High NaCl Diet Enhances Arterial Baroreceptor Reflex in NaCl-Sensitive Spontaneously Hypertensive Rats

David A. Calhoun, J. Michael Wyss, and Suzanne Oparil

Previous studies from our laboratory have shown that arterial baroreceptor reflex control of lumbar sympathetic nerve activity is blunted in the NaCl-sensitive spontaneously hypertensive rat (SHR-S) compared with either the NaCl-resistant spontaneously hypertensive rat (SHR-R) or the normotensive Wistar-Kyoto (WKY) rat. In the current study, the effect of dietary NaCl supplementation on arterial baroreceptor reflex control of lumbar sympathetic nerve activity and heart rate was assessed in SHR-S and control SHR-R and WKY rats. Male SHR-S, SHR-R, and WKY rats were fed diets containing either 1% or 8% NaCl beginning at 7 weeks of age and were studied at age 9-10 weeks. Arterial baroreceptor reflex-mediated changes in lumbar sympathetic nerve activity and heart rate were recorded in conscious, unrestrained rats during phenylephrine-induced (15-40 μg/kg/min) and nitroprusside-induced (15-300 μg/kg/min) changes in mean arterial pressure. SHR-S maintained on a 1% NaCl diet had blunted baroreceptor reflex control of lumbar sympathetic nerve activity during acute increases in MAP compared with SHR-R and WKY rats (p<0.05). After ingestion of the 8% NaCl diet, this blunting was absent, indicating enhancement of baroreceptor reflex control of lumbar sympathetic nerve activity. SHR-S maintained on a 1% NaCl diet also had blunted arterial baroreceptor control of lumbar sympathetic nerve activity during nitroprusside-induced decreases in mean arterial pressure compared with WKY rats, but this was not significantly altered during ingestion of the 8% NaCl diet. Arterial baroreceptor reflex-mediated control of heart rate during acute increases or decreases in MAP was blunted in SHR-S and SHR-R maintained on either diet compared with WKY rats (p<0.05). The NaCl-induced augmentation of arterial baroreceptor reflex control of lumbar sympathetic nerve activity in SHR-S would serve to buffer the NaCl-induced increase in mean arterial pressure. Further study is needed to elucidate its mechanism. (Hypertension 1991;17:363-368)

Studies of baroreceptor reflex function in animal models of hypertension suggest that high dietary NaCl exposure may modify baroreceptor reflex sensitivity. High dietary NaCl ingestion augments arterial and cardiopulmonary baroreceptor reflex-mediated sympathoinhibitory responses in salt-resistant Dahl (DR) rats. The sensitization of the arterial baroreceptor reflex in the DR rat has been attributed to alteration of the peripheral component of the baroreceptor reflex arc. In contrast, salt-sensitive Dahl (DS) rats have a blunted arterial and cardiopulmonary baroreceptor reflex-mediated control of heart rate and sympathetic nerve activity that is unaffected or exacerbated by dietary NaCl ingestion. The tendency of the DS rat to develop NaCl-induced hypertension may be related to the absence of the baroreceptor reflex sensitization observed in the DR rat.

Spontaneously hypertensive rats (SHR) develop hypertension independent of dietary NaCl intake. SHR from Taconic Farms, Germantown, N.Y., are NaCl-sensitive (SHR-S), manifesting an augmentation in blood pressure when fed a diet high in NaCl during the developmental phase of hypertension (age 7-12 weeks). This increase in blood pressure is associated with elevated levels of plasma catecholamines, consistent with increased sympathetic nervous system activity. Our laboratory has demonstrated that this NaCl-induced increase in MAP is
accompanied by a decreased turnover of norepinephrine in the anterior hypothalamic area (AHA), suggesting altered modulation of sympathetic output from this area.4-5 SHR from Charles River Breeding Laboratories, Wilmington, Mass., do not manifest NaCl-related changes in mean arterial pressure (MAP) and AHA norepinephrine turnover and, therefore, serve as hypertensive NaCl-resistant (SHR-R) controls for the SHR-S.5 Wistar-Kyoto (WKY) rats are likewise resistant to NaCl-related changes in MAP and AHA turnover and serve as normotensive NaCl-resistant controls.

