Increased Dietary Salt Enhances Sympathoexcitatory and Sympathoinhibitory Responses From the Rostral Ventrolateral Medulla

Julye M. Adams, Christopher J. Madden, Alan F. Sved, Sean D. Stocker

Abstract—Increased dietary salt exaggerates arterial blood pressure (ABP) responses evoked from the rostral ventrolateral medulla (RVLM). The present study determined whether these enhanced pressor responses were directly attributable to a greater increase in sympathetic nerve activity (SNA) and whether these enhanced responses were balanced by a greater responsiveness of RVLM neurons to inhibitory input. Male Sprague-Dawley rats were fed normal chow and given access to either water or a 1% NaCl solution for 14 days. Injection of L-glutamate (0.03, 0.1, 1.0, and 3.0 nmol) into the RVLM produced a significantly greater increase in renal SNA, splanchnic SNA, and ABP in rats drinking 1% NaCl versus water. Conversely, injection of the inhibitory amino acid y-aminobutyric acid (0.1, 1.0, and 10 nmol) into the RVLM produced significantly greater decreases in renal SNA, splanchnic SNA, and ABP of rats drinking 1% NaCl versus water. These enhanced SNA and ABP responses to L-glutamate and y-aminobutyric acid were not observed in rats drinking 1% NaCl for 1 or 7 days but were present in rats drinking 1% NaCl for 21 days. Moreover, the dietary salt-induced enhancement of both sympathoexcitatory and sympathoinhibitory responses from the RVLM persisted after the 1% NaCl solution was replaced with water for 1, but not 7, days. These findings indicate that the potentiated ABP responses observed previously are mediated by parallel changes in SNA, and these responses depend on a slowly developing and reversible form of neuronal plasticity. *(Hypertension. 2007;50:354-359.)*

Key Words: sympathetic nervous system ∙ blood pressure ∙ glutamate ∙ GABA ∙ sodium chloride

Elevated dietary salt intake is a contributing factor to the pathogenesis of hypertension in both humans and experimental animal models.1–4 Elevated dietary salt does not invariably increase arterial blood pressure (ABP) but does contribute to the development of arterial hypertension or exaggerate the severity of hypertension in salt-sensitive individuals and experimental models. Several mechanisms have been proposed to explain the salt sensitivity of ABP, including increased water and sodium retention with resultant expansion of blood volume or a neurogenically mediated increase in total peripheral resistance.1,3,4

The hypothesis that elevated dietary salt increases ABP through neurogenic mechanisms arises from several lines of evidence. First, salt-sensitive hypertension in both humans and experimental models is associated with elevations in sympathetic nerve activity (SNA).4 Second, Pawloski-Dahm and Gordon6 reported that excitation of neurons in the rostral ventrolateral medulla (RVLM) with microinjection of L-glutamate produced significantly larger increases in ABP of rats drinking 0.9% NaCl versus those drinking water for 14 days. The RVLM contains bulbospinal neurons that provide the major excitatory drive to sympathetic preganglionic vasomotor neurons in the thoracic and lumbar segments of the spinal cord.6,7 Moreover, elevated dietary salt enhanced the pressor response to several other distinct neuroactive compounds injected into the RVLM.8 Additional evidence indicates that the mechanism of these enhanced pressor responses resides within the RVLM, because vascular reactivity5,8,9 and the pressor response to spinal cord stimulation5,10 were not altered by increased dietary salt intake. Although these observations suggest that elevated dietary salt enhances pressor responses from the RVLM through greater increases in SNA rather than changes in peripheral mechanisms regulating ABP, there is no empirical evidence to directly support this notion.

The purpose of the present study was to determine whether the enhanced pressor responses evoked from the RVLM in rats with elevated dietary salt were directly attributable to a greater increase in SNA and whether these enhanced responses were balanced by a greater responsiveness of RVLM neurons to inhibitory input. Additional experiments were performed to characterize the time course of these enhanced responses and to determine whether these changes persisted after dietary salt intake was returned to normal levels.
Materials and Methods

Animals
Male Sprague-Dawley rats (Charles River Laboratories) weighing 250 to 300 g were housed individually in a temperature-controlled room (22°C to 23°C) with a 14-hour:10-hour light:dark cycle (lights on at 7 AM). Rats were fed standard rat chow containing 0.23% NaCl (Harlan Teklad Global Diet #2018) and were given access to deionized water for ≥7 days before experiments began. All of the experimental and surgical procedures conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Kentucky Institutional Animal Care and Use Committee.

