Endothelin ET\textsubscript{B} Receptor-Mediated Action on Systemic and Renal Hemodynamics and Urine Formation in Deoxycorticosterone Acetate-Salt-Induced Hypertensive Rats

Norio HASHIMOTO, Toshihiko KURO, Katsuya FUJITA, Satoshi AZUMA, and Yasuo MATSUMURA*  

Department of Pharmacology, Osaka University of Pharmaceutical Sciences, 4–20–1 Nasahara, Takatsuki, Osaka 569–1094, Japan. Received March 19, 1998; Accepted May 1, 1998

The pathophysiological role of endothelin ET\textsubscript{B} receptor-mediated action on systemic and renal hemodynamics and urine formation in deoxycorticosterone acetate (DOCA)-salt hypertensive rats was investigated. An intravenous bolus injection of a selective ET\textsubscript{B} receptor antagonist, BQ788 (1 mg/kg), produced a significant increase in mean arterial pressure (MAP) of DOCA-salt treated rats, whereas the agent-induced increase in MAP was less marked in normotensive sham rats. Administration of BQ788 caused a significant and sustained reduction in renal blood flow both in DOCA-salt and sham rats. No marked effects were observed on urine formation in both groups. Alternatively, a selective ET\textsubscript{A} receptor antagonist, FR139317 (10 mg/kg), produced a potent hypotensive effect, accompanied by significant renal vasodilation in DOCA-salt hypertensive rats, but these effects were partially reversed by the subsequent administration of BQ788. When renal perfusion pressure was protected from FR139317-induced hypotension by an aortic clamp, significant diuresis and natriuresis were observed, events partially reversed by the subsequent administration of BQ788. Our results indicate that the ET\textsubscript{B} receptor-mediated action tonically functions as a hypotensive and a renal vasodilatory factor and that these effects seem to be up-regulated in DOCA-salt hypertension. We also suggest that the ET\textsubscript{A} receptor blockade in DOCA-salt hypertensive rats unmasks the ET\textsubscript{B} receptor-mediated action which partially contributes to the antihypertensive effect induced by FR139317.

Key words: endothelin-1; endothelin receptor; ET\textsubscript{A} antagonist; ET\textsubscript{B} antagonist; deoxycorticosterone acetate-salt hypertension

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide isolated from porcine vascular endothelial cells.\textsuperscript{1,2} Physiological and pathophysiological responses to this peptide in various tissues are mediated by interaction with two types of receptors, the ET\textsubscript{A} and ET\textsubscript{B} receptor subtypes. Both receptor subtypes on smooth muscle cells mediate vasoconstriction, whereas the ET\textsubscript{B} receptor subtype on endothelial cells mediates vasodilation, possibly through the release of endothelium-derived relaxing factor.\textsuperscript{2} ET-1 may contribute to the maintenance or regulation of blood pressure through its potent vasoconstrictive effect.\textsuperscript{3} However, the pathophysiological role of this peptide in the development and/or maintenance of hypertension has not been fully elucidated.

There is accumulating evidence indicating that ET-1 is involved in the development and/or maintenance of deoxycorticosterone acetate (DOCA)-salt treated hypertension in rats.\textsuperscript{4,5} For example, immunoreactive ET-1 levels in aorta and mesenteric arteries\textsuperscript{6} and prepro ET-1 gene expression in these vessels\textsuperscript{7} were elevated in DOCA-salt hypertensive rats. We also noted that the ET-1 mRNA level in the kidney was elevated in this model of hypertension.\textsuperscript{8} In addition, Li et al.\textsuperscript{9} reported a significant smaller increase in blood pressure in DOCA-salt rats treated with a nonselective ET receptor antagonist than in vehicle-treated rats. More recently, we and others have demonstrated that, using this model, acute administration of a selective ET\textsubscript{A} receptor antagonist produces a potent hypotensive effect.\textsuperscript{10} We also noted that long-term treatment with this drug suppresses the development of hypertension and subsequent cardiovascular hypertrophy.\textsuperscript{11} Taken together, it seems likely that ET-1 could contribute to the development and maintenance of this type of hypertension, mainly via ET\textsubscript{A} receptors. However, there is little information about the possible involvement of ET\textsubscript{B} Receptor-mediated events in the pathogenesis of this model. We examined the effects of an ETB receptor antagonist on blood pressure, renal hemodynamics and urine formation in DOCA-salt hypertensive rats. In addition, we investigated whether the blockade of ET\textsubscript{A} receptors would unmask ET\textsubscript{B} receptor-mediated action in this model of hypertension.

