Enalapril Attenuates Downregulation of Angiotensin-Converting Enzyme 2 in the Late Phase of Ventricular Dysfunction in Myocardial Infarcted Rat

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Abstract—The early and long-term effects of coronary artery ligation on the plasma and left ventricular angiotensin-converting enzyme (ACE and ACE2) activities, ACE and ACE2 mRNA levels, circulating angiotensin (Ang) levels [Ang I, Ang-(1-7), Ang-(1-9), and Ang II], and cardiac function were evaluated 1 and 8 weeks after experimental myocardial infarction in adult Sprague Dawley rats. Sham-operated rats were used as controls. Coronary artery ligation caused myocardial infarction, hypertrophy, and dysfunction 8 weeks after surgery. At week 1, circulating Ang II and Ang-(1-9) levels as well as left ventricular and plasma ACE and ACE2 activities increased in myocardial-infarcted rats as compared with controls. At 8 weeks post-myocardial infarction, circulating ACE activity, ACE mRNA levels, and Ang II levels remained higher, but plasma and left ventricular ACE2 activities and mRNA levels and circulating levels of Ang-(1-9) were lower than in controls. No changes in plasma Ang-(1-7) levels were observed at any time. Enalapril prevented cardiac hypertrophy and dysfunction as well as the changes in left ventricular ACE, left ventricular and plasmatic ACE2, and circulating levels of Ang II and Ang-(1-9) after 8 weeks postinfarction. Thus, the decrease in ACE2 expression and activity and circulating Ang-(1-9) levels in late ventricular dysfunction post-myocardial infarction were prevented with enalapril. These findings suggest that in this second arm of the renin-angiotensin system, ACE2 may act through Ang-(1-9), rather than Ang-(1-7), as a counterregulator of the first arm, where ACE catalyzes the formation of Ang II.

Key Words: angiotensin-converting enzyme ■ myocardial infarction ■ renin–angiotensin system ■ remodeling ■ cardiac function

The renin–angiotensin system (RAS) is a more complex system than originally thought. A new angiotensin-converting enzyme (ACE), ACE2, has been recently identified as a homologue of ACE.[1] ACE2 is also a metalloprotease consisting of 805 amino acids with a considerable degree of homology to ACE (40% identity and 61% similarity).[2,3] ACE2 contains a single zinc-binding domain and is a carboxypeptidase, unlike somatic ACE, which contains 2 zinc-binding domains and is a dipeptidyl carboxypeptidase.[2] Both ACE2 and ACE are bound to the plasma membrane and must be cleaved to release the soluble enzyme.[2] Their cellular and tissue distributions are also different, in that ACE is expressed in the endothelium throughout the vasculature, whereas ACE2 is distributed to most tissues, including to the heart and kidney.[3] Analyses in vitro have shown that ACE2 cleaves angiotensin (Ang) I to Ang-(1-9), which is then cleaved by ACE to Ang-(1-7). However, ACE2 also cleaves Ang II to form Ang-(1-7). Because Ang-(1-7) is a potent vasodepressor peptide, its actions could counterbalance the vasopressor effect of Ang II.[4,5] ACE2 does not act on bradykinins and its activity is not inhibited by ACE inhibitors.[2]

Although a significant activation of the RAS system occurs after myocardial infarction,[6–10] the role of ACE2 and its main products [Ang-(1-9) and Ang-(1-7)] are still poorly understood. The importance of ACE2 in regulating cardiac function has been implicated by the phenotype of the ACE2 knockout mouse, showing left ventricular dilation, impaired contractility with upregulation of hypoxia-induced genes in the heart, suggesting a link to myocardial ischemia.[11] Studies on the expression of cardiac ACE2 between 1 to 28 days after myocardial infarction and its regulation by ACE inhibitors or Ang type 1 (AT_1) receptor blockers have yielded ambiguous results.[12,13] Ishiyama et al[12] reported that the expression of cardiac ACE2 did not change after 28 days of left anterior descending coronary artery (LCA) ligation, whereas the blockade of the type I angiotensin receptor (AT1-R) caused a 3-fold increase in

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TABLE 1. Short (1 Week) and Long-Term (8 Weeks) Effects of Coronary Artery Ligation and the ACE Inhibitor Enalapril on LV Function, Hemodynamic, and Morphometric Parameters

