Upregulation of Angiotensin-Converting Enzyme 2 After Myocardial Infarction by Blockade of Angiotensin II Receptors

Yuichiro Ishiyama, Patricia E. Gallagher, David B. Averill, E. Ann Tallant, K. Bridget Brosnihan, Carlos M. Ferrario

Abstract—We investigated in Lewis normotensive rats the effect of coronary artery ligation on the expression of cardiac angiotensin-converting enzymes (ACE and ACE 2) and angiotensin II type-1 receptors (AT₁a-R) 28 days after myocardial infarction. Losartan, olmesartan, or the vehicle (isotonic saline) was administered via osmotic minipumps for 28 days after coronary artery ligation or sham operation. Coronary artery ligation caused left ventricular dysfunction and cardiac hypertrophy. These changes were associated with increased plasma concentrations of angiotensin I, angiotensin II, angiotensin-(1–7), and serum aldosterone, and reduced AT₁a-R mRNA. Cardiac ACE and ACE 2 mRNAs did not change. Both angiotensin II antagonists attenuated cardiac hypertrophy; olmesartan improved ventricular contractility. Blockade of the AT₁a-R was accompanied by a further increase in plasma concentrations of the angiotensins and reduced serum aldosterone levels. Both losartan and olmesartan completely reversed the reduction in cardiac AT₁a-R mRNA observed after coronary artery ligation while augmenting ACE 2 mRNA by approximately 3-fold. Coadministration of PD123319 did not abate the increase in ACE 2 mRNA induced by losartan. ACE 2 mRNA correlated significantly with angiotensin II, angiotensin-(1–7), and angiotensin I levels. These results provide evidence for an effect of angiotensin II blockade on cardiac ACE 2 mRNA that may be due to direct blockade of AT₁a receptors or a modulatory effect of increased angiotensin-(1–7). (Hypertension. 2004;43:970-976.)

Key Words: angiotensin | receptors, angiotensin | angiotensin-converting enzyme | myocardial infarction | heart failure

The renin-angiotensin system (RAS) plays a key role in structural and functional remodeling after myocardial infarction (MI). Angiotensin (Ang) II is a major determinant in this process. Ang II stimulates cardiac hypertrophy and fibrosis in ischemic heart failure models, whereas Ang II blockade prevents development of left ventricular (LV) remodeling and hypertrophy after MI. Recently, a novel angiotensin-converting enzyme (ACE)-related carboxypeptidase, ACE 2, was identified in the human heart. ACE 2 degrades Ang I into Ang-(1–9) and Ang II into Ang-(1–7). Genetic inactivation of ACE 2 in mice resulted in severe cardiac dysfunction while cardiac and renal ACE 2 gene expression was reduced in three models of hypertension in which the ACE 2 gene mapped to quantitative trait loci previously detected by linkage analysis.

Characterization of the actions of angiotensin-(1–7) [Ang-(1–7)] demonstrated that the RAS consists of two biochemical arms: one generates Ang II via the action of ACE on Ang I, and the second generates Ang-(1–7) from either Ang I or Ang II via enzymes other than ACE. The discovery of ACE 2 and the demonstration that its catalytic efficiency is approximately 400-fold higher with Ang II as a substrate than with Ang I strengthened our hypothesis that this second arm of the system acts as a counter-regulator of the first arm. In further evaluation of this hypothesis, we determined in normotensive Lewis rats the effect of myocardial ischemia on the expression of cardiac ACE and ACE 2 both in the absence and in the presence of systemic blockade of Ang II type-1 receptors (AT₁-Rs) with losartan or olmesartan.

Methods and Materials

Animals
Male Lewis rats (250 to 300 g, Charles River Laboratory, Wilmington, Mass) were housed in individual cages (12-hour light/dark cycle) with ad libitum access to rat chow and tap water. Procedures complied with the policies implemented by our Institutional Animal Care and Use Committee.

Experimental Protocol
Seventy-eight normotensive rats (age range 8 to 10 weeks), randomly divided into 4 groups, were subjected to either sham-operation (Group I, n=20) or coronary artery ligation (CAL; n=58, Groups II-IV). Alzet osmotic minipumps (2MLA, Durect Corpora...
tion, Cupertino, Calif) were implanted subcutaneously under ketamine HCl/xylazine anesthesia (80/12 mg/kg IP, n = 67) 24 hours prior to either sham operation or administration of either vehicle or losartan. Rats randomized to olmesartan had the pump implanted within 4 hours after CAL. The vehicle (isotonic saline, Groups I and II), losartan (10 mg/kg per day, Group III), or olmesartan (0.1 mg/kg per day, Group IV) was infused continuously for 28 days after CAL. In 11 of the 58 rats undergoing CAL either the vehicle (saline, n = 4) or the AT2 antagonist (PD123319, 5 mg/kg every 12 hours, n = 7) was given by IP injection for the last 3 days of losartan administration.

