Antihypertensive Mechanisms Underlying a Novel Salt-Sensitive Hypertensive Model Induced by Sensory Denervation

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Abstract—A novel model of hypertension recently developed in our laboratory shows that neonatal degeneration of capsaicin-sensitive sensory nerves renders a rat responsive to a salt load with a significant rise in blood pressure. To determine the role of the renin-angiotensin system and the sympathetic nervous system in the development of hypertension in this model, newborn Wistar rats were given capsaicin 50 mg/kg SC on the first and second days of life. Control rats were treated with vehicle. After they were weaned, male rats were divided into 6 groups and subjected to the following treatments for 2 weeks: control + high sodium diet (4%) (CON-HS), capsaicin + normal sodium diet (0.5%) (CAP-NS), capsaicin + high sodium diet (CAP-HS), capsaicin + high sodium diet + losartan (10 mg/kg per day) (CAP-HS-LO), capsaicin + high sodium diet + prazosin (3 mg/kg per day) (CAP-HS-PR), and capsaicin + high sodium diet + hydralazine (10 mg/kg per day) (CAP-HS-HY). Levels of calcitonin gene–related peptide in dorsal root ganglia were decreased by capsaicin treatment ($P < 0.05$). Both tail-cuff systolic blood pressure and mean arterial pressure were higher in CAP-HS and CAP-HS-PR than in CON-HS, CAP-NS, CAP-HS-LO, and CAP-HS-HY ($P < 0.05$). The 24-hour urinary volume and sodium excretion were increased when a high sodium diet was given ($P < 0.05$), but they were lower in CAP-HS, CAP-HS-LO, CAP-HS-PR, and CAP-HS-HY than in CON-HS ($P < 0.05$). Urinary potassium excretion was not different among all 6 groups. We conclude that blockade of the angiotensin type 1 receptor with losartan but not antagonism of the $α_1$-adrenoreceptor with prazosin prevents the development of salt-sensitive hypertension induced by sensory denervation. Sensory denervation impairs urinary sodium and water excretion in response to a high sodium intake, regardless of blood pressure, suggesting that sensory innervation plays a direct role in regulating the natriuretic response to sodium loading. (*Hypertension. 1999;33[part II]:499-503.*)

Key Words: capsaicin ■ sodium, dietary ■ innervation, sensory ■ hypertension, salt-sensitive ■ renal circulation

It is well established that in a considerable number of essential hypertensive patients and patients with glomerulonephritis, primary aldosteronism, and diabetes mellitus, blood pressure is sodium sensitive.$^1$ Despite intensive research in this area, the cellular and molecular mechanisms underlying salt-sensitive hypertension are not well defined, and no pharmacological approaches have been developed that directly target the defect in salt handling. The existing genetic and experimental animal models of salt-sensitive hypertension, eg, Dahl salt-sensitive rats, Milan hypertensive rats, and deoxycorticosterone-salt hypertensive rats, have been widely used to explore underlying mechanisms linked to salt-sensitive hypertension.$^1$ Recently, we developed a novel salt-sensitive hypertensive model that is sensory nerve dependent. We found that capsaicin-induced degeneration of sensory nerves in rats impairs normal increase in urinary sodium and water excretion when a high sodium diet is given and renders the rats responsive to salt load with a significant and sustained rise in blood pressure.$^2$ This is the first decisive evidence that sensory innervation plays significant functional roles in antagonizing the development of salt-induced hypertension.

It has been established that sensory afferent fibers release a variety of vasodilator neuropeptides, eg, calcitonin gene–related peptide (CGRP) and substance P, peripherally in response to local stimuli.$^3$ These neuropeptides may directly affect blood pressure by modulating cardiovascular and renal functions.$^4$ Alternatively, the sensory nervous system may interact with the 2 powerful prohypertensive systems, ie, the renin-angiotensin and sympathetic nervous systems, to regulate blood pressure. For example, it has been shown that exogenous CGRP increases plasma renin activity in humans and stimulates renin release from isolated rat renal juxtaglomerular cells.$^5$ Furthermore, CGRP and substance P have been shown to influence sympathetic transmission in several peripheral systems, eg, rat mesenteric arterial bed and rat hepatic artery.$^6,7$ However, it is unknown whether the interaction between the sensory nervous system and the renin-an-
giotensin and sympathetic nervous systems plays a significant functional role in salt-induced hypertension. The present study was therefore designed to test the hypothesis that the development of salt-sensitive hypertension induced by sensory denervation is mediated by activation of the renin-angiotensin or sympathetic nervous systems.

