Function and Regulation of Endothelin-1 and Its Receptors in Salt Sensitive Hypertension Induced by Sensory Nerve Degeneration

Diana Z. Ye, Donna H. Wang

Abstract—To determine the role of endothelin-1 (ET-1) and its receptors in salt-sensitive hypertension induced by sensory nerve degeneration, selective ET_A antagonists (ABT-627) and ET_B antagonist (A-192621) were used. Newborn Wistar rats were given vehicle or 50 mg/kg capsaicin subcutaneously on the first and second days of life. After the weaning period, male rats were divided into eight groups, and subjected to the following treatments for 2 weeks: control + normal salt diet (Con+NS, 0.5%), control + high salt diet (Con+HS, 4%), control + high salt diet + ABT-627 (Con+HS+ABT-627), control + high salt diet + A-192621 (Con+HS+A-192621), capsaicin + normal salt diet (Cap+NS), capsaicin + high salt diet (Cap+HS), capsaicin + high salt diet + ABT-627 (Cap+HS+ABT-627), capsaicin + high salt diet + A-192621 (Cap+HS+A-192621). Both ABT-627 (5 mg/kg/d) and A-192621 (30 mg/kg/d) were given by oral gavage twice a day. Mean arterial pressure (MAP, mm Hg) was higher in Con+HS+A-192621 (141±11) than in Con+NS (94±10), Con+HS (95±5), and Con+HS+ABT-627 (97±6) (P<0.05). MAP was also higher in Cap+HS (152±6) and Cap+HS+A-192621 (180±7) than in Cap+NS (93±3) and Cap+HS+ABT-627 (104±5) (P<0.05), and it was higher in Cap+HS+A-192621 than in Cap+HS (P<0.05). Enzyme immunometric assay showed that ET-1 plasma concentration (pg/mL) was higher in Con+HS+A-192621 (7.59±0.78) than in Con+NS (2.68±0.56), Con+HS (2.50±0.92), and Con+HS+ABT-627 (3.54±0.79) (P<0.05). ET-1 plasma concentration was also higher in Cap+HS (8.95±2.16), Cap+HS+ABT-627 (9.82±1.22) and Cap+HS+A-192621 (10.97±0.57) than in Cap+NS (3.06±0.73) (P<0.05). We conclude that blockade of the ET_A receptor prevents the development of salt sensitive hypertension induced by sensory nerve degeneration, indicating that activation of the ET_A receptor by increased plasma ET-1 level contributes to elevation of blood pressure in this model. In contrast, blockade of the ET_B receptor leads to an increase in blood pressure in both normal and sensory nerve degenerated rats fed a high salt diet. These results suggest that ET_B plays an antihypertensive role in response to high salt intake under both normal and sensory nerve degenerated conditions. (Hypertension. 2002;39[part 2]:673-678.)

Key Words: endothelin ■ receptors, endothelin ■ hypertension, sodium-dependent

The endothelin-1 (ET-1) belongs to a family of endothelium-derived peptides. Endogenous ET-1 is a potent vasoconstrictor and plays a fundamental physiological role in maintenance of blood pressure in human.1 There are 2 distinct endothelin receptor subtypes, ET_A and ET_B.2,3 The ET_A receptor has a selectively higher expression in vascular smooth muscle cells.2 Endogenous ET-1 contributes to maintenance of the basal vascular tone and blood pressure via its action on the vascular smooth muscle ET_A receptor.4 ET_B receptors are mainly expressed in endothelial cells, as well as in vascular smooth muscle cells and renal epithelium.2,3,5,6 Endogenous ET-1 via binding to endothelial and renal ET_B receptors causes vasodilation and natriuresis that result in a decrease in blood pressure.7 The overall cardiovascular effect of endogenous ET-1 depends on the balance between ET_A- and ET_B-mediated effects.4

In addition to the well-known control by sympathetic nerves, peripheral vascular resistance is regulated by sensory nerves.7 For example, calcitonin gene–related peptide (CGRP), one of the sensory neurotransmitters, is a potent vasodilator and natriuretic factor.7 It has been shown that CGRP-containing nerves suppress vasconstriction mediated by sympathetic nerves.8 It has also been suggested that the defect in sensory vasodilator function may produce an imbalance that contributes to the development and maintenance of hypertension in spontaneously hypertensive rats (SHR).9,10,11 In contrast to this genetic model, we have developed a novel salt-sensitive hypertensive model that is sensory nerves-dependent.12,13 We demonstrate that neonatal degeneration of capsaicin-sensitive sensory nerves renders a rat responsive to a salt load with a significant increase in blood pressure.12,13 Moreover, we have shown that plasma renin activity is higher

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From the Department of Medicine, College of Human Medicine, Michigan State University, East Lansing, Mich.

