Effect of Dietary Sodium Intake on the Responses to Bicuculline in the Paraventricular Nucleus of Rats

Gerald F. DiBona, S.Y. Jones

Abstract—The tachycardic, pressor, and renal sympathoexcitatory responses produced by administration of the γ-aminobutyric acid antagonist bicuculline into the paraventricular nucleus of the rat were modulated by physiological alterations in the activity of the renin-angiotensin system. To understand the effects of physiological alterations in the activity of the renin-angiotensin system on the central regulation of RSNA, alterations in dietary sodium intake have been used to change the level of activity of the renin-angiotensin system in normal rats. When injected into the lateral cerebral ventricle or directly into the RVLM of normal rats fed a normal-sodium diet (NNa), the AT1 receptor antagonists losartan and candesartan decreased the basal level of RSNA and reset the arterial baroreflex regulation of RSNA to a lower level of arterial pressure. Both of these effects were greater in rats on a low-sodium diet (LNa) or in rats with congestive heart failure (CHF) with a higher level of arterial pressure. Both of these effects were greater in rats on a low-sodium diet (LNa) or in rats with congestive heart failure (CHF), both of which have increased activity of the renin-angiotensin system, and were not significant in rats on a high-sodium diet (HNa) with decreased activity of the renin-angiotensin system. These results suggest that physiologically produced local alterations in the activity of the renin-angiotensin system acting on AT1 receptors in the RVLM participate in regulation of the basal level of RSNA and in arterial baroreflex regulation of RSNA.

To test this hypothesis, HR, MAP, and renal sympathetic nerve activity (RSNA) were measured in normal rats fed a normal-sodium diet (NNa), the AT1 receptor antagonists losartan and candesartan decreased the basal level of RSNA and reset the arterial baroreflex regulation of RSNA to a lower level of arterial pressure. Both of these effects were greater in rats on a low-sodium diet (LNa) or in rats with congestive heart failure (CHF), both of which have increased activity of the renin-angiotensin system, and were not significant in rats on a high-sodium diet (HNa) with decreased activity of the renin-angiotensin system. These results suggest that physiologically produced local alterations in the activity of the renin-angiotensin system acting on AT1 receptors in the RVLM participate in regulation of the basal level of RSNA and in arterial baroreflex regulation of RSNA.

The purpose of the present study was to examine whether physiologically produced alterations in the level of activity of the renin-angiotensin system would affect excitatory synaptic inputs to sympathoexcitatory neurons in the RVLM that arise from activation of the PVN. To test this hypothesis, HR, MAP, and renal sympathetic nerve activity (RSNA) were measured in normal rats fed a normal-sodium diet (NNa), the AT1 receptor antagonists losartan and candesartan decreased the basal level of RSNA and reset the arterial baroreflex regulation of RSNA to a lower level of arterial pressure. Both of these effects were greater in rats on a low-sodium diet (LNa) or in rats with congestive heart failure (CHF), both of which have increased activity of the renin-angiotensin system, and were not significant in rats on a high-sodium diet (HNa) with decreased activity of the renin-angiotensin system. These results suggest that physiologically produced local alterations in the activity of the renin-angiotensin system acting on AT1 receptors in the RVLM participate in regulation of the basal level of RSNA and in arterial baroreflex regulation of RSNA.

Key Words: renin-angiotensin system ■ sympathetic nervous system ■ sodium, dietary ■ rats, inbred strains

The rostral portion of the ventrolateral medulla (RVLM) contains sympathoexcitatory neurons with spinal projections that play a major role in the tonic and phasic regulation of sympathetic nerve activity and arterial pressure. It is known that the synaptic regulation of these RVLM neurons involves glutamate and GABA receptors, and there is increasing evidence to support the involvement of angiotensin receptors. The RVLM contains a high density of angiotensin (Ang) II type 1 (AT1) receptors. The microinjection of Ang II into the RVLM increases arterial pressure and sympathoexcitatory activity, effects that are mediated by AT1 receptors. Under in vitro conditions, Ang II has a depolarizing postsynaptic effect on RVLM neurons that is mediated by AT1 receptors. Disinhibitory activation of the paraventricular nucleus (PVN) by microinjection of the GABA receptor antagonist bicuculline produced increases in heart rate (HR), arterial pressure (MAP), and renal sympathetic nerve activity (RSNA) that were attenuated by the microinjection of AT1 receptor antagonists into the ipsilateral, but not contralateral, RVLM. These results indicate that excitatory synaptic inputs to sympathoexcitatory neurons in the RVLM that arise from the activation of the PVN are mediated predominantly by the action of Ang II on AT1 receptors in the RVLM.