General Procedures
Rats were anesthetized with isoflurane (2% to 4% in 100% O₂) and instrumented with catheters in the femoral artery and vein. Then, isoflurane anesthesia was replaced with a mixture of α-chloralose (75 mg/kg, Sigma Aldrich) and urethane (750 mg/kg, Sigma Aldrich) given IV over 10 minutes. Rats were artificially ventilated with oxygen-enriched room air, paralyzed with gallamine triethiodide (25 mg/kg per hour at 25 °C in 5% dextrose, Sigma Aldrich), and end-tidal partial pressure of CO₂ was maintained between 4% and 5%. Body temperature was maintained at 37±1°C with a water-circulating pad. Renal and/or splanchnic postganglionic SNAs were recorded as described previously in our laboratory. An adequate depth of anesthesia was assessed by either the absence of a withdrawal reflex (before neuromuscular blockade) or a pressor response to a foot pinch. Supplemental doses of anesthetic (10% of initial dose) were given as necessary.

RVLM Microinjections
Rats were placed into a stereotaxic head frame with the incisor bar positioned 11 mm below the interaural line. A single-barrel glass micropipette (OD: 20 to 40 μm) angled 20° rostrally was lowered into the left RVLM at the following coordinates with reference to the dorsal surface and caudal tip of area postrema: 1.8 mm lateral, 1.8 mm rostral, and 2.8 mm ventral. Initially, L-glutamate (1 nmol) was injected into the RVLM at 3 different sites separated by 300 μm in the rostral-caudal plane to identify the site that produced the largest increase in ABP; subsequent injections were performed at these coordinates. Then, the pipette was removed, rinsed, and filled with glutamate or γ-aminobutyric acid (GABA). All of the injections (60 nL, dissolved in isotonic saline) were performed over 5 seconds using a pneumatic picopump. At the end of experiments, a blood sample (0.5 mL) was collected from the arterial line to determine hematocrit, plasma protein concentration, plasma Na⁺ and K⁺ concentration, and blood volume (for more detail, see the data supplement available online at http://hyper.ahajournals.org). Microinjection sites were marked with 2% Chicago Sky blue. Brainstems were sectioned at 50 μm using a cryostat, and injection sites were mapped onto standardized sections using the atlas of Paxinos and Watson.

Experimental Design
An initial set of experiments was performed to determine whether increased dietary salt intake enhances SNA responses produced by injection of L-glutamate or GABA into the RVLM. Rats were fed normal rat chow and given access to either 1% NaCl or deionized water as the sole source of fluid for 14 days. Food and fluid intake were measured daily. Once the RVLM was located functionally with L-glutamate (1 nmol), subsequent injections of L-glutamate (0.01, 0.1 and 3.0 nmol) were performed randomly with ≥5 minutes between injections. Separate animals were prepared similarly, except that GABA (0.03, 0.1, and 10 nmol) was injected into the RVLM. Both renal and splanchnic SNAs were not recorded simultaneously in every experiment. Because the changes in ABP to L-glutamate or GABA were not different whether one or both nerve recordings were successful, the data were combined.

Two additional sets of experiments were performed to characterize the time course of these enhanced responses. In the first experiment, rats were given access to normal chow and 1% NaCl solution for 1, 7, and 21 days (n=10, 11, and 7, respectively). In the second experiment, rats were given access to normal chow and 1% NaCl solution for 14 days. Then, the 1% NaCl solution was replaced with deionized water for either 1 (n=6) or 7 days (n=7). Once the RVLM was located functionally by injection of L-glutamate (1 nmol), subsequent injections of L-glutamate (0.1 nmol) or GABA (0.1 nmol) were performed randomly with a minimum of 5 minutes between injections.

