Scar and pulmonary expression and shedding of ACE in rat myocardial infarction

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Received 28 September 2001; accepted in final form 13 March 2002

Gaertner, Roger, Fabrice Prunier, Monique Philippe, Liliane Louedec, Jean-Jacques Mercadier, and Jean-Baptiste Michel. Scar and pulmonary expression and shedding of ACE in rat myocardial infarction. Am J Physiol Heart Circ Physiol 283: H156–H164, 2002. First published March 21, 2002; 10.1152/ajpheart.00848.2001.—We examined the topology of angiotensin-converting enzyme (ACE) mRNA expression, activity, and shedding in myocardial infarction-induced heart failure and sought to elucidate the source of the increased plasma ACE activity in this model. Three months after coronary ligation, lung, scar, and remaining viable left ventricular tissues were analyzed for ACE mRNA expression as well as tissue and solubilized ACE activity. ACE mRNA expression increased in the scar with respect to infarct severity, decreased in the lung, and remained unchanged in the left ventricle. ACE activity decreased in the lung and increased in the scar tissue and plasma. Shedding of ACE remained constant in the lung and increased in the scar. This study shows that ACE expression and activity is shifted from the pulmonary endothelium to the infarct scar tissue and that constancy of shedding in the lung and its increase in the scar are the source of the increased plasma ACE in congestive heart failure.

experimental heart failure; cardiac remodeling

EXPERIMENTAL MYOCARDIAL INFARCTION (MI) decreases left ventricular (LV) pumping ability by impairing both systolic and diastolic function. The absence of contractility in the infarcted region translates as a hemodynamic stress exerted on the scar tissue during systole (28, 33). Formerly thought to be inert, experiments show that the fibrous scar is a dynamic remodeling tissue (40) responding to systolic hemodynamic forces, which perhaps plays an active role not only in its own scarification but also in that of the remaining myocardium (12). In parallel, the increase in LV diastolic pressure has some early upstream consequences on the pulmonary circulation, including increase in pressure and vascular dimensions and decrease in endothelial shear stress (13). This decrease in shear stress on pulmonary endothelium leads to some endothelial dysfunction such as decrease in endothelial nitric oxide synthase expression (13) and increase in endothelin secretion (25).

Angiotensin-converting enzyme (ACE) is a ubiquitous ectoenzyme responsible for the conversion of the inactive angiotensin I (ANG I) to the active ANG II, an important mediator of myocardial and vascular remodeling. Inhibition of ACE has proven to be effective in decreasing mortality (34) and preventing remodeling (32, 43) in MI. ACE is an integral transmembrane protein, present also in the plasma in a solubilized form, shed by a zinc metalloprotease that cleaves the membrane-bound form in the stalk region (15, 30). In physiological conditions, the pulmonary endothelium is considered to be the main source of the plasma solubilized ACE (14). Nevertheless, in pathological conditions, ACE gene expression can shift from a predominant and constitutive endothelial expression to a more inducible expression in nonendothelial cells, providing evidence of cell activation, including smooth muscle cells and fibroblasts in response to hemodynamic forces (1, 3, 20) and monocytes during macrophage transformation (42). In a previous study, we (22) showed a decrease in ACE expression in the lung and an increase in its plasma concentration correlated with the severity of MI-induced heart failure in rats. Moreover, the presence of ACE in the scar (16, 24) mainly expressed by myofibroblasts (39) has been previously documented by in situ approaches.

In the present study, we further investigated the discrepancy between pulmonary and plasma ACE in heart failure in rats. We hypothesized that the increase in plasma ACE could be the result of a shift in ACE expression from the pulmonary endothelium to the scar and that the phenomenon of shedding could be responsible for the increased plasma ACE. We therefore examined ACE activity both in the tissues as well as in the supernatant of tissue explants, allowing us to investigate the shedding, and we compared ACE mRNA expression in the lung and scar, permitting us to explore the shift from a physiological to a pathological site of expression. Results were compared not only between MI and sham rats but also between the two

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First published March 21, 2002; 10.1152/ajpheart.00848.2001.

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basic classifications of MI rats: compensated and decompensated, the latter representing rats with a greater infarct severity.

