Evidence for Enhanced Distal Tubule Sodium Reabsorption in Chronic Salt-Depleted Dogs

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ABSTRACT In order to assess the renal tubular site(s) at which sodium reabsorption is enhanced in chronic sodium-depletion, seven normal dogs, six salt-depleted dogs, and three normal dogs receiving aldosterone were studied during a steady-state water diuresis under pentothal anesthesia and during progressive hypotonic saline diuresis. For both maintenance of the water diuresis and progressive hypotonic saline diuresis 0.45% NaCl was used. During the steady state water diuresis delivery of sodium to the diluting segment of the nephron as approximated by solute-free water clearance + sodium clearance/glomerular filtration rate (CnH20 + CnNa/GFR) was significantly lower in salt-depleted dogs compared to normal dogs with or without aldosterone. During progressive hypotonic saline infusion fractional free water excretion (CnH20/GFR) was similar in all three groups as CnH20 + CnNa/GFR increased up to 12-14 ml/min·100 ml GFR. Thereafter, CnH20/GFR continued to rise in virtually a straight line in salt-depleted dogs but leveled off in normal dogs with or without aldosterone. These data demonstrate that enhanced sodium reabsorption in the diluting segment of the nephron is an important determinant of the renal sodium retention in chronic extracellular volume contraction in dogs in addition to confirming the presence of increased proximal tubule sodium reabsorption in these animals.

INTRODUCTION

Recently there has been an increasing interest in the renal tubular site at which sodium reabsorption is enhanced in response to chronic extracellular fluid volume depletion (1-4). Using micropuncture techniques, Weiner et al. (1) found enhanced sodium reabsorption in the proximal tubule in rats with chronic volume depletion. However, Willis et al. (2) were unable to demonstrate increased proximal sodium reabsorption in salt-depleted dogs. More recently, studies by Stein and co-workers (3) showed that sodium transport in collecting ducts is enhanced with mild volume depletion while delivery of filtrate out of the proximal tubule was decreased with severe volume depletion. Mohammad et al. (4), utilizing clearance methodology, have suggested that chronic sodium depletion in the dog is associated with enhanced fractional sodium and water reabsorption primarily in the proximal tubule as these investigators could not demonstrate a significant alteration in distal tubular sodium reabsorption during hypotonic saline loading under hydrated conditions. In these studies, the natriuresis was relatively small in both normal and salt-depleted dogs, suggesting that distal reabsorption was almost complete in both groups. It is possible, therefore, that these investigators may not have achieved sufficient volume expansion to unmask a difference in the distal tubule between their normal and salt-depleted animals.

The present clearance experiments were designed to examine this possibility by infusing 0.45% saline both for establishing water diuresis and for producing progressive volume expansion in normal and chronic salt-depleted dogs. The results indicate that fractional sodium reabsorption is increased in both the proximal tubule and diluting segment of the nephron in the latter.
METHODS

All experiments were performed on female mongrel dogs weighing 15–25 kg. Seven normal dogs were maintained on a diet containing 60 meq of sodium and 40 meq of potassium per day. Six dogs were salt depleted by feeding a diet containing less than 10 meq of sodium and 40 meq of potassium per day for 7 days in addition to receiving furosemide, 40 mg intramuscularly, daily for the first 3 days. On the morning of the experiment each animal received an oral water load, amounting to 5% of body weight, via an orogastric tube. 1 h later, the animal was anesthetized with sodium Pentothal intravenously (1 mg/kg of a 2.5% solution). The level of anesthesia was maintained as light as possible with subsequent small doses of Pentothal. An endotracheal tube was inserted and respiration was regulated with a respirator supplying 60% oxygen. The urinary bladder was catheterized with a no. 16 Foley catheter, and double air washouts were used to ensure complete emptying. Blood samples were obtained through an Intracath secured into a femoral artery.

