High Sodium Intake Enhances Renal Nerve and Antinatriuretic Responses to Stress in Spontaneously Hypertensive Rats

JOHN P. KOEPEKE AND GERALD F. DiBONA

SUMMARY The effects of high sodium intake (drinking 0.9% NaCl for 15 days) on the increased renal sympathetic nerve activity and decreased urinary sodium excretion resulting from stressful environmental stimulation (air jet to head) were examined in conscious spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). On a normal sodium intake in SHR, air stress increased renal sympathetic nerve activity 77% and decreased urinary sodium excretion 28% without altering effective renal plasma flow or glomerular filtration rate. By contrast, in conscious SHR on high sodium intake, the same air stress caused a greater increase in renal sympathetic nerve activity (103%) and a greater antinatriuresis (42%) along with reductions in effective renal plasma flow and glomerular filtration rate. Surgical renal denervation prevented the antinatriuretic responses to air stress in other conscious SHR on high or normal sodium intake. In conscious WKY, air stress had no effect on renal sympathetic nerve activity or urinary sodium excretion, regardless of normal or high sodium intake. We conclude that the enhanced renal sympathetic nerve activity and antinatriuretic responses to air stress in conscious SHR on high sodium intake are dependent on a centrally mediated facilitation of sympathetic neural outflow to the kidney. The greater antinatriuretic response to air stress in conscious SHR than in WKY may reflect a greater genetic predisposition in SHR to increase renal sympathetic nerve activity during air stress. (Hypertension 7: 357–363, 1985)

KEY WORDS • renal sympathetic nerve activity • dietary sodium • environmental stress

STRESSFUL environmental stimulation has been used to study the neural control of renal excretory function in conscious spontaneously hypertensive rats (SHR) and dogs. Decreased urinary sodium excretion results from exposure of conscious SHR to air stress (see the preceding article by J. P. Koepke and G. F. DiBona on pages 350–356 of this issue), conscious dogs to aversive conditioning, and conscious humans to mental competition. The antinatriuretic response to aversive conditioning or air stress occurs in the absence of changes in glomerular filtration rate, which suggests an increased renal tubular sodium reabsorption (see the preceding article). Moreover, the antinatriuretic response to air stress is associated with increased renal sympathetic nerve activity and is abolished by surgical renal denervation or by intracerebroventricular administration of β-adrenergic receptor antagonists (see the preceding article). In addition, the increased renal sympathetic nerve activity and antinatriuresis resulting from stressful environmental stimulation is greater in conscious SHR than in normotensive Wistar-Kyoto rats (WKY). Similarly, humans with a parental history of hypertension respond to mental competition with a greater antinatriuresis than humans with no parental history of hypertension. Thus, the effects of air stress on urinary sodium excretion are mediated by the renal sympathetic nerves and are more pronounced in animals or individuals with a genetic predisposition toward hypertension.

Similar to stressful environmental stimulation, high sodium intake increases the responsiveness of the sympathetic nervous system to neurogenic stimuli to a greater degree in animals with a genetic predisposition toward hypertension. Responsiveness of abdominal sympathetic nerve activity to posterior hypothalamic...
stimulation is enhanced by high sodium intake in Dahl salt-sensitive rats and in deoxycorticosterone acetate (DOCA) hypertensive rats. High sodium intake may facilitate peripheral sympathetic neurotransmission as well. The responsiveness of vascular smooth muscle to norepinephrine is greater in stroke prone SHR on a high sodium intake than in those on a normal sodium intake.

In the present study, we tested the hypothesis that high sodium intake facilitates the antinatriuretic response to stressful environmental stimulation in conscious SHR and WKY. Moreover, we hypothesized that high sodium intake would facilitate the antinatriuretic response to stressful environmental stimulation to a greater degree in conscious SHR than in conscious WKY. Finally, we measured the renal sympathetic nerve activity response to stressful environmental stimulation in conscious SHR and WKY on normal and high sodium intakes to determine whether any facilitation of the antinatriuretic response could be attributed to enhanced renal sympathetic neural outflow from the central nervous system.

Materials and Methods

Male SHR and normotensive WKY rats 12 to 14 weeks of age were obtained from Taconic Farms, Inc. (Germantown, NY). The SHR and WKY were maintained for 15 days on either a normal sodium intake (SHR-NNa, WKY-NNa) or a high sodium intake (SHR-HNa, WKY-HNa). Normal sodium intake rats were given tap water, and high sodium intake rats were given isotonic saline to drink; both normal sodium and high sodium intake rats were given standard rat chow (Na content, 163 mEq/kg).

