ET\textsubscript{A} receptor blockade prevents renal dysfunction in salt-sensitive hypertension induced by sensory denervation

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Wang, Youping, Alex F. Chen, and Donna H. Wang. ET\textsubscript{A} receptor blockade prevents renal dysfunction in salt-sensitive hypertension induced by sensory denervation. Am J Physiol Heart Circ Physiol 289: H2005–H2011, 2005. First published July 1, 2005; doi:10.1152/ajpheart.00370.2005.—To test the hypothesis that activation of the endothelin type A (ET\textsubscript{A}) receptor contributes to decreased renal excretory function and increased blood pressure in sensory nerve-degenerated rats fed a high-salt diet, neonatal Wistar rats were given vehicle or capsaicin (CAP, 50 mg/kg sc) on the first and second day of life. After being weaned, vehicle or CAP-treated rats were fed a normal (NS, 0.5%) or a high- (HS, 4%) sodium diet for 2 wk with or without ABT-627 (5 mg·kg\textsuperscript{-1}·day\textsuperscript{-1}, a selective ET\textsubscript{A} receptor antagonist). Systolic blood pressure increased in CAP-treated rats fed a HS diet (CAP-HS) compared with vehicle-treated rats fed a HS diet (CON-HS, 145 ± 7 vs. 89 ± 5 mmHg, P < 0.05). Creatinine clearance and fractional sodium excretion (FEna) decreased in CAP-HS rats compared with CON-HS rats (creatinine clearance, 0.54 ± 0.05 vs. 0.81 ± 0.09 ml·min\textsuperscript{-1}·100 g body wt\textsuperscript{-1}; FEna, 8.68 ± 0.99 vs. 12.53 ± 1.47%, respectively; P < 0.05). Water and sodium balance increased in CAP-HS rats compared with CON-HS (water balance, 20.2 ± 1.5 vs. 15.5 ± 1.9 ml/day; sodium balance, 11.9 ± 3.1 vs. 2.4 ± 0.3 mg/day, respectively; P < 0.05). The endothelin (ET)-1 levels in plasma and isolated glomeruli increased by about twofold in CAP-HS rats compared with CON-HS rats (P < 0.05). ABT-627 prevented the decrease in creatinine clearance and FEna, the increase in water and sodium balance, and the increase in blood pressure in CAP-HS rats (P < 0.05). Therefore, the blockade of the ET\textsubscript{A} receptor ameliorates the impairment of renal excretory function and prevents the elevation in blood pressure in salt-sensitive hypertension induced by degeneration of sensory nerves, indicating that the activation of the ET\textsubscript{A} receptor impairs renal function and contributes to the development of a salt-induced increase in blood pressure in this model.

blood pressure; capsaicin; endothelin receptor; kidney

A high-salt diet has been implicated in the pathogenesis of hypertension, particularly in salt-sensitive individuals (34, 21). Despite intensive research in this area, the mechanisms underlying salt-sensitive hypertension are largely unknown. It is well established that the cardiovascular system receives dense innervation from the sensory nerve terminals that contain a variety of vasodilator neuropeptides, e.g., calcitonin gene-related peptide and substance P. Sensory afferent fibers release these vasodilator neuropeptides peripherally in response to local stimuli (38). Recently, we developed a novel salt-sensitive hypertensive model that is sensory nerve dependent. We found that capsaicin (CAP)-induced degeneration of sensory nerves renders a rat sensitive to a salt load with a significant increase in blood pressure (35). The study by Kopp et al. (20) has also shown that interruption of the afferent renal nerves impairs urinary sodium excretion and increases blood pressure in rats fed a high-salt diet, indicating that afferent renal nerves are important in preventing salt-induced increases in blood pressure. Moreover, we found that the salt-sensitive hypertension developed in sensory nerve-degenerated rats pretreated with CAP was prevented by sympathectomy or by blockade of the type 1 ANG II receptor (AT1), aldosterone receptor, or endothelin-A (ET\textsubscript{A}) receptors, suggesting that dysfunction of these prohypertensive neurohormonal systems occurred in neonatal CAP-treated rats fed a high-salt diet, leading to increased salt sensitivity of arterial pressure in these rats (14, 36, 42).

