Increased Dietary Salt Sensitizes Vasomotor Neurons of the Rostral Ventrolateral Medulla

Corinn M. Pawloski-Dahm, Frank J. Gordon

Excess dietary sodium is a major contributing factor to the incidence and severity of hypertension. However, the precise mechanism or mechanisms by which salt contributes to the severity of hypertension are unknown. The region of the rostral ventrolateral medulla (RVLM) is a principal brain stem locus critical for the regulation of arterial blood pressure by the sympathetic nervous system. The purpose of this study was to determine if excess dietary sodium chloride might alter the function or responsiveness of neurons in the RVLM. Male Sprague-Dawley rats were given either tap water or 0.9% sodium chloride solution to drink for 10 to 14 days. Excess sodium chloride did not affect baseline blood pressure. However, when neurons of the RVLM were stimulated by microinjections of L-glutamate, evoked increases in arterial pressure were potentiated in rats given sodium chloride. Augmented pressor responses could not be accounted for by increased vascular reactivity because both groups responded similarly to intravenously administered phenylephrine and norepinephrine. Additionally, electrical stimulation of descending spinal sympathoexcitatory axons produced identical pressor responses in both groups, indicating that altered synaptic transmission at central or peripheral neuroeffector junctions distal to the RVLM could not explain enhanced pressor responses produced by direct stimulation of RVLM cell somata. Finally, impaired arterial baroreceptor reflexes could not account for augmented RVLM pressor responses, as depressor and bradycardic responses produced by electrical stimulation of aortic baroreceptor afferents were not reduced in rats given excess dietary sodium chloride. These results indicate that increased dietary salt intake sensitizes RVLM sympathoexcitatory neurons and may predispose toward the exaggerated expression of hypertension, suggesting a potential link between salt, hypertension, and the brain. (Hypertension. 1993;22:929-933.)

KEY WORDS • blood pressure • brain stem • hypertension, sodium-dependent • sympathetic nervous system

The contribution of dietary sodium chloride (NaCl) to the pathogenesis of hypertension has been debated for almost half a century.1,2 Although an increase in NaCl intake is not invariably associated with elevated arterial blood pressure, a large subset of individuals exhibits fluctuations in arterial pressure when daily salt intake is varied.3-5 However, a diet high in salt alone is not sufficient to produce hypertension in otherwise normotensive individuals but instead plays a permissive role in enabling the exaggerated expression of hypertension.3 Salt sensitivity of blood pressure has been studied intensively, but the mechanism or mechanisms by which increased dietary salt imparts enhanced sensitivity to hypertensive stimuli are not known.

In several experimental models of hypertension, excess NaCl intake is essential for either the development or full expression of hypertension.6-11 Additionally, the central nervous system (CNS) and peripheral sympathetic nervous system contribute significantly to the maintenance of elevated arterial pressure in many of these salt-sensitive models.12-15 For instance, most forms of salt-sensitive hypertension can be prevented or reversed by destruction of various regions within the CNS.16-20 Furthermore, there is an enhanced contribution of the sympathetic nervous system to the maintenance of elevated arterial pressure in hypertension.7,10,17,18,21,22 Similarly, the sympathetic nervous system contributes to salt-sensitive hypertension in humans, as evidenced by inappropriately high levels of circulating norepinephrine23-25 and elevated peripheral vascular resistance5,26 coincident with sodium loading in salt-sensitive hypertensive individuals. Collectively, these observations provide substantial support for the contention that central mechanisms controlling sympathetic nervous system regulation of arterial blood pressure play an important role in the pathogenesis of salt-sensitive hypertension.

We hypothesized that increased dietary NaCl might predispose toward central sympathetic hyperresponsiveness. The region of the rostral ventrolateral medulla (RVLM) has come to be appreciated as a principal site responsible for the integration and regulation of sympathetic vasomotor tone.27,28 Electrical or chemical stimulation of the RVLM produces sympathetically mediated increases in arterial pressure and heart rate. Because of its pivotal role in cardiovascular regulation by the CNS, we hypothesized that sympathetically mediated increases in arterial pressure elicited by stimula-
tion of RVLM neurons would be potentiated by excess dietary NaCl.

Methods

Animals
Male Sprague-Dawley rats (Holtzman strain, 250 to 300 g; Harlan Sprague Dawley Inc, Indianapolis, Ind) were housed together and given free access to standard rat chow containing 1% NaCl (Purina Mills, Inc). For 10 to 14 days rats were given either tap water or isotonic saline (0.9% NaCl) ad libitum as their only source of fluid. All rats were treated in accordance with the guidelines for institutional animal care and use of Emory University.

Surgical Procedure
After 10 to 14 days of drinking either tap water or isotonic saline, all rats were anesthetized with urethane (1.2 to 1.3 g/kg IP) and prepared with femoral arterial and venous catheters for arterial blood pressure measurement and intravenous drug delivery, respectively. Rats were artificially respirated and paralyzed and were included in one of the following three protocols. During all experiments, arterial blood samples were obtained periodically for measurement of blood gases and pH. Respiratory rate and/or volume was adjusted for maintenance of blood gases and pH within normal limits.

