**ET_A Receptor Antagonist Prevents Blood Pressure Elevation and Vascular Remodeling in Aldosterone-Infused Rats**

Jeong Bae Park, Ernesto L. Schiffrin

**Abstract**—Increased endothelin-1 may be associated with elevation of blood pressure (BP) and promotion of vascular hypertrophy, especially in salt-sensitive hypertension. Mineralocorticoid hypertension has been associated with activation of the endothelin system. We evaluated whether in aldosterone-infused rats the selective endothelin type A receptor-antagonist BMS 182874 prevents BP elevation and vascular hypertrophy. Rats were infused with aldosterone (0.75 μg/h) subcutaneously via a mini-osmotic pump and were offered 1% NaCl in the drinking water. BMS 182874 (40 mg/kg in food) for 6 weeks. Systolic BP was monitored by the tail-cuff method, and vascular changes of mesenteric arteries were evaluated using a pressurized myograph. Aldosterone-infusion significantly increased BP to 151 ± 7 mm Hg compared with controls (108 ± 4 mm Hg, P < 0.01). BMS 182874 normalized BP (117 ± 4 mm Hg). Media cross-sectional area of aorta was significantly increased by aldosterone infusion (P < 0.05), and BMS treatment normalized it (P < 0.001). Aldosterone infusion increased media width and media-to-lumen ratio of mesenteric resistance arteries (17.6 ± 0.4 μm and 7.5 ± 0.4%) compared with controls (14.2 ± 0.5 μm, P < 0.01, and 5.9 ± 0.1%, P < 0.05). BMS 182874 normalized media and media-to-lumen ratio (15.1 ± 0.6 μm and 5.7 ± 0.1%, both P < 0.01). In conclusion, the endothelin type A receptor antagonist attenuated BP elevation and prevented vascular remodeling or hypertrophy of aorta and mesenteric resistance arteries in aldosterone-infused rats. These results suggest a role for endothelin-1 in BP elevation and structural alterations of large and small vessels in aldosterone and salt-induced hypertension. (Hypertension. 2001;37:1444-1449.)

**Key Words:** hypertension, sodium-dependent ■ endothelin ■ blood vessels ■ aorta ■ arteries ■ hypertrophy

Endothelin-1 (ET-1) gene expression is enhanced in blood vessels in deoxycorticosterone (DOCA)-salt hypertension.1–3 This mineralocorticoid and salt-dependent model of hypertension exhibits a severe degree of vascular hypertrophy and responds to endothelin antagonists with blood pressure (BP)—lowering and regression of hypertrophic remodeling of small arteries.4,5 It has accordingly been proposed that ET-1, which has hypertrophic and mitogenic properties,6,7 could play a role in the severe vascular hypertrophy present in salt-sensitive hypertensive rats.4,5 In addition to its vasoconstrictor action and hypertensive effects, vascular aldosterone may contribute to the pathophysiology of hypertension.8 A relationship between endothelin and aldosterone has been demonstrated in patients with primary aldosteronism.9 In the recently concluded Randomized Aldactone Evaluation Study (RALES),10 blockade of aldosterone improved cardiac mortality in heart failure patients, which could be mediated in part via blunting of ET-1 effects.11

We tested the hypothesis that the expression of the ET-1 gene in blood vessels of aldosterone-infused hypertensive rats would be enhanced and would result in exaggerated vascular hypertrophy as we previously showed in DOCA-salt hypertension.2–5 We also proposed that the endothelin type A (ET_A)-selective endothelin antagonist BMS 182874 would prevent BP elevation and vascular hypertrophy or remodeling in large and small arteries in this model.