After initial blood and urine samples were obtained, priming doses of inulin and p-aminohippurate (PAH) were administered. A sustaining solution of 0.45% saline containing sufficient inulin and PAH to maintain adequate blood levels was infused intravenously at 0.5 ml/min with a constant infusion pump for the remainder of the experiment. During the first part of the experiment water diuresis was maintained by infusing 0.45% saline intravenously at the rate of 1.5 ml/min above urine flow rate. After an initial 60-min equilibration period urine specimens were collected at 10-min intervals and a midpoint blood sample was obtained after every fourth clearance period. After a steady state of urine flow was achieved (three clearance periods with less than 5% variation in urine flow and osmolality), the rate of 0.45% saline infused was progressively increased (second part of the experiment) until the rate was 25–30 ml/min, at which time it was kept constant and 10-min clearance periods obtained until urine flow once again stabilized, at which point the experiment was terminated. In three normally fed dogs the exact same procedure was carried out except that each dog received 500 µg of aldosterone intravenously at the time of the oral water load followed by the infusion of 1 µg/min throughout the experiment.

In another group of studies, consisting of six normal dogs (two of which received aldosterone) and four salt-depleted dogs, after urine flow stabilized after progressive hypotonic saline loading, the peritoneal cavity was rapidly opened and the renal pedicles cross clamped. Both kidneys were immediately removed and partially frozen in dry ice-acetone. This entire procedure was accomplished in 2 min or less. Thereafter, each kidney was halved to expose the papillae. Two papillary tips, weighing 150–200 mg each, were removed from each kidney for analysis of papillary tip osmolality.

In a separate group of six normal dogs and six salt-depleted dogs maximum urine osmolality was determined after 24 h of food and fluid withdrawal and 16 h after the intramuscular injection of 5 U of pitressin tamate in oil. All urine and plasma specimens were analyzed for osmolality, sodium, and potassium according to the methods previously reported from this laboratory (5). Papillary tip osmolality was determined by the method of Appelboom et al. (6). The only modification was that the tissue was partially frozen (as described above) for ease of handling. The mean osmolality of the four papillary tip specimens taken from each dog was used as the papillary tip osmolality for that experiment. Inulin and PAH were determined on an AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N.Y.) by the methods of Fielddo and Stamey (7) and Harvey and Brothers (8), respectively. Inulin clearance was used as a measure of glomerular filtration rate (GFR) and PAH clearance (C_{PAH}) as a measure of effective renal plasma flow. Filtration fraction (FF) was calculated as GFR/C_{PAH}. Solute clearance (C_{solute}) was calculated as U_{solute}V/P_{solute} where U_{solute} and P_{solute} represent urine and plasma osmolality, respectively, and V is urine flow in milliliters per minute. Solute-free water clearance (C_{H_2O}) = V - C_{solute}. Sodium clearance (C_{Na}) = U_{Na}V/P_{Na} where U_{Na} and P_{Na} represent urinary and plasma sodium concentrations, respectively. The sodium load presented to the diluting segment was approximated by (C_{H_2O} + C_{Na}). In the text, tables, and figures, C_{H_2O}, C_{Na}, and C_{PAH} are corrected for a GFR of 100 ml/min.

The significance of the difference between means was analyzed by the Student's t test. Regression lines and correlation coefficients were calculated by the method of least squares.

RESULTS

After salt restriction and furosemide administration, the salt-depleted dogs had an average weight loss of 8.1% (ranging from 7 to 11%) and showed evidence of sodium retention as urinary sodium was less than 3 meq/liter in all six dogs the morning of the experiment. Mean hematocrit was 39±3% (SEM) in salt-depleted dogs, not significantly different from the mean hematocrit of 35±1 in normal dogs (P > 0.2). Mean plasma sodium was 137±2 in salt-depleted dogs, which was not significantly different from normal dogs, where the plasma sodium was 138±1. Mean plasma potassium was also not significantly different between these two groups of animals.

Table I summarizes the data in seven normal and six salt-depleted dogs during the steady-state water diuresis. U_{solute} was 81±3 in salt-depleted dogs and 91±10 mosmol/kg H2O in normal dogs, which was not significantly different. It was evident that there was sodium retention in salt-depleted dogs even after infusion of 0.45% saline for maintenance of the water diuresis. U_{Na}V was 48±12 meq/min and C_{Na} was 0.8±0.2 ml/min·100 ml GFR in salt-depleted dogs while U_{Na}V was 254±77 meq/min and C_{Na} was 4.3±1.4 ml/min·100 ml GFR in normal dogs (P < 0.02 for both). V was 9.5±1.5 ml/min·100 ml GFR in salt-depleted dogs and 18.0±2.6 ml/min·100 ml GFR in normal dogs (P < 0.02). C_{H_2O} and C_{Na} was also significantly lower in salt-depleted dogs, 7.4±1.2 compared to 16.1±2.4 ml/min·100 ml GFR in normal dogs (P < 0.01). There were no differences in GFR, C_{PAH}, and FF between these two groups of animals. Fig. 1