One group, comprised of 16 SHR and 13 WKY, underwent ketamine HCl anesthesia (150 mg/kg i.p.; Bristol Laboratories, Syracuse, NY) and was surgically prepared 24 to 48 hours before experimentation with catheters (Silastic 602-135, Dow Corning, Midland, MI) in the left jugular vein and right carotid artery. The venous and arterial catheters were tunneled to the back of the neck, filled with heparinized saline (1000 U/ml), and plugged with stainless steel pins. Through a suprpubic incision, a stainless steel urinary bladder catheter (18 gauge, 12.5 cm long), modified from that of Gellai and Valtin,9 was sutured into the urinary bladder, exteriorized, and secured to adjacent tissues. The urinary bladder catheter was obturated, and rats voided through the urethra until the experiment began. The venous catheter was used for saline infusions and blood sampling, the arterial catheter for arterial pressure and heart rate recordings, and the urinary bladder catheter for urine collections.

A separate group, comprised of 12 SHR and 12 WKY, was surgically prepared (pentobarbital sodium, 50 mg/kg i.p.) with renal nerve electrodes to record multifiber renal sympathetic nerve activity and with catheters in the jugular vein and carotid artery. Through a left flank incision, the left kidney was exposed by a retroperitoneal approach. With the use of a dissection microscope (25×), a renal nerve branch was dissected from the aortorenal junction and placed on a bipolar silver or platinum wire electrode (Cooner Wire Co., Chatsworth, CA). Renal sympathetic nerve activity was amplified (×10,000–50,000) and filtered (low, 30; high, 3,000 Hz) using a Grass P511 Bandpass Amplifier (Grass Instrument Co., Quincy, MA). The amplified and filtered signal was led to a Tektronix 5113 Oscilloscope (Tektronix Inc., Beaverton, OR) and Grass Model 7DA Polygraph for visual evaluation, to an audio amplifier/loudspeaker (Grass Model AM 8 Audio Monitor) for auditory evaluation, and to a rectifying voltage integrator (Grass Model 7P10). The voltage integrated signal was displayed on the Grass Polygraph. The quality of the renal sympathetic nerve signal was assessed during operation by examining the magnitude of decrease in recorded renal sympathetic nerve activity during sinoaortic baroreceptor loading with intravenous injections of norepinephrine (5 µg) and to increases in recorded renal sympathetic nerve activity during sinoaortic baroreceptor unloading by intravenous injections of acetylcholine (1 µg). When an optimal renal sympathetic nerve activity signal was observed, the recording electrodes were fixed to the renal nerve branch with Sil-Gel 604 (Wacker Chemie, Munich, W. Germany). The electrode cable was tunneled to the back of the neck and exteriorized, the flank incision was closed in layers, and 24 hours was allowed for recovery.

Another group of SHR (n = 14) underwent either renal denervation or sham renal denervation under ketamine anesthesia (150 mg/kg i.p.) 5 to 7 days before the experimental day. Through a midline laparotomy, bilateral renal denervation was performed by stripping the renal arteries and veins of adventitia, cutting the renal nerve bundles, and coating the vessels with a solution of 10% phenol in absolute ethanol, as previously described.11 Sham renal denervation was accomplished by exposing the renal arteries and veins, but leaving adventitia and renal nerve bundles intact. These rats also were implanted with jugular, carotid, and urinary bladder catheters 24 to 48 hours before experimentation.

On the experimental day, conscious SHR and WKY were placed in Lucite cylinders, which permitted forward and backward movement, and an isotonic saline infusion was started at a rate of 60 µL/minute. The arterial catheter was flushed and attached to a pressure transducer (P23Db, Statham, Oxnard, CA) zeroed at the center of the cylinder. For experiments with urine collections, a 3-cm polyethylene catheter was attached to the urinary bladder catheter and led to a collection beaker. For renal sympathetic nerve activity recording experiments, the renal nerve recording electrode cable was connected to a high impedance probe (Grass HIP 511), which in turn was connected to the bandpass amplifier. The quality of the renal nerve activity recording was tested with i.v. norepinephrine (5 µg) as on the surgical day. If the quality of the renal nerve activity recording was similar to that observed when
the electrode was implanted, then the experiment commenced.

