Enhanced Chloride Reabsorption in the Loop of Henle in Dahl Salt-Sensitive Rats

Richard J. Roman and Mary L. Kaldunski

This study examines the nephron segments contributing to the blunted pressure-natriuretic response in Dahl salt-sensitive rats. Urine and late proximal and early distal tubular fluid samples were collected from 16–20-week-old, inbred Dahl salt-sensitive (DS/Jr) and salt-resistant (DR/Jr) rats, and Dahl salt-sensitive (DS) and salt-resistant (DR) rats from the Brookhaven colony, that were maintained from birth on a low (0.3%) sodium chloride diet. Urine flow and sodium and chloride excretions were 65% less in the DS/Jr than in the DR/Jr rats when their kidneys were perfused at an equal renal perfusion pressure of approximately 150 mm Hg. The percentages of the filtered load of water and chloride remaining at the end of the proximal tubule were significantly greater in DS/Jr rats than in DR/Jr rats; however, the percentages of the filtered load of water and chloride reaching the early distal tubule were significantly less, by 29% and 77%, respectively. Fractional reabsorption of water and chloride in the loop of Henle of DS/Jr rats was twice that observed in DR/Jr rats. Similar results were obtained in DS and DR rats of the Brookhaven strain. Urine flow and sodium and chloride excretions were 60% lower in DS than in DR rats at a renal perfusion pressure of 135 mm Hg. Proximal tubular reabsorption of water and chloride was similar in DS and DR rats; however, the percentages of the filtered load of water and chloride reabsorbed in the loop of Henle were greater in DS than in DR rats. These results indicate that enhanced reabsorption of water and chloride in the loop of Henle contributes to the resetting of the pressure-natriuretic relations in DS and DS/Jr rats. In addition, a reduced glomerular filtration rate in DS/Jr rats, which impairs their ability to excrete sodium at normal levels of renal perfusion pressure, may explain the tendency of DS/Jr rats to develop mildly elevated arterial pressures even when fed a low salt diet. (Hypertension 1991;17:1018–1024)

Renal transplantation studies have indicated that some form of renal dysfunction underlies the development of hypertension in humans and genetically hypertensive rats. However, the factors responsible for resetting kidney function in hypertension and the nephron segments involved have not been identified. The pressure-natriuretic relation is altered before the induction of salt hypertension in Dahl salt-sensitive (DS) rats of both the Brookhaven and the inbred John Rapp (Jr) strains. Tubular reabsorption of water and electrolytes is enhanced in DS rats, and they require a higher renal perfusion pressure (RPP) to achieve the same fractional sodium excretion as control animals. The purpose of the present study was to identify the nephron segments contributing to the blunted pressure-natriuretic response in DS rats maintained from birth on a low (0.3%) sodium chloride diet.

Methods

Experiments were performed on nine inbred DS/Jr and nine Dahl salt-resistant (DR/Jr) rats, and on eight DS and seven DR rats of the Brookhaven strain. The animals were purchased from Harlan Industries (Madison, Wis.) and were maintained on a low (0.3%) sodium chloride diet from birth. The rats ranged from 16 to 20 weeks of age at the time of the study.

The rats were anesthetized with ketamine (30 mg/kg i.m.) and Inactin (30 mg/kg i.p.) and placed on a heated table to maintain temperature at 36.5°C. Cannulas were placed in the jugular vein for infusions, in the femoral artery for measurement of RPP, and in the ureter for the collection of urine. Studies were performed in rats undergoing a mild saline diuresis, because this is the experimental condition in which differences in the pressure-natriuretic responses of DS and DR rats were observed previously. The rats received an intravenous infusion of a...
0.9% sodium chloride solution containing 1% albumin at a rate of 100 μl/min throughout the experiment. [3H]Inulin (50 μCi/ml) was included in the infusion solution for measurement of glomerular filtration rate (GFR) and water reabsorption in the tubular fluid samples.

After surgery and a 50-minute equilibration period, RPP was set to the control arterial pressure measured in salt-sensitive rats, approximately 150 mm Hg in the inbred DS/Jr and DR/Jr rats, and 135 mm Hg in the DS and DR rats of the Brookhaven strain. RPP first was elevated by tying off the celiac and mesenteric arteries and then was returned to the desired level by tightening the clamp on the aorta above the renal arteries. After a 10-minute equilibration period, timed tubular fluid samples were collected from three or four late proximal tubules and three early distal tubules that were identified by observing the transit time of lissamine green dye after intravenous injection. Urine and plasma samples also were collected during two 30-minute clearance periods.