	1 Week		8 Weeks			
Parameter	S	MI	S	E-S	MI	E-MI
No.	12	11	17	14	16	13
BW, g	205±5	217±10	389±9	341±7†‡	370±9	344±9†‡
SBP, mm Hg	114±3	116±3	121±2	101±2†‡	119±2	106±2†‡
LVW/BW, mg/g	3.75±0.18	3.48±0.13	3.12±0.09	2.55±0.07†‡	3.45±0.11†	2.86±0.10†‡
LVP, content mg	10.6±2.4	9.7±1.9	10.4±0.1	13.6±1.3	32±5.7†	14.6±2‡
LVESD, mm	4.0±0.2	5.8±0.3*	5.2±0.1	5.0±0.1	6.6±0.3†	5.6±0.5†‡
LVEDD, mm	6.5±0.2	7.43±0.3*	7.4±0.2	7.1±0.1	8.5±0.2†	6.7±0.7‡
LVFS, %	38±2	22±2*	30±2	28±2	22±2†	24±1†
LVWT, mm	1.3±0.1	1.0±0.1*	1.5±0.1	1.5±0.1	1.1±0.1†	1.1±0.1†
LV infarct size, %	NA	27±1	NA	NA	29±1	28±1

Values are mean±SEM. LVWT indicates left ventricular infarcted wall thickness; NA, not applicable.
*$P<0.05$ vs S (1 week); †$P<0.05$ vs S (8 weeks); ‡$P<0.05$ vs MI (8 weeks).

ACE2 mRNA levels.[12] In contrast, Burrel et al[13] showed that both ACE and ACE2 mRNA levels increased at day 3 and 28 after myocardial infarction, and ramipril had no effect on cardiac ACE2 mRNA, which remained elevated in all areas of myocardial infarction in the rat heart.[13]

The long-term impact of myocardial infarction on the expression and activities of ACE, ACE2, and angiotensin peptide levels are not known, and it remains to be elucidated whether conventional ACE inhibitors change these parameters. The aims of the present study were to examine the early (1 week) and late (8 weeks) effects of LCA ligation on the ventricular function, plasma and left ventricular (LV) activities of ACE and ACE2, LV ACE2 mRNA levels, and circulating angiotensins and to investigate the influence of enalapril (E) treatment on these long-term effects of myocardial infarction.

Materials and Methods

Animals

Male Sprague Dawley rats (200±10 g) were housed in cages (12-hour light/dark cycle) with access ad libitum to rat chow and water. This investigation is compliant with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication N°85 to 23, revised 1985), and it was approved by an institutional review committee.

Experimental Protocol

Normotensive Sprague-Dawley rats, aged 6 to 7 weeks, were subjected to either sham-operation (S) or LCA ligation (MI) as previously described.[14] Briefly, the LCA was ligated between the pulmonary artery outflow and the left atrium with a 7-0 silk suture under aseptic conditions in anesthetized rats (ketamine HCl/xylazine 35/7 mg/kg IP). Sham-operated animals were treated similarly except that the suture was not placed around the LCA. Myocardial infarction was confirmed by electrocardiography 24 hours after surgery. Myocardial infarction produced by ligation of the LCA induced 33% mortality within the first 48 hours after the occlusion. We validated the experimental MI through 2 additional criteria: echocardiography and histological analysis as previously described.[15,16] The size of the infarction in survivors was determined by planimetry of the endocardial circumference of LV on histological sections. The development of new Q waves in EKG leads was associated with infarct size >29±1% (mean±SEM).

Experimental Design 1: Early-Term Effects of the LCA Ligation on Some Plasma and Cardiac RAS Components and Myocardial Function

Twenty-three adult normotensive rats, randomly divided into 2 groups, were subjected to either sham operation (S, n=12) or coronary artery ligation (MI, n=11). All rats were killed after 1 week. Final body weight (BW) was 200 to 220 g (Table 1).

Experimental Design 2: Long-Term Effects of the LCA Ligation on Some Plasma and Cardiac RAS Components, Myocardial Function, and Influence of ACE Inhibition

Sixty adult normotensive rats (final BW=340 to 390 g), randomly divided into 4 groups, were subjected to either sham operation (S, n=31) or coronary artery ligation (MI, n=29). S and MI rats were randomly assigned to receive either vehicle or enalapril (E, 10 mg/kg BW per day) by gavage for 8 weeks, starting 48 hours after the induction of MI.[17] Final BW was 340 to 390 g (Table 1).

Systolic blood pressure (SBP) was determined using the tail-cuff method by investigators blinded to the treatment group. LV function was assessed by bidimensional transthoracic echocardiography using a Sonos 5000 equipped with a 5 to 12 MHz sectorial electronic ultraband S12 Philips transducer. The following echocardiographic parameters were measured: LV end systolic diameter (LVESD), LV end diastolic diameter (LVEDD), LV fractional shortening (LVFS), and LV wall thickness. The animals were weighed before euthanization, and blood was collected in prechilled tubes containing heparin or guanidine thiocyanate alone, as described below. Plasma samples were stored at −80°C and assayed within 1 week. The hearts were quickly removed and freed from atrial tissues. The LVs were weighed, washed with cold saline solution, and frozen in liquid nitrogen.

