Pathophysiologica1 Roles of Endothelin-1 in Dahl Salt-Sensitive Hypertension

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Abstract—The purpose of the present experiment was to study the pathophysiologica1 roles of endothelin-1 (ET-1) in salt-sensitive hypertension with the use of Dahl salt-sensitive (DS) and salt-resistant (DR) rats. PreproET-1 mRNA expression was determin1 by reverse transcription–polymerase chain reaction. In the kidney, expression of preproET-1 mRNA was greater in DS rats on a normal salt diet compared with DR rats of the same age. In DS rats, the level of preproET-1 mRNA expression in kidney had a significant correlation with systolic blood pressure. The expression of preproET-1 mRNA in aorta and kidney was increased by 3-week high salt intake in DS rats but not in DR rats. Expression of preproET-1 mRNA and ET-1 levels in left ventricle was exaggerated by high salt intake in DS rats. However, there was no significant difference in plasma ET-1 levels between DS and DR rats regardless of salt intake. Pressor response curves for ET-1 in DS rats with or without high salt intake were significantly shifted to the left compared with those in DR rats. A single oral dose (3 to 10 mg/kg) of J-104132 (L-753 037), a potent, orally active mixed endothelin A and B (ET\textsubscript{A}/ET\textsubscript{B}) receptor antagonist, reduced blood pressure to normotensive levels in DS rats with high salt intake, and its action was maintained for $\geq$ 24 hours. In DS rats with normal salt intake, J-104132 (10 mg/kg) slightly but significantly decreased blood pressure. DR rats did not show obvious depressor responses to J-104132 (10 mg/kg) regardless of salt intake. These results suggest that ET-1 acts as one of the pathophysiologica1 factors in the development and maintenance of salt-sensitive hypertension, and a mixed ET\textsubscript{A}/ET\textsubscript{B} receptor antagonist could be useful in the treatment for salt-sensitive hypertension. (Hypertension. 1999;34:514-519.)

Key Words: endothelin ■ hypertension ■ rats, Dahl ■ kidney ■ receptors, endothelin

Endothelin-1 (ET-1) is known to have not only potent vasoconstrictive action but also mitogenic action on smooth muscle.\textsuperscript{1,2} With regard to salt-sensitive hypertension, Ferri et al\textsuperscript{3} recently demonstrated elevated plasma and urinary ET-1 levels in salt-sensitive hypertensive patients. Infusion of ET-1 with high salt intake but not with normal salt intake caused sustained hypertension in conscious rats.\textsuperscript{4} These results suggested that ET-1 may be involved in salt-sensitive hypertension.

Dahl salt-sensitive (DS) rats are genetically predisposed to hypertension, and an excess of dietary salt intake markedly enhances the development of hypertension in this model.\textsuperscript{5} The DS rat is therefore considered an animal model for salt-sensitive hypertension. Although the mechanism for the salt sensitivity and maintenance of hypertension with high salt ingestion in this strain has not been fully understood, abnormality in renal function is one of early changes before and during the course of hypertension.\textsuperscript{6} Similar to hypertension developed in DS rats, the salt-sensitive volume-loaded type of hypertension, such as that in deoxycorticosterone acetate (DOCA)–salt hypertensive rats, showed increased expression of preproET-1 mRNA in aorta, mesenteric artery, and kidney.\textsuperscript{7} In the kidney, ET-1 is not only a potent vasoconstrictor in renal vasculature\textsuperscript{8} but also causes mesangial cell contraction,\textsuperscript{9} which results in decreases in glomerular filtration rate and natriuresis.\textsuperscript{10,11} Therefore, the physiologica1 actions of ET-1 are complex, and enhanced activation of this system could contribute to the renal and vascular pathophysiologica1 of salt-sensitive hypertension.

Recently, several ET receptor antagonists demonstrated antihypertensive effects in DS rats.\textsuperscript{12,13} Although these results supported the hypothesis that ET-1 has pathophysiologica1 roles in salt-sensitive hypertension of DS rats, there was no direct measurement of production of ET-1 in various tissues and no relationship between the level of ET-1 in various tissues and development of hypertension in DS rats.

