Chronic ET\textsubscript{A} Receptor Blockade Attenuates Cardiac Hypertrophy Independently of Blood Pressure Effects in Renovascular Hypertensive Rats

Heimo Ehmke, Jörg Faulhaber, Klaus Münter, Michael Kirchengast, Rudolf J. Wiesner

Abstract—In isolated cardiac myocytes, the direct effects of angiotensin II on cellular growth and gene expression were shown to be mediated by endothelin via the endothelin subtype A (ET\textsubscript{A}) receptor. To determine whether this pathway is also involved in the cardiovascular adaptations to a chronic activation of the renin-angiotensin system in vivo, the effects of a selective ET\textsubscript{A} receptor antagonist (LU 127043) were investigated in adult rats with renal artery stenosis. Four groups of rats (n=107) were studied over a period of 10 days after surgery: (1) sham-operated animals with saline administration, (2) rats subjected to left renal artery clipping with saline administration, (3) sham-operated rats with LU 127043 administration, and (4) rats subjected to left renal artery clipping with LU 127043 administration. LU 127043 (50 mg/kg) or saline was given by gavage twice daily starting 1 day before the operation. In clipped rats with saline administration, plasma renin activity, the ratio of left ventricular weight to body weight, and mRNAs for β-myosin heavy chain and atrial natriuretic peptide were significantly elevated as early as 2 days after surgery. Blood pressure started to rise on the third postoperative day and attained a steady state hypertensive level by day 6. Blockade of ET\textsubscript{A} receptors had no effects on plasma renin activity or the time course of hypertension in clipped animals but completely prevented left ventricular hypertrophy and the re-expression of the β-myosin heavy chain and atrial natriuretic peptide genes on day 2. While the expressions of the β-myosin heavy chain and atrial natriuretic peptide genes were not different from saline-treated, clipped animals after day 4, the development of left ventricular hypertrophy remained markedly blunted (−50%) during ETA receptor blockade until day 10. These results show that a continuous blockade of ET\textsubscript{A} receptors significantly attenuates the development of left ventricular hypertrophy and, more transiently, fetal gene expression in the early phase of renovascular hypertension. Since neither blood pressure nor the increase in plasma renin activity was significantly altered by ETA receptor blockade, the inhibitory influences of the ET\textsubscript{A} receptor antagonist on left ventricular hypertrophy and gene expression were mediated most likely through a direct blockade of myocardial ET\textsubscript{A} receptors. (Hypertension. 1999;33:954-960.)

Key Words: gene expression ■ hypertension, renovascular ■ remodeling ■ renin-angiotensin system

One of the most potent stimuli for endothelin-1 (ET-1) release from cultured myocardial cells is angiotensin II (Ang II).\textsuperscript{1,2} Both Ang II and ET-1 induce hypertrophic growth and the re-expression of fetal genes in neonatal cardiac myocytes.\textsuperscript{1,3–6} In addition, mechanical stretch causes the secretion of Ang II\textsuperscript{1} and ET-1\textsuperscript{7} from cardiac myocytes, and it was shown that ET-1 potentiates the Ang II–induced activation of Raf-1 and mitogen-activated protein (MAP) kinase.\textsuperscript{8} Thus, in cultured cardiac myocytes, a complex interplay seems to exist between Ang II, mechanical load, and ET-1, which ultimately leads to cardiac hypertrophy and re-expression of fetal genes.

Increased plasma levels of Ang II, elevated blood pressure, and cardiac hypertrophy are characteristically observed in renovascular hypertension due to stenosis of 1 renal artery. This clinical form of secondary hypertension corresponds to the experimental 2-kidney, 1 clip hypertension model. Recent evidence has shown that renovascular hypertension is associated with left ventricular hypertrophy and re-expression of fetal genes.\textsuperscript{8,9} Similar cardiac alterations have been observed when circulating Ang II levels were elevated experimentally by chronic subcutaneous infusions.\textsuperscript{10,11}