Endothelin (ET)-1 is a potent vasoconstrictor peptide of 21 amino acids that is synthesized and released by endothelial cells (41). The physiological actions of the ET-1 are mediated by two receptor subtypes, i.e., ET\textsubscript{A} and endothelin type B (ET\textsubscript{B}) receptors. The interaction of ET-1 with ET\textsubscript{A} in vascular smooth muscle cells leads to vasoconstriction (12), whereas ET\textsubscript{B} expressed in endothelial cells mediates endothelium-dependent vasodilation via nitric oxide and prostacyclin (12). The kidney is one of the most sensitive organs to the stimulation of ET-1 (3, 18, 26). ET-1 is produced in the kidney, and both of its receptor subtypes ET\textsubscript{A} and ET\textsubscript{B} are present in the kidney (26). Thus ET-1 was thought to have potent and complex actions in the kidney, whereby it causes renal vasoconstriction leading to a reduction in glomerular filtration rate (GFR), renal blood flow, as well as sodium and water excretion (18, 19).

There is convincing evidence indicating that the ET system plays a vital role in the pathogenesis of salt-sensitive hypertension and hypertension-associated end-organ damage (32). The evidence has been mainly derived from experimental rat models of DOCA-salt hypertension (25) and the genetic Dahl salt-sensitive hypertensive rat model (4, 15). Considering that plasma ET-1 levels are remarkably increased in sensory nerve-degenerated rats fed a high-salt diet (42), it is unknown how elevated plasma ET-1 levels affect the renal ET system and renal function leading to the development of hypertension in this model. This study was therefore designed to define the role of ET-1, especially its effect mediated by the ET\textsubscript{A} receptor, in the impairment of the renal function and the development of hypertension in a salt-induced hypertensive model associated with sensory nerve degeneration.

METHODS

Animal experiments. Pregnant Wistar female rats (Charles River Laboratories, Wilmington, MA) were housed in the animal facility 1 wk before parturition. On the first and second day of life, neonatal rats...
received CAP (50 mg/kg sc) as previously described (35, 42). Control (CON) rats were treated with equal volumes of vehicle (5% ethanol and 5% Tween 80 in normal saline). After being weaned, 3-wk-old male rats were divided into six groups and subjected to the following treatments for 2 wk: CON + normal sodium diet (0.5%, CON-NS, n = 7 rats), CON + high-sodium diet (4%, CON-HS, n = 7 rats), CAP pretreatment + NS diet (CAP-NS, n = 7 rats), CAP pretreatment + HS diet (CAP-HS, n = 8 rats), CON + HS diet + ABT-627 (CON-HS-ABT-627, n = 7 rats), CAP pretreatment + HS diet + ABT-627 (CAP-HS-ABT-627, n = 8 rats). The rat food was purchased from Harlan Teklad Diets. ABT-627 (an ETA receptor antagonist) was diluted in drinking water at concentrations to deliver ~5 mg·kg⁻¹·day⁻¹. This dose of ABT-627 has been shown to be effective in blocking the ETA receptor in vivo (29, 37). All experiments were approved by the Institutional Animal Care and Use Committee at Michigan State University.

Systolic blood pressure. Indirect tail-cuff systolic blood pressures were measured 3 days before dietary treatment (day 0), and 6 and 12 days after the treatment with the use of a Narco Bio-Systems Electro-Sphygmomanometer (Austin, TX). The systolic blood pressure value for each rat was calculated as the average of three separated measurements at each session.

Metabolic study. After blood pressure measurements were taken, rats were housed individually in metabolic cages 1 day before the measurement for adaptation. Parameters of food and water intake and urine excretion were monitored for 24 h during the following day. Urinary sodium concentrations were determined by using a flame atomic absorption spectrophotometer (Instrumentation).

Collecting samples. Urine samples were collected for the measurement of creatinine and ET-1 levels 1 day before dietary treatment and 8 and 14 days after the treatment. Rats were euthanized by decapitation on day 14 after the treatment, and plasma samples were collected for the measurement of plasma creatinine and ET-1 levels. Fresh renal cortex was collected for glomeruli isolation.

