Role of Epinephrine in the Development of Hypertension in Dahl Salt-Sensitive Rats

Michael D. Johnson and Theodore A. Kotchen

The present experiments were designed to test the hypothesis that adrenal epinephrine contributes to the development of hypertension in the Dahl salt-sensitive (DS) rat. All studies were carried out in conscious male DS and Dahl salt-resistant (DR) rats weighing 200-240 g. An indwelling femoral arterial catheter was placed for blood sampling and measurement of blood pressure. After 5 days of either a high salt (7% NaCl) or a normal salt (1% NaCl) dietary regimen, DS and DR rats were subjected to an acute stress paradigm (graded electrical footshock). There were no differences in basal plasma catecholamine concentrations or in the acute pressor responses to graded footshock between the four substrain/diet groups. However, in both DS and DR rats, plasma epinephrine responses to acute footshock were greater on a 7% than on a 1% NaCl diet. Additional groups of DS rats were treated with an inhibitor of adrenal phenylethanolamine N-methyltransferase, SK&F 29,661 (1-2 g/kg body wt/day) or with vehicle. Three days after placement of an arterial catheter, rats were placed on a 7% NaCl diet, and blood pressure was measured daily for an additional 3 weeks. Although SK&F 29,661 treatment was effective in reducing adrenal epinephrine content and apparent release by approximately 80%, treatment did not alter the time course of salt-induced changes in blood pressure. We conclude that: 1) acute pressor responses to mild stress do not differ between DS and DR rats and are not enhanced by high dietary NaCl intake, 2) 5 days of high salt diet induces an apparent increase in adrenomedullary responsiveness to mild stress that is independent of DS or DR substrain, and 3) epinephrine of adrenal origin is not essential for the development of salt-sensitive hypertension in the DS rat. (Hypertension 1990;16:282-289)
Based on these findings, it has been suggested that a primary defect in the DS rat may be the inability to inhibit adrenal catecholamine synthesis under conditions of high dietary salt intake.14

The purpose of the present study was to evaluate the hypotheses that DS rats on a high salt diet exhibit an augmented adrenomedullary responsiveness to an acute stress and that adrenomedullary hyperresponsiveness contributes to the development of salt-sensitive hypertension in the DS rat. Because a high salt intake for 5 days has different effects on neural activity in DS and DR rats, we proposed that adrenal medullary and sympathetic neural hyperreactivity would be evident within that time frame. Specifically, we proposed that plasma epinephrine responses to acute mild footshock stress would be exaggerated in DS rats maintained on a high salt intake for 5 days. We also hypothesized that acute pressor responses to stress would be enhanced. We chose a graded electrical footshock paradigm as a reproducible method for creating a range of stress responses. Finally, we proposed that chronic blockade of adrenal epinephrine synthesis would prevent or attenuate the development of salt-sensitive hypertension in the DS rat. To test this latter hypothesis, DS rats were treated with an inhibitor of adrenal phenylethanolamine N-methyltransferase (PNMT), SK&F 29,661, to block the adrenal synthesis of epinephrine, and the time course of the development of salt-sensitive hypertension was examined. The results reported herein do not support our initial hypotheses.

Methods

Series I: Responses to Acute Stress in Dahl Rats

Animal preparation. Male DS and DR rats 3–4 weeks of age were purchased either from Brookhaven National Laboratories, Upton, N.Y., or from Harlan Sprague Dawley, Inc., Indianapolis, Ind. Rats were housed individually in the vivarium, with a 12-hour light/dark cycle, and food and water were provided ad libitum. Rats in series I were placed on a “normal” diet containing 1% NaCl (Purina Test Diets, Richmond, Ind.), and body weights were recorded three times a week.

When the rats had attained a body weight of 200–230 g (7–8 weeks of age), surgery was performed under methohexitol anesthesia (50 mg/kg i.p.) to implant a catheter (5 cm of Tygon 0.015 in. i.d. by 0.025 in. by 0.040 in.) in the right femoral artery. The catheter was tunneled subcutaneously, exiting at the back of the neck. Each rat was fitted with an ultrasuede vest that was attached to a stainless steel spring for protection of the catheter. The catheter was filled with a saline solution containing heparin (1,000 units/ml) and urokinase (100 units/ml) and was obturated.