The left anterior descending coronary artery was ligated between the pulmonary outflow tract and the left atrium with a 6-0 silk suture under aseptic conditions in unanesthetized rats (ketamine HCl/xylazine, 80/12 mg/kg IP; 150 mg/kg of ampicillin SC), mechanically ventilated (70 breaths/min) with room air (SAR-830/P, CWE Inc., Ardmore, Pa) through a tube inserted into their trachea. Onset of ventricular arrhythmias and the presence of myocardial Blanching distal to the suture confirmed successful ligation of the artery. After closing the thorax, rats were exsanguinated after recovery of spontaneous breathing. Sham-operated animals were intervened in the same manner but a suture was not placed around the coronary artery.

Hemodynamic measures were obtained 4 weeks after CAL or sham operation in halothane anesthetized rats (1.5%; Wyeth-Ayerst Laboratories, Gaithersburg, Md). Aortic pressure was measured with a catheter (PE-10 connected with PE-50, Clay Adams, Parsippany, NJ) inserted via a carotid artery. The catheter was later advanced into the left ventricle for measurement of left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP), the maximum rate of isovolumic pressure development (+dP/dt max) and decay (−dP/dt max), and heart rate (HR). Following collection of venous blood from the inferior vena cava, deeply anesthetized rats were euthanized by cardiopulmonary excision. The heart was rinsed in saline; the cardiac ventricles were separated from the atria, weighed, and cut transversely from apex to base. A 1 mm slice was then divided, frozen in liquid nitrogen and stored at −80°C. The left and right ventricles were fixed in formalin (4%) and paraffin embedded. Tissue sections (5 μm) and stained with picrosirius red (0.1% solution in saturated aqueous picric acid, Sigma Chemical Co., St. Louis, Mo). Infarct size, determined by planimetry, was calculated as the ratio of scar length to circumference from each of 3 slices. Measurements were expressed as a percentage of the total ventricular perimeter.

Biochemistry
Plasma concentrations of Ang I, Ang II, and Ang-(1–7) were determined by radioimmunoassay from blood collected into chilled tubes containing a mixture of 25 mmol/L ethylene-diaminetetraacetic acid (Sigma Chemical Co., St. Louis, Mo), 0.44 mmol/L 1,20-ortho-phenanthroline monohydrate, 1 mmol/L Na+ para-chloromercuribenzoate, and 3 μmol/L of WFML (rat renin inhibitor: acetyl-His-Pro-Phe-Val-Statine-Leu-Phe). Serum aldosterone concentrations were measured with a commercially available kit (Cot-A-Count Aldosterone, Diagnostic Products Corporation, Los Angeles, Calif).

Quantification of mRNA and Protein
One μg of RQ1 DNase-treated, total RNA, isolated from the noninfarcted portions of the left ventricle with the Trizol reagent (GIBCO BRL), was quantified by ultraviolet spectroscopy and reverse transcriptase-polymerase chain reaction assay was performed using the primers listed in Table 1. Amplification conditions (30 cycles) for measurements of AT1r and ACE 2 mRNAs were performed as follows: denaturation at 94°C for 60 seconds; annealing at 60°C for 60 seconds; and elongation at 72°C for 60 seconds, with a final elongation step at 72°C for 7 minutes. The ACE fragment was amplified for 32 cycles (annealing temperature of 62°C). Primers for the control EF1α sequence were added after 9 amplification cycles for AT1r and ACE 2, and after 6 cycles for ACE. Amplification products were separated on a 6% polyacryl-
respectively (P < 0.001). Activation of the RAS post-MI was also associated with a 3.6-fold rise in serum aldosterone concentration (Figure 1).