To understand the effects of physiological alterations in the activity of the renin-angiotensin system on the central regulation of RSNA, alterations in dietary sodium intake have been used to change the level of activity of the renin-angiotensin system in normal rats. When injected into the lateral cerebral ventricle or directly into the RVLM of normal rats fed a normal-sodium diet (NNa), the AT1 receptor antagonists losartan and candesartan decreased the basal level of RSNA and reset the arterial baroreflex regulation of RSNA to a lower level of arterial pressure. Both of these effects were greater in rats on a low-sodium diet (LNa) or in rats with congestive heart failure (CHF), both of which have increased activity of the renin-angiotensin system, and were not significant in rats on a high-sodium diet (HNa) with decreased activity of the renin-angiotensin system. These results suggest that physiologically produced local alterations in the activity of the renin-angiotensin system acting on AT1 receptors in the RVLM participate in regulation of the basal level of RSNA and in arterial baroreflex regulation of RSNA.
MAP, and RSNA responses to the activation of PVN were determined in normal rats fed LNa, NNa, and HNa diets and in rats with CHF.

Methods
Male Sprague-Dawley rats (weight, 250 to 300 g) were used for all experiments. All procedures in animals were performed in compliance with the University of Iowa Policies and Guidelines Concerning the Use of Animals in Research and Teaching and the Guide for the Care and Use of Laboratory Animals (NIH publication No. 93-23, revised 1985).

Dietary Preparation
Before the experiments, rats were allowed to equilibrate in individual metabolism cages for a minimum of 1 week while being fed 1 of 2 diets; the average time on the diets was 13 days. HNa rats had free access to normal-sodium rat pellet diet (Na\(^+\), 163 mEq/kg; Harlan Teklad 7001) and 0.9% NaCl drinking fluid. NNa rats had free access to normal-sodium rat pellet diet (Na\(^+\), 163 mEq/kg; Harlan Teklad 7001) and tap water drinking fluid. LNa rats had free access to sodium-deficient rat pellet diet (Na\(^+\), 1.6 mEq/kg; ICN) and tap water drinking fluid. With the exception of sodium content, the diets were similar.

Anesthesia
Rats were anesthetized with 50 mg/kg methohexital IP for the preparation of CHF rats or 50 mg/kg pentobarbital IP for long procedures (PVN microinjections).

CHF Protocol
With techniques previously described and validated for our laboratory, a branch of the left renal nerve was prepared for the measurement of RSNA.\(^7,13\) Postmortem RSNA was recorded for 30 minutes; this value was subtracted from all experimental values of RSNA. In the CHF rats, the heart was removed and weighed. The heart was then cut open and placed in a solution of 0.1 mmol/L phosphate buffer, pH 7.4, containing 4% paraformaldehyde. Subsequently, 50-μm-thick coronal sections of frozen brain tissue were made. Microinjections sites were identified on the basis of the deposition of Alcian blue dye and referred to standard anatomic structures of the brain stem according to the atlas of Paxinos and Watson.\(^14\)

Procedures

Catheterization
After anesthesia, the rats were placed on a heated surgical table that was thermostatically regulated to maintain rectal temperature at 37°C. An endotracheal tube was placed, and the rats were mechanically ventilated with room air. Polyethylene catheters were placed in a jugular vein and a carotid artery. The jugular vein catheter was connected to a syringe pump that delivered isotonic saline at a rate of 0.05 mL/min for the duration of the experiment. The arterial catheter was connected to an electronic strain-gauge pressure transducer (Statham P23Db), of which the pulsatile output drove a cardiograph (model P44; Grass) for the measurement of HR. Both HR and MAP were recorded on a direct writing ink recorder (model 7D; Grass).

