Exaggerated Vascular and Renal Pathology in Endothelin-B Receptor–Deficient Rats With Deoxycorticosterone Acetate–Salt Hypertension

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Background—Endothelin (ET)-1 plays an important role in the pathogenesis of deoxycorticosterone acetate (DOCA)-salt–induced hypertension. We evaluated the pathological role of ET_b receptors in DOCA-salt–induced hypertension, cardiovascular hypertrophy, and renal damage by using the spotting-lethal (sl) rat, which carries a naturally occurring deletion in the ET_b receptor gene.

Methods and Results—Homozygous (sl/sl) rats exhibit abnormal development of neural crest–derived epidermal melanocytes and the enteric nervous system, and they do not live beyond 1 month because of intestinal aganglionosis and intestinal obstruction. The dopamine β-hydroxylase (DbH) promoter was used to direct ET_b transgene expression in sl/sl rats to support normal enteric nervous system development. DbH-ET_b sl/sl rats live into adulthood and are healthy, expressing ET_b receptors in adrenal glands and other adrenergic neurons. When homozygous (sl/sl) and wild-type (+/+) rats, all of which were transgenic, were treated with DOCA-salt, homozygous rats exhibited earlier and higher increases in systolic blood pressure than did wild-type rats. Chronic treatment with ABT-627, an ET_A receptor antagonist, completely suppressed DOCA-salt–induced hypertension in both groups. Renal dysfunction and histological damage were more severe in homozygous than in wild-type rats. Marked vascular hypertrophy was observed in homozygous rats than in wild-type rats. Renal and vascular injuries were significantly improved by ABT-627. In DOCA-salt–treated homozygous rats, there were notable increases in renal, urinary, and aortic ET-1, all of which were normalized by ABT-627.

Conclusions—ET_b-mediated actions are protective in the pathogenesis of DOCA-salt–induced hypertension. Enhanced ET-1 production and ET_A–mediated actions are responsible for the increased susceptibility to DOCA-salt hypertension and tissue injuries in ET_b receptor–deficient rats. (Circulation. 2000;102:2765-2773.)

Key Words: endothelin ■ receptors ■ hypertension

Endothelin (ET)-1 is considered to participate in cardiovascular diseases, such as hypertension, vasospasm, atherosclerosis, and ischemia.1 ET-1 is implicated in the development and maintenance of hypertension because of its potent vasoconstrictor action.1 Other studies2–4 and our previous studies5,6 have found that ET-1 plays an important role in the pathogenesis of deoxycorticosterone acetate (DOCA)-salt–induced hypertension, on the basis of evidence showing that acute administration of ET_A–selective receptor antagonists or nonselective ET_A/ET_b receptor antagonists to DOCA-salt rats produces a hypertensive effect and that long-term treatment with these agents suppresses the development of hypertension. We have found that chronic treatment with ET_A–selective receptor antagonists improves tissue injuries in DOCA-salt rats.5,7 However, the pathological role of ET_b receptor–mediated action in the DOCA-salt model of hypertension remains controversial.8 A recent study indicates that nonselective ET_A/ET_b receptor antagonists cause decreases in blood pressure to a degree similar to that found with ET_A receptor–selective antagonists.9 We have found that chronic treatment with an ET_b receptor–selective antagonist to DOCA-salt rats leads to a deterioration in DOCA-salt–induced cardiovascular and renal injuries,7 thereby suggesting that blockade of this receptor could be harmful in such pathological conditions. To confirm this view, we used the spotting-lethal (sl) rat, which carries a naturally occurring deletion in the ET_b receptor gene,10 and we examined responses to DOCA-salt treatment.
The sl rat carries a naturally occurring 301-bp deletion in the 3′ end of the first exon of the ET_b gene. The deletion encompasses the first 2 transmembrane-spanning domains of the receptor and results in the activation of a cryptic splice donor site 15 bp upstream from the deletion. The shortened transcript does not encode a functional ET_b receptor. Homozygous (ssl/ssl) rats exhibit abnormal development of neural crest–derived epidermal melanocytes and of the enteric nervous system that is similar to that described in ET_b receptor–deficient mice. Therefore, the DβH-ET_b transgenic rats have dark eyes and pigmented coats only in small spots on their heads. Wild-type and heterozygous rats have pigmented heads, backs, and tails. To definitively differentiate these rats, polymerase chain reaction was performed on DNA isolated from tail biopsy specimens, as described.