METHODS

Experimental Design

Normotensive male Wistar rats (CERJ) were used in the study. Rats were housed in a temperature- and humidity-controlled environment on a 12:12-h light-dark cycle. Rats were fed standard rat chow and water ad libitum. MI of the LV was obtained by ligation of the left descending coronary artery while the rats were under general anesthesia (1 ml/kg ip ketamine [Imalgène 500, Merial] and 0.5 ml/kg ip xylazine [2% Rompun, Bayer]) and positive pressure ventilation, as described by Fishbein et al. (17). Sham-operated rats underwent the same surgical procedure with the exception of coronary ligation. Rats were examined by echocardiography during the week preceding the death. After 3 mo, all animals were euthanized under heavy anesthesia with the use of 6% pentobarbital sodium (70 mg/kg, Sanofi). The experimental protocol is in accordance with the Guide for the Care and Use of Laboratory Animals (Authorization No. 00577). The protocol is in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and performed under the authorization of the French Accreditation of Laboratory Animal Care (Authorization No. 00577).

Blood was collected in 10-ml prechilled tubes on citrate (11 mM) and the plasma separated by centrifugation at 2,500 g for 10 min at 4°C and frozen until used. The tissues were either immediately frozen in liquid nitrogen for mRNA analysis or put into sterile 15-ml polypropylene tubes containing 2-ml sterile RPMI without methionine or glutamine (Bio Media) for subsequent incubation. The heart was excised and weighed. The LV was removed and the scar (in infarcted rats) was carefully separated. Both were weighed and either frozen in liquid nitrogen or collected in sterile 15-ml polypropylene tubes as described above.

Echocardiographic Analysis

Echocardiography was performed 1 wk before death with the use of a commercially available echocographic system (Toshiba 6000, SSA 370A) equipped with a 14-MHz linear probe. Rats were lightly anesthetized with inhaled isoflurane, the chest was shaved, and the animals were placed in the supine position. Electrocardiographic electrodes were placed on inferior legs and on the neck for timing of intracardiac events by use of the single-channel electrocardiogram available on the imaging system. The interrogation depth was set at 3 cm.

Two-dimensional echocardiography. The LV was imaged in both parasternal long-axis and short-axis views at a frame rate of 120 Hz and transferred online to a computer as dynamic loops of 50 frames, allowing for off-line analysis (Ultrasound Image Workstation 300A, Toshiba). End-diastolic area was defined as the largest LV area and end systolic as the smallest area. Ejection fraction (EF) was calculated off-line by the modified Simpson’s method.

M-mode. M-mode tracings were recorded at the level of the papillary muscles and aortic valves with two-dimensional image guidance (Fig. 1). LV and left atrial (LA) cavity diameters were measured according to the recommendations of the American Society of Echocardiography (35).

Interobserver reproducibility of echographic parameters was assessed in all rats. Variability was expressed as the mean percent error derived as the absolute difference between the two measurements divided by the mean of the measurements (Table 1).

Tissue Explant Incubation

Incubation of tissue explants was performed under sterile conditions in FB-24 plaques containing 1-ml colorless RPMI with 1% penicillin-streptomycin-amphotericin B and 2% HEPES. Incubation lasted 24 h at 37°C, 5% CO₂. Supernatants were centrifuged (1,000 g for 15 min) and frozen at −20°C until used. The tissues were weighed and frozen at −20°C.

Infarct size determination. The surface area of the scar tissue was determined by tracing its circumference on millimeter graph paper, which was then cut and weighed (10).

