Effects of an NH₄Cl-induced metabolic acidosis on salt and water reabsorption in dog kidney

ROBERT SAFIRSTEIN, V. PHILIP GLASSMAN, AND VINCENT A. DIASCALA
Renal Service, Department of Medicine, Public Health Service Hospital, Staten Island, New York 10304


Five female mongrel dogs weighing 18-23 kg were studied. The dogs were untrained at the time of the initial study and tolerated the procedures well without discomfort. Food was withheld the day prior to study but dogs were allowed free access to water. On the day of study the dogs were weighed and placed, while awake, in a loose-fitting harness and 50 ml/kg of tap water was given by orogastric tube over a 15-min period. The bladder was emptied completely by suprapubic pressure and instillation of 10-20 ml of air 30 sec prior to the end of urine-collection periods. When a maximum urine flow rate was achieved, a sample of venous blood was collected in a heparinized syringe for immediate determination of pH and PCO₂ on a Radiometer Copenhagen pH meter 27 with gas monitor Radiometer Pco₂ electrode type E5036. The bicarbonate concentration in the blood was calculated using the Henderson-Hasselbalch equation—a pK₁ of 6.1 and solubility coefficient to convert CO₂ tension to H₂CO₃ of 0.0301 was used. After collection of these three control periods an infusion of 0.45% NaCl or 2.5% mannitol was begun. The infusion rates were 5-10 ml greater than urine flow rate. Five to fifteen minute urine collection periods were taken during the infusions with midpoint venous blood samples collected every other urine collection period or when a change in infusion rates occurred. The urine and blood osmolality, Na, K, Cl, urea, inulin, and PAH concentrations were determined using methods previously described from this laboratory (25). The study was terminated when an apparent maximal urine flow rate was achieved. The dogs were weighed again at the conclusion of the study. No anesthesia was necessary for any of the procedures, thus avoiding stimulation of ADH secretion during the study.

The degree of volume expansion achieved in the control and acidicotic groups was assessed in the following manner. The amount of dextrose water (43.1 ± 4.61 versus 47.6 ± 5.01 ml/kg), mannitol (107.6 ± 25.8 versus 100.7 ± 10.0 ml/kg), and saline (218.9 ± 4.06 versus 206.7 ± 6.23 mEq/kg) on a per kilogram basis was similar.

Hematocrits performed in dogs at maximum water hydration prior to acute saline infusion were 48% control and 46% acidicotic. At the conclusion of the acute saline infusion...
in these dogs the hematocrits were 42.5% control and 14% acidotic. Similarly, hematocrits at maximum water hydration prior to acute infusion of mannitol were 40.5% control and 39.5% acidotic. At the conclusion of mannitol infusion hematocrits dropped to 38% control and 37.5% acidotic. Serum sodium concentration at the point of maximum water hydration was 131 mEq/liter control and 130 mEq/liter acidotic. Finally, urinary osmolality at the point of maximum water hydration in control and acidotic groups did not differ significantly (Fig. 1). By these criteria, the degree of volume expansion and serum dilution is felt to be similar.

Seven to ten days later a metabolic acidosis was induced by use of the following protocol. A venous sample was taken prior to the induction of acidosis and pH and Pco2 were determined. A total of 20 mEq/kg of NH4Cl was given in two equally divided doses via orogastric tube for 3 consecutive days. It was determined in preliminary studies that the serum pH was stable after 48 hr of NH4Cl administration. Most dogs vomited at some time during the 3-day induction period. All dogs in which the acidosis was induced survived the induction period without changes in play behavior or feeding patterns. On the 4th day, at least 18 hr after the last dose of NH4Cl, the dogs were studied again using the same experimental design in the acidotic dogs as that used in the control dogs.

In addition, three dogs were studied under control and experimental hydropenic conditions. The dogs were fasted and deprived of water for 24 hr. At the end of this time the dogs were catheterized and a timed urine collection for urine flow rate and urine osmolality was obtained. A metabolic acidosis was induced in the aforementioned manner and the dogs were recatheterized and urine collected again.

Inulin, PAH, urea, Na, K, and Cl were determined by methods described previously (25). Standard clearance formulas were used with \( C_{\text{osm}} = \frac{V}{P_{\text{osm}}} \) and \( C_{\text{ClO}} = V - C_{\text{osm}} \). Statistical analyses used were the Student t test for paired data and analysis of covariance for regression coefficient (18).

