Impaired Reflex Response to Volume Expansion in NaCl-Sensitive Spontaneously Hypertensive Rats

Richard M. Thornton, J. Michael Wyss, and Suzanne Oparil

Abnormal baroreceptor reflex function that antedates or is a consequence of NaCl loading could contribute to the NaCl-induced exacerbation of hypertension in NaCl-sensitive spontaneously hypertensive rats (SHR-S). The current study tested the hypothesis that an impairment in cardiopulmonary baroreceptor reflex function exists in SHR-S before NaCl loading. The reflex response to volume expansion was compared in SHR-S, NaCI-resistant SHR (SHR-R), and normotensive Wistar-Kyoto (WKY) and Sprague-Dawley rats maintained on a normal NaCl diet. Conscious, free-moving SHR-S, SHR-R, WKY, and Sprague-Dawley rats were volume expanded with whole blood to 15% of blood volume within 6 minutes, and mean arterial pressure, heart rate, and lumbar sympathetic nerve activity were recorded. Heart rate and lumbar sympathetic nerve activity decreased significantly in SHR-R, WKY, and Sprague-Dawley rats after volume expansion. In contrast, in SHR-S neither heart rate after volume expansion nor lumbar sympathetic nerve activity was significantly different from levels before volume expansion. The blunted reflex response of heart rate and lumbar sympathetic nerve activity to volume expansion suggests impaired cardiopulmonary volume receptor function in SHR-S. This likely contributes to NaCl-induced hypertension in SHR-S on a high NaCl diet.

From the Hypertension Program of the Division of Cardiovascular Disease, Department of Medicine (R.M.T., S.O.) and the Department of Cell Biology and Anatomy (J.M.W.), University of Alabama at Birmingham, Birmingham, Alabama. Funded in part by grants HL-22544, HL-07457, and HL-35051 from the National Heart, Lung, and Blood Institute, National Institutes of Health and by the National Dairy Board in cooperation with the National Dairy Council. Address for correspondence: Richard M. Thornton, PhD, Department of Physiology, University of Alberta, 7-55 Medical Sciences Bldg., Edmonton, Alberta, Canada T6G 2H7. Received August 25, 1988; accepted in revised form July 5, 1989.

These neurochemical abnormalities, which are not seen in SHR-R or Wistar-Kyoto (WKY) control rats fed a high NaCl diet, may influence peripheral neurogenic mechanisms that modulate the NaCl-induced increase in blood pressure in SHR-S. Studies in other strains of rat have suggested that abnormalities in baroreceptor reflex control of the circulation may contribute to NaCl-sensitive hypertension. Both arterial and cardiopulmonary baroreceptor reflex sensitivity are reduced in prehypertensive Dahl salt-sensitive (DS) rats compared with Dahl salt-resistant (DR) rats on low NaCl diets.4–6 Additionally, NaCl loading in the DS rat does not alter cardiopulmonary or arterial baroreceptor reflex sensitivity, although sensitivity of the baroreceptor reflexes is increased in DR rats on a high NaCl diet.7–9 WKY rats, which are normally NaCl resistant, become NaCl sensitive when subjected to sinoaortic denervation, and hypertension develops when they are placed on a high NaCl diet.10 These findings suggest that abnormal baroreceptor reflex function is independent of NaCl loading and likely contributes to the development of NaCI-induced hypertension.

Whether SHR-S have a preexistent defect in baroreceptor reflex function that is therefore not a consequence of NaCl loading is unknown. If such a
defect exists, it may be involved in the pathogenesis of NaCl-sensitive hypertension in this strain of rat. The purpose of the current study was to determine if cardiopulmonary baroreceptor reflex function is impaired in SHR-S compared with SHR-R and normotensive WKY and Sprague-Dawley rats. All rats were maintained on normal NaCl diets to eliminate any effect that NaCl loading may have on the cardiopulmonary baroreceptor reflex. SHR-S, SHR-R, WKY, and Sprague-Dawley rats were subjected to volume expansion with whole blood, and reflex inhibition of lumbar sympathetic nerve activity (LSNA) and heart rate was assessed.