Evaluation of LV Hypertrophy

The degree of LV hypertrophy (LVH) was quantified by the relationship between LV weight (LVW), BW, LV protein (LVP) contents, and β-myosin heavy chain (β-MHC) protein levels as described previously.[14]

ACE Activity

ACE activity was measured fluorometrically after the hydrolysis of Z-phenyl-L-histidyl-L-leucine (Bachem Bioscience Inc) as described previously[18] and expressed in U/mL (1U=1 nmol L-histidyl-L-leucine/min). For assay of tissue ACE activity, 100 mg of LV were homogenized with buffer TN (Tris-HCl 50 mmol/L, pH 8.0; NaCl 1%).[18] The extract was centrifuged at 4°C, and the supernatant was recentrifuged at 15 000*g* (60 minutes at 4°C). The pellet was resuspended in buffer and centrifuged at 15 000*g* for 5 minutes at 4°C. Finally, the pellet was resuspended in TN buffer containing 8 mol/L 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS). Protein concentration was measured according to Bradford.[19] LV ACE activity was expressed in U/mg protein.

ACE2 Activity

ACE2 activity in plasma and homogenized LV was measured as described.[20] The assay is based on the use of the fluorogenic peptide substrate V (FPS V, 7-Mca-RPPGFSAFK(Dnp)-OH, R&D Systems). ACE2 re-

moves the C-terminal dinitrophenyl moiety that quenches the inherent fluorescence of the 7-methoxycoumarin group resulting in an increase in fluorescence in the presence of ACE2 activity at excitation and emission peaks of 320 nm and 405 nm, respectively. Samples containing ACE2 (up 10 μL) were incubated with 10 μmol/L FPS V, 1.2 mmol/L enalapril (to inhibit ACE activity), and reaction buffer (0.2 mol/L NaCl, 50 mmol/L Tris, 0.5 mmol/L $ZnCl_2$, pH 7.5) in a final reaction volume of 100 μL at 37°C. The change in fluorescence was monitored using a Turner TD-700 Fluorescence Reader (Molecular Devices). Total ACE2 activity was determined by subtracting the total activity in the presence of 1.2 mmol/L enalapril from the activity in the presence of 1.2 mmol/L enalapril and 10 mmol/L EDTA to chelate zinc. Specific ACE2 activity was expressed as pmol substrate converted to the product per unit time and normalized for protein content (U/mg protein) or volume (U/mL). Standard curves were generated using 0 to 50 μmol/L 7-methoxycoumarin-4-acetyl-Pro-Leu (Sigma). The specificity of ACE2 activity assay was evaluated using DX-600 (a specific ACE2 inhibitor, Phoenix Pharmaceutical).[20] Plasma ACE2 activity was inhibited by 95% with 1 μmol/L DX-600.

ACE and ACE2 mRNA Levels

DNAase-treated total RNA (1.5 μg), isolated from the non-infarcted portions of the LV with Trizol reagent, was quantified by ultraviolet spectroscopy. The RT-PCR assay was performed using the primers for ACE and ACE2 described by Ocaranza et al[18] and Ishiyama et al,[12] respectively. Amplification conditions for ACE were denaturation at 94°C for 1 minute, annealing at 60°C for 2 minutes, and elongation at 72°C for 3 minutes. For ACE2, amplification conditions were denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and elongation at 72°C for 1 minute. The cycle numbers for ACE and ACE2 were 30 and 40, respectively. Finally, an elongation at 72°C for 10 minutes was performed. After PCR, the amplification products were fractionated on a 1.5% (w/w) agarose gel and visualized by staining with ethidium bromide. Band intensities were quantified by computerized densitometry and normalized with respect to 18S RNA.

Plasma Angiotensins

Rats were anesthetized with a combination of ketamine (125 mg/kg) and xylazine (12.5 mg/kg) administered by intraperitoneal injection. Blood was collected from the inferior vena cava directly into a syringe containing 5 mL of 4 mol/L guanidine thiocyanate using a 25-gauge needle. Plasma for angiotensin peptide measurement was stored at −80°C until extracted with C18 Sep-Pak cartridges, and the peptides were acetylated according to the method of Campbell et al.[21] Acetylated Ang II, Ang I, Ang-(1-7), and Ang-(1-9) were assayed by use of high-performance liquid chromatography–based radioimmunoassay with amino-terminal-directed antisera kindly donated by Dr. DJ Campbell (St. Vincent's Institute of Medical Research, University of Melbourne, Fitzroy).[21]

Statistical Analysis

Results are shown as means±SEM. The differences in continuous variables were evaluated by *t* test or ANOVA analysis. Pearson and Spearman correlation analysis was used to assess the relationship between 2 variables. $P<0.05$ was considered statistically significant.

