Salt Supplementation Does Not Alter the Pressor Effect of Blocking Atrial Natriuretic Peptide in Nucleus Tractus Solitarii

Renhu Yang, Hongkui Jin, James Michael Wyss, Yiu-Fai Chen, and Suzanne Oparil

We have previously shown that microinjection of monoclonal antibody to atrial natriuretic peptide (ANP) into the caudal nucleus tractus solitarii causes a pressor response in salt-sensitive spontaneously hypertensive rats (SHR) fed a basal (1%) salt diet, suggesting that endogenous ANP in this region may be involved in the centrally mediated regulation of blood pressure in this model. The present study tested the hypothesis that the pressor effect of blocking endogenous ANP in caudal nucleus tractus solitarii is enhanced by dietary salt supplementation in salt-sensitive SHR. Monoclonal antibody to ANP (0.55 μg) in 50 nl artificial cerebrospinal fluid or control immunoglobulin G was microinjected into the caudal nucleus tractus solitarii of conscious salt-sensitive SHR, salt-resistant SHR, and Wistar-Kyoto rats fed 1% or 8% salt diets for 3 weeks. Microinjection of the monoclonal antibody into the caudal nucleus tractus solitarii evoked similar increases in mean arterial pressure in salt-sensitive SHR on both 1% and 8% salt diets and in salt-resistant SHR on a 1% salt diet but did not have an effect in Wistar-Kyoto rats. In contrast, microinjection of control immunoglobulin G into this brain area did not alter mean arterial pressure or heart rate in any experimental group. Thus, endogenous ANP in caudal nucleus tractus solitarii mediates tonic control of blood pressure in both salt-sensitive and salt-resistant SHR but not in Wistar-Kyoto rats, and this effect is independent of the salt sensitivity of hypertension and of dietary salt intake.

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Key Words: antibodies, monoclonal • natriuretic peptides, atrial • brain • microinjections • sodium, dietary • blood pressure

Our previous studies have demonstrated that salt-sensitive spontaneously hypertensive rats (SHR-S) from Taconic Farms (IBU3 colony, Germantown, N.Y.) exhibit significant increases in blood pressure and sympathetic outflow when fed a high-NaCl diet. In contrast, salt-resistant SHR (SHR-R) from Charles River Breeding Laboratories (Kingston, N.Y.) and normotensive control Wistar-Kyoto (WKY) rats do not develop these changes in blood pressure and sympathetic nervous system activity in response to dietary NaCl supplementation. We recently demonstrated that microinjection of monoclonal antibody (MAb) to atrial natriuretic peptide (ANP) into the caudal nucleus tractus solitarii (C-NTS) produces significant increases in blood pressure in SHR-S fed basal (1%) NaCl diets but not in WKY rats. Control injection of an equal volume of immunoglobulin G (IgG) into the C-NTS had no effect on blood pressure in SHR-S. Furthermore, control microinjection of the MAb into the hypoglossal nucleus, spinal trigeminal nucleus, or cuneate nucleus did not significantly alter blood pressure in either strain. These data suggest that endogenous ANP in C-NTS may be involved in the centrally mediated regulation of blood pressure in SHR-S.