Rats were placed in individual home cages in the laboratory after surgery where they remained for the duration of the study. A 12-hour light/dark cycle was provided. The cage consisted of an 8×11 in. Plexiglas and stainless steel footshock chamber (model 80000, Lafayette Instrument Co., Lafayette, Ind.) modified to provide food and water ad libitum. The catheter exited the top of the cage so that blood pressure could be recorded and blood samples could be drawn without disturbing the animals. Baseline mean arterial pressure and heart rate were recorded on the morning of the second day after surgery. Subsequently, half of the rats of each substrain (DS and DR) were placed on a high salt diet (7% NaCl) created by adding salt to the normal diet. The other half of the rats remained on the normal salt diet. After 5 days of either normal or high salt diet, an acute footshock experiment was performed with each rat as described below.

Acute footshock protocol. Basal blood pressure and heart rate were recorded from the unrestrained rat in its home cage, and a control blood sample (0.5 cc) was drawn. This and subsequent blood samples were replaced with an equal volume of 5% bovine serum albumin in saline. Each rat was then exposed to three consecutive 5-minute periods of electrical footshock at currents of 0.08, 0.15, and 0.30 mA (0.5 second duration, every 6 seconds). The levels of footshock intensity were chosen in preliminary experiments in which we observed the rats’ behavioral responses to these and other shock intensities. Shock level 1 (0.08 mA) was considered to be the minimum detectable intensity; the rats generally responded with no more than increased attentiveness. At level 2 (0.15 mA) the rats exhibited increased movement and occasional grooming behavior but no apparent distress. Level 3 (0.30 mA) was the minimum stimulus that would produce overt escape movements with each shock.

A blood sample (0.5 cc) was drawn at 4 minutes of each of the three 5-minute shock periods, and the shock was turned off during the last 30 seconds of each period so that blood pressure and heart rate could be recorded in the absence of shock-induced motor activity. Blood pressure and heart rate were monitored for an additional 10 minutes after termination of the shock protocol.

Blood samples from all experiments were transferred to chilled tubes containing EGTA and glutathione, and the plasma from centrifuged samples was stored at −70°C for later determination of catecholamine concentrations.

Restraint stress protocol. To evaluate the responses to a different type of acute stress, approximately half of the rats of each substrain were subjected to a restraint stress protocol on day 7 of high or normal salt diet (2 days after the acute footshock experiments). A basal blood sample was taken and blood pressure was recorded while the rat was resting quietly in its home cage. The rat was then transferred to a plastic rat restrainer typical of the type used for indirect measurement of blood pressure, which does not allow freedom of movement. Mean arterial blood
pressure was recorded, and a second blood sample was drawn after 4 minutes of restraint.

Series II: Effects of PNMT Inhibition on Development of Salt-Sensitive Hypertension in Dahl Salt-Sensitive Rats

Young male DS rats weighing 93 ± 2 g were purchased from Harlan Sprague Dawley and were housed in the vivarium. Rats in this series were placed on a "normal" salt diet containing 0.45% NaCl (Teklad, Madison, Wis.) on arrival. Starting the day after arrival and continuing throughout the study, half of the rats were treated daily by oral gavage with an inhibitor of adrenal PNMT (SK&F 29,661) at daily doses ranging from 1 to 2 mg/kg body wt. The drug was delivered as a single dose in distilled water vehicle (1 ml/kg); control rats were treated with the distilled water vehicle only. When the rats reached a body weight of 200–240 g and after 16–21 days of drug treatment, a femoral arterial catheter was implanted as described in series I. The rats were housed in the laboratory after surgery so that blood pressure could be measured daily via the indwelling catheter without disturbing the animal. After at least 3 days of daily basal blood pressure measurement while the rats were maintained on the normal salt diet, the dietary salt content was increased to 7% NaCl, and daily blood pressure measurements were continued for an additional 3 weeks.

Preliminary experiments revealed that the basal plasma epinephrine concentrations in three SK&F-treated rats were below the detectability limit of the assay (less than 7 pg/ml). To assess quantitatively the degree of blockade of adrenal epinephrine synthesis and release at the time of initiation of the high salt diet, animals in series II were exposed to a 4-minute period of electrical footshock (0.3 mA, 0.5 second duration every 6 seconds) on the final day of the 3-week high salt period, the rats were killed by ether anesthesia and decapitation, and the left adrenal gland was removed and homogenized in 5 ml of 1N HCl containing 5 mg/ml EDTA. After centrifugation, the supernatant was stored at −20°C for determination of tissue catecholamine content.