Enhanced Distal Tubule Sodium Reabsorption in Salt-Depleted Dogs
Table I
Summary of Results Obtained during the Steady-State Water Diuresis in Normal and Salt-Depleted Dogs

<table>
<thead>
<tr>
<th></th>
<th>Uosm</th>
<th>V</th>
<th>Csum</th>
<th>CH2O</th>
<th>Uosm</th>
<th>PN</th>
<th>CNa</th>
<th>CH2O + CNa</th>
<th>Uosm</th>
<th>PK</th>
<th>GFR</th>
<th>CPAH</th>
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<tr>
<td></td>
<td>molosmol/kg</td>
<td>ml/min -100 ml GFR</td>
<td>µeq/min</td>
<td>meq/liter</td>
<td>ml/min -100 ml GFR</td>
<td>µeq/min</td>
<td>meq/liter</td>
<td>ml/min</td>
<td>%</td>
<td></td>
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<tr>
<td>Normal (7)</td>
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<tr>
<td>Mean ±SEM</td>
<td>91 ±10</td>
<td>18.0</td>
<td>6.2</td>
<td>11.8</td>
<td>254</td>
<td>138</td>
<td>4.3</td>
<td>16.1</td>
<td>27</td>
<td>3.9</td>
<td>46.1</td>
<td>149.0</td>
<td>30.6</td>
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<tr>
<td>Salt depleted (6)</td>
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</tr>
<tr>
<td>Mean ±SEM</td>
<td>81 ±3</td>
<td>9.5</td>
<td>2.9</td>
<td>6.6</td>
<td>48</td>
<td>137</td>
<td>0.8</td>
<td>7.4</td>
<td>34</td>
<td>3.7</td>
<td>54.8</td>
<td>172.0</td>
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</tr>
</tbody>
</table>

P value NS <0.02 <0.05 <0.01 <0.02 NS <0.02 <0.01 NS NS NS NS NS

V, potassium excretion; PK, plasma potassium concentration.

illustrates the relationship between CH2O + CNa and cumulative sodium load, which is the total amount of sodium infused during the experiment up to and including the steady-state periods. It can be seen that mean CH2O + CNa was lower in salt-depleted dogs even with a significantly greater mean cumulative sodium load of 163 meq compared to 136 meq in normal dogs (P < 0.01).

Fig. 2 depicts the relationship between CH2O and CNa, CH2O + CNa, in normal, aldosterone-loaded normal, and salt-depleted dogs during the first part of the experiment and also during progressive hypotonic saline loading. At low levels of CNa, the increase in CH2O in salt-depleted dogs was similar to that of normal dogs. As CH2O + CNa increased beyond 12-14 ml/min·100 ml GFR, CH2O continued to rise progressively in salt-depleted dogs but tended to level off in normal dogs with or without aldosterone. The best-fit line for the relationship between CH2O and CNa + CNa in salt-depleted dogs is linear and represented by y = 0.74x + 1.00 (r = 0.96). This is significantly different from the curvilinear relationship in normal dogs represented by y = -0.022x + 1.064x - 0.033. In three normal dogs given aldosterone the relationship between CNa and CH2O + CNa was similar to normal dogs with the best-fit line represented by y = -0.021x + 1.050x - 0.442.

During progressive hypotonic saline loading, GFR, CPAH, and FF remained stable in all three groups with no significant differences noted. In addition, although a decrease in serum sodium and potassium was noted in the three groups, there were no differences among them.

Fig. 3 demonstrates the relationship between sodium and potassium excretion in normal, salt-depleted, and aldosterone-loaded dogs during hypotonic saline infusion. It is evident that almost throughout the range of sodium excretion, potassium excretion was greater in salt-depleted compared to normal dogs, although this became less evident at the higher rates of sodium excretion because of a tendency for potassium excretion to decrease in the former. The pattern of potassium excretion was similar in salt-depleted dogs and aldosterone-loaded dogs except that a decrease at higher rates of sodium excretion was not evident in the latter.

