Dietary Salt Intake Exaggerates Sympathetic Reflexes and Increases Blood Pressure Variability in Normotensive Rats

Sarah S. Simmonds, Jennifer Lay, Sean D. Stocker

Abstract—Previous studies have reported that chronic increases in dietary salt intake enhance sympathetic nerve activity and arterial blood pressure (ABP) responses evoked from brain stem nuclei of normotensive, salt-resistant rats. The purpose of the present study was to determine whether this sensitization results in exaggerated sympathetic nerve activity and ABP responses during activation of various cardiovascular reflexes and also increases ABP variability. Male Sprague–Dawley rats were fed 0.1% NaCl chow (low), 0.5% NaCl chow (medium), 4.0% NaCl chow (high) for 14 to 17 days. Then, the animals were prepared for recordings of lumbar, renal, and splanchnic sympathetic nerve activity and ABP. The level of dietary salt intake directly correlated with the magnitude of sympathetic nerve activity and ABP responses to electrical stimulation of sciatic afferents or intracerebroventricular infusion of 0.6 mol/L or 1.0 mol/L NaCl. Similarly, there was a direct correlation between the level of dietary salt intake and the sympathoinhibitory responses produced by acute volume expansion and stimulation of the aortic depressor nerve or cervical vagal afferents. In contrast, dietary salt intake did not affect the sympathetic and ABP responses to chemoreflex activation produced by hypoxia or hypercapnia. Chronic lesion of the anteroventral third ventricle region eliminated the ability of dietary salt intake to modulate these cardiovascular reflexes. Finally, rats chronically instrumented with telemetry units indicate that increased dietary salt intake elevated blood pressure variability but not mean ABP. These findings indicate that dietary salt intake works through the forebrain hypothalamus to modulate various centrally mediated cardiovascular reflexes and increase blood pressure variability. (Hypertension. 2014;64:00-00.) ● Online Data Supplement

Key Words: blood pressure ■ brain ■ hypernatremia ■ sodium ■ sympathetic nervous system

Several lines of evidence suggest that excess dietary salt (NaCl) intake adversely affects cardiovascular function independently of changes in arterial blood pressure (ABP).1-4 First, population studies indicate that normotensive humans with a greater level of salt intake have an increased risk for an adverse cardiovascular event.1,2 Second, evidence from humans and animal models indicates that excess dietary salt intake promotes endothelial and microvascular dysfunction,5,10 left ventricular hypertrophy,11 and fibrosis of the heart, kidney, and arteries,11 and enhances cardiovascular responses evoked by chemical excitation or inhibition of several brain stem structures.12-17 The latter effect has been largely attributed to a central sensitization of brain stem autonomic pathways as stimulation of the dorsolateral funiculus to activate downstream pathways produced similar changes in sympathetic nerve activity (SNA) or ABP or both of rats fed low-versus high-NaCl diets.13,17 Collectively, these observations suggest that dietary salt intake modulates the responsiveness or gain of central autonomic pathways to alter the functional regulation of SNA and ABP in normotensive, salt-resistant rats.

A limitation of the above-mentioned studies is that the experimental manipulations were largely limited to exogenous application of various neurotransmitters into brain stem nuclei. A key question is whether the ability of dietary salt to modulate the responsiveness of central autonomic circuits results in functional changes in the regulation of SNA and ABP during activation of physiological reflexes. Evidence of this idea arises from a limited number of studies that have reported greater pressor responses to stimulation of sciatic afferents,6,16 intracerebroventricular (ICV) injection of angiotensin II18 or hypertonic NaCl,19 and the exercise pressor reflex.20 In addition, the depressor response to electric activation of the aortic depressor nerve was also enhanced by increased dietary salt intake.17 Because the majority of these previous studies lacked direct recordings of SNA, the differences in ABP cannot be attributed to differences in SNA or the ability of dietary salt intake to alter the responsiveness of central autonomic circuits versus a change in vascular reactivity.