The isotonic saline infusion included inulin and para-aminohippurate (PAH) for determination of inulin and PAH clearances respectively. After 60 minutes of equilibration to the infusion, two consecutive 20-minute control periods were obtained, followed by a 20-minute stressful environmental stimulation period and then by two consecutive 20-minute recovery periods. For clearance experiments, 20 minute urine collections were made for each period, and venous blood samples (200 µL) were taken at the midpoints. Stressful environmental stimulation consisted of an air jet delivered to the top of the rat’s head through a tube located 4 to 5 cm in front of the rat. At the end of renal sympathetic nerve activity recording experiments, the quality of the renal nerve signal was again assessed with intravenous injections of norepinephrine (5 µg).

Finally, the rat was killed and postmortem renal nerve activity was recorded 30 to 45 minutes later as a measure of background noise; this value was subtracted from all values of renal sympathetic nerve activity.

Urinary volume was determined gravimetrically. Urine sodium concentration was measured by flame photometry (Model 143, Instrumentation Laboratories, Lexington, MA). Urine and plasma inulin and PAH concentrations were determined by the anthrone and ethylenediamine methods respectively. Glomerular filtration rate was measured as inulin clearance:

\[ C_{IN} = \frac{(V \times U_{IN})}{P_{IN}}, \]

where \( U_{IN} \) and \( P_{IN} \) are urine and plasma inulin concentrations respectively, and \( V \) is urine flow rate. Effective renal plasma flow was determined by PAH clearance:

\[ \left( \frac{V \times U_{PAH}}{P_{PAH}} \right) \]

Fractional sodium excretion was estimated by \( C_{Na}/C_{IN} \times 100 \), where \( C_{Na} \) is sodium clearance. Fractional water excretion was determined by \( V/C_{IN} \times 100 \).

Because the values of the two control periods were statistically similar, these values were averaged to give a single control period value. Similarly, the values from the two recovery periods were averaged to give a single recovery period value. All renal sympathetic nerve activity data were quantified for each experimental period. Statistical analyses were conducted with repeated measures analysis of variance (BMDP 2V) for main effects and interactions and Scheffe’s test for pairwise comparisons among means. Statistical significance was defined as \( p < 0.05 \).

### Results

**Renal Function and Mean Arterial Pressure**

High sodium intake augmented the antinatriuretic response to air stress in conscious SHR, but not in WKY (Figure 1). In fact, in WKY air stress had no effect on urine flow rate, urine sodium excretion, effective renal plasma flow, or glomerular filtration rate regardless of normal sodium or high sodium intakes (Table 1). The decreased urinary sodium excretion resulting from air stress in SHR-HNa (-42%) was greater \( p < 0.01 \) than the antinatriuretic response in SHR-NNa (-28%). Whereas no changes in urine flow rate (-8%), effective renal plasma flow (-2%), and glomerular filtration rate (-1%) occurred in SHR-NNa, these measures decreased during air stress in SHR-HNa (-34%, -24%, and -14% respectively).

Accordingly, the effects of air stress on the fractional excretions of sodium and water in SHR-HNa (-34% change from 3.47 ± 0.63%, \( p < 0.01 \), and -28% change from 3.22 ± 0.54%, \( p < 0.01 \) respectively) were greater \( p < 0.05 \) than in SHR-NNa (-22% change from 2.85 ± 0.58%, \( p < 0.05 \); and -2% change from 2.67 ± 0.57%, \( p = \text{not significant} \)). Urinary sodium excretion decreased \( p < 0.05 \) in sham renal denervated SHR on normal or high sodium intakes (30% from 2.2 ± 0.9 µEq/min/100 g body weight and 45% from 4.5 ± 1.0 µEq/min/100 g body weight respectively; \( n = 4 \)). In contrast, chronic bilateral renal denervation completely abolished the decreases in renal excretory and hemodynamic function resulting from air stress in conscious SHR-NNa and SHR-HNa. In fact, changes in sodium excretion during air stress after surgical renal denervation in SHR were not different from those in WKY.

