Abnormal Adrenal Catecholamine Synthesis in Salt-Sensitive Dahl Rats
KAROLY RACZ, OTTO KUCHEL, AND NGUYEN T. BUU

SUMMARY The possible role of catecholamines in the abnormal renal response to salt loading, a genetic defect resulting in hypertension in the salt-sensitive strain of Dahl rats, was investigated by measuring the adrenal synthesis of norepinephrine, epinephrine, and dopamine as well as their content in several tissues and the urinary excretion of these catecholamines as well as some of their metabolites at the height of salt-induced hypertension. We found that salt-sensitive Dahl rats, compared with salt-resistant Dahl rats, have a higher adrenal synthesis of [3H]norepinephrine following a pulse injection of [3H]tyrosine, a higher adrenal norepinephrine and epinephrine content but a lower kidney and heart ventricle content of dopamine and norepinephrine, and a decreased excretion of urinary dopamine, dihydroxyphenylacetic acid, 3-methoxytyramine, and homovanillic acid. These data suggest that the primary abnormality in salt-sensitive Dahl rats may be their inability to turn off, during high salt intake, their increased adrenal norepinephrine synthesis from dopamine. The abnormal catecholamine response of salt-sensitive Dahl rats to high salt intake indirectly suggests increased noradrenergic activity and decreased dopaminergic activity in the kidney, which may be important mechanisms in the sodium retention and hypertension of these rats. (Hypertension 9: 76-80, 1987)

KEY WORDS • Dahl rats • norepinephrine • dopamine • salt loading • hypertension

The salt-sensitive and salt-resistant Dahl rats are two lines of rats separated by selective breeding of Sprague-Dawley rats on the basis of their blood pressure (BP) response to salt ingestion. When kept on a high salt diet, the salt-sensitive rats (DS) manifest a lethal hypertension whereas the salt-resistant rats (DR) remain normotensive. Evidence supporting a role for the kidney in this model of hypertension derives primarily from cross-transplantation experiments conducted by Dahl and his colleagues.1 They found that transplantation of kidneys from DS into bilaterally nephrectomized DR resulted in higher BP compared with that observed in DR with kidneys transplanted from DR.1 Although these studies indicat-
**Materials and Methods**

Twenty-four male DS and DR from Brookhaven National Laboratory (Upton, NY, USA) were used in the experiments. The rats were delivered to our animal house a few days before weaning and fed an ordinary rat chow diet (Na⁺, 0.36%; K⁺, 1.08%) and water ad libitum for 10 days. After the 10-day accommodation period, the animals were fed a diet containing 8% NaCl (Ralston-Purina, Indiana City, IN, USA) for 5 weeks. Food and water were provided ad libitum, including the day of urine collections. Sodium intake was determined from daily measurement of consumed food. The systolic BP was measured by a tail cuff method. For each rat, 24-hour urinary collections were obtained by housing the rats in individual metabolic cages, 24 hours before the end of the experiment. The urine samples were subsequently analyzed for catecholamine (CA) content and CA metabolites as well as for sodium and creatinine.

At the end of the 5-week special diet, the DS and DR were divided into two groups receiving the same intravenous injection of [³H]tyrosine (700 μCi/kg body weight; specific activity, 46 Ci/mmol; Amersham Corp., Oakville, Ontario, Canada) and were killed by decapitation 15 minutes or 75 minutes after injection. Trunk blood samples were obtained for the determination of labeled and nonlabeled tyrosine in plasma. After decapitation, the adrenals, heart, and kidneys were removed and immediately frozen until homogenization.

Tissue CA content was determined by reverse-phase high-performance liquid chromatography with electrochemical detection. Aliquots of adrenal samples were further analyzed for [³H]NE, [³H]epinephrine (E), and [³H]dopamine (DA) after chromatographic separation by reverse-phase high-performance liquid chromatography with ultraviolet detection (Model 153 analytical ultraviolet detector; Altex Scientific, Berkeley, CA, USA), as described previously. The CA content in urine samples was determined radioenzymatically by the method of Da Prada and Zürcher. Urinary dihydroxyphenylacetic acid, methoxytyramine, homovanillic acid, and normetanephrine were analyzed by reverse-phase high-performance liquid chromatography with electrochemical detection, as previously described.

