Changes in cardiac ANG II postmyocardial infarction in rats: effects of nephrectomy and ACE inhibitors

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Leenen, Frans H. H., Vaclav Skarda, Baoxue Yuan, and Roselyn White. Changes in cardiac ANG II postmyocardial infarction in rats: effects of nephrectomy and ACE inhibitors. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H317–H325, 1999.—We evaluated in rats the time course of changes in cardiac versus plasma ANG I and II postmyocardial infarction (MI) and the effects of nephrectomy and angiotensin-converting enzyme (ACE) inhibitors on the early changes post-MI. Acute coronary artery ligation was induced in conscious rats using the two-stage model, and plasma and cardiac tissue were obtained shortly (6 h, 1 and 3 days) and chronically (1, 4, and 8–9 wk) after MI. In an additional group of rats, bilateral nephrectomy was performed 18 h before the coronary artery ligation, and samples were obtained at 6 h post-MI. Furthermore, in two additional groups of rats, treatment with enalapril and quinapril was started 3 days before the ligation, and samples were obtained at 1 or 3 days post-MI. In these groups of rats, plasma and left ventricular (LV) (infarct and infarct free) ANG I and II were measured by RIA after separation on HPLC. In control rats, plasma ANG I and II showed a clear increase at 6 h post-MI but subsequently only minor increases were observed. In contrast, LV ANG II showed major increases at 6 h and 1 day post-MI, which had returned to normal by 3 days in the infarct-free LV and after 1–2 wk in the infarct LV. LV ANG I showed a more gradual increase and remained elevated in the infarct up to 8–9 wk. Nephrectomy preceding the MI lowered ANG I and II in plasma but enhanced their increases in the heart at 6 h post-MI. Both ACE inhibitors decreased plasma ANG II associated with large increases in plasma ANG I. They also inhibited the increases in LV ANG II in both the infarct and infarct-free LV at 1 and 3 days post-MI with however no significant increase in LV ANG I. In conclusion, induction of a MI in conscious rats leads to rapid and marked, but only short-lived, increases in cardiac tissue ANG II in both the infarct and infarct-free parts of the LV. Pretreatment with ACE inhibitors, but not nephrectomy, blocks this increase. Local production appears to play a major role in the increases in cardiac ANG II post-MI.

Cardiac renin angiotensin system; ventricular remodeling; hemodynamics; coronary artery ligation; infarct

A myocardial infarction (MI) increases workload of the residual, spared myocardium, leading to increases in systolic and particularly diastolic wall stress (1). Depending on the extent and duration of the overload, progressive ventricular remodeling can develop resulting in left ventricular (LV) failure (21). Pivotal studies by Pfeffer and co-workers (20) in the rat model of MI produced by coronary artery ligation established that chronic treatment with angiotensin-converting enzyme (ACE) inhibitors improves ventricular function and survival. Because angiotensin II type 1 (AT₁) receptor blockers, at least in rats, have fairly similar positive effects (16, 27), the effectiveness of ACE inhibition likely relates to a large extent to inhibition of the renin-angiotensin system (RAS). The RAS has multiple effects on the cardiovascular system that can directly or indirectly enhance ventricular remodeling after MI. To what extent activation of the circulatory versus cardiac RAS or of both contributes to the remodeling post-MI is still unresolved. Several studies report only modest increases in plasma renin or plasma angiotensin II post-MI, until clear heart failure develops (4, 5, 29, 34). In the heart, ACE mRNA and ACE activity, as well as AT₁ receptor mRNA and its protein can markedly increase post-MI (7, 19, 22, 23, 31). LV renin mRNA showed 4-, 14- and 8-fold increases at 2, 4, and 7 days, respectively, after coronary artery ligation compared with sham-operated rats (18). Expression was again down to very low at 14 and 90 days post-MI (18). As assessed by in situ hybridization, at 4 days post-MI dense renin mRNA labeling was found only around the infarcted area, most probably in myofibroblasts and/or endothelial cells (18). From the increases in these components of the cardiac RAS, increased activity of the cardiac RAS has commonly been assumed (4, 7). However, whereas the gene expression of components of the cardiac RAS has been extensively studied post-MI, very little is known regarding changes in the biologically active compound of the system, i.e., cardiac angiotensin II. Duncan et al. (5) reported a significant increase in angiotensin II in the infarct area of the LV at 7 days after acute coronary artery ligation but no longer at 28 days. Yamagishi et al. (34) reported a significant increase in LV scar angiotensin II at 3 wk after acute coronary artery ligation. Treatment for 3 wk with the ACE inhibitor delapril lowered cardiac angiotensin II in the right ventricle (RV) and LV, but levels remained elevated in the LV scar (34).