Therefore, we sought to evaluate further the pathophysiologica1 role of ET-1 in salt-sensitive hypertension with the use of Dahl rats. To assess the production of ET-1 in vivo, we determined the expression of preproET-1 mRNA in the aorta, heart, and kidney reverse transcription–polymerase chain
reaction (PCR) and measured plasma and heart ET-1 levels in DS and Dahl salt-resistant (DR) rats with and without high-salt intake for 3 weeks. Blood pressure responses to ET-1 in Dahl rats with or without high salt intake were investigated to assess the vascular reactivity to ET-1 in vivo. Finally, the antihypertensive efficacy of a new orally active endothelin receptor antagonist, J-104132 (L-753 037), was evaluated in DS and DR rats. J-104132 is a potent mixed endothelin A and B (ET\(_A\)/ET\(_B\)) receptor antagonist with a long duration of activity \(^{14}\) and was used to determine the overall role of in vivo ET-1 in salt-sensitive hypertension by full blockade of both ET receptors.

**Methods**

**Animals**

All experimental procedures were performed according to the institutional guidelines for animal welfare. Male DS and DR rats were purchased from Seac Yoshitomi Ltd, Fukuoka, Japan. They were fed a normal pelleted animal chow (CE-2, containing 0.3% sodium, CLEA Japan Inc) ad libitum during the quarantine and accclimatization periods (at least 1 week). For salt intake, some of the rats were placed on a diet containing 8% NaCl (MF supplemented with 8% sodium chloride, Oriental Yeast Co, Ltd) for \(\approx 3\) weeks from 7 to 8 weeks of age. The remaining rats were fed normal animal chow.

**Determination of PreproET-1 mRNA in Aorta, Kidney, and Left Ventricle**

Systolic blood pressure was measured in DS and DR rats with or without salt intake by a tail-cuff method (BP-98A, Softron). The thoracic aorta, left kidney, and left ventricle were dissected under anesthesia with an injection of sodium pentobarbital (50 mg/kg IP), weighed, immediately frozen in liquid nitrogen, and stored at \(-80^\circ\)C until determination of preproET-1 mRNA. The expression of preproET-1 mRNA was analyzed by reverse transcription–PCR according to our previous reports.\(^{15}\)\(^,\)\(^{18}\) The tissue samples were identical to those that were used in the assay of preproET-1 mRNA levels. In brief, each tissue sample was homogenized with a Polytron homogenizer for 60 seconds in 10 volumes of 1 mol/L acetic acid that contained 10 \(\mu\)g/mL pepstatin (Peptide Institute) and immediately boiled for 10 minutes. The supernatant was stored at \(-80^\circ\)C until use. The supernatant was subjected to a sandwich enzyme immunoassay for ET-1. A sandwich enzyme immunoassay for ET-1 was performed with the use of immobilized mouse monoclonal antibody AWETN40, which recognizes the N-terminal portion of ET-1, and peroxidase-labeled rabbit anti-ET-1 C-terminal peptide (15–21) Fab.\(^{15}\)\(^,\)\(^{18}\)

**Determination of Plasma ET-1**

The animals were anesthetized with an injection of sodium pentobarbital (50 mg/kg IP) and were placed in the dorsal position. The right jugular vein and the abdominal aorta (through the left femoral artery) were cannulated with a polyurethane tube (MRE-033, 0.83 mm OD \times 0.35 mm ID, Micro-RENathane, Braintree Scientific, Inc) filled with normal saline.

After direct blood pressure determination, \(\approx 4\) mL of venous blood was drawn from the chronic indwelling catheter in the jugular vein, and the blood sample was placed into an ice-chilled tube (Neotube, Nipro) that contained EDTA-3K (3 mg/mL) and aprotinin (Trasylol, Bayer, 300 U/mL of blood). After centrifugation (3000 rpm, 10 minutes, \(4^\circ\)C), the plasma was separated and stored at \(-40^\circ\)C until ET-1 measurement.

To remove proteins from the plasma and to protect against the effects of hemolysis, 250 \(\mu\)L of each plasma sample and 1 mL of 95% of ethanol were mixed and centrifuged (12 000 rpm for 10 minutes at room temperature). The supernatant was separated and evaporated with ethanol by a centrifugal concentrator (VC-96N, TAITEC). The pellet was redissolved in 250 \(\mu\)L of assay buffer, and the concentration of ET-1 was measured by the ET-1 enzyme-linked immunosorbent assay method with commercially available kits (Biomedica Gruppe).

