Development of Hypertension Induced by Subpressor Infusion of Angiotensin II
Role of Sensory Nerves

Wei Wu, Yong Zhang, Jennifer R. Ballew, Gregory Fink, Donna H. Wang

Abstract—Long-term administration of a subpressor dose of angiotensin II (Ang II) leads to pressor hyperresponsiveness and slow development of hypertension. Our preliminary data show that mRNA expression for calcitonin-gene related peptide in dorsal root ganglia was significantly increased by subpressor infusion of Ang II. To determine the role of sensory nerves in the development of hypertension induced by subpressor infusion of Ang II, newborn Wistar rats were given 50 mg/kg SC capsaicin on the 1st and 2nd days of life. After the weaning period, male rats were divided into 4 groups and subjected to the following treatments for 2 weeks: capsaicin + Ang II (150 ng · kg⁻¹ · min⁻¹ SC by osmotic pumps, CAP-AII), capsaicin + vehicle (CAP), control + Ang II (CON-AII), and control + vehicle (CON). The results show that mean arterial pressure was significantly elevated in both Ang II–infused rats compared with non–Ang II–treated rats (P<0.05), and it was higher in CAP-AII than in CON-AII rats (P<0.05). The 24-hour urinary and sodium excretions were lower in CAP-AII than in CON-AII, CAP, and CON rats (P<0.05). These data demonstrated that sensory denervation exacerbates the development of hypertension and impairs renal excretory function when a subpressor dose of Ang II is given. These results indicate that activation of sensory nerves, either by Ang II or by other hormonal or hemodynamic factors, plays a compensatory role in promoting urine and sodium excretion and attenuating elevated blood pressure initiated by Ang II. (Hypertension. 2000;36:549-552.)

Key Words: Angiotensin II ■ peptides ■ hypertension, experimental

It is now clear that acute elevation of blood pressure induced by administration of pressor doses of Angiotensin (Ang) II is mainly caused by increased aldosterone production, salt and water retention, and resetting of the baroreceptor that results in increased sympathetic tone.¹⁻⁶ In addition, acute elevation of blood pressure has been shown to trigger compensatory mechanisms, for example, natriuresis and the local production of prostaglandins,⁷⁻⁸ which may attenuate the increase in blood pressure induced by pressor doses of Ang II. In contrast, the slow development of hypertension induced by long-term administration of subpressor doses of Ang II mimics the development of human hypertension to a greater extent than the administration of pressor doses. During the developmental stage of hypertension, altered aldosterone levels and salt and water retention cannot be detected.⁹⁻¹² Although intensive research has been conducted in this area, mechanisms underlying subpressor Ang II–induced hypertension are largely unknown.

There is evidence showing that the renin-angiotensin system interacts with sensory nerves to modulate cardiovascular function.¹¹ Sensory afferent nerves have cell bodies located in the dorsal root ganglia (DRG) and extend their processes to a variety of tissues including renal tubules, resistance arteries, and heart. It has been established that sensory afferent fibers release a variety of vasodilator neuropeptides, for example, calcitonin-gene related peptide (CGRP) and substance P, in response to local chemical and mechanical stimuli.¹⁴ These neuropeptides have been implicated to play a role in blood pressure regulation. Despite the fact that plasma levels of CGRP in humans are increased in response to Ang II infusion,¹³ the role of sensory nerves in the development of hypertension induced by Ang II remains unknown. This study was designed to test the hypothesis that sensory nerves play a compensatory role in attenuating the pressor action of Ang II.