Therefore, the purpose of the present study was several-fold. First, we simultaneously recorded SNA to several end-organs during activation of various sympathetic reflexes in animals fed low-, moderate-, and high-NaCl diets. Second, because a prior study reported that the ability of dietary salt intake to modulate centrally evoked responses depends on...
the integrity of the anteroventral third ventricular (AV3V) region, the above experiments were performed in animals with chronic AV3V lesions. Finally, if increased dietary salt intake exaggerates several cardiovascular reflexes in normotensive, salt-resistant rats, we hypothesized that dietary salt intake would alter blood pressure variability (BPV). Increased BPV has been identified as a predictor of subsequent cardiovascular disease.

Methods

All of the experimental procedures conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State College of Medicine.

Animals

Male Sprague–Dawley rats (225–275 g, Charles River Laboratories) were singly housed in a temperature-controlled room (22–23°C) with a 12:12 hour light:dark cycle. Sham or AV3V lesions were performed as described previously in our laboratory (Figure S1 in the online-only Data Supplement). After water and food intake returned to pre-surgical levels (+3–5 days), these animals and additional control rats were fed 0.1% NaCl chow (low, D17020, Research Diets) or 4.0% NaCl chow (high, D17013, Research Diets) with ad libitum access to deionized water for 14 to 17 days. Then, cardiovascular reflexes were evaluated as described below. Food and fluid intake were measured daily. In addition, we assessed a subset of cardiovascular reflexes in 2 other dietary NaCl groups. First, control animals were fed 0.5% NaCl chow (D14020401, Research Diets) to test the effect of a moderate NaCl diet. Second, control animals were fed 0.1% NaCl chow but water was replaced by 0.9% NaCl solution as prior studies have supplemented NaCl to the drinking water rather than chow. Again, the latter 2 groups were on the respective diet for 14 to 17 days.

Sympathetic Reflexes

After 14 to 17 days of the respective diet, sympathetic and cardiovascular responses to various reflexes were examined in Inactin-anesthetized rats at least 60 minutes after all surgical procedures were completed (please see the online-only Data Supplement). To maintain stable levels of SNA and ABP, only 3 to 4 reflexes were tested per animal. Responses to chemoreceptor stimulation or volume expansion were not tested in sham or AV3V-lesioned animals. (1) Somatosympathetic reflex: The sciatic afferent nerve was placed onto a bipolar, stainless steel electrode and stimulated electrically (1 ms pulse, 500 μA, 5 ms duration) over a range of frequencies (1, 2, 5, 10, 20 Hz) in a random order while animals were paralyzed with pancuronium (0.5 mg/kg, IV). (2) Chemoreceptor: Rats were ventilated with a hypoxic (10% O₂, 90% N₂, 30 s) or hypercapnic (33% O₂, 7% CO₂, 60 N₂, 30 s) gas mixture. Responses were tested twice separated by a minimum of 5 minutes. (3) Volume expansion: A double-lumen jugular catheter was placed into the vena-atrial junction as verified by the presence of dye in the third and fourth ventricle after injection of Evan’s Blue Dye (2 μL, 0.5%).

Analysis of BPV

BPV was analyzed in a separate group of rats chronically instrumented with PA-C40 telemetry units (Data Sciences International) and fed 0.1% and 4.0% NaCl chow at 2 week intervals (please see the online-only Data Supplement). Systolic, diastolic, and mean ABP and heart rate were sampled at 2 KHz and analyzed on a beat-to-beat basis using the Spike2 software for both day (1–3 PM) and night (1–3 AM) periods (lights on 7 AM–7 PM) at day 14 of the respective diet.

Statistical Analysis

All data are expressed as mean±SEM. Changes in SNA were calculated after background noise (assessed after nerve crush) was subtracted. Peak changes (1 s) were calculated for all responses, compared with a 30-s (sciatic, aortic depressor, cervical vagus, and chemoreceptor experiments) or 5-minute (volume expansion, central NaCl infusions) baseline segment. Data were analyzed by a 1- or 2-way ANOVA with repeated measures when appropriate. When significant F values were obtained, post hoc comparisons were performed using independent (between groups) or paired (within group) t tests with a layered Bonferroni correction. Linear regression analysis was performed using SigmaPlot. A P value <0.05 was significant.