MATERIALS AND METHODS

Animal Preparation Male Sprague-Dawley rats obtained from Japan SLC (Hamamatsu) and weighing 160–180 g were anesthetized with sodium pentobarbitral (50 mg/kg, i.p.) and a right kidney was removed via a right flank incision. After a 1-week postsurgical recovery period, the rats were treated twice weekly with DOCA suspended in corn oil, which was administered subcutaneously (15 mg/kg), and 1% NaCl added to the drinking water. Sham-operated rats (normotensive control) were uninephrectomized but not given DOCA or salt. Systolic blood pressure was monitored with a tail cuff and pneumatic pulse transducer. After 4 weeks of the above treatment, the rats with a systolic blood pressure over 180 mmHg were used for clearance studies.

Renal Clearance Study The rats were anesthetized with sodium thiobutabarbital (Inactin, 100 mg/kg) and placed on a surgical tray that maintained rectal temperature between 37°C and 38°C. After tracheotomy, the right femoral vein was cannulated for infusion of 0.9% saline containing 0.5% inulin (6 ml/h). The right and left femoral arteries were also cannulated to measure mean arterial pressure (MAP) and for blood sampling, respectively. After making an abdominal midline incision, the left kidney was exposed, and the renal artery was carefully stripped of connective tissue, followed by the application of 5% phenol in 70% ethanol to eliminate the influence of renal sympathetic nerves on renal function. An electromagnetic flow probe (1.0 mm in diameter, Nihon
Kohden, Tokyo, Japan) connected to a square-wave flowmeter (MFV-2100, Nihon Kohden) was positioned on the renal artery to measure renal blood flow (RBF). A polyethylene cannula was inserted into the ureter for urine collection. In one part of this study, a Blalock clamp was placed around the aorta, just below the origin of the left renal artery, to control renal perfusion pressure. In this case, MAP above the clamp was measured from a catheter inserted into the right carotid artery, and this served as an index of left renal perfusion pressure. MAP and RBF were continuously recorded on a polygraph (RM 6000, Nihon Kohden), throughout all the experiments. A 60–90 min period was allowed for stabilization of MAP, RBF and urine flow (UF).

**Experimental Protocol** In the first part of our experiments, we examined the effect of a bolus injection of BQ788 in seven DOCA-salt hypertensive rats and in five normotensive control rats. After an equilibration period, urine samples were collected during two 15 min control clearance periods. Following the control periods, BQ788 (1 mg/kg) was administered intravenously by slow bolus injection. This dose of BQ788 has been reported to abolish the depressor response to ET-1. During the first 5 min after injection, urine was not collected in order to take into account the "dead space" in the collection system. Following this, urine samples were collected during four consecutive 15 min periods (E1—E4). Blood samples (0.2 ml each) were obtained at 15 min before drug injection and at 20 min and 50 min after injection. The blood loss was replaced by injecting an equal volume of blood from donor rats and plasma was immediately separated by centrifugation. In the time-control study, vehicle was administered, and hemodynamic and excretory parameters were constant throughout all the periods. In the second part, we examined the effect of BQ788 on hemodynamic and excretory responses to the blockade of ET-A receptors. Following the two 15 min control periods, FR139317 (10 mg/kg), a specific ET-A receptor antagonist, was administered intravenously and urine samples were collected during the two 15 min clearance periods (E1 and E2). The hypotensive effect induced by this dose of FR139317 continued for at least 90 min. After the E2 period, BQ788 (1 mg/kg) or vehicle was injected intravenously and urine samples were collected during two 15 min periods (E3 and E4). During the first 5 min after each drug injection, urine was not collected in order to take account of the "dead space" of the urine collection system. In some rats, renal perfusion pressure was protected from FR139317-induced hypotension using an aortic clamp.

