Hormonal and Cardiac Effects of Converting Enzyme Inhibition in Rat Myocardial Infarction

Jean-Baptiste Michel, Anne-Laure Lattion, Jean-Loup Salzmann, Marie de Lourdes Cerol, Monique Philippe, Jean-Pierre Camilleri, and Pierre Corvol

To explain how converting enzyme inhibition could improve the prognosis in cardiac insufficiency, the effect of converting enzyme inhibition (CEI) by S9490-3 (Perindopril) treatment for 2 months (treated infarctions, n = 18) on hormonal plasma variables and the quantitative and qualitative changes in myocardium were studied in an experimental model of left ventricular infarction in rats (untreated infarctions, n = 18) and compared to a sham-operated control group (n = 15). Induction of myocardial infarction was associated with a transient decrease in blood pressure. CEI treatment maintained a lower blood pressure throughout the experimental period. Plasma renin concentration was not significantly increased in the untreated infarct group (155.4 ± 136.7 ng Al/ml/hr) as compared to the sham-operated group (47.6 ± 15.9 ng Al/ml/hr). Plasma aldosterone did not change in the three experimental groups. The plasma level of immunoreactive atrial natriuretic factor increased in the untreated infarct group (185 ± 245 pg/ml) as compared with the control group (76 ± 40 pg/ml) and was normalized by CEI (66 ± 60 pg/ml). Body weight was slightly decreased in both treated and untreated infarct groups, whereas the heart weight was significantly increased in the untreated group (1,540 ± 310 mg) and normalized by treatment (1,145 ± 180 mg) as compared with sham-operated controls (1,071 ± 80 mg). The combined atria and right ventricular mass was significantly increased in the untreated infarct group (660 ± 210 mg) and decreased by treatment (443 ± 106 mg) but was not completely normalized (controls, 343 ± 40 mg). Left ventricular isomyosin profiles were modified by myocardial infarction as compared with controls: V1 form decreased from 62.4 ± 9.4% in the sham-operated group to 41.6 ± 13.4% in the infarct group, and the V2 form increased from 13.0 ± 4.7% in sham-operated animals to 27.4 ± 11.8% in untreated infarct animals. CEI treatment partially, but significantly, reversed this modification of the isomyosin profile (V1, 53.0 ± 14.4%; V2, 17.5 ± 8.0%). Volume density of collagen was significantly increased in the untreated infarct rats (4.14 ± 0.81% versus 2.68 ± 0.49% in controls), and this was reversed by treatment (2.95 ± 0.66%). Messenger RNA encoding for atrial natriuretic factor, measured by dot blot hybridization, was significantly increased in both the atria and the ventricles in the untreated infarct group, and treatment by CEI partially reversed this increase. Thus, myocardial infarction profoundly modified several variables of peripheral circulation and quantitative and qualitative myocardial protein expression. CEI treatment largely reversed these changes but probably did not completely normalize them. (Circulation Research 1988; 62:641–650)

Left ventricular myocardial infarction in rats1 has been described as an experimental model of cardiac hypertrophy and overload that readily leads to cardiac insufficiency in relation to the size of the infarcted area.2 It has also been shown that converting enzyme inhibitor (CEI) does not change the size of the infarcted area, but it can improve the hemodynamic pumping ability of the heart and the survival curve of these animals.3 These experimental results have been recently confirmed in humans.4

The hormonal variables of the peripheral circulation and the quantitative and qualitative changes in the heart proteins have not been as extensively studied in this model as they have in cardiac hypertrophy secondary to pressure overload. Also, there have not been any studies on the effect of treatment by CEI on markers of structure and function of the peripheral circulation and on the heart itself.

To better understand how inhibition of the renin-angiotensin system is able to improve the prognosis in this experimental model, the response of the viable myocardium after myocardial infarction in rats has been examined in terms of the protective effects of an angiotensin CEI. Changes in blood pressure, plasma hormones involved in cardiovascular homeostasis, isoenzyme profile of left ventricular myosin, volume density of collagen within the viable myocardium, and atrial natriuretic factor (ANF) messenger RNA (mRNA) content are described 2 months after induction of a myocardial infarction, and the partial or complete reversal of these changes by chronic CEI are reported.

