Degeneration of Capsaicin-Sensitive Sensory Nerves Leads to Increased Salt Sensitivity Through Enhancement of Sympathoexcitatory Response

Donna H. Wang, Wei Wu, Keith J. Lookingland

Abstract—We have previously shown that neonatal degeneration of capsaicin-sensitive sensory nerves renders a rat responsive to a salt load with an increase in blood pressure and a decrease in natriuretic response. To test the hypothesis that the enhanced sympathoexcitatory response to a high salt intake contributes to the development of hypertension in this model, newborn Wistar rats were given 50 mg/kg capsaicin and/or 80 mg/kg guanethidine subcutaneously. Control rats were treated with vehicle. After the weaning period, male rats were grouped as the following and given a high sodium diet (4%) for 2 weeks: capsaicin and guanethidine coadministration (CAP-GUA), capsaicin only (CAP), guanethidine only (GUA), and vehicle control (CON). Norepinephrine concentrations in the atrium were significantly lower in CAP-GUA and GUA than in CON rats (P<0.05). Twenty-four–hour urine and sodium excretions were significantly lower in CAP than in CAP-GUA, GUA, and CON rats (P<0.05). Mean arterial pressure (mm Hg) was significantly higher in CAP (180±10) than in CAP-GUA (106±1), GUA (133±5), and CON (122±3) rats (P<0.05). Thus, sympathectomy restores the natriuretic response to a high salt intake and prevents the development of salt-sensitive hypertension induced by sensory denervation. These data indicate that sensory nerves counterbalance the prohypertensive effect of the sympathetic nerves to maintain blood pressure within normal range during salt loading. (Hypertension. 2001;37[part 2]:440-443.)

Key Words: denervation ■ sympathectomy ■ hypertension, sodium-dependent ■ sodium, dietary

Somatosensory input normally inhibits sympathetic nerve activity through the nucleus of the solitary tract pathway.1,2 Attenuation of this inhibition may lead to increased sympathetic nerve activity.3–5 Previous studies show that decreased responsiveness of sensory nerves contributes to increased renal sympathetic nerve activity and sodium retention in spontaneously hypertensive rats.6,7 Conversely, long-term ablation of sympathetic neurons is followed by an increase in the afferent innervation.8–10 These studies support the concept that an alteration of the normal balance between sensory and sympathetic nerves by eliminating either of these nerve populations will lead to hyperresponsiveness or hyperinnervation of the remaining nerve population.

In addition to the function traditionally known as “sensing” changes in the environment and transmitting the information to the central nervous system, sensory fibers have local effector function through releasing a variety of vasodilator neuropeptides, for example, calcitonin gene-related peptide (CGRP) and substance P, peripherally in response to local stimuli.10 The cell bodies of these afferent fibers are located in the dorsal root ganglia (DRG), in which sensory neurotransmitters are synthesized and stored. These sensory neurotransmitters may directly affect blood pressure by modulation of cardiovascular and renal function.10,11 For example, it has been shown that CGRP and substance P are not only potent vasodilators but also have direct and indirect effects on tubular ion transport resulting in natriuretic and diuretic actions.11–13 Moreover, degeneration of sensory nerves induced by capsaicin treatment leads to enhanced development of deoxycorticosterone-induced hypertension14 and 1-kidney, renal-wrap hypertension.15

In contrast to these studies that use experimental hypertensive rats, we have recently developed a model that contributes to the understanding of the primary role of sensory nerves in long-term blood pressure regulation. We found that neonatal treatment with capsaicin results in a marked decrease in CGRP levels in DRG and causes a normal rat to respond to a salt load with a significant rise in blood pressure.16 Furthermore, the increase in blood pressure can be prevented by blockade of the type 1 angiotensin II receptor (AT₁),17 indicating that the renin-angiotensin system is activated and plays a significant functional role in the development of hypertension in this model. The potential role of the sympathetic nervous system that interacts intimately with the sensory nervous system, however, has not been defined. The aim of this experiment is to test the hypothesis that neonatal degeneration of capsaicin-sensitive sensory nerves leads to increased salt sensitivity in terms of blood pressure regulation through enhancement of sympathoexcitatory response to salt load.
Body Weight (g) of Rats Before and After Dietary Treatment