Evaluation of mRNA Expression by Reverse Transcriptase-Polymerase Chain Reaction

Total RNA was extracted from LV, lung, and scar tissues using the method described by Chomczynski and Sacchi (11). RNA was quantified by optical density at 260 nm. Samples were migrated by electrophoresis (80 mV) to control the quality of the extracted RNA. For reverse transcription, 1 μg mRNA was used for the LV and scar and 500 ng for the lung. The mRNA was primed with 1 μg oligo-dT (Pharmacia Biotech) and incubated with Moloney murine leukemia virus reverse transcriptase (GIBCO) for 1 h at 37°C. Amplification of the cDNA was performed under the following conditions: 1.25 U Taq polymerase (GIBCO), 20 mM Tris-HCl (pH 8.0), 50 mM KC1, 0.2 mM 2-deoxynucleotide 5’-triphosphate, 10 pmol primers (ACE), 50 pmol glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 1.5 mM MgCl₂, 4 x 10⁵ counts/min [³²P]-labeled primer (NEN Life Science Products), and 3 μl cDNA in a 25-μl final volume. A thermal cycler (Techne) was used for amplification. For ACE, the primers used were 5’-AGAAGGCAAGGAGCTGATG-3’ (sense) and 5’-GACAAAGCCATGGAGGCTCAG-3’ (antisense), 64°C annealing temperature and 29 cycles for the LV, 29 for the lung, and 32 for the scar. For GAPDH, the primers used were 5’-GTGAAGTCCGAGTTCAACG-3’ (sense) and 5’-GGTGGAAGGCACGGTCTCTC-3’ (antisense), 55°C annealing temperature, and 22 cycles for the LV, 28 for the lung and 23 for the scar. Polymerase chain reaction (PCR) fragments were analyzed by 8% polyacrylamide gel electrophoresis and visualized by ethidium bromide staining. The bands were cut and digested with periodic acid at 25 mM for 2 h at 50°C and counted with a RackBeta scintillation counter after the addition of a scintillation solution (OptiPhase “HiSafe” 3, Wall). PCR amplification was verified to be exponential, and the amplification products were proportional to sample input. ACE mRNA expression was calculated by normalizing with respect to GAPDH mRNA expression.

ACE Activity Determination

Plasma and explant supernatants were tested directly, whereas an extraction was performed on tissues. Tissues were homogenized in Tris-HCl (pH 7.4) by potterization and subsequently centrifuged at 1,000 g for 10 min at 4°C. The supernatant was collected and centrifuged at 15,000 g for 10 min at 4°C. The supernatant was discarded and the pellet redissolved in 1 ml Tris-HCl with 8.5 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) (no CHAPS was added for the lung tissue). Samples were sonified for 20 s before being used.

The fluorometric assay protocol was based on the technique described by Freidland and Silverstein (18). Samples
were incubated for 10 to 60 min at 37°C in the presence of z-Phe-His-Leu-OH (Bachem) and Na-Tris-HCl (50 mM HCl, 1% NaCl, pH 8) in a 250-µl final volume. A negative control was included for each sample by addition of 25-µl enalaprilat (10⁻⁵ M). All controls and samples were done in duplicate. The reaction was stopped by the addition of 10% trichloroacetic acid (ProLabo) and centrifugation at 2,500 rpm for 10 min at 4°C. The supernatant was recuperated and incubated for 10 min at 37°C after addition of NaOH (0.28N) and o-phthaldialdehyde (Sigma). HCl (2N) was added to stop the reaction. Fluorometric measurements were done at 365 nm excitation and 500 nm emission (F-2000 Fluorescence Spectrophotometer, Hitachi).

Some tissue explants were incubated in the presence of GM-6001 (Calbiochem), a nonspecific metalloprotease inhibitor previously shown to prevent shedding of other integral membrane proteins (23, 38). Previous experiments in our laboratory indicated that a concentration of 1 mM GM-6001 inhibited the apparition of ACE activity in the explant supernatant, whereas not affecting ACE activity itself. These incubations were performed under the same conditions as previously described.

ACE activity in the tissue explant supernatants was normalized with respect to the total protein content in the incubated explant for the lung and total protein in the supernatant for the LV and scar, and in the tissues with respect to total protein (assayed using Bio-Rad total protein assay).

