Role of renin-angiotensin-aldosterone system in salt-sensitive hypertension induced by sensory denervation

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Huang, Yan, and Donna H. Wang. Role of renin-angiotensin-aldosterone system in salt-sensitive hypertension induced by sensory denervation. Am J Physiol Heart Circ Physiol 281: H2143–H2149, 2001.—To define the role of the renin-angiotensin-aldosterone system in a novel salt-sensitive model, neonatal Wistar rats were given capsaicin (50 mg/kg sc) on the first and second days of life. After weaning, male rats were divided into the following six groups and treated for 3 wk with: control + normal sodium diet (CON), CON + high-sodium diet (CON-HS), CON + HS + spironolactone (50 mg·kg⁻¹·day⁻¹, CON-HS-SP), capsaicin pretreatment + NS (CAP-NS), CAP-HS, and CAP-HS-SP. Radioimmunoassay shows that plasma renin activity (PRA) and plasma aldosterone level (PAL) were suppressed by HS, but they were higher in CAP-HS than in CON-HS and CON-HS-SP (P < 0.05). Both tail-cuff systolic blood pressure and mean arterial pressure were higher in CAP-HS than in all other groups (P < 0.05). Western blot did not detect differences in adrenal AT₁ receptor content. Therefore, insufficiently suppressed PRA and PAL in response to HS intake by sensory denervation may contribute to increased salt sensitivity and account for effectiveness of spironolactone in lowering blood pressure in this model.

capsaicin; sodium; dietary; innervation; plasma renin activity; plasma aldosterone level

IN ADDITION to the well-known afferent function of sensory nerves that transmit the information to the central nervous system, sensory nerves possess the local effector function via releasing a variety of vasodilator neuropeptides, e.g., calcitonin gene-related peptide (CGRP) and substance P, peripherally in response to local stimuli (11). The dorsal root ganglia are the prominent site of CGRP synthesis and contain cell bodies of primary afferent neurons that regulate blood pressure by modulating cardiovascular and renal function (7, 26). Treatment of newborn rats with an appropriate dose of capsaicin results in a selective and permanent destruction of up to 90% of peripheral unmyelinated afferent fibers (14) and leads to enhanced development of deoxycorticosterone-induced hypertension (13). Moreover, intrathecal administration of capsaicin in adult rats also selectively depletes spinal substance P and CGRP within small primary afferent nerve fibers (9) and leads to enhanced development of one-kidney renal wrap hypertension in the rat (2).

To investigate the mechanisms underlying salt-sensitive hypertension, we developed a novel salt-sensitive hypertensive model that is sensory nerve dependent (23, 24). We found that capsaicin-induced degeneration of sensory nerves renders a rat responsive to a salt load with a significant and sustained rise in blood pressure (23, 24). Furthermore, the increase in blood pressure can be prevented by blockade of the type 1 angiotensin II (ANG II) receptor (AT₁) in this model (23), indicating the renin-angiotensin system (RAS) is activated and plays a significant functional role in the development of hypertension in this model.

The RAS is a major regulator of the synthesis and secretion of aldosterone, an adrenocortical hormone. The classic physiological role of aldosterone is to promote unidirectional transepithelial sodium transport (6). It promotes salt and water reabsorption across a variety of epithelial tissues, the salivary gland, the intestine, the sweat gland, and the kidney. It has been shown that ANG II alters renal sodium and water reabsorption through its ability to stimulate the AT₁ receptor in the zona glomerulosa cells of the adrenal cortex to synthesize and secrete aldosterone (4, 8, 19). Although our data previously showed that AT₁ receptor blockade lowers blood pressure in a capsaicin-induced salt-sensitive model, it is not clear whether AT₁-mediated hypertensive effect is linked with abnormal synthesis and/or release of aldosterone. We hypothesize that the renin-angiotensin-aldosterone system (RAAS) is activated and plays a role in the development of salt-induced hypertension in sensory-denervated rats. Both the circulating and local RAAS activities were determined by measurement of plasma renin activity (PRA), plasma aldosterone level (PAL), and adrenal AT₁ receptor contents. The role of aldosterone in the regulation of blood pressure and renal function was assessed by chronic administration of aldosterone receptor antagonist spironolactone.

