Segmental Chloride Transport in the Dahl-S Rat Kidney During l-Arginine Administration

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The administration of the amino acid l-arginine prevents the development of hypertension in Dahl salt-sensitive (DS) rats (1,2). We have previously shown that this effect is associated with improvement in the blunted relationship between renal perfusion pressure and urinary sodium excretion that usually characterizes sodium handling in these animals (2). Neither the mechanism nor the tubule segment(s) responsible for the improvement in sodium handling induced by l-arginine has been identified. In previous studies, we, as well as Roman and Kaldunski, have reported that DS rats have greater chloride reabsorption in cortical loop segments than do Dahl salt-resistant (DR) rats when both are examined at equivalent renal perfusion pressures (3,4). This abnormality is present before the development of hypertension (4,5). Because l-arginine has no effect on renal sodium excretion in DR rats, it seemed reasonable to postulate that l-arginine's primary effect may be to correct the abnormality in cortical loop sodium chloride transport in DS rats. On the other hand, because pressure natriuresis is felt to be related to events occurring in juxtamedullary nephrons or the collecting duct (6-9), l-arginine could alter transport at sites beyond the distal convoluted tubule or in deep nephrons. This study was designed to examine fluid and chloride handling in the superficial cortical nephron segments of l-arginine or vehicle DS rats maintained on an 8% sodium diet to examine the location for l-arginine's effect on sodium excretion.

Methods

Male DS and DR Rapp rats (Harlan Sprague-Dawley, Indianapolis, IN) were maintained on tap water and standard rodent chow (20 μM Na/g of chow) for 72 h after arrival in our facility. All animals were housed according to institutional guidelines, and the studies were approved by the Animal Care and Use Committee of the University of Mississippi Medical Center. All rats were then placed on 8% sodium chow and given tap water to drink ad libitum. DS rats were given ip injections of either the hydrochloride salt of l-arginine (Sigma Chemical Co., St. Louis, MO) or its vehicle for 3 wk. DR rats received only the vehicle for l-arginine, because we have previously shown that L-arginine has no effect on blood pressure or sodium chloride handling in these animals (2). After 3 wk on the diet, rats were anesthetized with an ip injection of 5 sec-butyl-5 ethyl-2-thiobarbituric acid (Inactin; Promonto, Hamburg, Germany) in a dose of 80 mg/kg. Animals were placed on a thermostatically controlled animal table, and body temperatures were maintained at 37°C with a servo activated controller (Vestavia Scientific Co., Vestavia Hills, AL). After tracheostomy, PE-50 (Clay Adams, Parsippany, NJ) polyethylene catheters were placed in the femoral...
artery for the continuous measurement of mean arterial pressure and in the jugular vein for iv infusions. A flanged PE-50 polyethylene catheter was placed in the bladder for urine collection. From the start of surgery, isotonic Ringer's solution containing 5% polyfructosan (Inutest; Laevasan Gesellschaft, Linz, Austria) was infused at a rate of 1.5 mL/h. The abdomen was opened through a midline incision, and an ultramicroclamp was placed above the renal arteries for the manipulation of renal perfusion pressure. The left kidney was exposed through a subcostal incision and gently separated from the adrenal gland and perirenal fat. The kidney was placed in a plastic cup, and the upper ureteral segment was cannulated with PE-50 polyethylene tubing. Agar was placed around the kidney to form a well. The kidney was then bathed with mineral oil warmed to 37°C. To compensate for the reduction in plasma volume attendant on abdominal surgery, rats received a 1.2 mL/100 g body wt infusion of 5% albumin Ringer's solution during the surgical procedure. Additionally, all rats received an infusion of 154 mM sodium chloride containing 1% bovine serum albumin at a rate of 100 μL/min throughout the study. After the surgical procedure, cortical surface convolutions of three to four latest proximal and three to four earliest distal tubules were identified by observing the passage of three to four 0.05-mL boluses of 7.5% FD&C (Keystone Aniline and Chemical Co., Chicago, IL) green dye. In DS rat groups, one to two latest distal tubule segments were also identified.

After a 60-min surgical recovery period, tubule fluid samples were obtained in random order from the previously identified proximal, early distal, and in DS rats, late distal sites over a 90-min experimental period. The method of tubule fluid sampling has been described by us previously (3.5). Timed collections of tubule fluid were obtained for the determination of flow rate and inulin and chloride concentrations. Urine was collected under oil in preweighed vials for the duration of the experimental period. Blood was obtained at the beginning and end of the experimental period for the measurement of inulin, sodium, and chloride concentrations.