Correspondence to Donna H. Wang, MD, Department of Medicine, College of Human Medicine, Michigan State University, B338 Clinical Center, East Lansing, MI 48824. E-mail donna.wang@ht.msu.edu

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in sensory denervated rats than in sensory nerve-intact rats in response to high salt intake, and that blockade of the AT1 receptors prevents the development of hypertension in this model.13,14 Angiotensin (Ang II) has been shown to be a stimulus for ET-1 production both in vitro and in vivo.15,16,17 It is likely that the activated renin-angiotensin system activates the ET system that in turn contributes the development of hypertension in this model. The present study was therefore designed to test the hypothesis that the endothelin system is activated in the salt-sensitive hypertensive model induced by sensory nerve degeneration. This activation may be associated with increases in circulating ET-1 levels and blood pressure in these rats.

Methods

Animal Groups

Pregnant Wistar female rats (Charles River Laboratories Inc, Wilmington, Mass) were housed in the animal care unit for about 1 week before parturition. Newborn Wistar rats received vehicle (5% ethanol, 5% Tween 80 and 90% saline) or 50 mg/kg capsaicin (dissolved in 5% ethanol, 5% Tween 80 and 90% saline) subcutaneously on the first and second days of life as described.12,13 After the weaning period, male rats were divided into 8 groups, and subjected to the following treatments for 2 weeks: control + normal salt diet (0.5%, Con+NS, n = 7), control + high salt diet (4%, Con+HS, n = 7), capsaicin + normal salt diet (Cap+NS, n = 7), capsaicin + high salt diet (Cap+HS, n = 7), capsaicin + high salt diet + ABT-627 (Con+HS+ABT-627, n = 7), control + high salt diet + ABT-627 (Con+HS+ABT-627, n = 7), capsaicin + normal salt diet + Cap+HS+ABT-627, n = 7), capsaicin + high salt diet + Cap+HS+ABT-627, n = 7), capsaicin + high salt diet + A-192621 (Cap+HS+A-192621, n = 7), capsaicin + high salt diet + A-192621 (Cap+HS+A-192621, n = 7). The rat food was purchased from Harlan Teklad Diets. ABT-627 (5 mg/kg/d, an ETA receptor antagonist) and A-192621 (30 mg/kg/d, an ETB receptor antagonist) were dissolved in a 2-fold molar equivalent amount of 1 N NaOH and normal saline, and given by oral gavage twice a day 12 hours apart. These doses of ABT-627 and A-192621 have been shown to be effective in blocking the ETA and ETB receptor in vivo, respectively.18

Systolic Blood Pressure

Indirect tail-cuff systolic blood pressures were measured on day 0 (before dietary treatment), 5, 10, and 14 days after dietary and drug treatment in all rats by using a Narco Bio-Systems Electro-Sphygmomanometer (Austin, Tex). The systolic blood pressure value for each rat was calculated as the average of 3 separated measurements at each session.

Mean Arterial Pressure

At the end of 2-week dietary treatment, each rat was anesthetized with a single intraperitoneal injection of 80 mg/kg ketamine and 1 mg/kg xylazine, and carotid artery was catheterized for the measurement of mean arterial pressure (MAP), Three hours after surgery with rat fully awake and unstressed, the MAP was obtained by a Statham 231D pressure transducer (Gould) coupled to a Gould 2400s recorder. The MAP value for each rat was calculated as an average of continuous measurement during a 20-minute recording.