RSNA Electrode
Each rat was placed in the prone position with the head mounted in a stereotaxic apparatus. With techniques previously described and validated for our laboratory, a branch of the left renal nerve was placed for the measurement of RSNA.\(^7,13\)

PVN Microinjections
With the head of the rat placed in a stereotaxic frame, a small craniotomy was made near the bregma. Agents were dissolved in 0.9% NaCl and microinjected unilaterally into 1 PVN with coordinates of 1.8 mm caudal and 0.4 mm lateral to the bregma and 7.6 mm ventral.\(^14\) The PVN injection sites were randomly distributed between the left and the right side in each group of rats.

Microinjections were made with a 30-gauge needle within an outer 22-gauge guide cannula so that only the injection needle was lowered into the brain. Thirty minutes was allowed for equilibration after placement of the needle in the PVN. Microinjections were made in a volume of 40 nL of 0.1 nmol bicuculline during a 5-second period with a microsyringe pump connected to the needle via polyethylene tubing.\(^7\)

Experimental Protocol

Microinjections
HR, MAP, and RSNA were continuously recorded. After a control period of 5 minutes, a microinjection was made into the PVN (unilateral). Experimental period data were recorded for 9 minutes after the microinjection. At the end of each experiment, the injection sites were marked with 40 nL of 5% Alcian blue. In the CHF rats, the carotid arterial catheter was then advanced into the left ventricle for measurement of left ventricular end-diastolic pressure. Before the rats were killed with an overdose of pentobarbital, a sample of aortic blood was taken, and the plasma was separated and frozen for later determination of plasma renin activity (PRA). Postmortem RSNA was recorded for 30 minutes; this value was subtracted from all experimental values of RSNA. In the CHF rats, the heart was removed and weighed. The brain was removed and placed for >24 hours in a solution of 0.1 mmol/L phosphate buffer, pH 7.4, containing 4% paraformaldehyde. Subsequently, 50-μm-thick coronal sections of frozen brain tissue were made. Microinjections sites were identified on the basis of the deposition of Alcian blue dye and referred to standard anatomic structures of the brain stem according to the atlas of Paxinos and Watson.\(^14\)

Analytical Measurements
HR, MAP, and RSNA were digitized at 5 Hz and averaged during 1-second time bins. For each microinjection, the absolute values of HR, MAP, and RSNA during the control period were averaged and set to 100%. Experimental period data were expressed as a percent of the control period value. To take into account both the maximum change in HR, MAP, and RSNA from control and the duration of the response, the area under the curve (AUC) for the control period (0 to 60 seconds) was calculated (measured as percent control per second) and subtracted from the AUC for the experimental period (60 to 840 seconds).

PRA was measured through determination of the generation rate of Ang I per milliliter of plasma per hour with a radioimmunoassay and \(^125\)I-labeled Ang I as a tracer (Rianen; DuPont Medical Products). The sensitivity of the PRA assay was 0.05 pg · mL\(^{-1} \cdot h^{-1}\). The analytic coefficients of variation were 4.8% within assays and 12.6% between assays.

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### Basal Values of MAP, HR, RSNA, and PRA in the 4 Groups of Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>LNa (n=10)</th>
<th>NNa (n=9)</th>
<th>HNa (n=9)</th>
<th>CHF (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>100±5</td>
<td>101±4</td>
<td>108±5</td>
<td>93±5</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>364±13</td>
<td>358±19</td>
<td>301±6</td>
<td>379±22</td>
</tr>
<tr>
<td>RSNA, μV</td>
<td>1.82±0.14</td>
<td>1.50±0.12*</td>
<td>1.14±0.09</td>
<td>2.15±0.15</td>
</tr>
<tr>
<td>PRA, ng · mL(^{-1} · min^{-1})</td>
<td>18.2±1.9</td>
<td>2.9±0.6*</td>
<td>0.6±0.2</td>
<td>21.6±1.1</td>
</tr>
</tbody>
</table>

*P<0.05 or better vs all other groups within variable.
Statistical analysis was performed with ANOVA with the subsequent use of Scheffé’s method for simultaneous comparisons within groups and the subsequent use of the F ratio and modified statistic for nonsimultaneous comparisons between groups. A significance level of 5% was chosen. Data in the text, tables, and figures are expressed as mean±SEM.

