Hypothalamic Pressor Responses and Salt-Induced Hypertension in Dahl Rats

RUBEN D. BUÑAG, M.A., M.D., JASON BUTTERFIELD, B.S., AND SUSUMU SASAKI, M.D.

SUMMARY Interactions between abnormal salt intake and central sympathetic function were studied by recording pressor and sympathetic effects of hypothalamic stimulation in Dahl salt-resistant (DR) and salt-sensitive (DS) rats. All DS rats, including those fed a low-salt diet since weaning, became hypertensive by 11 weeks of age. Increased salt intake aggravated hypertension in DS rats without affecting blood pressure in DR rats. Basal sympathetic tone determined during urethane anesthesia, from the frequency of splanchnic nerve potentials as well as the magnitude of hypotension induced by α-adrenergic blockade with phentolamine, was consistently lower in low-salt DR rats than in any others. Pressor responses to electrical stimulation of the ventromedial hypothalamus, whether expressed as absolute or percent increases in mean pressure, were invariably enhanced in DS rats. On the other hand, attendant increases in sympathetic nerve firing were significantly higher in DS rats, but only when expressed as absolute changes and not when expressed as percent changes. Consequently, pressor and sympathetic responses became dissociated in magnitude such that low-salt DR rats which had the weakest pressor responses also had the highest percent increases in sympathetic nerve firing. Peripheral increases in cardiovascular reactivity were considered unlikely because pressor responses to drugs like norepinephrine, tyramine, and vasopressin, were unaltered. Although 5-week-old DS rats that had not been exposed to high-salt intake remained normotensive, basal sympathetic activity and pressor responsiveness to hypothalamic stimulation were already enhanced. Since sympathetic and hypothalamic enhancement occurred before any other changes could be detected it was considered possible that sympathetic hyperactivity of hypothalamic origin may be involved in initiating genetic hypertension. These results also suggest that induction of hypertension in DS rats might depend on genetic transmission of hypersensitivity not only to salt but also to stressful stimuli. (Hypertension 5: 460-467, 1983)

KEY WORDS • blood pressure • heart rate • high-salt diet • genetic hypertension • salt-sensitive Dahl rats • sympathetic hyperactivity • ventromedial hypothalamus

AFTER Dahl and his associates succeeded in breeding two rat strains with opposite genetic predispositions to salt-induced hypertension, they used parabiotic rats to show that the hypertension was due to a renal pressor substance. Other mechanisms were not ruled out, however, and it remains possible that sympathetic dysfunction may also be involved. In Dahl salt-sensitive (DS) rats maintained on high-salt intake, vasoconstrictor responsiveness to sympathetic nerve stimulation becomes selectively enhanced as does the fall in vascular resistance resulting after sympathetic denervation. Moreover, peripheral sympathectomy induced by either 6-hydroxydopamine or guanethidine prevents subsequent elevation of blood pressure and vascular resistance. Concomitant elevation in DS rats of renal vascular resistance seems unrelated to sympathetic overactivity, but it has further been suggested that sympathetic dysfunction occurs centrally rather than peripherally. Intracerebroventricular injections of angiotensin II or hypertonic saline elicit larger pressor responses in DS than in Dahl salt-resistant (DR) rats, and destruction of various hypothalamic areas attenuates the blood pressure elevation in DS rats.

Ordinary weanling rats that drink isotonic saline solution for 5 weeks develop mild hypertension together with enhanced pressor and sympathetic responses to hypothalamic stimulation. In the present studies we recorded hypothalamic responses in 11-week-old DR and DS rats that had been fed low- or high-salt diets. Additional experiments were done later in 5-week-old rats to measure hypothalamic responsiveness before basal blood pressures became markedly elevated.
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Methods

Forty-two male rats of the Dahl strain, half of which were DR while the rest were DS, were purchased together with two kinds of chow containing 0.4% and 8.0% sodium chloride (henceforth referred to as low-salt and high-salt diets respectively) from Brookhaven National Laboratory (Upton, New York). All rats were 3 weeks old upon arrival and were fed a 0.4% sodium chloride diet until they were 5 weeks old, after which two separate studies were performed. For the first study, 32 DR and DS rats were subdivided depending on dietary salt intake into four subgroups as follows: 1) eight DR rats on 0.4% NaCl; 2) eight DR rats on 8.0% NaCl; 3) eight DS rats on 0.4% NaCl; and 4) eight DS rats on 8.0% NaCl. These rats were fed the respective diets for 5 weeks and then terminal experiments were done to record sympathetic and cardiovascular responses to hypothalamic stimulation when the rats were 11 weeks old. For the second study, hypothalamic responsiveness was measured at 5 weeks of age in five DR and five DS rats.

