Salt Excretion and Vascular Resistance of Perfused Kidneys of Dahl Rats

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SUMMARY We used a cell-free, 5% albumin-containing bicarbonate saline solution to perfuse kidneys of salt-sensitive (S) and salt resistant (R) rats derived from Dahl's original strains. The animals had been maintained on diets whose salt content was either 8% (±Na) or 0.4% (−Na). On these regimens only S(±)Na rats become hypertensive. Glomerular filtration rate (GFR), urinary sodium excretion (NaE), renal vascular resistance (RVR), and filtration fraction were measured as perfusate pressure (P) was increased in stepwise fashion from the 80-100 to the 140-160 mm Hg range. Pressure-GFR and pressure-natriuresis curves for the S(−)Na kidneys were displaced to the right of R, so that for any given value of P both GFR and NaE were significantly less for S(−)Na than for R kidneys. Kidneys from hypertensive (S(±)Na) animals had even more markedly impaired filtration and salt excretion. Although R and S(−)Na kidneys had nearly the same RVR at the lowest perfusate pressures, only the S kidney showed an autoregulatory rise in RVR as perfusate pressure was increased. Filtration fraction did not change, so the rise in resistance probably reflects chiefly afferent arteriolar constriction. Thus, in comparison with R, perfused S kidneys show an intrinsic defect in salt excretion ascribable to a reduced filtered sodium load. The rightward shift of their pressure-GFR curves may be due to an exaggerated afferent arteriolar vasoconstrictor response to increase in perfusion pressure. (Hypertension 4:532-537, 1982)

KEY WORDS • salt excretion • renal vascular resistance • Dahl rats • autoregulation

In 1962, Dahl reported the development of two strains of rats having contrasting blood pressure responses to dietary salt. Rats of the salt-sensitive (S) strain regularly developed hypertension when maintained on a high salt diet, but remained normotensive when salt intake was low. Resistant (R) rats were normotensive regardless of their salt intake. Because of the impressive evidence that high salt intake leads to an elevated arterial blood pressure, Dahl rats have been extensively studied as possible models for human essential hypertension. The important findings to date are as follows: S rats have normal kidney function, do not show evidence of gross sodium excess even when maintained on a high salt diet, and, like hypertensive man, respond to a saline load with an "exaggerated natriuresis." A

There is nevertheless considerable evidence that the kidney, and in particular its ability to excrete sodium, is responsible for the development of salt hypertension in the Dahl rat. Dahl and coworkers transplanted kidneys between S and R rats, and found that S kidneys conferred salt-sensitive hypertension on R rats, and conversely that R kidneys rendered S rats immune to the pressor effects of high salt intake. Later, Tobian et al. showed that kidneys taken from normotensive S rats, when connected to the circulation of normal rats, required a higher perfusion pressure than R kidneys to excrete an equivalent amount of sodium. The kidneys showed, in other words, a rightward shift of their pressure-natriuresis curve. The S animals appear then to exemplify Guyton's hypothesis that hypertension represents an adaptive response on the part of the cardiovascular system to overcome a renal defect in salt excretion.

Experimental findings by Girardin et al. have recently cast some doubt on this satisfying and plausible hypothesis, however. Perfusing Dahl rat kidneys in an in vitro system, these workers found a right shift in the pressure-natriuresis curve only in kidneys taken from chronically hypertensive S animals. Rats of the S strain on a low-salt diet or on a high-salt diet for only 3 weeks had kidneys with pressure-natriuresis characteristics similar to those of R animals. They concluded that an intrinsic renal defect in salt excretion, although it might play a role in perpetuating established hyperten-
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Excretion, could not be the cause of blood pressure elevation in S Dahl rats. The findings of Girardin and coworkers suggest that either the observations of in vivo perfused kidneys were incorrect or that the defective natriuresis of S kidneys represents not a fixed intrinsic renal defect but an altered renal responsiveness to one or more humoral agents not present in an in vitro organ perfusion system. In light of this difference, we felt it important to publish our own findings on isolated perfused kidneys of Dahl rats. Like Tobian and coworkers, we found reduced sodium excretion by kidneys from normotensive, i.e., low-salt-eating S animals. In our preparation, significant hemodynamic differences between S and R kidneys appear to be the basis for their differing abilities to excrete sodium.

