Failure of Salt Loading to Inhibit Tissue Norepinephrine Turnover in Prehypertensive Dahl Salt-Sensitive Rats


SUMMARY To determine if alterations of electrolyte balance or sympathetic nervous system activity are present in Dahl salt-sensitive rats (DS) before the onset of hypertension, we compared electrolyte balances, extracellular fluid volume (inulin space), plasma volume (radiolabeled albumin), and norepinephrine turnover in peripheral tissues (heart and interscapular brown fat) in prehypertensive DS and Dahl salt-resistant rats (DR). Animals were maintained for 5 to 7 days on either a "normal" or high NaCl diet. Tissue norepinephrine turnover was evaluated by measuring the rate at which norepinephrine content decreased following tyrosine hydroxylase inhibition with α-methyl-p-tyrosine. Blood pressure was higher (p < 0.05) in DS (135 ± 2 [SE] mm Hg) than in DR (129 ± 2 mm Hg) and was not affected by the diets. Extracellular fluid volume and net Na+ and Cl− balances did not differ between DS and DR. However, plasma volume was greater in DS than in DR (p < 0.05). In both fat and heart, norepinephrine turnover was decreased by dietary NaCl loading in DR (p < 0.01), but not in DS. Thus, the tendency of the DS to become hypertensive with high NaCl intake may be related to the combined effects of an increased plasma volume and the failure of high dietary NaCl to inhibit peripheral sympathetic nervous system activity. (Hypertension 12: 568–573, 1988)

KEY WORDS • salt-sensitive hypertension • catecholamine turnover • plasma volume • sympathetic activity • Dahl rats

Dahl et al. established by selective breeding two lines of Sprague-Dawley rats characterized by different responses to a high salt diet.1, 2 The blood pressure of the Dahl salt-sensitive rat (DS) rises with increased NaCl intake, whereas that of the Dahl salt-resistant rat (DR) does not. DS excrete sodium less efficiently than do DR according to some3 but not all4 investigators, and evidence for an expansion of the extracellular fluid volume in the pathogenesis of hypertension in this salt-sensitive model is conflicting.5, 6

Alternatively, there is increasing evidence for the participation of the nervous system in the pathogenesis of salt-sensitive hypertension. Lesions of the paraventricular nuclei or the anteroventral third ventricle region protect against the development of hypertension in the DS and other models of salt-sensitive hypertension7, 8; chemical sympathectomy with guanethidine or 6-hydroxydopamine also prevents hypertension in DS fed a high NaCl diet.9, 10 Sympathetic nervous system activity was increased in several models of salt-sensitive hypertension that were studied after the development of hypertension.7, 9, 11–13 In the DS maintained on a low NaCl diet (and hence minimal blood pressure elevation), baroreceptor reflex control of heart rate and vascular resistance, measured in response to acute elevations of arterial pressure, is impaired, and it has been suggested that this contributes to the animal's tendency to become hypertensive.14–17 Cardiopulmonary baroreceptor reflex activity is also reportedly impaired in anesthetized, prehypertensive DS, as evidenced by a reduced capacity to inhibit efferent sympathetic nerve activity in response to volume expansion.18 Impaired baroreceptor reflex function in the DS has been attributed to a genetic abnormality in the peripheral afferent limb of the baroreceptor reflex arc.16, 19

The objective of this study was to determine if hypertension in the DS is related to an effect of
Dietary NaCl and Norepinephrine Turnover in Dahl Rats/Genain et al. 569
dietary NaCl on extracellular fluid volumes or peripheral sympathetic nervous system activity (or both). Animals were maintained on a high NaCl intake for a relatively short time to evaluate the effects of this intervention before the onset of hypertension. Specifically, we compared the effects of 5 days of dietary NaCl loading on extracellular fluid volume, plasma volume, and norepinephrine turnover in two tissues (interscapular brown fat and heart) in DR and prehypertensive DS.