The volume of the tubular fluid samples was determined by measuring sample length in capillary tubes. [3H]Inulin concentrations were determined using a liquid scintillation spectrophotometer. Tubular fluid chloride concentrations were measured using a microtitrator (model F-25, World Precision Instruments, New Haven, Conn.), and the chloride concentrations of urine and plasma samples were determined using a chloride meter (model 925, Corning Instruments, New Haven, Conn.), and the chloride concentrations averaged 64±6 meq/1 in DS/Jr rats and 90±6 meq/l in DR/Jr rats. The percentage of the filtered load of chloride in the terminal nephron (8±3%) than that of sodium was 100% and 65% greater in DS/Jr rats than in DR/Jr rats.

Table 1. Comparison of Renal Function in Inbred Dahl Salt-Sensitive and Salt-Resistant Rats (Rapp Strain) Maintained on a Low (0.9%) Salt Diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR/Jr rats (n=9)</th>
<th>DS/Jr rats (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>130±5</td>
<td>151±4</td>
</tr>
<tr>
<td>Renal perfusion pressure (mm Hg)</td>
<td>151±4</td>
<td>147±4</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min)</td>
<td>107±0.15</td>
<td>0.65±0.11*</td>
</tr>
<tr>
<td>Urine flow (μl/min)</td>
<td>97±17</td>
<td>40±7*</td>
</tr>
<tr>
<td>Na excretion (μequiv/min)</td>
<td>14±3±2.6</td>
<td>6.1±1.1*</td>
</tr>
<tr>
<td>Cl excretion (μequiv/min)</td>
<td>17±±2.5</td>
<td>7.2±1.4*</td>
</tr>
<tr>
<td>K excretion (μequiv/min)</td>
<td>2.5±0.4</td>
<td>1.0±0.2*</td>
</tr>
<tr>
<td>Fractional excretion H2O (%)</td>
<td>9.6±1.5</td>
<td>6.2±0.4*</td>
</tr>
<tr>
<td>Fractional excretion Na (%)</td>
<td>9.4±1.3</td>
<td>6.3±0.4*</td>
</tr>
<tr>
<td>Fractional excretion Cl (%)</td>
<td>14.8±1.8</td>
<td>10.5±0.7*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. DR/Jr, Dahl salt-resistant (Rapp strain); DS/Jr, Dahl salt-sensitive (Rapp strain). Body and left kidney weights averaged 232±14 and 1.0±0.1 g, respectively, in DR/Jr rats, and 267±13 and 1.3±0.1 g, respectively, in DS/Jr rats.

**Significant difference from corresponding value in DR/Jr rats.

**Results**

A comparison of renal function in inbred DS/Jr and DR/Jr rats is presented in Table 1. Mean arterial pressures were 20 mm Hg higher in DS/Jr than in DR/Jr rats maintained from birth on a low sodium diet. Baseline hematocrits (41±1% versus 43±2%) and plasma sodium (147±2 versus 145±2 meq/l) and chloride (113±4 versus 105±3 meq/l) concentrations were not significantly different in the DR/Jr and DS/Jr rats, indicating that the degree of volume expansion was similar in the two groups. Urine flow and sodium, potassium, and chloride excretions were 60% less in DS/Jr than in DR/Jr rats at an equivalent RPP of approximately 150 mm Hg. GFR was 39% lower in DS/Jr than in DR/Jr rats. Fractional excretions of sodium, chloride, and water were all lower, by approximately 30%, in DS/Jr than in DR/Jr rats.

The percentages of the filtered load of water and chloride reaching the late proximal tubule were significantly greater in DS/Jr than in DR/Jr rats (Figure 1); however, the percentage of the filtered load of chloride delivered to the early distal tubule was lower in DS/Jr than in DR/Jr rats. Distal tubular fluid chloride concentrations averaged 64±6 meq/l in DS/Jr rats and 90±6 meq/l in DR/Jr rats. The percentage of the filtered load of water delivered to the early distal tubule was not significantly different in DS/Jr and DR/Jr rats. Single nephron GFR measured from the distal tubular collection site was similar in DS/Jr and DR/Jr rats, and averaged 44±4 and 46±5 nl/min per gram kidney weight, respectively.