**Run-On Assay**

The transcription rate of the ACE gene was determined by a run-on assay, as previously performed in the laboratory (9). Lung nuclei were isolated at 4°C as described by Boggaram et al. (6). In vitro transcriptions were carried out in the pres-

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### Table 1. Interobserver variability and feasibility of Doppler parameters

<table>
<thead>
<tr>
<th></th>
<th>LVEDD</th>
<th>LA Diameter</th>
<th>EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feasibility observer 1, %</td>
<td>95.4</td>
<td>96.9</td>
<td>86.2</td>
</tr>
<tr>
<td>Feasibility observer 2, %</td>
<td>93.8</td>
<td>98.4</td>
<td>83.1</td>
</tr>
<tr>
<td>Interobserver error, %</td>
<td>3.7 ± 0.6</td>
<td>8.3 ± 1.7</td>
<td>7.3 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVEDD, left ventricular end-diastolic dimension; LA, left atrial; EF, ejection fraction.
ence of $4 \times 10^7$ isolated nuclei and [α-32P]CTP, as described by Mak et al. (27) with slight modifications. cDNA (10 μg) encoding for ACE and GAPDH, amplified in Bluescript KS plasmids and pGEM plasmids, respectively, were denatured and immobilized on a nylon filter (Hybond-N, Amersham). Empty plasmids were used as negative controls. Slot blots were then hybridized with equivalent amounts of radioactive RNA ($7 \times 10^6$ counts/min) obtained from lungs of myocardial rats or control rats. After hybridization, the blots were washed and exposed to Kodak Biomax MS film with an intensifying screen at −80°C for 7 days. The intensity of the band is proportional to the in vitro transcription rate.

Statistical Analysis

All results are expressed as the means ± SE. The statistics software package StatView 4.0 was used to calculate one-way analysis of variances for the comparison of biological parameters between the three experimental groups, post hoc Scheffé’s tests, and regression analyses. All tests used a α of 5%. Statistical significance was recognized when $P < 0.05$.

RESULTS

A total of 65 rats were used, of which 44 underwent coronary ligature. The operation created 27 rats that were classified as compensated and 17 as decompensated. Decompensated rats were identified as those presenting a pleural effusion. This classification was confirmed by echocardiography (see DISCUSSION). Half of the rats were exploited for mRNA analysis and the other half for explant incubation.

Heart Parameters

There was a slight tendency toward a reduction in body weight (BW) in rats with MI, which remained nonsignificant (536.0 ± 14 in sham, 516.7 ± 11 in compensated, 493 ± 15 g in decompensated rats). Heart weight increased with respect to infarct severity (1.58 ± 0.03 in sham, 1.66 ± 0.04 in compensated, and 2.13 ± 0.07 g in decompensated rats, $P < 0.0001$). In addition to the presence of pleural effusion, the classification of the rats as being compensated or decompensated was confirmed by an increase in right heart weight (heart weight – LV weight)/BW ratio (28), scar surface area, LV end-diastolic diameter (LVEDD)/BW (2, 26, 28), and LA diameter (37), and a decrease in the EF (37) (Table 2).

ACE mRNA Expression

The expression of ACE mRNA in the lung decreased significantly (2.93 ± 0.1 in sham, 1.9 ± 0.1 in compensated, and 1.4 ± 0.04 in decompensated rats, $P < 0.0001$) (Fig. 2) but increased in the scar tissue (1.1 ± 0.1 in compensated, 1.7 ± 0.3 in decompensated rats, $P < 0.05$). Lung ACE mRNA expression was negatively correlated with the LVEDD/BW ratio ($r = -0.38, F = 5.2, P < 0.05$) and LA diameter ($r = -0.66, F = 24.2, P < 0.0001$), whereas scar mRNA expression was positively correlated with LA diameter ($r = 0.54, F = 6.8, P < 0.05$). The remaining viable LV, used as a control in this study, showed no differences in mRNA expression between the groups (1.8 ± 0.1 in sham, 1.7 ± 0.1 in compensated, and 1.7 ± 0.1 in decompensated rats).

ACE Gene Transcription

Run-on experiments were performed to measure the transcription rate of the ACE gene in the lung tissue. Transcription rate of ACE in the lung, normalized with respect to that of the GAPDH gene, was decreased in infarcted rats (0.548 ± 0.119 in sham, 0.324 ± 0.016 in heart failure rats, $P < 0.05$) (Fig. 3).

ACE Activity in Plasma

ACE activity in the plasma increased with infarct severity (75.6 ± 5.3 in sham, 97.1 ± 7.0 in compensated, and 176.6 ± 8.3 nmol/ml in decompensated rats, $P < 0.0001$). This activity was positively correlated with both the LVEDD/BW index ($r = 0.54, F = 9.2, P < 0.01$) as well as LA diameter ($r = 0.56, F = 10.8, P < 0.01$) and negatively correlated with EF ($r = -0.69, F = 17.6, P < 0.001$; Fig. 4).

