Comparative Effects of Chronic Angiotensin-Converting Enzyme Inhibition and Angiotensin II Type 1 Receptor Blockade on Cardiac Remodeling After Myocardial Infarction in the Rat

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Background After myocardial infarction, the noninfarcted left ventricle develops reactive hypertrophy associated with a depressed coronary flow reserve, myocardial interstitial fibrosis, and reduced capillary density. The present study investigated the comparative cardiac effects of chronic angiotensin-converting enzyme (ACE) inhibition and selective angiotensin II type 1 receptor (AT\(_1\)) blockade in the rat model of myocardial infarction and failure.

Methods and Results Seven days after coronary ligation (MI), rats were randomized to enalapril (n=8; 500 μg·kg\(^{-1}·d^{-1}\)), losartan (n=9; 3 mg·kg\(^{-1}·d^{-1}\)), or placebo (n=8) and treated for 6 weeks. Sham-operated rats (n=10) served as controls. Coronary blood flow was measured with radiolabeled microspheres during baseline and maximal coronary dilation induced by dipyridamole (2 mg·kg\(^{-1}·min^{-1}\) over 10 minutes). Right and left ventricular (LV) weight was increased in infarcted rats compared with sham-operated animals and enalapril- and losartan-treated MI rats. Minimal LV and right ventricular coronary vascular resistance was increased in MI rats but normalized with enalapril and losartan (LV: sham, 8.9; MI-placebo, 12.7; MI-enalapril, 9.2; MI-losartan, 8.8 mm Hg·mL\(^{-1}·min^{-1}·g^{-1}\), all P<.05 versus MI-placebo). Interstitial fibrosis determined from perfusion-fixed hearts was increased in infarcted rats but reduced by both enalapril and losartan. Myocardial capillary density improved with enalapril and losartan. In separate groups treated as above, plasma and tissue ACE activity was determined and demonstrated significantly higher ACE activity in noninfarcted LV tissue of MI-placebo rats compared with sham (0.64 vs 0.27 nmol·mg protein\(^{-1}·min^{-1}\), P<.05). Enalapril and losartan reduced LV ACE activity (0.39 and 0.29 nmol·mg protein\(^{-1}·min^{-1}\), P<.05 versus MI-placebo).

Conclusions The present study demonstrates that both chronic ACE inhibition and AT\(_1\) receptor blockade (1) reduces cardiac hypertrophy, (2) restores minimal coronary vascular resistance in postinfarction reactive hypertrophy, and (3) attenuates the development of myocardial interstitial fibrosis in the noninfarcted LV. These results suggest that inhibition of generation of angiotensin II and AT\(_1\) receptor blockade are equally effective in preventing important features of ventricular remodeling after myocardial infarction. (Circulation. 1994;89:2273-2282.)

Key Words • infarction • hemodynamics • angiotensin • enzymes • losartan

Ventricular remodeling describes a series of pathological changes that occur after myocardial infarction and affect the infarcted and noninfarcted zone of the left ventricle (LV). This process includes infarct expansion as well as compensatory reactive hypertrophy and dilation of the noninfarcted LV. The latter is associated with depressed coronary flow reserve and the development of myocardial interstitial fibrosis with rearrangement of the extracellular matrix architecture. Angiotensin-converting enzyme (ACE) inhibition has been shown to prevent cardiac remodeling and to prolong survival in experimental myocardial infarction and in patients after myocardial infarction. This beneficial effect of ACE inhibitors has previously been attributed to the sustained reduction of preload and afterload and inhibition of neurohormonal activation. However, there is now evidence from experimental studies, supported by preliminary clinical data, that the cardiac tissue-type renin-angiotensin system is activated after myocardial infarction and failure. Moreover, two clinical megatrials have indicated that long-term ACE inhibition reduces the incidence of recurrent infarction irrespective of the degree of LV dysfunction, raising the possibility that an activated cardiac renin-angiotensin system, located predominantly within the endothelium of the coronary circulation, may be a target for ACE inhibitors. In this respect, the formation of angiotensin II, acting via specific angiotensin receptors (AT\(_1\) and AT\(_2\)), has been implicated in the development of vascular and ventricular remodeling through its proposed growth-promoting properties thereby stimulating myocyte hypertrophy and collagen synthesis. Consequently, it has been speculated that the beneficial effects of ACE inhibitors on cardiac hypertrophy are related to reduced levels of circulating and tissue angiotensin II. Conversely, more recent studies suggest that the inhibition