### Results

**Maximum water diuresis (Tables 1 and 2, Fig. 1).** After the induction of acidosis a consistent decrease in body weight was observed. All animals demonstrated a mean loss of 5% of body weight. Although there was a reduction in glomerular filtration rate, as measured by the clearance of inulin, the difference was not statistically significant. Clearance of PAH was similar in all animals, with a mean of 27.0 ± 26.0 in experimental groups. Inulin, PAH, urea, Na, K, and Cl were determined by methods described previously (25). Standard clearance formulas were used with \( C_{\text{osm}} = \frac{V}{P_{\text{osm}}} \) and \( C_{\text{ClO}} = V - C_{\text{osm}} \). Statistical analyses used were the Student t test for paired data and analysis of covariance for regression coefficient (18).

### Table 1. Weight, glomerular filtration rate, clearance of PAH, and plasma constituents of dogs at maximal water hydration

<table>
<thead>
<tr>
<th>C</th>
<th>E</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt, kg</td>
<td>90.2 ± 0.50</td>
<td>19.2 ± 0.67</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>14.0 ± 5.42</td>
<td>27.0 ± 3.72</td>
</tr>
<tr>
<td>Cea,m, ml/min</td>
<td>289.7 ± 27.0</td>
<td>295.5 ± 26.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.33 ± 0.01</td>
<td>7.18 ± 0.09</td>
</tr>
<tr>
<td>HCO3, mEq/liter</td>
<td>20.9 ± 1.08</td>
<td>10.3 ± 0.74</td>
</tr>
<tr>
<td>Na, mEq/liter</td>
<td>131 ± 1.48</td>
<td>130 ± 1.66</td>
</tr>
<tr>
<td>K, mEq/liter</td>
<td>3.21 ± 0.12</td>
<td>2.71 ± 0.12</td>
</tr>
<tr>
<td>Cl, mEq/liter</td>
<td>104 ± 1.65</td>
<td>116 ± 1.64</td>
</tr>
</tbody>
</table>

Values are means ± SE. Abbreviations are the same as in Table 1. Experimental refers to those dogs made acidotic with ammonium chloride.

### Table 2. Urinary electrolyte excretion and clearance rates at maximal water diuresis

<table>
<thead>
<tr>
<th>C</th>
<th>E</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uosm</td>
<td>44 ± 3.6</td>
<td>42 ± 2.7</td>
</tr>
<tr>
<td>UNa,V</td>
<td>17.8 ± 4.46</td>
<td>16.0 ± 5.16</td>
</tr>
<tr>
<td>Ua,V</td>
<td>10.3 ± 2.71</td>
<td>7.95 ± 1.71</td>
</tr>
<tr>
<td>UC,V</td>
<td>14.2 ± 2.90</td>
<td>60.2 ± 14.2</td>
</tr>
<tr>
<td>CNa</td>
<td>0.13 ± 0.03</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>CK</td>
<td>6.25 ± 0.85</td>
<td>3.00 ± 0.68</td>
</tr>
<tr>
<td>Cl</td>
<td>0.14 ± 0.03</td>
<td>0.51 ± 0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE. Abbreviations are the same as in Table 1.
from 10.1 to 12.7 ml/min per 100 ml GFR (P < .05). The rate of free water clearance increased from 8.5 to 10.8 ml/min per 100 ml GFR (P < .02). Urine flow rate and free water clearance, when unfactored for GFR, demonstrated the same results with V increasing from 8.6 to 10.0 ml/min (P < .05) during acidosis, and CH₂O increasing from 7.3 to 8.8 ml/min (P < .02).

Table 2 depicts urinary composition and electrolyte excretion rate during maximum water hydration. Urine osmolality was unchanged in control and experimental animals. There was no difference seen in the rates of sodium excretion or clearance. However, the rates of potassium excretion were decreased during the experimental condition.

**Hypotonic saline infusions** (Figs. 2 and 3). Data obtained during the infusion of hypotonic saline are plotted in Figs. 2 and 3. The correlation coefficients for linearity as well as the equations for first-order regression lines are included in inset. Figure 2 depicts the relationship between free water excretion (C_H₂O per 100 ml GFR), an index of sodium reabsorption in the diluting segment, and urinary flow rate (V per 100 ml GFR), an index of isosmotic fluid reaching the distal nephron (5, 8, 12, 13, 20, 21, 34). All values of urine flow rate and free water excretion during control and experimental conditions have been included in this and subsequent plots.