Tissue and Solubilized ACE Activity

After the extraction of the membranous fraction of the tissues, we measured the ACE activity normalized with respect to the total protein in the extract. ACE activity in the lung tissue decreased (1,192.3 ± 111 in sham, 928.9 ± 92 in compensated and 462.3 ± 68 nmol/mg total protein in decompensated rats, $P < 0.001$) in contrast to the increase found in the scar tissue (8.06 ± 1.3 in compensated, 13.59 ± 1.5 nmol/mg total protein in decompensated rats, $P < 0.05$). The

Table 2. Body weight and heart parameters

<table>
<thead>
<tr>
<th></th>
<th>Sham ($n = 21$)</th>
<th>Compensated ($n = 27$)</th>
<th>Decompensated ($n = 17$)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>536.0 ± 14</td>
<td>516.7 ± 11</td>
<td>493.4 ± 16</td>
<td>NS</td>
</tr>
<tr>
<td>HW, g</td>
<td>1.58 ± 0.03</td>
<td>1.66 ± 0.04</td>
<td>2.13 ± 0.07†</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>(HW−LV)/BW × 1,000</td>
<td>0.98 ± 0.05</td>
<td>1.17 ± 0.05</td>
<td>2.23 ± 0.02‡</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Scar surface, mm²</td>
<td>117.4 ± 14</td>
<td>168.9 ± 21‡</td>
<td>169.9 ± 21†</td>
<td>$P &lt; 0.05$</td>
</tr>
<tr>
<td>LVEDD/BW, mm/kg</td>
<td>19.60 ± 0.6</td>
<td>22.79 ± 0.6†</td>
<td>28.19 ± 1.21§</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>LA diameter, mm</td>
<td>5.1 ± 0.1</td>
<td>6.1 ± 0.2†</td>
<td>8.4 ± 0.41‡</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>75.3 ± 1</td>
<td>57.8 ± 3†</td>
<td>35.0 ± 3‡</td>
<td>$P &lt; 0.0001$</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>absent</td>
<td>absent</td>
<td>present</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of rats. HW, heart weight; BW, body weight; NS, not significant. *$P < 0.05$ vs. sham; †$P < 0.0001$ vs. sham; ‡$P < 0.05$ vs. compensated; §§$P < 0.0001$ vs. compensated as determined by Scheffé’s test.
activity in the lung tissue was negatively correlated with the LVEDD/BW ratio ($r = -0.67, F = 20.6, P < 0.0001$) and in the scar tissue, was negatively correlated with the EF ($r = -0.64, F = 6.4, P < 0.05$) (Fig. 4). Shed ACE activity remained unchanged in the lung (42.19 ± 8.6 in sham, 30.35 ± 3.0 in compensated, and 46.19 ± 8.9 nmol/mg total protein in decompensated rats) and in the LV (4.02 ± 0.9 in sham, 3.09 ± 0.4 in compensated, and 4.6 ± 0.6 nmol/mg protein in decompensated rats) explant supernatants.

The solubilized fraction, calculated as the ACE activity in the supernatants divided by the ACE activity in the tissue, did not change in the scar (449 ± 70 in compensated, 337 ± 39% in decompensated rats, not significant) and increased significantly in the lung (3.2 ± 0.7 in sham, 3.5 ± 0.5 in compensated, 8.3 ± 1.7% in decompensated rats, $P < 0.005$).

**Inhibition of ACE Shedding**

To determine whether or not the secretion of ACE into the tissue culture supernatants was the result of specific enzyme activity, we incubated lung, LV, and scar tissue in the presence of GM-6001, a nonspecific metalloprotease inhibitor. In the presence of 1 mM GM-6001, ACE activity in the supernatants was almost completely abolished (30.81 ± 3.4 vs. 2.86 ± 1.9 in the lung, 6.71 ± 2.8 vs. 2.3 ± 1.1 in the LV and 42.0 ± 4.0 vs. 5.3 ± 1.4 nmol/mg protein in the scar) (Fig. 5), whereas ACE activity in the tissues was conserved (data not shown).