**Pressor and Depressor Responses to Exogenous ET-1**

The rats, which were fed with normal or 8% NaCl diet for \(\approx 3\) weeks, were anesthetized with an injection of sodium pentobarbital (50 mg/kg IP) and were instrumented with a vascular cannula. The cannula was inserted into the lower abdominal aorta via the left femoral artery for the measurement of mean blood pressure. In addition, rats were also instrumented with a venous cannula for injection of ET-1. After surgery, the rats were returned to individual cages and allowed free access to food and water. The next day, the implanted arterial cannula was connected to a pressure transducer (DX-100, Ohmeda Pte Ltd) through a cannula swivel (375, Instech Laboratories, Inc). The signal from the pressure transducer was amplified by a blood pressure amplifier (AP-641G, Nihon Kohden) and recorded on a thermal array recorder (RTA-1200 mol/L, Nihon Kohden). ET-1 (Peptide Institute) was cumulatively injected in ascending order of dose with an interval of 0.5 to 2 hours through the tube inserted in the vein. Bolus injection of ET-1 causes an initial transient depressor response followed by a sustained pressor response. Peak depressor and pressor responses were measured in each animal.

**Effects of Acute ET\(_A\)/ET\(_B\) Receptors Blockade on Blood Pressure**

The DS and DR rats with or without 3-week salt intake had a femoral arterial cannula implanted. After overnight recovery, mean arterial pressure (MAP) was read from the digital output before treatment and 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 420, and 480 minutes and 24 hours after treatment of drugs. J-104132 (Banyu Pharmaceutical Co Ltd) was dissolved in a small amount of 0.1N NaOH solution (adjusted to pH 7.0 to 7.4 with 0.1N
but not DR rats. In left ventricle, preproET-1 mRNA levels increased expression of preproET-1 mRNA in kidney of DS than in DR rats with normal salt intake. High salt intake in DS than in DR rats. The magnitude of expression of with salt intake, preproET-1 mRNA was significantly greater difference did not reach statistical significance. However, this relationship was found in DR rats, except that of a weak negative linear correlation between systolic blood pressure and expression of preproET-1 mRNA in aorta (r = 0.644, P = 0.024).

Plasma and Left Ventricle ET-1 Levels
There were no significant differences in plasma ET-1 levels between groups (2.32 to 2.85 fmol/mL). ET-1 contents in left ventricle are shown in the Table. There were no differences in ET-1 levels between DS and DR rats with normal salt intake; however, 3-week high-salt intake increased ET-1 levels in DS rats but not in DR rats. This result was of the same amplitude as those shown in preproET-1 mRNA assay in left ventricular preparation (Figure 1C), and there was a significant correlation between ET-1 peptide levels and preproET-1 mRNA levels (r = 0.757, P < 0.01).

Pressor and Depressor Responses Induced by Exogenously Administered ET-1
The potencies of ET-1 to elicit pressor and depressor responses were determined by generating dose-response relationships to ET-1 in DS and DR rats with normal or high salt intake (Figure 3). The maximal pressor responses were not different between groups, while depressor responses were significantly greater in DS than in DR rats. When the potency were comparable between DS and DR rats when they were given a normal sodium diet, whereas high salt intake enhanced the expression of mRNA in DS rats but not in DR rats.

A positive linear correlation existed between systolic blood pressure and the magnitude of expressed preproET-1 mRNA in kidney (r = 0.831, P = 0.0008) but not in aorta (r = 0.4417, P = 0.151) and left ventricle (r = 0.396, P = 0.202) in DS rats with high and normal salt intake (Figure 2). However, no such relationship was found in DR rats, except that of a weak negative linear correlation between systolic blood pressure and expression of preproET-1 mRNA in aorta (r = 0.644, P = 0.024).

Physiological Variables in DS and DR Rats That Underwent Measurement of PreproET-1 mRNA in Tissue

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>Aorta/BW Ratio, %</th>
<th>Kidney/BW Ratio, %</th>
<th>LV/BW Ratio, %</th>
<th>ET-1 in LV, pg/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal salt DS</td>
<td>6</td>
<td>361.2±5.3*</td>
<td>0.0117±0.0004</td>
<td>0.4176±0.0124</td>
<td>0.2153±0.0059</td>
<td>254.9±11.3</td>
</tr>
<tr>
<td>High salt DS</td>
<td>6</td>
<td>344.5±5.2</td>
<td>0.0148±0.0009††</td>
<td>0.626±0.0109††</td>
<td>0.3002±0.0038††</td>
<td>447.6±46.9††</td>
</tr>
<tr>
<td>Normal salt DR</td>
<td>6</td>
<td>316.3±10.8</td>
<td>0.012±0.001</td>
<td>0.4045±0.0027</td>
<td>0.2073±0.0044</td>
<td>286.3±38.6</td>
</tr>
<tr>
<td>High salt DR</td>
<td>6</td>
<td>317.5±6.3</td>
<td>0.0107±0.0004</td>
<td>0.4398±0.0111</td>
<td>0.2432±0.0111</td>
<td>290.3±30.7</td>
</tr>
</tbody>
</table>

BW indicates body weight; SBP, systolic blood pressure; and LV, left ventricle.