Materials and Methods

Experimental Design

Normotensive male Wistar rats (Iffa Credo, Lyon, France), 10 weeks old and weighing 200 g, were used throughout the study. Rats were housed in our animal facility for 2 weeks before they were subjected to operative procedures.
Left ventricular infarction was produced by ligation of the left descending anterior coronary artery as previously described by Fishbein and coworkers and Pfeffer and coworkers. Briefly, each rat was anesthetized with ether, intubated, and ventilated by a positive pressure respirator (Harvard rodent respirator, Boston, Massachusetts). A left thoracotomy was performed, the heart was gently exteriorized, and the left atrium was retracted to facilitate the ligation of the descending anterior left coronary artery between the pulmonary infundibulum and left atrium. The heart was then replaced in its normal position, the lungs were inflated, and the wound was rapidly closed. Sham operations were performed in which the heart was everted from the thorax, but no suture was made around the coronary artery. The mortality rate of this procedure was 18.5% within the first 48 hours and 22% at the end of the first week. These animals, having died before the start of the treatment period, were excluded from the study.

One week after operation, animals with myocardial infarction were randomly divided into two groups. One of these groups (n = 22 rats) was treated by a daily gavage of S9490-3 (Perindopril), 2 mg/kg in 1 ml distilled water for 2 months. The other infarcted group (n = 21 rats) and the sham-operated group (n = 15 rats) received 1 ml distilled water by daily gavage. The experimental period was 2 months from the beginning of treatment. Systolic blood pressure was measured weekly by the tail-cuff method (W + W electronic recorder 8005, ApecLab, Bagneau, France) throughout the experimental period, including 1 week before the induction of myocardial infarction and 1 week after surgery before the beginning of treatment.

At the end of the experimental period, the three groups of animals were killed by decapitation. Blood samples were collected into tubes containing 200 μM EDTA from the unanesthetized rats at the time of decapitation. These samples were centrifuged for 15 minutes at 3,000g, and the plasma was removed and frozen at -70° C. At the same time, the heart was rapidly excised and weighed. Both atria were removed. The right ventricle was then cut flush from the septum, right ventricle, apex, and base of the left ventricle. The infarcted area, which was completely scarred 2 months after the coronary ligation, and the circumference of the left ventricular cavity were measured on each left ventricular apex. The infarct, and the transversal length of the transmural scarred tissue, the transversal length of tissue that was not involved with the coronary artery ligation.

**Heart Parameters**

**Left ventricular isomyosins.** Ventricular isomyosin profiles were determined on the left ventricular apexes of all the hearts. Crude tissue obtained from samples weighing approximately 100 mg was extracted according to the method described by Mercadier and coworkers. Polyacrylamide gel electrophoresis under non-dissociating conditions was performed according to Hoh and coworkers with modifications described by Mercadier and coworkers. Each gel [3.68% (wt/vol) acrylamide and 0.12% (wt/vol) N,N′-methylene-bis-acrylamide] was loaded with 20–40 μl of a 50-fold dilution of the crude extract. The temperature of the electrophoresis buffer was maintained at 3–4° C, and gels were run at a constant voltage of 14 V/cm for 20–22 hours. Densitometric tracings of the gels were obtained, and V1 and V2 isomyosins were expressed as the percentage of the total peak densitometric height (V1 + V2 + V3).

**Morphometric analysis of the myocardial collagen network.** The cross sections of each heart were obtained at 0.5 cm from the apex. The tissues were dehydrated, embedded in paraffin, and 5-μm sections were cut. The sections were stained with Sirius red as a 0.1% solution in saturated aqueous picric acid. The Sirius red staining technique has a good affinity for collagen fibers and gives pictures of high contrast as described by Junqueira and coworkers. This contrast was increased by using a 545-nm interference filter.

To achieve a crude estimate of infarct size, slides were examined with an automatic image analysis processor (NS 1500, Nachet, Evry, France) under a monozoom (×4 magnification) microscope. The area of transmural scarred tissue, the transversal length of the infarct, and the circumference of the left ventricular cavity were measured on each left ventricular section.

Each slide was examined under the microscope (×25 magnification) with the same automatic image analysis processor based upon morphological mathematical principles, and an algorithm was developed to analyze the collagen matrix of the heart. This algorithm measures the relative area occupied by collagen in each field. Thirty-five fields were analyzed in the subendocardial layer and 35 others in the subepicardial layer in the noninfarcted part of the wall of each left ventricle. The infarcted area, which was completely scarred 2 months after the coronary ligation, and the fibrosis-rich border zone were not included in this measurement. Analysis of fibrosis was performed only in viable tissue that was not involved with the coronary artery ligation.

