Differential Effect of Salt Loading on Sodium and Lithium Excretion in Dahl Salt-Resistant and -Sensitive Rats

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SUMMARY Fractional excretion of lithium, as a marker for proximal sodium reabsorption, was determined in normotensive Dahl S rats (susceptible to NaCl hypertension) and Dahl R rats (resistant to NaCl hypertension) before and following an acute sodium load. Baseline mean arterial pressures, inulin clearances, sodium excretion rates, and fractional lithium clearances were not different between the R and S rats. Following the salt loading and despite similar mean arterial pressures and degree of volume expansion, the glomerular filtration rate, urinary flow rates, and absolute sodium excretion rates were greater in R than S rats. The fractional excretion of lithium was also greater in R than S rats. These data demonstrate that, at equal mean arterial pressures, Dahl S rats have a reduced capacity for sodium excretion, and that this defect is present prior to the development of hypertension. Furthermore, the observation that these animals also have a lower fractional lithium excretion during volume expansion suggests that salt loading reduces proximal tubule reabsorption to a lesser extent in Dahl S than R rats. These data suggest that the subnormal sodium and water excretion observed after sodium loading in S rats may be partially due to an abnormality in proximal tubule sodium handling. (Hypertension 6: 420-424, 1984)

KEY WORDS • fractional lithium excretion • Dahl rats • volume expansion • renal sodium handling

ONE hypothesis to explain essential hypertension suggests that hypertensive individuals are more sensitive to the effects of dietary sodium than nonhypertensive individuals. Although the relationship of dietary sodium to human essential hypertension has been difficult to define precisely, increased sensitivity to dietary sodium ingestion is the mechanism for development of hypertension in several animal models such as the Dahl rat. These animals exist as two strains: a salt-sensitive (S) strain, which develops hypertension when ingesting a high sodium diet, and a salt-resistant (R) strain which is insensitive to salt intake. The etiology for the salt sensitivity in the S rats is uncertain. However, finding that salt sensitivity can be transferred from S to R animals by transplantation of an S strain kidney suggests that the kidney plays a major role in this phenomena. A partial explanation for this phenomena has been provided by Tobian et al. who demonstrated that for any given perfusion pressure, isolated perfused kidneys from S animals excrete less sodium than do kidneys from R animals. While several other groups of investigators have confirmed a reduced pressure natriuresis in isolated perfused kidneys from S rats, whether reduced sodium excretion also occurs in S rats in vivo has yet to be determined. Furthermore, there is some debate as to whether the reduction in sodium excretion observed in isolated perfused kidneys from S rats is a cause or an effect of the elevation in arterial pressure. Finally, the mechanisms responsible for the reduced pressure natriuresis observed in S rat kidneys has yet to be determined.

The current study was undertaken to examine whether the abnormalities in renal sodium handling observed in isolated perfused S rat kidneys also exist in vivo. Furthermore, since the proximal tubule reabsorbs the majority of the filtered sodium load and decreased proximal tubule reabsorption is a prominent effect of salt loading, we were interested in examining the response of the proximal tubule to salt loading in these two rat strains. This was performed using fractional lithium clearance, which accurately reflects
fractional delivery of sodium and water out of the proximal tubule. The results demonstrate that, at equivalent mean arterial pressures, S rats when compared to R rats had a reduced capacity to excrete a sodium load. The natriuretic handicap observed in S rats was associated with a lower glomerular filtration rate and lower fractional lithium excretion rate relative to R rats.

Materials and Methods

Male Dahl S and R rats weighing between 290 and 407 g were maintained on a sodium chloride-restricted diet (<3 Eq/g diet of both sodium and chloride; ICN Pharmaceutical Company, Cleveland, OH) from time of weaning. This diet resulted in comparable mean arterial pressures prior to study. Four hours before study, the animals were injected intraperitoneally with 0.8 ml/kg body weight (BW) of an isotonic lithium chloride solution. This produced serum lithium concentrations of 0.13 ± 0.01 mEq/liter in R rats and 0.12 ± 0.01 mEq/liter in S rats during hydropenia and 0.11 ± 0.01 mEq/liter in both R and S rats following sodium loading.

