Impaired Release of Atrial Natriuretic Factor in NaCl-Loaded Spontaneously Hypertensive Rats

HONGKUI JIN, YIU-FAI CHEN, REN-HUI YANG, QING CHENG MENG, AND SUZANNE OPARIL

SUMMARY Our previous studies demonstrated that NaCl-sensitive spontaneously hypertensive rats (SHR) of the Okamoto strain exhibit increased blood pressure and reduced noradrenergic input to the anterior hypothalamic area when fed high NaCl diets. The current study tested the hypotheses that 1) release of atrial natriuretic factor (ANF) into the plasma is impaired in NaCl-loaded SHR, a defect that would tend to elevate blood pressure, and 2) ANF levels in regions of brain involved in blood pressure regulation, such as the anterior hypothalamic area, are altered in SHR. SHR and control Wistar-Kyoto rats (WKY) were placed on 1% or 8% NaCl diets at age 7 weeks; 2 weeks later, ANF levels were measured in plasma, left and right atria, anterior hypothalamic area, ventral hypothalamic area, posterior hypothalamic area, pons, and medulla by radioimmunoassay. Blood for ANF assay was obtained from intra-arterial cannulas in conscious, unrestrained rats studied in the resting state. The 8% NaCl diet produced an increase in blood pressure in the SHR, but not in the WKY. Plasma ANF levels were significantly greater in WKY fed 8% NaCl than in WKY fed 1% NaCl, but dietary NaCl loading did not produce similar increases in plasma ANF in the SHR. Plasma ANF levels were not significantly different between SHR and WKY fed the 1% NaCl diet. The observation that dietary NaCl loading stimulated ANF release into the plasma in WKY but not in SHR suggests that the exacerbation in hypertension seen in NaCl-loaded SHR may be related to an impairment in ANF release. In addition, ANF stores were elevated in the anterior hypothalamic area of SHR fed either diet as compared with WKY. The role of this alteration in central nervous system ANF in the pathogenesis of NaCl-sensitive hypertension remains to be determined.

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KEY WORDS • atrial natriuretic factor • spontaneously hypertensive rats • salt sensitivity • plasma atrial natriuretic factor • brain regions

Atrial natriuretic factor (ANF), a family of peptide hormones isolated from mammalian atria, has potent natriuretic, diuretic, sympatholytic, vasodilator, and renin-suppressing and aldosterone-suppressing activities and is involved in the regulation of volume and electrolyte balance and blood pressure.1–10 Intravenous administration of ANF produces greater depressor and/or natriuretic and diuretic responses in spontaneously hypertensive rats (SHR) than in normotensive rats.11–12 Blood pressure and renal responses to ANF in NaCl-supplemented SHR have not been reported, however. Acute volume loading and atrial stretch are potent stimuli of ANF release in conscious normotensive rats and SHR,14–16 but the effects of chronic dietary NaCl loading on ANF release in the rat are less clear.17–19 Further, the role of circulating ANF in setting blood pressure and volume levels in chronically hypertensive rats fed diets of varying NaCl content is poorly understood.

Our previous studies have demonstrated that NaCl-sensitive SHR from Taconic Farms (SHR-S; IBU3 colony, Germantown, NY, USA) exhibit significant increases in blood pressure and sympathetic outflow and decreases in norepinephrine stores and release in the anterior hypothalamic area (AHA) when fed a high NaCl diet.20,21 Normotensive control Wistar-Kyoto rats (WKY) do not respond to dietary NaCl loading with either a pressor effect or alterations in AHA noradrenergic neuronal activity. We postulated that circulating ANF would increase in WKY fed a high NaCl diet, thus tending to maintain normal blood pressure through a depressor or vasodilator effect, but not in the

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were anesthetized with ether. Polyethylene catheters (PE-10 fused to PE-50) filled with heparin-saline solution (50 U/ml) were implanted into the abdominal aorta through the right femoral artery. Following catheter implantation, all rats were housed individually. Two days after implantation, tubing was connected with the femoral arterial catheter for blood sampling. At least 1 hour was allowed to pass before 1.0 ml of blood was collected from conscious, unrestrained, resting animals for ANF determination. The blood withdrawn was immediately replaced with an equal volume of 0.9% saline. Blood was placed in iced tubes containing 1.5 mg EDTA and 1 trypsin-inhibitor unit of aprotinin. Rats were then decapitated, and brain and left and right atri were removed quickly.

Twelve days after initiation of the special diets, rats were anesthetized with ether. Polyethylene catheters...
TABLE 1. Effects of NaCl Supplementation (2 Weeks) on Systolic Blood Pressure, Heart Rate, and Body Weight in SHR and WKY

<table>
<thead>
<tr>
<th>Group</th>
<th>1% NaCl</th>
<th>8% NaCl</th>
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<tbody>
<tr>
<td></td>
<td>SBP (mm Hg)</td>
<td>HR (beats/min)</td>
</tr>
<tr>
<td>SHR-S</td>
<td>154 ± 3* (19)</td>
<td>433 ± 14 (19)</td>
</tr>
<tr>
<td>WKY</td>
<td>113 ± 3 (20)</td>
<td>425 ± 13 (20)</td>
</tr>
</tbody>
</table>

Results represent means ± SEM. Numbers of animals are shown in parentheses. SBP = systolic blood pressure; HR = heart rate; SHR-S = NaCl-sensitive SHR.

