Effect of Sodium Intake on Sympathetic and Hemodynamic Response to Thermal Receptor Stimulation

Gerald F. DiBona, Susan Y. Jones

Abstract—Low dietary sodium intake increases central nervous system angiotensin activity, which increases basal renal sympathetic nerve activity and shifts its arterial baroreflex control to a higher level of arterial pressure. This results in a higher level of renal sympathetic nerve activity for a given level of arterial pressure during low dietary sodium intake than during either normal or high dietary sodium intake, in which there is less central angiotensin activity. Peripheral thermal receptor stimulation overrides arterial baroreflex control and produces a pressor response, tachycardia, increased renal sympathetic nerve activity, and renal vasoconstriction. To test the hypothesis that increased central angiotensin activity would enhance the responses to peripheral thermal receptor stimulation, anesthetized normal rats in balance on low, normal, and high dietary sodium intake were subjected to acute peripheral thermal receptor stimulation. Low sodium rats had greater increases in renal sympathetic nerve activity, greater decreases in RBF, and greater increases in renal vascular resistance than high sodium rats. Responses of normal sodium rats were between those of low and high sodium rats. Arterial pressure and heart rate responses were not different among dietary groups. Spontaneously hypertensive rats, known to have increased central nervous system angiotensin activity, also had greater renal sympathoexcitatory and vasoconstrictor responses than normotensive Wistar-Kyoto rats. These results support the view that increased central nervous system angiotensin activity alters arterial baroreflex control of renal sympathetic nerve activity such that the renal sympathoexcitatory and vasoconstrictor responses to peripheral thermoreceptor stimulation are enhanced. (Hypertension. 2003;41:261-265.)

Key Words: angiotensin I sodium I renal nerves I renal circulation

Stimulation of peripheral thermal receptors produces an increase in arterial pressure, heart rate, and renal sympathetic nerve activity (RSNA), with the latter resulting in a decrease in RBF.1-3 Thus, this reflex response overrides the normal arterial baroreflex control of heart rate and RSNA inasmuch as decreases in both heart rate and RSNA would be expected to follow an increase in arterial pressure. Compared with rats on normal or high dietary sodium intake, rats with low dietary sodium intake have increased basal RSNA and a resetting of the arterial baroreflex control of RSNA to a higher level of arterial pressure.5,5 The overall result is that at the same level of arterial pressure, the prevailing level of RSNA is higher in rats on low dietary sodium intake than in rats on normal or high dietary sodium intake. Although arterial baroreflex gain is not affected, this upward resetting represents an attenuation of overall arterial baroreflex control of RSNA. These effects are reversed by administration of either losartan or candesartan into either the lateral cerebral ventricle or the rostral ventrolateral medulla (RVLM).6 These results indicate that central angiotensin located in critical central nervous system cardiovascular regulatory nuclei is subject to normal physiological regulation by alterations in dietary sodium intake.6,7

As the full expression of the sympathetic responses to peripheral thermal receptor stimulation involves an overriding of arterial baroreflex control, it was proposed that the responses to peripheral thermal receptor stimulation would be enhanced in rats on a low dietary sodium intake compared with rats on a normal or high dietary sodium intake. As noted above, rats on a low dietary sodium intake have a higher level of RSNA at a given arterial pressure than rats on a normal or high dietary sodium intake, making it likely that their responsiveness to sympathoactivating stimuli would be greater. This hypothesis was tested in anesthetized rats in balance on low, normal, or high dietary sodium intake whose RSNA and hemodynamic responses to peripheral thermal receptor stimulation were measured. For comparison, spontaneously hypertensive rats (SHR) were also examined, as they have chronically increased central nervous system angiotensin activity and RSNA of genetic origin.

Methods

Male Sprague-Dawley (Harlan), Wistar-Kyoto (WKY, Taconic) and spontaneously hypertensive (SHR, Taconic) rats, weighing 300 to 350 g, were used for these studies. All procedures in rats were performed in compliance with the University of Iowa Policies and
Guidelines Concerning the Use of Animals in Research and Teaching and the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85–23, revised 1985).

Sprague-Dawley rats were housed in individual metabolic cages and divided into 3 dietary groups. All 3 dietary groups were given nominally sodium-free pellet food ad libitum (ICN, <0.9 μEq/g sodium). The low sodium group (LNa) received sodium-free distilled water as drinking fluid, the normal sodium group (NNa) received 50 mmol/L NaCl as drinking fluid, and the high sodium group (HNa) received 154 mmol/L NaCl as drinking fluid. The rats equilibrated on these different dietary sodium intake regimens for at least 1 week. On average, the NNa group drank ~35 mL fluid per day, which equates to a sodium intake of ~1.75 mEq/d. The HNa group drank ~60 mL fluid per day, which equates to a sodium intake of ~9.24 mEq/d. The LNa group drank ~20 mL of fluid, which equates to a sodium intake of ~0.0 mEq/d. As previously demonstrated in this laboratory, this dietary regime produces significant increases and decreases in plasma renin activity in LNa and HNa, respectively, compared with NNa.5,6 The WKY and SHR rats were housed in individual metabolic cages and given normal sodium pellet food and tap water as drinking fluid ad libitum.