Methods

Weanling rats of Dahl's S and R strains (Brookhaven National Laboratories, Upton, New York) were given diets of either 8% or 0.4% NaCl. Systolic blood pressures were taken at weekly intervals by tail plethysmography. The animals were designated S(+)Na, R(+)Na, S(-)Na, or R(-)Na according to their genetic susceptibility to hypertension and their sodium intake. After 6 to 8 weeks of the diet, they were anesthetized with sodium pentobarbital and given 1 ml of 10% mannitol in normal saline intravenously. Their right kidneys were then prepared for perfusion following previously described techniques. The kidneys and retroperitoneal structures were exposed through a midventral incision and the right ureter cannulated with a short length of PE 10 tubing. The adrenal artery, which arises from the right renal artery, was identified and ligated. Additional ligatures were placed loosely around the right renal and superior mesenteric arteries. The arterial cannula, a short thin-walled 19-gauge needle blunted at its end, was inserted into the mesenteric artery and advanced across the ligature to enter the aorta. Perfusion flow was then started and the cannula advanced into the right renal artery and tied in place. After cannulation, the kidney was excised and transferred to a thermostatically controlled perfusion chamber.

Renal arterial pressure was calculated from the pressure proximal to the arterial cannula (continuously monitored using strain gauge and recorder) and the previously determined flow-pressure relationships of the arterial cannula. Perfusion pressure could be varied over a range from approximately 80 to 160 mm Hg by adjusting the gas pressure applied to a sealed arterial reservoir. Perfusate leaving the kidney from the cut ends of the vena cava was continuously removed from the perfusion chamber by a roller pump and forced through a membrane filter (0.47 μM pore diameter) and then through a "lunge" consisting of two parallel 7 m coils of Silastic tubing (1.47 mm id, 0.2 mm wall thickness, Dow Corning Corporation Medical Products Division, Midland, Michigan) contained in a polyethylene jar filled with 95% O2-5% CO2. A second roller pump conducted the perfusate from the "lunge" into the pressurized arterial reservoir. Temperature at the kidney hilus was monitored by a thermistor probe and thermoregulator that controlled heating elements applied to the perfusion chamber, "lunge," and arterial reservoir. This device maintained a kidney temperature of 37°C to 38°C. Beginning 10 minutes after placement of the arterial cannula, glomerular filtration rate (GFR) and sodium excretion (NaE) were determined during three clearance periods, each at a different perfusate pressure spanning a range of 90 to 160 mm Hg. Urine was collected in preweighed vials, and perfusate samples were taken at the beginning of the first and at the end of the last clearance period. Perfusates and urine were analyzed for inulin, whose clearance was taken as the GFR, by the resorcinol method11 and for sodium by flame photometry. GFR and sodium excretion were normalized to the weight of the left (nonperfused) kidney. At the beginning and end of each clearance period, perfusate flow rate was measured by observing the volume change in the arterial reservoir with the inflow pump turned off. Renal vascular resistance was calculated as arterial pressure/perfusate flow rate in mm Hg/ml min−1/g kidney weight.

The medium used in these studies was a bicarbonate-saline solution having the following electrolyte composition (mM/liter): Na 140, K 5, Ca 1.0, Mg 1.2, HPO4 2.4, H2PO4 0.6, SO4 1.2, HCO3 25, Cl 108.6. When gassed with 5% CO2-95% O2, the solution maintained a pH of 7.35 to 7.45. Lactate, 8 mM, and d-glucose, 5.6 mM, were present as substrate in all media. Perfusates also contained inulin (100 mg/liter), and 5 g% bovine serum albumin (Fraction V, Miles Laboratories, Kankakee, Illinois). Protein concentrates used in preparing the media were dialyzed against three changes of distilled water for 72 hours and were titrated to pH 7.4. We realize that the sodium reabsorption by perfused rat kidneys can be enhanced if perfusate albumin concentration is raised to levels of 7.5 g/liter or if an assortment of amino acids is added to the perfusate. We elected not to make these additions because our object was to ascertain whether there are intrinsic differences between S and R kidneys that may be revealed during in vitro perfusion, not to study a perfused kidney whose function rivaled its in vivo counterpart.