Materials and Methods

Weanling male DR and DS were obtained from Brookhaven National Laboratories (Upton, NY, USA) and housed in individual metabolic cages to allow daily urinary collections for measurement of electrolyte excretion. Beginning at 9 weeks of age, rats received calibrated diets for 5 days before the experiments. "Normal" and high NaCl diets were prepared by adding 1% NaCl and 7% NaCl, respectively, to low NaCl chow (ICN, Cleveland, OH, USA). Blood pressure was measured every other day by tail plethysmography. During the 5 days on these diets, Na+ and Cl- balances were computed by subtracting urinary excretion from dietary intake. Na+ and Cl- concentrations were measured with a flame photometer.

At the completion of the balance study, norepinephrine turnover in peripheral tissues was measured by a nonisotopic method.20 The inhibitor of catecholamine biosynthesis, α-methyl-p-tyrosine methyl ester hydrochloride (Aldrich Chemical, Milwaukee, WI, USA), was administered intraperitoneally, and the rate of tissue norepinephrine depletion was evaluated. Each diet-strain group was divided into four subgroups, and one received only the saline vehicle. The other three groups were killed 6, 11, and 16 hours after receiving α-methyl-p-tyrosine. The α-Methyl-p-tyrosine methyl ester was dissolved in saline at a concentration of 200 mg/ml, and each rat received intraperitoneal injections of 0.33 ml of either saline adjusted to pH 4.0 with HCl or the α-methyl-p-tyrosine methyl ester solution (250 mg/kg). In addition, animals killed at 11 and 16 hours received a booster dose of α-methyl-p-tyrosine (125 mg/kg) 4 hours before death to assure that formation of norepinephrine was blocked during the entire period of study. Rats were killed by decapitation, and the hearts and samples of interscapular brown fat were quickly excised and frozen on dry ice. Samples were immediately homogenized by sonication in 1 N HCl containing 15 mM Na2EDTA, centrifuged for 45 minutes at 20,000 rpm, and supernatants were frozen at -70 °C until analysis. Tissue norepinephrine concentration was measured after separation on a high performance liquid chromatographic reverse-phase column (UltraspHERE; octadecylsilane, 4.6 mm; inside diameter, 25 cm; Altex, Berkeley, CA, USA) by electrochemical detection.21 Typically, 25 to 100-μl aliquots of samples were analyzed. Norepinephrine turnover was evaluated by linear regression analysis of the decline of the log norepinephrine tissue concentration over time after tyrosine hydroxylase inhibition.21 The rate constant of amine loss (k) was taken as the slope of log norepinephrine concentration versus time multiplied by 1/0.434.

In separate groups of identically treated animals, plasma volume and extracellular fluid volume (inulin space) were measured after 5 days of either normal or high NaCl intake. To avoid the potential difficulty of accurately quantitating a large amount of inulin in a small volume of urine collected in a short time, the inulin space was determined in nephrectomized rats. Animals were anesthetized with 100 mg/kg Inactin. The kidneys were rapidly removed through bilateral flank incisions, and care was taken to avoid blood loss. The femoral artery and jugular vein were catheterized with PE 50 tubing. A Hamilton syringe was filled with 4% inulin solution and weighed (weight was calculated to three decimal places). A 500-μl bolus was given in the venous catheter, which was then flushed with 250 μl of saline. The syringe was then weighed again to determine the exact amount of inulin solution given. An aliquot of the infusion solution was analyzed in triplicate to determine the exact concentration of the solution given. At 60 and 90 minutes after injection, 250-μl blood samples were taken to ensure that the final inulin concentration had reached equilibrium with the extracellular space. Extracellular fluid volume was determined from dividing the total amount of inulin injected by the equilibrated plasma inulin concentration. Inulin concentration was measured by the anthrone technique.22 Subsequently, in these same animals, 0.2 ml of radioiodinated serum albumin was injected to measure plasma volume. After a 10-minute equilibration period, three 0.2-ml blood samples were drawn, and the mean number of counts was used to compute plasma volume; adjustment was made for plasma trapping.23

Statistical significance was determined with analysis of variance, and specific comparisons were made using linear contrasts.24 Results in DS and DR were compared for each NaCl intake, and 1% and 7% NaCl intakes were compared for each strain. Values listed are means ± SE, and differences were considered significant for p values below 0.05.