**Plasma Measurements**

The plasma renin concentration of plasma samples was measured by radioimmunoassay (RIA) of angiotensin I generated by incubation with an excess of rat renin substrate. Plasma renin substrate activity was reflected by the level of angiotensin measured by incubation of the plasma with an excess of mouse submaxillary gland renin (20×10-3 Goldblatt U), and the angiotensin I liberated was measured by RIA as described previously. Plasma aldosterone was measured by direct radioimmunoassay after extraction of plasma on Extrelut 20 (Merck). Plasma atrial natriuretic factor was measured after solid phase extraction on Bond Elut cartridges by radioimmunoassay with commercially available antiserum (RAS 8798, Peninsula Laboratories, St. Helens, England), standard ANF (99-126) (Peninsula), and iodinated ANF (1-28, Amersham, Zürich, Switzerland). Plasma creatinine was measured in a Beckman automated analyser.

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**ANF mRNA Measurement**

The complete method for ANF mRNA measurement has been previously reported. Each group of organs, including left atria, right atria, left ventricular base, and right ventricle, was pooled and stored at −80°C. Total RNA was extracted from the atria and ventricles with the guanidine-cesium chloride method. Total RNA was determined by measurement of optical density at 260 nm.

Each RNA sample was diluted serially five or six times and dotted on a paper sheet (Glen Cove) membrane after denaturation with formaldehyde using a Hybridot manifold (BRL). Samples from the experimental group and from the control group were applied to each dot blot.

A greater amount of total ventricular RNA was applied, that is, 0.025–0.5 μg atrial RNA and 0.625–20 μg for ventricular RNA, to ensure a similar range of optical density for all samples. The blots were then prehybridized and hybridized according to the procedure of Thomas. The DNA probe was a synthetic 40-base oligodeoxyribonucleotide complementary to nucleotides (393–432 numbered from the first ATG triplet of the ANF cDNA; kindly provided by Dr. Wilhelm, Ciba-Geigy, Basel, Switzerland). Liver total RNA was included as a negative control.

The quantification of ANF mRNA was adapted from the method of Heinrich and coworkers as developed for assay of parathyroid hormone mRNA. The absorbances of the hybrid images were plotted against increasing amounts of total dotted RNA. At least three dot blots were performed for each sample from the treated groups and corresponding control groups.

**Statistical Methods**

Results are expressed as mean±SD. One-way analysis of variance and the Newman-Keuls test were used for mean comparison of one parameter under different experimental conditions. Linear regression curves and correlation coefficients were obtained by the least-squares method. Probability (p < 0.05) that the slope of the linear regression was different from zero and that the correlation coefficient (analysis of variance) was significant were determined for each dot blot. Two-way analysis of variance was performed to test the effect of organ, experimental procedure, and treatment on plasma aldosterone and to test the effect of the experimental procedure and treatment on the collagen in the subepicardial and subendocardial layers of the left ventricle.

**Results**

**Blood Pressure, Body Weight, and Mortality Rate**

Induction of a myocardial infarction in rats caused a significant fall in blood pressure (F = 40.5, p < 0.001) during the immediately postoperative period. However, in the absence of treatment, blood pressure later returned progressively toward control values (F = 2.65, p < 0.002) (Figure 1). In contrast, treatment with CEI maintained a lower blood pressure as compared with the sham-operated group and with the untreated infarcted group throughout the experimental period (F = 40.0, p < 0.001). The statistical comparisons between the three experimental groups during the experimental period are given in Table 1.

The experimental procedure was also associated with a delay in body weight increase in these young rats, which persisted throughout the experimental period in both the treated and untreated groups as compared with the sham-operated group (F = 63, p < 0.001).

During the 2-month period of treatment, there were three deaths in the untreated group of myocardial infarctions and four in the CEI-treated group. All these deaths were precocious, occurring in the first week of the treatment period, and were probably related to the extent of the infarction area. Data reported above were collected from the surviving animals that were killed 2 months later (n = 18 in both CEI-treated and untreated groups of myocardial infarctions).

**Plasma Variables**

Myocardial infarction was associated with significant changes in plasma hormone variables (Table 2). Plasma renin concentration did not significantly increase in the untreated infarcted group, and indirect assay of renin substrate showed a significant decrease in available angiotensinogen (p < 0.01). Nevertheless, plasma aldosterone did not significantly change in the untreated infarct group. The plasma level of ANF was significantly increased by the experimental procedure (p < 0.05). Plasma creatinine did not change significantly in this group as compared with controls.

