Effect of a Nonselective Endothelin Antagonist on Vascular Remodeling in Deoxycorticosterone Acetate–Salt Hypertensive Rats

Evidence for a Role of Endothelin in Vascular Hypertrophy

Jin S. Li, Richard Larivièrè, Ernesto L. Schiffrin

Abstract We have previously shown that the endothelin content in arteries of deoxycorticosterone acetate (DOCA)–salt hypertensive rats is increased. We designed this study to examine, using the new orally active nonselective endothelin receptor antagonist bosentan, whether this increase in vascular endothelin may contribute to elevated blood pressure and vascular hypertrophy in DOCA-salt hypertensive rats. Rats received bosentan (100 mg/kg body wt per day) for 3 weeks mixed with their food. Systolic blood pressure of DOCA-salt hypertensive rats rose to 197±5 mm Hg, and that of bosentan-treated DOCA-salt hypertensive rats was 177±4 mm Hg (P<.01). Mesenteric resistance arteries were studied on a wire myograph. The media width, ratio of media width to lumen diameter, and cross-sectional area of the media of resistance arteries of bosentan-treated DOCA-salt hypertensive rats were significantly smaller than those of untreated DOCA-salt hypertensive rats. The lumen diameter and cross-sectional area of the media of vessels of bosentan-treated rats were not different from those of uninephrectomized control rats. Vasoconstrictor responses, which were altered in DOCA-salt hypertensive rats, approached control in the bosentan-treated rats. We conclude that these results with a nonselective endothelin receptor antagonist may suggest a role for endothelin in the elevation of blood pressure and vascular hypertrophy and remodeling in DOCA-salt hypertensive rats. (Hypertension. 1994;24:183-188.)

Key Words • receptors, endothelin • resistance arteries • hypertrophy • endothelium • blood pressure • hypertension, mineralocorticoid

Endothelins comprise a family of potent vasoconstrictor peptides, endowed, as other similar peptides, with mitogenic properties. The role of endothelins in hypertension is unclear. Endothelin-1 (ET-1) is one of the best-studied and possibly one of the more important members of this family of peptides in peripheral tissues. Several studies have demonstrated that the circulating concentrations of ET-1 exhibit little or no increase in most models of hypertension in animals or in human essential hypertension. It has also been shown that the effects of ET-1 on blood vessels in experimental models of hypertension and in essential hypertensive humans are either normal or blunted. Thus, it has been difficult to postulate a role of ET-1 in hypertension.

We have recently demonstrated that in the deoxycorticosterone acetate (DOCA)–salt hypertensive rat the content of immunoreactive ET-1 is increased in aorta and mesenteric arteries despite normal circulating levels. Thus, endothelin secretion may occur abluminally, and exaggerated vascular production may not result in elevated plasma levels. Histochemical staining for endothelin was enhanced in endothelial cells in these vessels. In a subsequent study we found that the abundance of mRNA for ET-1 was significantly increased between three and five times in vessels of DOCA-salt hypertensive rats. In contrast to DOCA-salt hypertensive rats, spontaneously hypertensive rats (SHR) did not exhibit an increased endothelin content in vascular tissues. We were also impressed by the very important vascular hypertrophy present in blood vessels of DOCA-salt hypertensive rats, in contrast to the limited hypertrophy of blood vessels of SHR. We hypothesized that ET-1, which is known to have powerful hypertrophic and mitogenic properties, could play a pathogenic role in this vascular hypertrophy in addition to its vasoconstrictor action and perhaps be involved by these two mechanisms in the elevated blood pressure of DOCA-salt hypertensive rats.