The goal of the present experiments was to assess the importance of interactions between Ang II, mechanical load, and ET-1 in vivo. Since the rat myocardium predominantly expresses the endothelin subtype A (ET\textsubscript{A}) receptor,\textsuperscript{12,13} and selective ET\textsubscript{A} receptor blockade largely attenuated the hypertrophic effects induced by Ang II,\textsuperscript{1} mechanical load,\textsuperscript{7} and ET-1\textsuperscript{1} or endothelin-3 (ET-3)\textsuperscript{2} in cultured cardiac myocytes, we investigated the effects of chronic administration of the
orally active, selective ET<sub>α</sub> receptor antagonist LU 127043<sup>14,15</sup> on the temporal development of cardiac hypertrophy and fetal gene expression in renovascular hypertensive rats.

**Methods**

**Study Design**
Experiments were made in a total of 107 female Sprague-Dawley rats weighing 180 to 200 g (age, 9 weeks). Rats with renal artery stenosis and sham-operated animals received either saline or the specific ET<sub>α</sub> receptor antagonist LU 127043 (50 mg/kg) by gavage twice daily, starting 1 day before the operation. LU 127043 (Knoll AG; 2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid)<sup>13</sup> is an orally active, long-acting ET<sub>α</sub> receptor antagonist.<sup>14</sup> Two experimental series were performed. In the first series of experiments, animals were killed 2, 4, and 10 days after surgery (n=68). In the second series of experiments, animals were killed 10 days after surgery (n=39). In these animals, plasma renin activity (PRA) was determined at days 2, 4, and 10 after surgery by means of a chronically implanted arterial catheter. In all animals, systolic blood pressure was measured daily by the tail-cuff method. At the time of death, ratios of left ventricular to body weight were determined as a measure of left ventricular hypertrophy. Ratios of left kidney to right kidney weight were determined to evaluate successful stenosis of the renal artery,<sup>16</sup> and ventricular mRNA levels for β-myosin heavy chain (MHC) and atrial natriuretic peptide (ANP) were measured to determine myocardial fetal gene expression. All experiments were conducted in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals, US Department of Health and Human Services (NIH Publication No. 86-23), and were approved by local authorities.

**Renal Artery Clipping**
Renal artery stenosis was induced under anesthesia with ketamine/xylazine (100 mg/kg and 4 mg/kg, respectively). The left renal artery was exposed through an abdominal incision. A stainless steel wire (diameter, 0.3 mm) was placed next to the renal artery and tightly sutured with silk thread. Then the wire was removed, leaving the renal artery constricted to the size of the wire. Sham-operated animals underwent the same surgical procedure with the exception of suturing of the renal artery. To evaluate whether the operation had been successful, the kidneys were weighed after the death of the animals. On day 10, all animals with renal artery stenosis showed left kidney to right kidney weight ratios between 0.5 and 0.7 (see Table 2 for series 2; data not shown for series 1), indicating successful underperfusion but not ischemic atrophy of the left kidney.<sup>16</sup>

**Drugs**
Chronic ET<sub>α</sub> receptor blockade was induced by administration of LU 127043, the racemic parent drug of the active (+)-isomer LU 135252, a nonpeptide, selective ET<sub>α</sub> receptor antagonist. The selectivity of LU 127043 for ET<sub>α</sub> receptors in rabbit aorta, expressed as the ratio of the affinities for ET<sub>α</sub> over endothelin subtype B (ET<sub>B</sub>) receptors, is 161.<sup>14</sup> LU 127043 was dissolved in 0.1 mol/L NaOH, then diluted in saline to a final concentration of 20 μg/mL and buffered with 0.1 HCl to a pH of 8.0 to 8.5. Twice a day, 50 mg/kg body weight of LU 127043 was administered by gastric gavage with the rats under light ether sedation. Sham-treated rats received saline instead of the antagonist by the same procedure. In preliminary experiments in anesthetized rats, the hypotensive response to 0.3 μg/kg of the ET<sub>α</sub> receptor agonist sarafotoxin was still present after 30 mg/kg or 100 mg/kg of LU 135252 given 1 hour before by gastric gavage, whereas it was completely blocked after 20 mg/kg of the nonselective ET<sub>α</sub>/ET<sub>B</sub> receptor antagonist LU 224332, indicating that ET<sub>α</sub> receptor signaling was intact in the ET<sub>α</sub> receptor antagonist–treated animals in the present study. Plasma concentrations of ET-1 were found to be unaffected by chronic oral treatment with 100 mg/kg of LU 135252.<sup>17</sup> Corresponding to twice the dose of the ET<sub>α</sub> receptor antagonist used in the present study, which confirms the absence of significant ET<sub>α</sub> receptor blockade.