Data Analysis
All of the data are expressed as mean±SE. The 0.05-second peak SNA and ABP response was compared with a 30-second baseline segment immediately before the injection. Changes in integrated renal and splanchnic SNAs were calculated by subtracting background noise after hexamethonium (30 mg/kg, IV) and were expressed as a percentage change of the baseline value. All of the variables were analyzed by a 1- or 2-way ANOVA with repeated measures when appropriate. All of the posthoc tests were performed by independent or paired t tests with a layered Bonferroni correction. A P<0.05 was considered statistically significant.

Results
Increased Dietary Salt Enhances Both Sympathoexcitatory and Sympathoinhibitory Responses Evoked From the RVLM
A major goal of the present study was to determine whether the enhanced pressor response from the RVLM in rats with elevated dietary salt was directly attributable to a greater increase in SNA. Microinjection of L-glutamate dose-dependently increased renal SNA, splanchnic SNA, and mean ABP in rats drinking water or 1% NaCl for 14 days (Figure 1). Interestingly, the increase in renal SNA, splanchnic SNA, and mean ABP was significantly greater in rats drinking 1% NaCl versus water at each dose of L-glutamate tested. The duration, but not latency, of the SNA and ABP responses was significantly longer in rats drinking 1% NaCl (data not shown). The change in renal versus splanchnic SNA was not different within a group. Baseline mean ABP (110±3 versus 116±2 mm Hg) and heart rate (412±9 versus 399±9 bpm) did not differ between rats drinking water versus 1% NaCl, respectively, though the daily fluid intake was significantly greater in rats drinking 1% NaCl versus water (56.7±1.8 versus 35.3±1.0 mL; P<0.01).

A second goal of the present study was to determine whether increased dietary salt also enhanced sympathoinhibitory and depressor responses evoked by injection of GABA into the RVLM. Microinjection of GABA dose-dependently decreased renal SNA, splanchnic SNA, and mean ABP in rats drinking 1% NaCl or water for 14 days (Figure 2). Interestingly, the decrease in renal SNA, splanchnic SNA, and mean ABP was significantly greater in rats drinking 1% NaCl versus water for 14 days at each dose of GABA tested. The decrease in renal SNA versus splanchnic SNA was not different within groups. Again, the duration, but not latency, of the SNA and ABP responses was significantly longer in rats drinking 1% NaCl (data not shown).

Time Course of Enhanced Sympathoexcitatory and Sympathoinhibitory Responses
To characterize the time course of these enhanced responses, L-glutamate and GABA were injected into the RVLM of rats drinking 1% NaCl versus water for 7 days. As expected, the enhanced pressor response was greater in rats drinking 1% NaCl versus water. As shown in Figure 3, the duration, but not latency, of the SNA and ABP responses was significantly greater in rats drinking 1% NaCl versus water (56.7±1.8 versus 35.3±1.0 mL; P<0.01).
drinking 1% NaCl for 1, 7, and 21 days. The magnitude of the renal SNA and ABP responses of these rats was compared with rats that drank water or 1% NaCl for 14 days. Microinjection of glutamate and GABA produced similar changes in renal SNA and ABP of rats drinking 1% NaCl for 1 and 7 days versus control rats (Figure 3). However, the renal SNA and ABP responses to both L-glutamate and GABA were significantly greater in rats drinking 1% NaCl for 14 and 21 days versus those drinking water. There were no differences in these responses between rats drinking 1% NaCl for 14 and 21 days. Baseline mean ABP of rats drinking 1% NaCl for 7 and 21 days was slightly elevated versus control rats (control: 110±3, 1 day; 117±2, 7 days; 122±2, 21 days; 124±3 mm Hg; P<0.05). Baseline heart rate was significantly lower in rats drinking 1% NaCl for 1, 7, and 21 days versus control rats (see the data supplement).

To determine whether the enhanced responsiveness of RVLM neurons persisted after rats were returned to a normal salt intake, a separate group of rats drank 1% NaCl for 14 days followed by water for 1 or 7 days. Changes in renal SNA

Figure 1. A, Peak increase in mean ABP, renal SNA, and splanchnic SNA to injection of L-glutamate into the RVLM was significantly greater in rats drinking 1% NaCl (n=6 to 15) versus control rats drinking water (n=6 to 14) for 14 days. B, Individual example of ABP, mean ABP, and renal and splanchnic SNA after microinjection of 1 nmol of L-glutamate into the RVLM (see the data supplement for examples at other doses). *Significant difference vs control group at same dose (P<0.05). ▼, Microinjection of L-glutamate.