Statistical Methods
Values are given as mean±SEM. Statistical differences were evaluated by 1-way or repeated-measures ANOVA as appropriate, and post hoc comparison was done with Dunnett’s multiple comparison test (for comparison with 1 control) or Tukey’s multiple comparison test (for comparison between groups). The linear correlation between preproET-1 expression and blood pressure was ascertained by the least squares method. P < 0.05 was considered statistically significant. ED50 was determined by the sigmoid curve fitting to log dose-response data.

Results
Expression of PreproET-1 mRNA in Aorta, Kidney, and Left Ventricle
Blood pressure, body weight, and tissue weight in the study animals are summarized in the Table. Ingestion of a high-salt diet increased not only blood pressure but also tissue weight in DS rats. However, these parameters were not affected by salt intake in DR rats. Systolic blood pressure of DS rats with a normal diet was slightly higher than that of DR rats with normal and high salt intake.

Figure 1 shows the preproET-1 mRNA levels in aorta, kidney, and left ventricle in DS and DR rats with or without 3-week high-salt intake. In aorta, preproET-1 mRNA expression was 1.4-fold greater in DS than in DR rats, but this difference did not reach statistical significance. However, with salt intake, preproET-1 mRNA was significantly greater in DS than in DR rats. The magnitude of expression of preproET-1 mRNA in the kidney was 2-fold greater in DS than in DR rats with normal salt intake. High salt intake increased expression of preproET-1 mRNA in kidney of DS but not DR rats. In left ventricle, preproET-1 mRNA levels were comparable between DS and DR rats when they were given a normal sodium diet, whereas high salt intake enhanced the expression of mRNA in DS rats but not in DR rats.
of ET-1 to produce pressor responses was assessed by ED$_{50}$ (dose for 50% of maximal response), which indicates the dose that produces the response by 50% against full response, it was significantly greater in DS (0.029 and 0.018 nmol/kg) than in DR rats (0.089 and 0.078 nmol/kg) with normal and high salt intake, respectively. In addition, the ET-1 potencies for both pressor (0.029 versus 0.018 nmol/kg) and depressor responses (0.266 versus 0.123 nmol/kg) were enhanced by high salt intake in DS rats. Therefore, DS rats are more sensitive in terms of vascular reactivity to exogenous ET-1, and the reactivity is further increased by salt ingestion.

**MAP After Dosing of J-104132 in DS and DR Rats**

High salt intake significantly increased MAP in DS rats (MAP, 164±3 mm Hg [n=37] with high salt intake versus 119±2 mm Hg [n=14] with normal salt intake), but this was not the case in DR rats (MAP, 111±1 mm Hg [n=12] with high salt intake versus 109±2 mm Hg [n=14] with normal salt intake). With normal salt intake, MAP in DS rats was slightly higher than that in DR rats, but this difference was not statistically significant. A single oral administration of J-104132 (3 and 10 mg/kg) produced significant, dose-dependent reductions in MAP in conscious DS rats with high salt intake (Figure 4A). The antihypertensive effect of J-104132 was maintained for ≥24 hours. In DS rats with normal salt intake, J-104132 (10 mg/kg) elicited a slight but significant decrease in blood pressure (Figure 4B). In contrast, DR rats showed no obvious blood pressure lowering with J-104132 regardless of salt intake (Figure 4C and 4D).

**Discussion**

In the present study, the expression of preproET-1 mRNA in the kidney and aorta in DS rats was increased compared with DR rats even with normal salt intake. Enhanced expression of preproET-1 mRNA in both tissues was further increased by 3-week high salt intake. In addition, mRNA of preproET-1 and ET-1 levels in heart was increased by high salt intake in DS rats but not in DR rats. Blood pressure had a linear correlation with the magnitude of expression of preproET-1 mRNA in the kidney but not in either the aorta or the left ventricle in DS rats. The pressor responses to exogenous ET-1 were exaggerated in DS rats compared with DR rats regardless of salt intake. A mixed ET$_A$/ET$_B$ receptor antagonist, J-104132, significantly reduced blood pressure in DS rats but not in DR rats.

