Cardiac and Vascular Fibrosis and Hypertrophy in Aldosterone-Infused Rats: Role of Endothelin-1

Jeong Bae Park and Ernesto L. Schiffrin

Increased endothelin-1 (ET-1) or aldosterone may be associated with promotion of cardiovascular hypertrophy and fibrosis. We evaluated whether the selective ET_A receptor-antagonist BMS 182874 (BMS) prevents cardiac and vascular collagen deposition and hypertrophy in aldosterone-infused rats. Rats received subcutaneous aldosterone (0.75 µg/h) and 1% sodium chloride in drinking water with BMS (40 mg/kg per day in food) for 6 weeks. Heart and aorta were cross-sectioned and stained with Sirius red. Heart weight did not change with either aldosterone infusion or BMS treatment. Cardiac and aortic interstitial and perivascular collagen were quantified with videomorphometry. Aortic collagen and media cross-sectional area were significantly increased 3.5-fold (P < .01) and 1.13-fold (P < .05), respectively, with aldosterone infusion and decreased in BMS-treated rats (P < .05, P < .001, respectively). Aldosterone infusion increased interstitial and perivascular collagen in the left (1.6- and 2.7-fold, P < .05 and P < .01, respectively) and right ventricle (1.5- and 1.7-fold, P > .05 and P < .05, respectively). BMS prevented collagen deposition except for interstitial collagen in the right ventricle. Cardiac and aortic fibrosis were significantly increased in aldosterone-infused hypertensive rats. The ET_A receptor antagonist prevented cardiac and aortic collagen deposition and aortic hypertrophy. This suggests a role for ET-1 in fibrosis of heart and large vessels in conditions associated with mineralocorticoid excess. Am J Hypertens 2002;15:164–169 © 2002 American Journal of Hypertension, Ltd.

Key Words: Collagen, hypertension, myocardium, mineralocorticoid, endothelin receptor antagonist.

Cardiovascular alterations associated with hypertension are characterized by cardiovascular hypertrophy or remodeling associated with an increase in deposition of extracellular matrix (ECM) proteins such as collagen.1,2 The mechanism underlying these changes is unclear, but humoral factors, such as angiotensin II (Ang II),3,4 endothelin-1 (ET-1),5 or aldosterone3,6,7 may play a role. The accumulation of ECM in the cardiovascular system is involved in increases in wall stiffness and eventually in the development of heart failure.8 Indeed, increased stiffness of aorta is an independent predictor of cardiovascular mortality. The enhancement of collagen deposition in the myocardium and around coronary arteries has been demonstrated in other experimental models, including renovascular hypertension,9,10 after Ang II or aldosterone infusion,2,6,7,9 in spontaneously hypertensive rats (SHR),11 and recently in deoxycorticosterone (DOCA)-salt hypertensive rats.12,13

ET-1 plays a role in some models of salt-sensitive experimental hypertension, such as DOCA- or aldosterone-salt hypertensive rats. ET-1, whose expression in blood vessels is increased in these models,14–17 and blood pressure (BP) elevation, together participate in the hypertrophy of large and small arteries salt-sensitive hypertension. Administration of endothelin antagonists prevented the development of hypertension and reversed vascular hypertrophy in both large and small arteries,17 in part by blocking ET-1 hypertrophic and mitogenic properties.18,19 ET-1 also promotes growth of cardiomyocytes20 and collagen synthesis by cardiac fibroblasts.6 In addition, ET-1 is overexpressed in the heart in endothelin-dependent hypertension.21 This in turn increases procollagen I and III synthesis leading to abundant cardiac interstitial and perivascular fibrosis, as a result of an imbalance between the synthesis and degradation of collagen. Increased fibrosis will contribute to myocardial stiffness and contractile dysfunction, resulting in cardiac failure.

In the present study, we tested the hypothesis that ET-1...
plays a role in cardiac and aortic collagen deposition and hypertrophy in aldosterone-salt hypertensive rats, similar to that which we previously showed in DOCA-salt hypertensive rats, with the activation of ET<sub>A</sub> receptors.