<table>
<thead>
<tr>
<th>Time</th>
<th>CON</th>
<th>GUA</th>
<th>CAP</th>
<th>CAP-GUA</th>
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<td>n=6</td>
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<tr>
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<td>48±1</td>
<td>50±1</td>
<td>47±1</td>
</tr>
<tr>
<td>End</td>
<td>135±2</td>
<td>103±2 *</td>
<td>114±2 *</td>
<td>100±2†</td>
</tr>
</tbody>
</table>

Values are mean±SE. *P<0.05 vs CON, †P<0.05 vs CAP.

Methods

Animals
Pregnant Wistar rats (Charles River Laboratories Inc) were housed in the animal care unit for at least 1 week before parturition. Neonatal rats postnatally received capsaicin 50 mg/kg SC per day for 2 days and/or guanethidine 80 mg/kg SC per day for 2 weeks. Capsaicin is a selective toxin of sensory neurons, and guanethidine causes sympathoectomy. Control rats were treated with equal volumes of vehicle solution (5% ethanol, 5% Tween 80 in saline). After the weaning period, male rats were divided into 4 groups and fed a high (4%) sodium diet for 2 weeks: control vehicle (CON), guanethidine+vehicle (GUA), capsaicin+vehicle (CAP), and capsaicin+guanethidine (CAP-GUA). The food was purchased from Harlan Teklad Diets. At the end of the 2-week dietary treatment period, rats were anesthetized with a single intraperitoneal injection of 80 mg/kg ketamine and 1 mg/kg xylazine. The left carotid artery was catheterized for continuous measurement of mean arterial pressure (MAP, mm Hg) with Statham 231D pressure transducer (Gould) coupled to a Gould 2400s recorder 3 hours after surgery, with rats fully awake and unrestrained. The MAP value for each rat was calculated as an average of measurements during 20 minutes of recording.

Systolic Blood Pressure
Indirect tail-cuff systolic blood pressure was routinely obtained in all rats by a Narco Bio-System Electro-Spygmomanometer. The pressure was measured in conscious rats every 7 days for 14 days, beginning 1 day before dietary treatment. The blood pressure value for each rat was calculated as the average of 3 separate measurements at each session.

Water Intake, Urine Volume, and Urinary Sodium and Potassium Concentrations
Water intake and urine excretion were determined with the use of metabolic cages. These parameters were measured 1 day before the rats were killed. Urinary sodium and potassium concentrations were determined with a flame atomic absorption spectrophotometer (Instrumentation Laboratory Co) (kindly provided by Dr Gregory Fink, Michigan State University).

Norepinephrine Content Analysis
Animals were decapitated, and the atria were quickly removed and frozen at −70°C until assay. Norepinephrine (NE) concentrations were determined by high-performance liquid chromatography (HPLC) with electrochemical detection. On the day of the assay, samples were thawed and centrifuged for 30 seconds in a Beckman 152 Microfuge. Fifty microliters of the supernatant was injected into a C-18 reverse-phase analytical column (5-μm spheres; 250×4.6 mm; Biophsace ODS, Bioanalytical Systems), which is protected by a precolumn cartridge filter (5-μm spheres; 30×4.6 nm). The HPLC column was coupled to a single colormetric electrode conditioning cell in series with dual-electrode analytical cells (ESA). The conditioning electrode potential was set at +0.4 V; the analytical electrodes were set at +0.12 V and −0.31 V, respectively, relative to the reference electrodes. The HPLC mobile phase consisted of 1.0 mol/L phosphate-citrate buffer, pH 2.7, with 0.1 mmol/L EDTA, 0.35% sodium octylsulfate, and 20% methanol. The amount of NE in the samples was determined by comparing peak heights (determined by a Hewlett Packard Integrator, model 3393A) with those obtained from standards run on the same day.