**Methods**

**Animals**

Pregnant Wistar rats (Charles River Laboratories, Wilmington, Mass.) were housed in the animal care unit for at least 1 week before parturition. On days 1 and 2 of life, neonatal rats received capsaicin 50 mg/kg SC as described. Control rats were treated with equal volumes of vehicle solution (5% ethanol, 5% Tween 80 in saline). All treatments were performed with rats under ether anesthesia. After 3 weeks, male and female rats were separated, and only male rats were used in the present study. The rats were divided into 6 groups, pair-fed different sodium diets, and subjected to different drug treatments for 2 weeks: control (CON-HS), capsaicin + normal sodium diet (0.5%) (CAP-NS), capsaicin + high sodium diet (CAP-HS), capsaicin + high sodium diet + losartan (10 mg/kg per day) (CAP-HS-LO), capsaicin + high sodium diet + prazosin (3 mg/kg per day) (CAP-HS-PR), and capsaicin + high sodium diet + hydralazine (10 mg/kg per day) (CAP-HS-HY). The rat food was purchased from Harlan Teklad Diets. Antihypertensive drugs were given by oral gavage. The doses of these drugs were chosen based on previous studies showing that they are effective in antagonizing the development of hypertension in spontaneously hypertensive rats (SHR). At the end of the treatment period, rats were anesthetized with a single injection of ketamine 80 mg/kg and xylazine 1 mg/kg IP, and the carotid artery was catheterized. Mean arterial pressure (MAP) was measured with a Statham 231D pressure transducer (Gould) coupled to a Gould 2400s recorder ~3 hours after surgery with rats fully awake and unrestrained.

**Systolic Blood Pressure**

Indirect tail-cuff systolic blood pressures were routinely obtained in all rats by use of a Narco Bio-Systems Electro-Sphygmomanometer. The pressures were 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, Urinary Volume, and Urinary Sodium and Potassium Concentrations**

At the end of the 2-week treatment, 24-hour water intake and urinary excretions were determined in each of the 6 groups by use of metabolic cages. Urinary sodium and potassium concentrations were determined with the use of a flame atomic absorption spectrophotometer (Perkin-Elmer).

**Radioimmunoassay**

At the end of the experiment, the rats were killed by decapitation, and the cervical, thoracic, and lumbar dorsal root ganglia from each rat were immediately dissected and frozen in liquid nitrogen. To determine immunoactive CGRP content in the dorsal root ganglia, a commercially available rabbit anti-rat CGRP radioimmunoassay kit (Phoenix Pharmaceuticals) was used. This antibody has 100% cross-reactivity with rat a-CGRP and 79% with rat b-CGRP. There is no cross-reactivity with rat amylin, calcitonin, somatostatin, or substance P. The assay was performed as recommended by the supplier, and the total protein content was determined by the Bradford method (Bio-Rad).

**Statistical Analysis**

Values are mean±SE. Differences between groups were determined 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 6 groups before the dietary treatment (Table). Body weight increased significantly over the experimental period and was not significantly different among the 6 groups at the end of the experiment (Table). Thus, capsaicin and antihypertensive treatments do not alter somatic development of rats fed either a normal or a high sodium diet.

At the end of the experiment, tail-cuff systolic blood pressure was significantly higher in the CAP-HS rats than in CON-HS, CAP-NS, CAP-HS-LO, and CAP-HS-HY rats but was not significantly different between the CAP-HS and CAP-HS-PR rats (Figure 1). Systolic blood pressure was also significantly higher in the CAP-HS-PR than in CAP-NS and CAP-HS-LO rats (Figure 1). Direct measurement of MAP confirmed the results obtained from tail-cuff measurement, ie, MAP (millimeters of mercury) was significantly higher in the CAP-HS (143±4; n=7) rats than in CON-HS (115±3; n=5), CAP-NS (98±6; n=7), CAP-HS-LO (116±5; n=8), and CAP-HS-HY (117±4; n=6) rats but was not significantly different between the CAP-HS and CAP-HS-PR rats (130±3; n=8) rats. MAP was also significantly higher in the CAP-HS-PR than in CAP-NS rats. Thus, neonatal treatment with capsaicin increases blood pressure in rats fed a high sodium diet. Losartan and hydralazine, but not prazosin, prevent the development of salt-induced hypertension in capsaicin-treated rats.