Results

Hemodynamic and Morphometric Parameters and Left Ventricular Function

Table 1 summarizes BW, SBP, LVW/BW ratio, and LVP contents at death in the S and MI groups. In the MI group, LVW/BW ratio and LVP contents increased significantly at week 8, but not at week 1 after LCA ligation. As shown in Figure 1A and 1B, β-MHC protein levels were also increased in the MI group at week 8. BW was slightly depressed after week 8 post–myocardial infarction (5% decrease below the S group). There was, however, no change in SBP between these experimental groups. Functional studies performed in hearts showed significant increases in the LVESD and LVEDD indexes in the MI groups as compared with the S groups, with concomitant decreases in the LVFS and LV wall thickness

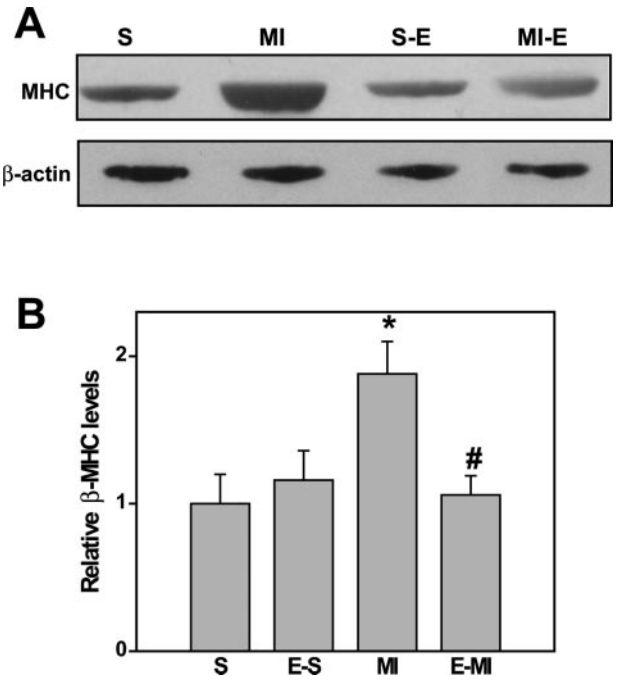

Figure 1. Long-term (8 weeks) effect of coronary artery ligation and the ACE inhibitor enalapril (E) on LV β-MHC protein levels. Cardiac samples were collected after 8 weeks in S rats, MI rats, and S or MI rats treated with enalapril (E-S, E-MI); β-MHC and β-actin protein levels were determined by Western blot as described in Material and Methods. The digitalized images were obtained by scanning the films (A). Then they were analyzed by the UN-SCAN-IT program software, and the values were expressed as fold over S (B). Data are mean±SEM. *$P<0.05$ vs S, and #$P<0.05$ vs MI.

values at both time points. LVW/BW ratio and SBP values decreased in the E-S and E-MI groups as compared with the MI (8 weeks) and S (8 weeks) groups. Enalapril also significantly decreased LVP and β-MHC protein levels in comparison to the MI (8 weeks) group (Table 1 and Figure 1A and 1B). Enalapril prevented the effect of LCA ligation on LVESD and LVEDD but not in LVFS and LV wall thickness parameters. The average infarct size was similar in the MI (1 week), MI (8 weeks), and E-MI groups.

Circulating and Left Ventricular ACE and ACE2 Activities

Figures 2 and 3 show ACE and ACE2 activities in plasma and LV extracts in MI and S rats. At week 1 after surgery, plasma ACE and ACE2 activities in the MI group were significantly higher than those in the S group (Figure 2). At week 8, however, plasma ACE2 activity was 45% less in the MI group than in the S group (Figure 2). As depicted in Figure 3, LV ACE activities increased significantly at weeks 1 and 8 post–myocardial infarction (63% and 100% increase above the S group values). Figure 3 also shows that LV ACE2 activities increased and decreased significantly at weeks 1 and 8 post–myocardial infarction (14% increase and 27% decrease above and below the sham-operated groups). As shown in Figure 4A and 4B, LV ACE and ACE2 mRNA levels increased and decreased 372% and 36%, respectively, in the MI (8 weeks) group, as compared with the corresponding control group. LV ACE2 mRNA levels positively correlated with ACE2 activity (Spearman

