Exacerbation of Hypertension by High Chloride, Moderate Sodium Diet in the Salt-Sensitive Spontaneously Hypertensive Rat

J. Michael Wyss, Maleewan Liumsiricharoen, Wanida Sripairojthikoon, David Brown, Richard Gist, and Suzanne Oparil

SUMMARY In salt-sensitive spontaneously hypertensive rats (SHR-S) of the Okamoto strain, dietary salt loading causes an exacerbation of hypertension that is associated with a decrease in noradrenergic input to the depressor neurons in the anterior hypothalamus. In the present study, the contribution of chloride to the salt-induced hypertensive response was examined in the SHR-S, in order to test the hypothesis that diets high in chloride but moderate in sodium elevate blood pressure in genetically predisposed subjects. SHR-S were fed diets high in NaCl (1.97% Na⁺, 2.93% Cl⁻; 5% NaCl), high in chloride (2.93%) but moderate in sodium (0.39%) or moderate in NaCl (0.39% Na⁺, 0.61% Cl⁻; 1% NaCl). After 2 weeks, rats on the high (5%) NaCl diet exhibited a significant elevation in blood pressure compared to rats on the moderate (1%) NaCl diet, and this elevation was maintained throughout the next 3 weeks. SHR-S on the high chloride diet were not significantly more hypertensive than 1% NaCl-fed SHR-S during the first 3 weeks, but during the fourth and fifth weeks, SHR-S on the high chloride diet displayed a significant exacerbation of hypertension. The diet-induced elevation in blood pressure in groups fed either the 5% NaCl or high chloride (compared to 1% NaCl) diets was associated with significant decreases in norepinephrine stores in the anterior hypothalamic region, but no other changes in monoamines or monoamine metabolites in this region or in the posterior hypothalamic region. The high chloride diet did not increase blood pressure in normotensive Wistar-Kyoto rats. These data demonstrate that diets high in chloride and moderate in sodium can increase blood pressure in SHR-S and suggest that this increase is related to a reduction in noradrenergic input to sympathoinhibitory neurons in the anterior hypothalamus.

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KEY WORDS • hypertension • hypothalamic catecholamines • salt sensitivity • anterior hypothalamic area • hypothalamus

In 1928, Addison carried out human studies to determine which of the ionic components of NaCl was responsible for the elevation in blood pressure in NaCl-sensitive individuals. He demonstrated that loading hypertensive patients with salts that did not contain NaCl, such as KCl, KBr, or K citrate, results in a fall in blood pressure, while administration of NaCl or NaBr significantly elevates blood pressure. These data were the first experimental evidence that strongly implicated the sodium ion as the crucial factor in salt-dependent hypertension. Subsequent studies have largely supported the primacy of the sodium ion in salt-induced or exacerbated hypertension, but increasing evidence has suggested that the anionic component of NaCl may also contribute to hypertension in several animal models and human hypertensive patients. Chloride and other halide ions by themselves do not appear to induce a rise in blood pressure, but in the deoxycorticosterone acetate (DOCA)–salt and Dahl salt-sensitive rat models, they appear to be necessary for the development of hypertension.

Recently we have demonstrated that diets high in NaCl cause a rapid (within 1 week) rise in blood pressure in 7-week-old Taconic Farms IBU3 spontaneously hypertensive rats of the Okamoto strain (SHR-S). This salt-induced augmentation of hypertension appears to be associated with a decrease in excitatory...
neurotransmitter release onto depressor neurons in the anterior hypothalamus. In the present study, we tested the hypothesis that in the SHR-S, diets high in chloride but moderate in sodium content can elevate blood pressure and that this elevation is associated with a decrease in anterior hypothalamic norepinephrine (NE) content. The results confirm the hypothesis and support the concept that the chloride ion contributes to the pressor effects of dietary NaCl.

Methods

Male SHR-S (IBU3 Colony) and normotensive Wistar-Kyoto rats (WKY) were obtained from Taconic Farms (Germantown, NY, USA) at 5 weeks of age. All rats were maintained four per cage in an environment having constant humidity (60 ± 5%), temperature (24 ± 1°C), and periods of light (0600–1800). One week after arrival, the SHR-S were caged individually, divided into three groups, and placed on one of the diets listed in Table 1. Chloride content was increased by adding 8.0 g of glycine chloride and 1.1 g of choline chloride (Sigma, St. Louis, MO, USA) to 100 g of the 1% NaCl diet (Ralston Purina, St. Louis, MO, USA). The mineral mix was adjusted to make the potassium and calcium content equivalent to that of the other two diets. The 5% NaCl and high chloride diets were reduced in sucrose to accommodate the addition of NaCl or chloride salts. All diets were assessed for sodium and chloride contents after ashing or nitric acid digestion.