Statistical Analysis
Values are expressed as mean±SEM. The data were analyzed by ANOVA followed by the Tukey-Kramer multiple comparison test. Differences were considered statistically significant at P<0.05.

Results
Body weight was not significantly different among the 4 groups before the dietary treatment (Table). Body weight increased significantly over the experimental period in all experimental groups. However, rats in the control group (CON) gained more weight than in the 3 treated groups (GUA, CAP, and CAP-GUA) by the end of the experiment.

Figure 1 shows that NE levels in rat atria were significantly higher in CAP than in CON, GUA, and CAP-GUA.

Figure 2 shows that tail-cuff systolic blood pressure was significantly higher in CAP than in CON, GUA, and CAP-GUA.
Figure 4. Ratio of 24-hour urine volume to water intake in each of 4 experimental groups. Values are mean±SEM; n=5 to 8 in each group. +P<0.05 vs CON; *P<0.05 vs GUA; #P<0.05 vs CAP.

GUA rats (P<0.05), beginning at the 7th day after dietary treatment and for the rest of the experimental period. Direct measurement of MAP at the end of the experiment confirmed the results obtained from tail-cuff measurement (Figure 3), that is, MAP was significantly higher in CAP than in CON, GUA, and CAP-GUA rats (P<0.05). These results indicate that guanethidine treatment prevents the increase of blood pressure induced by capsaicin and high salt treatment. Figure 4 shows the ratio of 24-hour urine volume to water intake. The ratio was significantly lower in CAP than in CON, GUA, and CAP-GUA rats (P<0.05). There was no significant difference among the latter 3 groups. Likewise, 24-hour urine Na+ excretion was significantly lower in CAP than in CON and CAP-GUA rats (P<0.05), and it was significantly lower in GUA than in CON rats (P<0.05, Figure 5). These results indicate that guanethidine treatment prevents the impairment of urinary volume and Na+ excretion induced by capsaicin and high salt treatment. In contrast, urinary K+ excretion was not significantly different among the 4 groups (Figure 6).

Discussion

Although it is well known that increased sympathetic drives contribute to increased salt sensitivity and blood pressure, knowledge about the mechanisms that lead to elevated sympathetic nerve activity remains incomplete. This experiment was designed to test the hypothesis that enhanced sympathoexcitatory response to a salt load occurs and contributes to increased salt sensitivity and blood pressure when sensory nerves are impaired by neonatal treatment of capsaicin. The data generated contain several distinct observations. Consistent with our previous findings, neonatal degeneration of capsaicin-sensitive sensory nerves renders the rat salt-sensitive in terms of blood pressure regulation.16,17 We have now shown for the first time that sympathectomy prevents the development of salt-sensitive hypertension induced by sensory denervation. Furthermore, increased salt sensitivity in capsaicin-treated rats is accompanied by the lack of an increment in urinary sodium and water excretion in response to a high salt intake.16,17 Again, we have now shown that sympathectomy restores the natriuretic response to a high salt intake in capsaicin-treated rats. This appears to be the first indication of a role for sensory nerves as a regulator of salt sensitivity and blood pressure through modulation of sympathetic nerve activity.

It is well known that blood pressure increases with age, especially during the fast growing period after weaning. Our data are consistent with this notion in which we found that systolic blood pressure increased with time in all 4 experimental groups. This age-related increase in blood pressure does not appear to associate with high salt intake because we have previously shown that systolic blood pressure increases with time in both control and capsaicin-treated rats fed either a normal or high sodium diet.16 However, capsaicin treatment that damages sensory nerves makes rats more susceptible to salt-induced elevation in blood pressure in light of the fact that blood pressure increases more in capsaicin-treated rats fed a high salt diet than in control rats fed a high salt diet. The mechanisms responsible for increased salt sensitivity induced by capsaicin treatment are unknown. It has been shown that sodium restriction increases whereas sodium loading decreases sympathetic activity.22 It is possible that a failure of suppression of sympathetic nerve activity after sodium loading occurs in capsaicin-treated rats.