Results

For purposes of presentation, results of control and AV3V-lesioned rats fed 0.1% or 4.0% NaCl are illustrated here, whereas data for those animals fed 0.5% NaCl chow or drinking 0.9% NaCl and tested for a subset of reflexes are located within the online-only Data Supplement. Because there were no differences in any variable between control versus sham-lesioned groups, the data sets were collapsed into a single control group for the respective diet. As expected, rats ingested significantly more NaCl and water when fed 4.0% versus 0.1% NaCl chow (Table). Importantly, daily NaCl intake did not differ between control versus AV3V-lesioned rats fed the same diet. Plasma [Na⁺] and [Cl⁻] were significantly higher in control and AV3V-lesioned rats fed 4.0% versus 0.1% NaCl.

Somatosympathetic Reflex

Electrical activation of the sciatic nerve produced frequency-dependent increases in mean ABP, heart rate, and lumbar, splanchnic, and renal SNA (Figure 1). These responses were significantly greater in control rats fed 4.0% versus 0.1% NaCl (Figure 1). Similar findings were observed in control rats fed 0.5% NaCl or drinking 0.9% NaCl (Figure S2). Moreover, a linear regression analysis revealed that there was a NaCl-dependent relationship as the magnitude of these responses progressively increased with the average daily intake of NaCl across rats fed 0.1%, 0.5%, and 4.0% NaCl (5 Hz: r²=0.412 for all variables, P<0.05). However, these differences were completely absent after AV3V lesions as AV3V-lesioned rats fed 4.0% NaCl displayed changes in SNA, heart rate, and mean ABP that were not statistically different from those found in control or AV3V-lesioned rats fed 0.1% NaCl (Figure 1).

Aortic Depressor Nerve

Sympathoinhibitory responses produced by stimulation of the aortic depressor nerve at higher frequencies (5–20 Hz) were significantly greater in rats fed 4.0% versus 0.1% NaCl (Figure 2). The only variable unaffected by dietary salt intake
was lumbar SNA; the decrease in lumbar SNA was not statistically different between rats fed 0.1% versus 4.0% NaCl. Similar findings were observed in control rats fed 0.5% NaCl or drinking 0.9% NaCl (Figure S3). Again, there was a NaCl-dependent relationship as the magnitude of these responses progressively increased with the average daily intake of NaCl across rats fed 0.1, 0.5, and 4.0% NaCl chow (5 Hz; \( r \geq 0.397 \) for all variables except lumbar SNA, \( P < 0.05 \)). These exaggerated sympathoinhibitory responses in rats fed 4.0% NaCl were absent in AV3V-lesioned rats (Figure 2).

**Vagal Afferent Nerve**

The decreases in mean ABP, heart rate, and splanchnic SNA during stimulation of vagal afferent nerves were significantly greater in rats fed 4.0% versus 0.1% NaCl (Figure 3). Although lumbar and renal SNA decreased in a frequency-dependent manner, the magnitude of these responses was not statistically different between rats fed 0.1% versus 4.0% NaCl. The responses were not tested in rats fed 0.5% NaCl or drinking 0.9% NaCl. Again, these differences were absent in AV3V-lesioned animals (Figure 3).

**Volume Expansion**

Acute volume expansion produced significant increases in mean ABP and right atrial pressure but decreased heart rate and renal SNA (Figure 4). The decreases in heart rate and renal SNA were significantly greater in rats fed 4.0% versus 0.1% NaCl. Splanchnic and lumbar sympathoinhibitory responses tended to be significantly greater in rats fed 4.0% versus 0.1% NaCl (\( P = 0.09 \), data not shown). Baseline right atrial pressure was not different between groups (0.1%: 3.61±0.22 versus 4.0%: 4.00±0.12 mmHg, \( P > 0.2 \)). The responses were not tested in control rats fed 0.5% NaCl or drinking 0.9% NaCl or AV3V-lesioned rats.