Results

Body weight, systolic blood pressure, and the weight of the left (unperfused) kidney for each of the four groups of animals are given in table 1. As expected, the blood pressures of S(+)-Na animals were markedly higher than any of the other groups. In addition, the blood pressures of both R(+)Na and S(-)-Na animals were slightly but significantly higher than R(-)Na. Body weights were similar for all four groups, but the kidney weight of S(+)Na animals was significantly increased above the others. Salt intake did not affect the GFR, filtration fraction, sodium ex-
TABLE 1. **Body Weight, Kidney Weight, and Systolic Blood Pressure on the Day of Kidney Perfusion for S and R Rats on High and Low Salt Intakes**

<table>
<thead>
<tr>
<th>Rat group</th>
<th>No.</th>
<th>Body wt (g)</th>
<th>Kidney wt (g)</th>
<th>Systolic BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (−)Na</td>
<td>5</td>
<td>325 ± 15</td>
<td>1.34 ± 0.06</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>R (+)Na</td>
<td>10</td>
<td>317 ± 8</td>
<td>1.42 ± 0.04</td>
<td>120 ± 1 *</td>
</tr>
<tr>
<td>S (−)Na</td>
<td>8</td>
<td>313 ± 7</td>
<td>1.36 ± 0.03</td>
<td>126 ± 2 *</td>
</tr>
<tr>
<td>S (+)Na</td>
<td>8</td>
<td>314 ± 9</td>
<td>1.71 ± 0.07†</td>
<td>193 ± 8‡</td>
</tr>
</tbody>
</table>

*Values significantly higher than R (−)Na, p < 0.01.
†Values significantly higher than S (−)Na, p < 0.01.

**R** = salt-resistant; **S** = salt-sensitive. Values are means ± SEM.

creatinine, or vascular resistance of the kidneys from R rats. Therefore, in the tables and figures that follow, the data for R (+)Na and R (−)Na animals have been combined.

As shown in table 2, where data have been grouped according to the perfusion pressure, both sodium excretion and GFR were lower for kidneys of hypertensive (S (+)Na) than those of the R animals. Kidneys from normotensive S animals had a higher salt excretion and GFR for any given range of perfusion pressure than their hypertensive cohorts. Nevertheless, GFR and sodium excretion were lower for the S (−)Na than for R kidneys. Differences among the kidneys of R, S (−)Na, and S (+)Na animals were also evident in the pressure-GFR and pressure-natriuresis curves in figures 1 and 2. For R and S (−)Na kidneys there was a direct linear relationship between both sodium excretion and GFR and the perfusion pressure. Note that the S (−)Na line is displaced to the right of R. In contrast, in S (+)Na kidneys neither GFR nor sodium excretion rose with increasing perfusion pressure, and the data points all lie well below the regression lines for kidneys from normotensive animals.

Also shown in table 2 are values of renal vascular resistance (RVR) for R, S (−)Na, and S (+)Na kidneys. At all perfusion pressures the resistances of R were less than those of either S (−)Na or S (+)Na kidneys, although the differences did not reach statistical significance in the lowest (80 to 100 mm Hg) pressure range. Unlike S kidneys, in which resistance increased sharply with a rising perfusion pressure, R kidneys maintained an essentially constant RVR (fig. 3).

In these experiments, filtration fraction was not noticeably influenced by perfusion pressure. The average value of 0.012 ± 0.001 mm Hg measured in the kidneys of the hypertensive (S (+)Na) rats was significantly less than the 0.025 ± 0.002 mm Hg in both R and S (−)Na kidneys.

![Image](http://hyper.ahajournals.org/)

**TABLE 2. Glomerular Filtration Rate (GFR), Perfusate Flow Rate (PFR), Sodium Excretion (UNaV), and Renal Vascular Resistance (RVR), Grouped According to Perfusate Pressure (P), for R, S (−)Na, and S (+)Na Kidneys**