Whether the increases in cardiac renin mRNA early post-MI (18) lead to biologically relevant increases in renin produced by cardiac tissue and therefore contribute to increases in cardiac angiotensin II post-MI has not yet been studied. Use of the classical one-stage model for acute coronary artery ligation leads to difficulties to interpret results because of the major confounding effects of anesthetic agents and surgical trauma resulting in marked stimulation of the RAS even in sham-operated rats (5, 14). In contrast, the two-stage model as described by Johnston et al. (10) and Schoemaker et al. (28) enables studies in conscious rats without these confounding influences.
The present study had three major objectives: 1) to assess changes in angiotensin II in the LV (infarct and infarct-free areas) versus plasma after acute coronary artery ligation in conscious rats using the two-stage model, with particular focus on the early period post-MI; 2) to assess the relevance of renal renin for the early changes in cardiac angiotensin II post-MI by bilateral nephrectomy before the MI; and 3) to assess the effects of treatment with ACE inhibitors with low (enalapril) versus high (quinapril) affinity for cardiac ACE on renal renin release.

**METHODS**

**Animals**

Male Wistar rats, 7–8 wk of age and weighing 200–250 g, were obtained from Charles River Breeding Laboratories (Montreal, Canada). Rats were given food and water ad libitum and kept on a 12-h light-dark cycle. After an acclimatization period of at least 4 days, the rats were randomly chosen for surgery for acute coronary artery ligation or sham ligation. During the chronic studies, animals were housed two per cage, and during the short-term studies, they were housed one per cage. Unless stated otherwise, surgical procedures were performed under halothane-nitrous oxide-oxygen inhalation anesthesia. All animals were treated in accordance with the procedures outlined in the “Guide for the Care and Use of Experimental Animals” endorsed by the Medical Research Council of Canada.

**Surgery for Application of Coronary Occluder**

Rats were anesthetized with 4% halothane and intubated with a number 14 catheter (Insyte, Becton Dickinson, Sandy, UT) through which 10% halothane in oxygen-nitrous oxide inhalation anesthesia. All animals were treated in accordance with the procedures outlined in the “Guide for the Care and Use of Experimental Animals” endorsed by the Medical Research Council of Canada.

**Actual Coronary Artery Ligation**

Seven to ten days after the open-chest surgery, we carefully pulled the occluder until it was no longer possible to move the occluder in relation to the outer guide. The exposed occluder was melted down with a cautery to form a bubble adjacent to the distal end of the outer guide tubing, fixing it in place. Animals allocated to sham surgery underwent the same procedures except that the coronary occluder was not placed around the left coronary artery.

**Experimental Protocols**

The experiments outlined below were performed in separate groups of rats. Hemodynamics and plasma versus cardiac angiotensin II were evaluated at 6 h and 1 and 3 days and 1, 4, and 8–9 wk after coronary artery ligation. LV pressures were only measured in the two (1 and 3 days) ACE-inhibitor experiments.

Six-hour studies. In rats with a (sham) coronary occluder positioned for 7–10 days, a PE-50 catheter was placed in the left carotid artery as described previously (33). About 18 h later and early in the morning, the coronary artery was (sham) ligated by pulling the occluder. Six hours after the ligation, we recorded resting blood pressure (BP) and heart rate in conscious unrestrained rats after they were allowed a 30-min rest period. We then collected a 2-ml arterial blood sample for plasma renin activity (PRA) and plasma angiotensin II assay. The rats were then euthanized with 2 M KCl (1 ml/rat), and the heart was removed.

In a separate experiment, in different groups of rats with a (sham) coronary occluder positioned for 7–10 days, we performed nephrectomy or sham nephrectomy via bilateral flank incisions 18 h before the coronary artery ligation. A PE-50 catheter was placed in the left carotid artery at the same time. The remaining of the protocol was similar as above.