**ICV Infusion of Hypertonic NaCl**

ICV infusion of 0.6 or 1.0 mol/L NaCl produced dose-dependent increases in mean ABP and lumbar SNA (Figure 5). Again, the magnitude of these responses was significantly greater in rats fed 4.0% versus 0.1% NaCl. Similar findings were observed in control rats fed 0.5% NaCl or drinking 0.9% NaCl (Figure S4). Again, there was a significant linear correlation between the changes in lumbar SNA and mean ABP.
and the average daily intake of NaCl across rats fed 0.1%, 0.5%, and 4.0% NaCl (1.0 mol/L; \( r^2 \leq 0.433 \) for mean ABP and lumbar SNA, \( P < 0.05 \)). AV3V lesion significantly attenuated this sympathoexcitatory response in rats fed 0.1% and 4.0% NaCl (Figure 5). In addition, there were no longer differences in the magnitude of these responses between AV3V-lesioned rats fed 0.1% versus 4.0% NaCl.

**Chemoreflex**

Chemoreceptor activation by ventilation with a hypoxic or hypercapnic gas mixture decreased mean ABP and increased SNA. However, the magnitude of these changes were not statistically different between control rats fed 0.1% versus 4.0% NaCl (Figure S5).

**Analysis of BPV**

In a final set of experiments, we analyzed ABP and heart rate during day and night periods of control rats instrumented with telemetry units and fed 0.1% or 4.0% NaCl at 2-week intervals. Mean ABP did not differ during day or night periods between rats fed 0.1% versus 4.0% NaCl (Figure 6); however, the standard deviation of beat-to-beat systolic ABP was significantly higher at night when rats were fed 4.0% versus 0.1% NaCl. Similar findings were observed with mean, diastolic, and systolic ABP (Table S2). Heart rate did not differ between dietary salt conditions (Table S2).

**Discussion**

The present study indicates that dietary salt intake modulates the responsiveness of central autonomic circuits to result in functional changes in the regulation of SNA and ABP. This conclusion is based on several novel observations to indicate that the level of dietary NaCl intake was directly correlated with the magnitude of the SNA and ABP responses during several experimental manipulations including (1) activation of sciatic afferents, (2) stimulation of aortic depressor nerve, (3) activation of vagal nerve afferents, (4) volume expansion, and (5) elevation in cerebrospinal fluid [NaCl]. The enhanced SNA and ABP responses in rats fed a high NaCl diet were prevented by prior lesion of the AV3V. Consistent with a sensitization of central autonomic pathways, a high-NaCl diet was also found to increase BPV independently of mean ABP. Altogether, these findings suggest that dietary NaCl intake, via the AV3V region, modulates the central gain of sympathetic circuits to alter the functional regulation of SNA and cardiovascular function in normotensive, salt-resistant rats.
Several experiments in the present study utilized electric stimulation of a peripheral nerve to control afferent neural input and directly assess central gain or responsiveness by changes in SNA and ABP. Rats fed 4.0% versus 0.1% NaCl displayed greater changes in SNA and ABP during electric stimulation of sciatic afferents, aortic depressor nerve, or vagal afferents. Similar exaggerated responses were observed in response to ICV infusion of hypertonic NaCl or volume expansion. These findings are consistent with previous observations in a high-NaCl diet enhanced baroreflex gain in rats and humans, the exercise pressor response, and sympathoinhibitory response to volume expansion. The novelty of the current findings is that dietary NaCl intake exaggerated the magnitude of the SNA response, thereby suggesting that the dietary NaCl affects central autonomic networks versus a change in vascular reactivity. Although it is difficult to define a normal NaCl intake in rodents, the SNA and ABP responses examined in a subset of reflexes displayed a NaCl-dependent relationship over a range of NaCl diets (0.1%, 0.5%, or 4.0% NaCl). In a few instances, dietary NaCl did not modulate the SNA response in all sympathetic nerves. The reason for this result is unclear but suggests a complex interaction. Altogether, these findings suggest that the level of dietary NaCl intake modulates the gain of central sympathetic networks to functionally alter the regulation of SNA and ABP in normotensive, salt-resistant animals.