Our laboratory has previously shown that arterial and cardiopulmonary baroreceptor reflex-mediated control of lumbar sympathetic nerve activity (LSNA) in SHR-S is impaired before exposure to high NaCl diet. SHR-S maintained on a 1% NaCl diet have blunted reflex control of LSNA during acute increases or decreases in MAP and during acute plasma volume expansion compared with WKY rats and Sprague-Dawley rats.6-7 In SHR-R maintained on a 1% NaCl diet, however, the sensitivity of arterial and cardiopulmonary baroreceptor reflex modulation of LSNA is identical to that observed in WKY and Sprague-Dawley controls.

The current study tested the hypothesis that, as in the DR rat, high NaCl exposure may enhance arterial baroreceptor reflex sensitivity in the SHR-R, thereby preventing NaCl-induced increases in MAP. Arterial baroreceptor reflex sensitivity was expected to remain blunted in SHR-S, facilitating the NaCl-induced increases in MAP.

Methods

Six-week-old male SHR-S (IBU-3 colony) and WKY rats were obtained from Taconic Farms. Six-week-old male SHR-R were obtained from Charles River Breeding Laboratories. Animals were housed four to a cage at constant temperature (24°C), humidity (60%), and 12-hour light/dark cycle. Rats were maintained on either normal (1%) or high (8%) NaCl rat chow (Purina Test Diets, Richmond, Ind.) containing 0.73% potassium.

After 2-3 weeks of diet, the rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) for placement of femoral catheters and lumbar nerve electrodes. Femoral artery and vein catheters made from PE-10 fused with PE-50 and were externalized between the scapulae. A midline abdominal incision was made, and the left lumbar sympathetic nerve was exposed inferior to the renal vein. Bipolar stranded stainless steel electrodes (Medwire, Mt. Vernon, N.Y.) were placed around the nerve for multifiber nerve traffic recording. The electrodes were connected by a Grass high gain impedance probe (P511, Grass Instrument Co., Quincy, Mass.) to a Grass P511 preamplifier where the signal was amplified (×20,000) and filtered (low frequency, more than 30 Hz; high frequency, less than 1,000 Hz). The signal was then fed into an oscilloscope (Tektronix 51113, Beaverton, Ore.) and Grass AM audiomonitor for evaluation. Once the signal was optimized, the electrodes were secured to the nerve with Wacker Sil Gel 604 (Wacker-Chemie Gmb, Munich, FRG). The electrode wires were externalized between the scapulae and fixed in place with dental cement along with the catheters. The abdominal incision was then closed.

Two days after instrumentation, and after determination of body weight, the arterial catheter was connected via a CP-02 pressure transducer (Century Technology, Inglewood, Calif.) to a Grass Model 7 polygraph for recording of MAP in the conscious, freely moving animal. Heart rate was recorded by a Grass cardiotach (7P44C). The signal from the lumbar recording electrodes was amplified and filtered as above and then rectified and integrated (Grass 7P10) before recording on the polygraph. The quality of nerve signal was assessed during an acute increase in MAP induced by the intravenous bolus injection of phenylephrine. Significant inhibition of nerve activity indicated a good signal. MAP, heart rate, and LSNA were recorded continuously throughout each experiment.

Each experiment consisted of determination of baseline MAP, heart rate, and LSNA during a 30-minute control period followed by continuous infusion of incremental doses of phenylephrine (5-40 µg/kg/min) to achieve a ramp increase in MAP over an ~4-5-minute infusion period. MAP, heart rate, and LSNA were then allowed to return to control values during a 30-minute stabilization period. After this stabilization period, nitroprusside was infused in incremental doses (13-300 µg/kg/min) to produce a ramp decrease in MAP over an ~4-5-minute infusion period. The total volume of infusate was less than 0.75 ml for each rat. After the nitroprusside infusion, each rat was killed by intravenous pentobarbital overdose, and postmortem nerve activity was recorded for 30 minutes. Thirty-minute postmortem nerve activity was subtracted from all measured LSNA values.