**Methods**

**Animal Experiments**

The study was conducted according to recommendations of the Animal Care Committee of the Clinical Research Institute of Montreal and the Canadian Council of Animal Care. Male Sprague-Dawley rats (Charles River, St. Constant, Québec, Canada) that weighed 200 g were studied. Rats, under anesthesia with 50 mg/kg ketamine and 5 mg/kg xylazine given intramuscularly, had a model 2002 mini-osmotic pump (Alza Corporation), which infused 0.5 μL/h for 2 weeks, implanted subcutaneously. The mini-osmotic pumps were replaced every 2 weeks, with the rats under anesthesia. The mini-osmotic pumps infused subcutaneously 0.75 μg/h d-aldosterone (Sigma Chemical Co) dissolved in 0.9% saline or the vehicle. All rats were offered 1% saline to drink. BMS 182874 (obtained from Dr James Powell, Bristol-Myers Squibb, Princeton, NJ) was offered in the drinking water (40 mg · kg⁻¹ · d⁻¹) throughout the experiment to one half of the aldosterone-infused rats. Systolic BP was measured weekly by the tail-cuff method and recorded on a model 7 polygraph fitted with a 7-P8 preamplifier and PCPB.
photoelectric pulse sensor (Grass Instruments Co). The average of 3 pressure readings was obtained. Rats were killed by decapitation at the end of the experiment, and half the thoracic aorta was fixed in Bouin solution and the rest was frozen in liquid nitrogen.

Study of Small Arteries

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 and placed in cold physiological salt solution (PSS) of the following composition: NaCl 120 mmol/L, NaHCO3 25 mmol/L, KCl 4.7 mmol/L, KH2PO4 1.2 mmol/L, MgSO4 1.2 mmol/L, CaCl2 2.5 mmol/L, EDTA 0.026 mmol/L, and glucose 5.5 mmol/L. A third-order branch of the mesenteric arterial tree (~2 mm in length) was carefully dissected 1 mm from the intestine and cleaned of all adherent connective tissue under a dissecting microscope. The arterial segments were mounted in a pressure myograph chamber, as previously described, and slipped onto 2-glass microcannulae. The axial length of the arterial segment was adjusted by carefully positioning the cannula until vascular walls were parallel without any stretch. Intraluminal pressure was set to 45 mm Hg with a servo-controlled pump. Vessels were then equilibrated for 1 hour with PSS that was bubbled with 95% air (21% O2) and 5% CO2 to give a pH of 7.4.

Endothelium-dependent and -independent relaxation was assessed by measuring the dilatory response of small arteries precontracted with norepinephrine (5 x 10^-6 mol/L) and sodium nitroprusside (10^-4 mol/L) to cumulative doses of acetylcholine (10^-7 to 10^-5 mol/L) and sodium nitroprusside (10^-7 to 10^-4 mol/L). Contractions to cumulative doses of ET-1 (10^-11 to 10^-7 mol/L) were also obtained. Thereafter, mesenteric resistance arteries were deactivated with Ca2+ -free PSS that contained 10 mmol/L EGTA for 30 minutes to eliminate tone. Lumen and media diameters were measured with the intraluminal pressure maintained at 45 mm Hg.

Study of Aorta

The aorta fixed in Bouin solution was processed for paraffin embedding in an automated system (SHANDON Citadel tissue processor). Serial sections (5-μm thick) of aorta were obtained. Tissue sections were dewaxed with ethanol and stained with Sirius red F3BA (0.5% in saturated aqueous picric acid) (Aldrich Chemical Company Inc). The media cross-sectional area (CSA) of aorta (magnification, ×10) was analyzed with an image analysis system (Northern Eclipse 5.0, EMPIX Imaging Inc). A single investigator blinded to the experimental groups performed the analysis.