High sodium intake had no effect on the mean arterial pressure or heart rate responses to air stress in either SHR or WKY. Mean arterial pressure and heart rate increased \( p < 0.01 \) in SHR-NNa by 13 mm Hg from 149 ± 8 mm Hg and 25 beats/minute from 423 ± 12 beats/minute respectively compared with the increases \( p < 0.01 \) in SHR-HNa of 9 mm Hg from 154 ± 4 mm Hg and 27 beats/minute from 429 ± 20 beats/minute. During recovery, mean arterial pressure and heart rate returned to control levels in both SHR-NNa (151 ± 8 mm Hg and 430 ± 14 beats/minute) and SHR-HNa (154 ± 3 mm Hg and 433 ± 20 beats/minute). Mean arterial pressure and heart rate were increased \( p < 0.05 \) by air stress in WKY-NNa (+4 mm Hg from 112 ± 6 mm Hg, and +15 beats/minute from 356 ± 20 beats/minute) and in WKY-HNa (+5 mm Hg from 117 ± 4 mm Hg and +15 beats/minute from 381 ± 13 beats/minute). Control measures of mean arterial pressure...
### TABLE 1. Renal Function Responses to Air Stress in Conscious Spontaneously Hypertensive Rats (SHR), SHR with Renal Denervation (SHR-DNX) and Wistar-Kyoto Rats (WKY) on Normal and High Sodium Diets

<table>
<thead>
<tr>
<th>Value</th>
<th>Normal Na</th>
<th>High Na</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHR (n = 8)</td>
<td>SHR-DNX (n = 6)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>149 ± 8</td>
<td>132 ± 9</td>
</tr>
<tr>
<td>Air</td>
<td>162 ± 10*</td>
<td>144 ± 7*</td>
</tr>
<tr>
<td>Air</td>
<td>151 ± 8</td>
<td>130 ± 11</td>
</tr>
<tr>
<td>V (μL/min/100 g BW)</td>
<td>15.8 ± 3.3</td>
<td>13.1 ± 1.2</td>
</tr>
<tr>
<td>Air</td>
<td>14.5 ± 3.2</td>
<td>18.1 ± 3.0</td>
</tr>
<tr>
<td>Air</td>
<td>15.8 ± 2.6</td>
<td>13.0 ± 1.5</td>
</tr>
<tr>
<td>UNaV (μEq/min/100 g BW)</td>
<td>2.5 ± 0.6</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Air</td>
<td>1.8 ± 0.4*</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>Air</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>ERPF (ml/min/100 g BW)</td>
<td>4.4 ± 0.5</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Air</td>
<td>4.3 ± 0.5</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Air</td>
<td>4.4 ± 0.6</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>GFR (ml/min/100 g BW)</td>
<td>0.87 ± 0.05</td>
<td>0.91 ± 0.07</td>
</tr>
<tr>
<td>Air</td>
<td>0.86 ± 0.05</td>
<td>0.89 ± 0.07</td>
</tr>
<tr>
<td>Air</td>
<td>0.90 ± 0.05</td>
<td>0.90 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SEM. C = control; R = recovery; MAP = mean arterial pressure; V = urine flow rate; UNaV = urinary sodium excretion; ERPF = effective renal plasma flow; GFR = glomerular filtration rate; BW = body weight; SHR-DNX = SHR with renal denervation; WKY = Wistar-Kyoto rats. *p < 0.01, †p < 0.05 compared with control.

and heart rate in SHR-NNa and WKY-NNa were similar to control measures in SHR-HNa and WKY-HNa respectively. Bilateral renal denervation or sham renal denervation did not affect the mean arterial pressure or heart rate responses to air stress in SHR-NNa or SHR-HNa. High sodium intake elevated control measures of urine flow rate and sodium excretion (p < 0.05), but had no effect on control measures of effective renal plasma flow, glomerular filtration rate, or mean arterial pressure. In SHR-HNa, control urine flow rate and sodium excretion were each 72% higher than in SHR-NNa. Control urine flow rate and sodium excretion, respectively, were 70% and 77% greater in WKY-HNa than WKY-NNa. Similarly, in SHR with bilateral surgical renal denervation, HNa increased control urine flow rate and sodium excretion by 50% and 165%, respectively, relative to renal denervated SHR or NNa.

### Renal Sympathetic Nerve Activity

Air stress increased renal sympathetic nerve activity only in SHR, but had no effect on renal sympathetic nerve activity in WKY (Figure 2). High sodium intake augmented (p < 0.05) the renal sympathetic nerve activity response to air stress only in SHR. In SHR-NNa, renal sympathetic nerve activity increased 77%, whereas in SHR-HNa renal sympathetic nerve activity increased 103%. In contrast, air stress failed to elicit a renal sympathetic nerve activity response in WKY on either high or normal sodium intake.
In these rats, air stress increased ($p < 0.01$) mean arterial pressure in SHR-NNa (+10 mm Hg), SHR-HNa (+9 mm Hg), WKY-NNa (12 mm Hg), and WKY-HNa (9 mm Hg; Table 2). The increases in mean arterial pressure during air stress were similar between rats on either high or normal sodium intake. Mean arterial pressure and heart rate returned to control levels during the recovery period.