Treatment with CEI activated the renin-angiotensin system as demonstrated by the large increase in plasma renin activity (p < 0.001) and by the large decrease in available plasma renin substrate (p < 0.001). Nevertheless, plasma aldosterone did not significantly change with treatment. Plasma ANF in this group was not significantly different from control, but differed significantly from that of the untreated infarcted group (p < 0.05). Plasma creatinine showed a small but significant increase compared with controls and rats with untreated infarctions (p < 0.05).
Table 1. Statistical Comparison of Systolic Blood Pressure Between the Different Groups During the Experimental Period

<table>
<thead>
<tr>
<th>Time</th>
<th>W₁</th>
<th>W₂</th>
<th>W₃</th>
<th>W₄</th>
<th>W₅</th>
<th>W₆</th>
<th>W₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>q=8.1</td>
<td>q=18.5</td>
<td>q=22.8</td>
<td>q=12.5</td>
<td>q=12.2</td>
<td>q=1.3</td>
<td>q=14.7</td>
</tr>
<tr>
<td>Controls vs. untreated infarctions</td>
<td>NS</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.05</td>
<td>NS</td>
<td>*p&lt;0.001</td>
</tr>
<tr>
<td>Sham-operated</td>
<td>q=2.5</td>
<td>q=22.4</td>
<td>q=41.1</td>
<td>q=50.6</td>
<td>q=28.3</td>
<td>q=16.7</td>
<td>q=41.9</td>
</tr>
<tr>
<td>Controls vs. CEI-treated infarctions</td>
<td>NS</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
</tr>
<tr>
<td>Untreated infarctions vs. CEI-treated infarctions</td>
<td>q=10.6</td>
<td>q=3.9</td>
<td>q=18.3</td>
<td>q=38.1</td>
<td>q=16.1</td>
<td>q=18</td>
<td>q=27.2</td>
</tr>
<tr>
<td>Controls vs. untreated infarctions</td>
<td>*p=0.05</td>
<td>NS</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
</tr>
</tbody>
</table>

All comparisons based on Newman-Keuls test.
W, week. W₁, 1 week before induction of infarction; W₀, 1 week after surgery; W₁, 1st week of treatment.

Heart Parameters

Absolute and relative weights. Body weight at the time of sacrifice was significantly different (p<0.05) in the three experimental groups as a result of the delay in growth described above (Table 3). Absolute heart weight and the ratio of total heart weight to body weight were significantly increased in the untreated infarcted group (p<0.001). The absolute ventricular weight and the ratio of the left ventricular weight to body weight were also significantly increased in the untreated infarcted group (p<0.001) as compared with controls, but the increase in this parameter did not reach a high level of significance in relation to the weight loss of the fibrous scarred infarction of the left ventricle (Figure 2). The weights of the myocardium hemodynamically upstream from the infarcted left ventricle, that is, left atria, right ventricles, and right atria, were significantly increased in their absolute weight as in their ratio to body weight (p<0.001).

The CEI treatment significantly decreased the absolute and relative heart weights as compared with the untreated infarcted group (p<0.001) to a value that remained significantly different from the sham-operated group (q=2.84, p<0.05). The absolute and relative values of the weights of left ventricles were also normalized by treatment. The absolute and relative values of both atria and right myocardium were significantly decreased by CEI as compared with the untreated infarcted group (p<0.01), but the relative value remained slightly higher than in the sham-operated group (p=0.05). Infarct size. There was no significant difference in the transmural scarred area (F=0.13, NS) or in the infarct transversal length (F=2.4, NS) between treated and untreated infarctions (Table 4). But infarctions induced a significant increase in left ventricular cavity circumference (F=12.44, p<0.001) as compared with controls, which was not significantly modified by CEI treatment (q=1.2, NS).

Isomyosin profile. The isoenzymic profile of left ventricular myosin was modified in the untreated infarcted group as compared with sham-operated controls (Table 5). The V₁ form decreased significantly (q=6.6, p<0.01), whereas the V₂ and V₃ forms showed the expected increase (q=6.3, p<0.01) (Figure 3).