**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) weighing 200 g were studied. Rats, under anesthesia with ketamine (50 mg/kg) and xylazine (5 mg/kg) given intramuscularly, were implanted subcutaneously with a model 2002 mini-osmotic pump (Alza Corporation, Palo Alto, CA) that infuses 0.5 mg/kg/h for 2 weeks. Intramuscular pumps were replaced every 2 weeks under anesthesia. The mini-osmotic pumps infused subcutaneously 0.75 μg/h d-aldosterone (Sigma Chemical Co., St. Louis, MO) dissolved in 0.9% saline or vehicle. All rats were offered 1% saline to drink. BMS 182874 (obtained from Dr. James Powell, Bristol-Myers Squibb, Princeton, NJ) was offered to half the aldosterone-infused rats in the drinking water (40 mg/kg/d) throughout the experimental period starting after mini-osmotic pump implantation. On the basis of previous experiments this dose was shown to prevent the increase in BP in aldosterone-infused rats. Systolic BP was measured weekly by the tail-cuff method and recorded on a model 7 polygraph and PCPB photoelectric pulse sensor (Grass Instruments Co., Cambridge, MA). The average of three pressure readings was obtained. Rats were killed by decapitation at the end of the experiment.

**Collagen Quantification in the Heart**

The thoracic aorta and heart fixed in Bouin solution were processed for paraffin embedding in an automated system (SHANDON Citadl tissue processor, Pittsburgh, PA). Serial cross-sections (5 μm thick) of both ventricles and aorta were obtained. Tissue sections were dewaxed with ethanol, and stained with the collagen-specific stain Sirius red F3BA (0.5% in saturated aqueous picric acid) (Aldrich Chemical Company, Inc., Milwaukee, WI). Interstitial collagen density of the left ventricle was evaluated throughout the inner third (subendocardial myocardium), the middle third (mid-myocardium), and the outer third (subepicardial myocardium) of the circumference of the left ventricle. From each of three nonconsecutive serial sections (which allowed convergence of results), about 10 fields in each region of the heart were randomly selected and recorded (magnification, ×20). Interstitial collagen in the right ventricle was evaluated in 15 to 20 fields. Perivascular collagen was determined by dividing the collagen area of the cross-section of coronary arteries by the luminal area of the vessel. Only intramyocardial vessels that appeared circular on cross section, 10 vessels from the left ventricle and 5 from the right ventricle, were analyzed. The severity of cardiac fibrosis was evaluated with the use of an image analysis system (Northern Eclipse 5.0, EM-PIX Imaging Inc., Mississauga, ON, Canada). A single investigator unaware of the nature of the experimental groups performed the analysis.

**Hypertrophy and Collagen Quantification in the Aorta**

The media cross-sectional area in Sirius red-stained aorta was analyzed by the image analysis system (magnification, ×10). Collagen content in intima and media of aorta were evaluated and averaged (magnification, ×40).

**Statistical Analysis**

Data are expressed as mean ± SEM. Statistical significance was assessed by one-way ANOVA followed by a Student Newman-Keuls test. Differences were considered significant at P < .05.

**Results**

**BP, Body, and Heart Weights**

As already reported, aldosterone-infusion significantly increased systolic BP to 151 ± 7 mm Hg compared to 108 ± 4 mm Hg of controls (P < .01) after 6 weeks. Treatment with the selective ET<sub>A</sub> receptor-antagonist BMS 182874 (BMS) resulted in prevention of BP elevation (117 ± 4 mm Hg). Body weight and relative heart weights were similar in treated and untreated aldosterone-infused hypertensive rats (Table 1).