Previous studies with endothelin-A (ETₐ) receptor antagonists such as BQ-123 have shown that these agents produce only a moderate lowering of blood pressure. Moreover, the presence of endothelin-B (ETₐ) receptors in vascular smooth muscle cells has recently been demonstrated. Thus, the moderate effect of ETₐ receptor antagonists may in part be due to the fact that ETₐ receptors are not being blocked. Recently, nonselective endothelin antagonists that block both ETₐ and ETₐ receptors have been developed. We therefore evaluated the effect of the nonselective endothelin antagonist bosentan, a potent, orally active, long-acting agent, on blood pressure and vascular hypertrophy of DOCA-salt hypertensive rats.
Methods

Materials
ET-1 (human, porcine) and arginine-vasopressin (AVP) were from Peninsula Laboratories. DOCA, acetylcholine, and norepinephrine were from Sigma Chemical Co. Bosentan was kindly provided by Dr Jean-Paul Clozel (Hoffmann-LaRoche). All agents were of the highest reagent grade available.

Animal Experiments
Animal experiments were performed following the recommendations of the Canadian Council for Animal Care and were approved by the Animal Care Committee of the Clinical Research Institute. Rats were housed under conditions of constant temperature (22°C) and humidity (60%) and exposed to a 12-hour dark/light cycle. DOCA-salt hypertension was induced by the method of Ormsbee and Ryan.23 Male Sprague-Dawley rats (Charles River Laboratories) were unilaterally nephrectomized under sodium pentobarbital anesthesia. Silicone rubber was impregnated or not with DOCA (200 mg per rat) and implanted in all experimental groups, and rats were offered 1% saline to drink. Systolic blood pressure was taken weekly by the tail-cuff method, after rats were warmed and under slight restraint, and recorded on a model 7 polygraph (Grass Medical Instruments) fitted with a 7-P8 preamplifier and a PCPB photoelectric pulse sensor. The average of three pressure readings was recorded. Starting the day of surgery, rats were offered bosentan (100 mg/kg body wt) mixed with powdered chow. This oral dose has been demonstrated to block the action of pressor doses of intravenously injected big ET-1 for more than 24 hours.22 Rats were studied 3 weeks after DOCA-salt rats became hypertensive (blood pressure higher than 150 mm Hg).

Preparation of Small Arteries
Mesenteric resistance arteries were studied as previously described.11,12 On the day of the experiment, rats were killed by decapitation. One rat was used each time, permitting two vessels of the same rat to be tested simultaneously and results averaged. Superior mesenteric arteries were taken from the part of the mesenteric vascular bed that feeds the jejunum 8 to 10 cm distal to the pylorus. A third-order branch 1 mm from a main stem was dissected from the mesentery and mounted in a 30-ml organ bath containing 10 ml of Krebs solution at 37°C. A 10 cm distal segment of vessel was used for all experiments. After cutting the vessel to the desired length, a 2-mm section was removed so that the vessel could be mounted on a myograph. The device rests on a lever mounted on a vertical arm. The vessel is attached at the bottom to a rigid support that can be adjusted to a straight horizontal position. The other end of the vessel is attached to a small transducer that converts length change to electrical potential, which is displayed on the x-axis of a recorder. Tension is measured by a load cell impregnated with silicon rubber and recorded on the y-axis of the recorder. The myograph is fitted with a 7-P8 preamplifier and a PCPB photoelectric pulse sensor. The average of three pressure readings was recorded. Starting the day of surgery, rats were offered bosentan (100 mg/kg body wt) mixed with powdered chow. This oral dose has been demonstrated to block the action of pressor doses of intravenously injected big ET-1 for more than 24 hours.22 Rats were studied 3 weeks after DOCA-salt rats became hypertensive (blood pressure higher than 150 mm Hg).