Blockade of Ang II receptors during the 28-day post-MI was associated with higher plasma levels of Ang I, Ang II, and Ang-(1–7) when compared with sham-operated or vehicle-treated CAL rats (Figure 1). Figure 1 shows that plasma Ang I levels in rats given olmesartan increased significantly above the values obtained in losartan-treated rats (P < 0.001). Plasma levels of Ang II were comparable in rats medicated with either losartan or olmesartan (P = 0.306), whereas a tendency for lower values of Ang-(1–7) in rats treated with olmesartan was not statistically significant (P = 0.06) (Figure 1). Chronic administration of either Ang II antagonist suppressed the elevations in serum aldosterone, which for the olmesartan-treated group was significantly less (P = 0.03) than for the group of rats given losartan (Figure 1). Pooled analysis of the relation between LVSP and plasma concentrations of Ang II and Ang-(1–7) showed that LVSP correlated inversely with Ang-(1–7) (r = –0.53, P < 0.001) and directly with Ang II (r = 0.73, P < 0.001).

Changes in Cardiac ACE and ACE 2 mRNA
Cardiac ACE mRNA was not different among the various experimental groups (Figure 2). Although cardiac ACE 2 mRNA did not change in MI vehicle-treated rats, both losartan and olmesartan elevated ACE 2 mRNA by an average of 97% and 42%, respectively. Myocardial AT₁-R mRNA was reduced 51% (P < 0.002) in vehicle-treated CAL rats (Figure 2). Both losartan and olmesartan treatments reversed the decrease in AT₁-R mRNA to values that were not different from those found in sham-operated rats (Figure 2). Figure 3 shows that ACE 2 mRNA was statistically correlated

Table 2. Hemodynamic Effects of Either Sham-Operation or Coronary Artery Ligation in Rats Given Vehicle or Angiotensin II Receptor Blockers

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I (Sham-Vehicle)</th>
<th>Group II (MI-Vehicle)</th>
<th>Group III (MI-Losartan)</th>
<th>Group IV (MI-Olmesartan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>373 ± 4</td>
<td>366 ± 4</td>
<td>371 ± 7</td>
<td>364 ± 5</td>
</tr>
<tr>
<td>Heart weight/body weight, mg/g</td>
<td>2.95 ± 0.03</td>
<td>3.68 ± 0.10*</td>
<td>3.15 ± 0.11†</td>
<td>3.24 ± 0.08†</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td>40 ± 1</td>
<td>43 ± 1</td>
<td>38 ± 1</td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>348 ± 6</td>
<td>300 ± 9</td>
<td>323 ± 8</td>
<td>319 ± 4</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>91 ± 1</td>
<td>74 ± 3*</td>
<td>66 ± 2†</td>
<td>62 ± 2†</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td>108 ± 1</td>
<td>89 ± 3*</td>
<td>83 ± 2</td>
<td>78 ± 2†</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>7 ± 1</td>
<td>22 ± 1*</td>
<td>17 ± 1†</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>LV +dP/dt max, mm Hg/s</td>
<td>4568 ± 156</td>
<td>2963 ± 132*</td>
<td>2596 ± 130</td>
<td>3426 ± 181‡</td>
</tr>
<tr>
<td>LV −dP/dt max, mm Hg/s</td>
<td>−4425 ± 180</td>
<td>−2603 ± 145*</td>
<td>−2397 ± 112</td>
<td>−2924 ± 175‡</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
MI indicates myocardial infarction; LV, ventricular; +dP/dt max, maximum rate of isovolumic pressure development; −dP/dt max, maximum rate of isovolumic pressure decay.
*P < 0.05 vs Sham-Vehicle, †P < 0.05 vs MI-Vehicle; ‡P < 0.05 MI-Losartan vs MI-Olmesartan.
Figure 2. Changes in the myocardial mRNA of angiotensin-converting enzyme (ACE), angiotensin-converting enzyme 2 (ACE 2), and Ang II receptor (AT₁α-R) in sham-operated (Sham-Veh) Lewis rats and those receiving either vehicle (MI-Veh) or Ang II antagonists (MI-Losartan; MI-Olmesartan) during 28 days after coronary artery ligation. Expression values for each mRNA were normalized to EF1α mRNA. Values are mean±SEM. *P<0.05 vs sham-operated animals (Sham-Veh); #P<0.05 vs rats given either vehicle (MI-Veh) or Ang II receptor blockers (MI-Losartan; MI-Olmesartan) after myocardial infarction (MI); ●P<0.05, MI-Losartan versus MI-Olmesartan.

with plasma Ang I, Ang II, and Ang-(1–7) levels. Figure 4 shows that concomitant administration of PD123319 in rats given losartan post-MI did not abolish the increase in ACE 2 mRNA and had no effect on ACE mRNA.