METHODS

Animals. Pregnant Wistar female rats (Charles River Laboratories; Wilmington, MA) were housed in the animal unit.

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for at least 1 wk before parturition. On the first and second day of life, neonatal rats received capsaicin (50 mg/kg sc) as previously described (23, 24). Control rats were treated with equal volumes of vehicle solution (5% ethanol, 5% Tween 80 in saline). After 3 wk, male rats were divided into the following six groups, pair-fed different sodium diets, and subjected to different drug treatments for 3 wk: control + normal sodium diet (0.5%, CON-NS), control + high-sodium diet (4%, CON-HS), control + high-sodium diet + spironolactone (50 mg/kg · day⁻¹ by oral gavage; CON-HS-SP), capsaicin pretreatment + normal sodium diet (CAP-NS), capsaicin pretreatment + high-sodium diet (CAP-HS), and capsaicin pretreatment + high-sodium diet + spironolactone (CAP-HS-SP). Spironolactone is an aldosterone antagonist, and its dose was chosen based on the previous study showing that it is effective in antagonizing the development of hypertension induced by ANG II in kininogen-deficient rats (12). The rat food was purchased from Harlan Teklad Diets. At the end of the 3-wk treatment period, rats were anesthetized with a single intraperitoneal injection of 50 mg/kg ketamine and 1 mg/kg xylazine, and the carotid artery was catheterized for the intraperitoneal injection of 80 mg/kg ketamine and 1 mg/kg xylazine, and the carotid artery was catheterized for the intraperitoneal injection of 80 mg/kg ketamine and 1 mg/kg xylazine. Blood pressures were measured in conscious rats every 7 days for 21 days, beginning 1 day before dietary treatment. The blood pressure value for each rat was calculated as the average of three separate measurements at each session.

**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 21 days, beginning 1 day before dietary treatment. The blood pressure value for each rat was calculated as the average of three separate measurements at each session.

**Water intake, urine volume, and urinary Na⁺ and K⁺ concentrations.** Water intake and urine excretion were routinely determined in each of six groups by use of metabolic cages. These parameters were measured every 7 days for 21 days, beginning 5 days after dietary treatment. Urinary Na⁺ and K⁺ concentrations were determined using a flame atomic absorption spectrophotometer (Instrumentation Laboratory) (kindly provided by Dr. Gregory Fink, Michigan State University).

**Radioimmunoassay.** The cervical, thoracic, and lumbar dorsal root ganglia were immediately dissected and frozen in liquid nitrogen. To determine immunoreactive CGRP content in the dorsal root ganglia, a commercially available rabbit anti-rat CGRP radioimmunoassay kit (Phoenix Pharmaceuticals) was used. The assay was performed as recommended by the supplier, and the total protein content was determined by using a protein assay kit (Bio-Rad). Plasma was separated and assayed with commercially available radioimmunoassay kits for PRA (Incstar) (21, 22) and for aldosterone (Diagnostic Products).

**Western blot assay.** The adrenal gland was homogenized in buffer containing 10 mmol/l Tris-HCl (pH 7.6) and 0.5 mmol/l MgCl₂ with protease inhibitors (10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mmol/l phenylmethylsulfonyl fluoride, and 1.8 mg/ml iodoacetamide). Homogenates were centrifuged at 20,000 g for 20 min at 4°C. The membrane pellets were suspended in lysis buffer containing 0.3 mol/l NaCl and 50 mmol/l Tris-HCl (pH 7.6) and 0.5% Triton X-100 with protease inhibitors, incubated on ice, and spun down at 14,000 g for 20 min at 4°C. The protein concentration was determined with a protein assay kit (Bio-Rad). After denaturation at 100°C for 10 min, 10-μg proteins from each sample were loaded on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to polyvinylidene fluoride membrane for 1 h at 100 V at 4°C. Membranes were blocked overnight at 4°C in 5% milk washing solution (50 mmol/l Tris-HCl, 100 mmol/l NaCl, and 0.1% Tween 20, pH 8.0). Membranes were incubated with rabbit polyclonal antibody against the AT₁ receptor (~50 kDa) (Santa Cruz) diluted 1:2,000 in blocking solution at room temperature for 1 h. Membranes were then washed and incubated with anti-rabbit horseradish-peroxidase-labeled secondary antibody diluted 1:5,000 in blocking solution for 1 h at room temperature. After being washed extensively, membranes were incubated with ECL Plus (Amersham) and exposed to film, which was immediately developed. The film was scanned by ScanJet 5370C (Hewlett-Packard). The intensity of the reaction was determined by using Image Quantity Program (Scion), and the final intensity was normalized to total protein content that was detected by Coomassie blue staining.