One- and three-day studies. Treatment with enalapril (250 mg/kg drinking water) or quinapril (200 mg/kg drinking water) was started 3 days before coronary artery ligation. For rationale for these doses, see Ruzicka et al. (25, 26). Water intake to assess actual drug intake was monitored throughout the study. Average water intake amounted to 140–150 ml/kg−1·day−1 before and 80–90 ml/kg−1·day−1 after coronary artery ligation. Average drug intake was therefore 35–40 mg·kg−1·day−1 for enalapril and about 30 mg·kg−1·day−1 for quinapril before the MI and 3–40% less after the MI.

In two separate experiments, at 1 or 3 days after the ligation and early in the morning, a PE-50 catheter was inserted into the LV via the right carotid artery and exteriorized on the neck. After a 4-h recovery period, resting LV end-diastolic pressure (LVEDP), LV peak systolic pressure (LVSP), and heart rate were assessed in conscious, unrestrained rats. This was followed by blood sampling and 2 M KCl (see Six-hour studies).

One, four, and eight to nine week studies. For these three separate experiments, at 1, 4, or 8–9 wk post-MI, a PE-50 catheter was placed in the left carotid artery. Arterial blood pressure and heart rate were measured in resting, conscious rats 24 h later. Subsequently, one or two arterial blood samples were collected for PRA and plasma angiotensin II assay. This was followed by 2 M KCl and removal of the heart.

**Sampling of Blood and Cardiac Tissue for Angiotensin Assays**

Arterial blood was collected into chilled microcentrifuge tubes containing EDTA-Na2 and 1,10-phenanthroline. After centrifugation, plasma was immediately extracted on a SepPak C18 cartridge (Millipore). The heart was rapidly excised and washed in ice-cold saline. After we removed the atria and great vessels, we blotted the ventricles dry and dissected the right ventricle along its septal insertion from the LV. In the chronic (≥7 days) experiments, the visibly necrotic area and intact tissue of the LV were then separated, weighed, and each placed into boiling 1 mol/L acetic acid. For the 6-h, 1- and 3-days studies, the LV area below the ligation suture, including the apex, was sampled as infarcted tissue. A team of two experienced workers practiced the whole procedure extensively; from KCl injection until placement of the LV into boiling acetic acid lasted <60 s for all hearts. Tissue samples were boiled for 15 min and then homogenized for 25 s. After
Assessment of Angiotensin I and II

Both the processing of samples and the actual assays were recently described in detail (26) and are here only summarized. Plasma and LV angiotensins were assessed by RIA after separation on HPLC. For this, angiotensins were separated on a CSC-Spherisorb-ODS2 C18 column, 15 x 0.46 cm with a 5-µm particle size. Fractions were collected every 1 min and divided into two pools containing the peaks of the two angiotensins. Sensitivity of the assays and recovery rates were previously reported (26). The coefficient of variation for interassay variance amounted to 14% for angiotensin I and 13% for angiotensin II, for intra-assay variance 8% and 5%, respectively.

PRA was determined by a RIA for angiotensin I, generated by incubation of plasma for 30 min at 37°C and pH 7.5.

Data Analysis

The MI ratio was defined as (weight of the infarcted area/total LV weight) times 100%. In the chronic (≥7 days) experiments, results from rats with an MI ratio <30% were excluded from the analysis. Results are expressed as means ± SE. Differences between groups at a given treatment period were evaluated by analysis of variance and Duncan’s multiple-range test (where applicable). P < 0.05 was considered statistically significant.

RESULTS

Acute coronary artery ligation resulted in MI ratios in the 40% range with no significant differences between groups at different time points (Table 1). Mortality was in the 40–50% range and reflected primarily early mortality (i.e., first hours post-MI) over the period of follow up. Mortality due to sham ligation was zero. LV weight showed a modest increase in the first few days post-MI, possibly reflecting interstitial edema (21), which was no longer present by 1 wk, but an increase in LV weight reappeared after 4 wk and 2 mo. RV weight showed a significant increase as of 1 wk post-MI. Body weight showed small decreases as of 1 wk post-MI (Table 1).

Acute coronary artery ligation in conscious rats caused a clear, significant decrease in systolic and diastolic BP at 6 h. LV peak systolic pressure was significantly decreased and LVEDP significantly increased at 1 and 3 days after coronary artery ligation (Table 2). During the more chronic phase, the extent of the decrease in BP appeared to diminish somewhat, but BP remained significantly decreased up to 2 mo post-MI. At no point did heart rate increase significantly.