The rats were then anesthetized with intraperitoneal injections of Inactin (Promonto GMBH, Hamburg, West Germany) in a dose of 100 mg/kg BW. They were then placed on a heated animal table and rectal temperatures maintained between 37° and 38°C using a servo-activated temperature controller (WSI Corporation, Yellow Springs, Ohio). Following tracheostomy, PE-50 polyethylene catheters were introduced into the right jugular vein for inulin infusion (Laevosan Gesellschaft, Linz, Austria) and into the left jugular vein for NaCl infusion. A PE-50 polyethylene catheter was placed in the right femoral artery for blood sampling and continuous blood pressure monitoring. This catheter was connected to a Model P23DC strain gauge (Statham Instruments, Hato Rey, Puerto Rico) and the arterial pressure recorded on a Grass Model 7D polygraph (Grass Instruments, Quincy, Massachusetts). A flanged PE-50 polyethylene catheter was placed into the bladder through a midline suprapubic incision for urine collections. Following surgery, an infusion of 5% inulin in 0.15 M sodium chloride was introduced at a rate of 0.5 ml/100g BW/hr and was continued through the study.

After a 30-minute surgical recovery period, urine was collected for 60 minutes to establish baseline renal function and electrolyte excretion rates. Following this collection, animals were infused over 30 minutes with 0.15 M sodium chloride (25 ml/kg BW). Urine was collected during this period and for 50 minutes following completion of the infusion. Blood was obtained at the midpoint of each urine collection for determination of hematocrit and serum inulin, lithium, sodium, and potassium concentrations.

Urine volume was determined by differences in the weight of preweighed glass vials. Sodium, potassium, and lithium concentrations in serum and urine were measured by flame photometry (Instrumentation Laboratories, Lexington, Massachusetts). Inulin concentrations in urine and serum were determined by the modified Anthrone method of Davidson and Sackner.

Determination of inulin, sodium, potassium, and lithium in blood and urine, and urinary flow rates permitted calculation of glomerular filtration rate and urinary excretion of sodium, potassium, and lithium by standard expressions. The increase in plasma volume during volume expansion was calculated from changes in hematocrit.

Statistical significance within each group was determined using the paired Student's t test. Statistical significance between groups was determined by using the Student's t test for unpaired data. A one-sided alternative hypothesis was used for the difference in lithium excretion between the two groups during saline loading. Saline loading is known to produce either no change or an increase in urinary sodium excretion in the rat. This increase is known to be higher in R rats3,5 or equal in R and S rats as well.

Results

There were no differences in mean arterial pressure, urinary flow rate, glomerular filtration rate, or absolute urinary sodium excretion between R and S rats prior to salt loading (Tables 1 and 2). Absolute urinary potassium excretion was also not different between R and S rats prior to sodium loading. Similarly, the initial fractional excretion of sodium and lithium was not different between R and S rats (Table 3). Fractional potassium excretion was also not different between R and S rat groups.

Following the sodium load, the mean arterial pressures and calculated change in plasma volume were similar in both R and S rat groups (Table 1). Sodium loading significantly increased urine volume and absolute sodium excretion in both groups (Table 2). However, the increase in urine flow rate and sodium excretion, as well as the final values for these parameters, were greater in R than S rats. Although glomerular filtration rate increased slightly in both R and S rats

| Table 1. Body Weight, Age, and Mean Arterial Pressure during and after Sodium Loading, and Degree of Plasma Volume Expansion following Infusion of Isotonic NaCl Solution |
|-----------------|----------|----------|----------|----------|----------|
| Rat group       | BW (g)   | Age (days) | MAP (mm Hg) | Plasma volume expansion |
| Dahl R (n = 8)  | 361 ± 13 | 88 ± 5    | 131 ± 6   | 123 ± 5   | 1.25 ± 0.04 |
| Dahl S (n = 13) | 362 ± 8  | 89 ± 4    | 131 ± 3   | 127 ± 3   | 1.20 ± 0.02 |