**Statistical analysis.** Values are expressed as means ± SE. The data were analyzed either by unpaired t-test or by two-way 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 six groups before the dietary treatment (Table 1). Body weight increased significantly over the experimental period in all experimental groups. However, rats in CAP-HS and CAP-HS-SP gained less weight than in CON-NS, CON-HS, and CON-HS-SP rats by the end of the experiment.

To confirm the effectiveness of neonatal capsaicin treatment, immunoactive CGRP content in the dorsal root ganglia from each of the six experimental groups was determined with the use of radioimmunoassay (Fig. 1). The results showed that CGRP content in dorsal root ganglia was significantly decreased in all capsaicin-treated rats compared with their respective control rats. Thus neonatal treatment with capsaicin results in depletion of CGRP in dorsal root ganglia of rats with or without antihypertensive drug treatment.

### Table 1. Body weight of rats before and after dietary and drug treatment

<table>
<thead>
<tr>
<th></th>
<th>CON-NS</th>
<th>CAP-NS</th>
<th>CON-HS</th>
<th>CON-HS-SP</th>
<th>CAP-HS</th>
<th>CAP-HS-SP</th>
</tr>
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<tbody>
<tr>
<td><strong>Beginning</strong></td>
<td>48 ± 3</td>
<td>44 ± 2</td>
<td>49 ± 5</td>
<td>49 ± 2</td>
<td>46 ± 2</td>
<td>46 ± 2</td>
</tr>
<tr>
<td><strong>End</strong></td>
<td>203 ± 13</td>
<td>187 ± 7</td>
<td>204 ± 12</td>
<td>202 ± 4</td>
<td>162 ± 6*</td>
<td>161 ± 7*</td>
</tr>
</tbody>
</table>

Values are means ± SE (in g); \( n = 8 \) rats for all groups. CON, control; NS, normal sodium diet; CAP, capsaicin; HS, high-sodium diet; SP, spironolactone. *\( P < 0.05 \) vs. CON-NS, CON-HS, and CON-HS-SP.
There was no significant difference in tail-cuff systolic blood pressure between control and capsaicin-treated rats fed a normal sodium diet. However, tail-cuff systolic blood pressure was significantly higher in CAP-HS compared with all the other high-salt-treated groups beginning at day 7 after dietary and drug treatments and continuing for the rest of the experiment (Fig. 2). Direct measurement of MAP at the end of the experiment confirmed the results obtained from the tail-cuff measurement (Fig. 3), i.e., MAP was significantly higher in CAP-HS than in all the other high-salt-treated groups. Thus neonatal treatment with capsaicin does not increase blood pressure in rats fed a normal sodium diet but leads to an elevation of blood pressure in rats fed a high-sodium diet. Furthermore, spironolactone, an aldosterone receptor antagonist, prevents the development of hypertension in this model.

In addition to its antihypertensive effects, the role of spironolactone in renal function was examined. Figure 4 shows that there was no significant difference in the ratio of the 24-h urinary volume to water intake between control and capsaicin-treated rats fed a normal sodium diet. However, on the fifth day after dietary and drug treatments and for the rest of the study period, this ratio was significantly lower in CAP-HS than in CON-HS and CON-HS-SP rats. These results indicate that neonatal treatment with capsaicin impairs proportional urinary excretion when rats are loaded with salt. Although the ratio in CAP-HS-SP was significantly lower than that in CON-HS and CON-HS-SP on the 5th and 12th day after dietary and drug treatments, there was no significant difference in this ratio among the three groups at the end of the experiment. Likewise, there was no significant difference in the 24-h urinary sodium excretion in control and capsaicin-treated rats fed a normal sodium diet. However, urine sodium excretion was significantly lower in CAP-HS than in CON-HS and CON-HS-SP on the fifth day after dietary and drug treatments and continued for the rest of the study period (Fig. 5). Although urine sodium excretion was initially low in CAP-HS-SP, there was no significant difference in urine sodium excretion among CON-HS, CON-HS-SP, and CAP-HS-SP on the 12th and 19th days after dietary and drug treatments. These data indicated that chronic spironolactone treatment alleviates renal functional impairment in capsaicin-treated rats fed a high-salt diet. In contrast, although urinary potassium excretion appears to be lower in capsaicin-pretreated rats than in control rats, there was no significant difference in urinary potassium excretion among the groups.