Analytical and Statistical Methods

Plasma epinephrine and norepinephrine concentrations were assayed by a commercially available radioenzymatic method (Amersham Corp., Arlington Heights, Ill.). A minimum detectable dose was determined for each catecholamine in each assay as the dose equivalent to a counting rate that was more than 2 SDs from that of a blank performed in quadruplicate. The mean minimum detectable doses of 21 separate assays were less than 0.30 pg for epinephrine and less than 0.35 pg for norepinephrine, corresponding to sensitivities of 6 and 7 pg/ml, respectively. The interassay coefficients of variation of a pool of rat plasma were 8% and 11% for epinephrine and norepinephrine, respectively. Adrenal tissue catecholamine contents in the rats of series II were analyzed by reverse-phase, high-performance liquid chromatography with electrochemical detection (Bioanalytical Systems, Inc., West Lafayette, Ind.), using 3,4-dihydroxybenzylamine as an internal standard. Adrenal tissue epinephrine contents are presented as micrograms of epinephrine per adrenal pair, assuming equal epinephrine contents of right and left adrenal glands.

Data were analyzed using a multivariate analysis of variance (ANOVA) program (Statsoft, Inc., Tulsa, Okla.). Data from the four strain/diet groups in series I were compared using a 2×2 factorial design (substrain and diet) with repeated measures on a third factor (time). Because the variances increased with increasing mean values for epinephrine and norepinephrine, catecholamine values were log/converted before statistical analysis. Changes with time within a group were determined by one-way ANOVA for repeated measures. In series II, blood pressure values for 3 consecutive days were averaged to reduce the daily variations while preserving the long-term trend. These data were also compared by multivariate ANOVA with repeated measures. Student's t test was used when only a single comparison was required.

Results

Series I

Table 1 shows resting mean arterial blood pressure before and after the 5-day dietary regimen, and the pressor responses to mild, graded footshock in the four strain/diet groups. Resting mean arterial blood pressure at the time of initiation of the high salt diet (day 0) was significantly higher in DS rats than in DR rats (115 ± 2 versus 100 ± 2 mm Hg, respectively; p < 0.001) even though the rats had been maintained on a 1% NaCl diet since weaning. Body weights of the DS and DR rats at the time of surgery were similar (211 ± 2 versus 212 ± 2 g, respectively), and there were no differences in weight gain between DS and DR rats on either dietary regimen. However, the normal salt rats of both substrains gained slightly more weight over the 5-day period than the high salt rats (normal salt, +16 ± 2 g; high salt, +5 ± 3 g). Resting blood pressure increased over the 5-day dietary treatment period only in the DS rats on the high salt regimen (+13 ± 3 mm Hg; p < 0.01), remaining unchanged in the DS normal salt and the DR high salt rats and declining slightly but significantly in the DR normal salt group. Because of the variations in the control blood pressures, pressor responses to footshock are presented as changes from the resting mean arterial blood pressure (day 5). Pressor responses increased gradually with each increment in stimulus intensity, declining quickly after termination of the last stimulus. The pressor response of DS rats on a high salt diet did not differ from that of any other group.

Resting heart rates in the four strain/diet groups, and the increments in heart rates during and after footshock, are shown in Table 2. Resting heart rates were significantly lower in DS rats on a high salt
TABLE 2. Resting Heart Rates in Conscious Dahl Rats and Changes in Heart Rate During and After Graded Electrical Footshock

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Resting HR (beats/min) 0 min</th>
<th>Change from resting HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60±16*</td>
<td>0.08</td>
</tr>
<tr>
<td>DR-high salt</td>
<td>9</td>
<td>418±9</td>
<td>16±10</td>
</tr>
<tr>
<td>DR-normal salt</td>
<td>8</td>
<td>436±10</td>
<td>16±5*</td>
</tr>
<tr>
<td>DS-normal salt</td>
<td>9</td>
<td>408±11</td>
<td>21±12</td>
</tr>
<tr>
<td>DS-high salt</td>
<td>9</td>
<td>443±9</td>
<td>6±7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, number of rats; DS, Dahl salt-sensitive rats; DR, Dahl salt-resistant rats.

*p<0.05; tp<0.001; tp<0.01 compared with the resting (0 time) value.
responded to restraint with a 50% greater increment in epinephrine than those maintained on a normal salt diet, whereas restraint-induced increments in plasma norepinephrine concentrations were not significantly different. Pressor responses to restraint stress (not shown) also were not different between the normal salt and the high salt groups, similar to the findings with footshock stress.

