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

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Abstract—To determine the role of endothelin-1 (ET-1) and its receptors in salt-sensitive hypertension induced by sensory nerve degeneration, selective ET₄ antagonist (ABT-627) and ET₉ 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 (99±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 ET₄ receptor prevents the development of salt sensitive hypertension induced by sensory nerve degeneration, indicating that activation of the ET₄ receptor by increased plasma ET-1 level contributes to elevation of blood pressure in this model. In contrast, blockade of the ET₉ 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 ET₉ 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, ET₄ and ET₉.²,³ The ET₄ 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 ET₄ receptor.⁴ ET₉ 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 ET₉ 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 ET₄- and ET₉-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 vasoconstriction 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...
Methods

Animal Groups
Pregnant Wistar female rats (Charles River Laboratories Inc, Wilm-
ington, Mass) were housed in the animal care unit for about 1 week before parturition. Newborn Wistar rats received vehicle (5% etha-
nol, 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. After the weaning period, male rats were divided into 8 groups, and subjected to the following treatments for 2 weeks: control (0.5% Con+NS, n=7), control + high salt diet (4%, Con+HS, n=7), control + high salt diet + ABT-627 (Con+HS+ABT-627, n=7), control + high salt diet + A-192621 (Con+HS+A-192621, n=7), capsaicin + normal salt diet (Cap+NS, n=7), capsaicin + high salt diet (Cap+HS, n=7), capsaicin + high salt diet + ABT-627 (Cap+HS+ABT-627, 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.

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 unstrained, 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 ex-
periment 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 decapi-
tation. 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 cervical, thoracic, and lumbar dorsal root ganglia from each animal were collected and stored at −80°C.

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/HS + A-192621, Cap/HS + A-192621) compared with rats treated with normal salt diet (Con/NS, Cap/NS), high salt diet (Con/HS, Cap/HS), or high salt diet plus ABT-627 (Con/HS + ABT-627, Cap/HS + ABT-627) (Figure 1). Tail-cuff systolic blood pressure was also significantly higher in Con/HS compared with Con/NS rats on day 5, but it was not different among Con/NS, Con/HS, and Con/HS + ABT-627 rats at any other time points. In capsaicin-treated groups, Cap/HS rats had significantly higher systolic blood pressure compared with Cap/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/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 significantly higher in Cap/HS (152 ± 6) and Cap/HS + A-192621 (180 ± 7) than in Cap/NS (99 ± 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). These results indicate that blockade of the ET\textsubscript{B} receptor with A-192621 increases blood pressure in both vehicle- and capsaicin-treated rats fed a high salt diet.

The ratio of the 24-hour urine volume to water intake was significantly increased in both vehicle- and capsaicin-treated rats when a high salt diet was given (Figure 2). However, neither ET\textsubscript{A} nor ET\textsubscript{B} receptor blockade altered this ratio in either vehicle- or capsaicin-treated rats fed a high salt diet (Figure 2). Likewise, the 24-hour urinary sodium excretion was significantly increased in both vehicle- and capsaicin-treated rats when a high salt diet was given (Figure 3), and neither ET\textsubscript{A} nor ET\textsubscript{B} receptor blockade altered urinary sodium excretion in either vehicle- or capsaicin-treated rats fed a high salt diet (Figure 3).

Circulating ET-1 was significantly increased after blockade of the ET\textsubscript{B} receptor with A-192621 in vehicle-treated rats fed a high salt diet (Con/HS + A-192621) compared with other 3 vehicle-treated groups (Con/NS, Con/HS, and Con/HS + ABT-627) (Figure 4). ET-1 plasma concentration was also significantly higher in Cap/HS, Cap/HS + ABT-627, and Cap/HS + A192621 rats compared with Cap/NS rats. These results indicate that the endothelin system is activated when the ET\textsubscript{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\textsubscript{A} or ET\textsubscript{B} receptor.