Our laboratory has previously shown that arterial and cardiopulmonary baroreceptor reflex-mediated control of lumbar sympathetic nerve activity in SHR-S maintained on a basal NaCl diet is impaired. SHR-S maintained on a 1% NaCl diet have blunted baroreceptor reflex control of lumbar sympathetic nerve activity during acute increases or decreases in mean arterial pressure and sympathetic nervous system activity in response to dietary NaCl supplementation. We recently demonstrated that microinjection of monoclonal antibody (MAb) to atrial natriuretic peptide (ANP) into the caudal nucleus tractus solitarii (C-NTS) produces significant increases in blood pressure in SHR-S fed basal (1%) NaCl diets but not in WKY rats. Control injection of an equal volume of immunoglobulin G (IgG) into the C-NTS had no effect on blood pressure in SHR-S. Furthermore, control microinjection of the MAb into the hypoglossal nucleus, spinal trigeminal nucleus, or cuneate nucleus did not significantly alter blood pressure in either strain. These data suggest that endogenous ANP in C-NTS may be involved in the centrally mediated regulation of blood pressure in SHR-S.
Because the C-NTS plays an important role in baroreceptor reflex-mediated control of blood pressure and sympathetic outflow, both of which are sensitive to dietary NaCl intake in the SHR model, the present study was designed to test whether the pressor effect of blocking endogenous ANP in the C-NTS with MAb is affected by dietary NaCl supplementation in SHR-S. We studied SHR-S on 1% and 8% NaCl diets, SHR-R on a 1% NaCl diet, and WKY rats on 1% and 8% NaCl diets. Microinjection of IgG purified from mouse ascites fluid into the C-NTS served as a vehicle control. We found that blockade of endogenous ANP in the C-NTS increased blood pressure to the same extent in SHR-S fed 1% and 8% NaCl diets and in SHR-R fed the 1% NaCl diet but had no effect in WKY rats on either diet. Thus, endogenous ANP in the C-NTS mediates tonic control of blood pressure in both SHR-S and SHR-R but not in WKY rats, and this effect is independent of the NaCl sensitivity of hypertension and of dietary NaCl intake.

Methods

Male SHR-S and normotensive WKY control rats were obtained from Taconic Farms (IBU-3 colony, Germantown, N.Y.) at the same age. All rats were maintained four per cage at constant humidity (65±5%), temperature (24±1°C), and light cycle (6 AM–6 PM). Three days after arrival, one half of the SHR-S and WKY rats were placed at random on an 8% NaCl diet (ICN Biochemicals Purina Chow with 8% NaCl, Costa Mesa, Calif.), and the other half remained on the 1% NaCl diet (Ralston-Purina Diet 5001, St. Louis, Mo.). SHR-R were placed on the 1% NaCl diet.

Statistical Analysis

Results are expressed as mean±SEM. Analysis of variance (ANOVA) was performed to assess the differences in MAP and HR responses to MAb KY-ANP-II among the five experimental groups and to compare differences over time in each group. Significant differences were then subjected to Neuman-Keuls post hoc analysis. A value of p<0.05 was considered significant.

Results

Twenty-five SHR-S, 20 WKY rats, and nine SHR-R were studied. Histological examination confirmed that
cannulas were properly placed in the C-NTS in 21 SHR-S (10 on the 1% NaCl diet and 11 on the 8% NaCl diet), 17 WKY rats (10 on the 1% NaCl diet and seven on the 8% NaCl diet), and eight SHR-R on the 1% NaCl diet. In one SHR-S and one WKY rat, the cannula entered the cerebellum; in two SHR-S and one WKY rat, the cannula was placed in the hypoglossal nucleus. In one SHR-S and one WKY rat, the cannula penetrated the superior cerebellar vessels; in one SHR-R, the cannula damaged NTS tissue. These four SHR-S, three WKY rats, and one SHR-R were excluded from the analysis of experimental results. Examination of 1% thionine-stained sections revealed that the extent of spread of the injectate was <200 μm (Figure 1). Neurons near the injection tip had normal morphology in Nissl-stained sections, indicating little damage at this site.

SHR-S and SHR-R on the 1% NaCl diet had significantly higher pretreatment MAP than WKY rats on either diet at the time of study (Table 1). High NaCl intake caused significant increases in MAP in SHR-S but not in WKY rats. There was no significant difference in MAP between SHR-S and SHR-R on the 1% NaCl diet. There was no difference in pretreatment HR among the five experimental groups (Table 1).