It is noteworthy that the present findings contradict a few previous studies in which dietary NaCl intake was not found to alter sympathetic and cardiovascular reflexes. In rabbits, 6-day access to 0.9% NaCl drinking solution did not alter renal SNA and cardiovascular responses to stressful stimuli (handling, gentle restraint, or nasopharyngeal activation with cigarette smoke). In rats, electrical stimulation of the aortic depressor nerve or cervical vagal afferents did not produce greater decreases in renal SNA and ABP of rats given access to 0.9% NaCl versus water for 1 week. Interestingly, previous studies from our laboratory have documented that enhanced SNA and ABP responses to rostral ventrolateral medulla (RVLM) activation are observed after 2 weeks of access to 0.9% NaCl diet. Therefore, the lack of a potentiation by increased NaCl intake in the above-mentioned studies may simply reflect a short duration of increased dietary NaCl.

The majority, if not all, of the reflexes tested in the current study depend on neurotransmission in the RVLM. Although prior studies have reported that increased dietary NaCl intake enhances sympathoexcitatory and sympathoinhibitory responses evoked from the RVLM, there is currently no direct evidence to indicate that the enhanced responses can be directly attributed to selective changes in the excitability of RVLM neurons versus other neuronal

**Figure 4.** Mean±SEM changes and peak 1 s responses in mean arterial blood pressure (ABP), heart rate (HR), right atrial pressure (RAP), and renal sympathetic nerve activity (SNA) during volume expansion of rats fed 0.1% vs 4.0% NaCl. *P<0.05 vs 0.1% NaCl.

**Figure 5.** Intracerebroventricular infusion of 0.6 or 1.0 mol/L NaCl produced significantly greater increases in mean arterial blood pressure (ABP) and lumbar sympathetic nerve activity (SNA) of rats fed 4.0% vs 0.1% NaCl. Lesion of the anteroventral third ventricular (AV3V) significantly attenuated these responses and abolished the differences between rats fed 0.1% vs 4.0% NaCl chow. *P<0.05 vs 0.1% NaCl; #P<0.05, 0.6 vs 1.0 mol/L NaCl within same diet; †P<0.05 vs control rats. HR indicates heart rate.

**Figure 6.** Mean±SEM of mean arterial blood pressure (ABP) and SD of systolic ABP of rats instrumented with telemetry and fed 0.1% or 4.0% NaCl for 2 weeks. ABP was analyzed on a beat-to-beat basis for day (1–3 PM) and night (1–3 AM) periods (lights on/off at 7 AM/7 PM). *P<0.05, †P<0.01.
populations. Indeed, the dependence of this effect on the AV3V region raises the possibility that dietary salt may affect multiple neuronal populations extending from the hypothalamus to the hindbrain. However, the absence of exaggerated responses to chemoreflex activation suggests that dietary salt intake does not modulate every central autonomic pathway or reflex.