<table>
<thead>
<tr>
<th>P (mm Hg)</th>
<th>81-100</th>
<th>101-120</th>
<th>121-140</th>
<th>141-160</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min/g kidney weight)</td>
<td>0.53 ± 0.09 (6)</td>
<td>0.69 ± 0.05 (10)</td>
<td>0.74 ± 0.06 (17)</td>
<td>0.90 ± 0.09 (11)</td>
</tr>
<tr>
<td>S (−)Na*</td>
<td>p &lt; 0.01</td>
<td>0.46 ± 0.06 (8)</td>
<td>0.60 ± 0.06 (16)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>S (+)Na</td>
<td>0.27 ± 0.10 (3)</td>
<td>0.29 ± 0.05 (9)</td>
<td>0.20 ± 0.09 (5)</td>
<td>0.21 ± 0.02 (6)</td>
</tr>
<tr>
<td>PFR (ml/min/g kidney weight)</td>
<td>24.5 ± 1.8</td>
<td>24.7 ± 1.5</td>
<td>30.0 ± 1.4</td>
<td>36.2 ± 1.5</td>
</tr>
<tr>
<td>R</td>
<td>24.5 ± 1.8</td>
<td>24.7 ± 1.5</td>
<td>30.0 ± 1.4</td>
<td>36.2 ± 1.5</td>
</tr>
<tr>
<td>S (−)Na</td>
<td>NS</td>
<td>19.5 ± 0.6</td>
<td>23.5 ± 1.2</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>S (+)Na</td>
<td>20.6 ± 1.1</td>
<td>19.9 ± 1.9</td>
<td>16.9 ± 4.0</td>
<td>17.9 ± 1.0</td>
</tr>
<tr>
<td>UNaV (µEq/min/g kidney weight)</td>
<td>7.8 ± 1.9</td>
<td>14.0 ± 2.4</td>
<td>21.0 ± 3.7</td>
<td>28.9 ± 3.5</td>
</tr>
<tr>
<td>R</td>
<td>7.8 ± 1.9</td>
<td>14.0 ± 2.4</td>
<td>21.0 ± 3.7</td>
<td>28.9 ± 3.5</td>
</tr>
<tr>
<td>S (−)Na*</td>
<td>NS</td>
<td>8.2 ± 1.6</td>
<td>17.8 ± 2.7</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>S (+)Na</td>
<td>3.8 ± 0.6</td>
<td>4.2 ± 1.3</td>
<td>4.7 ± 2.6</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td>RVR (mm Hg/ml m⁻¹/g kidney weight)</td>
<td>3.9 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>4.2 ± 0.1</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>R</td>
<td>3.9 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>4.2 ± 0.1</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>S (−)Na</td>
<td>4.3 ± 0.3</td>
<td>7.0 ± 0.3</td>
<td>6.5 ± 0.3</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>S (+)Na</td>
<td>4.6 ± 0.2</td>
<td>6.7 ± 0.8</td>
<td>9.0 ± 1.4</td>
<td>8.8 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SEM, for (N) clearance periods. p values for differences between the means are indicated.

*No clearance data were obtained for S (−)Na kidneys at the lower perfusion pressures. Not shown here are data for six clearance periods where perfusate pressure exceeded 160 mm Hg. These values are included in figure 2, where the data for S (−)Na kidneys have been regrouped in the intervals 130-140, 141-150, 151-160 + mm Hg.
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FIGURE 1. Pressure-natriuresis curves. The data points plotted are the mean values for each perfusion pressure group. The vertical and horizontal lines indicate 1 SEM. The equations, coefficients of correlation, and significance of the least squares regression lines are: R: $y = 0.364x - 25.7$, $r = 0.988$, $p < 0.01$; $S(-)Na$: $y = 0.453x - 53.6$, $r = 0.998$, $p < 0.05$; $S(+)Na$: $y = 0.014x - 0.25$, $r = 0.81$, NS.

FIGURE 2. Pressure-GFR curves. The equations, coefficients of correlation, and significance of the least squares regression lines are: R: $y = 0.006x - 0.016$, $r = 0.99$, $p < 0.02$; $S(-)Na$: $y = 0.007x - 0.475$, $r = 0.99$, $p < 0.05$; $S(+)Na$: $y = -0.001x + 0.417$, $r = 0.78$, NS.

FIGURE 3. Renal vascular resistance (RVR) as a function of perfusate pressure (P) for R, $S(-)Na$, and $S(+)Na$ kidneys. Vertical lines indicate SEM.