**Measurement of Plasma Renin Activity**
In 39 rats, a chronic catheter was implanted into the abdominal aorta caudal to the origin of the right renal artery and led to an incision in the neck. The catheters were implanted either immediately after clipping or sham clipping of the renal artery (for PRA determinations on days 2 and 4) or on the eighth day after the first operation (for PRA determinations on day 10). For measurement of PRA, animals were put into individual cages, and the catheter was connected to a syringe. Thirty minutes later, a 1-μL sample of blood was withdrawn from the awake, unstressed animal and replaced by an equal volume of saline. After centrifugation, the plasma was stored at –20°C. For the determination of PRA, plasma samples were incubated in the presence of 6 mmol/L EDTA, 1.6 mmol/L dimercaptopropanol, and 100 mmol/L N-tris(hydroxymethyl)-aminoethanesulfonate at pH 7.30 and 37°C for 60 minutes. The amount of angiotensin I (Ang I) formed was determined by radioimmunoassay.

**Determination of Blood Pressure**
Systolic blood pressure was measured between 10 AM and noon by the tail-cuff method in an environmental chamber heated to 35°C, starting on the first day after surgery. Each blood pressure value is the average of 2 consistent readings. During the week before the experiments, each animal was trained daily in the environmental chamber for 30 to 40 minutes. The validity of the tail-cuff method to detect changes in blood pressure after renal artery clipping was confirmed in a separate set of experiments in sham-operated and renovascular hypertensive rats by simultaneously recording blood pressure indirectly by the tail-cuff method and directly by means of a femoral catheter (data not shown).

**RNA Analysis**
After 2, 4, or 10 days, the animals were anesthetized as described above and weighed. The hearts were removed; left ventricles were weighed and freeze-clamped with stainless steel prongs at the temperature of liquid nitrogen and stored at –80°C for analysis. RNA was extracted from ventricles pulverized under liquid nitrogen.<sup>19</sup> It was confirmed that the probes used for mRNA analysis hybridized to a single band of the appropriate molecular weight by Northern blot analysis.<sup>9</sup> For quantification, RNA was blotted to nitrocellulose in serial dilutions (4, 2, 1 μg RNA per slot) with the use of a vacuum filtration slot blot apparatus. Blots were probed consecutively with cDNA probes specific for β-MHC mRNA (plasmid containing a 69-bp sequence of rat β-MHC mRNA kindly donated by Professor Schiaffino, Padua, Italy),<sup>16</sup> ANP mRNA (plasmid containing a 145-bp, PCR-derived sequence of rat preproANP mRNA kindly donated by Professor Forssmann, Hanover, Germany), and 28 S rRNA. The isolated cDNAs were labeled by the random priming method. Prehybridization (2 hours; 40% formamide, 5× SSC, 50 mmol/L phosphate buffer, pH 7.4, 10× Denhardt’s solution, 0.2% SDS, 100 μg yeast (RNA) and hybridization (16 hours, 50% formamide, 3× SSC, 10 mmol/L phosphate buffer, pH 7.4, 2× Denhardt’s solution, 0.2% SDS, 50 μg yeast (RNA) were performed at 42°C<sup>20</sup> Blots were washed 2×15 minutes with 2× SSC, 0.1% SDS at 42°C and exposed. Between hybridizations, the probes were stripped from the blots by shaking gently 4×5 minutes in boiling 0.01× SSC, 0.01% SDS. Complete removal of the probe was confirmed by autoradiography. After measurements of mRNA levels, it was confirmed that equal amounts of RNA had been blotted by hybridization of the blots to a cDNA specific for cytosolic 28 S rRNA. Autoradiographs of the slot blots were scanned densitometrically, and tissue levels of mRNAs were expressed as arbitrary densitometric units per 28 S densitometric units, with care taken that the signal was in the linear range for all measurements. The amounts of total RNA per unit mass of left ventricular tissue were determined by a quantitative extraction procedure in which the orcinol reaction was used to measure RNA content.<sup>21</sup>
Effects of renal artery stenosis and sham operation on systolic blood pressure in saline- and ET<sub>α</sub> receptor antagonist–treated animals. Shown are data obtained from all rats in series 1. Data from each group are presented as mean±SEM. *P<0.05, clipped vs sham-operated animals. 2K1C indicates 2-kidney, 1 clip; LU, LU 127043.