Results

Blood pressures were consistently higher (p < 0.01) in DS than in DR; however, no change of blood pressure was observed in DS maintained for 5 days on high NaCl (Table 1). Comparing DS and DR, net Na+, Cl−, and K+ balances did not differ in rats fed normal or high NaCl diets. The increases in Na+ and Cl− balances and the decreases in K+ balance were comparable in DR and in DS fed the high NaCl diet. Extracellular fluid volume was not affected by diet and did not differ significantly between DS and DR. In contrast, in rats on either...
Table 1. Arterial Pressure, Net Electrolyte Balances, Extracellular Fluid Volume, and Plasma Volume in Prehypertensive DS and DR Fed Normal or High NaCl

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal NaCl</th>
<th>High NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS (n=14)</td>
<td>DR (n=18)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>134.0±2</td>
<td>128.0±1*</td>
</tr>
<tr>
<td>Na+ balance</td>
<td>15.8±1.7</td>
<td>14.1±0.9</td>
</tr>
<tr>
<td>Cl− balance</td>
<td>13.7±0.9</td>
<td>12.8±0.8</td>
</tr>
<tr>
<td>K+ balance</td>
<td>21.8±1.8</td>
<td>21.9±1.1</td>
</tr>
<tr>
<td>ECFV (%)</td>
<td>17.7±1.4</td>
<td>16.2±0.9</td>
</tr>
<tr>
<td>Plasma volume</td>
<td>3.5±0.1</td>
<td>3.1±0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. SBP = systolic blood pressure; ECFV = extracellular fluid volume.

*p < 0.01, †p < 0.05, compared with DS fed the same diet.

In the present study, animals were fed 7% NaCl for 5 days, and blood pressure did not change during this time. Thus, we were able to evaluate the effect of high NaCl intake on electrolyte balance, volume, and neural activity in the absence of hypertension. As reported by others,9-26-27 in these 9-week-old animals the blood pressure of the DS was slightly higher than that of the DR. This blood pressure elevation may be attributed to the fact that animals were fed 1% NaCl chow from the time of weaning, and this is a relatively high NaCl intake for the rat.28 Dahl et al.29 have previously reported that blood pressures are higher in DS fed 1% NaCl than in animals fed 0.4% NaCl. However, we have observed (unpublished observation) that DS and DR fed 0.4% NaCl gain weight at a slower rate than animals fed 1% NaCl. Consequently, we did not study animals receiving lower NaCl intakes.

During the 5 days of this balance study, net Na+ and Cl− balances did not differ in DS and DR. Inulin space tended to be higher in DS than in DR, although this difference was not statistically significant. However, as with blood pressure, the plasma volume of DS was higher than that of DR fed 1% NaCl; plasma volume was not affected by 5 days of 7% NaCl intake. Other investigators have also reported that net Na+ balances do not differ in DR and prehypertensive DS,28 and Roman and Osborn30 observed a transient elevation of extracellular fluid volume in DS after 1 but not 3 days of high NaCl intake. Our results in prehypertensive animals are

In our experience, DS fed 7% NaCl become hypertensive within 2 to 3 weeks, but not sooner.25

In the experience, DS fed 7% NaCl become hypertensive within 2 to 3 weeks, but not sooner.25 In groups of animals not treated with α-methyl-p-tyrosine, tissue contents of norepinephrine in interscapular brown fat did not differ among DS or DR on normal or high NaCl intake (Table 2). In heart tissue, norepinephrine content was higher (p < 0.01) in DR on normal NaCl intake than in DR on high NaCl intake or in DS on either normal or high NaCl intake; heart norepinephrine content did not differ among the latter three groups.