To exclude the possibility that phenylephrine was stimulating cardiopulmonary receptors, the phenylephrine protocol was repeated in three SHR-R before and after bilateral sinoaortic denervation (SAD). After induction of anesthesia, arterial and venous femoral catheters and lumbar nerve recording electrodes were placed. Heart rate, MAP, and LSNA were recorded during phenylephrine-induced increases in MAP using the infusion protocol as above. After the phenylephrine infusion, a ventral midline incision was made in the neck and the trachea was cannulated. The left carotid bifurcation was exposed, the recurrent laryngeal and the aortic depressor nerves were sectioned, and the superior cervical ganglion was removed. Any remaining carotid baroreceptor afferents were destroyed by stripping the carotid bifurcation and painting with 10% phenol. This procedure was then repeated on the right side. After 30 minutes, the phenylephrine infusion
TABLE 1. Baseline Measurements for Rats Maintained on 1% or 8% NaCl Diet

<table>
<thead>
<tr>
<th>Strain/NaCl diet</th>
<th>n</th>
<th>MAP (mm Hg)</th>
<th>Weight (g)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-S/1%</td>
<td>5</td>
<td>137±3*</td>
<td>223±6*</td>
<td>399±25</td>
</tr>
<tr>
<td>SHR-S/8%</td>
<td>5</td>
<td>161±7†</td>
<td>234±5†</td>
<td>374±9</td>
</tr>
<tr>
<td>SHR-R/1%</td>
<td>6</td>
<td>144±4*</td>
<td>213±6*</td>
<td>375±15</td>
</tr>
<tr>
<td>SHR-R/8%</td>
<td>5</td>
<td>156±3†</td>
<td>223±3†</td>
<td>372±17</td>
</tr>
<tr>
<td>WKY/1%</td>
<td>6</td>
<td>110±2</td>
<td>285±9</td>
<td>382±6</td>
</tr>
<tr>
<td>WKY/8%</td>
<td>8</td>
<td>112±3</td>
<td>306±10</td>
<td>346±8*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. MAP, mean arterial pressure; HR, heart rate; SHR-S, NaCl-sensitive spontaneously hypertensive rats; SHR-R, NaCl-resistant spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

*Different from WKY/1%, p<0.01.
†Different from WKY/8%, p<0.01.
‡Different from SHR-S/1%, p<0.05.

Phenylephrine-induced increases in MAP produced significant reductions in heart rate and LSNA, and nitroprusside-induced decreases in MAP produced significant increases in heart rate and LSNA in all three rat strains. The slope of the ΔHR/ΔMAP relation during phenylephrine and nitroprusside infusion was significantly diminished in SHR-S and SHR-R maintained on the 1% NaCl diet compared with WKY rats, indicating blunting of arterial baroreceptor reflex control of heart rate in both SHR strains (p<0.05) (Tables 2 and 3; Figures 1A and 1B). This impairment in baroreceptor reflex control of heart rate was unaffected by 8% dietary NaCl exposure (Tables 2 and 3; Figures 1A and 1B). The average correlation coefficient for the ΔHR/ΔMAP relation for each of the diet groups was more than 0.90, indicating a high degree of linearity between heart rate and the change in MAP (Tables 2 and 3).

The slope of the ΔLSNA/ΔMAP relation during phenylephrine infusion was significantly reduced in SHR-S maintained on the 1% NaCl diet compared with SHR-R and WKY rats (p<0.05) (Tables 2 and 3; Figure 1C), indicating impaired sensitivity of arterial baroreceptor reflex control of LSNA. In SHR-S maintained on the 8% NaCl diet, the sensitivity of control of LSNA during phenylephrine infusion was augmented compared with 1% NaCl-fed SHR-S (p<0.05) (Table 2; Figure 1C). The sensitivity of baroreceptor reflex control of LSNA during nitroprusside infusion in 1% NaCl-fed SHR-S was also reduced compared with WKY rats, but was not significantly altered by the 8% NaCl diet (Table 3; Figures 1A and 1B).
The principal finding of this study was that high dietary NaCl exposure enhanced arterial baroreceptor reflex control of LSNA in SHR-S during phenylephrine-induced increases in MAP. Impairment of arterial baroreceptor reflex control of LSNA in SHR-S maintained on a 1% NaCl diet, as reported previously by this laboratory, was again observed. After dietary NaCl supplementation, however, this impairment was no longer present, suggesting that high dietary NaCl exposure enhances arterial baroreceptor reflex control of LSNA in SHR-S during acute increases in MAP. The slope of the baroreceptor reflex sensitivity curve relating change in LSNA to change in MAP during phenylephrine-induced increases in MAP was significantly increased in SHR-S maintained on an 8% NaCl diet compared with 1% NaCl-fed SHR-S. Baroreceptor reflex sensitivity during acute baroreceptor unloading with infused nitroprusside also tended to be enhanced in SHR-S maintained on an 8% NaCl diet compared with SHR-S maintained on 1% NaCl, but the difference did not reach statistical significance. Baroreceptor reflex sensitivity curves were statistically unchanged by high dietary NaCl exposure in SHR-R and WKY rats during both phenylephrine- and nitroprusside-induced changes in MAP.