Food and water were available ad libitum throughout the study. Twice weekly, between 0600 and 1100, systolic blood pressure and heart rate were measured in conscious, prewarmed, restrained rats by tail plethysmography. The median of five successive measurements was used as the estimate of blood pressure for each animal. Body weight was determined on the same day as the blood pressure measurement. Food and water intake were monitored daily during the 5 weeks of the study.

Five weeks after initiation of the diets, all rats were sacrificed by decapitation without anesthesia (0600–1100), and heart, kidney, and brain were quickly removed and weighed. Anterior hypothalamic region and posterior hypothalamic region were then dissected out. The dimensions of the anterior hypothalamic region were approximately 1 mm (rostrocaudal) by 2 mm (medialateral) by 2 mm (dorsovenral). The anterior hypothalamic dissection included the anterior hypothalamic area and segments of the ventral paraventricular, parviventricular, and retrochiasmatic nuclei. The dimensions of the posterior hypothalamic region dissected were approximately 1 mm (rostrocaudal) by 1.5 mm (medialateral) by 3 mm (dorsovenral). The posterior hypothalamic dissection included the posterior hypothalamic area and segments of the mamillary complex. After dissection, the brain regions were immediately frozen in liquid nitrogen and stored at −80°C.

For analysis of monoamine and monoamine metabolite content, each region was homogenized in 1 ml of 0.1 M acetic acid (pH 5.0) containing ascorbate oxidase and glutathione, and the reaction was terminated by adding 1 ml of 0.1 M perchloric acid containing 0.5 mM glutathione. Ascorbate oxidase reduces solvent-front interactions with NE by significantly lowering the concentration of ascorbic acid, which normally elutes in the solvent front and partially obscures the NE peak. The homogenates were centrifuged, and the resulting supernatants were filtered and assayed for catecholamines and metabolites by high performance liquid chromatography with electrochemical detection.

In a second experiment, WKY were placed on the 1% NaCl diet (n = 10) or the high chloride diet (n = 10) at 6 weeks of age, and blood pressure, heart rate, and food and water intake were measured as described above for the subsequent 5 weeks.

All statistical analyses were based on analysis of variance with appropriate tests post hoc as applicable. Significance was defined as p < 0.05. All animal procedures were in accordance with institutional guidelines.

Results

SHR-S

There were no significant differences in blood pressure among the three groups of SHR-S either immediately prior to (1% NaCl, 145 ± 5.2; 5% NaCl, 144 ± 2.5; high chloride, 145 ± 2.9 mm Hg) or 1 week after initiation of the diets (Figure 1). Two weeks after initiation of the diets, mean systolic pressure of SHR-S on the 5% NaCl diet was significantly elevated above that of SHR-S on either the 1% NaCl or the high chloride diet (see Figure 1). Blood pressure in the 5% NaCl
group continued to rise through Week 3, but displayed no further increase in Weeks 4 or 5. Despite this leveling off, the blood pressure of the 5% NaCl group remained significantly above that of the SHR-S fed 1% NaCl. In contrast, SHR-S on the high chloride diet did not develop significant increases in systolic pressure (compared to the 1% NaCl group) during the initial 3 weeks of feeding. However, during the fourth week, blood pressure rose sharply to levels well above that of SHR-S on the 1% NaCl diet, and also slightly above the blood pressures of SHR-S on the 5% NaCl diet. At week 5, blood pressure in the high chloride group remained significantly higher than that of SHR-S on the 1% NaCl diet.

To determine whether the augmentation of blood pressure seen in SHR-S on the high chloride diet was accompanied by an increase in heart rate, pulse rate was monitored in the three experimental groups. There were no differences between the heart rates of SHR-S on the 1% and 5% NaCl diets. The high chloride diet tended to reduce heart rate, and at Weeks 2 and 5 this reduction was significant ($p < 0.05$). At 4 and 5 weeks, water intake was significantly increased in the 5% NaCl and high chloride groups (compared to the 1% NaCl group). Water intake, urinary volume, and potassium balance were not significantly different between the 5% NaCl and high chloride groups at any time point examined. Furthermore, plasma sodium and chloride concentrations were not significantly different among the three groups following 5 weeks of the diets (sodium/chloride concentration: 1% NaCl group, 143.2 ± 2.4:104.5 ± 4.2; 5% NaCl group, 145.9 ± 2.2:106.3 ± 3.8; high chloride group, 144.8 ± 1.8:112.4 ± 4.5 mEq/L). Serum potassium concentrations and pH were not significantly different among the groups.