In the group of experiments in which papillary tip osmolality was determined, the mean CH2O + CNa at the time of sacrificing the animals was similar in all three groups of dogs, 23.6±1.3 in the four normal dogs, 21.8±2.1 in the two aldosterone-loaded dogs, and 22.9±1.4 ml/min·100 ml GFR in the four salt-depleted dogs. CH2O in normal dogs with or without aldosterone was similar, 12.7±7 and 13.0±0.8, respectively, but was significantly higher in salt-depleted dogs, 19.0±0.8 ml/min·100 ml GFR (P < 0.001). These findings are almost identical to the data depicted in Fig. 2. The mean papillary tip osmolality simultaneously measured was 387±9 in normal dogs, 370±22 in aldosterone-loaded dogs, and 409±16 mosmol/kg H2O in salt-depleted dogs, which are not statistically significantly different from each other.

Maximum urine concentration after 24 h of fasting and pitressin tannate in oil injection in six normal dogs was 1,732±153 and in six salt-depleted dogs it was 1,851±190 mosmol/kg H2O (P > 0.1).

FIGURE 1 Comparison of the relationship between sodium supply to the diluting segment (CH2O + CNa) and cumulative sodium load in normal dogs and salt-depleted dogs during the steady-state water diuresis. The mean for each group is shown ±1 SEM for both CH2O + CNa and cumulative sodium load.

DISCUSSION

In the present experiments, $C_{Na}$ during hypotonic saline diuresis was consistently greater in salt-depleted dogs compared to normal dogs as $C_{Na} + C_{H2O}$ increased beyond 16-18 ml/min·100 ml GFR (Fig. 2). Insofar as the clearance of free water is dependent primarily on sodium reabsorption in the diluting segment of the nephron, it follows that fractional sodium reabsorption is increased at this site in salt-depleted dogs. Since $C_{Na}$ may be suppressed by progressive volume expansion in dogs (9) possible differences in the degree of expansion of the extracellular fluid volume in these two groups must be considered. First, to achieve a comparable level of $C_{Na} + C_{H2O}$, salt-depleted dogs invariably received a greater cumulative sodium load. This is shown in Fig. 1 during the steady-state water diuresis, but it was also present during the progressive hypotonic saline infusion. Second, since sodium excretion was considerably less in salt-depleted dogs even at higher rates of distal delivery, there was a greater positive balance of sodium in this group. Thus, in salt-depleted dogs extracellular fluid volume expansion was probably greater than in normal dogs for each level of sodium supply to the diluting segment, yet $C_{Na}$ was greater in these animals.

Alternately, the greater $C_{Na}$ formation in salt-depleted dogs compared to normal dogs during hypotonic saline loading could be due to a decrease in the back diffusion of water in the collecting duct due to an alteration in collecting duct permeability to water or to a lesser gradient for water movement. However, urinary concentration under hydropenic conditions was similar in salt-depleted dogs compared to normal dogs, indicating that there was no alteration in the water permeability of the distal tubule and collecting duct. Moreover, papillary tip osmolality during water diuresis after hypotonic saline loading was similar in these animals compared to normal dogs with or without aldosterone so that there is no evidence for a lesser gradient. In fact, the lower solute excretion in salt-depleted dogs at higher levels of urine flow suggests that the gradient for water back diffusion in the collecting duct may be greater for these animals during progressive hypotonic saline loading.

The findings of enhanced distal sodium reabsorption after sodium depletion is in agreement with micropuncture data. Stein et al. (3) found that fractional sodium reabsorption was increased in early and late distal tubules as well as in the proximal tubule in severely volume-depleted rats whereas fractional reabsorption was enhanced only in collecting tubule in mild volume depletion. In addition, the difference in the handling of sodium in the distal nephron was noted only after volume expansion. In the face of sodium retention in mild volume depletion in the rat, Willis et al. (2) were unable to demonstrate greater fractional reabsorption in the proximal tubule and suggested the importance of the distal nephron in chronic regulation of sodium balance.