Materials and Methods

Male SHR-S of the Okamoto strain (IBU3 colony) and normotensive WKY control rats were obtained from Taconic Farms (Germantown, New York) at 8 weeks of age. Male SHR-R and normotensive Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts) at the same age. The rats were maintained four to a cage on a normal NaCl diet (1% NaCl) (diet 5001, Ralston Purina, St. Louis, Missouri) at constant humidity (60±5%), temperature (24±1°C), and light/dark cycle (12 hours on, 12 hours off).

One week after receiving the animals, the SHR-S, SHR-R, WKY, and Sprague-Dawley rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) for instrumentation. The femoral artery and vein were cannulated with PE-10 fused to PE-50 tubing for measurement of arterial blood pressure and infusion of blood, respectively. The catheters were exteriorized between the scapulae. A laparotomy incision closed.

Two days later, after measurement of body weight, the arterial cannula of freely moving animals was connected to a CP-02 pressure transducer (Century Technology, Inglewood, California) for recording of arterial pressure on a Grass Model 7 polygraph (Grass Instr. Co., Quincy, Massachusetts), where the signal was amplified (×20,000) and filtered (low 30 Hz, high 1000 Hz). The modified signal was fed into an oscilloscope (Tektronic 5113, Beaverton, Oregon) and Grass AM8 audiomonitor (Grass Instr. Co.) for evaluation. When an optimal signal was achieved, the electrodes were fixed in place with Wacker Sil Gel 604 (Wacker-Chemie Gimble, Munich, FRG). The wires were exteriorized between the scapulae and fixed in place, along with the catheters, and the incision closed.

Two days later, after measurement of body weight, the arterial cannula of freely moving animals was connected to a CP-02 pressure transducer (Century Technology, Inglewood, California) for recording of arterial pressure on a Grass Model 7 polygraph (Grass Instr. Co.). Heart rate was monitored by a cardiotach (model 7P44C, Grass Instr. Co.) triggered by the systolic pressure rise. For LSNA, the electrodes were connected as above and the modified signal fed into a Grass 7P10 rectifying voltage integrator (Grass Instr. Co.). The integrated signal was displayed on the polygraph. The quality of the nerve signal was assessed with an intravenous injection of norepinephrine (5 μg). If the signal decreased after norepinephrine administration and was of good quality, the experiment commenced. Mean arterial pressure, heart rate, and LSNA were recorded throughout each experiment.

To assess the compensatory response to volume expansion, blood pressure, heart rate, and LSNA were monitored during an infusion of whole blood that expanded blood volume by 15% within 6 minutes. Plasma norepinephrine was determined from blood samples drawn before and immediately after volume expansion.

An experiment consisted of a 30-minute control period, during which all measured parameters were stabilized, followed by a 6-minute period of progressive volume expansion during which 15% of a rat's blood volume (calculated on the basis of a blood volume ~7% of body weight as determined in our laboratory) was infused. Whole human blood collected from healthy normotensive donors was used for volume expansion. From an individual, 7-10 ml blood was drawn into a heparinized test tube and pooled with blood collected from several donors. The pooled blood was kept at 37°C in an open test tube for at least 15 minutes before use. During the control period and immediately after volume expansion, 0.7 ml blood was drawn from the arterial catheter for plasma norepinephrine determination. Samples were placed in iced tubes, centrifuged, and the plasma stored at −80°C for subsequent determination using a modification of the radioenzymatic method of Peuler and Johnson.11 Blood was replaced with 0.9% saline. After the final blood sample was taken, the rat was killed (overdose of sodium pentobarbital) and postmortem nerve activity recorded for 30 minutes. Postmortem activity was subtracted from all LSNA values during control and volume expansion periods.