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**Table 1.** Morphologic characteristic of aldosterone-infused rats treated with the selective ET<sub>A</sub> receptor antagonist BMS 182874

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Aldosterone</th>
<th>Aldosterone+BMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>7</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>BW (g)</td>
<td>419 ± 9</td>
<td>394 ± 11</td>
<td>393 ± 11</td>
</tr>
<tr>
<td>HW/BW (mg/g)</td>
<td>3.25 ± 0.06</td>
<td>3.66 ± 0.59</td>
<td>3.59 ± 0.15</td>
</tr>
<tr>
<td>Media CSA of aorta (×10&lt;sup&gt;5&lt;/sup&gt; μm&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>5.3 ± 0.2</td>
<td>6.0 ± 0.3*</td>
<td>4.6 ± 0.2†</td>
</tr>
</tbody>
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BW = body weight; HW = heart weight; CSA = media cross-sectional area.
* P < .05 v control rats; † P < .01 v aldosterone-infused rats.
Effect of ET<sub>A</sub> Receptor Antagonist BMS 182874 on Hypertrophy and Fibrosis of Aorta

Figs. 1 and 2 show that aldosterone infusion significantly increased media cross-sectional area of aorta (6.0 ± 0.3 × 10<sup>5</sup> μm<sup>2</sup>) compared to controls (5.3 ± 0.2 × 10<sup>5</sup> μm<sup>2</sup>, P < .05). BMS treatment prevented development of aortic hypertrophy (4.6 ± 0.2 × 10<sup>5</sup> μm<sup>2</sup>, P < .001). In the intima–media of aorta, collagen density was markedly increased to 15.2% ± 2.4% by aldosterone infusion (versus control of 4.3% ± 0.5%, P < .01). BMS decreased collagen deposition to 9.2% ± 1.2% (P < .05).

Effect of ET<sub>A</sub> Receptor Antagonist BMS 182874 on Cardiac Fibrosis

Myocardial fibrosis was enhanced by aldosterone infusion in the interstitial and perivascular regions of the left ventricle (1.6- and 2.7-fold, P < .05 and P < .01, respectively) and perivascularly in the right ventricle (1.7-fold, P < .05), compared to normotensive control rats (Figs. 3 and 4). Interstitial collagen density in the right ventricle did not increase significantly in aldosterone-infused rats (1.5-fold, P ≥ .05). BMS prevented cardiac collagen deposition except for interstitial collagen of the right ventricle (Fig. 4). Increase in interstitial collagen was very important in the subendocardial myocardium compared to the subepicardial or mid-myocardial regions of the left ventricle of aldosterone-salt rats (Fig. 5). Treatment with BMS prevented collagen deposition to the greatest degree in the subendocardial myocardium of the left ventricle after aldosterone infusion.

Discussion

We previously found that aldosterone infusion into normotensive rats increased tissue preproET-1 mRNA levels in large and small arteries, which in turn played a major role in ET-1-stimulated vascular hypertrophy and hypertension in vivo. Similarly, increased expression of cardiac ET-1 was demonstrated in DOCA-salt hypertensive rats, and it was also shown that ET-1 may be involved in cardiac hypertrophy in some experimental rat models. This study further demonstrates that aldosterone infusion significantly increases collagen deposition in the heart and aorta. Treatment with the ET<sub>A</sub>-selective endothelin receptor antagonist BMS 182874 prevented collagen deposition in the heart and aorta.