We report in the present study that ET_b receptor–deficient rats exhibit an exaggerated blood pressure sensitivity and enhanced tissue injuries to DOCA-salt treatment, suggesting that ET_b receptor–mediated actions are protective in the pathogenesis of DOCA-salt–induced hypertensive diseases. Furthermore, we examined the effect of chronic treatment with an ET-A-selective receptor antagonist.

**Methods**

**Animals**

The creation of DβH-ET_b transgenic rats has been described previously. Homozygous ssl/ssl rats have dark eyes and pigmented coats only in small spots on their heads. Wild-type and heterozygous rats have pigmented heads, backs, and tails. To definitively differentiate these rats, polymerase chain reaction was performed on DNA isolated from tail biopsy specimens, as described.

**Experimental Protocol**

Homozygous (ssl/ssl) and wild-type (+/+ ) rats (weighing 160 to 180 g, aged 6 weeks), all of which were DβH-ET_b transgenic, were unilaterally nephrectomized. After a 1-week postsurgical recovery period, the rats were treated twice weekly with DOCA suspended in corn oil, administered subcutaneously (15 mg/kg), and 1% NaCl was added to their drinking water. These rats were randomly divided into 2 groups and given ABT-627 (10 mg/kg per day, twice daily by gavage; Abbott Laboratories), which is a selective ETA receptor antagonist, or vehicle. The dose of ABT-627 was shown to abolish the ET-1–induced pressor effect. Control rats were uninephrectomized (UN) but not given DOCA and salt. Systolic blood pressure (SBP) was monitored once a week by the tail-cuff method or ABT-627 (10 mg/kg per day, twice daily by gavage; Abbott Laboratories), which is a selective ETA receptor antagonist, or vehicle. The dose of ABT-627 was shown to abolish the ET-1–induced pressor effect. Control rats were uninephrectomized (UN) but not given DOCA and salt. Systolic blood pressure (SBP) was monitored once a week by the tail-cuff method or ABT-627 (10 mg/kg per day, twice daily by gavage; Abbott Laboratories), which is a selective ETA receptor antagonist, or vehicle. The dose of ABT-627 was shown to abolish the ET-1–induced pressor effect. Control rats were uninephrectomized (UN) but not given DOCA and salt. Systolic blood pressure (SBP) was monitored once a week by the tail-cuff method or ABT-627 (10 mg/kg per day, twice daily by gavage; Abbott Laboratories), which is a selective ETA receptor antagonist, or vehicle. The dose of ABT-627 was shown to abolish the ET-1–induced pressor effect.

**Figure 1.** Time course for SBP in UN control rats (homozygous, n = 11; wild-type, n = 8) and DOCA-salt hypertensive rats treated with vehicle (homozygous, n = 9 to 12; wild-type, n = 9) or ABT-627 (homozygous, n = 8; wild-type, n = 8). A, Time course in homozygous rats. B, Time course in wild-type rats. Values are mean±SEM. *P<0.05 and **P<0.01 vs homozygous UN control rats; §P<0.01 vs homozygous DOCA-salt rats; #P<0.05 and ##P<0.01 vs wild-type UN control rats; and †P<0.01 vs wild-type DOCA-salt rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UN Control (n = 11)</th>
<th>DOCA-Salt (n = 9)</th>
<th>DOCA-Salt + ABT-627 (n = 8)</th>
<th>Wild-type Rats (n = 8)</th>
<th>DOCA-Salt (n = 9)</th>
<th>DOCA-Salt + ABT-627 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>304 ± 5</td>
<td>216 ± 12*†</td>
<td>248 ± 6§</td>
<td>303 ± 7</td>
<td>281 ± 6</td>
<td>286 ± 4</td>
</tr>
<tr>
<td>HW/BW, g/kg</td>
<td>2.83 ± 0.06</td>
<td>4.57 ± 0.19*†</td>
<td>3.71 ± 0.10*§</td>
<td>2.91 ± 0.06</td>
<td>3.62 ± 0.09§</td>
<td>3.18 ± 0.08§</td>
</tr>
<tr>
<td>LKWiBW, g/kg</td>
<td>5.42 ± 0.15</td>
<td>9.37 ± 0.41*‡</td>
<td>7.44 ± 0.37*§</td>
<td>5.23 ± 0.10</td>
<td>8.20 ± 0.21∥</td>
<td>6.61 ± 0.11∥†</td>
</tr>
<tr>
<td>Aortic weight, mg/cm</td>
<td>11.4 ± 0.3</td>
<td>14.7 ± 0.4*‡</td>
<td>11.7 ± 0.5§</td>
<td>11.2 ± 0.3</td>
<td>12.9 ± 0.5∥</td>
<td>11.9 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean±SE. BW indicates body weight; HW, heart weight; and LKWi, left kidney weight.