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of breakdown of bradykinin may contribute to the beneficial cardiac effects of ACE inhibitors.\textsuperscript{16} This mechanism may increase local tissue levels of bradykinin, which in turn stimulates the release of nitric oxide (which accounts for the biological activity of the endothelium-derived relaxing factor) and vasodilating prostaglandins.\textsuperscript{17} This pathway may also have important long-term effects in that nitric oxide may inhibit mitogenesis and proliferation of vascular smooth muscle cells.\textsuperscript{18} If the bradykinin pathway is involved in the hemodynamic and trophic effects exerted by ACE inhibitors, one would anticipate that chronic ACE inhibition is superior to selective AT\textsubscript{1} receptor blockade in preventing ventricular and vascular remodeling after myocardial infarction. Accordingly, the present study was designed to assess the long-term effects of ACE inhibition (enalapril) versus selective AT\textsubscript{1} receptor blockade (losartan) on reactive cardiac hypertrophy, coronary flow reserve, and structural myocardial alterations after myocardial infarction, including interstitial and perivascular fibrosis and capillary density.

**Methods**

**Experimental Preparations**

Infarction was produced in male Sprague-Dawley rats (body weight, 250 to 300 g) by left coronary artery ligation as previously described\textsuperscript{19} using the modification of McLean et al.\textsuperscript{20} Sham-operated rats served as controls. With this method, the 24-hour mortality rate was 40\% in the infarcted rats. All rats were fed standard rat chow and given water ad libitum. Subsequent experiments were started 7 weeks after surgery. Rats were screened for evidence of large myocardial infarction 7 days after surgery by surface ECG recording.\textsuperscript{3} Previous studies have shown that most rats identified in this manner have infarction of >40\% of the LV, elevated LV end-diastolic pressure and volume, and impaired systolic function.\textsuperscript{5} Five percent of infarcted rats died before randomization at day 7.

**Instrumentation**

Animals were anesthetized with halothane (1\% halothane in oxygen), and catheters (PE 50) were placed in the jugular vein, the tail artery, and via the right carotid artery in the LV as described recently.\textsuperscript{21} Catheter positions were verified by registration of typical pressure waves with pressure transducers (Statham ID 23 P). After closure, the rats were allowed to recover for at least 3 hours before experimental procedures were initiated. This time has been found to be sufficient to ensure a return to steady-state conditions in the rat.\textsuperscript{21}

**Regional Blood Flow Measurement**

To determine regional coronary blood flow and cardiac output, radioactive microspheres 15\pm 2 \( \mu m \) in diameter (New England Nuclear, Dreieich, Germany) were used according to the reference sample technique\textsuperscript{22} as adapted for use in rats.\textsuperscript{21} Regional blood flow was measured twice, before and after drug intervention, by injection of two different radioactive microspheres (\textsuperscript{16}Ce and \textsuperscript{103}Ru). The first measurement was taken for determination of baseline flow. To assess coronary vascular reserve, a second injection of microspheres was performed under maximal coronary dilation induced by dipyridamole (2 mg \cdot kg body wt\textsuperscript{-1} \cdot min\textsuperscript{-1} over 10 minutes) via the jugular vein.\textsuperscript{23} As reported by Wangler et al.,\textsuperscript{22} this dose and administration protocol produce maximal coronary dilation in the rat. At the end of the study, animals were anesthetized with ketamine (30 mg/kg) and phenobarbital (30 mg/kg). The heart then was fixed as detailed in the next section. At the end of the fixation procedure, organs and tissue samples were quickly removed, and all samples were immediately blotted, weighed, and transferred to a two-channel gamma-scintillation counter (Packard Minaxi Auto Gamma Counter, Packard Instruments) for determination of radioactivity levels as described by Karam et al.\textsuperscript{2} Blood flow data concerning the LV were obtained from the noninfarcted part of the LV.\textsuperscript{2} In sham-operated animals, coronary blood flow was measured in analogous portions of the LV.

**Hemodynamics**

Tracings from the LV and tail catheters were recorded and used to obtain heart rate, LV peak systolic pressure, LV end-diastolic pressure, and mean arterial pressure. Total vascular resistance was determined from mean arterial pressure and cardiac output data (mL \cdot min\textsuperscript{-1} \cdot kg\textsuperscript{-1}). Coronary vascular resistance was calculated by dividing mean arterial pressure recorded just before each microsphere injection by the corresponding coronary blood flow. With the exception of cardiac output, hemodynamic data were collected immediately before the microsphere injection.