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**Fig. 2.** Angiotensin-converting enzyme (ACE) mRNA expression as determined by reverse transcriptase-polymerase chain reaction (RT-PCR), expressed as a ratio of ACE over glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (in cpm). A: in the lung; B: in the scar; C: in the LV. *$P < 0.05$ vs. sham, **$P < 0.0001$ vs. sham, †$P < 0.05$ vs. compensated, as determined by a Scheffe’s test.

**Fig. 3.** ACE gene transcription in the lung tissue as determined by run on, and normalized with respect to GAPDH transcription and expressed in arbitrary units. pGEM, empty plasmid (negative control).
Immunohistochemistry

Immunohistochemistry showed a strong ACE staining in the medial portion of the infarct scar. Immunohistochemistry of ED-1 was negative in the scar and LV (not shown). Immunostaining for α-smooth muscle actin (α-SMA), a marker of myofibroblasts, showed a central localization within the scar (Fig. 6) and was negative in the remaining LV, except for the medial layer of coronary arterial vessels (not shown). With the exception of the coronary arteries, this expression was concurrent with that of ACE. Hematoxylin-eosin and Masson trichrome staining show that this expression is also concurrent with the presence of collagen fibers.

DISCUSSION

The objective of this study was to determine the topology of ACE expression in rats after MI and to determine whether or not ACE expression and shedding patterns vary with respect to infarct severity. Three months after MI, we measured ACE mRNA expression and activity in the lung, infarcted LV (scar), and remaining viable LV. We incubated tissue explants in serum-free medium and measured the ACE activity in the supernatants as well as in the plasma to ascertain tissue shedding of ACE. Transthoracic echocardiography has been shown as a useful technique for assessing LV remodeling after MI in the rat (2, 7, 19, 26, 36, 37). In the present study, the use of high frame rate echocardiography allowed high-quality recordings in two dimensions and M-mode (see Fig. 1). Low vari-
ability of LVEDD and LA diameter measurements was observed (Table 1). In case of inhomogeneous LV contraction, EF calculated by modified Simpson method is the best parameter to assess LV systolic function (35). This technique has been previously used in rats (45).

Quality of two-dimensional recordings was good enough to identify LV endocardial borders with an acceptable interobserver reproducibility (Table 1).

In this study, we confirm that plasma ACE activity is increased in experimental MI (22) and this in proportion to the LV dysfunction as demonstrated by the correlation to EF, LA diameter and LVEDD/BW ratio. We also show that the infarct scar is an important source of ACE. ACE activity in the scar tissue increased, parallel to an increase in ACE mRNA expression. ACE activity in the scar tissue is negatively correlated with EF, a key indicator of the LV dysfunction. We incubated the scar explants and dosed ACE activity in the culture supernatants to determine the shedding of ACE. Solubilized ACE activity increased in proportion to MI severity and with respect to LV systolic dysfunction as indicated by the negative correlation with EF. To ensure that the shedding of ACE into the explant culture medium was due to specific enzyme activity and not simply to tissue sufferance, we incubated the explants in the presence of GM-6001. This inhibitor is a broad-range metalloprotease inhibitor (5, 23), which, at the dose used, does not interfere with ACE activity itself. In the presence of GM-6001, ACE activity was no longer detected in the supernatants, confirming the role of a metalloproteinase in tissue ACE shedding in vivo.

Previous studies (22) in our laboratory have shown a decrease in lung ACE mRNA expression and tissue ACE activity in experimental heart failure in rats. In the present study, we confirm these results and also show by run-on experiments that the decrease in ACE mRNA in the lung is due to a reduction in ACE gene transcription. These changes are concurrent with the pulmonary endothelial dysfunction found in heart failure. As the heart failure syndrome progresses, the blood pressure in the pulmonary circulation increases and shear stress decreases (13). We (20) previously looked at the effects of stress on ACE expression in vitro and showed that diminished shear stress led to a decrease in ACE mRNA expression and activity. The ACE activity in the lung tissue was negatively correlated with the LVEDD/BW ratio, an indicator of the upstream consequences of LV diastolic dysfunction. We now show that the shedding of ACE from the lung tissue is unchanged in congestive heart failure rats. However, the solubilized fraction is significantly increased in the lung meaning that despite decreased ACE gene transcription and expression, the posttranslational maintenance of shedding from the pulmonary endothelium probably participates in the increase of plasma ACE associated with congestive heart failure.