Study Protocol for Small Arteries
After mounting, vessels were warmed to 37°C and allowed to equilibrate in PSS for approximately 30 minutes with the vessel internal circumference set to give a wall tension of 0.2 mN/mm². Media width was then measured using a Leitz-Diavert inverted light microscope at ×320 magnification at 12 different sites along the wall, which were then averaged. The vessel was mounted as a ring preparation on an isometric myograph (Living Systems Instrumentation). The dissection and mounting were performed in physiological salt solution (PSS) at room temperature. PSS had the following composition (mmol/L): NaCl 120, NaHCO₃ 25, KCl 4.7, KH₂PO₄ 1.18, MgSO₄ 1.17, CaCl₂ 2.5, EDTA 0.026, and glucose 5.5. K-PSS was PSS in which NaCl was replaced by KCl on an equimolar basis (total KCl concentration, 12.7 mmol/L). All solutions were bubbled with 95% O₂ and 5% CO₂ to give a pH of 7.40 to 7.45. Solutions were maintained at 37°C. After activation with each different agent, the vessels were washed with PSS for 15 minutes. Because of the persistent contraction elicited by ET-1, responses to ET-1 were always obtained at the end of the experiment.

Biochemical Methods
Blood was obtained from the neck during the first few seconds after decapitation, in tubes containing potassium EDTA, for measurement of plasma renin activity and ET-1. Immunoreactive endothelin was extracted from plasma by passage through C18 Sep-Pak cartridges and measured by radioimmunoassay as previously described.7,15,17 Plasma renin activity was measured by radioimmunoassay of angiotensin I generated during a 2-hour incubation of plasma at 37°C and pH 6.5 as described previously.7,15,17

Analysis of Data
Media thickness of blood vessels was obtained from media cross-sectional area assuming a constant media volume.13,14 For these calculations, media thickness was measured in unstretched vessels and lumen diameter in stretched vessels as previously described.13,14 Values are given as mean±SEM. Statistical differences were evaluated by two-tailed Student's t test or ANOVA followed by a Newman-Keuls test. Results were considered significantly different at a value of P<.05.

Results
Blood Pressure, Body and Heart Weights, Plasma ET-1, and Plasma Renin Activity
Systolic blood pressure rose in DOCA-salt hypertensive rats as expected. Rats receiving bosentan exhibited a significantly smaller rise in blood pressure, which nonetheless achieved hypertensive levels (Fig 1, Table 1). The body weight of bosentan-treated rats was smaller than that of DOCA-salt hypertensive rats, and both weighed less than uninephrectomized control rats. Heart weight and heart weight normalized for body weight were greater in DOCA-salt hypertensive rats, untreated or receiving bosentan, relative to normotensive rats (Table 1). Thus, there was no evidence that bosentan prevented left ventricular hypertrophy in treated rats.
Plasma ET-1 was significantly higher in bosentan-treated DOCA-salt rats (Table 1). Plasma renin activity was suppressed in DOCA-salt hypertensive rats as expected and remained equally suppressed in rats receiving bosentan.

**Morphological Characteristics of Resistance Arteries**

Mesenteric resistance arteries of DOCA-salt hypertensive rats exhibited a smaller lumen diameter and greater media width, media cross-sectional area, and media-to-lumen ratio, as expected, compared with normotensive rats (Table 2). Bosentan-treated DOCA-salt hypertensive rats presented a smaller increase in media width and ratio of media width to lumen diameter than untreated DOCA-salt hypertensive rats. The lumen diameter and cross-sectional area of the media of resistance arteries from bosentan-treated DOCA-salt hypertensive rats were similar to those of normotensive control rats.

**Vasoconstrictor Responses of Resistance Arteries**

Active tension and media stress of resistance arteries in response to increasing norepinephrine concentrations were blunted in untreated DOCA-salt hypertensive rats. Although developed tension was similar in bosentan-treated and normotensive rats, media stress in response to maximal norepinephrine concentrations was slightly lower in the former (Fig 2, left, and Table 3). The arteries of DOCA-salt hypertensive rats were less sensitive to norepinephrine, whereas those from bosentan-treated rats were similar to those from control rats (Table 3). In contrast to norepinephrine, tension responses to vasopressin were similar in the three groups (Fig 2, middle). However, because media width was greater in treated and untreated DOCA-salt hypertensive rats, media stress responses to vasopressin were blunted in both groups (Table 3). Responses to 100 nmol/L ET-1, the largest dose used, which usually provides a maximal response (Fig 2, right, and Table 3), were significantly depressed in untreated DOCA-salt hypertensive rats, as we have previously reported, and less blunted in bosentan-treated DOCA-salt hypertensive rats.

