Increased dietary salt intake enhances the exercise pressor reflex

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Yamauchi K, Tsuchimochi H, Stone AJ, Stocker SD, Kaufman MP. Increased dietary salt intake enhances the exercise pressor reflex. Am J Physiol Heart Circ Physiol 306: H450–H454, 2014. First published November 22, 2013; doi:10.1152/ajpheart.00813.2013.— Increased dietary salt in rats has been shown to sensitize central sympathetic circuits and enhance sympathetic responses to several stressors, including hyperinsulinemia, intracerebroventricular injection of angiotensin, and electrical stimulation of sciatic nerve afferents. These findings prompted us to test the hypothesis that increased dietary salt enhanced the exercise pressor reflex. Male Sprague-Dawley rats were fed 0.1% (low) or 4.0% (high) NaCl chow for 2 to 3 wk. On the day of the experiment, the rats were decerebrated, and the hind limb muscles were statically contracted for 30 s by electrically stimulating the cut peripheral ends of the L4 and L5 ventral roots. We found that contraction produced a significantly greater increase in mean arterial pressure of rats fed 4.0% (n = 26) vs. 0.1% (n = 22) NaCl (24 ± 2 vs. 15 ± 2 mmHg, respectively; P < 0.05). Baseline mean arterial pressure was not different between groups (0.1%, 77 ± 4 vs. 4.0% NaCl, 80 ± 3 mmHg). Likewise, the tension time indexes were not different between the two groups (P = 0.42). Section of the L4 and L5 dorsal roots greatly attenuated both the pressor and cardioaccelerator responses to contraction in both groups of rats, an effect showing that the responses were reflex in origin. Finally, electrical stimulation of the lumbar sympathetic chain produced similar increases in mean arterial pressure and decreases in femoral arterial blood flow and conductance between rats fed 0.1% vs. 4.0% NaCl diets. We conclude that increased dietary salt enhances the exercise pressor reflex.

static contraction; muscle afferents; autonomic nervous system

ACCUMULATING EVIDENCE SUGGESTS that increased dietary salt (NaCl) enhances the responsiveness of the sympathetic nervous system to a variety of central and peripheral stimuli (26) (17) (16). Surprisingly, this evidence includes stimuli that both increase and decrease the sympathetic outflow. With respect to the former, glutamate-induced stimulation of cell bodies in the rostral ventrolateral medulla (RVLM) or electrical stimulation of somatic afferent axons evoked greater increases in both mean arterial pressure and sympathetic nerve discharge in rats fed high versus low NaCl (26) (17) (2) (1). With respect to the latter, GABA-induced inhibition of the RVLM or stimulation of the aortic depressor nerve evoked a greater decrease in arterial blood pressure, heart rate, and/or sympathetic nerve activity in rats fed a high versus low NaCl diet (26) (16) (2). Consistent with this notion, increased levels of dietary NaCl have reported to increase baroreflex gain of sympathetic nerve activity in both rats (16) and humans (14).

The effect of a diet high in salt on the cardiovascular responses to exercise is not known. Two neural mechanisms are responsible for the increases in arterial pressure, heart rate, and sympathetic nerve discharge that are evoked by exercise. The first mechanism is central command (13, 20), and the second is the exercise pressor reflex (10, 22, 24). There is a large amount of evidence demonstrating that the exercise pressor reflex plays an important role in humans in evoking the cardiovascular responses to exercise (4, 5, 12, 25, 31, 32). Previous studies have demonstrated that the exercise pressor reflex, in part, depends on neurotransmission in the RVLM (8, 21). These findings prompted us to determine whether salt ingestion amplified the exercise pressor reflex in decerebrated, unanesthetized rats.

METHODS

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University, Hershey Medical Center. Adult male rats (Sprague-Dawley, n = 60, weighing 380 ± 6 g) were used in this study. Rats were housed in a temperature-controlled room (24 ± 1°C) with a 12-h:12-h light/dark cycle and were fed a standard rodent diet containing low NaCl (0.1%, D17020; Research Diets) or high NaCl (4.0%, D17013; Research Diets) for 2 to 3 wk before the experiment began. Food and tap water were available ad libitum throughout the study.

