Hydrostatic Pressure in Dahl Salt-Sensitive Rats

Tatsuya Kato, Salah Kassab, Fred C. Wilkins Jr, Kent A. Kirchner, Joey P. Granger

Abstract The ability of Dahl salt-sensitive (DS) rats to excrete a sodium load is significantly lower than that of Dahl salt-resistant (DR) rats. Because renal interstitial hydrostatic pressure (RIHP) is a major mediator of natriuresis in response to a sodium load, we proposed that the renal tubules of DS rats are less responsive to increases in RIHP than those of DR rats. To test this hypothesis, we determined the effect of direct increases in RIHP on renal excretory function in prehypertensive DS and DR rats. RIHP was directly increased by renal interstitial volume expansion via injection of 50 μL of a 2% albumin and saline solution into the renal interstitium through a chronically implanted renal interstitial catheter. RIHP, mean arterial pressure, glomerular filtration rate, urine flow rate, urinary sodium excretion, and fractional excretions of sodium, potassium, and lithium (an indicator of proximal tubule sodium handling) were measured before and after direct increases in RIHP in DS (n=8) and DR (n=8) rats. Baseline urine flow rate; urinary sodium excretion; fractional excretions of sodium, potassium, and lithium; RIHP; mean arterial pressure; and glomerular filtration rate were not different between DS and DR rats. Renal interstitial volume expansion in DS rats significantly increased RIHP (Δ4.7±0.8 mm Hg), urine flow rate (Δ14.5±3.4 μL/min), urinary sodium excretion (Δ2.62±0.62 μmol/min), and fractional excretions of sodium (Δ1.54±0.37%), potassium (Δ17.84±2.90%), and lithium (Δ19.68±3.52%). Renal interstitial volume expansion in DR rats also increased RIHP (Δ5.3±0.6 mm Hg), urine flow rate (Δ36.4±4.8 μL/min), urinary sodium excretion (Δ6.23±0.69 μmol/min), and fractional excretions of sodium (Δ3.56±0.46%), potassium (Δ37.85±3.75%), and lithium (Δ30.19±4.21%) significantly. Compared with DR rats, DS rats had significantly smaller increases in urine flow rate, urinary sodium excretion, and fractional excretions of sodium, potassium, and lithium in response to equivalent increases of RIHP. These data demonstrate that despite similar mean arterial pressure and glomerular filtration rate DS rats have smaller proximal tubule and whole-kidney natriuretic responses to direct increases in RIHP and that this defect is present before the development of hypertension. These results suggest that a reduced sensitivity of renal tubules to increases in RIHP in prehypertensive DS rats may contribute to their inability to excrete a sodium load. (Hypertension. 1994;23[part 2]:1082-1086.)

Key Words • rats, inbred • natriuresis • renal circulation

The kidneys play an important role in the long-term regulation of arterial pressure by maintaining sodium balance and extracellular fluid volume.1,2 Although results from a number of studies indicate that the kidneys of hypertensive animals have a reduced capacity to excrete sodium and water,1,2 the mechanisms for this abnormality have not been fully elucidated. The strain of Dahl salt-sensitive (DS) and salt-resistant (DR) rats developed by Dahl and coworkers3 has been especially useful as an animal model that closely resembles the subset of essential hypertensive patients who are known to be sodium sensitive. DS rats develop hypertension when ingesting a high sodium diet but remain normotensive on a low sodium diet. DR rats are insensitive to alterations in sodium intake. Although the exact mechanism responsible for the abnormal response to a sodium load is not clear, the finding that transplantation of prehypertensive kidneys from DS to DR rats produces hypertension4 would suggest that an intrinsic defect within the kidney may play a crucial role in the pathogenesis of salt-sensitive hypertension. It is now known that the natriuretic capacity is altered before the induction of salt hypertension in DS rats.5,6 Compared with DR rats, the ability of prehypertensive DS rats to excrete a medically administered saline load is significantly reduced.5,6 However, the exact mechanism mediating this sodium-retaining abnormality in DS rats in response to an acute saline load has not yet been elucidated. Renal interstitial hydrostatic pressure (RIHP) has been suggested to play a critical role in mediating volume expansion–induced natriuresis.7-10 Acute saline volume expansion is accompanied by increases in RIHP and renal sodium excretion. When RIHP is prevented from increasing before volume expansion, the natriuretic response is virtually abolished.7-8 RIHP is a major mediator of volume expansion–induced natriuresis, so it is possible that blunted increases in RIHP or decreased tubular responsiveness to RIHP could result in a reduced renal excretory response to saline loading. Recently, we have reported that the increases in RIHP did not differ in prehypertensive DS and DR rats after an acute saline load.6 However, the responsiveness of the renal tubules to directly increases in RIHP in DS and DR rats is unknown. The purpose of this study was to determine whether the blunted natriuretic response to a sodium load in DS rats is related to differences in response to increases in RIHP. To achieve this goal, we compared the effects of direct increases in RIHP on renal excretory function in prehypertensive DS and DR rats.