Figure 2. A, Peak decrease in mean ABP, renal SNA, and splanchnic SNA to injection of GABA into the RVLM was significantly greater in rats drinking 1% NaCl (n=6 to 9) vs control rats drinking water (n=6 to 11) for 14 days. B, Individual examples of ABP, mean ABP, and renal and splanchnic SNA after microinjection of 10 nmol of GABA into the RVLM (see the data supplement for examples at other doses). *Significant difference vs control group at same dose (P<0.05). ▼, Microinjection of GABA.
and mean ABP of these rats in response to L-glutamate or GABA were compared with those of control rats that drank deionized water or 1% NaCl for 14 days. Injection of L-glutamate and GABA into the RVLM of rats returned to water for 1 day produced exaggerated renal SNA and ABP responses compared with those of control rats (Figure 4). Indeed, the responses observed in these rats were not significantly different than those drinking 1% NaCl for 14 days. However, both renal SNA and ABP responses to both L-glutamate and GABA were not different between control rats and rats returned to water for 7 days after drinking 1% NaCl (Figure 4). Baseline mean ABP was slightly elevated in rats drinking 1% NaCl for 14 days followed by water for 1 day, but there were no differences in baseline heart rate (see the data supplement).

In each of the above experiments, there were no differences in the plasma Na\(^+\) and K\(^+\) concentration, plasma protein concentration, hematocrit, plasma or blood volume, and daily food intake. Rats given access to 1% NaCl ingested significantly larger amounts of fluid than those ingesting water (see the data supplement).

Figure 5 illustrates the location of injection sites in the RVLM for all of the rats used in the present study. Note that the injection sites were centered in the RVLM as defined by the triangular region located 0 to 500 \(\mu\)m caudal to the caudal tip of the facial nucleus and bordered dorsally by nucleus ambiguus, medially by the inferior olive or pyramidal tracts, and laterally by the spinal trigeminal nucleus. There was no apparent difference in the location of injection sites between rats drinking water or 1% NaCl.

**Discussion**

Previous studies suggest that elevated dietary salt alters the excitability of sympathetic-regulatory networks, especially those emanating from the RVLM. However, there was previously no empirical evidence to indicate that elevated dietary salt directly affected RVLM sympathetic-regulatory neurons and SNA rather than peripheral mechanisms regulating ABP. The present findings provide several new key observations: (1) elevated dietary salt enhances both SNA and ABP responses to injection of L-glutamate into the RVLM; (2) elevated dietary salt produced parallel changes in the responsiveness of RVLM neurons to GABA; (3) a chronic increase in dietary salt intake was required for these enhanced responses; and (4) the dietary salt-induced enhancement of SNA and ABP persisted for a short duration after the salt intake was returned to normal levels. These findings indicate that the potentiated pressor responses observed previously are likely mediated by parallel changes in SNA, and these responses depend on a slowly developing and reversible form of neuronal plasticity.

Previous studies have reported that increased dietary salt enhances the pressor responses to injection of L-glutamate and several other distinct excitatory neuroactive compounds into the RVLM. Because vascular reactivity has been shown repeatedly to be similar between rats on a low- versus high-salt intake, an exaggerated SNA response was hy-
somatic pressor response,8 a reflex that depends on glutamatergic and GABAergic tone even if release of either neurotransmitter has not changed. This enhanced responsiveness has functional significance as elevated dietary salt intake enhances the excitability of preganglionic neurons, electrical stimulation of the dorsolateral funiculus5 or spinal cord 10 produces excitability of preganglionic neurons), electrical stimulation in both renal and splanchnic SNA to micro-injection of L-glutamate at every dose tested. Although it is possible that the enhanced SNA and ABP responses are because of an altered responsiveness of sympathetic-regulatory circuits downstream of the RVLM (ie, altered excitability of preganglionic neurons), electrical stimulation of the dorsolateral funiculus5 or spinal cord10 produces similar increases in ABP between rats on a low- versus high-salt intake. This enhanced responsiveness has functional significance as elevated dietary salt intake enhances the somatic pressor response,8 a reflex that depends on glutamatergic neurotransmission in the RVLM.15