**Relaxation Responses of Resistance Arteries to Acetylcholine**

When norepinephrine-contracted resistance arteries were relaxed with increasing concentrations of acetylcholine, relaxation was impaired at the highest concentrations of acetylcholine in vessels from DOCA-salt hypertensive rats, as expected, and in bosentan-treated rats (Fig 3).

**Discussion**

This study shows that oral administration for 3 weeks of a nonselective endothelin antagonist, bosentan, results in a blunting of the rise in blood pressure of DOCA-salt hypertensive rats. This is associated with the absence of the vascular hypertrophy and remodeling, as well as the changes in response to vasoconstric-
tors, usually found in mesenteric resistance arteries in these hypertensive rats.

The role of endothelin in hypertension has not been elucidated. Although in some studies plasma ET-1 has been reported to be elevated in DOCA-salt hypertensive rats, in the present one as in previous reports from our laboratory, we have found similar levels in control and DOCA-salt hypertensive rats. Löffler et al. found that administration of the bosentan analogue Ro 46-2005 produced increases in circulating ET-1. In the present study DOCA-salt hypertensive rats receiving bosentan also had higher levels of plasma immunoreactive ET-1. We have also recently found that bosentan treatment of DOCA-salt hypertensive rats results in an increased expression of ET-1 and abundance of ET-1 mRNA in some vascular tissues (R.L. and E.L.S., unpublished observations, 1994). Löffler et al. suggested that the increase in circulating immunoreactive ET-1 by the bosentan analogue Ro 46-2005 was related to the sequestration of endothelin from endothelial ET$_B$ receptors because it occurred very rapidly. However, ET-1 gene expression also appears to be enhanced by bosentan in some vessels. The mechanism for this response remains to be established. Increased circulating and tissue endothelin levels indicate that bosentan does not exert part of its antagonism of the endothelin system by reducing endothelin production.

Previous results demonstrating enhanced ET-1 gene expression$^{13}$ and endothelin immunoreactivity$^{15}$ in blood vessels of DOCA-salt hypertensive rats suggested that endothelins could be involved in the mechanisms of elevated blood pressure in this hypertensive model. Furthermore, on the basis of the presence of the significant accumulation of immunoreactive endothelin in vessels of DOCA-salt hypertensive rats, which exhibit a very important degree of vascular hypertrophy,$^{13}$ and the absence of enhanced immunoreactive endothelin in vessels of SHR, which exhibit much less vascular hypertrophy,$^{11}$ we proposed that endothelins could play a role in vascular hypertrophy and remodeling in some forms of hypertension.$^{15,17}$ Our present results showing less elevation of blood pressure after administration of bosentan to DOCA-salt hypertensive rats suggest that endothelins may be involved in the development and maintenance of elevated blood pressure in this model of hypertension. It is unclear whether this effect relates to the vasoconstrictor and/or mitogenic and hypertrophic properties of endothelin.$^{3,25,26}$ The development of vascular hypertrophy (increased media cross section) and remodeling (decreased lumen and outer diameters of vessels) of resistance arteries was significantly attenuated in the bosentan-treated rats. This occurred even though blood pressure rose to a mean of 177 mm Hg in these rats, in contrast to a steeper rise in untreated DOCA-salt hypertensive rats to a mean of 197 mm Hg, a significant but not very large difference. Thus, it would appear that the difference in vascular hypertrophy and remodeling between the two groups was excessive for the difference in blood pressure. However, it is not possible in this study to completely dissociate blood pressure lowering and correction of structural damage of blood vessels.