Methods All experiments were performed in DS and DR rats implanted with an interstitial catheter. Two to 3 weeks after catheter implantation, the rats were anesthetized and prepared for the acute clearance experiment.

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Interstitial Catheter Design and Implantation

We have previously developed a technique for increasing renal interstitial volume directly to study the relation between RIHP and sodium excretion. The interstitial catheters used in the present study were made from polyethylene matrix material with 60-μm pores (Laborpor porous tubes, Bel Art Products) that is impermeable to solid tissue yet communicates with the interstitial fluid compartment. Each polyethylene matrix was cut into a cylinder 1.0 mm in diameter and 2.0 mm in length. A hole was drilled into one end of the polyethylene matrix so that tubing (PE-50) could be inserted into the hole. The tubing was permanently attached to the matrix with a combination of cement and silicone elastomer glue.

Eight- to 9-week-old male DS and DR rats of the Rapp strain were obtained (Harlan Sprague Dawley Co, Inc) and placed on a 0.3% sodium chloride diet (Teklad Test Diets, Harlan Sprague Dawley). The rats were allowed free access to tap water. Surgery and care of the rats were conducted in accordance with National Institutes of Health guidelines as approved by the Animal Care and Use Committee at the University of Mississippi Medical Center. After 1 week of delivery, the rats were anesthetized with pentobarbital sodium (30 mg/kg body wt IP) and implanted with renal interstitial catheters. The left kidney was exposed through a midline incision. One end of a stainless steel guide wire was inserted into the open end of the PE-50 tubing that was attached to the matrix. A small nick was made on the upper ventral pole of the left kidney, and the stainless steel guide wire was pushed into the nick and through the kidney. The matrix was pushed into the kidney parenchyma, and the PE-50 tubing leading from the matrix exited the dorsal side of the kidney. The interstitial catheter was flushed with isotonic saline to remove any residual blood from the capsule, and then the open end of the PE-50 tubing was sealed with a knot. The PE-50 tubing was then coiled and placed into the abdomen of the rat. After the right kidney was removed, the abdominal incision was closed and the rats were allowed to recover from surgery for 2 to 3 weeks. Gross examination of kidneys after completion of the study revealed that the matrix was located in the renal parenchyma at the corticomedullary junction.

Experimental Protocol

Eight DS and DR rats were studied. All rats were fasted overnight before experimentation and had continuous access to tap water. For the acute experimental study, the rats were anesthetized with thiobutabarbital (Inactin, Promonto GmbH) (100 mg/kg body wt IP) and placed on a thermostatically controlled warming table to maintain body temperature at 37°C. A tracheotomy was performed, and PE-240 tubing 3 cm long was inserted into the trachea to maintain an open airway. The left jugular vein was cannulated with PE-50 tubing for continuous intravenous infusion. The left carotid artery was also cannulated with PE-50 tubing for blood sampling and continuous arterial pressure monitoring. The carotid arterial catheter was connected to a model P23DC strain gauge, and mean arterial pressure (MAP) was recorded on a polygraph (MFE Instruments). A small midline incision was made and the bladder cannulated using flare-tipped, 3-cm PE-50 tubing for urine collection. The left kidney was exposed through a left flank incision, and the implanted interstitial catheter was connected to a pressure transducer for constant monitoring of RIHP. The renal interstitial catheter was checked for patency and responsiveness by determining the RIHP response to partial renal vein constriction.