Methods

Animal Groups
Pregnant Wistar rats (Charles River Laboratories Inc, Wilmington, Mass) were housed in the animal care unit for 1 week before parturition. On the first and second days of life, neonatal rats received 50 mg/kg SC capsaicin as described.¹⁵,¹⁶ Control rats were treated with equal volumes of vehicle solution (5% ethanol, 5% Tween-80 in saline). All

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treatments were performed with rats under ether anesthesia. We have previously demonstrated that neonatal treatment with this dose of capsaicin results in a remarkable decrease (6- to 7-fold) in CGRP levels in the DRG. After the weaning period, male and female rats were separated, and only male rats were used in this study. Male rats were divided into 4 groups and treated with following for 2 weeks: control + vehicle (CON, n=9), control + Ang II (CON-AII, n=12), capsaicin + vehicle (CAP, n=14), and capsaicin + Ang II (CAP-AII, n=14). Ang II was infused subcutaneously by osmotic pump (model 2002, Alza). The rate of delivery of Ang II was 150 ng/kg·min⁻¹. This dose of Ang II does not cause changes in plasma Ang II levels or acute increases in blood pressure. At the end of the 2-week treatment period, all of the rats in each group 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 a Statham 231-D pressure transducer (Gould) coupled to a Gould 2400S recorder. MAP was obtained 3 hours after surgery with rats fully awake and unrestricted.

Northern Blot Analysis
Total cellular RNA was isolated from DRG by the guanidine thiocyanate-phenol-chloroform extraction method. Approximately 40 µg of total cellular RNA from DRG was isolated. Five micrograms of total RNA for each sample was subjected to electrophoresis on denaturing formaldehyde-agarose gel. The fractionated RNAs were transferred to nylon membranes, and the blot was prehybridized for 5 hours at 42°C in hybridization buffer (50% deionized formamide, 5×Denhardt’s solution, 5×SSC, 0.5% SDS, and 200 µg/mL denatured salmon sperm DNA) and then hybridized with the [α-32P]-labeled CGRP probe for 18 to 20 hours at 42°C. The membrane was then washed successively in 2××1× and 0.5××SSC containing 0.1% SDS at 60°C. Blots were exposed to XAR-5 x-ray film (Eastman Kodak). The probe was then removed from the membrane and rehybridized with [α-32P]-labeled probe for 18s RNA as control. After exposure to x-ray film, autoradiographic signals were scanned with a laser densitometer (Ultrascan XL Laser densitometer). Relative gene expression was expressed as the ratio of CGRP mRNA to 18s rRNA.

Water Intake, Urine Volume, and Urinary Sodium and Potassium Concentrations
Water intake and urine excretions were determined in each of 4 groups by the use of metabolic cages. These parameters were measured 1 day before the animals were killed. Urinary sodium and potassium concentrations were determined with

Body Weight (g) of Rats at Beginning and End of Ang II Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>CON (n=9)</th>
<th>CON-AII (n=12)</th>
<th>CAP (n=14)</th>
<th>CAP-AII (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning</td>
<td>53±2</td>
<td>52±1</td>
<td>51±1</td>
<td>51±2</td>
</tr>
<tr>
<td>End</td>
<td>187±4</td>
<td>184±7</td>
<td>186±8</td>
<td>189±5</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Results
Body weights were not significantly different among the 4 groups at the beginning and the end of Ang II infusion (Table). Thus, neonatal treatment with capsaicin and/or Ang II does not alter somatic development of rats.

Subpressor infusion of Ang II for 2 weeks led to an increase in the level of CGRP mRNA in DRG (CON-AII, 1.33±0.05, n=5) when compared with that in the control rats (CON, 0.93±0.03, n=5, P<0.05), suggesting that hypertension induced by subpressor infusion of Ang II stimulates the synthesis or decreases the degradation of CGRP mRNA in DRG.

Twenty-four-hour urine volume to water intake is shown in Figure 1. The ratio was significantly lower in CAP-AII than in CON-AII, CAP, and CON rats (P<0.05). Likewise, 24-hour urinary sodium excretion (Figure 2) was significantly lower in CAP-AII than in CON-AII, CAP, and CON rats (P<0.05). In contrast, there was no significant difference in 24-hour urinary potassium excretion (Figure 3) among 4 groups (P>0.05). These results indicate that sensory denervation plus subpressor Ang II infusion impairs urinary and sodium excretions.

Figure 1. Twenty-four-hour urine volume to water intake. Values are mean±SEM; n=5 to 6. *P<0.05 vs CON, CON-AII, and CAP.