**Analytical Procedure** Urine and plasma inulin levels were measured using spectrophotometry (Hitachi 650-50, Tokyo, Japan), as described. Glomerular filtration rate (GFR) was calculated from the inulin clearance. Urine and plasma sodium concentrations were determined using a flame photometer (Hitachi 206D). Urine osmolality (Uosm) was measured by freezing-point depression with an auto-osmometer (Fiske, MA, U.S.A.).

**Drugs** FR139317 and BQ788 were kind gifts from Fujiwara Pharmaceutical Co., Ltd., Osaka, Japan and Banyu Pharmaceutical Company, Ltd., Tokyo, Japan, respectively. FR139317 was dissolved in 1 M NaOH and diluted with saline. BQ788 was dissolved in 10% nonionic polyoxymethylene surfactant (HCO-60, Nikko Chemicals Co., Ltd., Osaka, Japan) in saline. Other chemicals were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

**Statistical Analysis** All values were expressed as means±S.E.M. For comparisons between DOCA-salt hypertensive rats and normotensive control rats, the unpaired Student's t-test was used. Effects of drugs were analyzed by repeated measures one-way analysis of variance combined with Dunnett's multiple range test. Differences were considered significant at p<0.05.

**RESULTS**

The basal MAP of anesthetized DOCA-salt rats was significantly elevated compared with that of control rats (152±4 mmHg, n=26, vs. 103±5 mmHg, n=5; p<0.001). In DOCA-salt rats, there were marked reductions in RBF (2.89±0.22 ml/g per min vs. 6.26±1.04 ml/g per min; p<0.001) and GFR (0.53±0.06 ml/g per min vs. 1.09±0.09 ml/g per min; p<0.001), whereas renal vascular resistance (RVR) was markedly increased (61.33±6.44 mmHg/ml/g per min vs. 20.17±3.89 mmHg/ml/g per min; p<0.001). Basal levels of UF and urinary sodium excretion (Uosm,V) were also elevated in DOCA-salt treated rats (UF; 11.95±0.91 µl/g per min vs. 8.44±2.11 µl/g per min; p<0.05 Uosm,V; 2.13±0.24 µEq/g per min vs. 1.18±0.34 µEq/g per min; p<0.05).

Figure 1 shows changes in systemic and renal hemodynamics, and urine formation after intravenous injection of BQ788 to normotensive control rats. BQ788 produced a significant reduction in RBF. A maximum change was observed

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Fig. 1. Hemodynamic and Excretory Responses to Intravenous Injection of BQ788 (1 mg/kg) in Normotensive Control Rats

Values are means±S.E.M. for five rats. MAP: mean arterial pressure, RBF: renal blood flow, RVR: renal vascular resistance, GFR: glomerular filtration rate, UF: urine flow, Uosm,V: urinary excretion of sodium, FElo: fractional excretion of sodium. Statistically significant change from control period (+p<0.05, ++p<0.01).
during the E3 period (from a control value of 6.26±1.04 ml/g per min to 5.56±1.03 ml/g per min) and significant reductions were sustained throughout the experimental periods. Simultaneously, slight but significant (during E2, E3 periods) increases in RVR were observed after drug injection. There were no significant alterations in MAP, GFR and excretory parameters after injection of BQ788 to control rats, although MAP did tend to increase following drug injection.