The AV3V region and lamina terminalis play a pivotal role in body fluid homeostasis and cardiovascular regulation.3 AV3V lesions disrupt thirst, antidiuretic hormone secretion, and changes in SNA to body fluid homeostatic challenges.30,34 These lesions also prevent or attenuate several salt-sensitive models of hypertension35–39 and the enhanced SNA or ABP or both responses to stimulation of the RVLM of rats fed high-salt diets.12 In the present study, rats with chronic AV3V lesions fed 4.0% NaCl chow did not show exaggerated sympathoexcitatory and cardiovascular responses to any stimulus. In fact, the responses of AV3V lesioned rats were not different between those fed 0.1% versus 4.0% NaCl or control rats fed 0.1% NaCl. Consistent with a previous report,40 AV3V lesion attenuated the sympathetic or pressor or both responses to ICV infusion of hypertonic NaCl but there were no differences between dietary groups. These observations cannot be explained by differences in NaCl intake between control and AV3V lesioned animals. Altogether, these findings suggest that dietary salt intake affects neurons in the AV3V region to subsequently alter the excitability of central sympathetic circuits. The factor that links dietary salt intake to the AV3V region and gain of sympathoexcitatory responses is unknown; however, several studies have reported that dietary salt intake produces small changes in plasma electrolytes and osmolality.12,41 The present findings confirm this notion as plasma sodium and chloride concentrations were elevated in control and AV3V lesioned rats fed 4.0% NaCl versus control rats fed 0.1% NaCl. In addition, chronic ICV NaCl infusions have also been reported to exaggerate sympathoexcitatory and cardiovascular responses to air-jet stress in Dahl-resistant rats.19 Because the AV3V region contains osmosensitive neurons,42 it is interesting to speculate that dietary salt intake may activate these neurons through changes in electrolyte concentrations or osmolality. However, changes in dietary NaCl intake will also affect other circulating factors such as the renin-angiotensin system. Therefore, we cannot exclude the possibility that increases or decreases or both in such factors also contribute.

Accumulating evidence suggests that short- and long-term BPV are associated with the development of end-organ damage.21 Moreover, long-term BPV may predict adverse cardiovascular events. Numerous mechanisms contribute to BPV including modulation of central autonomic function, fluctuations in humoral systems, emotion, and behavior.21 Because dietary NaCl intake was found to exaggerate numerous sympathoinhibitory and sympathoexcitatory reflexes, we hypothesized that dietary NaCl intake may increase BPV independently of mean ABP. Indeed, BPV was significantly higher in rats fed 4.0% NaCl versus 0.1% NaCl at night but not during the day. The reason for the circadian effect is not clear but could be related to when the animals are active and plasma electrolyte concentrations are highest.12,41

Interestingly, these findings are consistent with population studies that indicate that normotensive humans with a greater level of salt intake have an increased risk of an adverse cardiovascular event.12,4

**Perspectives**

Although dietary salt intake is recognized as a contributing factor to the pathogenesis of hypertension,1,3,4,35–45 recent evidence suggests that dietary salt intake may increase the risk of adverse cardiovascular events independently of changes in ABP.1,4 The present study suggests that one such mechanism for this adverse effect may be a central sensitization of sympathoexcitatory circuits to result in exaggerated cardiovascular reflexes and increase BPV in normotensive, salt-resistant animals. Moreover, this central sensitization may also explain why dietary salt intake works with other factors (ie, angiotensin II) to synergistically increase ABP in chronic experimental models of hypertension. How dietary salt intake produces these adverse effects either in normotensive, salt-resistant animals or synergistically in salt-sensitive models is unknown.

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**Disclosures**

None.

**References**


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Dietary salt intake exaggerates sympathetic reflexes and increases blood pressure variability in normotensive rats

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Running Head: Dietary NaCl exaggerates sympathetic reflexes and BPV

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METHODS

General Procedures for Evaluation of Cardiovascular Reflexes. Animals were anesthetized with isoflurane (2-3% in 100% O₂), instrumented with a brachial arterial catheter and double-lumen jugular catheter, and prepared for recordings of lumbar, renal, and splanchnic SNA as described previously [1, 2]. After tracheal cannulation, animals were artificially ventilated to maintain end-tidal CO₂ between 3.5-4.5% by adjusting tidal volume (~1mL/100g BWT) or respiratory rate (60-80 bpm). Body temperature was measured rectally and maintained at 37±0.5°C with a water-circulating blanket. Once all surgical procedures were complete, isoflurane was replaced with Inactin anesthesia (120 mg/kg, IV). All variables were allowed to stabilize for at least 60 min before reflexes were examined. Then, plasma electrolytes were analyzed using blood (0.1mL) collected from the arterial catheter and 6+ I-STAT cartridges. Volume was replaced by isotonic saline.