After completion of surgery, the rats received a maintenance infusion of isotonic saline solution containing 25 mmol LiCl and 6.25% albumin and NaCl-ethanolamine (Isotex Diagnostics, 0.05 μCi/kg per minute) into the jugular vein at a rate of 2.5 mL/h (Syringe Infusion Pump 22, Harvard Apparatus).

The isotonic saline solution was infused for 60 minutes after surgery, and steady-state measurements of arterial pressure and RIHP were obtained during a 40-minute control clearance. The control period was followed by renal interstitial volume expansion (RIVE) with a 50-μL injection of 2% albumin in saline solution into the renal interstitium via the chronically implanted catheter. The PE tubing leading from the chronically implanted interstitial catheter was connected to a microcatheter via a 23-gauge needle for injection. This technique resulted in a stable increase in RIHP that was sustained over a 40-minute period. Five minutes after RIVE, a 40-minute experimental clearance was begun, and urine and plasma samples were collected. A 750-μL arterial blood sample was obtained at the midpoint of each urine collection for determination of plasma concentrations of sodium, potassium, and lithium.

After the rats were killed with a venous injection of potassium chloride, the left kidney was removed and weighed. Sodium, potassium, and lithium concentrations in plasma and urine were measured by flame photometry (IL-943, Instrumentation Laboratories). Glomerular filtration rate (GFR) and urinary excretions of sodium, potassium, and lithium were calculated from the determination of sodium, potassium, and lithium and radioactivities of 125I in plasma and urine. Fractional excretion of lithium was used to estimate proximal tubule handling of sodium.

Statistical Analysis

Results are expressed as mean±SEM. Statistical significance within each group was determined with the paired Student’s t test. Statistical significance between groups was determined with the Student’s t test for unpaired data. Differences were considered significant at a value of P<.05.

Results

The Table summarizes the effects of RIVE on renal function in DR and DS rats. At the time of acute studies, mean body weight was 304±7 and 285±10 g for DR and DS rats, respectively. Baseline MAP, GFR, RIHP, urine flow rate (UV), urinary sodium excretion (UNaV), urinary potassium excretion, and fractional excretions of sodium, potassium, and lithium were not different between DR and DS rats. Mean left kidney weight was 1.60±0.04 and 1.47±0.05 g for DR and DS rats, respectively.

Fig 1 compares the changes in RHP, UV, and UNaV in DR and DS rats in response to RIVE. The increases of RIHP after RIVE were not different in DR and DS rats (5.3±0.6 versus 4.7±0.8 mmHg), indicating that RIVE with an injection of 50 μL of 2% albumin in saline solution raised RIHP to a similar degree in both rat groups. The increases of UV and UNaV after RIVE were significantly greater in DR than DS rats (∆36.4±4.8 versus ∆14.5±3.4 μL/min, ∆6.23±0.69 versus ∆2.62±0.62 μmol/min, respectively). Despite the equivalent increases of RHP, the increases in UV and UNaV in DS rats were less than in DR rats by 60% and 58%, respectively.

Fig 2 compares the changes in fractional excretions of sodium, potassium, and lithium in DR and DS rats in response to RIVE. The increases of fractional excretions of sodium, potassium, and lithium after RIVE were significantly greater in DR than DS rats (∆3.56±0.46% versus ∆1.54±0.37%, ∆37.85±3.75% versus ∆17.84±2.90%, ∆30.19±4.21% versus ∆19.68±3.52%, respectively). Despite the same increases of RHP, the increases in fractional excretions of sodium and lithium in DS rats were lower than in DR rats by 57% and 35%, respectively.
Effect of Renal Interstitial Volume Expansion on Renal Function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dahl Salt-Resistant Rats (n=8)</th>
<th>Dahl Salt-Sensitive Rats (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>Control: 113±2</td>
<td>RIVE: 118±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: 115±5</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>1.16±0.06</td>
<td>1.22±0.06</td>
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<tr>
<td></td>
<td></td>
<td>1.15±0.08</td>
</tr>
<tr>
<td>RIHP, mm Hg</td>
<td>4.5±0.4</td>
<td>9.8±0.9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.6±0.9</td>
</tr>
<tr>
<td>UV, μL/min</td>
<td>15.4±2.3</td>
<td>51.8±4.8*</td>
</tr>
<tr>
<td></td>
<td>6.93±0.68*</td>
<td>31.0±5.2*</td>
</tr>
<tr>
<td>UNaV, μEq/min</td>
<td>0.65±0.10</td>
<td>0.86±0.27</td>
</tr>
<tr>
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<td>3.23±0.41*</td>
<td>3.58±0.83*</td>
</tr>
<tr>
<td>FENa, %</td>
<td>0.39±0.07</td>
<td>3.95±0.47*</td>
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<tr>
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<td>0.57±0.15</td>
<td>2.11±0.49*</td>
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<tr>
<td>FEK, %</td>
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<td>81.76±8.48*</td>
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<tr>
<td></td>
<td>49.49±9.26</td>
<td>67.30±8.38*</td>
</tr>
<tr>
<td>FEK, %</td>
<td>30.44±2.04</td>
<td>60.83±4.61*</td>
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<tr>
<td></td>
<td>30.40±5.00</td>
<td>50.08±4.93*</td>
</tr>
</tbody>
</table>