The fact that changes usually found in blood vessel structure in hypertensive rats$^{13}$ were much less evident in the bosentan-treated DOCA-salt hypertensive rats may suggest two conclusions: (1) lack of progression of vascular damage may indeed be related to blockade of the hypertrophic effects of endothelin, and (2) vascular hypertrophy and remodeling of these blood vessels do not play major roles in blood pressure elevation. With

### Table 3. Maximal Media Stress Responses and Sensitivity of Resistance Arteries

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Uni-Nx</th>
<th>DOCA-Salt</th>
<th>DOCA-Salt+Bosentan</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>5</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Media stress, kPa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>259±8.4</td>
<td>142±10.5</td>
<td>201±7.8§</td>
</tr>
<tr>
<td>AVP</td>
<td>282±14.1</td>
<td>217±14.3</td>
<td>233±7.7</td>
</tr>
<tr>
<td>ET-1</td>
<td>291±7.6</td>
<td>146±15.4</td>
<td>199±8.7§</td>
</tr>
<tr>
<td>pD$_2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>5.97±0.03</td>
<td>5.67±0.03</td>
<td>5.92±0.03海底</td>
</tr>
<tr>
<td>AVP</td>
<td>9.09±0.02</td>
<td>9.12±0.04</td>
<td>9.24±0.04海底</td>
</tr>
<tr>
<td>ET-1</td>
<td>7.78±0.10</td>
<td>7.83±0.02</td>
<td>8.08±0.04海底</td>
</tr>
</tbody>
</table>

Uni-Nx indicates uninephrectomized rats; DOCA, deoxycorticosterone acetate; NE, norepinephrine; AVP, arginine vasopres- sin; and ET-1, endothelin-1.

$^*$P<.01 vs DOCA-salt+Bosentan.

$^*$P<.05, $^*$P<.01 vs DOCA-salt.

$^*$P<.05, $^*$P<.01 vs Uni-Nx.
regard to the second conclusion, the present results may be taken to indicate that in fact vascular structural alterations contribute little to a mild elevation of blood pressure but contribute to the more severe elevation occurring in this rat group beyond a systolic blood pressure of 180 mm Hg. Thus, the severity of blood pressure elevation correlates with the presence of important vascular hypertrophy and remodeling, which then contribute to a vicious circle, with hypertrophy and remodeling aggravating blood pressure elevation. It could be speculated that when different mechanisms result in elevated blood pressure beyond certain levels, for example, 170 to 180 mm Hg systolic, ET-1 gene expression is turned on, as occurs in DOCA-salt hypertensive rats. Enhanced endothelin expression would result in vascular hypertrophy, which then could exaggerate the blood pressure elevation. Blockade of endothelin effects by bosentan would result in blunting the development of hypertrophy and remodeling or in regression of vascular structure toward normal. This reduces the vascular amplifying effect and impedes blood pressure elevation or produces a reduction of blood pressure to a mild form of hypertension, where it is maintained by mechanisms independent of vascular structure.

Previous results with BQ-123, an ET\(_A\) receptor antagonist, have demonstrated only moderate lowering of blood pressure in DOCA-salt hypertensive rats. Although the ET\(_A\) receptor was thought to be the endothelin receptor responsible for vasoconstriction and ET\(_B\) receptors were believed to be present in endothelium, where they elicited release of endothelin-derived relaxing factor, and not in vascular smooth muscle cells, the presence of ET\(_B\) receptors in vascular smooth muscle cells has now been demonstrated. The apparently superior effect of bosentan, which blocks both ET\(_A\) and ET\(_B\) receptors, suggests that the additional advantage of bosentan for blood pressure lowering may be due to its ability to block ET\(_B\) receptors. This remains to be definitively demonstrated. The mechanism for the effects of bosentan appears so far to be limited to blockade of ET\(_A\) and ET\(_B\) receptors. We have also found that bosentan does not block norepinephrine- or vasopressin-induced contraction of resistance vessels (unpublished observations, 1994), and in the present study it is shown not to affect plasma renin activity. Plasma renin activity is in any case suppressed in this hypertensive model, which is well known to be independent of renin. Other potential mechanisms for lowering blood pressure such as effects on sympathetic nervous activity require further investigation.