Figure 3. Scattergram of the relation among plasma concentrations of angiotensin I (Ang I), angiotensin II (Ang II), and angiotensin-(1–7) [Ang-(1–7)] as a function of ACE 2 mRNA.

Discussion

Left ventricular remodeling 28 days after MI was associated with cardiac dysfunction, compensatory cardiac hypertrophy, and stimulation of the RAS. We now report that the chronic phase of MI-induced cardiac remodeling decreased cardiac AT₁α-R mRNA without changes in cardiac ACE and ACE 2 mRNAs. While MI induced increases in plasma levels of Ang I, Ang II, and Ang-(1–7), examination of ratios as a function of Ang I demonstrated a relatively greater concentration of circulating Ang II compared with Ang-(1–7). Blockade of
AT1 receptors after CAL with 2 separate Ang II antagonists caused a large increase in ACE 2 mRNA to levels significantly higher than in sham- and vehicle-treated CAL rats. This increase was associated with restoration of AT1-R mRNA. In contrast, coadministration of PD123319 in rats given losartan had no effect on the increased ACE 2 mRNA.

The hemodynamic effects obtained after CAL agreed with those reported elsewhere.2,13 Loss of myocardial mass was associated with progression to heart failure characterized by increased LVEDP and decreased cardiac contractility. Several lines of evidence suggest that Ang II plays a critical role in mediating myocardial hypertrophy through direct effects on contractility, induction of growth-promoting genes, increased protein synthesis, and cell growth.1,3 In the mature heart, Ang II causes cardiac hypertrophy independent of its effect on blood pressure, whereas blockade of the RAS attenuates or reverses the cellular adaptations to pressure-overload.13,14 In contrast, Ang-(1–7) attenuated development of heart failure post-MI as well as acting as an antiarrhythmic factor during myocardial ischemia-reperfusion.15–17 Recently, we showed that cardiac myocytes, but not cardiac fibroblasts, contain a high density of Ang-(1–7) positive staining.18,19 Importantly, the myocyte content of Ang-(1–7) was significantly augmented in the functional myocardium of rats after CAL.18,19

Administration of either losartan or olmesartan caused partial reversal of cardiac hypertrophy and left ventricular dysfunction while further augmenting plasma angiotensin levels. This was associated with recovery of cardiac AT1a-R mRNA, increased cardiac ACE 2 mRNA, and no changes in cardiac ACE mRNA. Although 2 other studies20,21 found increased AT1-R mRNA levels between 24 hours and 7 days after MI, our data now suggest that this may be a transient phenomena due to the acute inflammatory response to ischemia,22 because at day 28 post-MI AT1-R mRNA was reduced by almost 50% in the noninfarcted portion of the heart. Consistent with this interpretation, cardiac AT1-R mRNA concentration and AT1-R density decreased in a canine model of pressure-overloaded hypertrophy and in human hearts post-MI.23,24 Reduction in AT1-R mRNA post-MI may be a compensatory mechanism in response to the increase in circulating Ang II and reduced plasma Ang-(1–7) levels. This interpretation agrees with the finding that AT1-R blockade reversed this process. Thus, different mechanisms may regulate AT1-R mRNA during the acute and chronic stages of ventricular remodeling post-MI.

ACE 2 is a carboxypeptidase insensitive to known ACE inhibitors.8–10 ACE 2 exhibits a high catalytic efficiency for the generation of Ang-(1–7) from Ang II since only dynorphin A and apelin 13 were hydrolyzed by ACE 2 with kinetics comparable to those of Ang II to Ang-(1–7) hydrolysis.11 Ablation of ACE 2 in mice caused severe cardiac dysfunction, a finding that suggests an important function of ACE 2 as a regulator of heart function.8 We now show for the first time that ACE 2 is unchanged during the process of ventricular remodeling post-MI but that sustained blockade of AT1-R with two different Ang II antagonists increases ACE 2 mRNA. While the data obtained with two different Ang II antagonists implicates the AT1a-R in the regulation of ACE 2 post-MI, the negative effect of coadministration of PD123319 on ACE 2 mRNA excluded the possibility that the underlying mechanism may involve a counter-regulatory effect of Ang II on AT1 receptors.13,25–27 The possibility that Ang II has a regulatory role of ACE 2 mRNA expression agrees with the demonstration that Ang II downregulates ACE 2 mRNA in cerebellar astrocytes in culture.28