Statistical Analysis
Statistical analysis was performed with the use of GRAPH-PAD PRISM software. All data obtained for each experimental variable were analyzed by 2-way ANOVA to determine whether treatment had any overall significant effects. If significance was detected, differences between groups were analyzed for each experimental day by 1-way ANOVA followed by Bonferroni’s test. Statistical comparisons of the results obtained for left ventricular weight to body weight ratios and total left ventricular RNA levels at day 10, which were combined from both experimental series, were made by the 2-tailed, unpaired Student’s t test. All data are expressed as mean±SEM. A value of P<0.05 was considered significant.

Results

Blood Pressure and Heart Rate
For the first 2 days after renal artery stenosis, systolic blood pressure remained at its control value in saline-treated animals. On the third day after clipping, blood pressure started to rise and was significantly increased by day 5 (190±11 mm Hg versus 145±4 mm Hg in sham-operated rats; P<0.05; Figure 1). Blood pressure further increased to 209±10 mm Hg on day 6 and then remained constant until day 10. A similar time course of hypertension after renal artery stenosis was found in animals treated with the ET<sub>α</sub> receptor antagonist, and the final level of blood pressure reached between days 6 and 10 was identical in ET<sub>α</sub> receptor antagonist– and saline-treated renovascular hypertensive rats (Figure 1). In both sham-operated groups, systolic blood pressure remained at constant levels over the 10-day period. Heart rate was similar in all experimental groups (Table 1).

Plasma Renin Activity
PRA rapidly increased in all animals with renal artery clamping (Figure 2). In saline-treated animals, PRA was elevated 3- to 4-fold by day 2 after clipping of the renal artery and then further increased to levels ~7-fold higher than in sham-operated animals by day 10. ET<sub>α</sub> receptor blockade did not significantly alter the activation of the circulating renin-