Chronic Cardiovascular Measurements in 11-Week-Old Awake Rats

With a photoelectric sensor (IITC Inc, Landing, New Jersey) that allows tail-cuff measurements in awake rats without preheating,12 systolic pressure, mean pressure, and heart rate were measured weekly during the first study as the rats grew from 4 through 10 weeks of age. The pressure level at which pulsations reappeared during cuff deflation was taken as the systolic pressure while that occurring later in the same cycle at peak oscillation was taken as the corresponding mean pressure. Each pressure value was obtained by averaging five individual readings. Heart rate was calculated by counting arterial pulsations recorded for 5 seconds with the cuff deflated and multiplying by 12.

Responses to Hypothalamic Stimulation in Anesthetized Rats

Each rat was anesthetized with urethane (100 mg/100 g, i.p.) to allow placement of a concentric stainless steel electrode 0.5 mm in diameter (NE-100; custom-made by Rhodes Medical Instruments, Woodland Hills, California) in the ventromedial hypothalamus. In 11-week-old rats, the stereotaxic coordinates used were: anteroposterior 6.0, lateral 1.0, and dorsoventral -3.7.13 In 5-week-old rats, to compensate for smaller head size we followed the procedure described by Sherwood and Timiras14 for young rats; in fixing the head to the stereotaxic frame, the top of the toothbar was placed at the same horizontal level as the center of the earbars, and the stereotaxic coordinates used were: anteroposterior 4.7, lateral 0.6, and dorsoventral 1.2. Catheters were inserted in all rats separately into the left femoral artery for recording blood pressure, and into the left femoral vein for drug injections. Pulsatile femoral pressure and sympathetic nerve activity were recorded continuously during graded hypothalamic stimulation and following intravenously-injected drugs.15 16 Hypothalamic stimulation was graded by using 10-second trains of 50–200 μA biphasic currents.

For recording sympathetic nerve activity, the inferior nerve bundle emerging from the coeliac ganglion was placed over a bipolar stainless steel electrode ( uninsulated tips 1 mm apart). Nerves and electrode tips were immersed in mineral oil. Spontaneous respiratory movements were abolished by paralyzing skeletal muscles with decamethonium bromide (Syncurine, 0.2 mg/100 g i.v.) and connecting the rat to an artificial respirator. Spike potentials were amplified (Grass P15AC amplifier Grass Medical Instruments, Quincy, Massachusetts) and recorded continuously on magnetic tapes, which were later played back into an amplitude analyzer (F. Haer and Company, Brunswick, Maine) to convert individual spikes into uniform pulses. The number of individual pulses per second was counted with a rate analyzer whose output was recorded as a histogram on an ink-writing recorder, converted to digital form by a computer interface, and printed by a programmed calculator.17

Brain Histology, Drugs, and Statistics

After every experiment, a 0.5 mA direct current was passed through the hypothalamic electrode for 10 seconds to produce a small lesion at its tip. Through a thoracotomy, a 15-gauge needle was inserted via the left ventricle into the ascending aorta, and 10% formalin was perfused into the brain as described by Wolf.18 The whole brain was then removed and stored in formalin (containing 1% potassium ferricyanide) until sectioning. Transverse sections (40 μm) stained with cresyl violet were compared with the atlas by Pellegrino et al.19 to locate lesion sites.

Drugs used were norepinephrine bitartrate (Levophed), 50, 100, and 200 mg; tyramine hydrochloride, 20 and 40 μg; arginine vasopressin (Parke-Davis), 5 mU; and phentolamine mesylate (Regitine), 0.5 mg. To ensure induction of complete α-adrenergic blockade, phentolamine was given in divided doses until repeated injection no longer produced any further fall in blood pressure. All doses are expressed in terms of the respective salts per 100 g body weight.