Analysis of PreproET-1 mRNA From Aorta

Expression of the ET-1 gene in aorta was studied by reverse transcription–polymerase chain reaction (RT-PCR). Total RNA was extracted from frozen aorta as previously described. RT was performed in a 30-μL volume that contained 1 μg of RNA, 1.5 μL of 10 mmol/L dNTP, 6 μL of BRL 53 buffer, 0.6 μL of oligo(dT)12–18 primer (0.5 mg/mL), 1.5 μL of 200 U/mL M-MLV reverse transcriptase (GIBCO-BRL), 0.9 μL of rRNasin (RNase inhibitor, 40 U/mL), and 3 μL of diethiothreitol (0.1 mol/L) for 1 hour at 37°C. The reaction was stopped by heating at 95°C for 5 minutes. Five microliters of the resulting cDNA mixture was amplified using specific primers. For amplification of the ET-1 gene cDNA, sense TTTTTTCTCCCTCTTCTTTC and antisense CTTCAACCTTCTTATTATTATT primers were used. For glyceraldehyde phosphate dehydrogenase (GAPDH), sense TATGATGACATCAAGAGGTGG and antisense ATGTCGTTGTCCCCACAC primers were used. PCR was conducted with an initial denaturing interval (95°C, 5 minutes) and then 30 sequence cycles; for preproET-1, 94°C (45 seconds), 47.5°C (30 seconds), and 72°C (1 minute); and for GAPDH, 94°C (45 seconds), 55°C (30 seconds), and 72°C (1.5 minutes). Amplification products were electrophoresed in 1.5% agarose gel that contained ethidium bromide (0.5 mg/mL). Bands that corresponded to RT-PCR products were visualized by UV light and digitized using Alphalmager software. Band intensity was quantified using ImageQuant (version 3.3, Molecular Dynamics) software. Under the conditions in which it was performed, RT-PCR allowed semiquantitative evaluation of preproET-1 and GAPDH mRNA with 0.1 μg of total RNA (Figure 1).

Measurement of Immunoreactive ET-1 in Plasma

Plasma ET-1 was measured by radioimmunoassay after extraction by passage through a C18 Sep-Pak cartridge as previously described. Data Analysis

Data are presented as mean ± SEM. Statistical analysis was performed using ANOVA for repeated measures. Differences between means were analyzed for “simple main effects” using a post hoc Student-Newman-Keuls test. P<0.05 was considered significant.

The remodeling index (the percent difference between internal diameters of hypertensive and normotensive vessels not attributable to growth) was calculated as follows: 100[(Di)h − (Di)n]/[(Di)h − (Di)remodel], in which (Di)h and (Di)n are the internal diameters of normotensive and hypertensive vessels, respectively, and (Di)remodel is the remodeled internal diameter. (Di)remodel = [(Di)h − 4CSA/π]1/2, in which (Di)h is the external diameter of hypertensive vessels and CSAa is the media CSA of normotensive vessels. The growth index

Figure 1. RT-PCR was performed with increasing concentrations of total RNA extracted from blood vessels (0.0067, 0.033, 0.167, and 0.333 μg). RT-PCR with 0.167 μg of original total RNA showed no saturation for either preproET-1 or GAPDH, and accordingly, a similar amount of original total RNA was used for the semiquantitative RT-PCR in the study. The correlation coefficient of both regressions was r² = 0.99 and r² = 0.98 for preproET-1 and GAPDH, respectively.
Body Weight, Plasma Potassium, and Immunoreactive Endothelin Levels of Aldosterone-Infused Rats Treated With the Selective ET<sub>A</sub>-Receptor-Antagonist BMS 182874

<table>
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<th>Parameters</th>
<th>Control</th>
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<tr>
<td>Number</td>
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<td>8</td>
<td>6</td>
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<tr>
<td>BW, g</td>
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<td>393±11</td>
<td>393±11</td>
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<tr>
<td>Plasma ET-1, pmol/L</td>
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<td>1.9±0.2</td>
<td>4.3±1.1</td>
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</table>

BW indicates body weight; BMS, BMS 182874.

*P<0.01.

was calculated as (CSA<sub>h</sub>−CSA<sub>n</sub>)/CSA<sub>n</sub>, in which CSA<sub>n</sub> and CSA<sub>h</sub> are the CSAs of normotensive and hypertensive vessels, respectively.