TABLE 1. Summary of Morphometric and Hemodynamic Data of Series 1

<table>
<thead>
<tr>
<th>Time of Death</th>
<th>Treatment</th>
<th>Number of Animals</th>
<th>Body Weight, g</th>
<th>LV Weight, g</th>
<th>Weight/BODY Weight, mg/g</th>
<th>Systolic Blood Pressure, mm Hg</th>
<th>Heart Rate, BPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2</td>
<td>Sham + saline</td>
<td>4</td>
<td>211±11.0</td>
<td>0.488±0.02</td>
<td>2.32±0.05</td>
<td>136±7</td>
<td>374±7</td>
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<tr>
<td></td>
<td>2K1C + saline</td>
<td>7</td>
<td>196±5.8</td>
<td>0.512±0.02*</td>
<td>2.61±0.09*</td>
<td>159±11</td>
<td>383±6</td>
</tr>
<tr>
<td></td>
<td>Sham + LU 127043</td>
<td>4</td>
<td>197±3.2</td>
<td>0.405±0.01</td>
<td>2.07±0.06</td>
<td>136±6</td>
<td>400±1</td>
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<tr>
<td></td>
<td>2K1C + LU 127043</td>
<td>7</td>
<td>194±5.2</td>
<td>0.434±0.01</td>
<td>2.17±0.05</td>
<td>153±7</td>
<td>362±14</td>
</tr>
<tr>
<td>Day 4</td>
<td>Sham + saline</td>
<td>4</td>
<td>211±2.9</td>
<td>0.470±0.01</td>
<td>2.23±0.07</td>
<td>146±5</td>
<td>383±9</td>
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<tr>
<td></td>
<td>2K1C + saline</td>
<td>7</td>
<td>198±4.7</td>
<td>0.524±0.01*</td>
<td>2.66±0.09*</td>
<td>164±12</td>
<td>380±9</td>
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<tr>
<td></td>
<td>Sham + LU 127043</td>
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<td>184±6.3</td>
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<td>2.22±0.06</td>
<td>146±7</td>
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<tr>
<td></td>
<td>2K1C + LU 127043</td>
<td>7</td>
<td>170±4.2</td>
<td>0.440±0.02</td>
<td>2.58±0.13</td>
<td>198±12†</td>
<td>390±9</td>
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<td>Day 10</td>
<td>Sham + saline</td>
<td>4</td>
<td>193±5.2</td>
<td>0.438±0.01</td>
<td>2.28±0.06</td>
<td>149±6</td>
<td>382±12</td>
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<td>175±6.1</td>
<td>0.564±0.02*</td>
<td>3.15±0.17*</td>
<td>216±14*</td>
<td>436±38</td>
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<tr>
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<td>Sham + LU 127043</td>
<td>6</td>
<td>186±5.1</td>
<td>0.427±0.02</td>
<td>2.29±0.08</td>
<td>145±2</td>
<td>395±15</td>
</tr>
<tr>
<td></td>
<td>2K1C + LU 127043</td>
<td>8</td>
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<td>0.465±0.02†</td>
<td>2.71±0.13†</td>
<td>218±12†</td>
<td>377±13</td>
</tr>
</tbody>
</table>

LV indicates left ventricular. Values are mean±SEM.

*P<.05, 2K1C + saline vs Sham + saline, 1-way ANOVA followed by Bonferroni’s test. †P<.05, 2K1C + LU 127043 vs Sham + LU 127043, 1-way ANOVA followed by Bonferroni’s test.
angiotensin system by renal artery stenosis, even though PRA tended to be higher on day 10 than in saline-infused clipped rats (48±9 versus 34±8 ng Ang I per milliliter per hour; P=0.3; Figure 2). In sham-operated animals, ET₁ receptor blockade had no effect on PRA.

Left Ventricular Hypertrophy

Left ventricular weight and the ratio of left ventricular weight to body weight were significantly increased by 5% and 13%, respectively, as early as 2 days after renal artery clipping in saline-treated animals (Figure 3; Table 1). No additional stimulation of cardiac growth was found on day 4, but between day 4 and day 10 the ratio of left ventricular weight to body weight further increased to a level 39% higher than that of sham-operated controls. The same degree of left ventricular hypertrophy was also observed in series 2 after 10 days of 2-kidney, 1 clip hypertension (Table 2). The early induction of left ventricular hypertrophy after renal artery stenosis was entirely blocked by ET₁ receptor blockade (Figure 3). By days 4 and 10, cardiac hypertrophy developed despite continuous ET₁ receptor blockade but remained significantly attenuated on day 10 (Figures 3 and 4A). The inhibitory effect of ET₁ receptor blockade on left ventricular growth was very similar in series 1 (251%; Table 1) and in series 2 (249%; Table 2), indicating a consistent influence of ET₁ receptor–mediated effects on myocardial growth.