Bilateral nephrectomy preceding the coronary artery ligation did not affect the changes in BP and LV weight at 6 h post-MI but caused a significant bradycardia (Table 3).

Treatment with either ACE inhibitor did not affect LVEDP and LVPSP at 1 day or LVPSP at 3 days post-MI but decreased LVEDP at 3 days (significant for enalapril, Table 2). The initial increase in LV weight post-MI was prevented by either ACE inhibitor (Table 1).

Table 1. Body weights, LV and RV weights, and MI ratios in rats postmyocardial infarction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Body Wt, g</th>
<th>LV Wt, mg/100 g body wt</th>
<th>RV Wt, mg/100 g body wt</th>
<th>MI Ratio, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h Post-MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>13</td>
<td>298 ± 5</td>
<td>193 ± 5</td>
<td>48 ± 1</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>MI</td>
<td>9</td>
<td>303 ± 7</td>
<td>236 ± 6*</td>
<td>50 ± 1</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>1 day Post-MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>10</td>
<td>315 ± 5</td>
<td>189 ± 4</td>
<td>47 ± 2</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>11</td>
<td>306 ± 5</td>
<td>205 ± 3*</td>
<td>44 ± 2</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>Enalapril</td>
<td>10</td>
<td>302 ± 4</td>
<td>186 ± 3†</td>
<td>44 ± 2</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>Quinapril</td>
<td>10</td>
<td>319 ± 6</td>
<td>192 ± 4†</td>
<td>45 ± 2</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>3 days Post-MI</td>
<td>9</td>
<td>378 ± 12</td>
<td>188 ± 7</td>
<td>49 ± 3</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>13</td>
<td>379 ± 8</td>
<td>167 ± 6</td>
<td>44 ± 2</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>MI</td>
<td>8</td>
<td>337 ± 9*</td>
<td>163 ± 4</td>
<td>54 ± 3*</td>
<td></td>
</tr>
<tr>
<td>1 wk Post-MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>8</td>
<td>446 ± 8</td>
<td>154 ± 5</td>
<td>39 ± 1</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>15</td>
<td>411 ± 10*</td>
<td>177 ± 4*</td>
<td>59 ± 6*</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>2 mo Post-MI</td>
<td>16</td>
<td>481 ± 9</td>
<td>157 ± 3</td>
<td>39 ± 1</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>19</td>
<td>476 ± 7</td>
<td>177 ± 4*</td>
<td>51 ± 3*</td>
<td>44 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. MI, myocardial infarction; LV, left ventricular; RV, right ventricular; LV weight includes infarcted and noninfarcted tissue. MI ratio, weight of infarcted area/total LV weight × 100%. MI ratio at 6 h (and at 1 and 3 days) are only rough estimates. *P < 0.05 vs. sham, †P < 0.05 vs. MI untreated.
Table 2. Changes in hemodynamic parameters in rats postmyocardial infarction

<table>
<thead>
<tr>
<th>Time Post-MI</th>
<th>LVEDP, mmHg</th>
<th>LVSP, mmHg</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>Heart Rate, beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h Post-MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>125 ± 3</td>
<td>92 ± 3*</td>
<td>95 ± 3</td>
<td>75 ± 4*</td>
<td>389 ± 5</td>
</tr>
<tr>
<td>MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>379 ± 14</td>
</tr>
<tr>
<td>1 day Post-MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0 ± 0</td>
<td>128 ± 1</td>
<td></td>
<td></td>
<td>391 ± 10</td>
</tr>
<tr>
<td>MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>12 ± 1*</td>
<td>96 ± 3*</td>
<td></td>
<td></td>
<td>367 ± 12</td>
</tr>
<tr>
<td>Enalapril</td>
<td>14 ± 2*</td>
<td>88 ± 5*</td>
<td></td>
<td></td>
<td>392 ± 14</td>
</tr>
<tr>
<td>Quinapril</td>
<td>10 ± 1*</td>
<td>98 ± 3*</td>
<td></td>
<td></td>
<td>393 ± 7</td>
</tr>
<tr>
<td>3 days Post-MI</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sham</td>
<td>1 ± 1</td>
<td>127 ± 4*</td>
<td></td>
<td></td>
<td>377 ± 8</td>
</tr>
<tr>
<td>MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>15 ± 3*</td>
<td>104 ± 4*</td>
<td></td>
<td></td>
<td>403 ± 12</td>
</tr>
<tr>
<td>Enalapril</td>
<td>9 ± 1*†</td>
<td>109 ± 1*</td>
<td></td>
<td></td>
<td>402 ± 19</td>
</tr>
<tr>
<td>Quinapril</td>
<td>12 ± 2*</td>
<td>104 ± 5*</td>
<td></td>
<td></td>
<td>391 ± 14</td>
</tr>
<tr>
<td>1 wk Post-MI</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>131 ± 2</td>
<td>102 ± 4</td>
<td>379 ± 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>101 ± 3*</td>
<td>82 ± 2*</td>
<td>396 ± 5</td>
<td></td>
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</tr>
<tr>
<td>4 wk Post-MI</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sham</td>
<td>140 ± 1</td>
<td>112 ± 2</td>
<td>386 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>114 ± 3*</td>
<td>92 ± 2*</td>
<td>387 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–9 wk Post-MI</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sham</td>
<td>137 ± 2</td>
<td>106 ± 2</td>
<td>387 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>117 ± 2*</td>
<td>92 ± 2*</td>
<td>390 ± 4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; for n see Table 1. LVEDP, LV end-diastolic pressure; LVSP, LV peak systolic pressure; Syst BP, systolic blood pressure; Diast BP, diastolic BP. *P < 0.05 vs. sham; †P < 0.05 vs. MI untreated.