The ratio of 24-hour urinary volume to water intake is shown in Figure 2. This ratio was significantly higher in all of the rats fed a high sodium diet than in rats fed a normal sodium diet. However, this ratio was significantly lower in the CAP-HS, CAP-HS-LO, CAP-HS-PR, and CAP-HS-HY rats than in CON-HS rats. These results indicate that neonatal treatment with capsaicin impairs proportional urinary excretion when rats are loaded with salt. Losartan, prazosin, and hydralazine do not prevent capsaicin-induced impairment of urinary excretion in response to a high sodium intake.
Likewise, 24-hour urinary sodium excretion was significantly higher in all of the rats fed a high sodium diet than in rats fed a normal sodium diet (Figure 3). However, urinary sodium excretion was significantly lower in the CAP-HS, CAP-HS-LO, and CAP-HS-PR rats than in CON-HS rats. Urinary sodium excretion was also slightly but not significantly lower in the CAP-HS-HY than in CON-HS rats. These results indicate that neonatal treatment with capsaicin impairs urinary sodium excretion when rats are loaded with salt. Losartan and prazosin do not prevent capsaicin-induced impairment of urinary sodium excretion in response to a high sodium intake. In contrast, urinary potassium excretion was not significantly different among the 6 groups, indicating that capsaicin and antihypertensive drugs do not alter urinary potassium excretion in rats fed either a normal or a high sodium diet (Figure 3).

Figure 3. Twenty-four hour urinary sodium and potassium excretion in each of the 6 experimental groups. Values are mean±SE; n=4 to 7 in each group. +P<0.05 vs CON-HS; *P<0.05 vs CAP-NS.

Discussion

We examined the role of the renin-angiotensin and sympathetic nervous systems in the development of salt-sensitive hypertension in rats. Capsaicin treatment during the neonatal period impaired sodium excretion and increased sodium sensitivity in adult rats. Losartan and prazosin did not prevent this effect. These results suggest that capsaicin-induced depletion of CGRP in the dorsal root ganglia may be involved in the development of salt-sensitive hypertension. Further studies are needed to elucidate the mechanism by which capsaicin affects sodium excretion and blood pressure regulation in rats.
hypertension induced by neonatal sensory denervation. The present study contains several distinct observations. First, losartan and hydralazine, but not prazosin, prevent the development of salt-sensitive hypertension induced by sensory denervation. Second, CGRP depletion in the dorsal root ganglia is similar in all capsaicin-treated rats, indicating that CGRP may not be responsible for differences in blood pressure in these rats. Finally, despite the effectiveness of antihypertensive treatment, sensory denervation impairs urinary sodium and water excretion in response to a high sodium intake. These findings have been collectively synthesized to indicate a role for the sensory nervous system, by interacting with other prohypertensive systems, in regulating blood pressure and natriuretic responses to salt load.

In contrast to existing genetic or experimental animal models of salt-sensitive hypertension, we recently developed a novel salt-sensitive hypertensive model that is sensory nerve dependent.\(^2\) We found that although neonatal treatment with capsaicin resulted in depletion of CGRP in the dorsal root ganglia of rats fed either a normal or high sodium diet, capsaicin-induced sensory denervation increased blood pressure only in rats fed a high sodium diet.\(^2\) These results provide conclusive evidence that sensory innervation plays significant functional roles in impeding the development of salt-induced hypertension. The question that remains to be answered, however, is the mechanisms by which sensory denervation renders the rats responsive to salt loading with a significant rise in blood pressure. It has been shown that sensory neurotransmitters, such as CGRP and substance P, are not only very potent vasodilators but also have direct and indirect effects on tubular ion transport resulting in natriuretic and diuretic actions.\(^13\)–\(^16\) It is possible that capsaicin depletes neurotransmitters (e.g., CGRP) in sensory nerve fibers, which leads to elimination or attenuation of vasodilatory and natriuretic responses to salt load and causes the rats to be salt sensitive in terms of blood pressure regulation. In support of this notion, it has been shown that bolus injection of CGRP\(^4\)–\(^7\), a specific CGRP receptor antagonist, produces dose-dependent increases in mean arterial pressure in deoxycorticosterone-salt hypertensive rats.\(^17\)