**Determination of Infarct Size**

The LV and septum were separated from the right ventricle (RV) (free wall), weighed, and fixed in 10\% formalin. Twenty-four hours later, the LV was cut into three transverse slices from apex to base. Three thin transversal slices of each piece were separated and used for histological examination. Thereafter, the remaining noninfarcted left myocardium was cut and prepared free of scar tissue and border zone. These three noninfarcted pieces of the LV were pooled and counted for radioactivity as described by Karam et al.\textsuperscript{2} The three thin transverse slices were stained with elastica van Gieson and mounted.\textsuperscript{4} With a digital image analyzer (MOP 2, Kontron, Munich, Germany), the endocardial and epicardial circumferences of the infarcted and noninfarcted LV were determined. The infarcted mean circumference (mean of endocardial and epicardial circumferences) of the three slices was summed and finally expressed as the ratio of summed mean circumference of the LV, similar to the method of Pfeffer et al.\textsuperscript{1,2}

**Experimental Protocol 1**

Seven days after surgery, infarct rats were randomly assigned to three groups and treated with either enalapril (500 \( \mu g \cdot kg \cdot body \; weight \textsuperscript{-1} \cdot d\textsuperscript{-1})),\textsuperscript{24} losartan (3 mg \cdot kg \cdot body \; weight \textsuperscript{-1} \cdot d\textsuperscript{-1}), or placebo for 6 weeks. Preliminary studies have shown that these two dosages caused similar reduction in arterial blood pressure in this model.\textsuperscript{22,25} Since the objective of the present study was to assess the effects of treatment on LV structure rather than short-term hemodynamic effects of ACE inhibition\textsuperscript{26} and AT\textsubscript{1} receptor blockade, the treatment regimen was discontinued 3 days before experiments started. Regional blood flow and hemodynamics were obtained in infarcted and sham-operated animals 7 weeks after surgery. During the 6-week treatment period, three animals died, two from the placebo group and one from the enalapril group. One additional animal from the losartan group died after instrumentation with an LV catheter.

**Fixation Procedure**

After hemodynamic parameters were obtained, animals were anesthetized with ketamine (30 mg/kg) and phenobarbital (30 mg/kg). In rapid succession, the abdomen was opened, the abdominal aorta was cannulated, and the heart was arrested in diastole by injection of KCl (1 mL/0.2 mol) directly into the LV.\textsuperscript{2} The chest was opened, the right atrium cut, and the coronary artery vascularly perfused with phosphate buffer (0.1 mol/L, pH 7.4) at a pressure equal to the in vivo measured mean arterial pressure. After perfusion with buffer, the coronary circulation was perfused with solution containing 2\% paraformaldehyde and 2.5\% glutaraldehyde, pH 7.2.\textsuperscript{2,24} After complete fixation, the heart was removed, the atria and great vessels were trimmed away, and the ventricles
were separated and weighed. For determination of interstitial fibrosis, medial thickness, perivascular fibrosis, and capillary density, the myocardium of the surviving noninfarcted LV was examined.

**Morphology and Morphometry**

Three coronal sections were embedded in paraffin and sequentially cut into slices 5 μm thick (Zeiss Microtome, Mainz, Germany), mounted onto slides, and stained with either sirius red, elastica van Gieson, or safranine methylene blue. Myocardial collagen volume fraction was determined by quantitative morphometry of the sirius red-stained sections. Each section was placed in a projection microscope (Ultraplan, Reichert, Austria) and scanned systematically at ×400 magnification. Based on their red levels of collagen fibers in direct light, the images were scanned systematically and computerized according to standard stereological principles previously reported by Riede et al. Collagen volume fraction was calculated as the sum of all connective tissue areas of the entire visual field divided by the sum of all connective tissue and muscle areas in the visual field of the section.

Perivascular and infarcted areas were excluded from this measurement. The results predict the proportion of myocardium occupied by fibrillar collagen. As previously reported by Brilla et al., total collagen volume fraction determined by this morphometric approach is closely related to hydroxyproline concentration of the LV (including perivascular fibrosis). Average medial wall thickness of intramural coronary arteries, ranging in diameter from 15 to 150 μm, was determined with an image analysis system (MOP 3, Kontron, Munich, Germany) in elastica van Gieson–stained sections. Wall thickness was calculated with the recently reported formula by Atherton et al:

\[
MT = [(VA + MA)/\pi]^{1/2} - (VA/\pi)^{1/2}
\]

where VA is vessel luminal area, MA is medial area, and MT is medial thickness. Medial area was defined as the area from the endothelium to the outer limit of the tunica media. All tissue sections were scanned systematically, and all arteries were photographed. The smallest diameter of the vessel, which was representative of luminal diameter, was used for determination. For each ventricular region, slides from all blocks were analyzed. Care was taken to exclude necrotic areas or areas with artificial separation. The lumens of all arteries were measured from the negatives, which were projected onto a screen to enlarge the images (×12). The negatives were also checked to verify whether any arteries were photographed more than once. For each section, a minimum of 10 vessels were found to be cut cross-sectionally; each was examined.