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/HS + A-192621, Cap/HS + A-192621) compared with rats treated with normal salt diet (Con/NS, Cap/NS), high salt diet (Con/HS, Cap/HS), or high salt diet plus ABT-627 (Con/HS + ABT-627, Cap/HS + ABT-627) (Figure 1). Tail-cuff systolic blood pressure was also significantly higher in Con/HS compared with Con/NS rats on day 5, but it was not different among Con/NS, Con/HS, and Con/HS + ABT-627 rats at any other time points. In capsaicin-treated groups, Cap/HS rats had significantly higher systolic blood pressure compared with Cap/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/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 significantly higher in Cap/HS (152 ± 6) and Cap/HS + A-192621 (180 ± 7) than in Cap/NS (99 ± 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). These results indicate that blockade of the ET\textsubscript{B} receptor with A-192621 increases blood pressure in both vehicle- and capsaicin-treated rats fed a high salt diet. Blockade of the ET\textsubscript{A} receptor with ABT-627 prevents the development of hypertension in capsaicin-treated rats fed a high salt diet.

The ratio of the 24-hour urine volume to water intake was significantly increased in both vehicle- and capsaicin-treated rats when a high salt diet was given (Figure 2). However, neither ET\textsubscript{A} nor ET\textsubscript{B} receptor blockade altered this ratio in either vehicle- or capsaicin-treated rats fed a high salt diet (Figure 2). Likewise, the 24-hour urinary sodium excretion was significantly increased in both vehicle- and capsaicin-treated rats when a high salt diet was given (Figure 3), and neither ET\textsubscript{A} nor ET\textsubscript{B} receptor blockade altered urinary sodium excretion in either vehicle- or capsaicin-treated rats fed a high salt diet (Figure 3).

Circulating ET-1 was significantly increased after blockade of the ET\textsubscript{B} receptor with A-192621 in vehicle-treated rats fed a high salt diet (Con+HS + A-192621) compared with other 3 vehicle-treated groups (Con+NS, Con+HS, and Con+HS + ABT-627) (Figure 4). ET-1 plasma concentration was also significantly higher in Cap+HS, Cap+HS + ABT-627, and Cap+HS + A192621 rats compared with Cap+NS rats. These results indicate that the endothelin system is activated when the ET\textsubscript{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\textsubscript{A} or ET\textsubscript{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 in sensory denervated rats and blockade of the ETB receptor increases blood pressure in both sensory denervated and sensory nerve-intact rats fed a high salt diet. These findings have several important implications and deserve further discussion.

We have recently provided evidence that intact function of sensory nerves is required for prevention of salt induced increase in blood pressure. We showed that neonatal degeneration of capsaicin-sensitive sensory nerves increases 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

![Figure 3. Twenty-four-hour urine sodium excretion in 8 groups of rats with different dietary and drug treatment. Values are mean±SE (n=5 to 7); *P<0.05 versus Con+NS or Cap-NS.](image)

![Figure 4. ET-1 plasma concentration in 8 groups of rats with different dietary and drug treatments. Values are mean±SE (n=4 to 5); in controls, *P<0.05 versus Con+NS, Con+HS, and Con+HS+ABT-627; in capsaicin-treated groups, *P<0.05 versus Cap+NS.](image)
ET<sub>a</sub> 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<sub>B</sub> receptor. In contrast, plasma ET-1 levels in sensory denervated rats fed a high salt diet are unaffected by blockade of either the ET<sub>a</sub> or ET<sub>B</sub> 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<sub>B</sub> 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<sub>a</sub> 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<sub>a</sub> receptor antagonists or nonselective ET<sub>a/b</sub> antagonists reduce blood pressure in several hypertensive models with overexpression of endothelins.22–26 In contrast, blockade of the ET<sub>B</sub> receptor exacerbates the development of hypertension in sensory denervated rats fed a high salt diet. Given the fact that blockade of either ET<sub>a</sub> or ET<sub>B</sub> receptors does not alter CGRP levels in DRG in sensory denervated or sensory nerve-intact rats, ET<sub>a</sub> or ET<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> receptor located on renal tubular epithelium may inhibit sodium and water reabsorption.29,30 In spite of the fact that blockade of the ET<sub>B</sub> 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<sub>a</sub> receptor has no effect on these variables. Our data are in agreement with a previous report in which blockade of the ET<sub>a</sub> or ET<sub>B</sub> 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<sub>a</sub>- and ET<sub>B</sub>-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<sub>a</sub> receptor prevents the development of salt sensitive hypertension induced by sensory denervation, indicating that the ET<sub>a</sub> receptor plays a pro-hypertensive role in this model. Blockade of the ET<sub>B</sub> receptor leads to an increase in blood pressure in both normal or sensory denervated rats fed a high salt diet, suggesting that the ET<sub>B</sub> receptor plays an antihypertensive role in response to a high salt intake under both normal and sensory denervated conditions.

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