In rats treated with α-methyl-p-tyrosine, tissue norepinephrine concentrations decreased over time in an exponential fashion in all diet-strain groups. In interscapular brown fat, the rate constant of norepinephrine loss (k) was less (p < 0.05) in DR (0.047 ± 0.012) than in DS (0.081 ± 0.012) fed a normal NaCl diet (Figure 1, see Table 2). Furthermore, the high NaCl intake resulted in a significant reduction (p < 0.01) of norepinephrine efflux in DR but not in DS. In heart tissue, in contrast with the results in fat, on normal NaCl intake the rate constant of norepinephrine efflux in DR (0.066 ± 0.008) was greater (p < 0.01) than in DS (0.027 ± 0.008) (Figure 2, see Table 2). However, as in fat, the rate of norepinephrine efflux was decreased (p < 0.0001) by high dietary NaCl in DR but not in DS.

Discussion

In our experience, DS fed 7% NaCl become hypertensive within 2 to 3 weeks, but not sooner.25

Table 2. Tissue Norepinephrine Content and Rate Constant of Norepinephrine Loss in Prehypertensive DS and DR Fed Normal or High NaCl

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal NaCl</th>
<th>High NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS</td>
<td>DR</td>
</tr>
<tr>
<td>Interscapular brown fat</td>
<td>1.66±0.18</td>
<td>1.41±0.09</td>
</tr>
<tr>
<td>k (hr⁻¹)</td>
<td>0.001±0.012*</td>
<td>0.047±0.012</td>
</tr>
<tr>
<td>Heart</td>
<td>0.59±0.03*</td>
<td>0.93±0.09</td>
</tr>
<tr>
<td>k (hr⁻¹)</td>
<td>0.027±0.008†</td>
<td>0.066±0.008</td>
</tr>
</tbody>
</table>

Values are means ± SE. NE = norepinephrine; k = rate constant of NE efflux.

*p < 0.05, †p < 0.01, compared with DR fed the same diet.

*p < 0.01, compared with normal NaCl diet in the same strain.
DIETARY NaCl AND NOREPINEPHRINE TURNOVER IN DAHL RATS/Genain et al. 571

FIGURE 1. Mean norepinephrine concentrations in interscapular brown fat of DR and prehypertensive DS that were either untreated (Time 0) or killed 6, 11, or 16 hours after being treated with α-methyl-p-tyrosine. Solid line depicts rats fed normal NaCl, and broken line depicts rats fed high NaCl.

FIGURE 2. Mean norepinephrine concentrations in heart tissue of DR and prehypertensive DS that were either untreated (Time 0) or killed 6, 11, or 16 hours after being treated with α-methyl-p-tyrosine. Solid line depicts rats fed normal NaCl, and broken line depicts rats fed high NaCl.

similar to those of Overbeck et al.,5 who reported that plasma volume, but not extracellular fluid volume, is expanded in hypertensive DS fed a high NaCl diet. Conceivably, an expanded plasma volume in the absence of a net increase of sodium balance may be related to a redistribution of fluid compartments resulting from alterations of vascular or interstitial compliance. Alternatively, measurement of net electrolyte balance may not be sufficiently sensitive to detect the relatively small differences that would account for the 20% increase of plasma volume that we observed in DS.

Sympathetic nervous system activity was assessed by the measurement of tissue norepinephrine turnover.21-31 Norepinephrine synthesis is proportional to sympathetic neuronal activity in a given tissue.21-33 Various techniques have been proposed to quantitate norepinephrine turnover in tissues based on the accumulation of L-dopa after inhibition of aromatic L-amino-acid decarboxylase,21 the decline of [3H]norepinephrine after its intravenous injection, or the measurement of the rate of decline of norepinephrine content after tyrosine hydroxylase inhibition,20-30 which was the approach used in the present study. None of these methods actually measures norepinephrine synthesis rate, and they each provide different absolute levels for norepinephrine turnover; however, they give comparable indices of changes in catecholamine turnover.21-33 In contrast to measurement of norepinephrine turnover, tissue catecholamine content does not reflect neuronal activity to that tissue, and, consistent with previous reports, the present study confirms that tissue catecholamine content does not necessarily reflect turnover rate.