**Results**

Body Weight, BP, Serum Potassium, and Plasma Endothelin

Body weight of aldosterone-infused hypertensive rats was slightly but not significantly lower than that of normotensive control rats (Table). Systolic BP of aldosterone-infused hypertensive rats was mildly but significantly elevated relative to that of normotensive control rats after 2 weeks of treatment and remained elevated thereafter (Figure 2). Treatment with the selective ET<sub>A</sub> receptor–antagonist BMS 182874 resulted in prevention of the development of hypertension in aldosterone-infused rats. Serum potassium was reduced in the aldosterone-infused rats and was normalized by BMS 182874 treatment. Plasma levels of immunoreactive endothelin showed no difference between the 3 groups.

Effects of ET<sub>A</sub> Receptor–Antagonist BMS 182874 on Structure and Function of Mesenteric Arteries

Aldosterone infusion resulted in an increased media width and media-to-lumen ratio (17.6±0.4 μm and 7.5±0.4%) compared with controls (14.2±0.5 μm, P<0.01, and 5.9±0.1%, P<0.05, respectively). BMS 182874 normalized media width and media-to-lumen ratio in aldosterone-infused rats (15.1±0.6 μm and 5.7±0.1%, both P<0.01). Media CSA was significantly increased from 11720±1006 to 14383±1091 μm<sup>2</sup> in aldosterone-infused rats (P<0.05) and tended to decrease under BMS 182874 (13721±1339 μm<sup>2</sup>) (Figure 3). Lumen diameter did not differ in the 3 groups. The remodeling index was −15% and the growth index was 23%, which indicated that the structural change was what has been denominated hypertrophic remodeling. Vasodilatory responses to acetylcholine or to a maximal dose (10<sup>−4</sup> mol/L) of sodium nitroprusside and vasoconstrictor responses to increasing concentrations of ET-1 (not shown) were similar to controls in aldosterone-infused rats without or with BMS 182874 treatment.

Effects of ET<sub>A</sub> Receptor–Antagonist BMS 182874 on Aorta

Aldosterone infusion significantly increased aortic media CSA (6.0±0.3×10<sup>5</sup> μm<sup>2</sup>) compared with controls (5.3±0.2×10<sup>5</sup> μm<sup>2</sup>, P<0.05). BMS 182874 treatment normalized the cross-section of aorta from aldosterone-infused rats (4.6±0.2×10<sup>5</sup> μm<sup>2</sup>, P<0.001, versus aldosterone-infused rats; Figure 4).

PreproET-1 Gene Expression in Aorta

Figure 5 shows results of RT-PCR analysis of RNA extracted from aorta. Although preproET-1 mRNA was significantly (P<0.05) increased 2.4-fold in aldosterone-infused hypertensive rats, BMS 182874 treatment did not change ET-1 gene expression.

**Discussion**

ET-1 has been proposed to play an important role in vascular hypertrophy in mineralocorticoid hypertension, such as in the DOCA-salt hypertensive rat<sup>4,5</sup>, as well as in genetic salt-sensitive Dahl rats<sup>13</sup> and in angiotensin II–induced hypertension in rats,<sup>18</sup> independent of the effect of BP elevation. In this study, we attempted to answer questions whether aldosterone infusion into normotensive rats would increase BP, expression of tissue ET-1 in blood vessels, and hypertrophy or remodeling of large and small arteries. If enhanced ET-1 gene expression is induced, will the altered vascular structure or high BP be prevented with an ET<sub>A</sub> receptor–antagonist
Our results demonstrate that in salt-loaded rats, aldosterone increases vascular ET-1 mRNA levels, which in turn mediate a major part of ET-1–stimulated vascular growth and hypertension in vivo. The blockade of the endothelin system at the level of ETA receptors prevented the development of hypertension and vascular hypertrophy in this experimental model. Interestingly, endothelin receptor antagonism also prevented the hypokalemia of the aldosterone-infused rats.