Data were routinely expressed as average ± SEM and results from 5-week-old rats were analyzed using two-tailed t tests for comparing means of independent samples;19 differences at a 5% level (p < 0.05) were considered significant. Analysis of variance was used to examine results from 11-week-old rats for possible changes occurring at different weeks or in response to various stimuli. Duncan’s multiple range test20 was applied to F ratios significant at 5% or less, to determine significance of differences between pairs of means. Percent changes, for which a normal distribution cannot be assumed, were compared using the Kruskal-Wallis21 nonparametric method for analysis of variance, and whenever chi-squares were significant, the Mann-Whitney test19 was used to determine significant differences between pairs of means.
Selective Hypertensive Effect of High-Salt Diets in DS Rats

Even before high-salt diets were introduced, blood pressure was already consistently higher in DS than in DR rats. Average systolic and mean pressures obtained with the tail-cuff method during the first 2 weeks (4th and 5th weeks of age) while all the rats were still receiving the low-salt diet were significantly higher in both DR and DS rats, whether on the low- or high-salt regimen, than the corresponding R-values obtained with the tail-cuff method during the first 2 weeks (4th and 5th weeks of age) while all the rats were still receiving the low-salt diet. At this time the pressure differences between DR and DS rats, whether on the low- or high-salt regimen, were larger than the corresponding R-values obtained with the tail-cuff method during the first 2 weeks (4th and 5th weeks of age) while all the rats were still receiving the low-salt diet. Average systolic and mean pressures obtained with the multiple range test at the 1% level on Week 7, and at the 5% level on Week 5 while those equal to or greater than 4.57 are significant at 1%.

Even before high-salt diets were introduced, blood pressure was already consistently higher in DS than in DR rats. Average systolic and mean pressures obtained with the tail-cuff method during the first 2 weeks (4th and 5th weeks of age) while all the rats were still receiving the low-salt diet were significantly higher in both DR and DS rats, whether on the low- or high-salt regimen, than the corresponding R-values obtained with the tail-cuff method during the first 2 weeks (4th and 5th weeks of age) while all the rats were still receiving the low-salt diet. At this time the pressure differences between DR and DS rats, whether on the low- or high-salt regimen, were larger than the corresponding R-values obtained with the tail-cuff method during the first 2 weeks (4th and 5th weeks of age) while all the rats were still receiving the low-salt diet. Average systolic and mean pressures obtained with the multiple range test at the 1% level on Week 7, and at the 5% level on Week 5 while those equal to or greater than 4.57 are significant at 1%.

Changes in body weight did not follow any consistent pattern and by the 10th week averaged (g ± SEM) 339 ± 8, 316 ± 9, 307 ± 8, and 306 ± 7 respectively (F ratio of 3.52 significant at 5% with DR rats on low-salt being heavier than either of the DS subgroups). Heart rates also did not differ between groups at any time; corresponding averages (bpm) measured during the 10th week were: 368 ± 6, 363 ± 8, 366 ± 16, and 366 ± 11 respectively (F ratio of 0.03 not significant).

Enhanced Pressor Responsiveness to Hypothalamic Stimulation in 11-Week-Old DS Rats

All rats were anesthetized with urethane to allow recording of sympathetic nerve potentials together with phasic intraarterial pressure during graded hypo-

<table>
<thead>
<tr>
<th>Variable measured</th>
<th>Low-salt</th>
<th>High-salt</th>
<th>F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Femoral blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systolic</td>
<td>135 ± 4</td>
<td>132 ± 5</td>
<td>161 ± 4</td>
</tr>
<tr>
<td>mean</td>
<td>103 ± 2</td>
<td>100 ± 3</td>
<td>115 ± 4</td>
</tr>
<tr>
<td>diastolic</td>
<td>83 ± 2</td>
<td>80 ± 3</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>B. Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>360 ± 13</td>
<td>356 ± 10</td>
<td>367 ± 7</td>
</tr>
<tr>
<td>C. Sympathetic nerve firing (spikes/sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 ± 2</td>
<td>36 ± 6</td>
<td>27 ± 2</td>
</tr>
</tbody>
</table>

Data expressed and analyzed as in table 1.
thalamic stimulation. Group differences in baselines for blood pressure and heart rate recorded directly from femoral catheters were the same as those detected indirectly with the tail-cuff method while the rats were awake. Systolic, mean, and diastolic pressures were significantly higher in DS rats on the high-salt diet than in any of the other three groups (table 2 A); differences obtained with the multiple range test by comparing average pressures from DS rats on high-salt with those from the other groups were all larger than the corresponding R values at the 1% level. Here again, the high-salt diet showed no discernible effect on blood pressure in DR rats, and none of the group differences in heart rate was significant (table 2 B). On the other hand, initial rates for frequency of sympathetic nerve firing were faster in all rats on the high-salt diet (table 2 C). For those on low-salt diets, firing rates were higher in DS than in DR rats (multiple range comparison significant at 5%).