Plasma Renin and Plasma Angiotensin II

PRA showed a significant increase at 6 h and only minor, not significant increases at 3 days, 4 wk, and 2 mo post-MI (Fig. 1). Plasma angiotensin II also showed a clear increase at 6 h post-MI and subsequently modest increases by 50–100%, which were significant at 1 and 4 wk and 2 mo post-MI (Fig. 2). Plasma angiotensin I showed more variability, and similar increases were not significant (1 and 3 days data, Table 4).

In the nephrectomy experiment, plasma angiotensin I and II were not elevated. In the nephrectomy + MI group, plasma angiotensin I and II also remained low (Table 3).

The two ACE inhibitors were similarly effective in lowering plasma angiotensin II in rats post-MI, significant at day 1 versus MI rats untreated (Fig. 3). These decreases in plasma angiotensin II were associated with 5- to 10-fold increases in plasma angiotensin I. These increases showed marked variation, but a tendency (not significant) for larger increases on enalapril versus quinapril is apparent at both time points (Table 4).

Cardiac Angiotensins Post-MI

In the infarct area, tissue angiotensin II already showed a marked increase at 6 h post-MI, which persisted at 1 and 3 days (Fig. 4). By 1 wk only a modest (P < 0.05) increase was still present, and by 4 and 8 wk post-MI no significant differences were present any-
more, all relative to LV angiotensin II in sham-operated rats. In the infarct-free part of the LV, tissue angiotensin II also rapidly increased and was markedly increased at 6 h and 1 day post-MI (Fig. 4). However, by 3 days this increase had already disappeared compared with LV angiotensin II in sham-operated rats.

Cardiac angiotensin I (Fig. 5) followed a somewhat different pattern than angiotensin II. In the infarct area, tissue angiotensin I increased over the first week and remained elevated (although less than at 3 and 7 days) throughout the whole period of follow up (i.e., up to 2 mo). In the infarct-free area of the LV, tissue angiotensin I increased less, and only significantly after 1 wk.

Nephrectomy and Cardiac Angiotensins Post-MI

Nephrectomy per se did not lower LV angiotensin II and even tended (P < 0.10) to increase LV angiotensin I. At 6 h post-MI, in the sham nephrectomy group LV angiotensin II was significantly elevated both in the infarct-free and infarct area. Changes in LV angiotensin I were similar but nonsignificant. In the nephrectomy group, 6 h post-MI, LV angiotensin II was also significantly increased, and in the infarct-free area LV angiotensin II was significantly higher compared with the group with MI and intact kidneys. LV angiotensin I followed a similar pattern but showed enhanced responses in both the infarct-free and infarct area in nephrectomized versus sham nephrectomized rats (Fig. 6).

ACE Inhibitors and Cardiac Angiotensin II Post-MI

In the infarct area and 1 day post-MI, both ACE inhibitors significantly lowered the high tissue angiotensin II, but levels remained elevated compared with the LV of sham-operated rats. At 3 days post-MI, levels were fully normalized by quinapril (Fig. 7). Angiotensin I levels in the infarct area were not affected by either ACE inhibitor at 1 or 3 days post-MI (Table 4).