CEI treatment also modified the isomyosin profile in the left ventricle as compared with the untreated infarcted group. The V₁ form was significantly higher (q=3.77, p<0.01), and the V₂ form was significantly lower (q=4.4, p<0.01). Nevertheless, the profile of myosin was not completely normalized by the treatment, as demonstrated by the significant difference in the V₂ form between sham-operated and CEI-treated groups (q=2.97, p<0.05) and in the ratio of V₁/V₂ in these two groups (0.21 in the sham-operated group versus 0.33 in the CEI-treated infarctions, p<0.05).

Collagen network. The infarct induced significant changes (F=21.5, p<0.01) in the subendocardial collagen networks of the unscarred left ventricle (Table 6). Experimental myocardial infarction induced a large increase in volume density of collagen in the subendocardial layer of the left ventricle (q=8.46, p<0.001) (Figure 4).

CEI treatment significantly decreased the volume density of collagen in subendocardium as compared with untreated infarcted group (q=7.34, p<0.001) and reached values that were not significantly different from controls.
from the collagen values measured in the sham-operated group ($q = 1.59$, NS).

ANF mRNA. Myocardial infarction of the left ventricle resulted in a significant ($p < 0.01$) increase in the amount of ANF mRNA in all four parts of the heart examined (Table 7 and Figure 5).

As shown by the slope of the dot blot, that is, the relation between specific mRNA encoding for ANF and total RNA present in the myocardium, there were similar, approximately fivefold, increases in the four parts of the myocardium examined as compared with the sham-operated control group.

Converting enzyme inhibition significantly decreased the slope of the dot blots ($p < 0.05$) as compared with the untreated infarcted group but did not completely normalize this variable as compared with sham-operated group ($p < 0.05$).

**Discussion**

In their studies on hemodynamic and life-curve consequences of myocardial infarctions, Pfeffer and coworkers have used the scarred area of myocardial tissue to classify the degree of cardiac insufficiency. In the present study, myocardial tissue was not used to determine the exact size of the infarcted area but was used to quantify other qualitative variables within the myocardium, such as collagen content, isomyosin profile, and ANF mRNA. Thus, each animal in both experimental groups of myocardial infarction was not classified according to the extent of myocardial infarction. Nevertheless, a crude estimate of transversal length (one dimension) of the infarct size was determined from the fixed transverse sections sampled at the middle part of each left ventricle. The absence of a significant difference in infarct size between treated and untreated animals and the absence of a significant difference in the death rate in the two groups during the experimental period indicated that the difference observed between the treated and the untreated groups was due more to the effect of CEI treatment than to a difference in the extent of myocardial injury. And thus, despite the probable heterogeneity in infarct size, the effect of the model and treatment could be assessed by comparison of the mean values of each variable.

Induction of myocardial infarction led to an immediate and significant decrease in systolic blood pressure.

**Table 3. Heart Weights and Body Weights at the Time of Sacrifice**

<table>
<thead>
<tr>
<th>Weight</th>
<th>Sham-operated controls (n = 15)</th>
<th>Untreated infarctions (n = 18)</th>
<th>CEI-treated infarction (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body (g)</td>
<td>432 ± 21</td>
<td>414 ± 29</td>
<td>400 ± 41*</td>
</tr>
<tr>
<td>Heart (mg)</td>
<td>1,071 ± 80</td>
<td>1,540 ± 310***</td>
<td>1,145 ± 180†††</td>
</tr>
<tr>
<td>Heart/body (mg/g)</td>
<td>2.47 ± 0.15</td>
<td>3.75 ± 0.81***</td>
<td>2.87 ± 0.42*††††</td>
</tr>
<tr>
<td>Left ventricular (mg)</td>
<td>728 ± 40</td>
<td>880 ± 10***</td>
<td>701 ± 60†††</td>
</tr>
<tr>
<td>Left ventricular/body (mg/g)</td>
<td>1.68 ± 7</td>
<td>2.14 ± 24***</td>
<td>1.73 ± 0.13†††</td>
</tr>
<tr>
<td>Heart - left ventricular (mg)</td>
<td>343 ± 40</td>
<td>660 ± 210***</td>
<td>443 ± 106†††</td>
</tr>
<tr>
<td>Heart - left ventricular/body (mg/g)</td>
<td>0.79 ± 0.19</td>
<td>1.59 ± 0.72***</td>
<td>1.11 ± 0.37††††</td>
</tr>
</tbody>
</table>

*Significant vs. controls, $p < 0.05$; **p < 0.01; ***p < 0.001.
†Significant vs. untreated infarctions, $p < 0.05$; ††p < 0.01; †††p < 0.001.