Somatosensory input normally inhibits sympathetic nerve activity through the nucleus of the solitary tract pathway.1,2 Attenuation of this inhibition caused by impairment of sensory nerve function leads to increased sympathetic nerve activity.3-5 It has been shown that decreased responsiveness of sensory neurons contributes to increased renal sympathetic nerve activity and sodium retention in spontaneously hypertensive rats.6,7 We have previously shown that neonatal treatment with capsaicin results in a remarkable decrease in CGRP levels in the DRG.16,17 Degeneration of sensory nerves induced by capsaicin would lead to elimination and/or attenuation of sensory inhibition of sympathetic nerve activity. As a result, elevation of blood pressure and suppression of natriuretic response to a high salt intake occur in this model.16,17 The fact that sympathectomy prevents the development of hypertension and restores the natriuretic re-

Figure 5. Twenty-four-hour urinary sodium excretion in each of 4 experimental groups. Values are mean±SEM; n=5 to 8 in each group. +P<0.05 vs CON; #P<0.05 vs CAP.

Figure 6. Twenty-four-hour urinary potassium excretion in each of 4 experimental groups. Values are mean±SEM; n=5 to 8 in each group.
response to a high salt intake in this model provides direct evidence that unsuppressed sympathetic drive caused by sensory denervation contributes to salt-induced elevation in blood pressure.

It is known that enhanced sympathetic drive to the kidney causes sodium and water retention and elevation in blood pressure.23,24 Thus, sympathectomy is expected to increase sodium and water excretion. However, urine sodium excretion was actually decreased in guanethidine-treated rats fed a high sodium diet compared with control rats fed a high sodium diet. This decrease in urine sodium excretion may be associated with compensatory activation of the renin-angiotensin system to prevent a fall in blood pressure caused by sympathectomy. On the other hand, the prohypertensive effect of sympathetic nerves in sensory denervated rats fed a high sodium diet does not appear to be mediated by activation of the α1-adrenergic receptor. We have previously shown that blockade of the α1-adrenergic receptor with prazosin has no effect on blood pressure and renal function in sensory denervated rats fed a high salt diet,17 indicating that the prohypertensive effect of sympathetic nerves is mediated by components other than the α1-adrenergic receptor. Future assessment of other α- and β-adrenergic receptor antagonists will help to define the role of various components of the sympathetic nervous system in the development of hypertension in this model.

In addition to its direct effect, altered sympathetic activity may influence renal function and blood pressure by interacting with the renin-angiotensin system. It is well known that increased renal sympathetic drive stimulates renin secretion through activation of the β1-adrenergic receptor.23,24 Activation of the renin-angiotensin system may synergistically contribute to increased blood pressure and decreased natriuretic response to a high salt intake in sensory-denervated rats. Indeed, we have shown that blockade of the AT1 receptor with losartan prevents the development of salt-sensitive hypertension induced by sensory denervation, providing direct evidence that the renin-angiotensin system plays a role in blood pressure regulation in this model.17 However, our previous data show that impaired natriuretic response is not reversed by losartan,17 indicating (1) that the antihypertensive effect of losartan is mediated by mechanisms (e.g., vasodilator mechanism) other than those that prevent the impairment of the renal function, and (2) that the sympathectomy-induced restoration of the renal function observed in the current study is not mediated by the renin-angiotensin system. Confirmation of these notions would rely on future direct measurements of plasma and tissue levels of various components of the renin-angiotensin system in guanethidine- and capsaicin-treated rats.

Conclusions
We have shown that sympathectomy prevents the development of hypertension and restores the natriuretic response to a high salt intake in capsaicin-treated rats. These data indicate that sensory nerves counterbalance the prohypertensive effect of sympathetic nerves to maintain blood pressure within normal range during salt loading. If this balance is disturbed as the result of degeneration of sensory nerves, it would lead to increased salt sensitivity in terms of blood pressure regulation through enhancement of sympathoexcitatory response to salt loading.

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