Figure 2 illustrates changes in systemic and renal hemodynamics, and urine formation after intravenous injection of BQ788 to DOCA-salt hypertensive rats. Following drug injection, significant increases in MAP and reductions in RBF were observed. A maximum increase in MAP (from a control value of 153±4 mmHg to 166±4 mmHg) was obtained during the E1 period and, thereafter, this effect diminished gradually to the baseline level. The pattern of changes in RBF was similar to that seen in control rats; RVR also significantly increased. There were no changes in GFR and urine formation after administration of this drug to DOCA-salt hypertensive rats.

Table 1 summarizes changes in systemic and renal hemodynamics after injection of BQ788 or vehicle following treatment with FR139317 in DOCA-salt hypertensive rats. FR139317 produced significant reductions in MAP and RVR and an increase in RBF, as reported. The injection of BQ788 produced a qualitatively similar effect to that seen following a single injection of BQ788 (increases in MAP and RVR, and a reduction in RBF). In BQ788-treated rats, no significant change in RBF was observed, unlike that seen following administration of FR139317 alone.

Finally, we examined the effect of BQ788 following treatment with FR139317 in DOCA-salt rats, when renal perfusion pressure was protected from FR139317-induced hypotension using an aortic clamp. As shown in Table 2, administration of FR139317 to DOCA-salt rats, under conditions such that the renal perfusion pressure remains constant, produced a sustained (>1 h) renal vasodilation, diuresis and natriuresis, but these responses were attenuated by injection of BQ788 (Table 3). During E4, there were no significant in-

Table 1. Systemic and Renal Hemodynamic Responses to FR139317 Administration, Followed by BQ788 Injection in DOCA-salt Hypertensive Rats

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MAP (mmHg)</th>
<th>RBF (ml/g·min)</th>
<th>RVR (mmHg/ml per g·min)</th>
<th>MAP (mmHg)</th>
<th>RBF (ml/g·min)</th>
<th>RVR (mmHg/ml per g·min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−30−0 (control)</td>
<td>152±9</td>
<td>2.84±0.35</td>
<td>62.8±13.0</td>
<td>156±6</td>
<td>2.71±0.32</td>
<td>68.2±13.9</td>
</tr>
<tr>
<td>5−20 (E1)</td>
<td>131±11***</td>
<td>3.15±0.34</td>
<td>47.4±10.8*</td>
<td>129±5**</td>
<td>2.86±0.33</td>
<td>52.6±9.9**</td>
</tr>
<tr>
<td>20−35 (E2)</td>
<td>115±7**</td>
<td>3.30±0.27</td>
<td>39.6±9.1**</td>
<td>119±5**</td>
<td>2.96±0.38</td>
<td>49.1±10.8**</td>
</tr>
<tr>
<td>40−55 (E3)</td>
<td>123±7**</td>
<td>3.11±0.32</td>
<td>45.5±9.8**</td>
<td>118±5**</td>
<td>3.00±0.38*</td>
<td>48.7±10.9**</td>
</tr>
<tr>
<td>55−70 (E4)</td>
<td>123±7**</td>
<td>3.02±0.32</td>
<td>46.5±10.9*</td>
<td>118±3**</td>
<td>3.12±0.38**</td>
<td>47.6±11.9**</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. of six (BQ788) and eight (vehicle) rats. *p<0.05, **p<0.01, compared with each control value (Dunnett's test). MAP, mean arterial pressure; RBF, renal blood flow; RVR, renal vascular resistance.