To ensure the accuracy of our vessel measurements, the effect of refraction in optical microscopic measurements was taken into account. Therefore, we used the correction formula for quantitative optical microscopic analysis previously reported by Chen et al to correct the geometric shape of intramural arteries (ie, circular or elliptical cross-cut sections).

All collagen surrounding an intramural cardiac small artery was considered perivascular fibrosis. Perivascular collagen was clearly distinguishable from interstitial collagen. The area of perivascular collagen was normalized to vessel luminal area of intramural resistance vessels in sirius red–stained sections. Capillary numerical densities (profile density) were determined from sections stained with safranine methylene blue at ×400 magnification. Tissue sections were systematically scanned, and all sampled areas were counted. Capillary density is expressed as number of profiles per square millimeter. Mean values in each group were obtained from average values in individual hearts. All histological measurements were made by a single observer.

**Experimental Protocol 2**

A separate group of animals, treated identically to protocol 1, were anesthetized with halothane (1% in oxygen), and a sternotomy was performed. A blood sample was taken from the LV, collected into a heparinized syringe, immediately centrifuged (300 rpm for 15 minutes), and then preserved in liquid nitrogen. The heart was rapidly removed, and atria and RVs were trimmed away. The infarcted area was excised; left and right ventricles were then put into liquid nitrogen. Thoracic aorta, lung, both kidneys, and quadriceps muscle were excised and stored in liquid nitrogen. In these animals, drug therapy was continued until the animals were killed.

**Determination of Plasma and Tissue ACE Activity**

Serum and tissue ACE activities were measured by the rate of generation of His-Leu from hippuryl-His-Leu substrate according to the previously described method of Cushman et al. After the samples were thawed and 0.3% Triton X-100 was added, the tissue was homogenized and the resulting suspension centrifuged. The supernatant (50 μL) was incubated for 1 or 3 hours with 150 μL sodium phosphate buffer (pH 7.4) containing 23.2 mg of hippuryl-histidyl-leucine (HHL) and 400 μL of radioactive 3H-labeled HHL (final concentration, 5 mmol/L). The rate of production of radioactive-labeled hippuric acid from HHL was measured with a radiochemical assay by β-counting. ACE activity in plasma is expressed in nmol·mL⁻¹·min⁻¹ and in tissue in nmol·mg protein⁻¹·min⁻¹.

**Statistical Analysis**

All data are given as mean±SEM. Differences between groups were evaluated by one way ANOVA, followed by Student-Newman-Keuls Test. Statistical significance was defined as P<.05.

**Results**

**Infarct Size and Reactive Hypertrophy**

The baseline characteristics of sham-operated and infarcted animals are given in Table 1. All animals developed large myocardial infarctions, with infarct size ranging from 37% to 55% of the LV circumference. The infarct sizes were similar for all three groups (Table 1). The RV and LV weights of placebo-treated infarct rats were markedly increased compared with sham-operated animals as well as compared with enalapril- or losartan-treated animals (Fig 1). Enalapril and losartan had similar effects on arterial blood pressure, cardiac output, and systemic vascular resistance (Table 1).

**Coronary Hemodynamics**

RV and LV blood flow at baseline was not statistically different in all four groups. During dipyridamole, coronary blood flow was impaired in infarcted animals compared with the sham, enalapril, and losartan groups. Losartan completely restored maximal dipyridamole-induced coronary flow. Similarly, maximal dipyridamole-induced RV flow was lower in the untreated infarct group (Table 2).

LV coronary vascular resistance at baseline was higher in the untreated infarct group compared with all three other groups (29.9±3 versus 20.8±2 for sham, 17.8±1 for the enalapril group, and 24.2±3 mm Hg · mL⁻¹·min⁻¹·kg⁻¹ for the losartan group, P<.05). Similarly, RV coronary vascular resistance at baseline was higher in the untreated infarct group (47.5±10 versus 29.8±3 for the sham group, 24.1±2 for the enalapril group, and 34.5±7 for the losartan group). The minimal coronary vascular...
resistance of RV and LV during dipyridamole was markedly elevated \( P < 0.05 \) in the untreated infarct group (Fig 2). Both enalapril and losartan restored the minimal coronary vascular resistance to normal values.

**Vascular and Interstitial Fibrosis**

Collagen volume fraction (%) was significantly increased in untreated infarct animals and reduced both by enalapril and losartan compared with untreated infarct rats but remained significantly elevated compared with sham-operated rats (Fig 3). There was a close relation between LV infarct size and collagen volume fraction (%) in untreated infarct rats \( n=8, r=.717, P < 0.044 \). Perivascular fibrosis normalized to vessel luminal area was similar in all three infarct groups (Table 3). Similarly, no significant differences between groups were observed for average vessel luminal diameter, medial wall thickness of intramural coronary arteries, and the ratio of medial area corrected by luminal area (Table 3).