Microinjection of MAb KY-ANP-II into the C-NTS resulted in significant increases in MAP in SHR-S on both diets and in SHR-R on the 1% NaCl diet but not in WKY rats on either diet (Figure 2, panel A). In SHR-S and SHR-R, the pressor responses to MAb KY-ANP-II began almost immediately after injection, reached maximal levels at 5 minutes, and returned to baseline by 40 minutes after injection. High NaCl intake did not significantly alter the pressor response to the microinjected MAb in SHR-S. There was no significant difference in the pressor response to the MAb KY-ANP-II between SHR-S on either diet and SHR-R on the 1% NaCl diet. Microinjection of MAb KY-ANP-II into the C-NTS did not alter HR significantly in any diet/strain group (Figure 2, panel B). Microinjection of control IgG into the C-NTS did not alter MAP or HR significantly in any diet/strain group (n=3 in each group). All control IgG injections were histologically verified as being within the C-NTS.

**Table 1.** Pretreatment Levels of Mean Arterial Pressure, Heart Rate, and Body Weight

<table>
<thead>
<tr>
<th>Strain/diet</th>
<th>MAP (mm Hg)</th>
<th>HR (bpm)</th>
<th>BW (g)</th>
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<tbody>
<tr>
<td>SHR-S 1% NaCl (n=10)</td>
<td>163.8±2.1*</td>
<td>382.0±8.9</td>
<td>232.9±1.7*</td>
</tr>
<tr>
<td>SHR-S 8% NaCl (n=11)</td>
<td>183.6±3.1**</td>
<td>381.8±8.7</td>
<td>232.5±2.6*</td>
</tr>
<tr>
<td>SHR-R 1% NaCl (n=8)</td>
<td>161.9±2.7*</td>
<td>385.6±10.8</td>
<td>232.4±4.7*</td>
</tr>
<tr>
<td>WKY 1% NaCl (n=10)</td>
<td>113.9±1.8</td>
<td>388.0±11.4</td>
<td>261.5±3.7</td>
</tr>
<tr>
<td>WKY 8% NaCl (n=7)</td>
<td>113.2±1.5</td>
<td>370.7±11.0</td>
<td>261.9±4.6</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HR, heart rate; BW, body weight; bpm, beats per minute; SHR-R, salt-sensitive spontaneously hypertensive rats; SHR-S, salt-sensitive spontaneously hypertensive rats; SHR-R, salt-resistant SHR; WKY, Wistar-Kyoto rats.

* *p<0.01 compared with WKY on either diet.
** tp<0.05 compared with 1% SHR-S or SHR-R.

**Discussion**

The present study demonstrated that microinjection of MAb KY-ANP-II into the C-NTS produced significant increases in MAP in SHR-S on either diet and in SHR-R on the 1% NaCl diet but not in WKY rats on either diet. There was no significant difference in the pressor response to the MAb among the three SHR groups. Control injection of an equal volume of IgG into the caudal NTS had no effect on MAP in any diet/strain group. These data suggest that endogenous ANP in the caudal NTS may be involved in the centrally mediated regulation of blood pressure in SHR-S and SHR-R but not in WKY rats and that this effect is independent of the NaCl sensitivity of hypertension and of dietary NaCl intake.

Previous studies have shown that ANP and its receptors are localized on cell bodies and nerve terminals in the NTS13-15 and that microinjection of ANP into NTS...
Figure 2. Graphs showing effects of microinjection of monoclonal antibody (MAb) to atrial natriuretic peptide into caudal nucleus tractus solitarii on mean arterial pressure (MAP) (panel A) and heart rate (HR) (panel B) in NaCl-sensitive spontaneously hypertensive rats (SHR-S), NaCl-resistant SHR (SHR-R), and Wistar-Kyoto (WKY) rats on 1% NaCl and 8% NaCl diets. **p<0.01, comparison between SHR and WKY groups over first 30 minutes (two-way analysis of variance). bpm, Beats per minute.