Proximal tubular reabsorption of water and chloride (Figure 2) was significantly reduced, by 19% and 32%, respectively, in DS/Jr rats compared with values measured in DR/Jr animals. In contrast, fractional reabsorption of water and chloride in the loop of Henle was 100% and 65% greater in DS/Jr rats than values observed in DR/Jr animals. DR/Jr rats reabsorbed 17±2% of the filtered load of water beyond the distal tubular collection site, whereas DS/Jr rats reabsorbed 12±6% of the filtered load of water in this portion of the nephron. Similarly, DR/Jr rats reabsorbed a greater percentage of the filtered load of chloride in the terminal nephron (8±3%) than DS/Jr rats (0.3±1%).

A comparison of renal function in DS and DR rats of the Brookhaven strain is presented in Table 2. Mean arterial pressures were similar in DS and DR rats. Baseline hematocrits (38±2% versus 41±1%) and plasma sodium (145±2 versus 149±2 meq/l) and chloride (108±2 versus 106±4 meq/l) concentrations were not significantly different in these animals. GFR also was not significantly different in the DS and DR rats. Urine flow and sodium, potassium, and chloride excretions were lower in DS than in DR rats when their kidneys were perfused at an equivalent RPP of approximately 135 mm Hg. In DS rats, fractional excretions of water, sodium, and chloride were approximately half of those observed in DR rats.
The percentages of the filtered load of water and chloride reaching the late proximal tubule (Figure 3) were similar in DS and DR rats. However, the fractions of the filtered load of water and chloride delivered to the early distal tubule were 26% and 59% lower in DS than in DR rats. Distal tubular fluid chloride concentrations were 55±6 meq/l in DS rats and 90±9 meq/l in DR rats. Single nephron GFR measured at the distal tubular site was similar and averaged 43±2 and 48±8 nl/min per gram kidney weight, in DR and the DS rats, respectively.

Fractional reabsorption of water and chloride in the proximal tubule were similar in DS and DR rats (Figure 4). In contrast, the percentages of the filtered load of water and chloride reabsorbed in the loop of Henle were 66% and 50% greater in DS than in DR rats. Reabsorption of water in the terminal nephron was not significantly different in DS and DR rats; however, the percentage of the filtered load of chloride reabsorbed in this portion of the nephron was four times greater in DR than in DS rats.

Discussion
The pressure-natriuretic response is abnormal before the induction of salt hypertension in DS rats of the Brookhaven and Rapp strains, but the nephron segments in which tubular reabsorption is altered have not been identified. In the present study, fractional excretions of sodium, chloride, and water were lower in inbred DS/Jr rats and in DS rats of the Brookhaven strain than the values observed in control animals when their kidneys were perfused at equivalent RPP. Thus, tubular reabsorption of water and electrolytes was enhanced in both groups of...
TABLE 2. Comparison of Renal Function in Dahl Salt-Sensitive and Salt-Resistant Rats (Brookhaven Strain) Maintained on a Low (0.3%) Salt Diet

<table>
<thead>
<tr>
<th></th>
<th>DR rats (n=7)</th>
<th>DS rats (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>126±3</td>
<td>131±2</td>
</tr>
<tr>
<td>Renal perfusion pressure (mm Hg)</td>
<td>139±4</td>
<td>133±3</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min-1-g kidney wt-1)</td>
<td>1.23±0.11</td>
<td>0.92±0.12</td>
</tr>
<tr>
<td>Urine flow (µl/min-1-g kidney wt-1)</td>
<td>103±24</td>
<td>34±8*</td>
</tr>
<tr>
<td>Na excretion (µeq/min-1-g kidney wt-1)</td>
<td>14.4±3.3</td>
<td>5.7±1.3*</td>
</tr>
<tr>
<td>Cl excretion (µeq/min-1-g kidney wt-1)</td>
<td>16.6±3.0</td>
<td>6.6±1.3*</td>
</tr>
<tr>
<td>K excretion (µeq/min-1-g kidney wt-1)</td>
<td>1.9±0.3</td>
<td>1.4±0.2*</td>
</tr>
<tr>
<td>Fractional excretion H2O (%)</td>
<td>7.9±1.0</td>
<td>3.9±0.6*</td>
</tr>
<tr>
<td>Fractional excretion Na (%)</td>
<td>7.6±1.0</td>
<td>4.0±0.7*</td>
</tr>
<tr>
<td>Fractional excretion Cl (%)</td>
<td>12.2±1.1</td>
<td>7.0±0.9*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. DR, Dahl salt-resistant; DS, Dahl salt-sensitive. Body and kidney weights averaged 250±15 and 1.0±0.1 g, respectively, in DR rats, and 263±16 and 1.1±0.1 g, respectively, in DS rats.