Capillary density was significantly reduced in untreated infarct rats compared with sham-operated animals. In the enalapril and losartan groups, the capillary density was increased compared with the untreated infarct group, but its value remained lower than that of the sham group (Fig 4).

**Plasma and Tissue ACE Activity**

Baseline characteristics of these separate groups of animals were similar to those of protocol 1. Although the majority of LV tissue was used for measurement of ACE activity, a crude estimate of infarct size assessed from one transversal slice from the middle part of each LV suggested similar infarct sizes from the three infarct

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**Table 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Sham-Operated Rats</th>
<th>Placebo</th>
<th>Enalapril</th>
<th>Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>423±14</td>
<td>416±12</td>
<td>435±7</td>
<td>434±9</td>
</tr>
<tr>
<td>RV weight, g/kg</td>
<td>0.72±0.04*</td>
<td>1.04±0.08*</td>
<td>0.74±0.06*</td>
<td>0.72±0.08*</td>
</tr>
<tr>
<td>Infarct size, %</td>
<td></td>
<td>44±3</td>
<td>43±3</td>
<td>40±3</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>368±14</td>
<td>364±17</td>
<td>343±10*</td>
<td>345±13*</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>103±8</td>
<td>101±6</td>
<td>86±3*</td>
<td>92±5*</td>
</tr>
<tr>
<td>CO, mL/min</td>
<td>425±38*</td>
<td>346±26</td>
<td>396±20*</td>
<td>382±22</td>
</tr>
<tr>
<td>SVR, mm Hg · mL⁻¹ · min⁻¹</td>
<td>0.26±0.03</td>
<td>0.30±0.08</td>
<td>0.21±0.01*</td>
<td>0.22±0.04*</td>
</tr>
</tbody>
</table>

RV indicates right ventricular; HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; CO, cardiac output; and SVR, systemic vascular resistance. Data are given as mean±SEM.

\* \( P < 0.05 \) vs placebo-treated infarct rats.

**Fig 1. Bar graphs showing left ventricular (LV) weight (left) and right ventricular (RV) weight (right) normalized to body weight (g/kg body weight). MI indicates placebo-treated infarcted animals; MI-Enal, enalapril-treated infarcted animals; MI-Los, losartan-treated infarcted animals; and Sham, sham-operated untreated animals. Data are given as mean±SEM.**

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TABLE 2. Effects of Enalapril and Losartan on Coronary Blood Flow

<table>
<thead>
<tr>
<th></th>
<th>Sham-Operated Rats</th>
<th>Placebo</th>
<th>Enalapril</th>
<th>Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left ventricle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.99±0.39*</td>
<td>3.55±0.29</td>
<td>4.73±0.39*</td>
<td>4.03±0.41</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>11.25±0.95*</td>
<td>8.21±0.37</td>
<td>9.93±0.84*</td>
<td>11.35±0.60*</td>
</tr>
<tr>
<td><strong>Right ventricle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.81±0.53*</td>
<td>2.85±0.49</td>
<td>3.55±0.27*</td>
<td>3.78±0.71*</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>9.12±0.89*</td>
<td>7.26±0.87</td>
<td>9.92±1.05*</td>
<td>10.65±1.38*</td>
</tr>
</tbody>
</table>

Data are mean±SEM (mL·min⁻¹·kg⁻¹). *P<.05 vs placebo-treated infarcted rats.

groups (untreated infarct, 38±4%; infarct-enalapril, 34±2%; infarct-losartan, 40±2%). Tissue ACE activity of RV and LV, aorta, and kidney were significantly increased in infarcted animals compared with sham-operated animals, whereas plasma ACE activity and skeletal muscle ACE activity were similar to the values in sham-operated animals. Infarcted rats treated either with enalapril or losartan showed a significant decrease of ACE activity in the noninfarcted LV, RV, aorta, kidney, and plasma compared with placebo-treated infarcted animals (Table 4). However, the degree of inhibition of tissue ACE activity was different in enalapril- and losartan-treated animals. Whereas enalapril was particularly effective in the kidney, the myocardial ACE activity was more inhibited by losartan. Only enalapril reduced skeletal muscle tissue ACE activity below levels observed in sham-operated rats.

Discussion

The salient finding of the present study is that, compared with ACE inhibition, long-term AT₁ receptor blockade is equally effective in preventing ventricular remodeling after myocardial infarction. Three lines of evidence support this conclusion. Both AT₁ receptor blockade and ACE inhibition (1) prevented the development of RV hypertrophy and, in part, of LV reactive hypertrophy, (2) restored coronary hemodynamics, and (3) attenuated the development of interstitial fibrosis of the noninfarcted hypertrophied LV. These results are consistent with the hypothesis that angiotensin II and its action via AT₁ receptors plays an important role for the development of cardiac hypertrophy, depressed coronary flow reserve, and interstitial fibrosis after myocardial infarction.