Stimulation of the Rostral Ventrolateral Medulla
Rats were placed in a stereotaxic device, and the ventral surface of the medulla was exposed. Multibarreled glass pipettes were placed into the RVLM, and microinjections were made using techniques described in detail previously. Microinjections into the RVLM of various doses of L-glutamate (GLU) (0.05 to 10 nmol/50 nL) were made over a period of 5 to 15 seconds, and changes in mean arterial pressure (MAP) and heart rate were recorded. Each rat received in random order two to five doses of GLU with at least 5 minutes allowed between each injection. Changes in MAP and heart rate produced by graded doses of phenylephrine (0.5 to 4 μg/kg IV) were also recorded in these rats. At the end of each experiment, 50 nL of 1.0% alcin blue dye was microinjected into the RVLM, and the rat was perfused transcardially with isotonic saline. Brains were removed and the ventral surface of the medulla was exposed. Multibarreled, 1-millisecond pulse duration) at graded frequencies (2 to 16 Hz) using bipolar hook electrodes, and changes in MAP and heart rate were recorded.

Stimulation of the Aortic Depressor Nerve
Through a ventral midcervical incision, the aortic depressor nerve was isolated and cut. The central end of the nerve was electrically stimulated for 15 seconds (5.0 V, 2.0-milliseconds pulse duration) at graded frequencies (2 to 16 Hz) using bipolar hook electrodes, and changes in MAP and heart rate were recorded.

Statistics
Data are expressed as mean±SEM and were analyzed by analysis of variance followed by the Newman-Keuls test for pairwise comparison between individual means. Criteria for statistical significance was set at a value of P<.05.

Results
Baseline MAP was not different between rats drinking water (96±2 mm Hg; n=16) and isotonic saline (94±4 mm Hg, n=18). Thus, increased dietary NaCl alone was not sufficient to elevate arterial blood pressure. Heart rate was slightly but significantly (P<.05 lower in rats drinking isotonic saline (354±8 beats per minute) compared with rats drinking water (388±15 beats per minute). Microinjections of graded doses of GLU into the RVLM produced dose-related increases in MAP and heart rate. Rats maintained on saline demonstrated greater pressor responses at every dose of GLU tested than did rats given water (Fig 1A). Tachycardic responses ranged between 5 and 30 beats per minute and were not significantly different between the two groups (Table). Autonomic ganglionic blockade with hexamethonium (30 mg/kg IV) or chlorisondamine (5 mg/kg IV) lowered arterial pressure to the same level in both rat groups (water: 50±2 mm Hg, n=16; NaCl: 53±3 mm Hg, n=18). In rats in which technical difficulties did not interfere, pressor responses (Fig 1A) evoked by the largest dose of GLU administered to each animal (range, 0.5 to 50 nmol/50 nL) were eliminated after ganglionic blockade and averaged 0±0 mm Hg for rats drinking water (n=12) and 0±1 mm Hg for rats drinking NaCl (n=13). These results demonstrate that cardiovascular responses produced by RVLM injections of GLU were neurogenically mediated. Furthermore, compared with rats drinking water, NaCl did not affect the sympathetic neural contribution to the maintenance of arterial blood pressure in these normotensive animals. Pressor responses produced by intravenous phenylephrine were identical in both rat groups (Fig 1B), as were reflex-mediated bradycardic responses (Table). Although we did not measure fluid and electrolyte balance in these animals, several previous studies have shown that substituting 0.9% to 1% NaCl for water as a drinking fluid has no effect on plasma sodium, plasma osmolality, plasma volume, or extracellular fluid volume and that sodium balance is achieved within 1 to 3 days after high salt consumption is begun.

Enhancement of RVLM pressor responses by excess dietary NaCl was not limited to one supplier's colony of...
Fig 1. Line graphs show changes in mean arterial pressure (MAP) produced by microinjections of L-glutamate (L-GLU) into the rostral ventrolateral medulla (A) and intravenous injections of graded doses of phenylephrine (B). Number of rats is shown in parentheses. Data are expressed as mean±SEM of responses elicited in rats drinking either tap water or 0.9% NaCl for 10 to 14 days before experimentation. *P<.05 NaCI vs water.

Changes in Heart Rate

<table>
<thead>
<tr>
<th>L-Glutamate in RVLM, nmol/50 nL</th>
<th>Water (n=4-8)</th>
<th>NaCl (n=4-9)</th>
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<tbody>
<tr>
<td>0.05</td>
<td>4±1</td>
<td>11±2</td>
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<tr>
<td>0.1</td>
<td>10±4</td>
<td>15±4</td>
</tr>
<tr>
<td>0.5</td>
<td>21±6</td>
<td>25±3</td>
</tr>
<tr>
<td>1.0</td>
<td>24±9</td>
<td>35±7</td>
</tr>
<tr>
<td>5.0</td>
<td>32±11</td>
<td>33±8</td>
</tr>
<tr>
<td>10.0</td>
<td>28±2</td>
<td>32±3</td>
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</table>

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<tr>
<th>Phenylephrine, µg/kg IV</th>
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<th>NaCl (n=8)</th>
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<tbody>
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<td>3.0</td>
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<td>-21±2</td>
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<tr>
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<th>Norepinephrine, µg/kg IV</th>
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<th>NaCl (n=5)</th>
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<tr>
<td>0.09</td>
<td>5±1</td>
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<tr>
<td>0.27</td>
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<tr>
<td>0.81</td>
<td>34±6</td>
<td>27±4</td>
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</table>

RVLM indicates rostral ventrolateral medulla. Values are mean±SEM and are shown in beats per minute.