*P<0.01 vs homozygous UN group; †P<0.01 and ‡P<0.05 vs wild-type DOCA-salt group; $P<0.01 vs homozygous DOCA-salt group; and ||P<0.01 vs wild-type UN group.
collected overnight by housing the rats in individual metabolic cages. After urine collection, the rats were exsanguinated, and arterial blood samples were obtained. The heart, left kidney, and aorta were excised and weighed. The thoracic aorta and left kidney were used for morphometric analysis. A portion of left kidney and aortic tissues was frozen separately for determination of tissue ET-1.

ET-1 Measurement

ET-1 was extracted from the kidney and aorta, according to our method.13 Radioimmunoassay for tissue ET-1 was performed with the use of ET-1 antisera (a generous gift from Dr Marvin R. Brown, University of California, San Diego), which does not cross-react with big ET-1. ET-1 concentrations in plasma and urine were determined by direct ELISA method without the extraction step (BIOMEDICA Gesellschaft mbH).

Analytical Procedures

Blood urea nitrogen (BUN), protein, and creatinine levels in plasma or urine were determined by use of the BUN-Test-Wako, Total Protein-Test-Wako, and Creatinine-Test-Wako (Wako Pure Chemical Industries, respectively). Urinary β-N-acetylglucosaminidase (NAG) activity, as an index of proximal tubule damage, was measured by using the synthesized substrate sodio-m-cresol sulfonphthaleinyl β-N-acetyl-d-glucosaminide. Urine and plasma sodium concentrations were determined by use of a flame photometer.

Histological Studies

The thoracic aorta and the left kidney of each rat were preserved stained with hematoxylin-eosin. Three different cross sections of chopped into small pieces, embedded in paraffin, cut at 4 μm, and stained with hematoxylin-eosin. Three different cross sections of each vessel placed under a microscope were photographed, and the vessel wall area, thickness, and wall-to-lumen ratio were determined by direct ELISA method without the extraction step (BIOMEDICA Gesellschaft mbH).

Figure 2. Comparative data on levels of plasma creatinine (Pcr, A), creatinine clearance (Ccr, B), BUN (C), and urinary excretion of protein (UproV, D) in UN control rats (homozygous [homo], n=11; wild-type [wild], n=8) and DOCA-salt hypertensive rats treated with vehicle (homo, n=9; wild, n=9) or ABT-627 (homo, n=8; wild, n=8). Values are mean±SEM. **P<0.01 vs homo UN control rats; §§P<0.05 and §§§P<0.01 vs homo DOCA-salt rats; ###P<0.01 vs wild UN control rats; and ††P<0.01 vs wild DOCA-salt rats.

Drugs

ABT-627 was dissolved in a mixture of 10% ethanol, 40% propylene glycol, and 50% distilled water. Other chemicals were obtained from Nacalai Tesque and Wako Pure Chemical Industries. ABT-62714 is the active enantiomer of the racemate A-127722,14 an orally active and highly potent ET₄-selective receptor antagonist.