Results

Baseline Data
MAP and HR did not differ among the 4 groups (Table). For both RSNA and PRA, the values were greater in LNa and CHF rats than in NNa rats, and values in NNa rats were in turn greater than those in HNa rats. In the CHF group, left ventricular end-diastolic pressure averaged 13.2±0.8 mm Hg in CHF rats compared with normal values of ∼3.0 mm Hg in sham-operated or control rats in our laboratory. Heart weight-to–body weight ratios averaged 0.80±0.04% compared with normal values of ∼0.40% in sham-operated or control rats in our laboratory.

Responses to Bicuculline
The responses of HR, MAP, and RSNA to bicuculline administered into the PVN are shown as percent of control in Figures 1 through 3. The peak HR responses (Figure 1) were small (∼2%), and there were no significant differences among the responses of the various groups of rats. The peak MAP responses (Figure 2) were slightly larger (10%), with the greatest response being seen in CHF rats; however, there were no significant differences among the responses of the various groups of rats. The peak RSNA responses (Figure 3) were the largest (70%), with the rank order of response magnitude being CHF>LNa>NNa>HNa; each group was significantly different from every other group (P<0.05 or better).

To take into account both maximum change in HR, MAP, and RSNA from control and the duration of the response, the response to bicuculline was calculated as increase in the AUC. For HR and MAP, although there was a trend for the responses to decrease from CHF and LNa to NNa to HNa, this was not significant (data not shown). However, as seen in Figure 4, the responses of RSNA exhibited a significant relationship such that CHF>LNa>NNa>HNa. Also, each group was significantly different from every other group (P<0.05 or better). In addition, the mean responses of RSNA (Figure 4) were correlated with the mean PRA values (Table) for the 4 groups (r=0.95, P<0.05).

Histological examination of the brain indicated that the majority of the centers of all injection sites were located either within or on the border of the PVN, extending from the level of 1.4 to 2.1 mm caudal to bregma (Figure 5).

Discussion
Previous investigators demonstrated that the injection of bicuculline into the PVN increased HR, MAP, and
In the present experiments, although the increases in RSNA were similar to those previously observed, the increases in HR and MAP were less than those previously observed. This may be accounted for by the use of pentobarbital anesthesia (compared with chloral hydrate, urethane, chloralose, and thiobutabarbitral in previous studies) and a relatively low dose of bicuculline (0.1 nmol). In addition, the HR and MAP responses are dependent on interactions between the changes in sympathetic nerve activity to the heart and vasculature and various cardiac and vascular mechanisms, some of which may be influenced by the changes in dietary sodium intake. Conversely, the RSNA responses represent directly measured, centrally regulated sympathetic outflow, not requiring interaction with peripheral mechanisms.

With respect to RSNA responses to bicuculline, compared with NNa rats, the response was increased in LNa rats and decreased in HNa rats. These dietary sodium–induced alterations in the RSNA responses to bicuculline were significantly correlated with the effects on the renin-angiotensin system, as reflected by PRA. Thus, LNa rats, with increased activity of the renin-angiotensin system, had increased RSNA responses to bicuculline, whereas HNa rats, with decreased activity of the renin-angiotensin system, had decreased RSNA responses to bicuculline. In addition, CHF rats, with an even greater increase in the activity of the renin-angiotensin system, had the greatest RSNA response to bicuculline.

Although there was a clear effect of dietary sodium intake on the RSNA response to bicuculline, this was less evident in the HR or MAP responses to bicuculline. With the relatively small HR and MAP responses to bicuculline of NNa rats, it
may have been difficult to detect further decreases in HNa rats, but this should not have hampered the detection of increases in LNa rats. Increased HR and MAP responses were not seen in LNa rats with increased activity of the renin-angiotensin system, but the HR and MAP responses to bicuculline were greatest in CHF rats with the greatest degree of activation of the renin-angiotensin system.