Discussion

The principal new finding of these experiments is that pressor responses evoked by stimulation of the RVLM are augmented by excess dietary NaCl. The excitatory amino acid GLU was used to stimulate RVLM neurons because GLU activates virtually all neurons in the CNS and selectively depolarizes cell somata without affecting axons of passage.33 Augmented RVLM pressor responses produced by increased dietary NaCl were observed in rats obtained from two different suppliers. This result indicates that potentiation of pressor responses by NaCl is reproducible across substrains of Sprague-Dawley rats and was not due to unique genetic factors peculiar to a particular colony of animals.
Although these results suggest that excess dietary NaCl might sensitize RVLM neurons to excitatory stimulation, several other mechanisms also might contribute to the augmented pressor responses we observed. Principal among these is an increase in vascular reactivity to adrenergic stimulation.36,37 This seems unlikely because pressor responses to intravenous phenylephrine were identical for rats drinking water or saline when baroreceptor reflexes were intact. Furthermore, in spinally transected animals in which reflex effects on the vasculature were removed, vascular reactivity to graded doses of norepinephrine, the endogenous neurotransmitter of sympathetic nerves, also was not affected by increased dietary NaCl. Therefore, increased vascular responsiveness cannot account for the enhanced pressor responses evoked by RVLM stimulation in rats ingesting excess NaCl.

Several mechanisms other than increased vascular reactivity might be responsible for enhanced pressor responses evoked by GLU stimulation of RVLM neurons in rats given excess NaCl. These include facilitated synaptic transmission potentially due to increased release of, decreased inactivation of, or enhanced postjunctional sensitivity to neurotransmitters in neural pathways efferent from the RVLM (eg, at sympathetic preganglionic neurons, sympathetic ganglia, or vascular muscle neuroeffector junctions). After transection of the spinal cord, electrical stimulation of descending spinal sympathoexcitatory axons allowed us to assess the possible contribution of these mechanisms without directly activating the cell somata or dendrites of RVLM cells. Although it is possible that spinal cord stimulation may have activated sympathoexcitatory pathways in addition to those originating from RVLM cell somata, it is the bulboспinal RVLM projection that appears to play the major role in central cardiovascular regulation.27,28 Pressor responses produced by electrical stimulation of the spinal cord were not different between rats drinking water and those drinking saline. This result suggests that augmented pressor responses produced by direct stimulation of RVLM neurons were probably not due to enhanced sympathetic neural transmission, either in the CNS or periphery, efferent from brain stem vasomotor neurons.

Finally, we examined the possibility that impaired arterial baroreceptor reflexes might contribute to the enhanced RVLM pressor responses observed in rats maintained on isotonic saline. If baroreceptor reflex-mediated feedback inhibition of RVLM neurons was impaired in rats drinking NaCl, this might explain the augmented pressor responses produced by GLU stimulation of the RVLM. However, reflex-mediated depressor responses produced by electrical stimulation of the aortic nerve were not impaired in rats drinking saline. This result is consistent with the finding that increased NaCl intake also did not reduce reflex-mediated bradycardia produced by raising arterial pressure with phenylephrine. Therefore, impaired baroreceptor reflexes cannot account for the potentiated RVLM pressor responses of rats consuming excess dietary NaCl.

In summary, the augmented pressor responses produced by direct activation of sympathoexcitatory neurons in the RVLM of rats consuming excess dietary NaCl cannot be attributed to enhanced vascular reactivity to adrenergic stimulation, alterations in synaptic transmission at neuroeffector junctions distal from the brain stem, or impaired arterial baroreceptor reflexes. We conclude that increased dietary NaCl specifically sensitizes the cell somata and/or dendrites of vasomotor neurons in the RVLM to excitatory stimulation. The mechanism or mechanisms contributing to this sensitization are unknown but could include, among other possibilities, changes in central neurotransmitter receptor number, affinity, or signal transduction characteristics as well as alterations in neural and/or humoral influences emanating from the CNS and/or periphery that might affect the function of RVLM neurons. It should be emphasized that rats drinking isotonic saline were not hypertensive but instead responded with greater increases in arterial pressure to GLU stimulation of the RVLM. Based on these results, we speculate that excess dietary NaCl consumption may predispose toward the development and maintenance of elevated arterial pressure in salt-sensitive hypertension by an action, at least in part, within the CNS.

Acknowledgments

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