Three groups of rats were examined. All rats were of comparable age at the time of study. The first group (N = 7) was DR rats treated with the vehicle for L-arginine administration. The second group of rats (N = 7) was DS rats that had received the vehicle for L-arginine administration. In these animals, renal perfusion pressure was reduced to the level of the L-arginine-treated DS rats before the surgical recovery period by tightening the aortic clamp. The third group of rats (N = 8) was DS rats that received daily ip injections of L-arginine in a dose of 300 mg/kg body wt. The last dose of L-arginine was administered approximately 18 h before the acute study. At the end of the experimental period, animals from all groups were euthanized by exsanguination while still under anesthesia, and the kidneys were removed and weighed.

Analytical Techniques

Urinary flow rate was determined by change in weight of preweighed vials. Chloride concentrations in serum and urine were analyzed amperometrically. Inulin concentration in urine and plasma was determined by the diphenylamine method of Walser et al. (10). Tubule fluid volume was measured in constant-bore glass tubing with a microslide comparator (Gaertner Scientific, Chicago, IL). Tubule fluid inulin concentration was determined by the method of Vurek and Pegram (11). Tubule fluid chloride concentration was determined by electrometric titration according to the second method of Ramsey et al. (12).

Analysis of Data

Because of the genetic contamination of Dahl-Rapp rats in early 1993, only data from rats obtained before February 1993 were used for analysis. The determination of the concentrations of inulin, sodium, and chloride in blood and urine and urinary flow rate permitted the calculation of whole-kidney GFR and urinary excretion rates of sodium and chloride according to standard expressions. The measurement of flow rate and inulin and chloride concentrations in tubule fluid samples allowed the determination of single-nephron GFR (SNGFR) and fractional delivery rates of chloride and fluid to puncture sites as previously described (3). The fraction of filtered chloride reabsorbed between two nephron segments, A and B, was calculated according to the following expression:

$$\frac{([TF/P]_{Cl/IN} - [TF/P]_{Cl/IN}^P) \times 100}{[TF/P]_{Cl/IN}^P}$$

where ([TF/P]_{Cl/IN}) is the tubule fluid to plasma chloride and inulin ratio. This calculation was performed for each individual animal from the mean values obtained in that individual animal. Absolute chloride reabsorption was then calculated in each individual animal from the mean values for early distal SNGFR nephron filtered load, and appropriate value for fractional reabsorption were determined in that animal. In all statistical treatments, N represents the number of animals and not tubules. Analysis of variance was used to determine statistical significance between groups. If analysis of variance indicated that a statistical significance existed, then a Student-Newman-Keuls test was used to determine statistical significance among the three groups (13). Statistical significance was set at the P < 0.05 level.

RESULTS

The whole-kidney and blood values in each of the three study groups are summarized in Table 1 and Figure 1. Mean arterial pressure was less (P < 0.05) in L-arginine-treated DS rats than in vehicle-treated DS rats and not different (P = not significant [NS]) from mean arterial pressure in DR rats. Renal perfusion pressure was not different (P = NS) among any of the three groups. Inulin clearance determined at approximately equivalent renal perfusion pressures (Table 1) tended to be slightly less in vehicle-treated DS rats than in other groups, but this did not reach statistical significance. Absolute and fractional urinary sodium excretion rates were greater (P < 0.05) in L-arginine-treated DS rats than in vehicle-treated DS rats. Sodium excretion was not different (P = NS) between L-arginine-treated DS rats and DR rats. Urinary chloride excretion demonstrated a similar pattern (Figure 1). There were no differences in plasma sodium or chloride concentrations among the three groups. SNGFR determined from either proximal or distal puncture sites was not different (P = NS) among any of the three experimental groups (Figure 2).

Absolute and fractional chloride delivery rates to the latest accessible segment of the proximal convoluted tubule were not different between any of the three rat groups (Table 2). Fractional proximal chloride reab-
TABLE 1. Mean arterial pressure, inulin clearance, and electrolyte excretion rates in Dahl rats maintained on 8% sodium intake and given L-arginine or its vehicle for 3 wk