Figure 2. Twenty-four-hour urinary sodium excretion. Values are mean±SEM; n=5 to 6. +P<0.05 vs CON, CON-AII, and CAP.
MAP (Figure 4) was significantly elevated in both Ang II–infused rats compared with non–Ang II–infused rats, and it was higher in CAP-AII than in CON-AII rats (P<0.05). These results show that sensory denervation exacerbates the development of hypertension induced by subpressor infusion of Ang II.

**Discussion**

We examined the role of sensory nerves in the development of hypertension induced by Ang II. This study contains several distinct observations. First, subpressor infusion of Ang II stimulates the synthesis of sensory neurotransmitter CGRP in DRG. Second, sensory denervation impairs urinary and sodium excretion in rats infused with subpressor Ang II. Finally, sensory denervation exacerbates the development of hypertension induced by subpressor infusion of Ang II. These findings have been collectively synthesized to indicate a role for sensory nerves in regulating blood pressure and renal excretory function in subpressor Ang II–infused rats.

We have previously demonstrated that neonatal treatment with capsaicin results in a remarkable decrease (6– to 7-fold) in CGRP levels in the DRG and causes a normal rat to respond to a salt load with a significant and sustained rise in blood pressure. Furthermore, we have shown that blockade of the type I Ang II receptor (AT1) with losartan prevents the development of hypertension induced by sensory denervation and sodium loading. These studies suggest that sensory denervation activates either the local or circulating renin-angiotensin system that plays a role in the development of salt-sensitive hypertension induced by sensory denervation. On the other hand, the present study indicates that subpressor infusion of Ang II increases the synthesis of sensory neurotransmitter CGRP that may act in response to increased Ang II or blood pressure per se. Given the fact that sensory denervation enhanced the development of hypertension induced by Ang II infusion, the increase in CGRP synthesis appears to be a compensatory response to attenuate the increase in blood pressure induced by Ang II. Taken together, our previous and present studies indicate a close interaction between the renin-angiotensin system and sensory nervous system to modulate blood pressure and cardiovascular function.

It is well known that salt and water balance is unchanged in experimental animals treated with small or subpressor doses of Ang II. Our findings that urinary and sodium excretion is not altered by subpressor infusion of Ang II, regardless of increased blood pressure, support these results. In contrast to the findings in these rats that receive Ang II only, sensory denervation leads to disturbed renal excretory function in subpressor Ang II–infused rats. These facts indicate that normal sensory innervation preserves the excretory function of the kidney in hypertension induced by subpressor infusion of Ang II. These data are consistent with the results showing that renal nerves promote sodium excretion in hypertension induced by pathophysiological infusion of Ang II and provide a rationale for future investigation of sensory neural control of the renal function in physiological and pathophysiological conditions.

The mechanisms by which sensory denervation impairs the renal excretory function in Ang II–infused rats are unknown. However, several possibilities exist: (1) sensory denervation results in decreased synthesis and release of sensory neurotransmitters that are very potent vasodilators and diuretic and natriuretic factors that act as depressors; (2) sensory denervation activates epithelial sodium channels to increase reabsorption of sodium from renal tubules and distal colon (our unpublished observations support this possibility); and (3) altered sensory feedback from the kidney to the brain causes generalized increase in sympathetic discharge, promoting vasoconstriction and sodium and water retention. All of these actions may provoke the unbalance of the renal function and render the rats responsive to Ang II infusion with decreased urinary and sodium excretion.

In conclusion, we have shown that neonatal degeneration of capsaicin-sensitive sensory nerves increases the Ang II initiated hypertension. These results indicate that activation of sensory nerves, either by Ang II or by other hormonal or hemodynamic factors, plays a compensatory role in promoting urine and sodium excretion and attenuating elevated blood pressure initiated by Ang II. Future investigation that elucidates molecular mechanisms responsible for activation or inhibition of the sensory nervous system may provide insights into the pathogenesis of hypertension and hypertension-induced organ damage.

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**References**


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