On the day of the experiment, rats were anesthetized with sodium pentobarbital (50 mg/kg IP); an oral endotracheal tube was inserted, and mechanical ventilation with room air was instituted. A jugular pentobarbital (50 mg/kg IP); an oral endotracheal tube was inserted, and mechanical ventilation with room air was instituted. A jugular vein was catheterized for the administration of additional anesthetic (10 mg/kg IV per hour) and isotonic saline at 0.05 mL/min. A carotid artery was catheterized for the measurement of arterial pressure (AP; pulsatile=P, mean=M) and heart rate (HR). Through a left flank incision, the left renal nerve bundle was dissected free and placed on a bipolar silver wire electrode to which it was fixed with Silgel (Wacker Chemie); the nerves were left intact. A noncannulating electromagnetic flow probe (1.5 mm circumference) was placed around the left renal artery and connected to an electromagnetic flowmeter (Carolina Medical Electronics). After surgery, a 45-minute period was allowed for equilibration and stabilization.

The control period consisted of 20 minutes of continued recording of AP, HR, RBF and RSNA. The peripheral thermal receptors were stimulated by insertion of the rat’s tail in 51°C water (as previously performed in this laboratory),2,3 and recording was continued for an additional 20 minutes (experimental period). Then, the renal nerve recording electrode was connected to an electrical stimulator (Grass S88) and the renal nerves were stimulated at 15 V, 2 ms, and 4 Hz, with a reduction in RBF of <50% taken to indicate that the renal nerves were intact and functional. Rats that failed this test were excluded. At the conclusion of the experiment, the rat was given an overdose of sodium pentobarbital, after which postmortem signals were recorded for 20 minutes.

### Data Analysis
AP, both pulsatile and mean, was recorded by an electronic pressure transducer (Statham). Heart rate was determined with a cardiometer (Grass 7P4) driven by the pulsatile arterial pressure waveform. RSNA was bandpass-filtered between 30 and 1000 Hz, amplified ×20 000, rectified, and integrated with a 20-ms time constant. RBF, both pulsatile and mean, was recorded by the electromagnetic flowmeter, the output of which was low-pass–filtered below 10 Hz by the built-in analog filter. Renal vascular resistance (RVR)=AP/RBF. The outputs of the pressure transducer, the cardiometer, electromagnetic flowmeter, and RSNA integrator were led to a Grass Model 7D polygraph recorder for graphic output and to VHS tape by a pulse code modulation adapter (Vetter Model 4000A PCM Recording Adapter) for later offline analysis.

All data signals were corrected for the postmortem signals. Analog AP, HR, RSNA, RBF, and RVR (as a calculated value) signals were sampled from tape at 20 Hz. For the 20-minute control period, all 24 000 points were averaged for each variable. The average for each variable in the control period was set to 100% and the responses to peripheral thermal receptor stimulation were expressed as percent of control. To take into account differences in both magnitude and duration of the responses to peripheral thermal receptor stimulation, the responses were calculated as change in the area under the time curve calculated by use of the trapezoidal rule.

Statistical analysis was performed with ANOVA, with the subsequent use of the Scheffé method for simultaneous comparisons within groups and the subsequent use of the F ratio and modified statistic for nonsimultaneous comparisons between groups.1 A significance level of 5% was chosen. Data in the text, tables, and figures are expressed as mean±SEM.

### Results
#### Effect of Dietary Sodium Intake in Normal Rats
The control values for the measured variables for each of the 3 dietary sodium intake groups are shown in Table 1. HR was significantly greater in LNa than HNa (but not NNa). While acknowledging the difficulties of comparing multifiber recordings of sympathetic nerve activity between groups, the measured values of RSNA were significantly greater in LNa than both NNa and HNa, confirming previous observations.4 The time courses of the responses of the different variables to peripheral thermal receptor stimulation exhibited a similar pattern among the 3 dietary sodium intake groups. The increases or decreases (in the case of RBF) achieved their peak or nadir in the following order: RSNA, 1 to 10 seconds; AP, 15 to 20 seconds; RBF, 30 to 60 seconds; RVR, 30 to 60 seconds. The HR responses (data not shown) were similar to those previously observed2,3 with the peak in HR occurring slightly after that of AP and before that of RBF. Thereafter, the values of each variable returned toward their control period levels, reaching them within 10 minutes. Figure 1 shows the RSNA and RBF responses for one LNa and one HNa for the first 180 seconds after peripheral thermal receptor stimulation. RSNA was greater in LNa than HNa for the initial 30 seconds and was similar thereafter. The nadir in RBF was lower and occurred somewhat later in LNa than in HNa, but whereas RBF returned progressively toward control in HNa, it remained decreased in LNa.