The salt-loaded rat infused with aldosterone is a model that has in the past been used to investigate cardiac fibrosis. We previously demonstrated that in DOCA-salt hypertensive rats, which have an important ET-1–dependent component, there is abundant interstitial and perivascular fibrosis of the heart, similar to that found in the salt-loaded rat infused with aldosterone. For this reason, we have investigated the salt-loaded aldosterone-infused rat in search of an endothelin-dependent component. Indeed, 6-week administration of aldosterone resulted in hypertrophic remodeling of small mesenteric arteries and aorta. There are no previous in vitro studies demonstrating that aldosterone stimulates ET-1 mRNA expression in endothelial or smooth muscle cells. This study demonstrates, for the first time, that aldosterone is able in vivo to increase vascular ET-1 tissue concentrations in comparison to salt-loaded control rats. Plasma levels of the peptide did not increase in the aldosterone-infused rat. The discrepancy between tissue and plasma ET-1 concentrations in our model is not dissimilar from the difference between the markedly elevated ET-1 tissue levels in small mesenteric arteries but not plasma levels after 2-week administration of angiotensin II. In the DOCA-salt hypertensive rat, tissue levels of ET-1 message or peptide are significantly increased, but plasma levels of immunoreactive endothelin will be found elevated in some but not in other series of experiments.

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**Figure 3.** Lumen diameter, media width, media CSA, and media-to-lumen ratio of mesenteric arteries from normotensive control rats and aldosterone-infused rats treated or not treated with BMS 182874. Vascular parameters were measured at a constant intraluminal pressure of 45 mm Hg. Results are presented as mean±SEM. *P<0.05 vs control, ***P<0.001 vs control, and ++P<0.01 vs Aldo.

**Figure 4.** Media CSA of thoracic aorta assessed from histological sections. Aldosterone infusion into normotensive rats significantly increased aortic media CSA, and BMS 182874 treatment normalized it. Results are presented as mean±SEM. *P<0.05 vs control, ++P<0.01 vs Aldo.

**Figure 5.** PreproET-1 mRNA content of aorta. Aldosterone infusion increased ET-1 message 2.4-fold, which was not prevented by concomitant treatment with BMS 182874. Results are presented as mean±SEM. Arbitrary units are the ratio of abundance of RT-PCR products of ET-1 mRNA and GAPDH mRNA. Number 4 in each group. *P<0.05 vs control.
These results support the hypothesis that ET-1 is a paracrine-autocrine, locally acting system, in which the peptide is secreted mainly abuminally. Small changes in plasma concentrations of immunoreactive endothelin, which were found but that did not achieve statistical significance, may relate to displacement of the peptide from the receptor in tissues to the circulation.

The function of small blood vessels was not altered in aldosterone-infused rats. Vasodilation in response to acetylcholine was not impaired, and vasoconstriction to ET-1 was unchanged in vessels from aldosterone-infused rats. Treatment with the ET₃ antagonist did not influence the function of mesenteric arteries in these rats. This finding is different from isolated aortic or mesenteric rings from DOCA-salt or from salt-sensitive Dahl rats, in which markedly reduced endothelium-dependent relaxations to acetylcholine and attenuated constriction to ET-1 could be demonstrated. The reason for these disparate results is not clear. A possible explanation may relate to the magnitude of BP elevation in aldosterone-infused rats (systolic BP, 150±7 mm Hg) compared with the more severe elevation of BP found in DOCA-salt or Dahl-salt sensitive rats (systolic BP, 190±4 mm Hg). Functional alterations in small vessels may be a later manifestation compared with BP elevation or structural changes. The structural alterations of mesenteric arteries from SHR and subcutaneous resistance arteries from mild hypertensive patients occurred much earlier and were more prevalent than endothelial dysfunction. Small artery remodeling may precede most clinically relevant manifestations of target organ damage.