The presence of aldosterone receptors on large arteries such as aorta and in the heart, and the recent discovery...
of vascular synthesis and cardiac synthesis of aldosterone suggest that this hormone plays a significant role in the regulation of the cardiovascular structure. The role of endogenous aldosterone in large artery fibrosis and hypertrophy in hypertension is presently uncertain. The mineralocorticoid receptor antagonist spironolactone has antifibrotic properties. An effect of aldosterone on adventitial fibroblasts is a potential explanation for increased collagen synthesis induced by aldosterone, as demonstrated on cardiac fibroblasts. Aldosterone increases vascular and cardiac preproET-1 mRNA levels, which mediates partly by transforming growth factor-β1, ET-1-stimulated cardiac fibrosis in DOCA-salt hypertensive rats, and vascular growth in this model and the aldosterone salt-loaded rat. Blockade of ET$_A$ receptors prevented the development of hypertension, vascular hypertrophy, and cardiac fibrosis in both experimental models. Mineralocorticoids may also potentiate the action 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. Although the development of cardiac fibrosis and left ventricular hypertrophy concomitant with BP increase in the aldosterone-salt model may indicate a role for BP elevation, cardiac fibrosis occurs in both ventricles, whereas atrial natriuretic peptide gene expression is stimulated only in the left but not the right ventricle. Furthermore, prevention of cardiac fibrosis by subhypotensive doses of spironolactone supports a BP independent effect of aldosterone in the induction of cardiac fibrosis. In the present study BP increase was prevented by the ET$_A$ antagonist. This raises the question whether the fact that BP did not increase prevented cardiac fibrosis in aldosterone-infused ET$_A$ antagonist-treated rats. In this model cardiac fibrosis affects both the right and the left ventricle, which is more typically a hormonal than a hemodynamic effect, as discussed above. This has allowed investigators in this field to attribute cardiac fibrosis in aldosterone-infused rats to direct aldosterone effects rather than to BP elevation. By the same token, correction of fibrosis with ET$_A$ antagonism in both right and left ventricles may be attributed with the same confidence to blockade of the action of ET-1 rather than to BP control. In this particular study and in contrast to experiments of other investigators, aldosterone plus salt treatment was not associated with cardiac hypertrophy. Rats did not undergo unilateral nephrectomy in the present study, which may result in lesser BP elevation than previous studies. Thus, differences in protocol and its duration (6 weeks in this study v 8 weeks in studies from other investigators), may explain the absence of cardiac hypertrophy in the present work.

Aldosterone infusion caused little evidence of cardiac hypertrophy, but slight and significant elevation in BP and marked accumulation of collagen. Collagen deposition was increased to the same extent in the right and left ventricles as previously reported in other studies. This suggests that in aldosterone-infused rats, extracellular matrix deposition is more dependent on neurohormonal influences such as the activation of sympathetic nerves or the endothelin system than on pressure overload or the degree of left ventricular hypertrophy. Blood pressure elevation may have less influence on cardiac collagen deposition than on development of cardiac hypertrophy, in contrast to aldosterone, similar to what occurs in DOCA hypertensive rats. Because in the heart, ET-1 overexpression appeared to occur mainly in endothelium of blood vessels, ET-1 produced in blood vessels of the heart may influence interstitial fibroblasts and stimulate collagen production, but could conceivably have only a minor effect on cardiomyocytes.

Perivascular cardiac fibrosis generally appears first, followed later by interstitial fibrosis in experimental hypertension associated with unilateral renal ischemia. This is similar to the present findings that showed more accumulation of perivascular collagen than interstitial collagen in the aldosterone-infused rat. However, Brilla et al showed that aldosterone infusion in uninephrectomized rats drinking 1% NaCl induced marked accumulation of interstitial collagen in both ventricles, and to a lesser extent, or not at all, an increase in perivascular collagen. Interestingly, DOCA has been shown to increase mainly perivascular fibrosis, whereas aldosterone, for a similar BP increase, increased both interstitial and perivascular fibrosis. The ET$_A$ antagonist treatment of aldosterone-infused hypertensive rats completely prevented development of hypertension and interstitial fibrosis of the left ventricle and perivascular collagen in both ventricles and in aorta, but interstitial fibrosis of the right ventricle was unaffected. Prevention of fibrosis in the myocardium may occur with a different time course in different animal models and in both ventricles, suggesting that different mechanisms may be involved.

Cardiac fibrosis occurred in aldosterone-infused hypertensive rats predominantly in the subendocardial and to a lesser extent in the mid-myocardial or subepicardial myocardium of the left ventricle, as previously found in DOCA-salt hypertensive rats. In DOCA-salt hypertensive rats increased density of small arterioles, 20 μm in lumen diameter, and capillary rarefaction occurred mainly in the subendocardial myocardium, which was in part...
corrected by treatment with an ET<sub>A</sub> antagonist. 36 Vascular overproduction of ET-1 may be a mechanism shared by cardiac fibrosis and vascular changes in endothelin-dependent hypertension in rats. Increased arteriolar density may increase coronary vascular resistance as arteriolar segments become longer. Decreased capillary density and fibrosis may compromise oxygen and nutrient supply to cardiac myocytes, contributing to hypoxia in this area of the myocardium, which is most vulnerable. Whereas he-
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