Fig. 1. Immunoactive calcitonin gene-related peptide (iCGRP) content in the dorsal root ganglia of rats in each of the 6 experimental groups. CON, control; NS, normal salt diet; HS, high-salt diet; SP, spironolactone; CAP, capsaicin. Values are means ± SE; n = 6 to 7 rats in each group. *P < 0.01: CAP-NS vs. CON-NS; **P < 0.01: CAP-HS or CAP-HS-SP vs. CON-HS and CON-HS-SP.

Fig. 2. Systolic blood pressure in each of the 6 experimental groups. Values are means ± SE; n = 6 to 8 rats in each group. *P < 0.01: CAP-HS vs. CON-NS, CON-HS, CON-HS-SP, and CAP-HS-SP.

Fig. 3. Mean arterial pressure in each of the 6 experimental groups. Values are means ± SE; n = 5 to 7 rats in each group. *P < 0.01: CAP-HS vs. CON-HS, CON-HS-SP, and CAP-HS-SP.

Fig. 4. Ratio of 24-h urinary volume to water intake in each of the 6 experimental groups. Values are means ± SE; n = 6 to 8 rats in each group. *P < 0.01: CAP-HS vs. CON-HS and CON-HS-SP.
among groups at any time (Fig. 6). These results suggested at least two possibilities: 1) the failure to detect a significant change may be related to insufficient number of animals studied, and 2) capsaicin treatment selectively impairs natriuretic response to a high salt intake without damaging other renal function.

To assess the circulating RAS activity, PRA was measured by radioimmunoassay (Fig. 7). Capsaicin pretreatment has no effect on PRA in rats fed a normal sodium diet. As expected, PRA was suppressed when a high-salt diet was given. Furthermore, PRA was significantly higher in CAP-HS than in CON-HS and CON-HS-SP rats. These data indicate that the circulating RAS is insufficiently suppressed by a high salt intake in capsaicin-pretreated rats. There was no significant difference in PRA between CAP-HS and CAP-HS-SP rats.

The RAS is one of the most important pathways controlling aldosterone synthesis and secretion. Figure 8 shows that the change of PAL correlates with that of PRA, i.e., capsaicin pretreatment has no effect on PAL in rats fed a normal sodium diet, but increases PAL in rats fed a high-salt diet. Again, there was no difference in PAL between CAP-HS and CAP-HS-SP rats. These data indicated that activation of the circulating RAS leads to an increase in PAL, which contributes to salt-induced increases in blood pressure in this model.

ANG II regulates aldosterone synthesis and secretion via activation of the AT1 receptor in the adrenal gland. We therefore assessed AT1 receptor content in each of the experimental groups (Fig. 9). We found that there was no significant difference in AT1 receptor expression in the adrenal gland among groups. These results indicated that increased PAL is not due to an increase in the adrenal AT1 receptor levels in this model.

**DISCUSSION**

We examined the role of aldosterone in the development of salt-sensitive hypertension induced by sensory denervation. The present study shows that PRA and PAL are significantly higher in sensory-denervated rats fed a high-salt diet than in sensory nerve-intact rats fed a high-salt diet. Furthermore, the expression of AT1 receptors in the adrenal gland is not altered by either capsaicin or high-salt treatment or by the combination of the two. These data indicate that the circulating RAAS is insufficiently suppressed by salt load in sensory-denervated rats, resulting in increased salt
sensitivity in terms of blood pressure regulation. Moreover, the aldosterone receptor blocker spironolactone prevents the development of the hypertension.