β-MHC and ANP Gene Expression

Levels of mRNAs for β-MHC and ANP (normalized to 28S rRNA) were significantly elevated after renal artery clipping in saline-treated animals compared with sham-operated con-
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Figure 5. Effects of renal artery stenosis and sham operation on the ratios of \(\beta\)-MHC to 28 S cytosolic ribosomal RNA and ANP mRNAs to 28 S cytosolic ribosomal RNA in saline- and ET\textsubscript{A} receptor antagonist-treated animals. For each clipped group, the expression ratios were normalized to the mean value of the appropriate sham-operated group. Data from each group are presented as mean ± SEM. * \(P<0.05\), clipped vs sham-operated animals; † \(P<0.05\), ET\textsubscript{A} receptor antagonist–treated vs saline-treated animals with renal artery stenosis.

The activation of both genes occurred early (day 2), and mRNA levels remained relatively constant until day 10, with 1.8- to 2.8-fold increases for \(\beta\)-MHC mRNA and 1.7- to 2.7-fold increases for ANP mRNA. ET\textsubscript{A} receptor blockade completely prevented the activation of \(\beta\)-MHC and ANP gene expression on day 2 (Figure 5). On days 4 and 10, however, both genes were activated by renal artery stenosis to approximately the same degree as in saline-treated animals (1.6- to 3.2-fold increases for \(\beta\)-MHC mRNA, 1.9- to 2.9-fold increases for ANP mRNA; Figure 5) despite continued ET\textsubscript{A} receptor blockade and a significantly diminished hypertrophic response (Figures 3 and 5; see also Figure 4). In all sham-operated groups, the ratio of \(\beta\)-MHC mRNA to 28 S rRNA and ANP mRNA to 28 S rRNA decreased considerably (50% to 60%) between day 2 and day 4.

Total RNA levels increased in parallel with myocardial hypertrophy and were 1.4-fold higher on day 2 and 1.7-fold higher on day 10 after renal artery clipping in saline-treated animals compared with sham-operated controls. For each clipped group, the expression ratios were normalized to the mean value of the appropriate sham-operated group. Data from each group are presented as mean ± SEM. * \(P<0.05\), clipped vs sham-operated animals; † \(P<0.05\), ET\textsubscript{A} receptor antagonist–treated vs saline-treated animals with renal artery stenosis.

Discussion

The growth-promoting effect of Ang II on cultured neonatal cardiomyocytes has been attributed to a stimulation of the autocrine secretion of ET-1.\textsuperscript{1} In addition, mechanical stretch has been shown to directly increase the release of ET-1 from myocardial cells.\textsuperscript{7} The results of the present study show that a continuous blockade of ET\textsubscript{A} receptors significantly attenuates the development of left ventricular hypertrophy and, more transiently, fetal gene expression in the early phase of renovascular hypertension. The effects of the ET\textsubscript{A} receptor blockade on myocardial growth and fetal gene expression were particularly pronounced during the first 2 days after renal artery stenosis, when systolic blood pressure was still at its control level. Renal artery stenosis rapidly increases circulating Ang II because of the decrease of renal perfusion pressure in the clipped kidney and stimulates sympathetic activity through an activation of afferent renal nerves.\textsuperscript{22} It has been recently shown that chronic infusions of either Ang II\textsuperscript{10} or norepinephrine\textsuperscript{23} can induce left ventricular hypertrophy and fetal gene expression within 1 to 3 days in rats. These early effects of Ang II infusions were independent of changes in blood pressure,\textsuperscript{10} suggesting that they resulted from a direct action of Ang II on cardiac myocytes. Similar phenotypic changes have been observed in cultured myocytes after the addition of Ang II\textsuperscript{1,5} or catecholamines.\textsuperscript{24,25} The hypertrophic effects of Ang II on cultured myocytes could be completely blocked by either the specific ET\textsubscript{A} receptor antagonist BQ 123 or coadministration of an antisense mRNA directed against the coding region of prepro-ET-1.\textsuperscript{1} Together with these observations, the data presented here support the view that the left ventricular adaptations during the early neurohumoral phase of renovascular hypertension require intact myocardial ET\textsubscript{A} receptor–activated signaling pathways.