Analysis of Blood Pressure Variability (BPV). Rats were anesthetized with isoflurane, instrumented with PA-C40 telemetry units (Data Sciences International) in the abdominal aorta as described previously [3] and treated with buprenex (0.05 mg/kg, sc) caprofen (5mg/kg, sc), and ampicillin (100mg/kg, sc). Then, animals were returned to the home cage for 1 week and fed 0.1% NaCl. Then, animals were fed 0.1% NaCl or 4.0% NaCl for 14 days. On day 14, ABP was sampled at 2kHz. Then, animals were switched to the other diet for 14 days, and the sampling was repeated. Mean and SD of mean, diastolic, and systolic ABP and heart rate were analyzed by Spike2 software for the day (1-3 PM) and night (1-3 AM) periods (lights on 7AM-7PM).

REFERENCES

Figures S1. (A) Representative example of histology for control and A3V3-lesioned animals. Scale bar: 500 μm. Schematic drawings of electrolytic lesions for rats fed 0.1% NaCl (B) or 4.0% NaCl (C) chow. The lesion boundary is outlined in black. Coordinates are in reference to Bregma [4]. Abbreviations: LV, lateral ventricle; DBB, diagonal band; AC, anterior commissure; OVLT, organum vasculosum of the lamina terminalis; MnPO, median preoptic nucleus; 3V, 3rd ventricle; OC, optic chiasm; f, fornix; SFO, subfornical organ; PVH, hypothalamic paraventricular nucleus.
Figure S2. Peak (1s) changes in mean ABP, heart rate, and lumbar, splanchnic and renal SNA during electrical stimulation of sciatic afferent nerve in rats fed 0.1% NaCl, 0.5% NaCl, 4.0% NaCl, or given access to 0.9% NaCl drinking solution for 14-17 days. *P<0.05 versus 0.1% NaCl. #P<0.05, 0.5% versus 4.0% NaCl
Figure S3. Peak (1s) changes in mean ABP, heart rate, and lumbar, splanchnic and renal SNA of rats fed 0.1% NaCl, 0.5% NaCl, 4.0% NaCl, or given access to 0.9% NaCl drinking solution during electrical stimulation the aortic depressor nerve at 5, 10, and 20 Hz. *P<0.05 versus 0.1% NaCl, #P<0.05 between 0.5% vs 4.0% NaCl. Although electrical stimulation of the aortic depressor nerve at 1 and 2 Hz produced decreases in all variables, there were no differences across groups in any variable (data not shown).
Figure S4. ICV infusion of 0.6M or 1.0M NaCl produced significantly greater increases in mean ABP and lumbar SNA of rats fed 4.0% NaCl chow or 0.9% NaCl drinking solution versus 0.1% NaCl chow. *P<0.05 versus 0.1% NaCl;
Figure S5. Peak (1s) changes in mean ABP, heart rate, end-tidal CO₂ and lumbar, renal, and splanchnic SNA of rats fed 0.1% NaCl or 4.0% NaCl during 30s ventilation with hypoxic or hypercapnic gas mixture. ANOVA revealed that there were no differences in any variable between groups.
Table S1. Characteristics of control rats fed 0.5% NaCl or drinking 0.9% NaCl solution.

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Values are mean±SEM. *P<0.05 versus rats fed 0.1% NaCl, #P<0.05 versus rats fed 4.0% NaCl.
Table S2. Arterial blood pressure and heart rate values for rats instrumented with telemetry units and fed 0.1% or 4.0% NaCl for 2 weeks.

<table>
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<td>SD of Mean ABP, mmHg</td>
<td>7.1±0.6</td>
<td>7.5±1.0</td>
<td>†</td>
<td>7.7±0.7</td>
</tr>
<tr>
<td>Heart Rate, bpm</td>
<td>355±7</td>
<td>395±3</td>
<td>†</td>
<td>344±10</td>
</tr>
<tr>
<td>SD of Heart Rate, bpm</td>
<td>27±3</td>
<td>43±8</td>
<td></td>
<td>23±4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<0.05 vs 0.1% within same cycle, †P<0.05 vs Day within same diet