Effect of ACE Inhibition and AT₁ Receptor Blockade on Cardiac Hypertrophy

For interpretation of the absolute and relative weights of the LV after myocardial infarction, the loss of myocardium related to infarction needs to be considered. It has been shown that myocyte hypertrophy occurs in the noninfarcted LV of the rat. The increased LV weights in the untreated infarct group, despite large infarcts and considerable loss of myocardium (replaced...
by a thin wall of scar tissue), strongly suggest that reactive hypertrophy of the noninfarcted LV occurred, consistent with numerous previous reports.\(^2\)\(^-\)\(^4\) Consequently, the reduced LV weights of the enalapril- and losartan-treated groups indicate that the reactive hypertrophy was partially prevented or reduced. However, it is unlikely that a complete regression (or prevention) occurred, since the LV weights of the enalapril and losartan infarct groups were similar to those of the sham group, although the latter did not lose myocardial tissue, whereas in the infarcted groups the infarcted area was replaced by scar tissue of low mass density.

The RV weight appears to be a useful marker of the overload hypertrophy induced by the model. The effectiveness of losartan in preventing RV hypertrophy (similar to enalapril) suggests that the unloading effect of losartan was comparable to that of enalapril, consistent with the observations of Raya et al.\(^4\)\(^1\) Thus, although the reduction of cardiac hypertrophy by both enalapril and losartan would favor a role of angiotensin II and its action on cardiac AT\(_1\) receptors for the development of reactive LV hypertrophy, the concomitant decrease in the loading state of the ventricle represents a contributing factor. Therefore, the present study cannot establish whether the impact of these two agents on cellular morphology (eg, myocyte hypertrophy) is related to blockade of cardiac AT\(_1\) receptors or mediated indirectly by their interferences with the peripheral actions of these agents, such as the decrease in tissue vascular and renal ACE activity. However, recent data obtained in the rat infarct model indicate that cardiac tissue angiotensin II levels are reduced, whereas circulating angiotensin II levels are increased, during AT\(_1\) receptor blockade, supporting the role of cardiac-derived angiotensin for the development of ventricular hypertrophy.\(^4\)\(^2\) Moreover, AT\(_1\) blockade reduced LV hypertrophy in a mouse model of aortic stenosis, when peripheral unloading of the LV cannot occur.\(^4\)\(^3\) Finally, two previous

### TABLE 3. Morphological Characteristics of Intramural Left Ventricle Resistance Vessels

<table>
<thead>
<tr>
<th>Infarcted Rats Treated With</th>
<th>Sham-Operated Rats</th>
<th>Placebo</th>
<th>Enalapril</th>
<th>Losartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>10</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>MT, (\mu)m</td>
<td>11.04±0.43</td>
<td>13.11±0.7</td>
<td>13.8±0.67</td>
<td>12.96±0.79</td>
</tr>
<tr>
<td>MA, (10^3\times\mu)m(^2)</td>
<td>3.4±0.85</td>
<td>3.9±0.35</td>
<td>4.6±0.58</td>
<td>4.4±0.47</td>
</tr>
<tr>
<td>MA/LA, %</td>
<td>11.04±1.43</td>
<td>14.20±1.36</td>
<td>13.84±1.56</td>
<td>12.96±0.79</td>
</tr>
<tr>
<td>LD, (\mu)m</td>
<td>112±27</td>
<td>96±7</td>
<td>108±14</td>
<td>101±7</td>
</tr>
<tr>
<td>PVF/LA, %</td>
<td>0.86±0.06</td>
<td>0.83±0.04</td>
<td>0.74±0.05</td>
<td>0.73±0.04</td>
</tr>
</tbody>
</table>

\(MT\) indicates medial thickness; \(MA\), medial area; \(MA/LA\), ratio of medial area normalized to luminal area; \(LD\), minimal luminal diameter; and \(PVF/LA\), ratio of perivascular fibrosis normalized to luminal area. Data are given as mean±SEM.
Effect of ACE Inhibition and AT1 Receptor Blockade on Coronary Hemodynamics

The potential effects of ACE inhibition on the coronary circulation has recently gained clinical attention in light of the surprising observation in two multicenter trials in which ACE inhibitors attenuated the incidence of recurrent myocardial infarction, irrespective of the degree of LV dysfunction.4,10 In the present study, we investigated the potential of ACE inhibitors to restore the depressed coronary flow reserve in postmyocardial reactive hypertrophy.3 Since arterial pressure is usually decreased in animals with large infarctions and both losartan and enalapril further reduced blood pressure, minimal coronary vascular resistance was calculated for evaluation of treatment effects on coronary hemodynamics.