Discussion

These experiments, like those of Tobian and co-workers,

suggest that the hypertension in $S$ rats is due, at least in part, to the kidneys' abnormal propensity for salt retention. The resultant sodium excess might elevate the blood pressure through any of a variety of mechanisms including volume expansion,15 "waterlogging" of arteriolar walls,16 or stimulation of the production of a natriuretic hormone that has a "side effect" of increasing vascular reactivity.17 18

The reasons for the salt-retaining bias for $S$ kidneys are not known. The experiments of Tobian et al.7 failed to reveal significant hemodynamic differences between $S$ and $R$ kidneys; the observed discrepancies in salt excretion would therefore have to be ascribed to differences in tubular sodium reabsorption. Enhanced sodium reabsorption by $S$ kidneys might represent a fixed intrinsic property of their tubules or an altered responsiveness to humoral substances present in the circulation of the perfusor animals.

In the present experiments, where the kidneys were perfused in vitro with a cell-free medium that lacked hormones affecting vasomotion or tubular transport, differences between the kidneys of $S(-)Na$ and $R$ animals were also apparent. In comparison with $R$, the $S(-)Na$ kidneys displayed a right shift of the pressure-GFR as well as the pressure-natriuresis curves. The right shift in the pressure-natriuresis function resulted not from increased tubular sodium reabsorption but from a reduced filtered sodium load. As can be seen in table 2, $S(-)Na$ kidneys showed comparable relative reductions in GFR and sodium excretion.

Perfused $S$ and $R$ kidneys also differed in vascular responses to changes in perfusate pressure. As shown in table 2 and figure 3, the kidneys from normotensive
(as well as hypertensive) S animals underwent a steep rise in vascular resistance as perfusate pressure was increased. Thus, the kidneys displayed an intrinsic capacity for autoregulation. In contrast to S, R kidneys showed no rise in vascular resistance with increasing perfusate pressure. Since S(—)Na kidneys have a lower GFR but the same filtration fraction as R kidneys, we may infer that the afferent arterioles participate in the autoregulatory vasoconstriction. If the efferent arterioles alone were to constrict, filtration fraction would be expected to rise.† Afferent arteriolar participation in the autoregulatory process is consistent with the results of studies on Munich-Wistar rats. In this strain, which has surface glomeruli accessible to micropuncture, it has been shown that autoregulatory resistance adjustments occur chiefly in the afferent arteriole, for glomerular capillary pressure is maintained in the face of a fall in arterial blood pressure. These results suggest that a critical difference between S and R kidneys may reside in the afferent arterioles, S vasculature having an exaggerated and R a depressed vasoconstrictor response to increased intravascular pressure.

Some recently published data are in conflict both with our present study and that of Tobian et al. Girardin and coworkers failed to find a right shift of the pressure-natriuresis curve of in vitro perfused kidneys of normotensive S rats. Humoral factors affecting the function of in vivo perfused kidneys, but absent from in vitro perfusates, might in some as yet undefined manner be responsible for the discrepancy between these results and those of Tobian and co-workers. The difference between the two in vitro studies is less easily explained. The medium of Girardin et al. included 20 volumes percent bovine red blood cells, was enriched with amino acids, and contained vasopressin and aldosterone. It is difficult to see how these additions to the simpler medium used in the present experiments might have masked the intrinsic differences in vascular resistance and glomerular dynamics.

Both in vitro studies are, however, in agreement in showing a right shift in the pressure-GFR and pressure-natriuresis curves of S(+)Na animals. The low GFR and filtration fraction observed after 7 or more weeks of high salt intake in our study and by Girardin et al. is most probably due to glomerular damage caused by the blood pressure elevation. Rats with long-standing salt-induced hypertension have glomerular lesions consisting of hyalinization, basement membrane thickening, and endothelial and epithelial cell proliferation. Less severe lesions (mesangial thickening and intraepithelial deposition of PAS-positive material) are present in the glomeruli of S rats after only 5 weeks of high salt intake.

In summary, kidneys from nonhypertensive S rats, whether perfused with the blood of living animals or with an artificial perfusate, manifest intrinsic abnormalities that distinguish them from R kidneys and may interfere with their ability to excrete salt. Increased tubular sodium reabsorption is most clearly demonstrated in kidneys connected to the circulation of living perfusor animals. An exaggerated autoregulatory increase in afferent arteriolar resistance, which blunts the rise in filtered sodium load accompanying increased perfusion pressure, becomes apparent when the perfusion is carried out in an in vitro system. Either of these functional abnormalities, if present in the intact animal, would cause salt retention in S rats and lead, in turn, to the development of arterial hypertension when a high sodium diet is ingested.

References
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