Table 2. Hemodynamic and Excretory Responses to FR139317 Administration in DOCA-salt Hypertensive Rats, with Constant Renal Perfusion Pressure

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MAP (RPP) (mmHg)</th>
<th>HR (beats/min)</th>
<th>RBF (ml/min/g)</th>
<th>RVR (mmHg/ml per g·min)</th>
<th>GFR (ml/min)</th>
<th>UF (µl/g·min)</th>
<th>U_{Na}V (µEq/g·min)</th>
<th>FEPa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>−15−0 (control)</td>
<td>150±4</td>
<td>322±14</td>
<td>2.14±0.15</td>
<td>71.8±4.7</td>
<td>0.76±0.10</td>
<td>9.87±1.37</td>
<td>1.92±0.17</td>
<td>1.54±0.17</td>
</tr>
<tr>
<td>5−20 (E1)</td>
<td>149±4</td>
<td>326±12</td>
<td>2.35±0.19*</td>
<td>65.6±4.8*</td>
<td>0.76±0.09</td>
<td>17.27±3.10**</td>
<td>3.02±0.26**</td>
<td>2.45±0.28**</td>
</tr>
<tr>
<td>20−35 (E2)</td>
<td>144±4</td>
<td>330±10</td>
<td>2.50±0.18**</td>
<td>59.6±4.0**</td>
<td>0.82±0.12</td>
<td>16.95±2.89**</td>
<td>3.12±0.24**</td>
<td>2.42±0.33**</td>
</tr>
<tr>
<td>40−55 (E3)</td>
<td>145±4</td>
<td>336±11</td>
<td>2.57±0.19**</td>
<td>56.9±3.6**</td>
<td>0.77±0.10</td>
<td>18.38±2.74**</td>
<td>3.10±0.31**</td>
<td>2.39±0.26**</td>
</tr>
<tr>
<td>55−70 (E4)</td>
<td>144±4</td>
<td>336±12</td>
<td>2.53±0.17**</td>
<td>56.2±3.5**</td>
<td>0.78±0.10</td>
<td>17.88±2.63**</td>
<td>2.93±0.19**</td>
<td>2.32±0.20**</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M. of seven rats. *p<0.05, **p<0.01, compared with each control value. MAP, mean arterial pressure; RPP, renal perfusion pressure; HR, heart rate; RBF, renal blood flow; RVR, renal vascular resistance; GFR, glomerular filtration rate; UF, urine flow; U_{Na}V, urinary excretion of sodium; FEPa, fractional excretion of sodium.
creases in UF and UNaV and a reduction in RVR.

**DISCUSSION**

Previous studies in our laboratory and by others using a selective ET_A receptor antagonist clearly demonstrated that ET-1 and ET_A receptors play an important role in development and maintenance of DOCA-salt-induced hypertension in rats.\(^8,9,10,11\) However, the functional role of ET_B receptors in this model is not fully understood. ET_B receptors on endothelial cells are functionally linked to vasodilation\(^12,13\) possibly through the release of endothelium-derived relaxing factor/nitric oxide (NO). On the other hand, it has been reported that ET_B receptors on smooth muscle cells are involved in vasoconstriction.\(^15,16\) In the kidney, ET_B receptor-mediated vasoconstrictor and vasodilator responses have been observed.\(^17-19\) Furthermore, several reports have indicated functional roles for ET_B receptors in the renal tubular reabsorption of sodium and water.\(^20-22\) The present study was designed to evaluate the possible involvement of ET_B receptors in hemodynamic and excretory abnormalities of DOCA-salt-induced hypertensive rats. In addition, we investigated whether ET_A receptor blockade would unmask ET_B receptor-mediated events in this model of hypertension.

An intravenous injection of a selective ET_B receptor antagonist, BQ788, produced a significant increase in the MAP of DOCA-salt hypertensive rats, whereas the agent-induced increase in MAP was less potent in normotensive control rats. A sustained reduction in RBF induced by BQ788 was observed in both DOCA-salt and control rats. Our results also demonstrated that a selective ET_A receptor antagonist, FR139317, produced a potent hypotensive effect accompanied by a significant renal vasodilation in DOCA-salt hypertensive rats, as reported.\(^9\) These effects were partially reversed by the subsequent administration of BQ788. FR139317-induced diuresis and natriuresis, observed under conditions where renal perfusion pressure was constant,\(^22\) were also attenuated by the subsequent injection of BQ788. Thus, it seems likely that ET_B receptor-mediated events tonically function as hypotensive and renal vasodilatory factors and that these effects are up-regulated in DOCA-salt hypertensive rats. Furthermore, our results indicate that FR139317-induced actions on excretory responses in DOCA-salt rats are partially due to the unmasking effects of ET_B receptor-mediated events.