The method of Westerink and Wirix was adopted for the plasma tyrosine assay, with the following modifications: eluents of Sephadex G-10 columns were analyzed by reverse-phase high-performance liquid chromatography with ultraviolet detection, and the radioactivity of the column eluent fraction corresponding to tyrosine was counted for the determination of [³H]tyrosine.

Data are presented as the mean ± SE. The statistical significance of the differences between mean values of DS and DR were analyzed using Student's t test.

**Results**

Illustrated in Table 1 are the results for determination of systolic BP, body weight, sodium intake, and urinary sodium excretion in DS and DR fed a high salt diet. No differences in body weight and urinary sodium excretion were found during weekly measurements (unpublished results) in the 3-week period of high salt intake. After being fed 8% NaCl for 5 weeks, the DS had a markedly elevated systolic BP (210 ± 8 mm Hg) while the DR remained normotensive (systolic BP, 116 ± 1.6 mm Hg). The mean body weight was also significantly (p < 0.05) different between the two strains of rats (293 ± 9 and 344 ± 8 g in DS and DR, respectively). Although the sodium intake — and the food intake — was the same in both groups of rats, the urinary sodium excretion was (but only at the end of the experiments) significantly lower in DS than in DR, probably because of the severity of hypertension. The comparable food intake also suggests that the decreased weight of DS, which was not apparent at the onset of the 8% NaCl diet (weight, 159 ± 3 and 160 ± 5 g in DS and DR, respectively), was not due to decreased food consumption.

The DS receiving food with high sodium content for 5 weeks had significantly (p < 0.01) higher adrenal NE and E content as compared with the DR fed the same diet (NE in DR and DS, 29 ± 1.2 and 35 ± 1.8; E in DR and DS, 139 ± 2 and 155 ± 2 nmol/pair, respectively), but the adrenal DA content was similar in both groups of rats. Figure 1 shows that when the specific

**Table 1.** Systolic Blood Pressure, Body Weight, Sodium Intake, and Urinary Sodium Excretion in Dahl Rats After 5 Weeks of High Salt Diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR</th>
<th>DS</th>
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<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>116 ± 1.6</td>
<td>210 ± 8*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>344 ± 8</td>
<td>293 ± 9*</td>
</tr>
<tr>
<td>Sodium intake (mmol/24 hr)</td>
<td>40.5 ± 0.7</td>
<td>40.1 ± 0.7</td>
</tr>
<tr>
<td>Urinary sodium excretion (mmol/24 hr)</td>
<td>29.9 ± 1.4</td>
<td>22.7 ± 1.8*</td>
</tr>
</tbody>
</table>

Data are means ± SE of groups of 12 rats. *p < 0.05, compared with values in DR.

**Figure 1.** Specific activities of adrenal dopamine, norepinephrine, and epinephrine in DS and DR 15 and 75 minutes after a pulse injection of [³H]tyrosine (700 μCi/kg body weight). The weight of adrenals was 47 ± 1.5 and 62 ± 1.7 mg (p < 0.001) in DR and DS, respectively. Each point represents the mean for five to six rats, and the vertical line is 1 SE. Single (p < 0.05) and double (p < 0.01) asterisks indicate significant difference between groups. CA = catecholamine.
activities of adrenal CA were compared in DS and DR receiving a pulse injection of [3H]tyrosine (700 μCi/kg body weight), the accumulation of [3H]NE was significantly higher in DS than in DR, indicating an increased rate of conversion of adrenal [3H]DA to [3H]NE in the DS. However, the decline of specific activity of adrenal DA between 15 and 75 minutes postinjection was similar in both groups of rats (see Figure 1). There was no difference in 3H-labeled or nonlabeled tyrosine in plasma between the two groups of rats (Table 2).