<table>
<thead>
<tr>
<th>Rat</th>
<th>MAP (mm Hg)</th>
<th>RPP (mm Hg)</th>
<th>Cln (μL/min per g kidney wt)</th>
<th>UNaV (nmol/min per g kidney wt)</th>
<th>FeNa (%)</th>
<th>UCIV (nmol/min per g kidney wt)</th>
<th>PNa (mmol/L)</th>
<th>PCI (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR (N = 7)</td>
<td>130 ± 1</td>
<td>130 ± 1</td>
<td>1,144 ± 171</td>
<td>9,211 ± 1,305</td>
<td>5.27 ± 0.67b</td>
<td>9,338 ± 1,313</td>
<td>159 ± 3</td>
<td>122 ± 2</td>
</tr>
<tr>
<td>DS Vehicle</td>
<td>167 ± 7</td>
<td>124 ± 3</td>
<td>861 ± 45</td>
<td>2,793 ± 428</td>
<td>2.07 ± 0.28</td>
<td>3,128 ± 462</td>
<td>157 ± 3</td>
<td>118 ± 2</td>
</tr>
<tr>
<td>DS L-Arginine (N = 8)</td>
<td>129 ± 3b</td>
<td>127 ± 3</td>
<td>1,121 ± 178</td>
<td>9,370 ± 904b</td>
<td>6.14 ± 1.02b</td>
<td>8,897 ± 476b</td>
<td>157 ± 2</td>
<td>118 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SE; N, number of rats studied; MAP, mean arterial pressure; RPP, renal perfusion pressure; Cln, inulin clearance; UNaV, absolute urinary sodium excretion; FeNa, fractional urinary sodium excretion; UCIV, absolute urinary chloride excretion, PNa, plasma sodium concentration; PCI, plasma chloride concentration. Cln and electrolyte excretion rates were determined at renal perfusion pressures listed in the second column. b P < 0.05 versus DS vehicle.

![Fractional Urinary Chloride Excretion](image)

Figure 1. Fractional urinary chloride excretion in DR rats (R), DS rats (S), and DS rats treated with L-arginine (L-ARG) daily for 3 wk. Rats were studied at equivalent renal perfusion pressures. * P < 0.05 versus DS rats.

![SNGFR](image)

Figure 2. SNGFR in DR rats, DS rats, and DS rats treated with L-arginine. See legend to Figure 1 for an explanation of abbreviations.

![Inhibitor Study](image)

L-arginine and DS rats that have received vehicle. Fractional loop segment chloride reabsorption was greater (P < 0.05) in both DS rat groups than in DR rats (Figure 3). Fractional loop segment chloride reabsorption was almost identical in vehicle-treated and L-arginine-treated DS rats (58.4 ± 1.4 versus 58.9 ± 3.9%; P = NS). Fractional loop segment fluid reabsorption tended to be greater in both DS rat groups compared with DR rats, but this difference did not reach statistical significance (Figure 4). Fractional fluid reabsorption in the loop segment was not different between vehicle-treated and L-arginine-treated DS rats.

There were no differences in absolute or fractional chloride delivery to the late distal tubule puncture site between vehicle-treated and L-arginine-treated DS rats (Table 3). Fractional chloride reabsorption in the distal tubule was almost identical between these two DS rat groups (DS, 5.0 ± 1.6% versus DS L-arginine, 5.4 ± 3.4%; P = NS) (Figure 3). Fractional fluid reabsorption in the distal tubule was likewise not different between vehicle-treated and L-arginine-treated DS rats (Figure 4).

DISCUSSION

In previous work, we have shown that either the acute or the chronic administration of L-arginine to DS rats restores the abnormal pressure-natriuresis relationship that usually characterizes these animals (2). This response, however, was not observed with equivalent amounts of D-arginine and could be prevented by the concomitant administration of the nitric oxide synthase inhibitor L-NAME. L-arginine, however, had no effects on pressure natriuresis in DR rats. This study confirms the central findings of our previous work, that at equivalent perfusion pressures, urinary sodium chloride excretion is greater in DS rats receiving L-arginine than in DS rats receiving L-arginine vehicle and not different from DR rats. This study also extends these findings by examining fluid and chloride handling in cortical nephron segments in these groups. The results show that SNGFR in cortical nephrons and,
perfusion pressure and sodium excretion in the DS rat results from an effect on sodium chloride transport beyond the distal convoluted tubule, perhaps in the collecting duct. Alternatively l-arginine could increase delivery out of juxtamedullary nephrons.

The mechanism by which l-arginine increases sodium chloride excretion in DS rats remains to be determined. We have previously shown that l-arginine improves the autoregulation of GFR in the DS rat (2). In those studies, as in this investigation, whole-kidney GFR was slightly greater in l-arginine-treated DS rats compared with vehicle-treated DS rats at each individual renal perfusion pressure examined. Although these differences did not reach statistical significance, increases in these parameters are likely to be important contributors to the overall improvement in renal sodium chloride excretion observed in l-arginine-treated rats. On the other hand, we, as well as Roman and Kaldunski, have shown that the cortical loop segment of DS rats has enhanced chloride reabsorption relative to that of DR rats (3,4). Furthermore, this finding is present before exposure to a high-salt diet and before the development of hypertension (4,5). We have proposed that this abnormality may be important for the blunted natriuretic capacity and the attenuated pressure-natriuresis relationship found in DS animals. This study confirms that loop chloride reabsorption is greater in hypertensive DS rats than in DR rats when both are examined at equivalent renal perfusion pressures. Interestingly and unexpectedly, the chronic administration of l-arginine to DS rats had no effect on loop chloride reabsorption, even though it prevented the development of hypertension and increased urinary sodium chloride excretion to rates not different from those observed in DR rats. In fact, loop chloride reabsorption was almost identical in DS rats receiving l-arginine or its vehicle and in both circumstances was greater than that observed in DR rats (Figure 3). Thus, a reduction in loop chloride uptake is not a requirement for either the prevention of hypertension or the improvement in sodium chloride excretion that follows l-arginine administration to the DS rat.