**Figure 2. Typical appearance of the scarred and unscarred area within the left ventricle in the three experimental groups. Note the increase in septum thickness in the untreated infarct group as compared with sham-operated controls and the lack of thickening in the CEI-treated infarct group.**

**Table 4. Infarction Size**

<table>
<thead>
<tr>
<th>Infarction size</th>
<th>Sham-operated controls (n = 15)</th>
<th>Untreated infarctions (n = 18)</th>
<th>CEI-treated infarctions (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmural scarred area (mm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarction length (mm)</td>
<td>9.62 ± 4.29</td>
<td>9.19 ± 2.79</td>
<td></td>
</tr>
<tr>
<td>Left ventricular cavity circumference (mm)</td>
<td>12.9 ± 2.1</td>
<td>20.0 ± 3.7*†</td>
<td>21.5 ± 7.7*</td>
</tr>
</tbody>
</table>

***Significant vs. controls, $p < 0.001$.**
in all the infarcted animals. Systolic blood pressure did not remain low in the untreated experimental group, but it tended to return toward control values, probably as a result of the activation of peripheral pressor systems and the compensatory hypertrophy of the noninfarcted left ventricle, which can produce more force with less cells. In humans, the decrease in blood pressure induced by myocardial infarction has a vital prognostic value. If Fletcher and coworkers have demonstrated that the decrease in blood pressure observed after ligation of the coronary artery was proportional to the size of the myocardial infarct in both hypertensive and normotensive animals. In this study, the abrupt fall in blood pressure after induction of the myocardial infarction illustrates the same phenomenon. By inhibiting the renin-angiotensin system, CEI prevented the return of blood pressure to normal after myocardial infarction and thus decreased the left ventricular loading.

The plasma level of the renin-angiotensin system may partly explain the effect of treatment. In humans and in experimental animal models, congestive heart failure is associated with an activation of the renin-angiotensin system, as suggested in the present study by the increase in plasma renin concentration and by the proportional decrease in available renin substrate. In this situation, converting enzyme inhibition by blocking the transformation of angiotensin I in angiotensin II suppresses the negative feedback of angiotensin II on renin release and thus produces a large increase in the plasma level of renin activity and the consumption of renin substrate. However, the decrease in plasma angiotensin II concentration observed with CEI treatment could prevent the normal stimulatory effect of angiotensin II on the hepatic biosynthesis of renin substrate.

Surprisingly, the plasma aldosterone level did not increase in the untreated infarcted group and did not decrease in the treated group. One explanation could be the effect of ANF on the secretion of aldosterone. In our experiment, as in an earlier study, the level of ANF was increased in this model. The values found in this study are lower than those reported by Tsunoda and coworkers. This difference could be due to the lack of precaution taken during the collection of blood samples: no protease inhibitor, no quick freezing of the plasma, etc. Nevertheless, the increase in mRNA encoding for ANF confirms the probability that levels of circulating ANF were greatly increased in this model. ANF acts directly to inhibit the secretion of renin and the secretion of aldosterone. Thus, in our study, ANF could be one of the factors decreasing aldosterone secretion despite the increase in plasma renin activity.

### Table 5. Left Ventricle Isomyosin Profiles

<table>
<thead>
<tr>
<th>Isomyosin (%)</th>
<th>Sham-operated controls (n=15)</th>
<th>Untreated infarctions (n=18)</th>
<th>CEI-treated infarctions (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vi</td>
<td>62.4±9.4</td>
<td>41.6±13.4**</td>
<td>33.0±14.4*†††</td>
</tr>
<tr>
<td>Vt</td>
<td>24.6±4.5</td>
<td>31.0±4.3**</td>
<td>29.5±7.5</td>
</tr>
<tr>
<td>Vv</td>
<td>13.0±4.7</td>
<td>27.4±11.8**</td>
<td>17.5±11.8†††</td>
</tr>
</tbody>
</table>

*Significant vs. controls, p<0.05; **p<0.01.
†Significant vs. untreated infarctions, ttp<0.01.