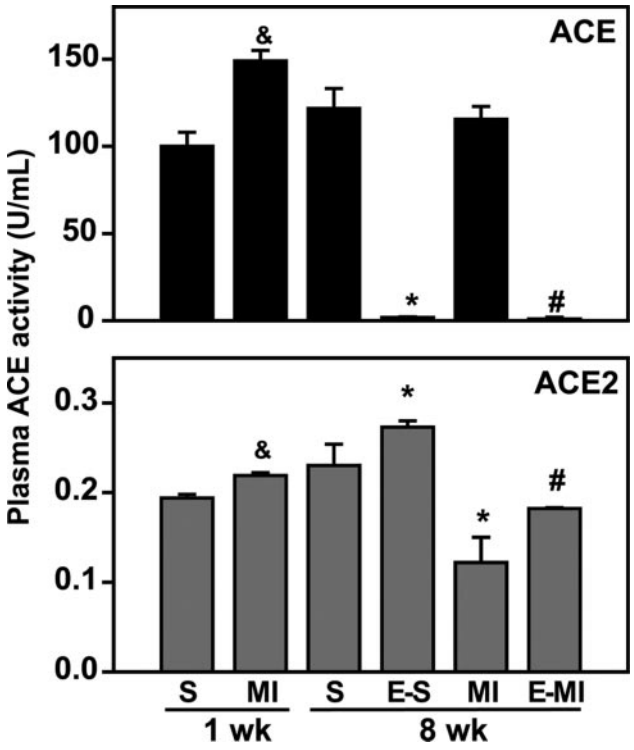

Figure 2. Early (1 week) and long-term (8 weeks) effects of coronary artery ligation and the ACE inhibitor enalapril (E) on plasma ACE and ACE2 activities. Plasma samples were collected after 1 or 8 weeks in S rats, MI rats, and S or MI rats treated with enalapril (E-S, E-MI), and ACE activities were determined as described in Material and Methods. Data are mean±SEM. &$P<0.05$ vs S (1 week), *$P<0.05$ vs S (8 weeks), and #$P<0.05$ vs MI.

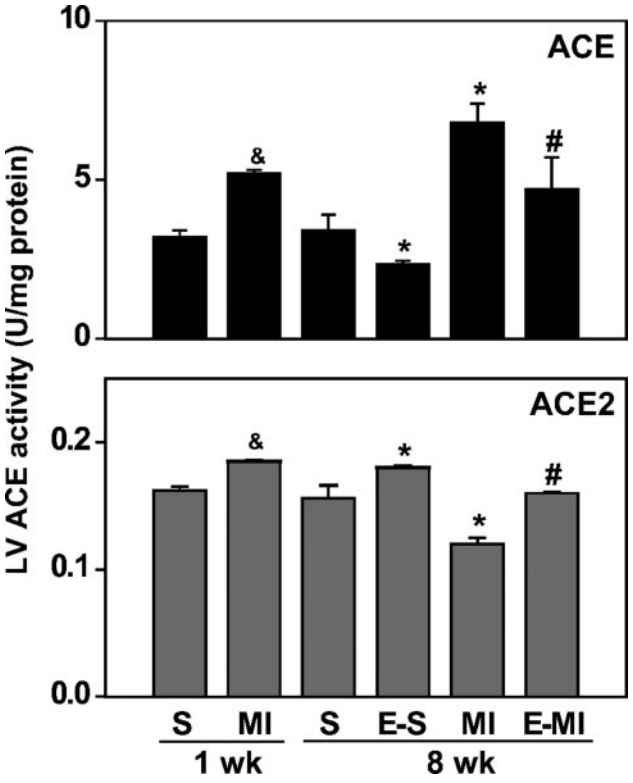

Figure 3. Early (1 week) and long-term (8 weeks) effects of coronary artery ligation and the ACE inhibitor enalapril (E) on LV ACE and ACE2 activities. Samples were collected after 1 or 8 weeks in S rats, MI rats, and S or MI rats treated with enalapril (E-S, E-MI), and tissue ACE activities were determined as described in Material and Methods. Data are mean±SEM. &$P<0.05$ vs S (1 week), *$P<0.05$ vs S (8 weeks), and #$P<0.05$ vs MI.

r=0.561, $P<0.04$). Enalapril completely prevented the changes induced by the 8-week LCA ligation on the ACE and ACE2 in the LV as well as in plasma ACE2 (Figures 2 and 3). Similarly, the myocardial infarction–induced upregulation and downregulation for ACE mRNA and ACE2 mRNA, respectively, was significantly prevented by enalpril (Figure 4).

Plasma Angiotensin Levels

Both at weeks 1 and 8 after LCA ligation, MI rats had lower plasma Ang I levels, associated with higher plasma Ang II levels and Ang II/Ang I ratio. In contrast, plasma Ang-(1-7) concentrations were not different from those of sham-operated rats (Table 2). At week 1, MI rats showed increased circulating Ang-(1-9) levels and Ang-(1-9)/Ang I ratio, whereas the plasma levels of Ang-(1-9) in the MI group at 8 week were not different from the controls. As shown in Table 2, the treatment of MI rats for 8 weeks with enalapril modified the effects of LCA ligation on circulating levels of Ang I, Ang II, and Ang-(1-9) levels. In S rats, enalapril increased and decreased circulating levels of Ang-(1-9) and Ang II, respectively (Table 2). The values of the ratios on Ang-(1-9)/Ang I and Ang-(1-7)/Ang II increased, whereas the Ang II/Ang I ratio decreased in the E-MI group as compared with the MI group (Table 2).