Series II

Figure 2 shows arterial blood pressure before and during the first 3 weeks of a high salt diet in DS rats receiving daily oral doses of either SK&F 29,661 or vehicle. There were no differences in blood pressure between the treated and untreated rats at any time. Because all rats were maintained on a normal salt diet during the 16–21 days of drug or vehicle treatment before initiation of the high salt diet, the data suggest that SK&F 29,661 does not alter blood pressure under normal salt conditions as well. Therefore, separate normal salt control groups of SK&F-treated and vehicle-treated DS rats were not considered necessary.

Basal plasma epinephrine concentrations were below the detectability limit of the assay in the SK&F-treated group. Plasma epinephrine concentrations during footshock were 405±100 pg/ml in vehicle-treated rats and 91±16 pg/ml in the rats receiving SK&F 29,661. Despite this significant attenuation of the stress-induced plasma epinephrine concentration, neither the acute pressor response nor the plasma norepinephrine concentrations were different between vehicle-treated and SK&F-treated rats during footshock. Mean arterial blood pressure increased 21±2 and 26±3 mm Hg in the vehicle-treated and the SK&F-treated rats, respectively, during footshock, and plasma norepinephrine concentrations were also similar (476±82 and 408±107 pg/ml, respectively).

Analysis of the adrenal glands at the time the rats were killed revealed that SK&F 29,661 treatment reduced adrenal gland epinephrine content by approximately 80% and caused a modest adrenal hypertrophy. Adrenal epinephrine contents were 31.0±1.2 and 6.0±0.6 µg/adrenal pair in vehicle-treated and SK&F-treated rats, respectively, and total adrenal masses were

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma epinephrine (pg/ml)</th>
<th>Plasma norepinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrestrained</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal salt</td>
<td>35±10</td>
<td>259±33</td>
</tr>
<tr>
<td>High salt</td>
<td>45±15</td>
<td>873±80</td>
</tr>
<tr>
<td>Restricted</td>
<td>154±20</td>
<td>814±61</td>
</tr>
<tr>
<td>High salt</td>
<td>225±26*</td>
<td>1,065±116</td>
</tr>
<tr>
<td></td>
<td>119±20</td>
<td>811±97</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, number of rats; DS, Dahl salt-sensitive rats; DR, Dahl salt-resistant rats.

*p<0.05 compared with the normal salt group.
Mean phenylethanolamine N-methyltransferase were not different among the four substrate/diet groups. Second, circulating concentrations of catecholamines in the basal, resting state were not different between DS and DR rats on a normal salt intake. Finally, plasma catecholamine responses to acute stress in our experiments may simply be an indication that the adrenal pool of releasable epinephrine is greater under conditions of salt loading. That high dietary sodium intake can potentiate the sympathetic nervous system and produce enhanced catecholamine release during stress has been demonstrated previously.\(^{15,16}\) The conditions under which this occurs and whether there are any differences between the genetic strains of salt-sensitive rats and their salt-resistant controls remains controversial. We were unable to show any differences between the stress responses of DS and DR rats on a normal salt diet and after 5 days of a high salt intake at a time when blood pressure is just beginning to increase in the DS rat. In contrast, McCarty and Saavedra\(^{19}\) reported that hypertensive DS rats that have been maintained on a high salt intake from weaning until 10 weeks of age show a greater increment in plasma norepinephrine concentration after mild stress than do the nonhypertensive, low salt DS rats. Furthermore, the same laboratory\(^{16}\) reported that the inbred strain of salt-sensitive rat (the SS/Jr rat) responds to mild stress with an increase in both norepinephrine and epinephrine, whereas the corresponding control SR/Jr rat does not. The SS/Jr rats became hypertensive on a normal salt intake; therefore, the increased responsiveness to stress in these animals could be secondary to the hypertension rather than a primary event.