Surgical preparation. Rats were anesthetized with a mixture of 4% isoflurane and 100% oxygen. The right jugular vein and common carotid artery were cannulated (PE-50) for the delivery of fluids as well as for the measurement of arterial blood pressure, respectively. The carotid arterial catheter was connected to a pressure transducer (model P23 XL; Statham). Heart rate (HR) was calculated beat to beat from the arterial pressure pulse (Gould Biotach). The trachea was cannulated, and the lungs were ventilated mechanically (Harvard Apparatus). Arterial blood gases and pH were measured by an automated blood-gas analyzer (model ABL-80, Radiometer). PCO2 and arterial pH were maintained within normal range by either adjusting ventilation or by intravenous administration of sodium bicarbonate (8.5%). A rectal temperature probe was inserted, and the core body temperature of the animal was maintained at 37° to 38°C by a water-perfused heating pad and a lamp. The rat was then placed in a Kopf stereotaxic frame. Dexamethasone (0.2 mg) was injected intravenously just before the decerebration procedure to minimize brainstem edema. The left common carotid artery was tied off, and a precollicular decerebration was performed. The plane of section was less than 1 millimeter anterior to the superior colliculi. All neural tissue rostral to the section was removed, bleeding was controlled, and the cranial cavity was packed with cotton. In our experiments, rats were decerebrated instead of anesthetized because the preponderance of the evidence indicates that anesthesia prevents the exercise pressor reflex in this species (29).

We performed a laminectomy to expose the lower lumbar and sacral portions of the spinal cord (L2-L5). The rat was then secured in a customized spinal frame by clamps placed on the rostral lumbar vertebrae and pelvis. Using the skin on the back, we formed a pool that was filled with warm (37°C) mineral oil. The dura was cut and reflected allowing visual identification of the spinal roots. The left L4 and L5 ventral roots were identified and cut close to their exits from the spinal cord. The calcaneal bone of a left hind limb was severed, and the triceps surae muscles were isolated. Once the surgeries were completed, the anesthesia was withdrawn, and the lungs were ventilated with room air. A recovery period of 60 min after decerebration...
Experiment 2. A second experiment was performed to assess whether the dietary NaCl altered the vascular responsiveness to sympathetic neural input. Animals were prepared as described above. In addition, the lumbar sympathetic chain was isolated through a ventral midline incision and placed onto a stimulating electrode. A flow probe (Transonic) was placed on the right femoral artery. After animals stabilized, we measured arterial pressure and right femoral flow while stimulating the lumbar sympathetic chain (1 ms pulse, 50 μA) for 1 min at 1, 2.5, and 5 Hz in a randomized order.

Measurement of electrolytes. In a subset of animals, plasma electrolytes (Na⁺, Cl⁻, and K⁺) were analyzed in rats fed 0.1% or 4.0% NaCl using a blood collected from the carotid arterial catheter and an ABL-80 analyzer (Radiometer America, Westlake, OH).

Data analysis. Arterial blood pressure, HR, and tension developed by the triceps surae muscles were recorded with a Spike 2 data acquisition system (CED; Cambridge) and stored on a computer hard drive (Dell). Mean arterial pressure is expressed in millimeters of mercury and HR in beats per minute (beats/min). The tension-time index (TTI) was calculated by integrating the area between the tension trace and the baseline level (Spit 2) and is expressed in kilogram-seconds (kg·s).

All values are expressed as means ± SE. Statistical analyses of arterial pressure, heart rate, and tension development were performed with unpaired t-tests. Statistical analysis of femoral vascular conductance was performed with a one-between-one within ANOVA in which dietary salt intake was the between subjects main effect and frequency of the pulses applied to the sympathetic chain was the within subjects main effect. The criterion for statistical significance was set at P < 0.05.

RESULTS

Baseline mean arterial pressure did not differ between rats fed a 0.1% vs. 4.0% NaCl diet (Fig. 1A). The high-salt diet tended to decrease baseline heart rate (Fig. 1B), although the effect was not significant (P = 0.06). There were also no differences in plasma sodium, chloride, or potassium concentration between rats fed a 0.1% vs. 4.0% NaCl diet (Table 1).

To test whether a high-salt diet enhances the exercise pressor reflex, we measured the pressor and cardioaccelerator responses to static contraction of the hind limb muscles in decerebrate rats. Static contraction significantly increased mean arterial pressure (P < 0.05) and heart rate (P < 0.05) above baseline levels in rats fed 0.1% or 4.0% NaCl. Interestingly, we found that the pressor and cardioaccelerator responses to static contraction were significantly greater in rats fed 4.0% NaCl (25 ± 2 mmHg, 17 ± 1 beats/min, n = 26; Fig. 1) versus those fed 0.1% NaCl (16 ± 2 mmHg, 11 ± 1 beats/min, n = 22; Fig. 1). The tension-time index was not different between groups (Fig. 1C).

