallel of the well-documented changes in ultrastructure and function in proximal tubular cells, including increased activity of the Na^+/H^+ antiport and the Na^+/K^- pump, which have been described *in vitro* (20–24). The increase in transport capacity is seen in various nephron segments but primarily occurs in the proximal tubule (7,20). The activity of Na^+/H^+ exchanger in the remaining kidney was found to be increased following 24 h after uninephrectomy in rats (23,24). Authors have demonstrated that antiport activity measured in cortical brush-border membrane vesicles rapidly increases within the 30 minutes after contralateral uninephrectomy (24). However, the signal that triggered antiport activation in the remaining kidney still also unknown. In the present study, the accentuated

fall in the proximal sodium reabsorption after 10-day treatment with ammonium chloride may have blunted in acidotic rats the natriuretic response to uninephrectomy observed in pair-fed animals. These results could be explained by the maximal Na^+/H^+ exchanger expression or activity after acidotic stimuli. The present study also emphasizes the importance of poor nutrition as an inhibitor of linear growth. Our data show a significant inhibition of renal growth in uninephrectomized pair-fed as compared to uninephrectomized acidotic rats (Figure 1). This corroborates earlier data by Philipps et al (25) which showed that calorie limitation in neonatal rats reduced IGF-I and II in serum, liver and brain.

Previous studies have been shown that alterations in acid-base balance modify renal gluconeogenesis. Metabolic acidosis stimulates it in a variety of preparations by increase of phosphoenolpyruvate carboxykinase mRNA cloning and enzyme activity (26,27). There are many indications that gluconeogenesis and the reabsorption of Na^+ are reciprocally related. Investigation has demonstrated that American opossum kidney (OK cells), respond to the stimulus of acidosis with increased glutamine metabolism and ammonium formation (28). Previous studies using OK cells have demonstrated that acidosis decrease the activity of Na^+/H^+ exchanger thereby increasing intracellular H^+ (29). Isolated proximal tubules studies showed that enhanced glutamine metabolism and ammonia production are linked to increased gluconeogenesis (30). On the other hand, maneuvers that inhibit of Na^+/K^+ATPase and thereby sodium tubule transport by the kidney stimulate gluconeogenesis (31,32). Thus, as metabolic acidosis may result in a lower filtered load of bicarbonate and consequently less bicarbonate is reabsorbed; and a decreased Na^+/H antiporter activity associated with stimulated gluconeogenesis. These features take together, it should lead to less dissipation of the electrochemical gradient for Na^+ resulting in a decreased reabsorption of Na^+ so as observed in present study. Additionally, the present findings suggest that in energy-requiring processes, renal growth, sodium transport and gluconeogenesis, may compete for energy availability in the nephron tubule, and could explain the striking natriuresis observed in ours study. Our results indicate that a transient increase in ion delivery out of the proximal tubule is a constant feature of the response of the remaining kidney to uninephrectomy in the rat, but that the underlying changes in proximal tubular function vary with the time elapsed and systemic acid-base status. Therefore, an increased sodium reabsorption in the proximal and distal nephrons can compensate partially the early, altered renal sodium handling in acidotic rats. Under our experimental conditions instead of previous studies (4,33), the fractional potassium excretion was also increased during acidosis. Many factors have been proposed as being important influences on the renal excretion of potassium including blood pH, potential across luminal membrane, sodium delivery to the distal tubule and urinary flow rate (34). Finally, the kaliuresis of uninephrectomized rats can be explained as a consequence of a markedly increased rate of potassium secretion into the accessible region of the distal tubule, mainly in acidotic rats.

While the present study provides fresh insights into the response of the renal Na^+ excretion and chronic metabolic acidosis, it should be emphasized that it was conducted on whole-kidney function in metabolic cages using unanesthetized, unrestrained rats. In conclusion, the present study demonstrated that chronic metabolic acidosis in rats induces a sustained increase of renal weight and ion excretion. This altered renal Na^+ handling and K^+ excretion may result from a reciprocal relationship between tubular metabolic pathway stimuli and ion transport. Further studies are required to investigate the acidosis involvement on functional kidney response.

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