Values are means ± SEM. n = number of observations; BW = body weight; MAP = mean arterial pressure; cont = control; inf = infusion.
following the sodium load, in neither case did it reach statistical significance. Similarly, the change in glomerular filtration rate was not different between the two groups. Final glomerular filtration rate, however, was significantly greater in R than S rats. Potassium excretion was increased in both R (1.75 ± 0.46 → 3.46 ± 0.58 μEq/min; p < 0.001) and S (1.66 ± 0.17 → 2.63 ± 0.36 μEq/min; p < 0.005) rat groups. There was no difference in final potassium excretion rates between groups.

Fractional excretion of sodium and lithium were increased following sodium loading in both groups (Table 3). Fractional potassium excretion was also increased following sodium loading in the R rat (13.0% ± 2.3% → 27.9% ± 4.0%; p < 0.0005) and S rat (17.0% ± 1.6% → 26.4% ± 2.8%; p < 0.005). The increment in the fractional excretion of sodium and potassium was not different between Dahl R and S rats. However, both the increase in fractional lithium excretion and final fractional lithium excretion rate were greater in Dahl R than S rats.

Thus, despite equal salt loading and comparable degrees of volume expansion, R rats increased sodium and water excretion to a greater extent than S rats.

### Discussion

The results of the current study demonstrate that, at equivalent mean arterial pressures, S rats excrete less of a given sodium load than do R rats. Furthermore, the reduction in sodium excretion observed in the S group occurred at a blood pressure level considered normotensive for the rat. Therefore, the reduction in natriuretic capacity in these animals was present prior to the elevation in mean arterial pressure. The observation that reduced natriuretic capacity observed in isolated perfused kidney preparations from S rats also exists in vivo might have been predicted from previous studies by Iwai and co-workers. They found that administration of hydrochlorothiazide to Dahl rats maintained on a high sodium intake significantly increased sodium excretion only in rats of the S strain. This suggested that, for equivalent sodium intakes, S rats retained more sodium than their R counterparts. The finding that S rats excrete sodium less efficiently than R rats and that this defect occurs prior to the onset of hypertension is consistent with the hypothesis that the reduction in urinary sodium excretion is the cause for hypertension in the S rat group.

Although these differences have not always been statistically significant, they tend to be greater at higher perfusion pressures or under conditions of high salt intake. Maude and Kuo-Lo felt that the lower glomerular filtration rate was the primary mechanism accounting for the reduced sodium excretion from S rat kidneys. Tobian and associates, on the other hand, felt that the difference between glomerular filtration rates from R and S rat kidneys was not of sufficient magnitude to account for the differences in urinary sodium excretion.

### Table 3. Fractional Excretion of Sodium and Lithium before and after Sodium Loading

<table>
<thead>
<tr>
<th>Rat group</th>
<th>FeNa (%)</th>
<th>FeLi (%)</th>
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<tbody>
<tr>
<td></td>
<td>Cont</td>
<td>Inf</td>
</tr>
<tr>
<td>Dahl R</td>
<td></td>
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</tr>
<tr>
<td>(n = 8)</td>
<td>0.09</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>±0.02</td>
<td>±0.42</td>
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<tr>
<td></td>
<td>(p &lt; 0.025)</td>
<td>(p &lt; 0.005)</td>
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<tr>
<td>Dahl S</td>
<td>0.09</td>
<td>0.54</td>
</tr>
<tr>
<td>(n = 13)</td>
<td>±0.02</td>
<td>±0.15</td>
</tr>
<tr>
<td></td>
<td>(p &lt; 0.005)</td>
<td>(p &lt; 0.0005)</td>
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</tbody>
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Values are means ± SEM. n = number of observations; FeNa = fractional excretion of sodium; FeLi = fractional excretion of lithium; Δ = difference between control and infusion.