Statistical Analysis

Values were expressed as mean±SEM. For statistical analysis, we used the unpaired Student t test for 2-group comparison (homozygous versus wild-type rats). Multicomparisons within groups were performed by 1-way ANOVA followed by a Bonferroni multiple comparison test. Histological data for the kidney were analyzed by the Kruskal-Wallis nonparametric test combined with a Steel-type multiple comparison test. For all comparisons, differences were considered significant at P<0.05.
Results

Changes in SBP
Basal SBPs (before the DOCA-salt treatment) of homozygous (s/s) and wild-type (+/+ rats were 131.3±1.3 mm Hg (n=31, P<0.001 versus wild-type) and 122.9±1.3 mm Hg (n=25), respectively. As shown in Figure 1, SBP of both groups was progressively elevated by treatment with DOCA and salt, but hypertensive effects were much more pronounced in homozygous than in wild-type rats (respective values were 156.7±3.4 versus 133.0±3.7 mm Hg at 1 week, P<0.01; 188.5±5.2 versus 144.3±3.7 mm Hg at 2 weeks, P<0.01; 208.1±5.5 versus 151.6±3.7 mm Hg at 3 weeks, P<0.01; and 206.0±4.5 versus 164.3±4.8 mm Hg at 4 weeks, P<0.01). Three of the 12 homozygous rats died at 3 weeks, although we could not determine the cause of death.

Table 2. Histopathologic Assessment of Kidneys in UN Control and DOCA-Salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Histopathologic Changes/Grade</th>
<th>UN Control (n=11)</th>
<th>DOCA-Salt (n=9)</th>
<th>DOCA-Salt + ABT-627 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Tubular dilatation and atrophy</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Proteinaceous casts in tubuli</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fibrinoid-like necrosis in glomeruli</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Interstitial cell infiltration</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Thickening of small arteries</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are number of animals with histopathologic changes. Grades are as follows: intact (–), minimal (+), mild (++), moderate (+++), and severe (+++). *P<0.01 vs homozygous UN group; †P<0.01 vs wild-type DOCA-salt group; ‡P<0.01 vs homozygous DOCA-salt group; and §P<0.01 vs wild-type UN group.

UN control rats from both groups showed no significant changes from each basal SBP value. In both homozygous and wild-type rats, daily oral administration of ABT-627 suppressed almost completely the development of hypertension induced by DOCA-salt, although a significant increase in SBP was observed only at 4 weeks in homozygous rats compared with UN control rats.

Body, Heart, Kidney, and Aorta Weights
In homozygous rats, the body weight gain in vehicle-treated DOCA-salt rats was much less than that in UN control rats (Table 1). ABT-627 treatment led to recovery of losses. When heart and left kidney weights were corrected by body weight, there were significant increases in each organ weight-to-body weight ratio in vehicle-treated DOCA-salt rats. Aortic weight showed a significant increase by the

Figure 4. Representative light micrographs of renal tissues obtained from homozygous (homo) and wild-type (wild) UN control and DOCA-salt hypertensive rats treated with vehicle or ABT-627. Arrowhead and arrow indicate proteinaceous casts in tubuli and fibrinoid-like necrosis in glomeruli, respectively. Original magnification ×80.
DOCA-salt treatment. These increments were significantly suppressed by ABT-627 administration. Qualitatively similar findings were obtained in wild-type rats, although changes induced by DOCA-salt and ABT-627 treatments were less.

Renal Function
Figures 2 and 3 illustrate changes of renal function parameters at the end of the experimental period. Plasma creatinine in homozygous rats revealed high levels compared with levels in wild-type rats. In homozygous rats, DOCA-salt treatment slightly increased plasma creatinine and BUN, whereas the level of creatinine clearance decreased markedly, showing diminished glomerular function. These changes were overcome by ABT-627 administration. In wild-type rats, no significant alterations in renal parameters were observed. DOCA-salt treatment produced significant increases in the urinary excretion of protein in both groups, although increases in the homozygous group were greater. ABT-627 treatment markedly suppressed this increase in the urinary excretion of protein in both groups (Figure 2). As shown in Figure 3, NAG, the fractional excretion of sodium, and the urinary excretion of sodium were markedly elevated by DOCA-salt treatment in both homozygous and wild-type rats, and the level of each parameter was significantly higher in homozygous rats compared with wild-type rats. ABT-627 administration suppressed the DOCA-salt–induced changes.

Histological Findings in Kidney
Wild-type DOCA-salt rats revealed relatively mild damage characterized by tubular dilatation and atrophy, proteinaceous casts in tubuli, fibrinoid-like necrosis in glomeruli, interstitial cell infiltration, and thickening of small arteries. These lesions were significantly reduced by ABT-627 treatment.