Alternatively, the sensory nervous system may interact with other neurohormonal systems that change during salt load to regulate blood pressure. It is well known that salt balance is the major physiological regulator of the activity of the renin-angiotensin system, i.e., salt loading suppresses whereas salt deficiency activates the renin-angiotensin system. Abnormal regulation of either the circulating or local renin-angiotensin system due to sensory denervation may contribute to the development of salt-induced hypertension. Indeed, our results show that blockade of the type I angiotensin II receptor (AT\(_1\)) by losartan prevents the development of hypertension induced by sensory denervation and sodium loading, suggesting that interaction between the sensory nervous system and the renin-angiotensin system plays significant functional roles in antagonizing the development of salt-induced hypertension. Confirmation of this would require direct measurements of plasma and tissue levels of the various components of the renin-angiotensin and sensory nervous systems. However, when we consider the fact that capsaicin equally depletes CGRP in the dorsal root ganglia of rats with different levels of blood pressure, CGRP may be ruled out as a factor that contributes to the blood pressure–lowering effects of losartan. Moreover, the nonspecific vasoconstrictor hydralazine shows antihypertensive effects similar to those of losartan, suggesting that blockade of AT\(_1\)-mediated vasoconstriction by losartan may not be the only mechanism responsible for decreased blood pressure in this model.

In addition to the renin-angiotensin system, the sympathetic nervous system is another powerful prohypertensive system that may be affected by sensory denervation. It has been shown that permanent destruction of capsaicin-sensitive afferent neurons leads to an increase in the transmitter content and/or innervation density of sympathetic nerve endings.\(^18\)–\(^19\) Conversely, long-term ablation of sympathetic neurons is followed by an increase in the afferent innervation.\(^18\)–\(^22\) 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 hyperinnervation of the remaining nerve population.\(^23\) Despite the possibility that the sympathetic nerve density and transmitter contents may increase by sensory denervation in the present study, blockade of the \(\alpha\)-adrenoreceptor by prazosin does not prevent the development of hypertension. Several possibilities exist. First, it is possible that the dose of prazosin we used is not high enough to decrease blood pressure in this model. However, it has been shown that, in SHR, prazosin (0.03 to 3 mg/kg per day) given orally resulted in dose-dependent reductions in blood pressure.\(^11\) Thus, the dose of prazosin (3 mg/kg per day) used in the present study is the maximal dose that effectively decreases blood pressure in SHR. These results indicate that the \(\alpha\)-component of the sympathetic nervous system either does not contribute to the development of hypertension in this model or is necessary for preventing salt-induced hypertension. The study conducted by Osborn et al\(^24\) supports the latter possibility. Osborn et al\(^24\) have shown that blockade of the \(\alpha\)-adrenoreceptor with prazosin leads to the development of salt-sensitive hypertension, indicating that the \(\alpha\)-adrenoreceptor may be a determinant in salt-induced increase in blood pressure. Future assessments of not only \(\alpha\)- and \(\beta\)-adrenoreceptor antagonists but also AT\(_1\) and AT\(_2\) receptor antagonists in both vehicle- and capsaicin-treated rats will help to define the role of various components of both the sympathetic nervous system and the renin-angiotensin system in the development of hypertension in this model.

We have previously shown that the prohypertensive effects of capsaicin in rats fed a high sodium diet are accompanied by decreased urinary volume and sodium excretion without affecting urinary potassium excretion.\(^2\) The present study confirms these findings and suggests that capsaicin may selectively impair the natriuretic response to a high salt intake. Unexpectedly, however, losartan and hydralazine do not prevent the impairment of urinary sodium and water excretion even though they prevent the development of hypertension in this model. These results suggest that, regardless of blood pressure, intact sensory innervation is necessary for normal natriuretic response to sodium loading and that the antihypertensive effects of losartan and hydralazine may be
mediated by mechanisms (eg, vasodilatory mechanism) other than those that prevent the impairment of urinary sodium and water excretion.

In conclusion, we have shown that losartan and hydralazine, but not prazosin, prevent the development of salt-sensitive hypertension induced by sensory denervation. Moreover, regardless of the effectiveness of antihypertensive treatment, sensory denervation impairs urinary sodium and water excretion in response to a high sodium intake. Further research on the interaction of the renin-angiotensin system, the sympathetic nervous system, and the sensory nervous system may enhance our understanding of the regulation of blood pressure and the pathogenesis of salt-sensitive hypertension.

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References

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