Peripheral organ weight to body weight ratios were elevated by the high chloride diet to levels significantly greater than those of the other two groups (see Table 1). The mean heart weight to body weight ratio for the high chloride group was significantly elevated above ratios for the other two groups, but the mean heart weight to body weight ratios of the 1% and 5% NaCl groups were not significantly different from one another. In contrast, the mean kidney weight to body weight ratio was significantly greater in the 5% NaCl group than in the 1% NaCl group, and this measure was elevated further in the high chloride group. The lower mean body weight of the high chloride group contributed to the observed elevations in organ weight to body weight ratios. At the initiation of the study, all groups had similar body weights, but the high chloride group ate very small amounts of food during the initial 3 days of the study, and as a result lost weight (from 120 to 96 g). Weight gain in the three groups was similar over the next 5 weeks (see Table 1).

Monoamine and monoamine metabolite contents of the anterior hypothalamic region of SHR-S on the three diets are shown in Figure 2. The NE content of the anterior hypothalamic region was significantly reduced in both the high chloride and the 5% NaCl groups compared to the 1% NaCl group. In contrast, there were no significant differences among groups in dopamine or serotonin levels or in levels of their metabolites in the anterior hypothalamic region. Furthermore, there were no significant between-group differences in stores of NE, dopamine, serotonin, or their metabolites in the posterior hypothalamic region. Mean NE content of the posterior hypothalamic region was: 1% NaCl group, 1722 ± 169; 5% NaCl group, 1685 ± 170; high chloride group, 1785 ± 158 pg/mg tissue wet weight).

### Table 1. Electrolyte Content of the Three Diets, Body Weights Before and During the Experiment, and Final Organ Weight and Water Content

<table>
<thead>
<tr>
<th>Variables</th>
<th>1% NaCl (n = 12)</th>
<th>5% NaCl (n = 12)</th>
<th>High Cl⁻ (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food electrolyte content by assay (mEq/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.17</td>
<td>0.84</td>
<td>0.17</td>
</tr>
<tr>
<td>K⁺</td>
<td>0.10</td>
<td>0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>0.17</td>
<td>0.82</td>
<td>0.84</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 0</td>
<td>124 ± 3</td>
<td>123 ± 4</td>
<td>120 ± 3</td>
</tr>
<tr>
<td>Week 1</td>
<td>132 ± 3</td>
<td>133 ± 3</td>
<td>96 ± 2*</td>
</tr>
<tr>
<td>Week 3</td>
<td>177 ± 2</td>
<td>173 ± 3</td>
<td>139 ± 3*</td>
</tr>
<tr>
<td>Week 5</td>
<td>205 ± 5</td>
<td>199 ± 6</td>
<td>168 ± 5*</td>
</tr>
<tr>
<td>Heart wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In grams</td>
<td>0.76 ± 0.03</td>
<td>0.76 ± 0.03</td>
<td>0.71 ± 0.02</td>
</tr>
<tr>
<td>In grams per kg body wt</td>
<td>3.71 ± 0.07</td>
<td>3.82 ± 0.06</td>
<td>4.19 ± 0.07*</td>
</tr>
<tr>
<td>Left kidney wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In grams</td>
<td>0.68 ± 0.01</td>
<td>0.74 ± 0.01*</td>
<td>0.92 ± 0.02*</td>
</tr>
<tr>
<td>In grams per kg body wt</td>
<td>3.32 ± 0.07</td>
<td>3.73 ± 0.06*</td>
<td>5.51 ± 0.09*</td>
</tr>
<tr>
<td>Daily water intake, Weeks 4 and 5 (ml)</td>
<td>32 ± 3</td>
<td>45 ± 4*</td>
<td>47 ± 5*</td>
</tr>
</tbody>
</table>

* $p < 0.05$ compared to the 1% NaCl diet; † $p < 0.05$ compared to the 5% NaCl diet.
WKY

In a second study, the response of normotensive WKY to the high chloride diet was tested. Previous studies from this laboratory had demonstrated that the blood pressure of WKY does not respond to an 8% (compared to 1%) NaCl diet. In the current study, 10 WKY were fed the high chloride diet for 5 weeks beginning at 6 weeks of age, while 10 control animals received the 1% NaCl diet. Prior to initiation of the diets, systolic blood pressures were not significantly different (1% NaCl group, 117 ± 5.5; high chloride group, 125 ± 4.3 mm Hg). Five weeks after initiation of the diets, blood pressures in the two groups were slightly lower than the prediet pressures, and were not significantly different from one another (1% NaCl group, 110 ± 2.6; high chloride group, 109 ± 3.5 mm Hg). At the termination of the study, WKY on the high chloride diet were lighter (222 ± 6 g) in body weight than animals on the 1% NaCl diet (275 ± 7 g). The high chloride diet resulted in significantly elevated kidney weight to body weight ratios (1% NaCl group, 3.38 ± 0.02; high chloride group, 5.2 ± 0.08 g) but did not significantly affect heart weight. Plasma sodium (1% NaCl group, 140.3 ± 0.3; high chloride group, 141.1 ± 0.8 mEq/L) and chloride (1% NaCl group, 102.1 ± 2.2; high chloride group, 102.5 ± 3.3 mEq/L) were not significantly altered by the diets.