### Table 6. Collagen Network Within the Myocardium of the Left Ventricle

<table>
<thead>
<tr>
<th>Volume density of collagen (%)</th>
<th>Sham-operated controls (n=15)</th>
<th>Untreated infarctions (n=18)</th>
<th>CEI-treated infarctions (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subendocardium</td>
<td>2.68±0.49</td>
<td>4.14±0.81***</td>
<td>2.95±0.66†††</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>2.86±0.63</td>
<td>3.50±1.07</td>
<td>2.93±0.69</td>
</tr>
</tbody>
</table>

*Significant vs. controls, ***p<0.001.
†Significant vs. untreated infarctions, †tp<0.01.
In contrast, ANF seems to be unable to totally block the increase in renin secretion but could limit its increase in this model. Thus ANF, by limiting the level of activation of the renin-angiotensin system, could act as one of the regulators of afterload in myocardial infarction. Treatment with CEI decreased the plasma level of ANF and seemed to normalize it, but the level of expression of the gene encoding for ANF in the heart was not completely normalized by treatment, demonstrating the relative insensitivity of the plasma assay of such a peptide compared with methods that better describe the level of biosynthesis and thus the overall activation of the system.

As in other experimental situations, the beneficial hemodynamic effect of CEI in myocardial infarction was associated with a slight increase in creatinine plasma. This phenomenon is probably due to the blockade of the renin-angiotensin system that could participate in the regulation of renal filtration in this model.

The absolute and relative myocardial weights for each cavity of the heart obtained in this study confirm the results reported by Pfeffer and coworkers. The absolute and relative weights of the left ventricle are difficult to interpret because of the low mass density of the scarred area compared with normal or hypertrophied cardiac muscle. Nevertheless, Anversa and coworkers have shown that a mixed concentric and eccentric hypertrophy occurs in the noninfarcted part of the left ventricular myocardium. Concentric hypertrophy of the left ventricular myocardium usually represents an adapted response of the myocardium to pressure overload that tends to normalize systolic stress, whereas eccentric hypertrophy appears as an adaptive mechanism to volume overload that tends to counteract the increase in end-diastolic wall stress normalizing the stroke volume but decreasing the ejection fraction. The absolute and relative weights of the myocardium hemodynamically upstream of the deficient left ventricle, that is, left atria, right ventricle, and right atria, appear to be predominant markers of the overload hypertrophy induced by the model.

In this model, the isoenzyme profile of left ventricular myosin was modified toward more of the V3 form and toward less of the V1 form. This change is associated with a lower velocity of shortening of the cardiac fiber but corresponds to a myothermal economy for the cardiac contraction. In myocardial infarction, this shift in the isomyosin profile could appear as a phenomenon adapted to the change in the geometry of the left ventricular cavity, which induces an increase in the stress despite a normal blood pressure.

**TABLE 7. ANF mRNA in the Four Regions of the Myocardium**

<table>
<thead>
<tr>
<th>ANF mRNA</th>
<th>Sham-operated controls</th>
<th>Unrekt untreated infarctions</th>
<th>CEI-treated infarctions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 18)</td>
<td>(n = 18)</td>
</tr>
<tr>
<td>Total RNA extracted (µg RNA/g tissue)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right atrium</td>
<td>212</td>
<td>180</td>
<td>131</td>
</tr>
<tr>
<td>Left atrium</td>
<td>226</td>
<td>136</td>
<td>108</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>191</td>
<td>216</td>
<td>232</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>258</td>
<td>246</td>
<td>194</td>
</tr>
<tr>
<td>ANF mRNA (slope)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right atrium</td>
<td>107.5 ± 4.0</td>
<td>530.3 ± 23.4**</td>
<td>360.3 ± 22.8*†</td>
</tr>
<tr>
<td>Left atrium</td>
<td>81.1 ± 5.1</td>
<td>583.9 ± 44.6**</td>
<td>373.6 ± 29.2*†</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.4 ± 0.1</td>
<td>2.5 ± 0.1**</td>
<td>1.3 ± 0.2*†</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>0.8 ± 0.2</td>
<td>3.5 ± 0.6**</td>
<td>3.1 ± 0.6*</td>
</tr>
</tbody>
</table>

All data based on pooled-tissue samples, average of 3 dot blots per group.