Electrical stimulation of the ventromedial hypothalamus with graded currents produced increases in mean blood pressure and neural firing rate accompanied by decreases in heart rate. During each 10-second period of stimulation, neural firing accelerated immediately to attain peak increases during the first 5 seconds after which it subsided slightly, but still stayed well above the baseline level. Attendant pressor and bradycardic responses began soon after neural firing increased (fig. 1), with magnitude of all three effects being directly related to the current strength applied to the hypothalamus. For current strengths of 100 µA or more, pressor responses were invariably stronger in DS than in DR rats regardless of the diet (table 3 A). Conversely, corresponding levels of bradycardia were weaker in DS than in DR rats no matter what the diet was (table 3 B). Although increases in neural firing rate generally tended to be higher in DS rats, the differences were not pronounced and were significant only for DS rats on the high-salt diet (table 3 C).

### Table 3. Sympathetic and Cardiovascular Responses to Ventromedial Hypothalamic Stimulation in Anesthetized 11-week-old Dahl Rats

<table>
<thead>
<tr>
<th>Current (µA)</th>
<th>Low-salt DR</th>
<th>High-salt DR</th>
<th>Low-salt DS</th>
<th>High-salt DS</th>
<th>p of F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1 ± 0</td>
<td>1 ± 0</td>
<td>2 ± 1</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>13 ± 3</td>
<td>16 ± 1</td>
<td>30 ± 3†</td>
<td>30 ± 4†</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>150</td>
<td>18 ± 2</td>
<td>18 ± 1</td>
<td>37 ± 1†</td>
<td>39 ± 3†</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>200</td>
<td>21 ± 1</td>
<td>22 ± 2</td>
<td>38 ± 2†</td>
<td>42 ± 3†</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Data are average changes from baselines given in table 2, with pressor responses indicating increases in mean femoral pressure.

*Significantly different from DR rats on either diet at the 5% level using Duncan's multiple range test.

†Significantly different from DR rats on either diet at the 1% level using Duncan's multiple range test.

FIGURE 1. Cardiovascular and neural effects of stimulating the ventromedial hypothalamus in urethane-anesthetized rats. Panel A is from a DR rat and panel B from a DS rat; both rats had previously been fed a high-salt diet. Tracings from top to bottom of phasic femoral pressure (mm Hg), heart rate (1/min), original analog signal of sympathetic nerve activity, and histogram showing frequency of sympathetic nerve firing (spikes/sec). Large arrows in each panel indicate onset of 10-sec period of hypothalamic stimulation with numbers immediately below each arrow signifying current strengths (µA) used for stimulation. Small arrows in the last panel indicate where the histogram went off scale because of very high increases in firing frequency.
TABLE 4. Pressor and Sympathetic Nerve Responses to Hypothalamic Stimulation in 11-Week-Old Dahl Rats Expressed as Percent Changes

<table>
<thead>
<tr>
<th>Current (μA)</th>
<th>Low-salt DR</th>
<th>High-salt DR</th>
<th>Low-salt DS</th>
<th>High-salt DS</th>
<th>p of Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Increase in mean pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>15 ± 2</td>
<td>16 ± 1</td>
<td>27 ± 3</td>
<td>21 ± 2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>150</td>
<td>19 ± 2</td>
<td>18 ± 2</td>
<td>32 ± 2</td>
<td>28 ± 3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>200</td>
<td>21 ± 2</td>
<td>22 ± 2</td>
<td>33 ± 2</td>
<td>31 ± 3</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

B. Increase in sympathetic nerve firing

<table>
<thead>
<tr>
<th></th>
<th>Low-salt DR</th>
<th>High-salt DR</th>
<th>Low-salt DS</th>
<th>High-salt DS</th>
<th>p of Chi square</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 462 ± 68</td>
<td>204 ± 46</td>
<td>359 ± 65</td>
<td>322 ± 44</td>
<td>&lt; 0.1</td>
<td></td>
</tr>
<tr>
<td>150 751 ± 119†</td>
<td>280 ± 43</td>
<td>470 ± 89</td>
<td>419 ± 65</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>200 808 ± 125†</td>
<td>303 ± 43</td>
<td>544 ± 96</td>
<td>461 ± 76</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from low-salt DR rats at the 5% level.
†Significantly different from high-salt DR rats at the 1% level.