Increased dietary salt intake does not invariably increase SNA and ABP. In fact, evidence from both humans and laboratory animals indicates that elevated dietary salt either does not affect or inhibits SNA.5,8,16–18 However, the present findings together with previous reports5,8,13 indicate that increased dietary salt intake enhances the responsiveness of RVLM neurons to excitatory amino acid inputs. Therefore, this enhanced glutamate responsiveness may be expected to increase SNA unless it is balanced by a decreased glutamate release, an increased GABAergic input, or a parallel change in the responsiveness of RVLM neurons to inhibitory input. The present findings provide strong support for this latter possibility, because rats drinking 1% NaCl for 14 days displayed greater sympathoinhibitory responses at every dose of GABA injected into the RVLM. Similar responses have been observed in rats maintained on 8% versus 1% NaCl chow.8 Together with the present findings, these observations suggest that increased dietary salt intake may directly alter the excitability or sensitivity of RVLM sympathetic-regulatory neurons through a general change in membrane conductance rather than an increase in receptor density and/or neurotransmitter clearance. Such possibilities include changes in K+ conductance, because this would permit an enhanced responsiveness to a number of excitatory and inhibitory synaptic inputs.

The dietary salt-induced enhancement of SNA and ABP responses from the RVLM appears similar to the enhanced pressor and depressor responses reported in water-deprived rats.19 Water deprivation increases plasma osmolality resulting in elevated sympathetic outflow through activation of a glutamatergic pathway from the hypothalamic paraventricular nucleus to the RVLM.11,19,20 Because of the apparent differences between rats ingesting salt versus those deprived of water, it is not clear whether the enhanced responsiveness of RVLM neurons in both conditions is mediated by similar mechanisms. First, sympathetic outflow is elevated during water deprivation,11,21 and this depends on excitatory amino acid receptor activation within the RVLM.19 In contrast, elevated salt intake either decreases5,8,16–18 or does not affect SNA.5,8 Second, it is not known whether the enhanced pressor and depressor responses evoked from the RVLM in water-deprived rats reflect exaggerated SNA responses.19 Third, water-deprived rats have an elevated plasma osmolality and sodium concentration,19 whereas we did not observe a difference in plasma sodium concentration or blood volume between rats ingesting water versus 1% NaCl despite a daily increase in sodium intake (≈2.7 mEq Na+ versus ≈12.4 mEq Na+). However, elevated dietary salt intake has been reported to increase plasma Na+ concentration when comparisons are made within individual rats.22,23 Such a mechanism would suggest that peripheral or central sodium sensors may activate circuits that alter the excitability of RVLM neurons. We also cannot exclude the possibility that factors secondary to the elevated salt intake (ie, circulating angiotensin II levels) mediate the enhanced responsiveness.

One important question is whether increased dietary salt selectively alters the excitability of neurons within the RVLM versus a more generalized effect on sympathetic-regulatory networks. In this regard, excitation of neurons in the nucleus that a chronic increase in dietary salt is needed, because the exaggerated SNA and ABP responses to t-glutamate or GABA were not observed in rats drinking 1% NaCl for 1 or 7 days. A similar time course has been observed in rats fed 1% and 8% NaCl chow (S. Ito and A.F. Sved, unpublished observations, 1997). Moreover, these changes persisted for a short duration after dietary salt intake was returned to normal levels as the enhanced SNA and ABP responses were observed in rats that drank 1% NaCl for 14 days followed by water for 1, but not 7, days. Altogether, the time course of these responses suggests that some form of neuronal plasticity (ie, gene expression, receptor density, or neurotransmitter clearance) may underlie the effect of increased dietary salt on the excitability of RVLM neurons. It is noteworthy that the pressor responses to injection of other distinct neuroactive compounds into the RVLM are enhanced in rats maintained on 8% versus 1% NaCl chow.8 Together with the present findings, these observations suggest that increased dietary salt intake increases in ABP between rats on a low- versus high-salt intake. This enhanced responsiveness has functional significance as elevated dietary salt intake enhances the somatic pressor response,8 a reflex that depends on glutamatergic neurotransmission in the RVLM.15