In the present study, we showed that ACE mRNA expression did not change in the remaining viable myocardium in MI rats with respect to infarct severity or with respect to sham-operated rats. ACE activity was below detection limits in LV tissue and remained very low and unchanged in the explant supernatants. Other studies have looked at ACE expression in the LV after MI, both in experimental rat models as well as in the human hearts, and have given conflicting results. Passier et al. (31) showed that ACE mRNA expression in rats increased within 5 days postinfarct and subsequently decreased to baseline levels by day 90. However, in this same study, Passier et al. showed that the
ACE activity in the LV was significantly increased by day 7 and remained high through to day 90. The possible reason for this discrepancy is that care was not taken to separate the scar tissue from the rest of the LV, and that scar was probably the predominant source of the measured tissular ACE activity in this experimental situation.

The loss of contractile tissue and the subsequent dilation and remodeling of the LV results in hemodynamic alterations and increased stress-strain forces acting on the infarct scar. Our laboratory (8) previously showed that in the hypertrophied heart (induced by renovascular hypertension) the induction of ACE mRNA and activity was not found in isolated cardiomyocytes, but instead in pooled noncardiomyocyte cells. We have also shown that induction of ACE expression occurs primarily in smooth muscle cells and fibroblasts in response to hemodynamic stress (3). These observations are in agreement with our current results, which indicate that ACE expression and activity are increased only in the scar tissue principally composed of myofibroblasts (41). These data are in agreement with a previous study by Yamada et al. (44) showing a high density of ACE in valve leaflets in the normal rat heart. This tissue is similar to the infarct scar in that it is mainly composed of myofibroblast and is subjected to high hemodynamic stress at each systole (29). Sun et al. (39) have shown a colocalization of ACE with α-SMA in the scar of MI rats, a result that we confirm in the present study. We also confirm that this expression is concurrent with the accumulation of collagen (40).

Our study also shows that ACE activity in the scar culture supernatants is correlated with LV systolic dysfunction (represented by EF). The EF is also correlated with ACE plasma levels. Combined with the observation that the absolute values of shed ACE activity are increased in decompensated rats, probably due to the increase in the hemodynamically stressed scar area, it appears likely that the scar participates in the observed increased plasma ACE.

Postinfarct remodeling involves not only the scarification of the necrosed tissue, but also the remaining viable myocardium. Small accumulations of collagen remote to the infarct scar can represent up to two-thirds of the total collagen content in the infarcted human heart, and it is these accumulations that are believed to be the major component of the adverse structural remodeling found in ischemic cardiomyopathy (4). Increases in tissular ACE activity in the scar may have an important role in local collagen accumulation as well as that of the surrounding viable myocardium. The importance of local tissue ACE expression is underlined by Higaki et al. (21), who showed that local transfection of human ACE into the LV results in tissue hypertrophy and increased collagen content.

In conclusion, this study shows that there is a shift in ACE mRNA expression and activity from the lung to the infarct scar in experimental heart failure and this in proportion to the severity of the infarct. The increase in scar tissue occurred in parallel with an augmentation of solubilized ACE, shed from the cell membrane by the action of a zinc metalloprotease. In contrast, despite the decrease in ACE expression, ACE posttranslational shedding is maintained in the lung. This increase in ACE shedding from the scar and its maintenance from the lung could be responsible for the increase in plasma ACE activity observed in this experimental model. Therefore, the shift of ACE expression in congestive heart failure and its increased level in the plasma provides evidence of the dual hemodynamic consequences of the increased tensile stress-strain relationship in the scar and decreased shear stress on the pulmonary endothelium.

This study was supported by Institut National de la Santé et de la Recherche Médicale and by a grant from the Fondation de France.

REFERENCES

13. Driss AB, Devaux C, Henrion D, Duriez M, Thuillez C, Levy BI, and Michel JB. Hemodynamic stresses induce endothelial dysfunction and remodeling of pulmonary artery in ex-