Since hypothalamic responses may have been altered by baseline differences existing prior to stimulation, increases in mean pressure and sympathetic nerve firing produced by stimulation with 100, 150, and 200 μA currents were recalculated as percent increases from the respective baselines (table 4). Pressor responses remained stronger (as they were when expressed as absolute changes) in DS than in DR rats. However, the apparent elevation in spikes/sec for sympathetic nerve firing in DS rats disappeared. Instead, percent increases in sympathetic nerve firing became appreciably higher in DR rats on low-salt diet than in any others. Hence, although hypothalamic stimulation produced smaller pressor responses in these rats, because their basal levels of sympathetic nerve firing were initially lower, resulting increases in neural firing were more pronounced in DR rats on low-salt intake than in others. Based on this, it seems unlikely that enhancement of hypothalamic pressor responsiveness was caused by augmented sympathetic discharges.

Blood Pressure Responses to Injected Drugs in 11-Week-Old Rats

To determine whether pressor responsiveness to injected drugs had also been altered, blood pressure was recorded during intravenous injections of norepinephrine, tyramine, or vasopressin. Magnitude of pressor responses to all these drugs was larger in DS than in DR rats when expressed as absolute increases in mm Hg (table 5 A), but when recalculated as percent increases to compensate for initial differences in baseline mean pressure, none of the differences was significant (table 5 B).

Upon subsequent induction of α-adrenergic blockade by intravenous injection of phentolamine, the blood pressure fall in mm Hg was weakest in DR rats on low-salt diet and strongest in DS rats on high-salt diet, with some intervening group differences being statistically significant (table 5 A). For instance, the hypotensive effect in DS rats on high-salt (−93 ± 5 mm Hg from table 5 A) was significantly greater than

<table>
<thead>
<tr>
<th>Drug injected</th>
<th>Doses (100 g)</th>
<th>Low-salt DR</th>
<th>High-salt DR</th>
<th>Low-salt DS</th>
<th>High-salt DS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Mean pressure increases (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Norepinephrine</td>
<td>50</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>100</td>
<td>12 ± 2</td>
<td>13 ± 1</td>
<td>20 ± 1</td>
<td>19 ± 1</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>22 ± 2</td>
<td>24 ± 1</td>
<td>29 ± 1*</td>
<td>27 ± 2*</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Tyramine</td>
<td>20</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
<td>10 ± 2</td>
<td>11 ± 1*</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>40</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
<td>18 ± 3</td>
<td>18 ± 2</td>
<td>&lt; 0.1</td>
<td></td>
</tr>
<tr>
<td>Vasopressin</td>
<td>5</td>
<td>24 ± 2</td>
<td>21 ± 2</td>
<td>32 ± 3</td>
<td>33 ± 2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>0.5</td>
<td>−45 ± 6</td>
<td>−63 ± 4*</td>
<td>−74 ± 5</td>
<td>−93 ± 5</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

B. Mean pressure increases (%)

<table>
<thead>
<tr>
<th>Drug injected</th>
<th>Doses (100 g)</th>
<th>Low-salt DR</th>
<th>High-salt DR</th>
<th>Low-salt DS</th>
<th>High-salt DS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>50</td>
<td>8 ± 1</td>
<td>6 ± 1</td>
<td>11 ± 1</td>
<td>9 ± 2</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>100</td>
<td>12 ± 2</td>
<td>13 ± 1</td>
<td>17 ± 1</td>
<td>14 ± 1</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Tyramine</td>
<td>20</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>ns</td>
</tr>
<tr>
<td>40</td>
<td>12 ± 1</td>
<td>13 ± 1</td>
<td>15 ± 1</td>
<td>14 ± 1</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Vasopressin</td>
<td>5</td>
<td>23 ± 2</td>
<td>22 ± 2</td>
<td>27 ± 2</td>
<td>24 ± 2</td>
<td>ns</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>0.5</td>
<td>−44 ± 6</td>
<td>−61 ± 2*</td>
<td>−64 ± 3*</td>
<td>−66 ± 4*</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Data obtained from the same rats and presented as in table 3.
*Significantly different from low-salt DR rats at the 5% level.
†Significantly different from low-salt DR rats at the 1% level.
§Significantly different from high-salt DR rats at the 5% level.
§§Significantly different from high-salt DR rats at the 1% level.
that in DS rats on low-salt (−74 ± 5 mm Hg; multiple range test significant at the 5% level). However, when these same hypotensive effects were expressed as percent changes, the only significant difference was that low-salt DR rats were less responsive than those in any other groups (table 5 B; upon comparing low-salt DR rats with those in other groups, z values obtained with the Mann-Whitney test were significant at the 5% level). These results, therefore, confirm the differences shown previously by comparing baselines in sympathetic nerve firing (table 2): namely, that sympathetic tone was lower in DR rats on low-salt diet than in other rats.