A potential role of Ang-(1–7) in stimulating ACE 2 mRNA cannot be totally excluded since the increases in urinary Ang-(1–7) produced by dual inhibition of ACE and neprilysin in spontaneously hypertensive rats are accompanied by a marked rise in kidney ACE 2 mRNA.29 In our experiments, plasma Ang II and Ang-(1–7) levels correlated significantly with cardiac ACE 2 mRNA whether the data were pooled for all groups studied or selected from animals given losartan or olmesartan. Although we did not investigate the molecular stimuli accounting for the upregulation of ACE 2 after blockade of the AT1-R, the consistent and highly significant increases in ACE 2 mRNA after AT1 blockade suggests that ACE 2 may contribute to the beneficial effects of Ang II blockade after MI. Since ACE mRNA was unaffected by blockade of AT1 receptors, these data further demonstrate that ACE and ACE 2 are regulated differentially following AT1-R blockade.

We also showed for the first time that activation of the RAS after MI is associated with augmented plasma Ang-(1–7) concentrations that correlated inversely with LVSP and

![ACE mRNA](image)

![ACE2 mRNA](image)
mean arterial pressure. Loot et al\textsuperscript{17} reported that in rats systemic infusion of Ang-(1–7) preserved cardiac function, coronary perfusion, and aortic endothelial function following induction of MI while in the isolated perfused rat heart Ang-(1–7) restored contractility and reduced the occurrence of ventricular fibrillation after coronary artery occlusion.\textsuperscript{15,16} That these effects of Ang-(1–7) may reflect a paracrine or autocrine action of the peptide is suggested by our demonstration of Ang-(1–7) in myocytes of Lewis rats\textsuperscript{18,19} and the finding of intracardiac formation of Ang-(1–7) from Ang I or Ang II in the interstitium of the left ventricle of a dog.\textsuperscript{20} The demonstration that attenuation of ventricular dysfunction and remodeling was associated with further increases in circulating Ang-(1–7) that correlated with perfusion pressure provide further evidence that Ang-(1–7) may function to oppose the mechanisms stimulating cardiac remodeling post-MI.

While increased afterload sets into motion a structural and hemodynamic response of the left ventricle, other studies showed that ACE inhibitors and Ang II antagonists reversed ventricular remodeling by mechanisms independent of changes in blood pressure.\textsuperscript{2,20} In agreement with these findings, we showed that ACE 2 mRNA did not correlate with changes in either arterial or left ventricular pressures. These data suggest that the consequences of increased ACE 2 expression following blockade of Ang II receptors may affect signaling mechanisms involved in cardiac remodeling rather than ventricular hemodynamics. In support of this hypothesis, Ang-(1–7) inhibits vascular neointima proliferation by a mechanism independent of blood pressure.\textsuperscript{31}

In assessing the effects of AT\textsubscript{1}-R blockade with 2 different Ang II antagonists, we excluded the possibility that the ACE 2 response was drug-specific because uricosuric\textsuperscript{32} and inhibition of platelet aggregation\textsuperscript{33} effects of losartan have not been reported with other Ang II antagonists. Although olmesartan reduced mean arterial pressure and LVSP more than losartan, the difference was not statistically significant. However, olmesartan treatment improved left ventricular contractility, whereas losartan had no effect. Olmesartan produced significantly higher levels of plasma Ang I but comparable increases in plasma Ang II or Ang-(1–7) levels in MI-treated rats when compared with CAL rats given vehicle. In contrast, serum aldosterone inhibition was significantly higher in rats given losartan. Quantitative rather than qualitative differences in the response between the two agents may be related to the doses employed, although in these experiments both drugs were given at concentrations shown to inhibit the effects of Ang II.\textsuperscript{34}

**Perspectives**

Suppression of Ang II plays an important role in preventing ventricular hypertrophy and cardiac dysfunction after MI. Blockade of Ang II receptors after CAL reversed cardiac hypertrophy and augmented cardiac ACE 2 and AT\textsubscript{1}-R mRNA independent of its effects on blood pressure and infarct size. These data suggest that the beneficial effects of Ang II blockade on cardiac remodeling are accompanied by upregulation of AT\textsubscript{1}-R and increased expression of ACE 2. The data obtained here support the hypothesis that this second arm of the RAS acts as counter-regulator of the first arm wherein ACE catalyzes the formation of Ang II.\textsuperscript{35,36} The data further suggest that upregulation of ACE 2, through increased conversion of Ang II into Ang-(1–7) may counterbalance the vasopressor effects of ACE that are mediated by Ang II formation.

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**References**

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