### Discussion

Stressful environmental stimulation in conscious SHR increased renal sympathetic nerve activity and decreased urinary sodium excretion without altering effective renal plasma flow or glomerular filtration rate. The antinatriuretic effect was abolished by bilateral surgical renal denervation. These findings suggest that the antinatriuretic response to stressful environmental stimulation increased renal tubular sodium reabsorption, which in turn was mediated by increased efferent renal sympathetic nerve activity. In sharp contrast, stressful environmental stimulation had no effect on renal sympathetic nerve activity or urinary sodium excretion in conscious WKY. These results support the study of Lundin and Thoren,\(^1\) in which stressful environmental stimulation caused a much greater increase in renal sympathetic nerve activity and antinatriuresis in conscious SHR than in conscious WKY. A new finding of the present study is that a high sodium intake enhances the magnitude of the renal sympathetic nerve activity and antinatriuretic responses to stressful environmental stimulation in conscious SHR. This enhancement was selective in that the mean arterial pressure and heart rate responses were not increased; however, high sodium intake had no additional effect on renal sympathetic nerve activity or urinary sodium excretion responses to stressful environmental stimulation in conscious WKY.

Therefore, it appears that conscious SHR are not only more sensitive to the effects of stressful environ-

---

**TABLE 2. Renal Sympathetic Nerve Activity and Mean Arterial Pressure Responses to Air Stress in Conscious Spontaneously Hypertensive Rats and Wistar-Kyoto Rats on Normal and High Sodium Diets**

<table>
<thead>
<tr>
<th>Value</th>
<th>SHR (n = 6)</th>
<th>WKY (n = 6)</th>
<th>SHR (n = 6)</th>
<th>WKY (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>167 ± 9</td>
<td>109 ± 6</td>
<td>158 ± 10</td>
<td>109 ± 4</td>
</tr>
<tr>
<td>Control</td>
<td>177 ± 9*</td>
<td>121 ± 6*</td>
<td>167 ± 9*</td>
<td>118 ± 4*</td>
</tr>
<tr>
<td>Air</td>
<td>168 ± 7</td>
<td>115 ± 7</td>
<td>156 ± 10</td>
<td>111 ± 3</td>
</tr>
<tr>
<td>Recovery</td>
<td>6.2 ± 0.6</td>
<td>7.0 ± 0.6</td>
<td>5.9 ± 0.4</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td>RSNA (integrator resets/min)</td>
<td>10.9 ± 1.0*</td>
<td>7.7 ± 0.6</td>
<td>13.6 ± 1.0*</td>
<td>7.0 ± 0.9</td>
</tr>
<tr>
<td>Control</td>
<td>6.2 ± 0.5</td>
<td>6.8 ± 0.5</td>
<td>7.5 ± 0.5</td>
<td>6.7 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SEM. MAP = mean arterial pressure; RSNA = integrated renal sympathetic nerve activity; SHR = spontaneously hypertensive rats; WKY = Wistar-Kyoto rats. *$p < 0.01$ compared with control.
These results indicate that the effects of high sodium on the increases in mean arterial pressure and heart rate were unaffected by the high sodium intake. Similarly, control levels of mean arterial pressure and study, 15 days on the high sodium intake had no effect on the antinatriuretic response in humans as well.

The pathophysiology of hypertension is accelerated by high sodium intake alone or by stressful environmental stimulation alone, but the combination of high sodium intake and stressful environmental stimulation is more effective in producing hypertension than either factor alone. The development of hypertension was more rapid and severe in SHR exposed to territorial stress plus a high sodium intake (3% NaCl for drinking) than when either territorial stress or high sodium intake was presented alone. In similar fashion, hypertension was produced in conscious dogs over several weeks only when they were exposed to aversive conditioning plus high sodium intake. Whether high sodium intake and stressful environmental stimulation exacerbate the pathophysiology of hypertension by increased renal sympathetic nerve activity resulting in antinatriuresis and increased sodium retention, as observed during short-term exposure to these stimuli in the present study, remains to be examined.

Acknowledgments
We thank Linda L. Sawin for excellent technical assistance.

References
High sodium intake enhances renal nerve and antinatriuretic responses to stress in spontaneously hypertensive rats.
J P Koepke and G F DiBona

Hypertension. 1985;7:357-363
doi: 10.1161/01.HYP.7.3.357
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/7/3_Pt_1/357

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/