*p<0.05, compared with respective values of WKY group.
†p<0.01, compared with respective values of 1% NaCl control group.

Results

SHR-S fed the 8% NaCl diet had significantly higher blood pressures than SHR-S fed the 1% NaCl diet (Table 1). After 2 weeks on the special diets, systolic pressure in the 8% NaCl group averaged 21 mm Hg more than in the 1% NaCl group (p<0.01). In contrast, blood pressures of WKY showed no change in response to dietary NaCl supplementation. The 8% NaCl diet did not influence heart rate significantly in any experimental group. Body weights of WKY were significantly greater than those of SHR-S fed either 1% or 8% NaCl diets. There was no difference in body weight between the 1 and 8% NaCl groups within each strain.

Dietary NaCl supplementation was associated with a significant increase in plasma ANF levels in WKY but not in SHR-S (Figure 1). After 2 weeks of the 8% NaCl diet, SHR-S had significantly lower plasma ANF levels than did WKY fed the same diet. In contrast, there was no significant difference in plasma ANF between WKY and SHR-S fed the 1% NaCl diet.

There were no significant differences in ANF content of left or right atria either between SHR-S and WKY fed the same diet or between 1 and 8% NaCl rats within the same strain (Figure 2).
ANF content of the AHA in SHR-S was significantly higher than in WKY for both diet groups (Table 2). There was no significant difference in ANF content of the AHA between the 1 and 8% NaCl groups within either strain. Further, there were no significant differences in ANF content of the PHA, VHA, pons, or medulla among the four experimental groups (see Table 2). ANF stores in the hypothalamus were inhomogeneously distributed: three to five times as much ANF (expressed as ng/g wet wt) was found in the VHA as in the AHA or PHA.

Discussion

The current study demonstrated that plasma ANF levels were not significantly increased in SHR-S after 2 weeks of a high NaCl (8%) diet, suggesting that chronic dietary NaCl loading does not stimulate ANF release in this hypertensive model. In contrast, in control WKY, plasma ANF was increased by 60% after 2 weeks of the high NaCl regimen. The high NaCl diet produced an increase in blood pressure in the SHR-S, but not in the WKY. Plasma ANF levels were not significantly different in conscious, unrestrained, young male SHR-S and WKY fed a basal (1%) NaCl diet and studied in a resting state. ANF stores were elevated in the AHA, a brain region that modulates sympathetic outflow and gives rise to a depressor response when stimulated, in SHR-S on both diets as compared with WKY. These data provide preliminary evidence that both peripheral and central nervous system levels of ANF are altered in the SHR-S. Further study is needed to elucidate the functional role of those alterations in the pathogenesis of spontaneous hypertension and its exacerbation by NaCl loading in this model.

Numerous studies have shown that acute or chronic NaCl loading causes significant increments in plasma ANF levels in normotensive rats, suggesting that NaCl loading induces release of ANF from the atria into the circulation. This effect has generally been attributed to volume expansion with resultant right atrial stretch, although an independent effect of the sodium ion per se cannot be ruled out. Our finding of increased circulating ANF levels following 2 weeks of oral salt loading in WKY is consistent with this body of knowledge, and failure of the young SHR-S to elevate plasma ANF levels in this situation suggests that the SHR-S have an impairment in ANF release during the developmental phase of hypertension. Our results are consistent with the recent report of Haass et al., who found that 4-week-old male SHR showed a tendency toward blunting of ANF release in response to acute volume expansion with isotonic saline (NaCl, 2.6 mEq/kg i.v. in 1 ml over 2 minutes) compared with age-matched WKY. Although the NaCl-induced increments in ANF levels were not statistically significant due to the small number of animals studied (six in each group) and the large variance in the data, the mean change in plasma ANF in the SHR was 43% less than that in the WKY, suggesting that the difference would have attained statistical significance if more animals had been studied. Further, the threshold for ANF release after acute volume expansion with blood was three times higher in 4-week-old SHR than in WKY in this study. Interestingly, the difference in responsiveness of ANF release to both NaCl loading and blood volume expansion disappeared when 16-week-old SHR and WKY were studied. This finding may correspond to the lack of NaCl sensitivity of blood pressure in SHR-S older than 14 weeks of age that we noted in a previous study. In addition, the mechanism by which ANF release is impaired in dietary-NaCl-loaded SHR-S is unknown. One possibility is that the atria of SHR-S may be less distensible than the atria of WKY, reducing the amount of stretch produced by a given level of volume expansion. It has been shown that the dynamic distensibility of left atrial wall is significantly decreased in SHR as compared with WKY. Thus, it seems likely that volume expansion induced by dietary NaCl loading causes less stretch of the atrial wall in SHR-S than in WKY or that more atrial stretch is required to elevate circulating ANF levels in SHR-S than in WKY. Further study is needed to provide direct evidence for this explanation in our experimental model.