Isolated glomeruli preparation. Glomeruli were isolated from the renal cortex with the use of a sieving method as described previously (23). Briefly, the freshly isolated renal cortex was cut into 2- to 3-mm³ pieces. The tissue was passed through a 0.5-mm stainless steel grid using a flattened pestle, and the steel grid was then rinsed with 2 ml of ice-cold HBSS containing (in mmol/l) 137 NaCl, 5.4 KCl, 0.4 MgSO₄, 0.5 MgCl₂, 1.25 CaCl₂, 0.44 KH₂PO₄, 0.33 Na₂HPO₄, and 4 NaHCO₃ pH 7.4. The homogenate was successively passed through 105-µm and 53-µm mesh nylon sieves without pressing and washed with ice-cold HBSS. Glomeruli retained on the last sieve were collected to determine the number of glomeruli under a light microscopy. The purity of the preparations from the renal cortex was >95%. The glomerular suspension was centrifuged at 2,000 g for 5 min. The supernatant was discarded, and the pellet was resuspended in ~100 to 150 µl of 1 M acetic acid containing 10 µg/ml of pepstatin A. Renal tissues were kept at 4°C during the procedure. Finally, samples were heated to 100°C for 10 min, chilled, and stored at −80°C for ET-1 assay (31).

Creatinine assay. Plasma and urine creatinine concentrations were determined by an improved Jaffe creatinine assay kit (BioAssay Systems). Briefly, the samples and creatinine standards were added to a plate and incubated with working reagent supplied by the manufacturer. After 20 min, the plate was read at 490 nm by an absorbance microplate reader (Molecular Devices).

Radioimmunoassay. ET-1 levels in plasma, urine, and isolated glomeruli were determined by the use of an anti-rat ET-1 RIA kit (Peninsula). Plasma samples were applied to Sep-Pak C18 columns for purification of peptide, as recommended by the manufacturer. The antibody mainly cross-reacted with ET-1 (100%) but also with Big ET-1 (17%), ET-2 (7%), and ET-3 (7%).

Statistical analysis. All values are means ± SE. One-way ANOVA followed by a Bonferroni’s test for multiple comparisons was used to evaluate the difference in creatinine clearance, FEna, and ET-1 levels among groups. Comparisons between groups at each experimental time point were determined by the use of two-way ANOVA followed by a Bonferroni’s test. Differences were considered statistically significant at P < 0.05.

RESULTS

As shown in Fig. 1, tail-cuff systolic blood pressure was significantly higher beginning on day 6 after the dietary treatment and continuing for the rest of the experiment in the CAP-HS group compared with all the other groups. On day 12 after the dietary treatment, tail-cuff systolic blood pressure (mmHg) was significantly increased in CAP-HS (145 ± 7) compared with CON-NS (96 ± 6), CON-HS (89 ± 5), CAP-NS (90 ± 4), CON-HS-ABT-627 (99 ± 7), and CAP-HS-ABT-627 (106 ± 6) (P < 0.05). Thus neonatal treatment with CAP did not increase blood pressure in rats fed a NS diet but led to an elevation in blood pressure in rats fed a HS diet. Moreover, chronic blockade of the ETA receptor with ABT-627 prevented the development of hypertension in CAP-HS rats but had no effect on blood pressure in CON-HS rats.

Figure 2 summarizes the changes in body weight and metabolic parameters during the study. Body weight was not significantly different among groups before the dietary treatment (Fig. 2A). All animals gained weight during the study period in a similar pattern that was independent of CAP or dietary treatment. Food intake was similar among all groups during the 2-wk dietary treatment period (Fig. 2B). Also, water intake, urine volume, and sodium excretion were not different among groups before the treatment (Fig. 2C–E). These parameters were significantly elevated in rats fed a HS diet with or without CAP treatment compared with rats given a NS diet. However, urine volume and sodium excretion were significantly lower in CAP-treated rats fed a HS diet compared with control rats fed a HS diet on day 8 (urine volume, 71 ± 6 vs. 88 ± 4 ml/day; sodium excretion, 16 ± 4 vs. 26 ± 3 meq/day; P < 0.05) and day 14 (urine volume, 73 ± 7 vs. 97 ± 11 ml/day; sodium excretion, 15 ± 5 vs. 29 ± 4 meq/day; P < 0.05) after the treatment. The decrease in urine volume and sodium excretion in CAP-HS rats was prevented by chronic treatment with ABT-627 in these rats. As shown in Fig. 3, water and sodium balances were significantly higher in
CAP-HS rats beginning on day 8 after the treatment and increased to 20.2 ± 2 ml/day (water balance) and 11.9 ± 3.1 meq/day (sodium balance) on day 14 compared with CON-NS (13.6 ± 1.8 ml/day and 1.8 ± 0.3 meq/day, respectively), CON-HS (15.5 ± 1.9 ml/day and 2.4 ± 0.3 meq/day, respectively), CAP-NS (13.0 ± 1.5 ml/day and 1.9 ± 0.2 meq/day, respectively), CON-HS-ABT-627 (15.2 ± 1.8 ml/day and 2.2 ± 0.2 meq/day, respectively), and CAP-HS-ABT-627 (16.2 ± 1.6 ml/day and 3.5 ± 1.5 meq/day, respectively) (P < 0.05).