Discussion

Our main findings were: (1) circulating and LV activities of ACE2 are downregulated in the long-term phase of LV dysfunction in MI rats, being prevented by a conventional ACE inhibitor; (2) in contrast, both in plasma and in LV, ACE and ACE2 activities increased early (1 week) after LCA occlusion; (3) plasma Ang-(1-9) levels significantly increased when MI rats or sham-operated rats were treated with enalapril for 8 weeks; and(4) circulating Ang-(1-7) levels did not change in any phase after myocardial infarction. Taken together, these data suggest a significant interaction between ACE and ACE2 in the late phases of dysfunctional infarcted heart.

After the occlusion of the LCA, we distinguished 2 functional and neurohormonal states. The earliest was characterized by LV dysfunction without development of LVH and with a substantial activation of the circulating and cardiac RAS. Our experiments confirmed that some RAS components are increased or activated.[6,8,10] Plasma and LV ACE activity and circulating levels of Ang II were higher at week 1 after myocardial infarction. Several studies have shown that Ang II plays a critical role in myocardial remodeling through direct effects on contractility, induction of growth-promoting genes, increased protein synthesis, and cell growth.[22–24] In this study, we showed that circulating and LV ACE2 activities are significantly increased. Ferrario's group was the first to investigate the regulation of ACE2 after myocardial infarction,[12] describing no effect of myocardial infarction on ACE2 mRNA levels on day 28 as compared with sham-operated rats. In contrast, Burrell et al[13] have recently described a marked myocardial expression of ACE and ACE2 in injured tissues as compared with the viable area at day 3 after myocardial infarction.[13] Whereas, by day 28, increases, relative to controls, in both ACE and ACE2 mRNA were observed in the viable myocardium of MI rats. Our data are fully consistent with these latter observations.