The BQ788-induced systemic and renal vasoconstriction observed in DOCA-salt hypertensive rats was more potent than that seen in normotensive control rats. This observation corresponds to reported results,\(^23\) showing that Ro 46-8443, an ET_B receptor antagonist, produced a marked increase in blood pressure in DOCA-salt and spontaneously hypertensive rats (SHR), compared with normotensive rats. In addition, Clozel and Breu\(^24\) reported that the pressor effect of Ro 46-8443 in SHR was abolished by pretreatment with a NO synthase inhibitor. Taken together, ET_B receptor-mediated hypotensive activities, probably via the production of NO, appear to be up-regulated to protect against various vasoconstrictive stimuli, including ET-1, in the hypertensive state. This hypothesis is supported by a report\(^25\) suggesting that the endothelium compensates for the increased contractile response of vascular smooth muscle in DOCA-salt rats by increasing NO production. However, Gellai et al.\(^26\) stated that vasoconstrictive effects induced by ET_B receptor blockade could be at least partially due to activation of vasoconstrictive ET receptors. Further studies are required to clarify the precise mechanisms of the pressor effects induced by ET_B receptor blockade.

Of particular interest in the present study is the effect of ET receptor antagonists on urine formation. The administration of FR139317 to DOCA-salt hypertensive rats, where renal perfusion pressure was protected from agent-induced hypertension, produced marked diuresis and natriuresis. This suggests that ET-1 actions via the ET_A receptor are responsible for water and sodium retention in this type of hypertension. Several investigators have noted that an intravenous injection of ET-1 produced significant reductions in UF and UNaV.\(^27-28\) We found that the FR139317-induced diuretic and natriuretic effects were partly attenuated by ET_B receptor blockade with BQ788, thereby suggesting that the administration of FR139317 unmasks the ET_B receptor-related diuretic and natriuretic responses in DOCA-salt hypertensive rats. In contrast to DOCA-salt hypertensive rats, FR139317 or BQ788 produced no significant effect on urine formation in normotensive control rats, thereby indicating that endogenous ET-1 does not play a role in regulating water and sodium excretion under normal conditions.

It has been reported that ET-1 inhibits arginine vasopressin (AVP)-induced water reabsorption in the collecting duct of the rat.\(^29\) This effect is probably due to the ET_B receptor-mediated inhibitory action on AVP-stimulated cyclic-AMP accumulation in the collecting duct.\(^29\) The intrarenal administration of sarafotoxin S6c (S6c), a selective ET_B receptor ag-
onist, resulted in a diuretic response with no change in sodium excretion. In contrast, other investigators noted that the infusion of S6c into the renal artery increased both UF and fractional excretion of sodium with little effect on renal hemodynamics, thereby suggesting that ETB receptor stimulation inhibits sodium reabsorption at the tubular level. Taken together, it seems likely that stimulation of ETB produces an increasing effect on water excretion by inhibiting AVP-mediated water reabsorption. Whether the ETB receptor modulates sodium excretion remains uncertain.

In conclusion, the prevailing response to endogenous ET-1 via the ETB receptor is vasodilation in both DOCA-salt hypertensive and normotensive rats. This effect may be up-regulated to protect against ET and/or other factor-mediated vasoconstriction in DOCA-salt-induced hypertension. Our results also showed that selective ETA receptor antagonist-induced antihypertensive effects are partially due to unmasked ETB receptor activation. Therefore, we suggest that the treatment with a selective ETA receptor antagonist is likely to be more beneficial for subjects with mineralocorticoid-dependent hypertension, than using a nonselective ETB antagonist.

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