The slopes of these lines were significantly different at the 0.025 level, with the slope of the acidotic group exceeding that of the control group. A significant difference exists for similar equations relating C_H₂O to V without regard to GFR: the equation for the control group being \( y = 14.48 \log x - 5.78 \), and that for experimental \( y = 19.23 \log x - 10.21 \) (P < .005). In Fig. 3 an attempt was made to evaluate Na reabsorption in the diluting segments (C_na) as a function of distal delivery of sodium, C_H₂O + C_Na (8, 13, 20–22). Again, C_H₂O rose at a greater rate during acidosis, the change in slopes being significantly increased (P < .001).

When absolute values for C_H₂O and C_H₂O + C_Na are used again, the rate of change of C_H₂O is increased during acidosis, slopes differing significantly at the 0.01 level. Equations for this relationship are control \( y = 0.52x + 3.96 \), and experimental \( y = 0.66x + 3.16 \).

**Mannitol studies.** To assess C_H₂O generation over a greater range of osmolar clearance (5) and at a time when intratubular sodium concentration is depressed (7), hypotonic mannitol was used to augment distal sodium delivery to the diluting site(s). During hypotonic mannitol infusion V is an overestimate of the amount of sodium reaching the distal nephron by the extent to which mannitol replaces sodium in the tubular fluid in attaining isotonicity. Therefore, C_H₂O plus C_Na more accurately reflects the distal sodium load (13). Figure 4 depicts the relationship of C_H₂O per 100 ml
GFR and $C_{H_2O} + C_{Na}$ per 100 ml GFR in the same five dogs under control and experimental conditions. During acidosis, as in the saline experiments, distal sodium reabsorption as reflected by $C_{H_2O}$ generation rose more steeply as distal sodium supply increased. When $C_{H_2O}$ and $C_{H_2O} + C_{Na}$ are plotted without reference to 100 ml GFR, the rate of increase of $C_{H_2O}$ during acidosis is once again significantly greater than controls; the equations being $y = 16.39 \log x - 7.18$ control, and $y = 22.51 \log x - 10.8$ experimental.

Hydropenic studies (Table 3). To determine if the difference in urinary flow rate and $C_{H_2O}$ is due to a defect in the permeability of water at water-clearing sites (5), three dogs were compared under hydropenic conditions during control and acidosis. Neither $V$ nor $U_{osm}$ in acidotic animals showed changes consistent with altered water permeability.

### DISCUSSION

Studies in animals undergoing water diuresis have afforded a model by which differential proximal and distal salt and water reabsorption may be assessed (5, 14, 17). In this model at maximum hydration, urine flow rate, $V$, is an estimate of isosmotic fluid leaving the proximal tubule and $C_{H_2O}$ is an estimate of sodium reabsorption in the diluting segment(s) (5, 17). Any condition which depresses proximal reabsorption of fluid and sodium would be expected to increase urine flow rate and free water generation. This approach has been used widely to assess the influence of a variety of agents and factors on sodium and water reabsorption (1-3, 5, 13, 14, 17, 21, 24). During water hydration in the present studies urine flow rate under experimental conditions was 16% greater than the control urine flow rate. This increase was accompanied by a 21% increase in the solute free water clearance. When these functions were corrected for 100 ml of glomerular filtration rate, the percentage changes were +25% and +20%, respectively.

As pointed out by Eknovan et al. (5), the use of $V$ to assess proximal tubular function may be significantly misleading in instances of inhibition of sodium reabsorption in the diluting segment(s). This is true because of the resultant loss of medullary hypertonicity and diminished water reabsorption in the descending limb of the loop of Henle (5). For that reason distal sodium reabsorption was examined under solute loading. During saline infusion $C_{H_2O}$ increased as a function of $V$ in both experimental and control conditions. As seen from Fig. 2, up to 38% of the total volume of the glomerular filtrate was excreted under experimental conditions; at the same time $C_{H_2O}$ increased in a nearly linear manner. In Figs. 3 and 4 $C_{H_2O}$ again increased as a function of increasing sodium delivery, both in control and experimental conditions, without depression of distal sodium reabsorption, as measured by $C_{H_2O}$ excretion. Therefore, no inhibition of distal sodium reabsorption at the diluting site(s) is uncovered during the metabolic acidosis.

A second source of error is a change in the permeability of the nephron to water as a result of the experimental condition. A decrease in the permeability to water along the nephron would result in changes in urine flow rate not indicative exclusively of sodium reabsorption in the proximal tubule. During hydropenia the finding of similar maximal urine osmolality and urine flow rate before and after acidosis is evidence that water permeability was not affected by the acidosis. The increase in urine flow rate in the present studies with subsequent increase in $C_{H_2O}$ excretion then indicates depression of proximal tubular epithelial salt and water reabsorption. These findings agree with other data demonstrating depressed proximal reabsorption of sodium in rats undergoing a similar NH4Cl-induced acidosis (11, 19).