Another possibility for the differences between laboratories may be related to the conditions of the experiments, as plasma catecholamine concentrations are extremely sensitive to stress. Walker et al\(^{20}\) reported that cardiovascular and renal hemodynamics remain altered in conscious rats after recovery from surgery and suggested that sympathetic outflow may be enhanced during the recovery period. The experiments discussed above\(^{15,16}\) were performed on the second day after surgical preparation; basal epinephrine concentrations reported in their animals are approximately 10-fold higher than the basal concentrations in our experiments. Our experiments were performed 7 days after surgery, when the animals had gained weight (+12±3 g) compared with the presurgical value. In other nonhypertensive strains of rats, plasma epinephrine concentrations in conscious, unstressed rats have been reported as 295±43 pg/ml on the first day after surgery\(^{18}\) and 20–30 pg/ml on the seventh day.\(^{19,20}\)

The observation that DS rats exhibit greater stress-induced increments in heart rate than DR rats confirms similar findings by Friedman et al.\(^{21}\)
The mechanism underlying this effect is unclear. Although Friedman et al.21 hypothesize that the differences in heart rate might be due to greater sympatho-adrenomedullary reactivity in DS than in DR rats, our data suggest that this is not the case as plasma epinephrine responses did not differ between DS and DR rats on either diet. Several groups of investigators have reported that baroreceptor reflex control of heart rate is impaired in DS rats compared with DR rats.22-25 Thus, it seems plausible that, given equivalent acute pressor responses to stress, baroreceptor-mediated suppression of stress-induced increments of heart rate would be less effective in DS rats than in DR rats.

If epinephrine facilitates norepinephrine release in this model, then one might anticipate that facilitated norepinephrine release would be observed during high salt intake, in conjunction with the higher plasma concentrations of epinephrine. Examination of Table 3 reveals that this is the case only at the highest level of stimulation, and only in the DR rats (1,043±97 versus 711±72 pg/ml; p<0.05). It is possible that inhibitory countermechanisms are present in the DS rats on the high salt diet that are not present in the DR rats under the same dietary conditions. Indeed, Tam et al.26 have demonstrated that the number of renal α2-adrenergic receptor binding sites increases by 600% in DS rats ingesting a high salt diet but remains unchanged in DR rats under the same conditions. Whether differences in prejunctival α2 inhibitory activity can account for the observed differences in norepinephrine between the two groups remains speculative at this time.

To further evaluate the role of epinephrine in the development of salt-sensitive hypertension in the DS rat, additional experiments were conducted in which adrenal epinephrine synthesis was blocked with an inhibitor of adrenal PNMT before and during administration of a high salt diet. SK&F 29,661 has been shown to be an effective peripherally acting PNMT inhibitor that can be administered orally without apparent toxic effects.27,28 In the present experiments SK&F 29,661 produced inhibition of adrenal epinephrine synthesis, as judged by approximately 80% reductions of both adrenal epinephrine content and stress-induced increments in plasma epinephrine concentration. Nevertheless, SK&F 29,661 treatment had no effect on resting blood pressure while the rats were maintained on a normal dietary salt intake, did not alter acute stress-induced increments in blood pressure, and did not affect the time course of the development of salt-sensitive hypertension. Together, the data do not support the hypothesis that circulating epinephrine plays an important role in the development of salt-sensitive hypertension in the DS rat. It should be noted, however, that SK&F 29,661 is a peripherally acting PNMT inhibitor that does not cross the blood-brain barrier; thus, these data do not exclude a role for central epinephrine in the initiation of hypertension in this model.

Iwai et al.29 demonstrated some years ago that adrenalectomized DS rats fail to develop hypertension when fed a high salt diet. Though their data do not necessarily imply that abnormal adrenal function causes hypertension in these animals, they do imply that intact adrenals are necessary for salt-induced hypertension to develop. Our data suggest that the necessary component of adrenal function is not the secretion of epinephrine but point instead to the possible importance of adrenal cortical steroids. Although DS rats exhibit altered patterns of adrenal steroid synthesis,30-32 the role of adrenal steroids in the development of hypertension in the DS rat remains uncertain.

In summary, basal plasma catecholamine concentrations were not different between conscious DS and DR rats and were unaffected by 5 days of high dietary salt intake. High dietary salt intake did not alter either acute pressor responses or plasma norepinephrine responses to footshock stress in either DS or DR rats. High dietary salt intake increased plasma epinephrine responsiveness to footshock stress and to restraint stress in both DS and DR rats. Finally, chronic blockade of adrenal epinephrine synthesis with an inhibitor of adrenal PNMT failed to alter the time course of salt-induced increases in blood pressure in DS rats. We conclude that epinephrine of adrenal origin is not essential for the development of salt-induced hypertension in the DS rat. Whether epinephrine contributes to the development of other models of genetic or induced hypertension remains to be determined.

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