Our finding that plasma ANF levels did not increase in SHR-S fed an 8% NaCl diet contrasts with an earlier report that SHR exhibit an increase in circulating ANF after consuming a high NaCl intake for 2 weeks. Our findings differ from those of the latter study in several respects. First, blood was collected from decapitated rats in the earlier study and from intra-arterial cannulas in conscious, unrestrained, resting rats in the current one. Second, rats in the earlier study were much older (20 weeks of age) than the animals used in the current study (9 weeks). Previous studies from our laboratory have demonstrated that SHR lose NaCl sensitivity in the established phase (> 14 weeks) of hypertension.

### Table 2: ANF Content in Brain Regions

<table>
<thead>
<tr>
<th></th>
<th>AHA</th>
<th>PHA</th>
<th>VHA</th>
<th>Pons</th>
<th>Medulla</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>% NaCl</td>
<td>% NaCl</td>
<td>% NaCl</td>
<td>% NaCl</td>
<td>% NaCl</td>
</tr>
<tr>
<td>SHR-S</td>
<td>24.7±2.7*</td>
<td>27.4±3.2*</td>
<td>22.3±4.3</td>
<td>15.6±2.8</td>
<td>80.7±17.7</td>
</tr>
<tr>
<td>WKY</td>
<td>14.7±2.3</td>
<td>15.2±2.0</td>
<td>15.4±3.1</td>
<td>13.0±3.0</td>
<td>79.4±14.5</td>
</tr>
</tbody>
</table>

Results represent means ± SEM (in ng/g). Numbers of animals are shown in parentheses. AHA, PHA, VHA = anterior, posterior, and ventral hypothalamus area, respectively; SHR-S = NaCl-sensitive SHR.

*p < 0.05, compared with respective values of WKY group.
Third, rats in the earlier study consumed their supplemental NaCl as 1% saline, thus receiving a smaller dietary NaCl load than our 8% NaCl-fed rats. The response of circulating ANF to dietary NaCl supplementation appears to be dependent on the magnitude and duration of the NaCl load as well as on the strain and age of the rats studied and the method of blood collection.

Our finding that plasma ANF levels do not differ significantly between SHR-S and WKY fed a basal (1%) NaCl diet confirms the previous report of Haass et al.16 that basal circulating levels of ANF are similar in young and adult conscious, unrestrained SHR and WKY from which blood is sampled through intraperitoneal cannulas. In contrast, significantly increased (compared with WKY) plasma ANF levels have been reported in anesthetized or decapitated SHR.2, 19, 26, 32 Plasma ANF data obtained from anesthetized or decapitated rats must be interpreted with caution, since various anesthetics and different methods of blood collection alter plasma levels of ANF.15, 16, 33

Immunoreactive ANF has been detected in various brain regions by RIA of extracted tissue and by immunocytochemistry.23, 34-37 Immunocytochemical data indicate that ANF-containing cell bodies are present primarily in the preoptic-hypothalamic areas, but also in the amygdala, mesencephalon, and pons.15, 38 The largest collection of ANF-containing cell bodies is found in the hypothalamus, adjacent to the anteroventral tip of the third ventricle, a region involved in the development and maintenance of experimental hypertension and in fluid and electrolyte balance.3, 15, 39 This region is essentially the same as the AHA dissected in our study.

In the current study, ANF stores were elevated in the AHA of SHR-S on both diets as compared with WKY. There were no other differences between strains or diet groups in ANF in any other brain region. The AHA contains neurons that have sympathoinhibitory and depressor effects when excited by norepinephrine inputs. Reductions in norepinephrine activity in the AHA would be expected to decrease inhibition of sympathetic outflow and thereby cause blood pressure to rise. Previous studies from our laboratory have shown that SHR-S fed an 8% NaCl diet for 2 weeks exhibit increased blood pressure, increased sympathetic outflow, and reduced norepinephrine release in the vicinity of sympathoinhibitory neurons in the AHA compared with control SHR-S fed a 1% NaCl diet.20, 21 WKY do not manifest these NaCl-induced changes. The demonstrations that ANF inhibits norepinephrine release from nerve terminals in the periphery4, 6 and reduces membrane excitability of neurons in a number of brain regions18 suggest that ANF in the central nervous system (especially in the AHA) may act as a neuromodulator or neurotransmitter, providing a possible mechanism linking increased ANF stores in the AHA and NaCl-sensitive hypertension in the SHR-S. If ANF in the AHA acted as an inhibitory neuromodulator, reducing norepinephrine release from nerve terminals in this region, the peptide could participate in the pathogenesis of the NaCl-induced hypertension in SHR-S. Further studies of the direct effects of ANF on norepinephrine release by AHA neurons in vivo and in vitro are needed to test this hypothesis.

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