To confirm the contribution of muscle afferents to the pressor and cardioaccelerator responses during static contraction of rats fed 0.1% and 4.0% NaCl, we cut the L4 and L5 dorsal roots and repeated the static contraction. As expected, the pressor and cardioaccelerator responses to static contraction were significantly different between rats fed 0.1% NaCl versus those fed 4.0% NaCl.

Table 1. Plasma electrolytes of rats fed a low (0.1%) or high (4.0%) NaCl diet for 2 to 3 wk

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>Plasma [Na⁺], mM</th>
<th>Plasma [Cl⁻], mM</th>
<th>Plasma [K⁺], mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>14</td>
<td>136.6 ± 1.6</td>
<td>99.9 ± 1.1</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>High</td>
<td>16</td>
<td>139.8 ± 1.5</td>
<td>102.4 ± 1.2</td>
<td>4.8 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE.
tion were greatly attenuated by transection of the L4 and L5 dorsal roots in both groups (Fig. 2).

A final set of experiments was performed to determine whether the greater pressor and cardioaccelerator responses to static muscle contraction in rats fed 4.0% NaCl could be attributed to a greater vascular responsiveness to sympathetic neural input. Electrical stimulation of the lumbar sympathetic chain in rats fed 0.1% or 4.0% NaCl produced frequency-dependent increases in mean arterial pressure and decreases in femoral blood flow and conductance (Fig. 3). However, there were no differences in these responses between rats fed 0.1% vs. 4.0% NaCl at any frequency.

**DISCUSSION**

We have shown that the pressor and cardioaccelerator responses to static contraction of the hind limb muscles in rats fed a high-salt diet for 2 to 3 wk were significantly larger than the pressor and cardioaccelerator responses to contraction of these muscles in rats fed a low-salt diet for the same period of time. As expected and as shown many times before, the pressor and cardioaccelerator responses to contraction were greatly attenuated by cutting the L4 and 5 dorsal roots. When considered together, these two findings demonstrate that the exercise pressor reflex in rats is amplified by salt ingestion.

Several factors might be responsible for the salt-induced amplification of the exercise pressor reflex in our experiments. The first is sensitization of the brainstem circuitry that increased the sympathetic outflow to the vasculature and decreased the parasympathetic outflow to the heart. Neurons in the RVLM may be particularly important in this regard because their stimulation with glutamate evoked larger pressor and sympathetic nerve responses in rats fed a high- versus low-salt diet (26) (17) (2). In contrast, electrical stimulation of the

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**Fig. 2.** The pressor (A) and cardioaccelerator (B) responses to static contraction before (white bars) and after (black bars) cutting L4 and L5 dorsal roots in rats fed low (0.1%, n = 5) or high (4.0%, n = 7) NaCl diet. #Significant difference (P < 0.05) between before versus after dorsal root transection.

**Fig. 3.** The pressor (A), femoral blood flow (B), and femoral artery vascular conductance (C) responses to lumber nerve stimulation (1-ms pulses of 50 μA at randomized frequencies of 1, 2.5, or 5 Hz for 1 min) in rats fed low (0.1%: ○, n = 6) or high (4.0%: □, n = 6) NaCl diet. There were no statistical differences in any variable at any frequency between rats fed low versus high NaCl diets.
dorsolateral funiculus to activate descending spinal sympathoexcitatory neurons evoked identical increases in sympathetic nerve activity and arterial blood pressure in rats fed low- or high-salt diets (3) (26). In addition, electrical stimulation of sciatic nerve afferents produced greater increases in renal sympathetic nerve activity and/or arterial blood pressure of rats fed a high- versus low-salt diet (17). Similarly, the sympathoexcitatory response to hyperinsulinemia is enhanced in rats fed a high-salt diet (17). Interestingly, the sympathoexcitatory responses to activation of sciatic afferents or insulin is mediated by neurotransmission in the RVLM (7, 19). When considered together, the above evidence suggests the larger pressor reflex is best explained by a salt-induced sensitization of medullary circuits controlling the sympathetic outflow in rats fed a high-salt diet.