It is well known that aldosterone is one of the most important mineralocorticoid hormones produced by the zona glomerulosa cells of the adrenal cortex, which retains salt (4, 8, 19). One of the important pathways controlling aldosterone synthesis and secretion is the RAS. The stimuli, such as low blood pressure and low Na\(^+\) concentrations at the macula densa segment of the distal tubule, lead to increased secretion of renin that cleaves angiotensinogen secreted by the liver to ANG I. ANG I then is cleaved by the angiotensin-converting enzyme to ANG II, the effector molecule of the RAAS. ANG II increases aldosterone synthesis and secretion via stimulation of the AT\(_1\) receptor in the adrenal gland. We have previously shown that blockade of the AT\(_1\) receptor with either candesartan or losartan prevents the development of salt-sensitive hypertension in this model (23). The antihypertensive effects of these AT\(_1\) receptor antagonists may be due to inhibition of ANG II-induced aldosterone release. Indeed, we now find that blockade of the aldosterone receptor with spironolactone prevents the development of hypertension in this model.

We have previously shown that salt-induced hypertension in sensory-denervated rats is associated with impairment of the renal function. The current study confirmed this finding by showing that the natriuresis response to a high-salt intake is impaired in capsaicin-treated rats. Interestingly, chronic blockage of the aldosterone receptor with spironolactone seems to alleviate renal functional impairment, considering the fact that spironolactone normalizes decreased urine sodium and water excretion in capsaicin-treated rats fed a high-salt diet by the end of the experiment. It is well established that aldosterone increases the reabsorption of sodium and secretion of potassium by regulating the Na\(^+\)-K\(^+\)-ATPase and epithelial Na\(^+\) channels located in a variety of tissues, including the collecting ducts of the kidney, sweat glands, salivary glands, and intestine (3, 17). Moreover, aldosterone has vascular actions. For example, aldosterone enhances ion permeability in vascular smooth muscles to reset baroreceptors and amplifies local vasoconstrictor systems, which has been suggested to play a role in the development of hypertension both in the reduced renal mass model and the mineralocorticoid-salt model (10, 18, 20, 25). It is conceivable that activation of the aldosterone receptor plays a role in the development of hypertension in

![Fig. 6. Twenty-four-hour urinary potassium excretion measured at 5th, 12th, and 19th days in each of the 6 experimental groups. Values are means ± SE; n = 6 to 8 rats in each group.](http://ajpheart.physiology.org/)

![Fig. 7. Plasma renin activity in each of the 6 experimental groups. Values are means ± SE; n = 5 to 6 rats in each group. *P < 0.05: CAP-HS vs. CON-HS and CON-HS-SP.](http://ajpheart.physiology.org/)
this model via altering both the natriuresis and vascular response to a high salt intake.

Whereas the fact that both AT1 and aldosterone receptor antagonists lower blood pressure indicates that salt-sensitive hypertension induced by sensory denervation is a RAAS-dependent model, direct measurements of PRA and PAL confirm this notion. We found that, although markedly suppressed by high salt intake, PRA and PAL are significantly higher in sensory-denervated rats fed a high-salt diet than in sensory nerve-intact rats fed a high-salt diet. Our investigation strongly suggests that the circulating RAAS was insufficiently suppressed by salt load in sensory-denervated rats, resulting in increased salt sensitivity in terms of blood pressure regulation. From these data, it is not surprising that blockade of the binding of elevated aldosterone to its receptor prevents the development of hypertension in this model.

In addition to the circulating RAAS, a potential contribution of local RAAS to blood pressure in this model cannot be discounted. It is known that the AT1 receptor is the predominant receptor in the adrenal gland and accounts for most known physiological consequences of ANG II binding, i.e., the aldosterone synthesis and secretion (1, 8, 15, 19). It has been shown that AT1 receptors in the adrenal gland are upregulated with sodium deficiency (5) but not changed by a high sodium intake (16). The present study shows that the AT1 receptor content in the adrenal gland is not altered either by capsaicin or high salt treatment or by the combination of the two in this salt-sensitive hypertension model. These data...
indicate that the elevated aldosterone level in this model results from an increase in circulating ANG II levels rather than an increase in AT1 receptor content in the adrenal gland.

In conclusion, we have shown that spironolactone prevents the development of salt-sensitive hypertension induced by sensory denervation. The antihypertensive effect of spironolactone may associate with an improvement of the renal function in this model. Moreover, PRA and PAL were insufficiently suppressed by salt loading in rats neonatally treated with capsaicin. The higher PRA and PAL in these rats may contribute to increased salt sensitivity and account for effectiveness of spironolactone in lowering blood pressure.

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