All values presented are expressed as mean±SEM. LSNA during volume expansion is expressed as percent change from nerve activity during the control period. To compare the compensatory response to volume expansion among the four groups of rats, mean arterial pressure, heart rate, and LSNA were compared at control and 2, 4, and 6 minutes of expansion (5%, 10%, and 15% volume expansion) by analysis of variance, followed by the Student-Neuman-Keuls multiple comparison test. To compare LSNA and heart rate during volume expansion to control (pre-expansion) levels in a particular strain of rat, an analysis of variance was performed followed by Dunnett's multiple comparison test. Plasma norepinephrine values were compared among the four groups at control and after volume expansion with the same statistical test. The response of plasma norepinephrine to volume expansion was compared by a
Table 1. Body Weight, Mean Arterial Pressure, and Heart Rate Before Volume Expansion for the Four Groups of Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-S</td>
<td>206±3</td>
<td>127±3*</td>
<td>384±6</td>
</tr>
<tr>
<td>SHR-R</td>
<td>205±4</td>
<td>138±5*t</td>
<td>406±12</td>
</tr>
<tr>
<td>WKY</td>
<td>212±4</td>
<td>104±3</td>
<td>379±6</td>
</tr>
<tr>
<td>C-D</td>
<td>245±4e</td>
<td>104±3</td>
<td>455±12†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=10 for SHR-S and WKY rats; n=9 for SHR-R and C-D rats. MAP, mean arterial pressure; HR, heart rate; SHR-S, NaCl-sensitive spontaneously hypertensive rats; SHR-R, NaCl-resistant SHR; WKY, Wistar-Kyoto rats; C-D, Sprague-Dawley rats.

*Significantly different from WKY and C-D rats (p<0.01).
†Significantly different from SHR-S (p<0.05).
‡Significantly different from SHR-S, SHR-R, and WKY rats (p<0.05).

paired Student’s t test. In all statistical tests, significance was attained if p<0.05.

Results

Body weight was similar in SHR-S, SHR-R, and WKY rats, but Sprague-Dawley rats were significantly heavier (Table 1). Pretreatment mean arterial pressure was significantly higher in both hypertensive strains compared with the normotensive groups and in SHR-R compared with SHR-S (Table 1). Pretreatment heart rate was higher in the Sprague-Dawley group than in the other three groups, which had similar heart rates (Table 1).

In response to volume expansion, LSNA and heart rate began to fall by the time 5% of blood volume had been infused in the SHR-R, WKY, and Sprague-Dawley groups. Heart rate and LSNA continued to decrease throughout volume expansion in these three groups (Figure 1). LSNA at 5, 10, and 15% volume expansion was significantly (p<0.01) different from control (before volume expansion) values in the SHR-R, WKY, and Sprague-Dawley groups. Heart rate at 10 and 15% volume expansion was significantly (p<0.01) different from control values for these three groups. In contrast, during volume expansion, neither LSNA nor heart rate was significantly different from control levels (before volume expansion) in the SHR-S group (Figure 1). LSNA was significantly different in SHR-S from the other three groups at 10 and 15% of volume expansion (Figure 1). Heart rate was significantly different in SHR-S from SHR-R, WKY, and Sprague-Dawley rats at 5, 10, and 15% of volume expansion. Mean arterial pressure increased by 15 mm Hg immediately after 5% volume expansion in SHR-S and remained elevated throughout the experiment (Figure 1). In contrast, the other three groups experienced only a small (3–7 mm Hg) rise in mean arterial pressure at 5% volume expansion. Mean arterial pressure remained elevated by about 5 mm Hg in Sprague-Dawley rats throughout the period of study, and it continued to increase in SHR-R and WKY rats during progressive volume expansion. Mean arterial pressure reached the same level in WKY rats as in SHR-S after 10 and 15% of blood volume expansion (Figure 1).

Before volume expansion, plasma norepinephrine was not different among the four groups (Table 2). After expansion, norepinephrine levels tended to decrease in the normotensive WKY and Sprague-Dawley rats. Norepinephrine tended to increase in both hypertensive groups after volume expansion. The increase was statistically significant in SHR-S but not in SHR-R.
TABLE 2. Plasma Norepinephrine Before and After Volume Expansion and the Percent Change Resulting from Volume Expansion for the Four Groups of Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>NE before VE (pg/ml)</th>
<th>NE after VE (pg/ml)</th>
<th>Percent change (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR-S</td>
<td>180±25</td>
<td>271±45*</td>
<td>29±51</td>
</tr>
<tr>
<td>SHR-R</td>
<td>204±21</td>
<td>259±21</td>
<td>21±42</td>
</tr>
<tr>
<td>WKY</td>
<td>200±24</td>
<td>157±22</td>
<td>39±15</td>
</tr>
<tr>
<td>C-D</td>
<td>269±29</td>
<td>225±32</td>
<td>7±5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=7 for SHR-S, SHR-R, and C-D rats; n=10 for WKY rats. NE, norepinephrine; VE, volume expansion; SHR-S, NaCl-sensitive spontaneously hypertensive rats; SHR-R, NaCl-resistant SHR; WKY, Wistar-Kyoto rats; C-D, Sprague-Dawley rats.