In rats fed a normal NaCl diet, norepinephrine turnover rate was greater in DS than in DR interscapular brown fat, whereas the converse was true in heart tissue. We have no obvious explanation for this apparently discrepant result in these two tissues. In heart tissue, the steeper slope in DR may be related to the higher basal norepinephrine content; however, we cannot exclude the possibility that the lower turnover in DS is related to plasma volume expansion. Nevertheless, in both tissues, norepinephrine turnover rates were decreased by high dietary NaCl intake in DR, but not in prehypertensive DS. These results suggest that a high NaCl intake inhibits sympathetic outflow in the DR but not in the prehypertensive DS.

Consistent with this observation, Saavedra et al.27 have previously reported that dietary NaCl loading results in decreased activity of two catecholamine-forming enzymes (tyrosine hydroxylase and dopamine β-hydroxylase) in the adrenal glands of DR but not of hypertensive DS. High salt diets have also been reported to enhance afferent discharge of aortic baroreceptors and to augment sympathoinhibitory responses to volume expansion (cardiopulmonary baroreceptors) in DR but not in DS.35-37 High dietary NaCl also reduces peripheral sympathetic function in the normotensive Wistar rat.38 Taken together, these observations are consistent with the hypothesis that protection against the development of hypertension in DR fed high NaCl is related to inhibition of sympathetic nervous system activity.
In contrast, in the DS dietary NaCl loading has been reported to potentiate the increment of vascular resistance in response to sympathetic nerve stimulation and electrical stimulation of the ventromedial hypothalamus, to increase the rate of basal neural firing of the splanchic nerve, and to exacerbate impairment of baroreceptor reflex control of heart rate. Augmentation of cardiac norepinephrine turnover rate by NaCl loading, after the onset of hypertension, has also been reported in other models of salt-dependent hypertension. Thus, the predisposition of the DS to become hypertensive may be related to the combination of an expanded plasma volume and an inability of dietary NaCl to inhibit sympathetic nervous system activity. Salt sensitivity in hypertensive humans has also been attributed to the failure of high salt intake to suppress plasma norepinephrine concentration or other indices of sympathetic activity. This study does not address the mechanism by which high NaCl intake inhibits sympathetic outflow in the DR. Conceivably, the sensor may be a subtle increase of right atrial pressure that occurs in response to dietary NaCl loading in the rat. Alternatively, neural responses to different salt intakes may be related to a central effect of dietary NaCl.

In summary, in animals maintained on a normal (normal) NaCl intake, both blood pressure and plasma volume were higher in DS than in DR. Neither blood pressure nor plasma volume was affected by intake of 7% NaCl for 5 days. However, in two separate tissues (interscapular brown fat and heart), the rate of NE turnover was decreased after 5 days of 7% NaCl for 5 days. However, in two separate tissues (interscapular brown fat and heart), the rate of NE turnover was decreased after 5 days of 7% NaCl intake in DR but not in DS. We suggest that the higher plasma volumes and blood pressures seen with normal NaCl intake are related to the fact that 1% NaCl is a high NaCl intake for the rat. In combination with an expanded plasma volume, the failure of the 7% NaCl intake to inhibit sympathetic outflow may account for the subsequent development of hypertension in DS fed a 7% NaCl diet.

Acknowledgment

The authors thank Vickie Zahradnik for secretarial assistance.

References


Failure of salt loading to inhibit tissue norepinephrine turnover in prehypertensive Dahl salt-sensitive rats.
C P Genain, S R Reddy, C E Ott, G R Van Loon and T A Kotchen

_Hypertension_. 1988;12:568-573
doi: 10.1161/01.HYP.12.6.568

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/12/6/568

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at: http://hyper.ahajournals.org/subscriptions/