RIVE indicates renal interstitial volume expansion; MAP, mean arterial pressure; GFR, glomerular filtration rate; RIHP, renal interstitial hydrostatic pressure; UV, urine flow rate; UNaV, urinary sodium excretion; UU, urinary potassium excretion; FENa, fractional excretion of sodium; FEK, fractional excretion of potassium; and FEl, fractional excretion of lithium. Values are mean±SEM; n=number of animals.

*Statistically different from control measurements (P<.05).

Discussion

The results of the current study demonstrate that despite equivalent MAP and GFR DS rats have a reduced proximal tubule and whole-kidney natriuretic response to direct increases in RIHP compared with DR rats. The blood pressure level in these animals is...
considered normotensive for the rat, so this defect in DS rats is present before the development of hypertension. Before the development of hypertension, DS rats exhibit a blunted response to extracellular volume expansion. In response to an acute saline load, prehypertensive DS rats excrete significantly less sodium than DR rats.5,9 Reasons if the apparent that the proximal tubule may be an important nephron site responsible for the blunted natriuretic response to acute saline expansion in prehypertensive DS rats. However, the exact mechanism mediating this sodium-retaining abnormality in DS rats has not yet been elucidated. We and others have suggested that RIHP plays a critical role in mediating volume expansion–induced natriuresis.9,10 Acute saline volume expansion is accompanied by a reduction in peritubular colloid osmotic pressure and increases in peritubular capillary hydrostatic pressure, RIHP, and renal sodium excretion.5,6,9,14,15 When RIHP is prevented from increasing during volume expansion, the natriuretic response is virtually abolished.7,9 Furthermore, decapsulation of the kidney in volume-expanded rats prevents increases in RIHP after elevations in renal perfusion pressure and markedly attenuates pressure natriuresis.16 As RIHP is a major mediator of volume expansion–induced natriuresis, it is possible that blunted increases in RIHP or decreased tubular responses to RIHP could result in a reduced renal excretory response to saline loading.

Recently, we have examined the effect of an acute saline load on RIHP and U_aV in DS and DR rats.6 Saline loading increased U_aV more in DR rats than in prehypertensive DS rats, but the increases in RIHP did not differ between these animals. The fact that RIHP is elevated equivalently after saline loading in prehypertensive DS and DR rats suggests that abnormalities in the transmission of RIHP are not responsible for the blunted natriuresis to an acute saline load in DS rats. However, differences in the sensitivity of the renal tubules to increases in RIHP between DS and DR animals could be another possible mechanism to account for the differences in sodium excretion during saline loading. With the use of a protocol in which RIVE resulted in an increase in RIHP of 4 to 5 mm Hg, which is comparable to that observed after acute saline infusions,7,8 RIVE caused similar increases in RIHP in DS and DR rats (4.7±0.8 versus 5.3±0.6 mm Hg). These changes in RIHP were associated with increases in UV, U_aV, and fractional excretion of sodium in both groups. However, compared with DR rats, the increases in UV, U_aV, and fractional excretion of sodium were lower in DS rats by 60%, 58%, and 57%, respectively. Thus, prehypertensive DS rats have a defect in the translation of increases in RIHP to increases in sodium excretion.