*Significantly different from value before volume expansion.

Discussion

The principal finding of this study was that NaCl-sensitive SHR-S on a normal NaCl diet had an impaired compensated reflex response to acute volume expansion, as neither LSNA nor heart rate decreased during volume expansion. In contrast, SHR-R and normotensive WKY and Sprague-Dawley rats maintained on normal NaCl diets showed substantial decreases in both heart rate and LSNA in response to volume expansion. This suggests that SHR-S have an abnormality in cardiopulmonary baroreceptor reflex control when compared with SHR-R or normotensive WKY and Sprague-Dawley rats that is independent of NaCl loading. In the current study, SHR-R had a higher mean arterial pressure than SHR-S, ruling out the possibility that the abnormal cardiopulmonary baroreceptor reflex control observed in SHR-S compared with SHR-R was merely a result of baroreceptor reflex resetting in response to elevated arterial pressure. Although results of this study indicate impaired cardiopulmonary baroreceptor reflex function in SHR-S, defects in arterial baroreceptor reflex function may also exist in this strain, as mean arterial pressure increased 20 mm Hg during volume expansion without an accompanying decrease in LSNA or heart rate. To confirm the presence of an abnormality in arterial baroreceptor reflex function, additional studies are needed. Further, current data do not define the anatomic locations of the defects in the cardiopulmonary reflex arc in SHR-S. These abnormalities could reside in the baroreceptors themselves or in the vessel wall where the sensory fibers are located,12,13 in afferent projections from the baroreceptors, in central integration sites,14 or in efferent autonomic pathways.

Since SHR-S and SHR-R were obtained from different suppliers, it is possible that differences between the two strains in response to volume expansion were related not only to differences in NaCl sensitivity but also to the different sources of the two strains. However, the two strains of SHR used appear to be closely related. Taconic Farms SHR and Charles River SHR were initially seeded 1 year apart from National Institutes of Health SHR stock at least 30 generations ago. SHR from these two sources show genetic homogeneity,15 and the growth rate and time course for development of hypertension are similar for the two strains of SHR when maintained on normal NaCl diets.16 Additionally, preliminary DNA fingerprinting of SHR from the colonies used in this study, carried out on DNA extracted by Dr. Allen Naftilan of our laboratories, showed no obvious genetic difference between SHR-S and SHR-R.17 Therefore, differences in response to volume expansion would likely be related to differences in NaCl sensitivity, and use of the SHR-R as a NaCl-resistant control for the SHR-S strain would not be confounded by the different sources of the two strains.

Evidence from studies of another strain of rat with genetically mediated salt sensitivity, the DS rat, suggests that both cardiopulmonary and arterial baroreceptor reflexes are impaired, compared with control DR rats even before initiation of oral NaCl supplementation.4,5,8 In these studies, arterial baroreceptor reflex-mediated bradycardia, hindlimb vasodilation, and inhibition of renal and splanchnic sympathetic efferent nerves were less pronounced in DS than in DR rats.4,5,8 Additionally, in arterial baroreceptor-denervated prehypertensive DS rats, volume expansion caused a greater increase in left ventricular end diastolic pressure but lesser inhibition of splanchnic sympathetic nerve activity than in DR rats,4 indicating an impaired cardiopulmonary baroreceptor reflex. Further, after NaCl loading, there was increased sensitivity of both the cardiopulmonary baroreceptor reflex and the arterial baroreceptor reflex in the DR but not in the DS rat.7-9

In the prehypertensive DS rat, the impairment in baroreceptor reflex function appears to reside at the baroreceptor level. Recordings made from the cut peripheral end of the aortic depressor nerve showed that for equal increases in arterial pressure, aortic depressor nerve activity increased more in DR than in DS rats.18 In contrast, graded stimulation of the cut central end of the aortic depressor nerve produced similar decreases in arterial pressure and lumbar sympathetic nerve activity.18 Thoren et al19 concluded that the impairment of cardiopulmonary baroreceptor reflex function in the DS rat is the result of a resetting of left atrial sensory endings to a higher threshold in DS than in DR rats. This resetting appeared to be independent of increases in atrial or arterial pressures and of changes in cardiac distensibility.