*Significant difference from corresponding value in DR rats.

salt-sensitive rats under the present experimental conditions.

The micropuncture results indicate that the site of the enhanced tubular reabsorption of water and chloride in these animals is in the loop of Henle. Our findings are consistent with a recent report that elevated chloride reabsorption in the loop of Henle was responsible for the inability of euvoletic hypertensive DS rats of the Brookhaven strain to excrete sodium as well as DR rats when RPP was reduced to a normotensive level.9 However, this experiment failed to establish a cause and effect relation, because the rats were already hypertensive at the time of the study. The new information in the present study is that the abnormality in loop chloride transport in DS rats preceeds the development of hypertension and that inbred DS/Jr rats maintained on a low salt diet exhibit a similar abnormality in loop function. In addition, our study was performed in volume-expanded rats, and RPP was acutely increased in the DR rats to the level seen in the DS rats, because differences in the pressure-natriuretic responses of DS and DR rats are potentiated under these experimental conditions.4

Chloride is actively transported with sodium and potassium in the thick ascending limb, and tubular fluid chloride concentration falls below that of plasma in this segment.10 Our finding that the chloride concentration of early distal tubular fluid was 30 meq/l lower in both strains of DS versus DR rats suggests that the thick ascending limb is the likely site for the enhanced loop chloride reabsorption. The thick ascending limb and thin ascending limb of Henle are relatively impermeable to water.10

Thus, the enhanced reabsorption of water in the loop of Henle of the DS rats probably is due to increased passive water abstraction from the pars recta or the thin descending loop of Henle. Because the driving force for water reabsorption in these segments is the cortical outer medullary osmotic gradient, which is dependent on active chloride transport in the thick ascending limb, the elevated water reabsorption in the loop of Henle of DS rats probably is secondary to the enhanced chloride reabsorption in the thick ascending limb.

In vitro experiments have indicated that sodium and chloride reabsorption in the thick ascending limb is stimulated by vasopressin, calcitonin, and parathyroid hormone via a cyclic AMP-dependent pathway10,11 and is inhibited by prostaglandin E2.12,13 None of these substances, however, has been shown to play a major role in the physiological adjustments of loop chloride transport in response to volume expansion14,15 or changes in sodium intake.10 In the absence of definitive information about the factors...
that regulate sodium chloride reabsorption in the thick ascending loop of Henle in vivo, it is difficult even to speculate about the mechanism that alters loop chloride reabsorption in DS rats. The defect could be in the neural, hormonal, or autacoid regulation of transport in this nephron segment; it could be at the level of signal transduction; or it could be related to genetic abnormalities in the synthesis or activity of the Na\(^+\)-K\(^+\) (2Cl\(^-\)) cotransporter or Na\(^+\),K\(^+\)-ATPase.

GFR was reduced by 39% in the DS/Jr rats. The fall in the filtered load of water and electrolytes contributed to the blunted pressure-natriuretic response observed in these animals. The reduction in GFR in DS/Jr rats may be due to the progressive glomerular disease recently described by Sterzel et al.\(^{16}\) In support of this possibility, we found that the surface of the kidney of DS/Jr rats had a "splotchy" appearance, and there were atrophic nephrons that had prolonged proximal tubular transit times (>20 seconds). The collection rate and single nephron GFR measured in these nephrons usually was very low (<15 nI/min). However, the mean single nephron GFR reported in the present study was not significantly different in DS/Jr and DR/Jr rats. The reason that the single nephron GFR results did not reflect the reduced GFR in the DS/Jr rats is because samples collected from nephrons with prolonged transit times were not included in the analysis. Therefore, our results reflect values obtained from the healthy nephron population in DS and DR rats.