Increased dietary salt intake does not invariably increase SNA and ABP. In fact, evidence from both humans and laboratory animals indicates that elevated dietary salt either does not affect or inhibits SNA.5,8,16–18 However, the present findings together with previous reports5,8,13 indicate that increased dietary salt intake enhances the responsiveness of RVLM neurons to excitatory amino acid inputs. Therefore, this enhanced glutamate responsiveness may be expected to increase SNA unless it is balanced by a decreased glutamate release, an increased GABAergic input, or a parallel change in the responsiveness of RVLM neurons to inhibitory input. The present findings provide strong support for this latter possibility, because rats drinking 1% NaCl for 14 days displayed greater sympathoinhibitory responses at every dose of GABA injected into the RVLM. Similar responses have been observed in rats maintained on 8% versus 1% NaCl chow (C.J. Madden and A.F. Sved, unpublished observations, 2000). This parallel change in the responsiveness of RVLM neurons to inhibitory input permits a balance between glutamatergic and GABAergic tone even if release of either neurotransmitter has not changed.

The specific mechanism underlying the enhanced responsiveness of the RVLM during increased dietary salt is currently unknown. Findings from the present study indicate
tractus solitarius and the caudal ventrolateral medulla produces potentiated depressor responses in rats with an elevated dietary salt.\textsuperscript{8,13,14} Because these responses depend on GABAergic neurotransmission in the RVLM\textsuperscript{6,7} and the present findings clearly show that elevated dietary salt enhances GABAergic responses from the RVLM, it is likely that the potentiated responses evoked from these other regions are completely attributable to the enhanced responsiveness of the RVLM. This also explains the potentiated depressor responses to stimulation of the aortic depressor nerve in rats drinking 0.9\% NaCl.\textsuperscript{1} Interestingly, preliminary data suggest that increased dietary salt enhances the pressor response to electrical stimulation of the lateral parabrachial nucleus but not the anterior hypothalamus\textsuperscript{24}; the former but not the latter response depends on neurotransmission within the RVLM.\textsuperscript{15} Collectively, these observations indicate that increased dietary salt may selectively affect the excitability of RVLM neurons rather than a more generalized alteration in sympathetic-regulatory networks.

**Perspectives**

Salt-sensitive hypertension in humans and experimental animal models is associated with elevations in sympathetic vasomotor tone. Together with previous observations,\textsuperscript{5,8} the present study indicates that increased dietary salt enhances SNA and ABP responses to injection of both l-glutamate and GABA into the RVLM. The enhancement of both excitatory and inhibitory responses may explain why increased dietary salt, by itself, does not increase SNA and ABP or alter the balance of inhibitory or excitatory tone within RVLM. More importantly, the ability of elevated dietary salt to alter the excitability or gain of RVLM neurons now permits a small change in synaptic drive or balance of excitatory and inhibitory tone to produce a large increase in SNA and ABP. Support for this model arises from studies performed in Dahl salt-resistant and -sensitive rats in which elevated dietary salt potentiated the pressor responses to injection of l-glutamate in Dahl-resistant rats, but blockade of excitatory amino acid receptors did not lower ABP.\textsuperscript{25} However, injection of kynurenic acid in the RVLM of Dahl salt-sensitive rats fed 8\% NaCl chow significantly lowered ABP.\textsuperscript{25} Whether similar synaptic mechanisms within the RVLM underlie neurogenically mediated increases in total peripheral resistance and ABP in other forms of salt-sensitive hypertension remains to be investigated.

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**Disclosures**

None.

**References**

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**Figure I.** Individual examples of ABP, mean ABP, and renal and splanchnic SNA in response to microinjection of (A) 0.01, (B) 0.1, and (C) 3 nmol L-glutamate into the RVLM of rats drinking water or 1% NaCl for 14 days. Summary data are presented in Figure 1 of the manuscript. ▼ injection of glutamate
Figure II. Individual examples of ABP, mean ABP, renal SNA and splanchnic SNA in response to microinjection of (A) 0.03 and (B) 0.1 nmol GABA into the RVLM of rats drinking water (control) or 1% NaCl for 14 days. Summary data are presented in Figure 2 of manuscript. ▼ injection of GABA.