Functional changes may be more prominent in more severe forms of hypertension than in milder forms of hypertension, experimental or human. Recently, a study in humans has suggested that blockade of mineralocorticoid receptors with spironolactone could improve endothelium-dependent relaxation, possibly by improving bioavailability of nitric oxide through reduction in vascular wall oxidative stress. This could imply that aldosterone may be able to affect ET-1 production by reducing nitric oxide bioavailability, because nitric oxide may inhibit ET-1 generation.

It is difficult to differentiate between the structural consequences of BP reduction and the direct influence of endothelin blockade on vascular structure independent of BP reduction. In the present experiments, this differentiation cannot be made. Hemodynamic parameters have an impact on vascular structure. However, other studies using endothelin receptor antagonists have been able to demonstrate pressure-independent effects of ET-1 on vascular hypertrophy.

Hence, stimulation of vascular endothelin production represents a pressure-independent mechanism of vascular hypertrophy in aldosterone-infused rats.

The mechanism of increase of ET-1 production in aldosterone-infused hypertensive rats remains unclear. Vasopressin, the levels and effects of which were shown in the past to be enhanced in DOCA-salt hypertension, may play a role in stimulation of ET-1 expression. Mineralocorticoids may potentiate effects of vasopressin on ET-1 expression in blood vessels and heart, or alternatively, mineralocorticoids may stimulate directly or indirectly vasopressin, which in turn may stimulate ET-1 expression. This remains undefined. Mineralocorticoid receptors have been found in endothelial and vascular smooth muscle cells in the aorta and pulmonary artery of rabbit. In myocyte and endothelial cells of the heart, the presence of mineralocorticoid receptors was also detected. This finding supports the possibility of direct aldosterone actions in the heart and blood vessels. At the level of resistance artery, a direct action of aldosterone has been proposed. Rizzoni et al reported vascular remodeling (increased media-to-lumen ratio and narrowed lumen) in resistance arteries from gluteal subcutaneous tissue taken from patients with primary aldosteronism. The present study is the first to suggest that effects previously attributed to direct actions of aldosterone may in fact be mediated by ET-1. Interestingly, in other models of hypertension, such as the transgenic (mREN2)27 rat in which a secondary form of aldosteronism occurs in response to angiotensin II stimulation of the adrenal, recent studies have demonstrated that endothelin antagonism with the same ET₃ antagonist used in the present study did not induce regression of cardiovascular remodeling. Several explanations are possible, but one could be that elevation of aldosterone in that model is not sufficient within the time limits of the experimental paradigm to stimulate ET-1 production. A similar finding has been reported in the 2-kidney, 1-clip Goldblatt hypertensive rat, another angiotensin II–dependent hypertensive model with secondary aldosteronism in which there is neither vascular overexpression of preproET-1 nor response of vascular remodeling to an endothelin receptor antagonist. Of interest in this regard is the normalization of serum potassium in the aldosterone-treated rats by the endothelin receptor antagonist, the mechanism for which remains to be investigated but may involve an effect of renal ET-1 in the distal tubule of the kidney, in which ET-1 has been shown to influence natriuresis.

In conclusion, in salt-loaded, aldosterone-infused hypertensive rats, large and small artery hypertrophy and hypertension associated with increased vascular ET-1 mRNA were observed. These changes were abrogated if rats were treated with ET₃ receptor antagonism. This suggests that vascular hypertrophy and the increase in BP induced by aldosterone in vivo are mediated at least in part by increased production of endogenous ET-1, which activates ET₃ receptors that contribute to the observed changes in the cardiovascular system. The blockade of the endothelin system may therefore exert beneficial effects on both large and small arteries in conditions in which aldosterone blockade has been demonstrated to be efficacious.

Acknowledgments

This work was supported by grant 37917 and a group grant from the Medical Research Council of Canada and the Canadian Institutes of Health Research to the Multidisciplinary Research Group on Hypertension. The authors are grateful to Suzanne Diebold for excellent technical assistance.

References


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Hypertension. 2001;37:1444-1449
doi: 10.1161/01.HYP.37.6.1444

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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