The present study shows for the first time that long-term inhibition of the renin-angiotensin system both by ACE inhibition and at the level of the AT1 receptor improved minimal coronary vascular resistance. It is possible that the beneficial effect of ACE inhibition or AT1 receptor blockade on coronary hemodynamics is, in part, due to the reduction of reactive hypertrophy, resulting in an improved myocardial capillary density (capillaries per myocardium). However, it appears unlikely that the regression of LV hypertrophy can solely account for the complete restoration of minimal coronary vascular resistance, since LV capillary density and interstitial fibrosis were only partially restored. Moreover, coronary flow is regulated predominantly by coronary resistance vessels ranging in diameter from 80 to 200 μm. Thus, besides restoring myocardial capillary density, the beneficial effects of ACE inhibition on coronary hemodynamics should be related to functional or structural changes of coronary resistance vessels. The present study demonstrates that, in contrast to cardiac hypertrophy associated with hypertension, structural alterations do not occur in this normotensive model of post-myocardial infarction reactive hypertrophy, whereas our previous studies have documented endothelial dysfunction in this setting.47 Although vascular function in the coronary circulation was not tested in the present study, recent work indicates that ACE inhibitors are able to improve aortic endothelial dysfunction in this rat model of myocardial infarction.48 In addition, losartan has been shown to elicit endothelium-dependent dilation in the coronary circulation.49 Thus, it is conceivable that an improvement in endothelial function of coronary resistance vessels might have contributed to the beneficial effects of enalapril and losartan on coronary hemodynamics, in particular since Holtz et al50 have recently shown that the increase in coronary blood flow elicited by dipyridamole is partially endothelium dependent. Finally, both long-term ACE inhibition and AT1 receptor blockade reduce LV end-diastolic pressure,41 which might contribute to the decrease in minimal coronary vascular resistance. Regardless of the underlying mechanisms, this beneficial effect of ACE inhibition and AT1 receptor blockade may have important clinical implications, e.g., for patients with chronic heart failure after myocardial infarction. Previous clinical studies have focused only on the impact of ACE inhibitors on the coronary circulation in hypertensive patients, demonstrating improvement in coronary flow reserve, most likely by reducing medial hypertrophy of coronary resistance vessels,51 which is thought to occur in response to high arterial pressure rather than secondarily to myocyte hypertrophy.

Effect of ACE Inhibition and AT1 Receptor Blockade on Myocardial Interstitial Fibrosis

Although vascular remodeling of coronary resistance vessels does not occur in postinfarction reactive hypertrophy, the myocardial remodeling process includes increased interstitial fibrosis of the noninfarcted myocardium, particularly in the subendocardial layers.52 Chronic ACE inhibition partially restores the collagen network in
blockade is similarly effective in reducing myocardial collagen content. Recent data suggest that alteration of collagen phenotypes is independent of blood pressure and may be dependent on neurohumoral factors such as angiotensin, aldosterone, or norepinephrine. Similarly, humoral factors, in particular angiotensin II and aldosterone, appear to regulate the accumulation of collagen in cardiac hypertrophy associated with increased circulating levels of angiotensin II or aldosterone. The present observation that AT₁ receptor blockade and ACE inhibition are equally effective in preventing interstitial fibrosis in vivo is consistent with the hypothesis that angiotensin II and its action on AT₁ receptors are involved in increased interstitial fibrosis in post-myocardial infarction reactive hypertrophy. Indeed, there is evidence from in vitro studies that angiotensin II can stimulate cultured adult rat cardiac fibroblast growth and increase collagen synthesis through activation of AT₁ receptors. In contrast to cardiac hypertrophy associated with renovascular hypertension, plasma renin activity, plasma aldosterone, and presumably plasma angiotensin II levels are not necessarily elevated in the chronic compensated phase in the rat infarct model. Therefore, one has to assume that local cardiac angiotensin II levels are increased. There is accumulating evidence that the cardiac renin-angiotensin system is activated in the noninfarcted LV in this model. Besides increased cardiac ACE activity documented in the present study as well as in previous studies, recent investigations have shown increased cardiac expression of angiotensinogen, ACE, and AT₁ receptor protein in this infarct model. Both long-term treatments were associated with decreased LV ACE activity. Thus, consistent with recent preliminary data, we would assume that the inhibition of the cardiac renin-angiotensin system was associated with decreased cardiac activities of angiotensin II.