Discussion

These results demonstrated that diets high in chloride and moderate in sodium can exacerbate hypertension in the SHR-S and that this rise in blood pressure may depend, in part, on reductions in noradrenergic input to the anterior hypothalamic area. The rise in blood pressure exhibited by SHR-S on the high chloride, moderate sodium diet was delayed in comparison to that displayed by SHR-S on the high (5%) NaCl diet. In both cases, the elevation in blood pressure was associated with increased water intake.

These data support previous demonstrations that the halide ion plays an important role in salt-sensitive hypertension in animals and humans. In Sprague-Dawley rats treated with intramuscular DOCA and fed a sodium-deficient diet, administration of NaCl is associated with the development of hypertension, while equimolar amounts of sodium bicarbonate, sodium ascorbate, or a combination of bicarbonate and ascorbate do not effect major changes in blood pressure. The difference in blood pressure response was not attributable to alterations in sodium or potassium balance, weight gain, or caloric intake. A study recently completed by Whitescarver and colleagues demonstrated that chronic high dietary intake of sodium provided as a mixture of sodium bicarbonate, phosphate, and amino acids did not produce hypertension in the Dahl salt-sensitive rat, while a diet containing equimolar amounts of sodium provided as NaCl was associated with the development of hypertension. There were no significant differences between groups in plasma volume, arterial pH, plasma sodium, potassium, chloride, ionized calcium, or creatinine concentrations or in renomedullary prostaglandin E₂ production.

Whitescarver and associates were unable to detect any alterations in blood pressure in the Dahl salt-sensitive rat during administration of a diet similar to the high chloride diet used in the present study. In the Whitescarver study, high NaCl intake did not elevate blood pressure in Dahl salt-sensitive rats until Week 7 of the diet. In the present study, the high chloride diet took twice as long as the 5% NaCl diet to elevate blood pressure in the SHR-S. If this delay is characteristic of pressor responses to high chloride diets, then in the Whitescarver study one might expect that the high chloride diet would not have effected a rise in blood pressure until Week 14. The study was terminated at Week 11. Alternatively, it is possible that the defective mechanism underlying the chloride sensitivity of SHR-S is not shared by Dahl salt-sensitive rats.

Several studies have demonstrated that neurons in the anterior hypthalamic area have a depressor function. Electrical stimulation of this area reduces blood pressure and heart rate, direct injections of α₂-adrenergic receptor agonists into the area depress the cardiovascular system; and injection of 6-hydroxydopa-
mine or placement of electrolytic lesions in this region leads to hypertension.\textsuperscript{13, 14} Together, these data demonstrate that increased NE input to the anterior hypothalamic area depresses the cardiovascular system. Conversely, reductions in NE input to the anterior hypothalamic area would be expected to decrease sympathoinhibition and thereby elevate blood pressure.

Intake of both high chloride and 5\% NaCl diets resulted in a significant reduction in NE content in the anterior hypothalamic region of SHR-S. This confirms our previous demonstration of a reduction in NE stores in the anterior hypothalamus of SHR-S provided a high (8\%) NaCl diet.\textsuperscript{7} Our previous study also showed reduced levels of the primary central nervous system terminal metabolite of NE, 3-methoxy-4-hydroxyphenylglycol, and a reduced rate of NE release in this area.\textsuperscript{7} Thus, both the present data and our previous studies suggest that high NaCl diets may, at least in part, elevate blood pressure by selectively reducing NE input to sympathoinhibitory anterior hypothalamic neurons in the SHR-S. Altered NE levels were not present in anterior hypothalamic areas of WKY or any other brain region of WKY following 8\% (compared to 1\%) NaCl feeding, and there was no NaCl-induced pressor response in WKY.\textsuperscript{7} In contrast, in the current study, neither high NaCl nor high chloride diets altered levels of NE or other monoamines or their metabolites in the posterior hypothalamus of SHR-S. It will be necessary in future studies to confirm our interpretation of the data by using more dynamic techniques. Our preliminary data demonstrate that turnover of NE is reduced in the anterior hypothalamus of NaCl-loaded SHR-S but not in that of WKY controls.

In summary, these studies demonstrated that diets high in chloride and moderate in sodium can elevate blood pressure in SHR-S and suggest that this augmentation of hypertension is associated with a decrease in excitation of sympathoinhibitory hypothalamic neurons.

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