Thus, filtered loads of sodium and chloride were not different between vehicle-treated and l-arginine-treated DS rats. There were also no differences in chloride and fluid reabsorption in the proximal tubule, loop segment, or distal convoluted tubule between DS rats receiving l-arginine and DS rats receiving l-arginine vehicle. Thus, these data suggest that the effect of l-arginine to increase urinary sodium excretion and restore the relationship between renal perfusion pressure and sodium excretion in the DS rat results from an effect on sodium chloride transport beyond the distal convoluted tubule, perhaps in the collecting duct. Alternatively l-arginine could increase delivery out of juxtamedullary nephrons.

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Chen and associates have recently shown that dietary L-arginine administration prevents the hypertensive nephrosclerosis that occurs in DS rats maintained on a high-sodium diet (14). On the basis of their findings, the greater urinary sodium chloride excretion observed in DS rats chronically treated with L-arginine in our study could have resulted from the prevention of structural renal damage. On the other hand, we have previously shown that the acute iv administration of L-arginine improves blood pressure and normalizes sodium excretion even in hypertensive DS rats (5). Thus, the improvement in sodium chloride excretion after chronic L-arginine administration in this study may have resulted from a combination of the prevention of structural damage and specific effects on electrolyte transport.

The finding that L-arginine improves the relationship between pressure and sodium excretion without altering chloride, and presumably sodium, reabsorption in the cortical proximal tubule, loop, or distal convoluted tubule would be consistent with our current understanding of the mechanisms and tubule segments responsible for the pressure-natriuresis relationship. Kunau and Lameire demonstrated that, in normotensive rats, acute increases in renal perfusion pressure increase urinary sodium excretion without altering tubular sodium reabsorption up to the superficial late distal tubule (7). In subsequent studies, Haas and associates, as well as Roman, have shown little or no effect of changes in renal perfusion pressure on delivery out of cortical proximal tubule segments (8,9). Roman and Zou have recently proposed that changes in renal perfusion pressure influence tubular reabsorption through changes in medullary hemodynamics and renal interstitial hydrostatic pressure (15). Furthermore, the direct administration of L-arginine analogs that inhibit nitric oxide synthase reduces renal interstitial pressure and urinary sodium excretion when infused directly into the renal medulla (16). This occurs without altering blood pressure or GFR. The relationship between renal perfusion pressure and renal interstitial hydrostatic pressure has been found to be abnormal in hypertensive DS rats and is improved by L-arginine administration (17). Husted and Stokes have shown that cells from the inner medullary collecting duct of kidneys from prehypertensive DS rats transport more sodium than do cells obtained from a similar location in DR rats (18). L-Arginine analogs have been reported to alter transport-dependent oxygen consumption in suspensions of inner medullary collecting duct cells in vitro (19). Thus, L-arginine could improve sodium excretion and perhaps pressure natriuresis by altering sodium reabsorption and sodium transport in the renal medulla. Additional studies will be necessary to determine which sites account for the improvement in pressure natriuresis observed during L-arginine administration.

Finally, most investigators attribute the effects of L-arginine on blood pressure and renal function to its ability to enhance nitric oxide production (1,2,20). Whether the improvement in blood pressure and sodium excretion found in DS rats is related to changes in nitric oxide production or release was not examined in this study. Westberg and associates have reported that urinary NO₂ + NO₃ excretion, a putative index of nitric oxide activity, is lower in DS rats than in DR rats after exposure to a high-salt diet and is increased by L-arginine administration (21). We have previously shown that the improvements in these parameters can be prevented by the administration of N⁶-nitro-L-arginine methylester (2). Furthermore, the improvement in blood pressure during L-arginine administration to DS rats is associated with significant increases in urinary cGMP excretion, the final messenger for the inhibition of sodium reabsorption in terminal nephron segments (1). Nitric oxide has also been shown to play an important role in the regulation of pressure natriuresis in normal animals (21–24). Thus, it seems reasonable to suggest that the improvement in sodium excretion in DS rats after L-arginine administration in this study was also mediated through the nitric oxide pathway.

In summary, this study confirms our previous work that L-arginine given to DS rats maintained on a high-salt diet prevents the development of hypertension and improves their ability to excrete sodium chloride. The improvement in sodium chloride excretion is not the result of enhanced delivery from cortical tubule segments nephrons but may result from the inhibition of transport in the collecting system. Alternatively, L-arginine could increase delivery from juxtamedullary nephrons.

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