Although the effectiveness of AT₁ blockade in attenuating the increase in myocardial collagen content is consistent with the in vitro observations that angiotensin II stimulates the synthesis of collagen by cultured rat fibroblasts, it is possible that the preventive effect of losartan on interstitial fibrosis was not exclusively attributed to AT₁ receptor blockade. In fact, a surprising finding of the study is that chronic AT₁ receptor blockade was associated with decreased plasma and tissue ACE activities, including the noninfarcted LV. Similarly, a preliminary communication reported that cardiac ACE activity was reduced by chronic AT₁ receptor blockade in a rat model of pressure overload by aortic banding, whereas short-term administration of AT₁ receptor blockers did not suppress cardiac ACE activity. The latter is consistent with the pharmacological characterization of these agents, indicating that losartan and other AT₁ receptor antagonists do not have ACE inhibiting properties, ie, do not interfere directly with ACE. The present study was not designed to elucidate the underlying mechanism for the depressed tissue ACE activities during chronic AT₁ receptor blockade. Conceivably, cardiac ACE activity decreases over time secondarily to an improvement in systemic hemodynamics exerted by these agents. In addition, or alternatively, a negative feedback mechanism may operate, ie, acting via the AT₂ receptor. Recent data suggest that plasma angiotensin II levels are increased during therapy with AT₁ receptor antagonists. Angiotensin II has been shown to cause a negative feedback on the gene expression of the tissue ACE. Studies are under way to address this issue by assessing the cardiac converting enzyme gene expression after chronic AT₁ receptor blockade. Regardless of the underlying mechanisms, the decreased myocardial ACE activity during chronic AT₁ receptor blockade may contribute to the overall effect of these agents on central and coronary hemodynamics and the degree of myocardial interstitial fibrosis. In the face of effective blockade of the AT₁ receptor, any additional benefit must be provided by an alternative pathway, ie, by bradykinin and subsequently nitric oxide.

Mechanisms by Which ACE Inhibition Prevents the Development of Myocardial Interstitial Fibrosis

It has been proposed that the cardiac effects of ACE inhibition on hypertrophy and/or collagen content are, in part, due to the reduced breakdown of bradykinin. This concept is supported by recent studies of Linz and Schölvens, demonstrating that a specific β₂-kinin receptor antagonist abolishes the antihypertrophic effect of chronic ACE inhibition, whereas losartan was less effective in preventing cardiac hypertrophy in the pressure-overload rat model of aortic banding. However, these findings may be model specific. Notably, AT₁ receptor blockade was more effective than ACE inhibition in blunting the hypertrophic response in a volume-overload rat model, raising the possibility that angiotensin II generated via an angiotensin II–forming enzyme resistant to ACE inhibition acts as trophic factor. Importantly, both the pressure-overload and volume-overload rat models are associated with an activated plasma renin-angiotensin system. In contrast, plasma renin activity is not elevated in the chronic phase of the rat infarct model. Importantly, cardiac ACE activity during chronic AT₁ receptor blockade was not measured in previous studies and, therefore, the potential contribution of a negative feedback during AT₁ receptor blockade on tissue ACE activity remained unsettled. In light of the present findings and the observations from other experimental models, it appears that the relative contributions of angiotensin II and bradykinin to the antihypertrophic effect of ACE inhibition depends on the degree of activation of the systemic and tissue renin-angiotensin systems as well as the experimental model. Moreover, inhibition of both pathways in vivo, ie, the bradykinin breakdown and the AT₁ receptor, may not be additive in their effects during long-term therapy.

In summary, the results of this study indicate that chronic ACE inhibition and AT₁ receptor blockade are similarly effective in (1) reducing reactive hypertrophy after myocardial infarction, (2) restoring minimal coronary resistance in postinfarction reactive hypertrophy, and (3) attenuating the development of myocardial interstitial fibrosis in the noninfarcted LV. These results are consistent with the hypothesis that angiotensin II and its action via cardiac AT₁ receptors play an important role for the development of reactive hypertrophy, depressed coronary flow reserve, and interstitial fibrosis.
after myocardial infarction. However, the decrease in vascular and renal tissue ACE activity during long-term AT₁ receptor blockade suggests that long-term interference at the level of the AT₁ receptor is associated with adjustments of other components of the renin-angiotensin system. Thus, the cardiac effects of AT₁ receptor blockade in the rat infarct model may be due to multiple actions and interactions, including blockade of cardiac and peripheral AT₁ receptors and feedback or secondary downregulation of tissue ACE activities. Finally, the beneficial effects of inhibiting the generation or action of angiotensin II on coronary hemodynamics in postinfarction reactive hypertrophy may have important clinical implications, given the preventive effect of ACE inhibitors on recurrent infarction in patients after infarction.

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