The unexpected result that high NaCl exposure enhanced arterial baroreceptor reflex sensitivity in SHR-S but not SHR-R is contrary to both our original hypothesis and previously reported findings in Dahl rats. High dietary NaCl ingestion has been reported to augment arterial baroreceptor reflex sensitivity in DR rats and to have no effect or to exacerbate arterial baroreceptor reflex sensitivity in DS rats.1-3 The contrasting effects of NaCl exposure on baroreceptor reflex sensitivity noted in SHR and Dahl rats may be related to differing experimental techniques or to different mechanisms of arterial baroreceptor reflex dysfunction. In the Dahl studies, normal and high dietary NaCl groups of anesthetized, female rats were used to compare differences in afferent aortic depressor nerve activity.1 In the current study, normal and high dietary NaCl groups of fully conscious (48 hours after anesthesia), male SHR were used to compare differences in efferent LSNA. These differences in experimental design may account for some of the apparent inconsistencies in experimental results.

In addition to differences in experimental design, the contrasting effects of NaCl exposure on baroreceptor reflex sensitivity in SHR and Dahl rats may be due to differences between strains in the mechanisms of baroreceptor reflex dysfunction. Aortic depressor nerve activity is diminished in prehypertensive DS rats (before high dietary NaCl exposure) during phenylephrine infusion compared to DR rats, indicating impairment of the arterial baroreceptor reflex at the level of the peripheral baroreceptor.8 Stimulation of the aortic depressor nerve produces equivalent changes in heart rate, MAP, and LSNA in DS and DR rats, suggesting no concomitant central defect in arterial baroreceptor reflex function. However, after high dietary NaCl exposure, aortic depressor nerve activity is enhanced in DR rats compared with DS rats during phenylephrine infusion, indicating augmentation of peripheral baroreceptor sensitivity.1 NaCl exposure does not affect aortic depressor nerve activity in DS rats, indicating persistent impairment of peripheral baroreceptor function. In

### Table 3. Linear Regression Values and Correlation Coefficients for ΔLSNA/ΔMAP and ΔHR/ΔMAP During Nitroprusside Infusion for Rats Maintained on 1% or 8% NaCl Diet

<table>
<thead>
<tr>
<th>Strain/NaCl diet</th>
<th>ΔLSNA/ΔMAP (% control/mm Hg)</th>
<th>ΔHR/ΔMAP (beats/min/mm Hg)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-S/1%</td>
<td>-1.49±0.35*</td>
<td>-0.91±0.03</td>
<td>-0.91±0.28†</td>
</tr>
<tr>
<td>SHR-S/8%</td>
<td>-2.44±0.55</td>
<td>-0.97±0.01</td>
<td>-1.49±0.23‡</td>
</tr>
<tr>
<td>SHR-R/1%</td>
<td>-2.06±0.12</td>
<td>-0.98±0.01</td>
<td>-2.55±0.31†</td>
</tr>
<tr>
<td>SHR-R/8%</td>
<td>-1.56±0.34</td>
<td>-0.95±0.01</td>
<td>-1.62±0.31‡</td>
</tr>
<tr>
<td>WKY/1%</td>
<td>-3.08±0.63</td>
<td>-0.96±0.02</td>
<td>-4.86±0.55</td>
</tr>
<tr>
<td>WKY/8%</td>
<td>-2.91±0.37</td>
<td>-0.98±0.01</td>
<td>-3.80±0.47</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. LSNA, lumbar sympathetic nerve activity; MAP, mean arterial pressure; HR, heart rate; SHR-S, NaCl-sensitive spontaneously hypertensive rats; SHR-R, NaCl-resistant spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

* Different from WKY/1%, p<0.05.
† Different from WKY/1%, p<0.01.
‡ Different from WKY/8%, p<0.01.
contrast, aortic depressor nerve stimulation produces smaller changes in heart rate, MAP, and splanchnic sympathetic nerve activity in SHR than in WKY rats, indicating a central impairment of arterial baroreceptor reflex function.9