**Are Pressor Responses to Hypothalamic Stimulation Also Enhanced in 5-Week-Old Rats?**

To answer this question, femoral arterial pressure and sympathetic nerve potentials were recorded during hypothalamic stimulation in 5-week-old rats that had been fed a low-salt diet since they were weaned. There were no significant differences in arterial pressure between DR and DS rats (table 6), although basal heart rate and frequency of sympathetic neural firing were both significantly higher (p-values < 0.05) in DS rats. Despite the absence of differences in baseline blood pressure, however, pressor responsiveness to hypothalamic stimulation was already markedly enhanced. Magnitude of absolute increases in both mean pressure and sympathetic nerve firing was consistently more pronounced in DS than in DR rats at all current strengths used for hypothalamic stimulation (table 7). Since there were no differences in baseline pressure, pressor responses did not have to be recalculated, but increases in sympathetic nerve firing expressed as percent changes showed no consistent differences between DR and DS rats. Bradycardia during stimulation with 100–200 μA currents tended to be less in DS rats but group differences were not significant. Though ineffective in DR rats, 50 μA currents still elicited appreciable pressor, bradycardiac, and sympathetic effects in DS rats (table 7). There were no differences between DR and DS rats in magnitude either of pressor responses to injections of norepinephrine, tyramine, or vasopressin, or of hypotensive responses to α-adrenergic blockade with phentolamine (table 8). Thus, at 5 weeks of age, baselines for sympathetic activity and pressor response to hypothalamic stimulation were already enhanced in DS rats, even though basal blood pressure and cardiovascular reactivity were still essentially the same as in DR rats.

**Postmortem Verification of Hypothalamic Electrode Placement**

Electrode tips were invariably located in the ventromedial hypothalamus adjacent to the fornix, median forebrain bundle, and anterior and lateral hypothalamic areas. Average stereotaxic coordinates in either 11-week-old (table 9 A) or 5-week-old (table 9 B) rats differed only slightly between groups, thereby indicating that electrodes had been positioned similarly in the different rat groups.

**Discussion**

What is transmitted genetically in DS rats must be a predisposition to hypertension which is worsened by,
but not totally dependent on high salt intake. This would explain why hypertension developed in all 11-week-old DS rats including those that had been fed a low-salt diet since weaning (Tables 1 and 2). An increase in salt intake evidently aggravates hypertension selectively in DS rats because introduction of the high-salt diet did not alter blood pressure in DR rats but augmented the elevation attained in DS rats of the same age. These findings are not unexpected since they simply confirm those Dahl et al. reported long ago.12,13

Direct relationships between dietary salt and the sympathetic nervous system have often been implied on the basis of chemical determinations of norepinephrine, either in plasma30 or in tissue turnover.25 By using electrophysiological techniques like those used here, Takishita and Ferrario26 showed that at any given blood pressure level, integrated sympathetic activity recorded from postganglionic renal nerves was lower in sodium-depleted than in normal dogs. Although the effects of salt-loading were undetermined, our data agree with theirs in that sympathetic nerve firing was weaker in all rats (whether DR or DS) on a low-salt diet than on a high-salt diet (Table 2; all R values obtained using Duncan’s test were significantly larger than the corresponding differences at 5%). Additionally, DS rats must be inherently predisposed to sympathetic overactivity because even when salt intake was restricted, they still had higher neural firing rates (Table 2) and connective differences at 5%). Additionally, DS rats must be inherently predisposed to sympathetic overactivity because even when salt intake was restricted, they still had higher neural firing rates (Table 2) and larger hypertensive responses to alpha-adrenergic blockade with phentolamine (Table 5) than DR rats.