The present data are at variance with the clearance data of Mohammad et al. (4). These authors found that
CH2O per CH2O + Cna was unchanged after sodium depletion during hypotonic saline loading in dogs. In these studies, water diuresis was produced by the infusion of 2.5% glucose and progressive volume expansion achieved by infusion of hypotonic saline so that overall volume expansion was much less than in the present experiments. As a result, urinary sodium excretion remained low in both sodium-depleted and normal dogs in contrast to the present experiments, indicating that distal tubule sodium reabsorption was not significantly inhibited in either group in their experiments.

Stein et al. (9), demonstrated that CH2O attained a maximal with marked natriuresis when water diuresis and progressive volume expansion were achieved with 0.45% saline in normal dogs. In contrast, when 2.5% mannitol was infused, CH2O progressively increased and a maximum was not noted. In other clearance studies by Barton et al. (10), in which water diuresis was induced with 1.5% dextrose infusion followed by 0.45% saline, there was no evidence of inhibition of distal transport in normal dogs. More recently, Bennett (11) has clarified previous conflicts in the literature by demonstrating that if sufficient extracellular fluid volume expansion is achieved in normal dogs inhibition of distal reabsorption will take place. This author noted that increased distal delivery of sodium achieved without volume expansion was not associated with a limit in distal sodium reabsorption.

In our studies, both water diuresis and progressive volume expansion was achieved with hypotonic saline, and sodium excretion, even during the steady-state water diuresis, was greater in both groups of animals (Table I) compared to the studies of Mohammad et al. (4). It is likely, therefore, that there was a greater degree of extracellular fluid volume expansion in our dogs and that distal sodium reabsorption reached a maximum rate in normal dogs, unmasking the persistent distal sodium reabsorption in salt-depleted dogs even at high rates of delivery of filtrate to the diluting segment.

The studies in normal dogs pretreated with aldosterone demonstrate that the mineralocorticoid does not alter CH2O formation even at high levels of filtrate delivery to the diluting segment. Therefore, it seems unlikely that a mineralocorticoid effect could account for the enhanced sodium reabsorption in the water clearing site after sodium depletion. Since plasma sodium concentration was similar in normal and salt-depleted dogs throughout the experiment, augmented CH2O formation in the latter could not be accounted for by a greater degree of hyponatremia (12). Finally, there were no differences in GFR, CKNa, FF, or serum potassium concentration between the two groups.

Potassium excretion was appreciably higher in salt-depleted dogs at each level of sodium excretion compared to normal dogs (Fig. 3). When aldosterone was administered to normal dogs, potassium excretion was similar to salt-depleted dogs, suggesting that this effect in the latter was secondary to increased aldosterone activity. Of interest is the fact that potassium excretion tended to decrease in salt-depleted dogs at high levels of sodium excretion almost approaching that noted in normal dogs, whereas potassium excretion remained high throughout in aldosterone-loaded normal dogs. These findings suggest that aldosterone activity decreased in salt-depleted dogs as volume expansion increased.

CH2O + Cna, which may be used as an approximation of the fraction of sodium escaping reabsorption in the proximal tubule, was significantly lower in salt-depleted dogs than normal dogs during the steady-state water diuresis even with a greater cumulative sodium load (Table I and Fig. 1), suggesting enhanced proximal sodium and water reabsorption associated with chronic sodium depletion. This finding is similar to that of Mohammad et al. (4) in spite of the fact that water diuresis was sustained in the present experiments with hypotonic saline which apparently decreased proximal tubule sodium reabsorption.

In summary, the present studies demonstrate that enhanced sodium reabsorption in the diluting segment is an important determinant of the renal sodium retention in chronic extracellular fluid volume contraction. This is not meant to imply that alterations in proximal reabsorption do not play a major role in sodium retention in chronic sodium depletion. However, it seems clear from the present experiments that even when enhanced sodium reabsorption in the proximal tubule of chronic salt-depleted dogs is partially overcome by progressive volume expansion with hypotonic saline loading which is associated with an increase in sodium delivery to the diluting segment, this portion of the tubule continues to avidly reabsorb sodium and would appear to be the final regulator of urinary sodium excretion in these animals.

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