Sample Collection

Water intake and urine excretion was determined at the end of the experiment in each of the 8 groups by use of metabolic cages. Urinary sodium concentrations were determined using a flame atomic absorption spectrophotometer (Instrumentation Laboratory Co) (kindly provided by Dr. Gregory Fink, Michigan State University). At the end of the experiment, rats were sacrificed by decapitation. Blood samples were collected in chilled EDTA tubes. Plasma was separated by centrifugation at 1,600g for 10 minutes at 4°C and stored at -80°C. The cerebral, thoracic, and lumbar dorsal root ganglia from each animal were collected and stored at -80°C. The cerebral, thoracic, and lumbar dorsal root ganglia from each animal were collected and stored at -80°C.

<table>
<thead>
<tr>
<th>Group</th>
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<th>End</th>
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<tr>
<td>Con+NS (n = 5)</td>
<td>57 ± 4</td>
<td>172 ± 4</td>
</tr>
<tr>
<td>Con+HS (n = 6)</td>
<td>54 ± 1</td>
<td>171 ± 7</td>
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<tr>
<td>Con+HS+ABT-627 (n = 6)</td>
<td>55 ± 1</td>
<td>173 ± 5</td>
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<tr>
<td>Con+HS+A-192621 (n = 6)</td>
<td>54 ± 0</td>
<td>171 ± 8</td>
</tr>
<tr>
<td>Cap+NS (n = 7)</td>
<td>56 ± 3</td>
<td>176 ± 5</td>
</tr>
<tr>
<td>Cap+HS (n = 5)</td>
<td>50 ± 4</td>
<td>154 ± 18</td>
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<tr>
<td>Cap+HS+ABT-627 (n = 7)</td>
<td>50 ± 1</td>
<td>165 ± 7</td>
</tr>
<tr>
<td>Cap+HS+A-192621 (n = 6)</td>
<td>55 ± 2</td>
<td>157 ± 10</td>
</tr>
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Con indicates control rats; Cap, capsaicin pre-treated rats; NS, normal sodium diet (0.5%); HS, high sodium diet (4.0%); ABT-627, ETα receptor antagonist; A-192621, ETβ receptor antagonist.

Enzyme Immunoassay Kit (EIA)

Plasma ET-1 concentration was detected by using Human ET-1 Enzyme Immunoassay Kit (Assay Designs, Inc). This kit detects ET-1 levels in biological fluids of human, bovine, canine, murine, porcine and rats samples. Peptides were extracted from 0.5 mL plasma by C18 Sep-Pak column and reconstituted in 220 μL assay buffer supplied with EIA kit. Polyclonal antibody to ET-1 was preimmobilized on a microtiter plate on purchase. The ET-1 standards and reconstituted peptides were added to the plate. After the plate was incubated overnight at 4°C, it was washed as described in the protocol provided by the Assay Designs, Inc. A rabbit polyclonal antibody to ET-1 conjugated to Horseradish peroxidase was reconstituted in the buffer provided with the EIA kit and added to the plate. This labeled antibody can bind to the ET-1 captured on the plate. Substrate was then added to react with the labeled antibody. Stop solution was added after incubating in dark at room temperature for 30 minutes. The plate was then read at 450 nm by an absorbance microplate reader (Molecular Devices). The ET-1 concentration of plasma was calculated from the ET-1 standard curve. The cross-reactivities for ET-1 (1–31), ET-2 (1–21), and other related compounds were 100%, 3.32%, and <1%, respectively.

Radioimmunoassay (RIA)

To determine CGRP content in the dorsal root ganglia (DRG) in each rat, radioimmunoassay (RIA) was performed using a commercially available rabbit-anti-rat CGRP RIA kit (Phoenix Pharmaceuticals). This antibody has 100% cross-reactivity with rat CGRP and 79% with rat β-CGRP. There is no cross-reactivity with rat amylin, calcitonin, somatostatin, or substance P. Total protein content in each sample was determined by the Bradford method (Bio-Rad Laboratories), and was used to normalize the CGRP content per sample.