In DS rats of the Brookhaven strain, the mean GFR was 25% lower than the value observed in DR rats, but this difference was not significant because GFR was highly variable in the DS rats. In three of eight of the DS rats, GFR was reduced by 50%, as we saw in the DS/Jr strain. In the remaining animals, however, GFR was identical to the values observed in the DR rats. Moreover, single nephron GFR was similar in the DS and the DR rats, and unlike the DS/Jr rats, there were few nephrons with prolonged transit times in the DS rats of the Brookhaven strain. Arterial pressure also was similar in DS and DR rats of the Brookhaven strain. This observation suggests that the low GFR in DS/Jr rats, which impairs their ability to excrete sodium at normal levels of RPP, may explain the development of mild hypertension in these animals even when they are maintained on a low salt diet.

The micropuncture results indicate that DS rats of both the Brookhaven and Rapp strains reabsorb very little chloride in the nephron segments beyond the early distal tubule. Inhibition of chloride transport in this portion of the nephron opposed, but could not compensate for, the enhanced loop reabsorption in DS rats. Aldosterone, prostaglandins, and atrial natriuretic peptide are factors that regulate electrolyte reabsorption in the collecting duct.\(^{10}\) The inhibition of chloride transport in the terminal nephron of DS rats probably is not due to an enhanced influence of prostaglandin E\(_2\), because the synthesis of this substance is reduced in the kidney of DS rats.\(^{17}\) Aldosterone levels also are reduced in DS rats.\(^{18}\) However, it is unlikely that collecting duct chloride transport is inhibited because of a mineralocorticoid deficiency, because net plasma mineralocorticoid activity actually is elevated in DS rats because of enhanced production of 18-hydroxy-11-deoxycorticosterone.\(^{18}\) Circulating levels of atrial natriuretic factor are elevated in DS rats,\(^{19,20}\) but they are hyporesponsive to the effects of this substance on sodium excretion and its ability to increase renal cyclic GMP levels.\(^{21-23}\) The present finding that chloride reabsorption is inhibited at the site of action of atrial natriuretic peptide offers a possible explanation for the lack of a natriuretic response to this substance in DS rats. It remains to be determined whether the inhibition of electrolyte reabsorption in
the terminal nephron in DS rats is due to an adaption to elevated atrial natriuretic peptide levels or to chronic sodium retention.

Proximal tubular reabsorption of chloride (and presumably sodium) and water were significantly inhibited in DS/Jr rats in which GFR was reduced but not in DS rats of the Brookhaven strain in which GFR was relatively normal. This suggests that, to maintain salt balance in the face of a reduction in GFR, inhibition of proximal tubular reabsorption in the DS/Jr rats might be secondary to suppression of the factors that normally regulate sodium transport in this portion of the nephron. Physical factors, renal nerve activity, and angiotensin II are the major controllers of proximal tubular reabsorption. Because filtration fraction, papillary blood flow, and renal interstitial pressure are similar in DS/Jr and DR/Jr rats, it is unlikely that differences in physical factors are responsible for the inhibition of proximal tubular transport in the DS/Jr rats. Also, the renal nerves probably are not involved, because renal denervation does not affect the excretion of a sodium load or the development of salt hypertension in these animals. Plasma renin activity is reduced in DS rats.

Because angiotensin II stimulates sodium transport in the proximal tubule, inhibition of proximal tubular reabsorption in DS rats may be due to lower intrarenal levels of angiotensin II. The chronic suppression of plasma renin activity in DS rats might be due to the enhanced loop chloride transport, because the macula densa cells that regulate renin release are modified thick ascending limb cells.

In summary, enhanced reabsorption of water and chloride in the loop of Henle contributes to the resetting of the pressure–natriuretic relation in inbred DS/Jr rats and DS rats of the Brookhaven strain and may explain the predisposition of these animals to develop hypertension when challenged with a high sodium diet. GFR also is reduced in DS/Jr rats. A reduction in filtered load, which further impairs the ability of these rats to excrete water and electrolytes at normal levels of RPP, may contribute to the development of elevated arterial pressures in DS/Jr rats maintained on a low sodium diet.

References


**KEY WORDS** • kidney • renal circulation • renal hypertension • chloride • kidney tubules • Dahl rats
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