The second factor that might be responsible for the salt-induced amplification of the exercise pressor reflex is the reactivity of the vasculature to neurotransmitter released by sympathetic postganglionic terminals. To address this factor, we stimulated the lumbar sympathetic chain while measuring changes in femoral artery blood flow and arterial pressure. We found that the decrease in femoral artery conductance evoked by stimulation of the lumbar sympathetic chain was the same in rats fed a high- versus a low-salt diet. These findings are consistent with previous reports that increased dietary salt does not alter vascular reactivity to sympathetic stimulation in rats (17, 18, 26). Altogether, these observations suggest that the changes in the vasculature to neurotransmitter release cannot explain the amplification of the exercise pressor reflex in rats fed a high- versus low-salt diet.

The third factor is salt-induced sensitization of the group III and IV afferents comprising the sensory arm of the exercise pressor reflex (11, 22). At present there is no evidence that salt ingestion sensitizes these afferents to any stimulus arising in contracting skeletal muscle. There is evidence, however, that muscle metabolites can sensitize thin fiber afferents to a variety of stimuli. For example, cyclooxygenase metabolites of arachidonic acid are known to increase the mechanical sensitivity of group III afferents to static contraction (28). Similarly, bradykinin is known to increase the responsiveness of the group III afferents to mechanical distortion of their receptive fields (23).

Although dietary salt intake is a contributing factor to the pathogenesis of hypertension, in the present study the rats fed a high-salt diet did not display an elevated arterial blood pressure versus those fed a low-salt diet. Indeed, the standard laboratory rat is salt resistant (30). Our finding is consistent with previous reports concerning the effect of salt ingestion on baseline arterial pressure in either Wistar-Kyoto (16) or Sprague-Dawley rats (17, 26). In addition, salt ingestion in our experiments had a tendency to decrease baseline heart rate, with the effect approaching but not reaching the criterion level for significance. This too is consistent with previous reports showing that salt ingestion decreased baseline heart rate, an effect which was at times significant (27) and at other times not significant (17). We speculate that the cause of the baseline cardiac slowing in rats fed high-salt diets may have been a sensitized baroreflex (26) (16). Alternatively, the cause may have been an expanded plasma volume, which in turn increased baseline discharge of the aortic and cardiac baroreceptors in rats fed a high-salt diet.

How does the ingestion of dietary sodium amplify the exercise pressor reflex? In short, the answer is unknown. However, a recent study reported that chronic lesion of either the anteroventral third ventricle region or the organum vasculosum of the lamina terminalis prevented the exaggerated sympathoexcitatory and pressor responses to RVLM injection of glutamate or angiotensin II in rats ingesting isotonic saline versus water (1). The same study reported that rats drinking isotonic saline versus water had significantly higher plasma sodium concentration at night but not during the day (1). Although the ingestion of 4% vs. 0.1% NaCl chow tended to raise plasma sodium (and chloride) concentration by ~3 mM in the present study, the values were not statistically significant ($P = 0.07$). Plasma osmolality was not measured. Using a within-subject design, Habeker and colleagues (15) reported significant differences in plasma sodium concentration of rats ingesting a sodium-deficient diet (<0.02%) versus a high-sodium diet (4% NaCl). These differences were similar in magnitude to differences in the present study (~3 mM) and were much larger at night than during the day. Interestingly, previous studies have reported that neurons of the lamina terminalis can detect changes in osmolality less than 1% (9). Altogether, these observations raise the possibility that the ingestion of excess dietary NaCl raises plasma sodium concentrations or osmolality (during the day or night) to activate neurons of the lamina terminalis and alter the excitability of sympathetic regulatory neurons. Future experiments are needed to test this possibility.

In conclusion, we have shown that the exercise pressor reflex is amplified by salt ingestion in rats. Our finding may have some clinical relevance in that an amplified reflex pressor response to static exercise is likely to increase myocardial oxygen demand by forcing the heart to pump against a higher afterload. In individuals with severe coronary artery disease, such a situation might be detrimental, causing ischemia and possibly fibrillation. Likewise, in individuals with peripheral artery disease salt ingestion would amplify the exercise pressor reflex, which is already exaggerated by the lack of an adequate arterial blood supply to the contracting limb muscles (6).

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GRANTS

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REFERENCES