In the infarct-free area of the LV, both ACE inhibitors blunted the increase in angiotensin II at day 1 post-MI, more clearly for quinapril than for enalapril. At 3 days post-MI, angiotensin II levels were back to normal, and these were not further lowered by either ACE inhibitor (Fig. 7). Similar to the infarct area, in the infarct-free LV the ACE-inhibitors caused only minor, nonsignificant increases in tissue angiotensin I (Table 4).

DISCUSSION

The present study provides several major new findings regarding the cardiac RAS post-MI in rats. First, whereas plasma renin and angiotensin II show only modest increases during the first 2 mo post-MI, angio-
tensin II in the infarct and infarct-free parts of the LV markedly increases during the first few days of post-MI but then normalizes. Second, bilateral nephrectomy preceding the MI does not inhibit the increase in cardiac angiotensin II, even enhances the increase. Third, both ACE inhibitors lower plasma angiotensin II post-MI associated with major increases in plasma angiotensin I. In the infarct and infarct-free parts of the LV, the ACE inhibitors also prevent most of the increases in tissue angiotensin II but without a further increase in tissue angiotensin I.

**Circulatory RAS**

Neurohumoral activation post-MI has been well documented both in humans and in animals. Early increases in plasma renin and angiotensin II, as reported in humans (15, 24, 29), can be caused by, for example, hypotension and sympathetic activation. So far, no studies have assessed this time course in the equivalent rat model, i.e., induction of MI in the conscious rat as used in the present study. Similar to humans, plasma angiotensin II quickly (at +6 h) increased post-MI, but only minor/modest increases were noted as of 1 day until 2 mo. Because blood pressure remains lower (Table 2) and sympathetic drive increased (13), it is possible that other mechanisms inhibiting renin release, such as an elevated atrial natriuretic peptide (32), may offset in part the effects of activating mechanisms.

Both ACE inhibitors decreased plasma angiotensin II at 1 and 3 days post-MI, associated with marked increases in plasma angiotensin I. The latter likely reflects less negative feedback on renal renin release by the lower angiotensin II levels. The substantial increase in the ratio of plasma angiotensin I to angiotensin II is consistent with a high degree of inhibition of circulatory/endothelial ACE by either ACE inhibitor (11).
Cardiac RAS

The cardiac tissue angiotensin II levels rapidly and markedly increased after acute coronary artery ligation in conscious rats. The extent of these increases was fairly similar in the infarct and infarct-free areas of the LV at 6 h and 1 day post-MI and substantially more than the modest increase in plasma angiotensin II. Evidence for compartmentalized regulation of angiotensin II within the heart is apparent during the more chronic phase post-MI. Thus tissue angiotensin II in the infarct-free, LV normalizes by 2–3 days and remains normal at 1, 4, and 8 wk post-MI. In contrast, in the infarct tissue, angiotensin II was still clearly elevated by 3 days, to a minor extent by 7 days, and normal at 4 and 8 wk post-MI. Thus even within the LV regulation of angiotensin II appears to occur differently in the infarct versus infarct-free area. Similar changes were reported by Duncan et al. (5) at 1 and 4 wk post-MI, using the one-stage model. In the study by Yamagishi et al. (34), tissue angiotensin II in the infarct-free LV was also normal at 3 wk post-MI but still threefold elevated in the infarcted LV. This more persistent increase in angiotensin II in the infarct may relate to their method of collection of the heart, which took up to 7 min (34), that may have lead to aspecific activation and degradation.

Whereas plasma angiotensin I and angiotensin II followed a similar pattern post-MI, in the heart the pattern of changes in tissue angiotensin I versus angiotensin II appears to differ. Thus angiotensin I in the infarct area and to a less extent in the infarct-free area remains elevated up to 2 mo post-MI. This increase in the ratio of tissue angiotensin I to angiotensin II is suggestive for less effective ACE activity within the cardiac tissue. This is a rather unexpected finding considering the many studies reporting increases in ACE mRNA and/or activity in the LV post-MI (6, 7, 19, 30). One may speculate that post-MI-elevated ANP inhibits cardiac conversion of angiotensin I to angiotensin II (12).