The salt-sensitive hypertension in DS rats may originate, at least in part, from renal dysfunction. The pressure-natriuresis relationship is blunted before the development of hypertension in DS rats and is worsened by salt intake, which is a mechanism that could contribute to high salt sensitivity and consequent salt-sensitive hypertension. In the present study, the magnitude of expression of preproET-1 mRNA was greater in the kidney than in the aorta or the left ventricle in DS rats. The pressor responses to exogenous ET-1 were exaggerated in DS rats compared with DR rats regardless of salt intake. A mixed ET$_A$/ET$_B$ receptor antagonist, J-104132, significantly reduced blood pressure in DS rats but not in DR rats.
the ET\textsubscript{A}/ET\textsubscript{B} receptor antagonist SB 209670 significantly increased urinary sodium excretion in DS rats with high salt intake but not in DR rats, and the ET\textsubscript{A} selective antagonist A-127722 partially corrected the attenuated pressure natriuresis in DS rats. These findings suggest that ET-1 is one of the important factors that participate in mechanisms that underlie blunted pressure natriuresis in DS rats.

ET-1 is not only a potent vasoconstrictor but a potent mitogen for vascular smooth muscle cells, cardiac myocytes, and glomerular mesangial cells. In the present study, tissue weights of left ventricle, aorta, and kidney, in which the expression of preproET-1 mRNA was enhanced by high salt intake in DS rats, were significantly greater than those in other groups. ET-1–induced hypertrophy and/or proliferation may partly contribute to the increases in tissue weights, although contribution of other factors, such as norepinephrine and mechanical stress caused by high blood pressure, cannot be excluded in this study.

Exogenous ET-1 caused an initial transient decrease followed by a sustained increase in blood pressure in rats. Although the pattern of cardiovascular responses was not altered in DS rats with or without high salt intake, the pressor response to ET-1 mediated by ET\textsubscript{A}/ET\textsubscript{B} receptors was exaggerated in DS rats regardless of high salt intake. This result indicates that the vascular reactivity to ET-1 in DS rats was exaggerated compared with that in DR rats regardless of salt intake. Goligorsky et al reported that glomerular mesangial cells and vascular smooth muscle cells obtained from prehypertensive DS rats exhibited an exaggerated intracellular calcium response to ET-1 and enhanced contraction of vascular rings in DS rats. However, it has been reported that after establishment of hypertension, vascular reactivities to ET-1 are attenuated in DS rat mesenteric artery, which suggests downregulation of ET-1 receptor. However, the cardiovascular effects of ET-1 in DS rats have not been tested. It has been reported that responses to norepinephrine are increased markedly after 1 to 3 weeks of high-salt diet in DS rats and that the enhanced responses to norepinephrine are reversed after 6 weeks of a high-salt diet. The activation of the sympathetic nervous system has been demonstrated in DS rats. Thus, the present results may indicate that both production of and receptor sensitivity to ET-1 were enhanced after 3 weeks of high salt intake in DS rats. The maximal depressor responses to ET-1 were enhanced without changing the sensitivity in DS rats with or without high salt intake. Although these enhanced responses may be due to high baseline blood pressure in DS rats, enhanced vasodilatory mechanisms through activation of ET\textsubscript{B} receptor in endothelial cells or compensatory dilatory mechanisms cannot be ruled out in the present study.

In DS rats, preproET-1 mRNA in the aorta and kidney before salt intake (without hypertension) was 1.4-fold and 2-fold greater than in DR rats, respectively. Furthermore, the pressor response to ET-1 in DS rats without salt intake was
significantly enhanced compared with that in DR rats. However, there was no significant difference in plasma ET-1 levels between DS and DR rats. On the basis of these results, it is likely that a mechanism for salt-sensitive hypertension in DS rats might be due to local activation of the ET-1 system in tissues.

J-104132 produced long-lasting antihypertensive effects in hypertensive DS rats, which has been reported with other endothelin receptor antagonists. ET receptor antagonists are also effective as antihypertensive agents in other salt-sensitive hypertensive models, such as the DOCA-salt rat. ET-1 content in the aorta, mesenteric vascular bed, and kidney was also increased dramatically in DOCA-salt hypertensive rats. Furthermore, infusion of ET-1 at a dose that caused no hypertension in rats fed a normal salt diet produced hypertension in rats fed a high-salt diet. These results strongly support the theory that ET-1 contributes to the development and maintenance of salt-dependent hypertension.

In conclusion, results from the present experiment indicate that the enhanced activity of the ET system is apparent in DS rats with high salt intake, as follows: (1) greater expression of preproET-1 mRNA in tissues, (2) enhanced sensitivity to pressor responses by ET-1, and (3) full antihypertensive efficacy when both ETA and ETB receptors are inhibited by J-104132. These results suggest that ET-1 is an important pathophysiological factor in the development and maintenance of salt-sensitive hypertension and that blockade of ETA/ETB receptors could have clinical benefit for treatment of salt-sensitive hypertension.

References


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