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**Figure 5.** Representative light micrographs showing cross sections of thoracic aortas obtained from homozygous (homo) UN control and homozygous DOCA-salt hypertensive rats treated with vehicle or ABT-627.
More severe histopathologic changes were observed in homozygous DOCA-salt rats. ABT-627 treatment markedly and significantly improved these changes (Table 2). Typical photographs of different experimental groups are shown in Figure 4.

**Morphological Analysis of Aorta**

Figures 5 and 6 show typical examples of representative cross sections of the aorta obtained from one each of UN control and DOCA-salt (with or without ABT-627) rats in both the homozygous (Figure 5) and wild-type (Figure 6) groups. Increase in vascular medial thickness (wall thickness), a characteristic finding for hypertensive arterial hypertrophy, was evident in vehicle-treated DOCA-salt rats from both homozygous and wild-type groups. Treatment with ABT-627 suppressed this vascular change induced by DOCA-salt. There were significant increases in wall thickness, wall area, and the wall-to-lumen ratio in vehicle-treated DOCA-salt rats compared with UN control rats, and the effects were more marked in homozygous than in wild-type rats. Wall thickness and the wall-to-lumen ratio were significantly greater in DOCA-salt–treated homozygous rats than in wild-type rats. In both groups, ABT-627 markedly decreased these parameters, and the observed values did not significantly differ from those for UN control rats (Table 3).

**Renal, Urinary, Aortic, and Plasma ET-1 Contents**

When ET-1 contents in renal tissues of UN control rats were determined, there were significant increases in homozygous compared with wild-type rats (0.402 ± 0.033 versus 0.263 ± 0.030 ng/g tissue, respectively; P < 0.01). DOCA-salt treatment markedly increased the renal ET-1 content in homozygous rats, and this increase was abolished by ABT-627 administration. No significant changes occurred in the renal ET-1 content in wild-type rats. In contrast, DOCA-salt treatment significantly increased aortic ET-1 content in both homozygous and wild-type rats, although the extent in wild-type rats was small (from 1.090 ± 0.501 to 6.270 ± 1.310 ng/g tissue in homozygous rats, P < 0.01; from 0.484 ± 0.108 to 1.070 ± 0.098 ng/g tissue in wild-type rats, P < 0.01). These increases were abolished by ABT-627 administration. There were no differences in plasma ET-1 levels in homozygous

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UN Control (n=11)</th>
<th>DOCA-Salt (n=9)</th>
<th>DOCA-Salt+ABT-627 (n=8)</th>
<th>UN Control (n=8)</th>
<th>DOCA-Salt (n=9)</th>
<th>DOCA-Salt+ABT-627 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall thickness, μm</td>
<td>127±4</td>
<td>174±4†</td>
<td>139±9‡</td>
<td>123±2</td>
<td>149±5§</td>
<td>127±3†</td>
</tr>
<tr>
<td>Wall area, mm²</td>
<td>0.677±0.031</td>
<td>0.921±0.025*</td>
<td>0.708±0.045‡</td>
<td>0.676±0.015</td>
<td>0.853±0.033§</td>
<td>0.712±0.024†</td>
</tr>
<tr>
<td>Wall-to-lumen ratio</td>
<td>0.361±0.008</td>
<td>0.530±0.017†</td>
<td>0.379±0.020§</td>
<td>0.324±0.004</td>
<td>0.401±0.016§</td>
<td>0.324±0.006†</td>
</tr>
</tbody>
</table>

Values are mean±SE.

*P < 0.01 vs homozygous UN group; †P < 0.01 vs wild-type DOCA-salt group; ‡P < 0.01 vs homozygous DOCA-salt group; and §P < 0.01 vs wild-type UN group.
and wild-type UN control rats. In homozygous rats, DOCA-salt treatment did not affect plasma ET-1 levels, but there were notable increases with ABT-627 administration. In the wild-type rats, slight but significant increases in plasma ET-1 level were observed. Urinary ET-1 content was significantly increased by treatment with DOCA-salt in both homozygous and wild-type rats, although observed values were higher in homozygous rats. ABT-627 efficiently suppressed these increases in both groups (Figure 7).