Statistical Analysis

Data analysis was performed by ANOVA (parametric test) followed by the Tukey-Kramer multiple comparison test (systolic blood pressure, MAP, urine Na+ excretion, and ET-1 plasma concentration). For nonparametric comparison, data analysis was performed by Kruskal-Wallis test (ratio of urine to water intake, CGRP, and ETα and ETβ content) followed by the Dunn’s multiple comparison test. Differences were considered statistically significant at P < 0.05.

Results

Although body weight tended to be lower in Cap+HS, Cap+HS+ABT-627, and Cap+HS+A-192621 than in Cap+NS and all vehicle-treated rats at the end of the experiment, there was no significant difference in body weight among all 8 groups (Table). It is possible that less food intake to some extent occurred in the former 3 groups resulting in somewhat lighter body weight in these rats.
In both vehicle- or capsaicin-treated groups, tail-cuff systolic blood pressure was significantly higher beginning at day 5 after the dietary treatment and continuing for the rest of the experiment in the rats treated with high salt diet plus A-192621 (Con/H11001 HS/A-192621, Cap/H11001 HS/A-192621) compared with rats treated with normal salt diet (Con/H11001 NS, Cap/H11001 NS), high salt diet (Con/H11001 HS, Cap/H11001 HS), or high salt diet plus ABT-627 (Con/H11001 HS/ABT-627, Cap/H11001 HS/ABT-627) (Figure 1). Tail-cuff systolic blood pressure was also significantly higher in Con/H11001 HS compared with Con/H11001 NS rats on day 5, but it was not different among Con/H11001 NS, Con/H11001 HS, and Con/H11001 HS/ABT-627 rats at any other time points. In capsaicin-treated groups, Cap/H11001 HS rats had significantly higher systolic blood pressure compared with Cap/H11001 NS rats beginning at day 10 and continuing for the rest of the experiment. Consistent with that obtained from the tail-cuff measurement, mean arterial blood pressure (MAP) was significantly higher in Con/H11001 HS/A-192621 (141±11) than in Con/H11001 NS (94±10), Con/H11001 HS (95±5), and Con/H11001 HS/ABT-627 (97±6) (P<0.05). MAP was also significantly higher in Cap/H11001 HS (152±6) and Cap/H11001 HS/A-192621 (180±7) than in Cap/H11001 NS (99±3) and Cap/H11001 HS/ABT-627 (104±5) (P<0.05), and it was higher in Cap/H11001 HS/A-192621 than in Cap/H11001 HS (P<0.05). These results indicate that blockade of the ET_b receptor with A-192621 increases blood pressure in both vehicle- and capsaicin-treated rats fed a high salt diet.

Circulating ET-1 was significantly increased after blockade of the ET_b receptor with A-192621 in vehicle-treated rats fed a high salt diet (Con/H11001 HS/A-192621) compared with other 3 vehicle-treated groups (Con/H11001 NS, Con/H11001 HS, and Con/H11001 HS/ABT-627) (Figure 4). ET-1 plasma concentration was also significantly higher in Cap/H11001 HS, Cap/H11001 HS/ABT-627, and Cap/H11001 HS/A192621 rats compared with Cap/H11001 NS rats. These results indicate that the endothelin system is activated when the ET_b receptor is blocked in a normal rats fed a high salt diet, and in capsaicin-treated rats fed a high salt diet with or without blockade of the ET_a or ET_b receptor.
CGRP content in DRG was dramatically decreased in capsaicin-treated rats compared with vehicle-treated rats, confirming the effectiveness of capsaicin treatment (Figure 5). However, no significant difference was observed among 4 vehicle-treated groups or 4 capsaicin-treated groups. Thus, neonatal treatment with capsaicin results in depletion of CGRP in DRG with or without endothelin receptors antagonist treatment.