produces significant increases in the firing rate of NTS neurons associated with reductions in arterial pressure in anesthetized Wistar rats.\(^{16,17}\) In the latter studies, the majority of ANP-responsive sites (85%) were located between 0.55 mm rostral and 1.5 mm caudal to the obex, corresponding to the site of termination of baroreceptor and chemoreceptor afferents.\(^{17}\) It has also been shown that the single units excited by microinjection of ANP into NTS are excited by activation of arterial baroreceptors and inhibited by baroreceptor unloading.\(^{17}\) Furthermore, studies from our own laboratory have demonstrated that administration of exogenous ANP into the C-NTS blunts the bradycardiac response to systemic administration of phenylephrine.\(^{18}\) Together, these findings suggest that ANP in the NTS participates in baroreceptor reflex activation. Our observation that blockade of endogenous ANP in C-NTS with MAb KY-ANP-II caused rapid-onset pressor responses in SHR-S fed a basal NaCl diet supports this interpretation.\(^{4}\) The pressor response to microinjection of the anti-ANP antibody decreased progressively in magnitude with increasing distance rostral in the NTS, suggesting that the neuronal population involved was in the baroreceptor reflex pathway.

The finding that injection of MAb KY-ANP-II into the C-NTS has a pressor effect in SHR-S and SHR-R but not in WKY rats is consistent with previous evidence that ANP stores and receptor numbers are altered in SHR compared with WKY control rats. Studies from a number of laboratories have demonstrated that the ANP content of the hypothalamus,pons, and septum is significantly elevated in SHR compared with age-matched WKY controls.\(^{19-23}\) Furthermore, intravenous injection of ANP into the intact rat or application of ANP to brain slices in vitro causes greater increases in cGMP levels in hypothalamus and brainstem of SHR than of WKY rats.\(^{22,23}\) Thus, levels of ANP-sensitive particulate guanylate cyclase activity in the hypothalamus and brain stem appear to be greater in SHR than in WKY rats. The NTS contains a particularly high density of ANP binding sites,\(^{25,26}\) and NTS neurons display a significantly greater increase in cGMP after administration of ANP to slice preparations than do most other neurons in the central nervous system.\(^{27}\) Although the functional significance of these alterations in endogenous brain ANP and its second messenger with respect to cardiovascular regulation has only begun to be studied, the current results lend further support to the hypothesis that the central ANP system is altered in hypertensive rats.

The location of the neurons that innervate the C-NTS remains incompletely defined. Although in the rat the nodose ganglion contains ANP,\(^{28,29}\) and ANP-positive neurons have been identified in dorsal root sensory ganglia,\(^{30}\) it has yet to be demonstrated that the vagal afferents to the NTS use ANP as a neurotransmitter. Several areas that project to the NTS contain ANP immunoreactive-positive neurons, as do some NTS neurons themselves.\(^{25,21}\) Any of these could be responsible for the effects observed in response to microinjections of ANP or Mab KY-ANP-II into the C-NTS of SHR-S, but further studies are needed to define the neuronal pathways involved.

Combined with the results of previous immunocytochemical and electrophysiological studies of ANP and its receptors in NTS, the present finding that blockade of endogenous ANP in C-NTS elicits a pressor response in SHR is consistent with the hypothesis that NTS neurons are tonically activated by endogenous ANP in this model of hypertension. This would tend to buffer the hypertension in SHR, and the hypertension would become more severe when the ANP was removed, as by administration of an anti-ANP antibody. The absence of a pressor response to blockade of endogenous ANP in NTS of WKY rats suggests that NTS neurons are not tonically activated by ANP in the normotensive WKY rat. Tonic activation of the central baroreceptor reflex arc by ANP in the SHR could lead to blunting of baroreceptor reflex responsiveness to stimulation by volume expansion and phenylephrine infusion in SHR compared with WKY rats,\(^{5}\) contributing to the central defect in the baroreceptor reflex pathway previously described in SHR.\(^{32}\) The observation that the pressor responsiveness to blockade of endogenous ANP in the NTS was not altered by dietary NaCl supplementation in SHR-S is consistent with previous findings that sensitivities of the arterial and cardiopulmonary reflexes are shifted in opposite directions by dietary NaCl supplementation in this model, likely leading to no net change in overall baroreceptor reflex sensitivity. This, plus the finding that the magnitude of the pressor response...
response to injection of anti-ANP antibody into the NTS was the same in SHR-S on both diets as in SHR-R, suggests that tonic control of blood pressure by endogenous ANP in NTS of SHR is independent of the NaCl sensitivity of hypertension and of dietary NaCl intake.

References