Creatinine clearance, a parameter representing GFR, and FE_{Na}, a parameter representing tubular sodium excretion, were shown in Fig. 4. On day 14 after the treatment, creatinine clearance (in ml·min⁻¹·g kidney wt⁻¹) was significantly reduced in CAP-HS rats (0.29 ± 0.03) compared with CON-NS (0.51 ± 0.05), CON-HS (0.59 ± 0.08), CAP-NS (0.58 ± 0.09), CON-HS-ABT-627 (0.61 ± 0.07), and CAP-HS-ABT-627 (0.48 ± 0.07) rats (P < 0.05). Thus decreased creatinine clearance in CAP-HS rats was abolished by chronic blockade of the ETA receptor with ABT-627. FE_{Na} was increased by HS intake in CON-HS (12.53 ± 1.47%), CAP-HS (8.68 ± 0.99%), CON-HS-ABT-627 (13.42 ± 1.42%), and CAP-HS-ABT-627 (11.35 ± 1.34%) groups compared with CON-NS (0.46 ± 0.06%) and CAP-NS (0.53 ± 0.07%) groups (P < 0.05). However, FE_{Na} was significantly lower in CAP-HS rats compared with CON-HS rats (P < 0.05). The decrease in FE_{Na} in CAP-HS rats was abolished by chronic blockade of ETA receptor with ABT-627.

As shown in Fig. 5, the glomerular ET-1 level (in pg/1,000 glomeruli) was greater in CAP-HS (1.95 ± 0.24) and CAP-
HS-ABT-627 (2.28 ± 0.30) groups compared with CON-NS (1.08 ± 0.27), CON-HS (1.00 ± 0.21), CAP-NS (1.15 ± 0.27), and CON-HS-ABT-627 (0.95 ± 0.16) groups (P < 0.05). Thus the increase in glomerular ET-1 content in CAP-HS rats was not affected by chronic blockade of the ETA receptor with ABT-627 rats (P > 0.05).

On day 8 and 14 after the treatment, immunoreactive ET-1 excretion was similar in rats fed a NS diet with or without CAP treatment (Fig. 6), and HS diet alone or in combination with CAP treatment with or without ABT-627 significantly increased ET-1 excretion compared with that in CON-NS and CAP-NS rats. On day 14 after the treatment, ET-1 excretion (in pmol/day) was greater in CON-HS (2.4 ± 0.3), CAP-HS (2.2 ± 0.2), CON-HS-ABT-627 (2.6 ± 0.3), and CAP-HS-ABT-627 (2.8 ± 0.3) rats compared with CON-NS (0.4 ± 0.1) and CAP-NS (0.3 ± 0.1) rats (P < 0.05). The increased ET-1 excretion in CAP-HS or CON-HS rats was not affected by chronic blockade of the ETA receptor, indicating that the increased ET-1 excretion is mainly due to a HS diet that is independent of CAP or ABT-627 treatment.

Plasma ET-1 levels (in pg/ml) were higher in CAP-HS (4.3 ± 0.7) and CAP-HS-ABT-627 (4.7 ± 0.7) groups compared with CON-NS (1.9 ± 0.5), CON-HS (2.1 ± 0.4), CAP-NS (1.8 ± 0.4), and CON-HS-ABT-627 (2.3 ± 0.5) groups (P < 0.05). In addition, there were no significant changes in plasma sodium and potassium and creatinine concentrations among groups by the end of the 2-wk treatment (data not shown).