Discussion

We investigated the pathological role of the ETB receptor in DOCA-salt–induced hypertension and related tissue injuries with the use of rescued ETB-deficient rats.12 These rats express ETB receptors in the adrenal glands and other adrenergic neurons, but they are ETB deficient in other tissues, but most important is the deficiency in the kidney, vascular endothelium, and vascular smooth muscle.13 In addition, we preliminarily confirmed that ETB receptor–mediated responses, endothelium-dependent vasorelaxation, endothelium-independent vasoconstriction, and diuretic action were not observed in these rats. Thus, rescued ETB-deficient rats are a useful tool in evaluating the pathophysiological roles of ETB receptors, particularly in renal and/or cardiovascular diseases. In the present study, these rats clearly exhibited an exaggerated blood pressure sensitivity to DOCA-salt treatment compared with the sensitivity in wild-type rats, as noticed in our preliminary report,17 in which homozygous rats treated with DOCA-salt exhibited an earlier onset of hypertension than did heterozygous and wild-type rats. In addition, the ETB-deficient rats had enhanced cardiovascular hypertrophy and worsening of renal dysfunction and tissue damage after the DOCA-salt treatment. These findings lead us to propose that ETB receptor–mediated actions are protective in the pathogenesis of DOCA-salt–induced hypertension.

Long-term treatment with selective ETA receptor antagonists or nonselective ET(A)/ET(B) receptor antagonists prevents DOCA-salt–induced hypertension and related tissue injuries, such as vascular hypertrophy, in a qualitatively similar fashion.4,6,7,9,18 Chronic treatment with selective ETA receptor antagonists attenuates the development of hypertension and renal injury in Dahl salt-sensitive rats19 and in salt-loaded stroke-prone spontaneously hypertensive rats.20 Thus, although it is unknown which type of antagonist is favorable for the treatment of these hypertensive models, there is general agreement that ETA receptor–mediated action plays an important role in the development of salt-dependent hypertension and associated tissue injury.8 We have recently found that chronic treatment of DOCA-salt rats with A-192621, an orally active and highly potent ETB-selective receptor antagonist,16 leads to an exaggerated deterioration of cardiovascular and renal injuries,7 thereby suggesting that the blockade of this receptor subtype is harmful in such pathological conditions. In addition, the hypertensive effect induced by an intravenous bolus injection of Ro 46-8443, a selective ETB receptor antagonist, in DOCA-salt rats was greater than that in UN control rats.21 Renal vasoconstrictor effects induced by BQ-788 were also enhanced in DOCA-salt hypertensive rats.22 These findings suggest that the lack of ETB-mediated systemic and renal vasodilative activities is at least partly responsible for the deterioration of DOCA-salt–induced hypertensive diseases observed in ETB-deficient rats.

Exaggerated blood pressure sensitivity and enhanced cardiovascular and renal injuries observed in ETB-deficient homozygous rats were markedly suppressed by the daily administration of ABT-627, a potent ETB-selective receptor antagonist.16 Previous studies, including ours, have demonstrated that ET-1 content and ET-1 mRNA expression were increased by treatment with DOCA-salt in both homozygous and wild-type [wild], n=8) and DOCA-salt hypertensive rats treated with vehicle (homo, n=8; wild, n=9) or ABT-627 (homo, n=8; wild, n=8). Values are mean±SEM. **P<0.01 vs homo UN control rats; §§P<0.01 vs homo DOCA-salt rats; ##P<0.05 and ###P<0.01 vs wild UN control rats; and †P<0.05 and ‡P<0.01 vs wild DOCA-salt rats.
is essential for the protection from DOCA-salt–induced hypertensive injuries, irrespective of the presence of the ET\textsubscript{A} receptor. This view might explain the findings that selective ET\textsubscript{A} receptor antagonists and nonselective ET\textsubscript{A}/ET\textsubscript{B} receptor antagonists similarly improve DOCA-salt–induced hypertension and related tissue injuries.\textsuperscript{4,6,7,9,18} Further studies on tissue distribution, localization, density, and affinity of ET\textsubscript{A} receptors in the ET\textsubscript{B}–deficient rats are required to clarify the mechanisms of increased susceptibility to ET\textsubscript{A}–mediated ET-1 action in these animals.