*Significant vs. controls.
†Significant vs. untreated infarctions, p<0.05.
Collagen is also a classic qualitative marker associated with concentric cardiac hypertrophy. While the significance of this increase in collagen content and its relation to the function of the left ventricle remain unclear, increased collagen content can affect local conduction within the heart. Accumulation of nonhomogenous collagen is probably one of the structural changes that increases the arrhythmogenic risk associated with various models of cardiac hypertrophy. Recently, Bakth et al have shown that in diabetic dogs the threshold of ventricular fibrillation was decreased with an accumulation of collagen and a hypersensitivity to catecholamines. Accumulation of collagen, particularly in the subendocardium could impair the diffusion of a depolarizing wave throughout the myocardium and thus facilitate reentrant activity and arrhythmias. In human pathology, the arrhythmogenic risk is also frequently associated with myocardial fibrosis in primitive cardiomyopathies and in myocardial infarction. In experimental models, the hydroxyproline assay was the conventional method of evaluating the collagen content of tissues. Recently, some authors have proposed morphological methods that can be automated by computerization to quantify the collagen within the heart. These methods can be applied to the heart of humans or of rats. In the present study, as in an earlier study of concentric and eccentric hypertrophy, we have used such a method to quantify the collagen network in the subendocardial layer, as in other models of concentric hypertrophy secondary to hypertension. If collagen were synthesized only by fibroblasts in the heart, this increase could be related to an activation of fibroblast (or other cell types?) activity during the increase in systolic stress that leads to an increase in the tendon-like support of hypertrophied myocytes.

Bioisometric of ANF can also be used as a qualitative marker of cardiac hypertrophy. We have recently shown that ANF mRNA markedly increases in the volume-overloaded left ventricle. The same phenomenon of ventricular myocardial recruitment for transcriptional increase of ANF gene expression in the experimental myocardial infarction is described here. Quantification of this increase by measuring the slope of the linear-regression curve obtained between specific hybridization of mRNA encoding for ANF and the increasing amounts of total mRNA extracted from the myocardium could be considered as a method of relative rather than absolute quantification. In experimental conditions such as induction or regression of cardiac hypertrophy, the relative amount of a specific messenger RNA versus other RNA could also be modified in a complicated manner, and recruitment phenomena without increase in cell number requires that the change in cardiac mass be taken into account. Nevertheless, the ANF gene expression is markedly increased in experimental myocardial infarction, and this methodological approach seems to be a more sensitive description of the activation level of the system than in the plasma determination of immuno-reactive peptide. The latter depends more on immediate contingencies such as stress, time, methods of blood collection, conservation of the peptide in plasma, and quality of extraction, and these factors probably explain the large variance of the plasma results.

Treatment with CEI reversed all the quantitative and qualitative variables measured in the heart but did not completely normalize them as compared with sham-operated controls. CEI reversed the cardiac increase in mass, the isoenzyme profile of cardiac myosin, and the collagen content in the left ventricle, and it decreased the ANF gene expression in the four parts of the myocardium. However, it did not completely normalize heart variables other than collagen. Converting enzyme has a beneficial hemodynamic effect in myocardial infarction in decreasing blood pressure by inhibiting the renin-angiotensin system, as it does in experimental hypertension and in normotension.

Angiotensin II has a direct positive chronotropic and inotropic effect on the myocardium, and inhibition of the renin-angiotensin system does not act as a direct inotropic agent. Thus, inhibition of converting enzyme acts on the function of the heart as a pump by only decreasing the load on each cardiac fiber. In myocardial infarction, the loss of contractility of the scarred area of the left ventricle leads to enlargement of the left ventricular cavity during systole and diastole without changing the area of the aortic orifice. In the absence of vasodilator treatment, the efficiency of each contraction to generate flow at an organ level decreases and
induces compensatory mechanisms in the heart, such as hypertrophy and an isomyosin shift, which tend to maintain stroke volume and decrease myocardial energetnic consumption. Simultaneously, compensatory mechanisms develop in the peripheral circulation and allow blood pressure to normalize, but this results in a permanent increase in ventricular stress that corresponds to a permanent overload on each cardiac fiber. The degree of overload depends on the size of the myocardial infarction and is probably correlated with the resulting change in ventricular geometry. Despite the absence of effect on the infarct size, CEI, by decreasing blood pressure, decreases the load on each remaining cardiac fiber and thus permits the reversal of quantitative and qualitative markers of cardiac overload. Nevertheless, the decrease in load induced by CEI does not lead to the complete normalization of all the variables within the heart. In particular, CEI seems to have more effect on myocardial markers of systolic events, such as isozyme profile and subendocardial volume density of collagen rather than on myocardial markers of diastolic events such as recruitment of ventricular myocytes for ANF biosynthesis.

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