In the present study, baseline GFR was not different between DS and DR rats. Furthermore, RIVE had no effect on GFR in either group. Thus, the attenuation of the natriuretic response in DS rats occurred without changes in GFR, which implies that the effect of increases in RIHP could not be ascribed to major changes in the filtered load of sodium under the present experimental conditions. If the differences in sodium excretion observed in our study are not attributable to differences in filtered load, then differences in net tubular sodium reabsorption in response to increases in RIHP must exist between DS and DR rats. As lithium appears to be handled like sodium in the proximal tubule and is not appreciably reabsorbed in other nephron segments, fractional lithium excretion can be used as a marker for fractional delivery of sodium out of the proximal tubule.11 Our current study demonstrated that baseline fractional excretion of lithium was not different between DR and DS rats and that increases in RIHP were associated with significant increases in fractional excretion of lithium in both groups. Moreover, lithium clearance increased to a greater extent in DR than DS rats. The fact that fractional lithium excretion was not different between DR and DS rats before increases in RIHP suggests that enhanced sodium reabsorption is not fixed intrinsic property in DS rat proximal tubules. This finding is consistent with previous studies5,17 demonstrating that proximal tubular reabsorption is similar in prehypertensive DS and DR rats. Our results also suggest that prehypertensive DS rats have a blunted proximal tubule response to direct increases in RIHP. This reduced proximal tubule sensitivity to increases in RIHP in prehypertensive DS rats may in part be responsible for their inability to excrete a sodium load.

The reduced proximal tubule sensitivity may not totally account for the differences in sodium excretion between DS and DR rats. Recently, Kirchner18 reported that the lower U_aV was associated with increased loop chloride reabsorption in hypertensive DS rats when renal perfusion pressure was reduced to that of DR rats by aortic constriction. Moreover, Roman and Kaldunski19 suggested that enhanced reabsorption of water and chloride in the loop of Henle contributes to the resetting of the pressure-natriuretic relation in prehypertensive DS rats. Although it may be possible that increases in RIHP affect nephron sites beyond the proximal tubule, no studies to date have examined whether these nephron sites are responsive to direct increases in RIHP. Further studies must be undertaken to define the relation between changes in RIHP and distal nephron function in the reduced natriuretic capacity in DS rats.

Although the exact mechanism whereby RIHP affects the tubular reabsorption of sodium and water is unknown, it may be related to alterations in sodium permeability in the tight junctions of the proximal tubules and/or release of renal autacoids such as prostaglandins, which inhibit sodium reabsorption.10 It is possible that the blunted natriuretic response to increases in RIHP in DS rats may be due to differences in tight junction permeability to sodium between prehypertensive DS and DR rats. Further studies will be necessary to quantitatively assess the effect of direct increases in RIHP on sodium backleak across the proximal tubule.

Recent studies indicate that the intrarenal prostaglandin system may have an important influence on the pressure-natriuresis mechanism.19,23 Direct support for a role of renal prostaglandin in mediating the effects of RIHP was provided by Pawlowska et al.24 Inhibition of prostaglandin synthesis prevented the increase in urinary excretion of prostaglandin E2 and markedly attenuated the natriuretic response to direct increases in RIHP caused by RIVE. In addition to these findings, Kinoshita and Knox25 also recently reported that direct
increases in RIHP inhibited proximal sodium reabsorption and this inhibition could be blocked by prostaglandin synthesis inhibitors. Thus, renal prostaglandin E₂ may mediate the effect of increases in RIHP on the proximal tubule. Previous studies suggest that the synthesis of prostaglandin E₂ is reduced in the kidneys of DS rats compared with DR rats at prehypertensive and hypertensive phases.26-28 The decreased synthetic capacity of renal prostaglandin E₂ may also explain the blunted response to increases in RIHP on the proximal tubule in DS rats.

In summary, prehypertensive DS rats compared with DR rats have a blunted natriuretic response to increases in RIHP, and this defect is associated with less proximal tubule response and determined by fractional lithium clearance. As RIHP is a major mediator of natriuresis in response to a sodium load, these results suggest that a reduced sensitivity of the proximal tubule and possibly other nephron sites to increases in RIHP in prehypertensive DS rats may be responsible for their inability to excrete a sodium load.

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