These results suggest that physiological alterations in the activity of the renin-angiotensin system affect the responses to excitatory synaptic inputs to sympathoexcitatory neurons in the RVLM that arise from PVN activation. It is known that these responses to PVN activation are also markedly attenuated by the administration of AT1 receptor antagonists into the RVLM. These results suggest that endogenous Ang II acting locally on AT1 receptors in the RVLM contributes to these responses to PVN activation. This interpretation is in agreement with the observation that responses to the injection of AT1 receptor antagonists into the RVLM are substantially influenced by changes in dietary sodium intake that physiologically altered the activity of the renin-angiotensin system. Thus, the decreases in RSNA were most marked in LNa rats wherein PRA was stimulated and least evident in HNa rats when PRA was suppressed. Taken together, these results suggest that alterations in dietary sodium intake influence the amount of Ang II present in the RVLM, being highest with LNa rats and lowest with HNa rats. The sources for this endogenous Ang II could be nerve fibers in the RVLM, in which Ang II immunoreactivity has been demonstrated. Because the PVN contains neurons that are immunoreactive for Ang II and the PVN projects directly to the RVLM, it may be that PVN activation results in the release of Ang II from the terminals (located in RVLM) of axons that originate from Ang II–containing cell bodies in the PVN. Alternatively, local brain Ang II may be formed from angiotensinogen, produced by astrocytes, and released into the extracellular fluid. This would allow the possibility that Ang II locally formed in the RVLM could be taken up into nerve terminals and subsequently released. Whether the source of the endogenous Ang II in the RVLM is Ang II–containing projections from the PVN, local synthesis, or other, the present results suggest that it is capable of being modulated by physiological alterations in dietary sodium intake sufficient to modulate the activity of the circulating renin-angiotensin system (ie, PRA).

Alternative possibilities derive from the consideration of general ligand-receptor interactions in other tissues. Thus, changes in dietary sodium intake, via alterations in the prevailing concentration of Ang II in RVLM, would be expected to influence the number of AT1 receptors (ie, receptor upregulation or downregulation). Alternatively, these changes in either Ang II concentration or the number of AT1 receptors could occur at the level of the PVN itself. For example, an increase in the prevailing concentration of Ang II in PVN could increase the tonic activity of PVN neurons that project to RVLM sympathoexcitatory neurons and increase their degree of activation after the removal of GABAergic inhibition.

It must be acknowledged, however, that an analysis based on an understanding of Ang II–AT1 receptor interactions in tissues outside the central nervous system may not rigorously apply to the central nervous system. Difficulties in the measurement of local tissue concentrations of components of the renin-angiotensin system in the central nervous system have not permitted a clear understanding of the effects of alterations in dietary sodium intake on activity of the renin-angiotensin system in the central nervous system.

A LNa diet is known to increase both the plasma and cerebrospinal fluid concentrations of Ang II in humans. That the levels are not correlated suggests that the regulation of cerebrospinal fluid Ang II concentration is independent of that of plasma, raising the possibility that it reflects the activity of the brain renin-angiotensin system. In dogs with 2-kidney, 1-clip hypertension, there were parallel increases in plasma and cerebrospinal fluid Ang II concentrations. However, in dogs made hypertensive with the intravenous administration of Ang II during a 7-day period, cerebrospinal fluid Ang II concentrations were unaffected, whereas plasma Ang II concentrations were markedly increased. Thus, the extent to which measurement of the components of the renin-angiotensin system in peripheral blood provides information on the activity of the brain renin-angiotensin system is unclear.

Further evidence that the influence of dietary sodium alterations on the ligand-receptor interaction may be different in the central nervous system comes from studies in rats that were fed LNa, NNa, and HNa diets. Whole brain AT1a receptors were increased with a LNa diet compared with a NNa diet. AT1a receptors were increased, however, with a HNa diet compared with a NNa diet.

In conclusion, the present study demonstrates that the tachycardic, pressor, and renal sympathoexcitatory responses to PVN activation are significantly influenced by physiological alterations in the activity of the renin-angiotensin system produced by changes in dietary sodium intake. Because these responses are known to be mediated by the action of local Ang II on AT1 receptors in the RVLM, it appears that alterations in dietary sodium intake that act via changes in the activity of the renin-angiotensin system participate in this local process in the RVLM.

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