In contrast to renal ET-1 content in homozygous rats, renal ET-1 content in wild-type rats was not increased by DOCA-salt treatment. We previously noted that ET-1 mRNA expression but not ET-1 content increased in the kidney of DOCA-salt hypertensive rats.\textsuperscript{5,26} One possible explanation for the discrepancy between gene and peptide expression is that the renal tissues are abundant in ET-1–degrading enzymes, such as neutral endopeptidase.\textsuperscript{27,28} On the basis of the finding that the urinary ET-1 level, which is known to reflect the renal ET-1 expression,\textsuperscript{29} was significantly increased by the DOCA-salt treatment, it seems likely that ET-1 production in the kidney is upregulated in DOCA-salt–treated wild-type rats. In homozygous rats, the augmentation of renal ET-1 production may overcome the capacity for enzymatic degradation, leading to higher tissue content. This view may be applicable to aortic ET-1 content.

DOCA-salt–induced cardiovascular hypertrophy and renal injury were markedly augmented in ET\textsubscript{B}–deficient homozygous rats compared with wild-type rats. It is unclear whether the augmentation results from exaggerated blood pressure sensitivity to DOCA-salt treatment. In our recent study,\textsuperscript{7} daily administration of A-192621 caused greater deterioration of the DOCA-salt–induced cardiovascular and renal injuries, with no changes in SBP. Thus, the above augmentation seems to be independent of blood pressure. Elevated blood pressure itself has been known to play a major role in cardiovascular hypertrophy and remodeling. However, there is growing evidence that the cardiovascular hypertrophy and remodeling are not simply a response to elevated blood pressure and that various vasoactive substances, such as angiotensin II, are implicated in the development of these structural changes.\textsuperscript{30} ET-1 has potent mitogenic and hypertrophic properties, mainly via the stimulation of ET\textsubscript{A} receptors.\textsuperscript{31} On the other hand, ET\textsubscript{B} receptor–mediated action may protect against cardiovascular hypertrophy via endothelial NO generation, which inhibits mitogenesis and the proliferation of vascular smooth muscle cells.\textsuperscript{32} It is reasonable to consider that the augmentation of cardiovascular hypertrophy in ET\textsubscript{B}–deficient homozygous rats is attributable to both an increase in ET\textsubscript{A}–mediated hypertrophic activity and a lack of ET\textsubscript{B}–mediated antihypertrophic activity.

We recently noted that chronic treatment with A-192621 to DOCA-salt rats failed to further increase the blood pressure.\textsuperscript{7} This is in conflict with the present finding but may be explained by an increased ET\textsubscript{B}–mediated ET-1 action in ET\textsubscript{B}–deficient homozygous rats. In addition, the exaggerated blood pressure sensitivity in homozygous rats was observed at an early phase of DOCA-salt treatment (1 to 2 weeks). In our previous study,\textsuperscript{7} A-192621 was administered 2 weeks after the start of DOCA-salt treatment. Thus, ET\textsubscript{B}–mediated protection from DOCA-salt–induced hypertension may be most important at an early phase.

Blood pressure assessment of homozygous rats before DOCA-salt treatment revealed a higher level than that seen in wild-type rats. The lack of ET\textsubscript{B}–mediated ET-1 activity (eg, vasodilatory mechanisms) and/or an increase in ET\textsubscript{A}–mediated action may be involved in this phenomenon. Another possible explanation is that elevated blood pressure may be related to the enhanced salt sensitivity of these animals.\textsuperscript{13} Several studies indicate that ET\textsubscript{B} receptor stimulation inhibits sodium reabsorption at the tubular level,\textsuperscript{22,23} suggesting a role of ET\textsubscript{B} receptors in natriuretic mechanisms. It is possible that an ET\textsubscript{B}–deficient condition leads to the enhancement of tubular sodium reabsorption, which is known to be a causal factor of salt-sensitive hypertension.\textsuperscript{34}

We conclude that ET\textsubscript{B} receptor–mediated actions are protective in the pathogenesis of DOCA-salt–induced hypertension. Enhancements of ET-1 production and ET\textsubscript{A}–mediated actions are responsible for the increased susceptibility to DOCA-salt–induced hypertension and tissue injuries in ET\textsubscript{B}–deficient rats.

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References


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