Neither the changes in blood pressure nor the increases in PRA after renal artery stenosis were significantly altered by ET\textsubscript{A} receptor blockade. This suggests that the inhibitory influences of the ET\textsubscript{A} receptor antagonist on left ventricular hypertrophy and gene expression were mediated by a direct effect on cardiomyocytes and were not secondary to blood pressure effects or an inhibition of the renin-angiotensin system. The development of renovascular hypertension was also found to be unaffected by chronic blockade of both ET\textsubscript{A} and ET\textsubscript{B} receptors with the nonselective ET antagonist bosentan.\textsuperscript{26} In contrast, when Ang II was administered exogenously, chronic ET\textsubscript{A} receptor blockade nearly completely prevented the increases in blood pressure.\textsuperscript{27,28} The reason for these different responses to endogenous or exogenous Ang II elevation is not clear but may be related to different kinetics of elevation of circulating Ang II or to additional mechanisms activated by renal artery stenosis. Similar to the present study, combined ET\textsubscript{A}/ET\textsubscript{B} receptor blockade did not alter the stimulation of the renin-angiotensin system after renal artery stenosis,\textsuperscript{29} indicating that chronic endothelin receptor blockade has no major effect on the renin-angiotensin system. In contrast, exogenous administration of ET-1 acutely inhibits renin release in rats in vivo\textsuperscript{30} and in vitro,\textsuperscript{31} and renal ischemia rapidly increases renal ET-1 mRNA levels.\textsuperscript{32} Furthermore, we have recently observed a potentiation of pressure-dependent renin release after acute ET\textsubscript{A} receptor blockade in conscious dogs (H. Berthold et al, unpublished data, 1998). Accordingly, compensatory mechanisms may restore the normal activity of the renin-angiotensin system during chronic endothelin receptor antagonism.

The development of left ventricular hypertrophy remained markedly blunted during ET\textsubscript{A} receptor blockade by day 10, whereas the expression of the \(\beta\)-MHC and ANP genes, which
is high in the fetal ventricle and becomes repressed during postnatal development, was only transiently affected after renal artery stenosis. In previous studies on the role of ET-1 in pressure overload cardiac hypertrophy, alterations of the hypertrophic responses induced by ET receptor antagonists were always found to be paralleled by similar changes in fetal gene expression.23,33,34 The present results suggest that the intracellular signal transduction pathways that mediate the phenotypic changes of cardiac hypertrophy may be differentially modulated by endogenous ET-1. In vitro studies have demonstrated that Ang II activates independent intracellular signaling pathways to induce an overall stimulation of protein synthesis or more specific changes in myocardial gene expression.35 The upregulation of ANP and β-MHC gene expression is mediated by an Raf-1- and MAP kinase–dependent pathway.36 Activation of this signaling cascade by Ang II has been recently shown to be strongly potentiated by ET-1.7 On the other hand, cardiac hypertrophy results from an accelerated rate of overall protein synthesis, which requires an increase of de novo ribosome biogenesis.37 ET-1 has been shown to increase the transcription rate of rDNA through phosphorylation of an rDNA transcription factor, the upstream binding factor.38 Since the transcription of rDNA constitutes the rate-liming step for ribosome biogenesis in cardiac myocytes, under certain conditions a continuous stimulation by ET-1 may be required to induce the maximal growth response. Consistent with this notion, in the present study total left ventricular RNA levels, which provide an estimate of myocardial rRNA levels, were significantly lower in clipped rats subjected to ETa receptor blockade than in saline-treated controls.

In conclusion, the present study demonstrates that the endothelin system participates in the phenotypic alterations of the left ventricular myocardium during the early phase of renovascular hypertension. Similar observations of a growth-promoting role of endogenous ET-1 have been reported recently for pressure overload–induced33,34 and norepinephrine-induced35 left ventricular hypertrophy. Together with these findings, the present observations indicate that activation of myocardial ETa receptors is a common signaling pathway that is stimulated independently by different primary growth-promoting stimuli—mechanical load, catecholamines, and Ang II—during the development of cardiac hypertrophy.

Acknowledgments

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References


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