* p < 0.01, compared to Dahl S rats.
† p < 0.005, compared to Dahl S rats.
In accord with the isolated perfused kidney studies, we found that the glomerular filtration rate in vivo was greater in R than S rats, but this only reached statistical significance following salt loading. However, since the difference in glomerular filtration rate between R and S rats following sodium loading was 37% while the difference in urinary sodium excretion was 179%, it would seem that the differences in glomerular filtration rate are too small to completely account for the differences in sodium excretion. If the differences in sodium excretion observed in our study cannot be attributed solely to differences in filtered load, then differences in net tubular sodium reabsorption must also exist between R and S animals.

Since a major response to sodium loading is a reduction in proximal tubule salt handling might be another possible mechanism accounting for the attenuated natriuresis in S 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. Our current study demonstrated that fractional excretion of lithium was not different between R and S rats under baseline conditions, but that lithium clearance increased to a greater extent in R than in S rats following a salt load. If lithium is an adequate marker for proximal reabsorption in our current study, then this would suggest that salt loading suppressed proximal tubule reabsorption less in S than in R animals. The explanation for this is not apparent. It does not appear that enhanced sodium reabsorption in S rat proximal tubules is a fixed intrinsic property of these structures, since fractional lithium excretion was not different between R and S rats prior to salt loading. This is consistent with micropuncture studies demonstrating similar tubule fluid to plasma inulin ratios (TF/P inulin) between proximal tubules of isolated perfused R and S kidneys obtained from rats ingesting a low sodium diet.

It also seems unlikely that the differences in proximal reabsorption between R and S rat groups can be attributed solely to differences in volume expansion, since comparable changes in hematocrit occurred following salt loading in both groups.

Single nephron glomerular filtration rate, however, can be a major determinant of the rate of proximal tubule fluid reabsorption through the mechanism of glomerulotubular balance. Although glomerulotubular balance is attenuated by salt loading, it is possible that the lower glomerular filtration rate that followed salt loading in S animals might account for the higher proximal tubular reabsorption in this group. The observations in our present study do not exclude more distal nephron segments as the primary site for the reduced natriuretic response that follows salt loading in S rats. Knox and co-workers have demonstrated that increased proximal sodium delivery is not necessarily a prerequisite for natriuresis and that distal nephron segments participate in the sodium excretion that follows volume expansion. Diezi et al. have demonstrated inhibition of collecting duct sodium transport in response to volume loading, while Sheth and co-workers have demonstrated that the rapid antinatriuresis that occurs during recovery from volume expansion is modulated primarily through enhanced distal tubule sodium chloride reabsorption. Thus, differences in distal tubule sodium chloride transport between volume expansion could be another explanation accounting for the difference in urinary sodium excretion observed between S and R strains.

Finally, Tobian and co-workers have recently presented evidence that the enhanced sodium reabsorption in S rats might represent altered responsiveness to a humoral substance modulating sodium transport. The site or sites affected by this agent are uncertain. Clearly, further studies must be undertaken to define the relationship between changes in glomerular filtration rate, proximal tubule reabsorption, and distal nephron function in the reduced natriuretic capacity observed in S rats.

In summary, nonhypertensive S rats, when compared to R rats, have a reduced in vivo capacity to excrete a salt load. This natriuretic handicap is associated with a lower glomerular filtration rate and greater proximal tubule reabsorption, as determined by fractional lithium clearance. Since the natriuretic handicap is demonstrable only following salt loading, it appears not to be a fixed intrinsic abnormality of S rat kidneys. Furthermore, as the natriuretic handicap following salt loading is present in normotensive S animals, it may participate in the development of arterial hypertension in this strain.

Acknowledgments

The authors thank Kathy T. Holder for technical assistance and Carolyn Davis and Cassandra Fountain for secretarial help in the preparation of this manuscript.

References