Figure 2 shows the data for each variable calculated as area under the curve. Peripheral thermal receptor stimulation increased RSNA in all 3 dietary sodium intake groups (Figure 2A). The increase in RSNA in LNa was significantly greater than that in HNa. The increases in AP were similar in all 3 dietary sodium intake groups (Figure 2B). The decrease in RBF in LNa was greater than that in NNa, whereas RBF in HNa increased. This is explained by the fact that although the peak decreases in RBF were relatively similar (LNa 23%,
NNa 24%, HNa 20%), the decreases were sustained over time in LNa and NNa, whereas in HNa RBF had an early return (on average, at 6 minutes) to the control value followed by an overshoot to values greater than control for the remainder of the experimental period. This emphasizes the importance of analyzing the data as area under the curve, to take into account the full change over the entire time course of the response. When the RBF responses were subjected to ANOVA, RBF in NNa was significantly different \( (P<0.05) \) from RBF in LNa over the time interval from 36 to 180 seconds and from RBF in HNa over the time interval from 35 to 180 seconds. RVR increased in all 3 dietary sodium intake groups, with the increase in LNa being significantly greater than those in either NNa or HNa (which were not different from each other, Figure 2D).

**WKY and SHR**

The control values for the measured variables for WKY and SHR are shown in Table 2. Values for AP, HR, RVR, and RSNA are significantly greater in SHR than in WKY, whereas RBF values were similar.

Figure 3 shows the RSNA and RBF responses for one WKY and one SHR for the first 540 seconds after peripheral thermal receptor stimulation. The immediate peak in RSNA is greater in SHR than WKY. Thereafter, RSNA in WKY returns to the control level at \( \approx 120 \) seconds, whereas RSNA

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**Table 2. Control Period Values for WKY and SHR**

<table>
<thead>
<tr>
<th>Variable</th>
<th>WKY (n=9)</th>
<th>SHR (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP, mm Hg</td>
<td>117±2</td>
<td>159±2*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>305±13</td>
<td>361±14*</td>
</tr>
<tr>
<td>RBF, mL/min</td>
<td>6.58±0.13</td>
<td>6.61±0.15</td>
</tr>
<tr>
<td>RVR, mm Hg/mL per minute</td>
<td>17.8±0.4</td>
<td>23.3±0.7*</td>
</tr>
<tr>
<td>RSNA, mV/20 ms</td>
<td>21.6±2.1</td>
<td>45.0±4.0*</td>
</tr>
</tbody>
</table>

WKY indicates Wistar-Kyoto rats; and SHR, spontaneously hypertensive rats. \( ^*P<0.05 \) for SHR vs WKY.
in SHR decreases more slowly and remains above RSNA in WKY throughout. AP followed a similar pattern as RSNA. Whereas the early nadir in RBF is similar in WKY and SHR, RBF returns toward the control level within 120 to 180 seconds in WKY, whereas RBF in SHR remains decreased below control throughout. These observations emphasize the importance of analyzing the data as area under the curve, to take into account the magnitude of the change over the entire time course of the response. When the RBF responses were subjected to ANOVA, RBF in SHR was significantly different (P < 0.05) from RBF in WKY over the time interval from 89 to 540 seconds.

The HR responses (data not shown) were similar to those previously observed, with the peak in HR occurring slightly after that of AP and before that of RBF.

The group data for RSNA, RBF, and AP (area under the curve) are shown in Figure 4. RSNA increased 25% more in SHR than WKY. RBF decreased 69% more in SHR than WKY, and AP increased 22% more in SHR than WKY (all P < 0.05).

**Discussion**

These results indicate that the sympathetic and hemodynamic responses to peripheral thermal receptor stimulation are influenced by alterations in dietary sodium intake, which are known to produce physiological changes in the activity of the renin-angiotensin system. The LNa rats with presumably stimulated renin-angiotensin activity had a greater increase in RSNA, a greater decrease in RBF, and a greater increase in RVR compared with other dietary sodium intake groups with normal or suppressed renin-angiotensin system activity. Thus, in response to a standard peripheral reflex stimulation, LNa rats had a greater renal sympathoexcitatory response, which was associated with a greater renal vasoconstrictor response. In the LNa rats, the increased central activity of the renin-angiotensin system is known to produce a resetting and/or attenuation of arterial baroreflex control of RSNA. This in turn facilitates the renal sympathoexcitatory response to peripheral thermoreceptor stimulation, a maneuver known to override arterial baroreflex control.