Vasoconstrictor responses of blood vessels were corrected in bosentan-treated DOCA-salt hypertensive rats. It is unclear at present whether this rather paradoxical effect, which we have previously found with other antihypertensive agents, is a direct effect of the drug or whether it is a result of the correction of vascular structure. It is possible that this effect is a nonspecific consequence of the correction of the abnormalities of blood vessels that result from elevated blood pressure, with improvement in the contractility of vascular smooth muscle, and not necessarily a specific beneficial effect of bosentan. Responses to ET-1 were depressed in DOCA-salt hypertensive rats, as we have described in resistance vessels and conduit arteries in this hypertensive model, and were practically normalized in resistance arteries under treatment with bosentan in the current study. Thus, the blocking effect of bosentan was not present in vitro when the vessels were studied. ET-1 has a higher affinity than bosentan for the endothelin receptors. It is therefore not surprising that bosentan is easily washed away during preparation of vessels and that ET-1 effects are not blocked in arteries from the bosentan-treated DOCA-salt hypertensive rats.

It is well known that DOCA-salt hypertensive rats present an impairment of endothelium-dependent relaxation, which is also found in the present study. Interestingly, although constrictor responses were corrected by bosentan treatment, relaxation elicited by acetylcholine remained blunted. Since blood pressure was still elevated in the bosentan-treated rats, it is possible that this interfered with potential beneficial effects of bosentan on the endothelium.

In contrast to vascular hypertrophy and remodeling, the ratio of heart weight to body weight was unaltered in the bosentan-treated DOCA-salt rats despite the lowering of blood pressure. This may indicate that endothelin is not involved in an important way as a hypertrophic factor in the myocardium. Other factors with which bosentan does not interfere may play important roles in left ventricular hypertrophy. Together with the blood pressure, which remains within the hypertensive range, such factors may not allow a regression of cardiac hypertrophy.

Rats receiving bosentan in their food did not grow as much as the DOCA-salt hypertensive rats and much less than controls. We did not study the mechanism for this effect. ET-1 has potent effects on the kidney, where it may reduce renal blood flow and glomerular filtration rate. There may be an important contribution for ET\(_B\) receptor-mediated vasoconstriction in the kidney, which together with ET\(_A\)-mediated effects is also antagonized by bosentan. Thus, bosentan treatment may result in less water and salt retention in this model of volume-expanded hypertension, which could play a role in the lack of weight gain in the treated rats. Since the difference in weight with DOCA-salt hypertensive rats was not very large and cardiac hypertrophy still occurred while vascular hypertrophy and remodeling were blunted, it appears unlikely that this fact played a major role in the findings being reported.

In conclusion, treatment of DOCA-salt hypertensive rats with the nonselective endothelin antagonist bosentan blunted the development of DOCA-salt hypertension and resulted in an attenuation of vascular hypertrophy and remodeling in mesenteric resistance arteries and of the abnormalities of vasoconstrictor responses usually found in these blood vessels, whereas cardiac hypertrophy was unaffected. These results suggest that ET-1 may play a role in the elevation and maintenance of blood pressure and in vascular hypertrophy and remodeling in DOCA-salt hypertensive rats. In apparent opposition to this proposal, a recent report has shown that targeted disruption of the mouse ET-1 gene, which results in the death of homozygotes, produced elevated blood pressure in heterozygotes. This would appear to argue against a possible role of ET-1 in hypertension. However, it is likely that in normal conditions low concentrations of endothelin stimulate endothelial ET\(_B\) receptors and contribute to the
basal release of endothelium-derived relaxing factors, which play a role in maintaining normal vascular tone. Disruption of the ET-1 gene with a reduction in the production of ET-1 in heterozygotes could result in a decrease of ET-1-stimulated generation of endothelium-derived relaxing factors and thus in apparently paradoxical vasoconstriction and elevation of blood pressure. On the other hand, if excess endothelin is produced, as in blood vessels of DOCA-salt hypertensive rats and perhaps in other forms of hypertension, this could result in vasoconstriction, vascular hypertrophy, and hypertension.

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