As shown in Figure 2, the kidney DA and NE contents were significantly lower in DS than in DR after 5 weeks of high dietary salt intake (DA, 64 ± 4 and 44 ± 3; NE, 793 ± 36 and 379 ± 22 pmol/g tissue in DR and DS, respectively). In DS, the ventricular DA and NE contents also were significantly decreased (DA, 65 ± 4 and 40 ± 2 pmol/g tissue; NE, 3.6 ± 0.12 and 1.7 ± 0.14 nmol/g tissue in DR and DS, respectively). In addition, the NE content in the atria of DS and DR showed differences similar to those of the kidney and ventricle (6.4 ± 0.2 and 4.5 ± 0.2 nmol/g tissue in DR and DS, respectively), although the atrial DA content was not different between the two strains of rats.

Although DS excreted more total (free plus sulfoconjugated) NE than did DR (16.6 ± 1.8 vs 11.8 ± 0.9 nmol/24 hr in DR; p<0.05), their excretions of DA and its metabolites were significantly lower than those in DR (Table 3). When the urinary excretions were normalized for creatinine excretion, the differences in DA and dihydroxyphenylacetic acid excretions between the two groups of rats still existed and the NE and normetanephrine excretions in DS were significantly increased (see Table 3).

### Discussion

A current hypothesis postulates that salt loading may influence renal sodium handling through actions involving the peripheral sympathetic nervous system with the release of NE and DA, two CAs with opposing actions, being of crucial importance. The results of the present study indicate that the rate of synthesis of [3H]NE in the adrenals of DS was increased without a corresponding difference in the adrenal DA labeling or plasma [3H]tyrosine between the two groups of rats. The lack of difference in adrenal DA labeling between the two groups of rats contrasts sharply with the increased synthesis of adrenal NE in DS, since increased NE synthesis is expected to be associated with an accelerated turnover of its precursor, DA. A likely possibility was, however, that a difference in the release or metabolism, or both, of adrenal DA could obscure the strain differences in adrenal DA labeling between the two groups of rats. We assessed this possibility by measuring urinary excretion of DA and its metabolites, dihydroxyphenylacetic acid, 3-methoxytyramine, and homovanillic acid, which indicated significantly lower values in DS than in DR. Alternatively, differences in the DA labeling may be obscured by differences in adrenal tyrosine content in these rats. Although we did not measure the adrenal tyrosine content, the absence of differences in the specific activity of plasma tyrosine between the two groups of Dahl rats makes such a possibility unlikely. It is therefore likely that during high salt intake the adrenal CA synthesis of the two strains of Dahl rats differs in that DS use more DA for the synthesis of NE compared with DR, resulting in an increased adrenal NE synthesis as well as a decreased adrenal DA release and metabolism in DS. This suggests the importance of a possible abnormality in adrenal dopamine-ß-hydroxylase, which was shown to be increased in DS. In our study, the lower DA content in kidneys and ventricles of DS, as compared with those of DR, also supports the possibility that DS may have a marked depletion of the tissue DA pool during high salt intake.

Previous studies showed that DS have a reduced ability to inhibit sympathetic nervous system activity in response to volume expansion. This observation may explain our findings of increased adrenal NE synthesis and urinary free plus sulfoconjugated NE excre-

### Table 2. Specific Activities of Tyrosine in Plasma 15 and 75 Minutes After the Injection of [3H]Tyrosine, 700 μCi/kg

<table>
<thead>
<tr>
<th>Postinjection time (min)</th>
<th>DR</th>
<th>DS</th>
</tr>
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<tbody>
<tr>
<td>15</td>
<td>815 ± 74</td>
<td>862 ± 27</td>
</tr>
<tr>
<td>75</td>
<td>144 ± 12</td>
<td>150 ± 20</td>
</tr>
</tbody>
</table>

Specific activities (expressed in fmol [3H]-labeled/nmol total tyrosine) are means ± SE of groups of five to six rats. Between-group differences were not significant. Plasma tyrosine concentration was 74.6 ± 5.4 (SE) nmol/ml in DR and 73.5 ± 9.9 nmol/ml in DS.
tion in DS. It is likely, however, that other mechanisms are also involved in the abnormal CA synthesis and metabolism in those rats. The urinary excretions of NE and its metabolites may derive from a number of different sources, including renal neuronal and renal or other nonneuronal elements. Although NE released from renal nerves generally is considered to represent the major catecholaminergic mechanism in the regulation of renal function, we found increases in urinary NE and normetanephrine excretions in DS only after the correction of those values with creatinine excretion.