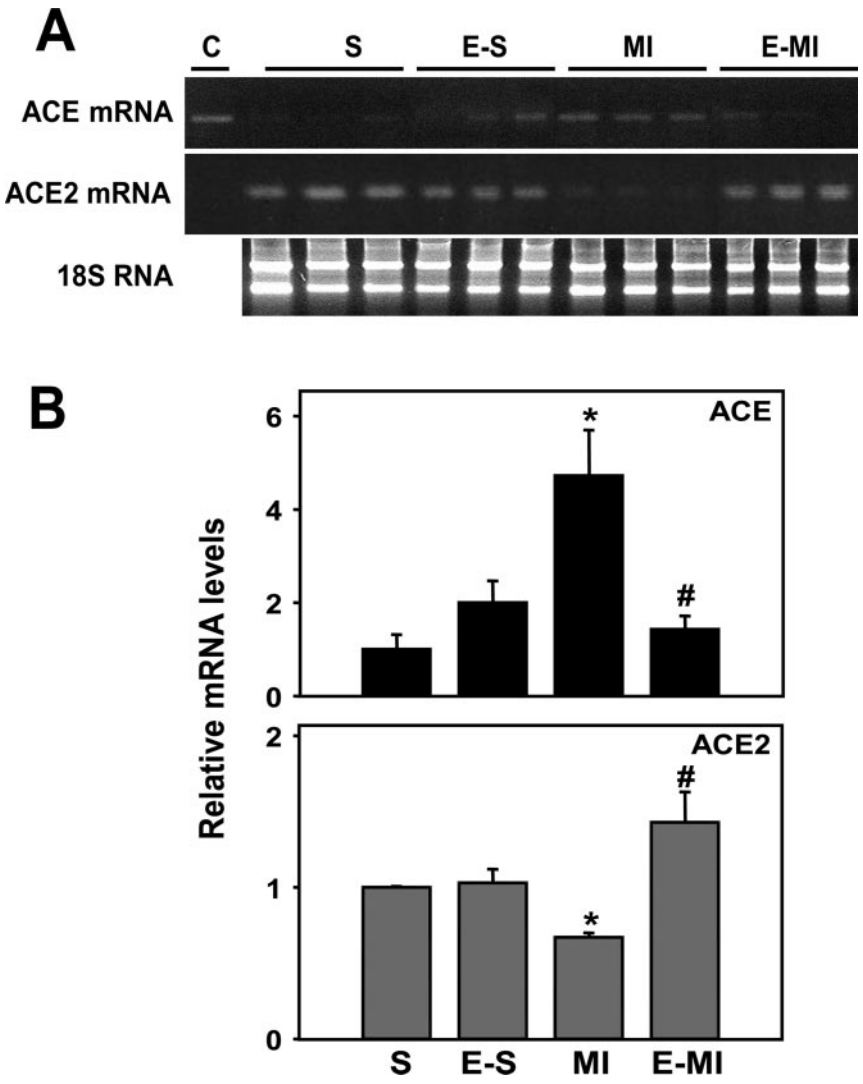

Figure 4. Long-term (8 weeks) effects of coronary artery ligation and the ACE inhibitor enalapril (E) on LV ACE2 mRNA levels. A, Samples were collected after 8 weeks in lung (C, control tissue for ACE) or from hearts obtained from S rats, MI rats, and S or MI rats treated with enalapril (E-S, E-MI). DNAase-treated total RNA was isolated with Trizol, and the integrity of 18S RNA was assessed on agarose gel (A). Band intensities were quantified by computerized densitometry and normalized with respect to 18S RNA (B). Data are mean±SEM. *$P<0.05$ vs S, and #$P<0.05$ vs MI.

Unlike ACE, ACE2 exhibits a high catalytic activity in the generation of Ang-(1-7) from Ang II or in the production of Ang-(1-9) from Ang I (reviewed in Reference 25). To confirm the significance of circulating ACE2 in our studies, we determined the plasma concentrations of Ang-(1-7), Ang-(1-9), and Ang II. Our data showed that the circulating levels of Ang-(1-9), but not Ang-(1-7) levels, increased 1 week after myocardial infarction with respect to sham-operated rats. These findings were correlated with a higher ACE2 activity in plasma and in the LV. Two reports from Ferrario's group have shown that cardiac myocytes exhibit intense Ang-(1-7)–positive staining and that the content of Ang-(1-7) seemed to be significantly upregulated in the functional myocardi-

TABLE 2. Early (1 Week) and Long-Term (8 Weeks) Effects of Coronary Artery Ligation on Circulating Levels of Ang I, Ang II, Ang-(1-7), and Ang-(1-9)

	1 Week		8 Weeks			
Parameter	S	MI	S	E-S	MI	E-MI
No.	12	11	12	11	12	12
Ang I, fmol/g	37.2±4.0	21.6±2.0*	28.4±4.0	32.9±1.1	16.2±2.2†	34.9±4.3‡
Ang-(1-7), fmol/g	5.5±1.0	4.2±0.4	4.1±0.8	3.4±0.9	3.2±0.4	4.4±1.3
Ang-(1-9), fmol/g	4.0±0.8	8.4±0.9*	5.2±0.8	27.4±8.5†	5.6±0.7	32.2±9.2†‡
Ang II, fmol/g	21.7±3.0	44.1±6.0*	23.5±3.0	17.5±1.2†	31.3±2.1†	20.6±4.7‡
Ang II/Ang I	0.58±0.1	2.04±0.31*	0.83±0.10	0.53±0.09†	1.93±0.21†	0.59±0.08†‡
Ang-(1-9)/Ang I	0.11±0.01	0.43±0.05*	0.19±0.02	0.83±0.09†	0.37±0.05†	0.92±0.10†‡
Ang-(1-7)/Ang II	0.24±0.07	0.11±0.01*	0.16±0.02	0.19±0.01	0.10±0.01†	0.21±0.03†‡

Values are mean±SEM.
*$P<0.05$ vs S (1 week); †$P<0.05$ vs S (8 week); ‡$P<0.05$ vs MI (8 weeks).

um of rats after myocardial infarction.[26,27] This last observation is not consistent with our findings on circulating Ang-(1-7) levels, neither with our changes in circulating and LV ACE2 activity nor with unchanged cardiac ACE2 mRNA levels reported by Ishiyama et al after 28 days post–LCA ligation.[12] Almost all attention has been focused on Ang-(1-7) instead of Ang-(1-9). It has been proposed that Ang-(1-7) may counteract the effects of Ang II through actions on stimulation of NO synthase and bradykinins.[28] Ang-(1-7) seems to have effects on the peripheral cardiovascular system and myocardium.[29–31] Our data demonstrated that circulating levels of Ang-(1-9) increased significantly after 1 week post–myocardial infarction. However, it remains to be seen whether Ang-(1-9) itself has a direct biological effect on cardiac cells or indirect actions through the generation of different metabolites from this peptide. In humans, ACE2 catalytic efficiency is 400-fold higher with Ang II as a substrate than with Ang I.[4] Campbell et al have described the failure of Ang-(1-9) levels to increase in response to increased Ang I levels in human coronary circulation, suggesting a minor role for ACE2 in Ang I metabolism.[32] However, the levels of Ang-(1-7) were also more linked to those of Ang I than to Ang II.[32] Taken together, all this evidence is more consistent with the formation of Ang-(1-7) by endopeptidase-mediated metabolism of Ang I rather than by ACE2-mediated metabolism of Ang II.[32]