We conclude from the above findings on the changes in LV angiotensin I and angiotensin II versus changes in plasma angiotensin I and II post-MI that the cardiac levels are clearly locally regulated and that within the heart levels in infarct and infarct-free areas show different regulation. The present study does not address which mechanisms contribute to such differential regulation or which population(s) of cells may contribute to such local regulation. Cardiac renin mRNA increases clearly within 2 days post-MI, but this increased expression is only short lived and has already disappeared by 14 days post-MI (18). The present results on the time course of cardiac angiotensin II post-MI are consistent with the time course of cardiac renin mRNA post-MI. The nephrectomy experiment indicates that uptake of renal renin (3) is not required for the initial increase in cardiac angiotensin II post-MI. This finding suggests that the increase in cardiac renin mRNA post-MI leads to biologically relevant increases in cardiac renin and angiotensin II. Of interest, increases in cardiac angiotensin I and II post-MI were even enhanced in nephrectomized rats. One may speculate that in normal hearts, renin taken up from the plasma suppresses cardiac renin production and that in the absence of renal renin, cardiac renin becomes more responsive.

ACE Inhibitors and Cardiac RAS

Pretreatment with either ACE inhibitor clearly blunted the increases in LV angiotensin II post-MI. This inhibition was found in both the infarct and infarct-free areas of the LV, consistent with a study by Johnston et al. (9) who showed an inhibition of cardiac ACE in both areas by enalapril. In the present study, quinapril tended to be more effective than enalapril (i.e., infarct-free LV angiotensin II at 1 day and LV infarct angiotensin II at 3 days). However, these differences did not reach statistical significance and require further study. At day 1 post-MI, the increase in LV infarct angiotensin II was only partially prevented and clear increases persisted. Further studies with higher doses of the ACE inhibitors will be needed to assess whether more complete inhibition of this increase is possible. It appears likely that this early marked increase in LV tissue angiotensin II post-MI exerts biological effects, for example, for wound healing. To what extent these are beneficial or detrimental for (long-term) outcome can be assessed by initiation of blockade either before the MI or 1–2 wk post-MI.

Whereas the ACE inhibitors caused marked increases in plasma angiotensin I, cardiac tissue angiotensin I showed only minor changes. A similar dissociation of plasma and LV angiotensin I was found in rats with aortocaval shunt (see Table 1, Ref. 26). These findings are further consistent with local control of cardiac angiotensin II levels and that a cardiac reninlike enzyme is not subject to negative feedback control by local angiotensin II levels. Iwai et al. (8) recently showed that quinapril does not cause a further induction of renin mRNA in the balloon-injured artery and concluded that renin gene expression in medial smooth muscle cells is regulated differently than in juxtaglomerular cells. Assessment of the effects of ACE inhibitors on cardiac renin mRNA and cardiac renin activity post-MI is essential to substantiate such a conclusion for the heart.

Methodological Considerations

Assessment of angiotensin I and II by HPLC and RIA represents a standard biochemical technique assuming antibodies of sufficient quality. Plasma and cardiac angiotensin II in control rats were fairly consistent across experiments with low variability (see means ± SE). Angiotensin I showed somewhat more variability and results of the 3-day experiment show higher values due to spreading of the peaks. As we previously discussed (26), the sampling part of the method is critical to preserve angiotensins at the level that most likely reflects the actual (patho) physiological processes. The early changes post-MI were assessed after induction of
a MI in conscious rats, which eliminates the major confounding effects of anesthetics and extensive surgery. The two-stage model clearly appears essential to detect the early changes post-MI; use of the one-stage model resulted in high cardiac and plasma levels at 1–2 days postsurgery in both sham and MI rats (5). To stop ischemia-activated proteases, cardiac tissue was placed into boiling acetic acid within 1 min after KCl injection. This approach has, however, the disadvantage that infarct size can only be assessed in a global fashion. However, the clear decreases in BP, the early increase in LVEDP, and the development of RV hypertrophy (20) are consistent with the induction of moderate to large size infarcts.

In conclusion, the present study demonstrates that the induction of a MI in conscious rats leads to rapid and marked increases in cardiac tissue angiotensin II in both the infarct and infarct-free parts of the LV. However, these increases are short lived, and cardiac angiotensin II is back to normal by 3 days post-MI in both the infarct and infarct-free parts of the LV.

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