Had LNa rats had a greater renal vasoconstrictor response to peripheral thermal receptor stimulation in the absence of an increased renal sympathoexcitatory response; it could be argued that this could be related to a peripheral effect of increased circulating angiotensin, facilitating norepinephrine release from renal sympathetic nerve terminals by a presynaptic action of angiotensin on AT1 receptors. However, this was not the case, as directly measured RSNA increased in LNa. Although it cannot be completely excluded that a portion of the enhanced renal vasoconstrictor response may be due to such a peripheral action of angiotensin, it is clear that the greater increase in RSNA indicates that there is an important central action of the LNa, probably mediated by an increase in activity of the renin-angiotensin system within the central nervous system. These findings are in agreement with previous evidence that LNa increases central nervous system angiotensin activity. When AT1 receptor antagonists were injected either into the lateral cerebral ventricle or the RVLM, the decreases in RSNA and AP were correlated with the degree of activation of the renin-angiotensin by the different dietary sodium intakes. Thus, the decreases in RSNA and AP were greater in LNa, in which plasma renin activity was stimulated, and least in HNa, in which plasma renin activity was suppressed. In addition, the arterial baroreflex control of RSNA was reset to a lower arterial pressure, with the magnitude of the shift being greatest in LNa and least in HNa. As the responses to peripheral thermal receptor stimulation indicate that the normal arterial baroreflex regulatory mechanism is being overridden, then it is likely that any existing attenuation of arterial baroreflex regulation of RSNA would facilitate the renal sympathoexcitatory response to peripheral thermal receptor stimulation. Therefore, in LNa, it is likely that the higher basal level of RSNA as well as the upward shift of the arterial baroreflex control of RSNA contributed to the enhanced renal sympathoexcitatory response to peripheral thermoreceptor stimulation.

Similar findings of an increased renal sympathoexcitatory and vasoconstrictor response to peripheral thermal receptor stimulation were observed in SHR. The SHR is a genetic model of hypertension characterized by an increased basal level of RSNA and an arterial baroreflex relation, which is shifted substantially upward to the higher level of arterial pressure. In addition, there is increased central nervous system angiotensin activity, based on the demonstration that injection of candesartan or valsartan into the RVLM decreased both lumbar sympathetic nerve activity and arterial pressure in SHR but not in WKY. Given that these characteristics are similar to those observed in LNa, it was not surprising that the response to peripheral thermal receptor stimulation in SHR was similar to that in LNa.

Although a role for angiotensin in the RVLM has been difficult to demonstrate in normal rats with presumably normal central renin-angiotensin system activity, it is evident that under circumstances in which the renin-angiotensin system is stimulated, as with LNa and in SHR, it is clear that angiotensin in the RVLM is supporting both the level of peripheral (renal, lumbar) sympathetic nerve activity and the
arterial pressure. As a consequence, therefore, the arterial baroreceptor relation between arterial pressure and sympathetic nerve activity is shifted to a higher level of arterial pressure. Although arterial baroreflex gain is not altered, this situation can be considered an attenuation of arterial baroreflex function in that for a given level of arterial pressure sympathetic nerve activity is higher than when the renin-angiotensin system is not stimulated. The importance of this attenuation can be seen when interventions are applied that secondarily engage the arterial baroreflex to exert a buffering action, for example, peripheral thermal receptor stimulation. As the peripheral stimulus is the same, it is evident that the enhanced renal sympathoexcitatory response seen in both LNa and SHR, compared with LNa and WKY, respectively, reflect the reduced capacity for arterial baroreflex buffering when the central renin-angiotensin system activity is increased.

**Perspectives**

When faced with decreased dietary sodium intake, the organism mobilizes those several mechanisms involved in the normal compensatory response of the kidney which is to increase renal tubular sodium reabsorption (ie, decrease urinary sodium excretion) so as to achieve sodium balance. RSNA and the activity of the circulating renin-angiotensin system are two such potent renal sodium-retaining mechanisms that are activated by decreased dietary sodium intake. It now appears that these two mechanisms have a central nervous system interaction wherein decreased dietary sodium intake increases central renin-angiotensin system activity in important cardiovascular sympathoexcitatory nuclei (eg, RVLM), which contributes to the increase in RSNA. Although this interaction is beneficial for the important task of achieving sodium balance, the arterial baroreflex control of RSNA is altered so that for the same level of arterial pressure the level of RSNA is greater than that seen during normal or increased dietary sodium intake. The functional consequences of this upward resetting of the arterial baroreflex control of RSNA may not be great in the absence of challenges requiring the buffering ability of the arterial baroreflex. However, when challenged by an intervention that normally engages compensatory arterial baroreflex buffering so as to modulate the final response, the functional consequences are seen to be larger in proportion to the magnitude of upward resetting of arterial baroreflex control of RSNA.

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**References**

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