Localization of the arterial baroreceptor reflex defect to the peripheral arterial baroreceptor in Dahl rats and to the central component of the baroreceptor reflex arc in SHR suggests that the mechanisms of baroreceptor reflex impairment are different in the two strains. These different mechanisms of baroreceptor reflex dysfunction may account for the disparate effects of NaCl exposure on arterial baroreceptor reflex sensitivity in SHR and Dahl rats. It is possible that high dietary NaCl exposure increases the sensitivity of the peripheral arterial baroreceptor in DR rats and the gain of the central component of the baroreceptor reflex in SHR-S. Comparison of heart rate, MAP, and sympathetic nerve activity responses to aortic depressor nerve stimulation in SHR-S fed 1% and 8% NaCl diets will be necessary to test this hypothesis.

Sensitization of the arterial baroreceptor reflex by dietary NaCl in SHR-S would tend to reduce the severity of NaCl-induced hypertension. Failure of this mechanism to prevent NaCl-induced hypertension suggests that other countervailing influences are involved. One such influence may be persistent blunting of the cardiopulmonary baroreceptor reflex, which has previously been shown by our laboratory to be impaired in SHR-S before high NaCl exposure.6 In addition, our laboratory has shown that the right atrial pressure is chronically elevated in SHR-S regardless of NaCl intake.10 If the cardiopulmonary
baroreceptor reflex remained blunted in the SHR-S during dietary NaCl supplementation, it would facilitate the NaCl-induced increases in MAP. Determination of the effects of dietary NaCl supplementation on cardiopulmonary baroreceptor reflex function in SHR-S will be necessary to evaluate this hypothesis.

The current study did not exclude the possibility that the observed NaCl-induced enhancement in arterial baroreceptor reflex sensitivity was a consequence of the higher MAP in the SHR-S maintained on the 8% NaCl diet. This seems unlikely, as 1) increased pretreatment MAP would be expected to blunt, rather than enhance, baroreceptor reflex sensitivity and 2) SHR-S maintained on the 1% NaCl diet had impaired arterial baroreceptor reflex control of LSNA compared with SHR-R and WKY rats in spite of an MAP similar to SHR-R and significantly higher than WKY rats. If the observed arterial baroreceptor reflex augmentation were purely a pressure phenomenon, SHR-S fed a 1% NaCl diet would not be expected to have a blunted arterial baroreceptor reflex compared with SHR-R and WKY rats.

It has recently been noted that high dose phenylephrine may stimulate cardiopulmonary baroreceptor reflexes and, therefore, may not be selective for arterial baroreceptor reflex function. To exclude this possibility, three SHR-R were subjected to the phenylephrine infusion protocol before and after bilateral SAD. Before SAD, significant reductions in heart rate and LSNA were noted. After SAD, no significant changes in heart rate or LSNA occurred, which confirmed the completeness of the SAD and excluded significant stimulation of cardiopulmonary receptors. These results suggest that at the doses used in the current study, phenylephrine did not significantly stimulate cardiopulmonary receptors.

Arterial baroreceptor reflex control of heart rate was blunted in SHR-S and SHR-R independent of dietary NaCl intake. The slope of the baroreceptor reflex curves relating change in heart rate to change in MAP was reduced in SHR-S and SHR-R maintained on a 1% NaCl diet compared with WKY rats, indicating impaired arterial baroreceptor reflex control of heart rate, as has been demonstrated by other investigators. Arterial baroreceptor reflex control of heart rate remained blunted in both SHR-S and SHR-R maintained on the 8% NaCl diet, indicating the absence of an enhancing effect of dietary NaCl supplementation on heart rate control in either SHR strain. This is not inconsistent with the enhancing effect of dietary NaCl supplementation on arterial baroreceptor reflex control of LSNA in SHR-S, since arterial baroreceptor reflex suppression of heart rate in conscious rats is predominantly parasympathetically mediated.

In summary, the present study has demonstrated that high dietary NaCl intake augmented arterial baroreceptor reflex-mediated control of LSNA in SHR-S during phenylephrine-induced increases in MAP. Localization of this effect to the peripheral or central component of the central arterial baroreceptor reflex arc will be the objective of future studies.

References


KEY WORDS • baroreceptors • sodium-dependent hypertension • sympathetic nervous system • spontaneously hypertensive rats
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