In the long-term phase of our model, LVH development was associated with marked myocardial dysfunction assessed by a reduction in LVFS and increases in LVESD and LVEDD parameters. These effects were similar to those described by Johnston et al[16] The ratio lung weight/body weight was similar between the MI and sham groups, implicating no congestive chronic heart failure (data not shown). There are no studies assessing the relationship between ACE and ACE2 activities during the late phase of cardiac remodeling and dysfunction associated with myocardial infarction in the rat. Our results showed that the systemic RAS was still activated at week 8 after myocardial infarction. Plasma and LV ACE activities and circulating levels of Ang II were significantly higher in the 8-week MI rats than in the corresponding controls. The ratio Ang II/Ang I was consistent with this observation. However, the most novel result was the significant decrease in ACE2 activity both in plasma and in the LV at the long-term-phase post–myocardial infarction. These results were different from those detected at short-term-phase post-LCA and from the previous work of others, who evaluated ACE2 expression within the time frame of 1 to 28 days after myocardial infarction.[12,13] Decreased ACE2 activity correlated with a downregulation on ACE2 mRNA levels, however circulating concentrations of Ang-(1-9) were similar to controls. Again, no changes in plasma levels of Ang-(1-7) were observed during this time.

The beneficial effects of ACE inhibitors in slowing the deterioration of the failing heart are at least in part, attributable to the prevention of downstream events for Ang II formation, such as elevated vascular tone, heart and vessel remodeling, and salt and water retention.[23] Our results are consistent with the previous findings[33] reporting that ACE inhibitors did not improve fractional LV shortening but attenuated myocardial hypertrophy and remodeling in this experimental model.[34,35] A new pharmacological mechanism of action for ACE inhibitors has recently been proposed, based on the observations that ACE also degradates Ang-(1-7).[36,37] Ferrario's group found a significant increase in ACE2 mRNA levels after AT1-blockade for 4 weeks in rats after myocardial infarction.[12] More recently, using spontaneously hypertensive rats, this group described that losartan increases both ACE2 mRNA levels and ACE2 activity whereas ACE inhibitors only upregulated ACE2 mRNA without changes in enzymatic activity.[38] Our data clearly showed an influence of the ACE inhibitor enalapril on ACE2 in plasma and LV, which was not found by Burrell et al using ramipril.[13] It remains unclear whether this ACE inhibitor-dependent increase in ACE2 activity was directly attributable to its pharmacological action or to improved cardiac function through Ang II suppression.

Our data also showed that the levels of plasma and cardiac ACE2 could be regulated at a transcriptional level. However, future experiments should clarify whether this effect was a consequence of a lower transcription in the ACE2 gene or increased degradation of ACE2 mRNAs. ACE also hydrolyzes bradykinins, potent vasodilators, and cardioprotective agents. Because bradykinins are more readily hydrolyzed by ACE than Ang I,[39] the net therapeutic effect of ACE inhibitors may reflect both diminished Ang II and increased bradykinin levels. Further work using specific antagonists for AT_1 or BK1 receptors, alone or combined with ACE inhibitors, will help elucidate the role of Ang-II, bradykinin, and Ang-(1-9) on the regulation of the expression and activity of ACE2. The beneficial effects of ACE inhibitors can be also attributed to the activation of a distinct ACE signaling cascade rather than to the changes in Ang II and bradykinin levels.[40] An alternative mechanism to explain our novel findings of ACE inhibitors on ACE2 and Ang-(1-9) levels could be related with the direct effects of ACE inhibitors on ACE itself[41] and may also explain why enalapril did not provoke the same effects than ramipril.

The main limitations in the present study are: (1) We failed to differentiate between the infarct area and the viable myocardial tissues. Burrell et al described tissue- and cell-specific alterations of ACE2 expression, and these differences were observed to be time-dependent.[12] (2) We did not also measure cardiac angiotensins that could have an independent regulation other than the circulating endocrine RAS. (3) Although we studied the short- and long-term effect of LCA on heart function and some components of RAS using animals at the same initial state of development and corresponding controls, we cannot discard an age factor in our study. The adult rats fully grown for a 1 week time point could have also been used. However, Deten et al have established that heart function and molecular biological parameters were comparable in young adult and aged rats after chronic myocardial infarction.[41]

Perspectives

We observed a selective decrease in the expression and activity of ACE2 in the late myocardial remodeling after myocardial infarction, associated with myocardial dysfunction. Importantly, these effects were prevented with enalapril. These findings support the hypothesis that, in this second arm of the RAS, ACE2 through Ang-(1-9) instead of Ang-(1-7), could act as a counterregulator of the first arm, where ACE catalyzes the formation of Ang II.

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Disclosures

None.

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