Sodium loading by dietary high salt ingestion or by saline infusion has been shown to be associated not only with decreases in renal sympathetic nervous activity, but also with increases in urinary DA excretion. Although dopaminergic nerves are not known to be present in rat kidneys, the source of increased urinary DA excretion during a high salt diet may be an increased conversion of circulating L-dopa by the kidney or an inhibition of dopamine-β-hydroxylase by high salt intake. These salt-induced changes in combination with a consequently increased urinary DA excretion and decreased urinary NE excretion may participate in the facilitation of renal excretion of sodium during salt loading, since DA increases natriuresis and thereby opposes the effect of NE on renal sodium excretion. It is therefore possible that the difference in renal sodium handling between DS and DR could be accounted for by a difference in their CA responses to a high salt diet: DS may be unable to adjust appropriately their renal noradrenergic and dopaminergic activities to salt ingestion. Although the respective roles of high urinary NE and low urinary DA during high salt intake in DS are difficult to establish, both abnormalities may be important in the sodium retention and salt-sensitive hypertension in these rats.

In the present study, we found high adrenal NE and E concentrations in DS while the NE content in other tissues was lower in DS than in DR. The increase of NE and E in the adrenals of DS probably was not due to a strain difference in the weight of the medullary tissue, since a previous report indicated a similar medullary weight in DS and DR when the animals were fed the same diet. Although the high adrenal E and NE content in DS was thought to indicate an increased adrenal E and NE synthesis, the relationship between tissue content and synthesis of the respective CAs has to be interpreted with care; the present experiments revealed that despite the elevated adrenal E content in the DS, the synthesis of [3H]E as well as the urinary E excretion was not different between the DS and DR. It is also probable that the low kidney and heart NE concentrations in DS are not an indication of a decreased NE synthesis in those tissues. According to previous studies, decreased kidney and heart NE concentrations also occur in other models of experimental hypertension that are associated with an increased sympathetic tone. A low tissue content and, possibly, an increased turnover of NE in the kidneys and heart of DS could result from an increased sympathetic activity in those organs, as has already been demonstrated in DS. Consistent with this possibility are the findings of increased urinary excretion of free plus sulfoconjugated NE, which could indicate an increased NE release not only from the adrenals but also from other peripheral tissues.

In summary, the present study demonstrated marked differences in the adrenal synthesis of NE, the tissue content of DA and NE, and the urinary excretion of DA and its metabolites between DS and DR after 5 weeks of high salt intake. The data indirectly suggest that an increased noradrenergic activity in the kidney, combined with a decreased dopaminergic activity, may be closely related to the salt sensitivity and hypertension of DS.

Acknowledgments

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References


<table>
<thead>
<tr>
<th>Catecholamine</th>
<th>Urinary excretion (nmol/24 hr)</th>
<th>Urinary excretion (nmol/mmol creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DR</td>
<td>DS</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>15.7 ± 0.8</td>
<td>15.7 ± 1.7</td>
</tr>
<tr>
<td>Normetanephrine</td>
<td>14.9 ± 1.0</td>
<td>14.3 ± 1.1</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>6.5 ± 0.5</td>
<td>4.9 ± 0.8</td>
</tr>
<tr>
<td>Dopamine</td>
<td>142 ± 8.2</td>
<td>67 ± 9.4*</td>
</tr>
<tr>
<td>DOPAC</td>
<td>256 ± 11</td>
<td>156 ± 11.8*</td>
</tr>
<tr>
<td>3-methoxytyramine</td>
<td>21.6 ± 1.3</td>
<td>13.7 ± 1.6*</td>
</tr>
<tr>
<td>Homovanillic acid</td>
<td>277 ± 6.8</td>
<td>204 ± 14.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE of groups of 12 to 24 rats. DOPAC = dihydroxyphenylacetic acid. *p < 0.05, compared with values in DR.
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