Despite this reduction of proximal tubular reabsorption, sodium excretion remains at low levels. The clearance of sodium was 0.13 ml/min during acidosis, representing excretion of 0.16% of the filtered load. These parameters of sodium excretion did not differ significantly from the control value (0.15 and 0.17%, respectively). This is similar to other instances of decreased proximal sodium reabsorption without change in urinary sodium excretion and indicates increased distal reabsorption of sodium (4, 12, 21). Figures 2-4 suggest such an augmentation of distal sodium reabsorption.

The weight of the animals was uniformly reduced; the mean change in weight representing 5-7% of total body weight. Sartorius, Roenmoucle, and Pits (15) and others have attributed this loss of weight during ammonium chloride loading to a reduction in the extracellular fluid volume (11). Accompanying this loss of weight in the present studies was a small decrease in the glomerular filtration rate. It is likely, therefore, that extracellular fluid volume was reduced under experimental conditions.

Attention has been focused recently on the effects of reduced pH on the transport of sodium in epithelia (6, 9, 16, 23). A large reduction in the pH of mucosal bathing solutions is associated with a reduction in the transepithelial transport of sodium. Several mechanisms may account for this inhibition. First, luminal acidification may decrease the permeability to a whole series of cations including sodium (6, 23). Second, acidification may interfere with sodium motility into and through the cell (23). Third, changes in intracellular pH may affect energy-generating steps essential to transport of sodium (6, 22). And fourth, changes in the ionic constituents (for example, decreased HCO3- of peritubular and tubular fluid secondary to the acidosis itself may account for the inhibition (26).

The relationship of peritubular bicarbonate concentration and sodium reabsorption was explored by Ullrich (26). In these studies, net transtubular Na+ transport decreases

<table>
<thead>
<tr>
<th>Dog</th>
<th>$U_{osm}$</th>
<th>$V$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 C</td>
<td>1,303</td>
<td>0.10</td>
</tr>
<tr>
<td>1 E</td>
<td>1,570</td>
<td>0.14</td>
</tr>
<tr>
<td>1 C</td>
<td>1,543</td>
<td>0.90</td>
</tr>
<tr>
<td>1 E</td>
<td>1,525</td>
<td>0.10</td>
</tr>
<tr>
<td>1 C</td>
<td>1,670</td>
<td>0.16</td>
</tr>
<tr>
<td>1 E</td>
<td>1,683</td>
<td>0.16</td>
</tr>
<tr>
<td>Mean</td>
<td>1,503</td>
<td>0.15</td>
</tr>
<tr>
<td>Mean</td>
<td>1,593</td>
<td>0.13</td>
</tr>
</tbody>
</table>

$U_{osm}$ = urinary osmolality; $V$ = rate of urine flow.
as the HCO₃⁻ concentration in fluids perfusing peritubular capillaries decreases. The reduction of serum bicarbonate in the present studies to 50% of the control values falls well within the levels of bicarbonate at which depression of transtubular sodium transport was seen in the capillary perfusion studies of Ullrich (26).

In summary, the present study provides indirect evidence that an ammonium chloride-induced acidosis causes a decreased reabsorption of sodium and water in the proximal tubule of dogs. The increased load of salt to the distal nephron is reabsorbed completely without net change in salt excretion from control states. Although the mechanism of its action has not been investigated here, it is suggested that the metabolic acidosis itself plays an important role in the changes seen.

REFERENCES


The authors are grateful to Dr. Stephen Rudich, Miss Joan Niezgodski, and Mr. Raphael Saccone for their excellent technical assistance. We also appreciate the help of Mrs. Iris Quintavalli, Mrs. Judy O'Donovan, and Miss Elaine Allen in the preparation of the manuscript.

This research was supported by a grant from the Public Health Service, Federal Health Programs Service, Health Services and Mental Health Administration, P-Y.72-19.

R. Safirstein and V. F. Glassman are Public Health Service Postdoctoral Research Fellows, Federal Health Service, Health Services and Mental Health Administration.

Present address of R. Safirstein: The Mount Sinai Hospital, New York City 10029.

Picaso address requests for reprints to: V. A. DiScala, M.D., Public Health Service Hospital, Staten Island, New York 10304.

Received for publication 5 June 1972.