In contrast to the findings in the DS rat, an impairment in arterial baroreceptor reflex control in SHR has been localized to the central nervous system.14 Stimulation of the aortic depressor nerve in decerebrate or urethane-anesthetized SHR caused an attenuated inhibition of splanchnic sympathetic nerve activity compared with similarly treated WKY rats.14 The location of the impairment in cardiopulmonary baroreceptor reflex activity in SHR-S compared with SHR-R, whether at the sensory endings...
or central nervous sites or both, remains to be determined.

Our finding of reduced plasma norepinephrine levels at the end of volume expansion in WKY and Sprague-Dawley rats, but not in SHR-S and SHR-R, supports the finding that sympathetic nerve activity, as indicated by LSNA and heart rate, decreased in response to volume expansion in the normotensive strains. Additionally, as expected from the decrease in sympathetic nerve activity, the increase in mean arterial pressure during volume expansion was small in Sprague-Dawley rats. However, in WKY rats, mean arterial pressure increased by 20 mm Hg at the end of volume expansion, even though LSNA, heart rate, and norepinephrine levels were decreased. This paradoxical rise in mean arterial pressure in WKY rats suggests that perhaps some other defect exists in response to volume expansion in that strain.

In the hypertensive strains, plasma norepinephrine levels tended to increase at the end of volume expansion, whereas the other indexes of sympathetic nerve activity, LSNA and heart rate, decreased during volume expansion in the SHR-R and did not change in the SHR-S. This increase in one index of sympathetic activity, even though the other indexes decreased or remained unchanged during volume expansion, is difficult to explain. Although measurements of LSNA and heart rate are direct indexes of sympathetic nerve activity, these measures may not reflect sympathetic activity in all vascular beds. In contrast, plasma norepinephrine concentration is a measure of total sympathetic nervous system activity, although plasma levels of norepinephrine are dependent not only on release from nerve terminals, but also on release from the adrenal medulla and the rate of clearance from the plasma. Even though LSNA and heart rate decreased in the SHR-R with volume expansion, the increase in mean arterial pressure, along with the slight increase in plasma norepinephrine, suggests that there may have been an inappropriate increase in sympathetic nerve activity in some other (than lumbar sympathetic) nerve distribution. This suggests the presence of a defect in the reflex response to volume expansion in other sympathetic beds. In the SHR-R, the rapid increase in mean arterial pressure, along with the increase in norepinephrine during volume expansion, without a concomitant decrease in LSNA and heart rate, strongly supports the contention that SHR-S have a defect in cardiopulmonary baroreceptor reflex function compared with the SHR-R and the two normotensive strains. It would be of interest to determine whether the difference in reflex function between the SHR-S and the SHR-R is as evident in another sympathetic (i.e., renal) distribution.

The impairment in cardiopulmonary reflex response to volume expansion in the SHR-S could be related to greater cardiac hypertrophy or hemodynamic abnormalities compared with the SHR-R. Alternatively, the reflex impairment could be linked to preexisting genetic factors. Because we did not measure cardiac filling pressure or heart weight, it is impossible to distinguish between underlying genetic factors, cardiac hypertrophy, and hemodynamics as possible causes of the abnormality in the cardiopulmonary baroreceptor reflex in the SHR-S.

In summary, this study demonstrated an impaired compensatory reflex response to volume expansion in SHR-S. This defect is independent of NaCl loading and not merely a result of elevated arterial pressure. This abnormality in cardiopulmonary baroreceptor reflex function may well contribute to the exacerbation of hypertension that occurs in the SHR-S when placed on a high NaCl diet.

Acknowledgments

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**KEY WORDS** • volume expansion • sympathetic nervous system • heart rate • baroreceptor reflex • NaCl-sensitive spontaneously hypertensive rats
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R M Thornton, J M Wyss and S Oparil

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