DISCUSSION

Although we have previously shown that plasma ET-1 levels are high in salt-sensitive hypertensive rats induced by neonatal CAP treatment, the role of ET-1 in mediating the changes in renal function in this model has not been defined. This study extends our previous findings by demonstrating that the inhibitory effects of ABT-627, a highly selective ETA receptor antagonist, on the development of hypertension in sensory nerve-degenerated rats fed a HS diet are associated with the improvement in renal hemodynamics and excretory function. These results indicate that elevated endogenous ET-1 and subsequent activation of the ET_A receptors exert antinatriuretic actions that may contribute to salt-induced elevation in blood pressure in the sensory nerve-degenerated model.

It is well established that long-term increases in arterial pressure are associated with the impairment of renal excretory function (11). Our finding in CAP-treated rats is that HS intake...
leads to a decrease in renal sodium and water excretion and an increase in blood pressure, indicating that the kidneys of CAP-treated rats have a reduced capability to excrete sodium and water and that the pressure-natriuresis curve right shifted. Furthermore, chronic treatment with the ET<sub>A</sub> receptor antagonist prevents the decrease in renal sodium and water excretion and the increase in blood pressure in sensory denervated rats fed a HS diet, indicating that ET<sub>A</sub> receptor blockade prevents the right shift of the pressure-natriuresis curve and leads to a chronic reduction in arterial pressure in this model. Our results agree with the previous studies (15, 16, 25) showing that ET-1 plays an important role in the development of salt-induced hypertension including DOCA-salt hypertension and genetic Dahl salt-sensitive hypertension. However, the studies by Allcock et al. (1) and Fujita et al. (9) show that either long-term or acute administration of an ET<sub>A</sub> receptor antagonist has no effect on renal function in DOCA-salt hypertensive rats. The discrepancy is unknown but may relate to different methodologies, including the dose, duration, and route of the drug administration in these hypertensive models. In CAP-treated rats fed a HS diet, we found that the increased ET-1 levels in plasma and glomeruli occurred in association with the decreases in GFR and renal excretory function. These results agree with a previous study (7) showing that systemic infusion of ET-1 in a high dose results in a profound antinatriuretic and antidiuretic effect, apparently secondary to a decrease in GFR and renal blood flow (RBF). Wilkins and colleagues (39, 40) also showed that systemic administration of ET-1, at a dose that increases circulating levels of ET-1 by two- to threefold, led to an increase in renal vascular resistance, a decrease in GFR and pressure natriuresis, and hypertension.

The kidney contains both ET<sub>A</sub> and ET<sub>B</sub> receptors that mediate the renal hemodynamic and excretory effects of ET-1 (19). Previous studies (10, 27, 28) have shown that renal cortical vasoconstriction induced by ET-1 appears to be mediated by the ET<sub>A</sub> receptor. Consistent with the studies by Gurbanov et al. (10), Pollock et al. (27, 28) reported that the renal vasoconstriction in response to a high dose of ET-1 was mediated by ET<sub>B</sub>-like receptors, whereas lower doses were mediated by ET<sub>A</sub> receptors. In addition to the increased ET-1 levels in plasma and glomeruli, the reduction in GFR and renal excretory function was abolished by blockade of the ET<sub>A</sub> receptor in the present study, suggesting that ET-1 may regulate the renal hemodynamic and excretory function via the ET<sub>A</sub> receptors.