Evidence implicating central neurogenic mechanisms in Dahl hypertension has heretofore been obtained by recording the effects of brain destruction rather than brain stimulation. Hypertension in DS rats has been inhibited by destroying the anteromedial hypothalamus,8 the paraventricular nuclei,9 or the anterolateral third ventricle (AV3V) region.10 Although electrical stimulation of the suprachiasmatic nuclei does not affect blood pressure even when cervical sympathetic activity has been markedly inhibited,25 Goto et al.9 found hypertension in DS rats inexplicably enhanced by suprachiasmatic lesions. Notwithstanding this discrepancy, however, the results of brain lesion studies to date seem generally compatible with elimination of an increased sympathetic drive emanating from the hypothalamus.

The ventromedial hypothalamus could be a probable central site for initiating sympathetic hyperactivity because according to the current hypothesis proposed for regulation of plasma insulin concentrations,26,27 the level of sympathetic activity is primarily determined in the ventromedial hypothalamus. In line with this, lesions of the ventromedial hypothalamus have recently been shown to lower norepinephrine turnover rates in various tissues and organs.28 However, evidence derived from these and other lesion studies remains inconclusive since neural destruction includes not only cell bodies at the lesion site but also fibers originating from elsewhere. Because central regulation of blood pressure involves a complex interaction of facilitatory and inhibitory pathways at various levels of the neuraxis,29 the present findings are compatible with either increased facilitatory or decreased inhibitory inputs on sympathetic pathways descending from the hypothalamus through synapses in the medulla, spinal cord, or thoracolumbar chains.

Our results further indicate that in DS rats increases in basal sympathetic activity and hypothalamic pressor responsiveness precede any detectable changes in either baseline blood pressures or pressor responses to drugs. At 5 weeks of age, both the initial rates of sympathetic nerve firing (Table 6) and pressor responses to hypothalamic stimulation (Table 7) were already stronger in DS than in DR rats. The absence of appreciable differences in pressor responses to drugs (Table 8) indicates that peripheral cardiovascular reactivity was unchanged. Considered altogether these findings could mean that a centrally-induced sympathetic hyperactivity occurs even before blood pressure becomes elevated. Once hypertension has developed, assessment becomes more difficult because the blood pressure elevation can obscure underlying mechanisms. For instance, along with enhanced pressor responses to hypothalamic stimulation (Table 3A) in 11-week-old rats we also found seemingly substantial increases in attendant sympathetic nerve firing (Table 3C) and responses to pressor drugs (Table 5A). But when the data were recalculated as percent changes to compensate for initial baseline differences, then the only finding that remained significant was the enhancement of hypothalamic pressor responses in DS rats (Table 4A).

Inasmuch as neither increased sympathetic firing nor increased cardiovascular reactivity can account for the enhancement in hypothalamic pressor responses, some other mechanism must be responsible. Perhaps enhancement results from impaired baroreflexes since baroreflex regulation of heart rate30 as well as of vascular resistance31 becomes abnormal in DS rats. This may be the cause of reduced bradycardia during hypothalamic stimulation (Table 3), but since faulty buffering should augment responsiveness to all pressor stimuli32
it is difficult to explain why our responses to norepinephrine, tyramine, or vasopressin, were unaffected (table 5 B). Alternatively, enhanced hypothalamic activity could have accelerated baroreceptor resetting since the hypothalamus normally inhibits reflex bradycardia.32

Consistent enhancement of pressor responses to hypothalamic stimulation, regardless of dietary salt intake (tables 3 A and 4 A) or age (table 7), represents our most cogent finding because it implies that DS rats are inherently hypersensitive not only to salt but also to stressful stimuli. Others have likewise shown that DS rats compared with DR rats, respond to approach-avoidance conflict with larger increases in blood pressure,33 and to handling or immobilization stress with larger increments in plasma norepinephrine.34 Although DS rats were originally derived from a Sprague-Dawley strain, while Okamoto and Aoki35 bred their spontaneously hypertensive rats (SHR) from a Wistar strain, our results show that both strains share at least two common characteristics, namely: aggravation of hypertension with increased salt intake,36 and enhanced sympathetic responsiveness to hypothalamic stimulation.17,37 Exactly how much each characteristic contributes to the final blood pressure elevation is difficult to judge, but their mutual existence in two separate models of genetically-derived hypertension reinforces the suggestion that both traits may have been transferred genetically.

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