**Discussion**

In light of the fact that the ET system may be intimately involved in the control of salt sensitivity, we investigate the function and regulation of ET-1 and its receptor subtypes in salt sensitive hypertension induced by sensory nerve degeneration in the current study. We found that plasma ET-1 levels and blood pressure are increased in sensory denervated rats fed a high salt diet. Blockade of the ETA receptor prevents salt induced-increase in blood pressure only when a high salt diet is given. Moreover, we have shown that plasma renin activity is higher in sensory denervated rats than in sensory nerve-intact rats in response to a high salt intake, indicating that plasma renin activity is insufficiently suppressed by salt load in the former one. Increased activity of the renin-angiotensin system may stimulate the ET system in this model. It has been shown that Ang II stimulates gene expression and release of ET-1 in isolated vascular smooth muscle and endothelial cells. Also, elevated ET-1 stimulated by Ang II augments contractility of Ang II in resistance arteries of spontaneously hypertensive rat. Our finding that plasma ET-1 levels are increased in sensory denervated rats fed a high salt diet is consistent with the hypothesis that the activated renin-angiotensin system increases the synthesis and release of ET-1 in this model.

Whereas increased synthesis and release of ET-1 may elevate circulating ET-1 levels, plasma ET-1 concentration reflects the dynamic balance of production and removal of ET-1. It is well known that the ETB receptor functions as a clearance receptor of ET-1 to participate in regulating circulating ET-1 levels. Indeed, blockade of the ETB but not...
ET$_A$ receptor dramatically increases plasma ET-1 levels in sensory nerve-intact rats fed a high salt diet, indicating that elevated plasma ET-1 levels in these rats is the result of a decrease in internalization and clearance of ET-1 due to blockade of the ET$_B$ receptor. In contrast, plasma ET-1 levels in sensory denervated rats fed a high salt diet are unaffected by blockade of either the ET$_A$ or ET$_B$ receptor. Taken together, these results indicate that elevated plasma ET-1 levels in sensory-denervated rats fed a high salt diet are the result of increased production and ET$_B$ receptors are not effectively clearing ET-1 from the circulation in these rats.

Regardless the causes responsible for elevated circulating ET-1 levels, blockade of the ET$_A$ receptor prevents the development of hypertension in sensory denervated rats fed a high salt diet. Our data are consistent with the observations that selective ET$_A$ receptor antagonists or nonselective ET$_A$/ET$_B$ antagonists reduce blood pressure in several hypertensive models with overexpression of endothelins.22–26 In contrast, blockade of the ET$_B$ receptor exacerbates the development of hypertension in sensory denervated rats fed a high salt diet. Given the fact that blockade of either ET$_A$ or ET$_B$ receptors does not alter CGRP levels in DRG in sensory denervated or sensory nerve-intact rats, ET$_A$ or ET$_B$ antagonist-induced changes in blood pressure are less likely to be mediated by changes in CGRP levels in these rats. In view of the fact that the ET$_B$ receptor on vascular endothelium releases nitric oxide and prostaglandins to produce vasodilation in many vascular beds,27,28 it is possible that elimination of these beneficial effects due to blockade of the ET$_B$ receptor accounts for further increase in blood pressure in sensory denervated rats fed a high salt diet.

In addition to its vasodilatory effect that regulates peripheral vascular resistance as well as contributes to the natriuretic and diuretic actions of ET-1, the ET$_B$ receptor located on renal tubular epithelium may inhibit sodium and water reabsorption.29,30 In spite of the fact that blockade of the ET$_B$ receptor increases blood pressure in both sensory denervated or sensory nerve-intact rats fed a high salt diet, urine excretion and sodium excretion are not altered in these rats. Similarly, blockade of the ET$_A$ receptor has no effect on these variables. Our data are in agreement with a previous report in which blockade of the ET$_A$ or ET$_B$ receptor produces no further changes in urine excretion and sodium excretion in control rats fed a high salt diet.18 Taken together, these results indicate that changes in blood pressure in both sensory denervated or sensory nerve-intact rats may depend on changes in vascular function resulting from the balance between ET$_A$- and ET$_B$-mediated effects rather than rely on changes in renal function.

In conclusion, we have shown that the ET system is activated in salt sensitive hypertension induced by sensory denervation. Blockade of the ET$_A$ receptor prevents the development of salt sensitive hypertension induced by sensory denervation, indicating that the ET$_A$ receptor plays a pro-hypertensive role in this model. Blockade of the ET$_B$ receptor leads to an increase in blood pressure in both normal or sensory denervated rats fed a high salt diet, suggesting that the ET$_B$ receptor plays an antihypertensive role in response to a high salt intake under both normal and sensory denervated conditions.

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