Infusion of “pressor” concentrations of ET-1 is associated with a decrease in urinary sodium excretion resulting from severe reduction in both GFR and RBF (7, 39, 40). However, infusion of ET-1 at supressor doses (10<sup>−11</sup>-10<sup>−9</sup> M) in isolated perfused rat kidney caused an increase in urinary sodium excretion with a decrease in GFR and RBF (8). Furthermore, the study by Hsieh et al. (13) has demonstrated that the enhanced synthesis of ET-1 in the renal medulla correlates with urinary sodium excretion in the early stage of DOCA-salt-treated rats, suggesting that renal medullary ET-1 is involved in water-electrolyte balance. Therefore, the effect of ET-1 on sodium and water excretion depends on the dose and source of ET-1. In low doses or when produced locally in tubular epithelial cells, ET-1 decreases the reabsorption of salt and water via the activation of the ET<sub>B</sub> receptor. This is due to the ET<sub>B</sub> receptor that mediates renal tubular excretory effects of ET-1 (19). In the present study, we found that urinary ET-1 excretion was increased by HS intake with or without CAP treatment, which may reflect increased renal medullary ET-1 production (2, 5, 6). However, fractional sodium excretion was significantly reduced in CAP-HS rats compared with CON-HS rats, indicating that the renal tubular reabsorption was increased in CAP-HS rats. Importantly, decreased fractional sodium excretion can be prevented by blockade of the ET<sub>A</sub> receptor, indicating that increased tubular reabsorption is mediated by the activation of the tubular ET<sub>A</sub> receptor. The study by Rothermund et al. (30) showed that the activation of the renal ET system in conjunction with an increased ET<sub>A</sub>-to-ET<sub>B</sub> receptor ratio is attributed to the reduction in renal sodium excretion in salt-sensitive spontaneous hypertensive rats, suggesting that the ET<sub>A</sub> and ET<sub>B</sub> receptor balance plays a crucial role regulating renal sodium excretion. Taken together, our data indicate that ET<sub>A</sub> and ET<sub>B</sub> receptor imbalance combined
with increased ET production is responsible for the impairment of renal tubular sodium handling in CAP-HS rats. Further studies are necessary to examine the changes in renal medullary ET receptor subtype density and affinity in CAP-treated rats fed a HS diet.

Although glomerular ET-1 levels were increased in CAP-HS rats, we found that the ET-1 content in the renal cortex of CAP-treated rats was not increased by a HS diet (data not shown). The findings agree with previous studies (1, 22, 24) showing that preproET-1 mRNA expression in the renal cortex was not increased in DOCA-salt-treated rats, albeit ET-1 mRNA expression and ET-1 content were elevated in vascular tissues of DOCA-salt hypertensive rats. It is well known that vessels and glomeruli make up a small portion of the kidney tissues. Thus the possible explanation is that changes in the ET-1 content in glomeruli were not detected when using the whole cortical tissue. In addition, we found that in CON-HS rats, urinary but not plasma ET-1 levels were increased. This result is consistent with previous studies (2, 6) showing that urinary ET-1 concentrations are several times higher than in plasma and are not correlated with plasma ET-1 levels and GFR. Furthermore, there is evidence showing that only a negligible amount of labeled ET-1 infused into the systemic circulation could be detected in urine (5). These results support the conceptions that the kidney does not clear ET-1 from the circulation and that urinary ET-1 is mainly derived from the kidney.

In conclusion, neonatal degeneration of CAP-sensitive sensory nerves causes rats to respond to a salt load with a sustained rise in blood pressure and a reduction in renal sodium and water excretion. Chronic ETA receptor blockade ameliorates the reduction in renal excretory function and prevents the elevation in blood pressure. These findings indicate that the activation of the ETA receptor plays a crucial role in the impairment of renal function in salt-sensitive hypertension induced by degeneration of sensory nerves.

**Perspectives.** It is well documented that ET-1 plays an important role in salt-sensitive hypertension, including the DOCA-salt-induced hypertension, Dahl salt-sensitive hypertension, and salt-sensitive spontaneous hypertension (4, 15, 25, 30). Moreover, it has been shown (15) that reduced renal excretory function in Dahl salt-sensitive hypertensive rats was largely overcome by treatment with ETA-selective receptor antagonists, suggesting that the ET system and activation of ETA receptors are involved in the renal dysfunction in Dahl salt-sensitive hypertension. However, the mechanisms responsible for the activation of the ET system remain to be elucidated. There is accumulating evidence showing that a defect in the sensory nervous system exists in spontaneously hypertensive rats and Dahl-salt-sensitive hypertensive rats (33, 17). Given the fact that ET-1 and the activation of ETA receptors contribute to the development of hypertension and renal dysfunction in sensory nerve-degenerated rats fed a HS diet, it is conceivable that sensory nerve dysfunction may contribute to the activation of the ET system in these models of hypertension. Moreover, it is reasonable to speculate that agents that modulate the sensory nervous system may be beneficial in treating hypertension as well as in improving the renal dysfunction in certain models of salt-sensitive hypertension.

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