Local Angiotensin II Generation in the Rat Heart
Role of Renin Uptake

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Abstract—To elucidate the local effects of renin in the coronary circulation, we examined local angiotensin (Ang) I and II formation, as well as coronary vasoconstriction in response to renin administration, and compared the effects with exogenous infused Ang I. We perfused isolated hearts from rats overexpressing the human angiotensinogen gene in a Langendorff preparation and measured the hemodynamic effects and the released products. We also investigated cardiac Ang I conversion, including the contribution of non-angiotensin-converting enzyme–dependent Ang II–generating pathways. Finally, we studied Ang I conversion in vitro in heart homogenates. Renin and Ang I infusion both generated Ang II. Ang II release and vasoconstriction continued after renin infusion was stopped, even though renin disappeared immediately from the perfusate. In contrast, after Ang I infusion, Ang II release and coronary flow returned to basal levels.

Ang I conversion (Ang II/Ang I ratio) was higher after renin infusion (0.109 ± 0.027 versus 0.026 ± 0.003, 15 minutes, P<.02) compared with infused Ang I. Remikiren added to the renin infusion abolished Ang I and II; captopril suppressed only Ang II, whereas an AT₁ receptor blocker did not affect Ang I and II formation. All the drugs prevented renin-induced coronary flow changes. Total cardiac Ang II–forming activity was only partially inhibited by cilazaprilat (4.1 ± 0.1 fmol · min⁻¹ · mg⁻¹) and on a larger extent by chymostatin (2.6 ± 0.3 fmol · min⁻¹ · mg⁻¹) compared with control values (5.6 ± 0.4 fmol · min⁻¹ · mg⁻¹). We conclude that renin can be taken up by cardiac or coronary vascular tissue and induces long-lasting local Ang II generation and vasoconstriction. Locally formed Ang I was converted more effectively than infused Ang I. Furthermore, the comparison of in vivo and in vitro Ang I conversion suggests that in vitro assays may underestimate the functional contribution of angiotensin-converting enzyme to intracardiac Ang II formation. (Circ Res. 1998;82:13-20.)

Key Words: transgenic rat • angiotensin • human renin • cardiac renin-angiotensin system • chymase-like activity

Angiotensin II plays a major role in cardiovascular homeostasis, including the regulation of blood pressure, salt balance, and tissue remodeling. Ang I and Ang II are produced not only in the blood compartment but also locally in tissues. In fact, the tissues are the major site of Ang I and Ang II formation, and the release of locally formed Ang II contributes to the circulating levels of these peptides. All the components of the renin-angiotensin system have been detected in the heart, indicating that the heart is not only a target but also a site of endocrine or paracrine Ang II formation. Local Ang II formation may result from the interaction of renin and Aogen produced within the vessel wall or from the uptake and retention of renin and Aogen from the plasma, on the cell surface, or the interstitial fluid. Many studies addressing the cardiac effects of Ang II have been performed by infusing Ang I or Ang II into the coronary circulation. In vivo, the cascade leading to Ang formation is stimulated by the enzyme renin. Even though it seems not to be produced by cardiac tissue, renin may be taken up to react locally with its substrate, Aogen.

There is a current controversy regarding the major pathway for Ang II formation in the human heart. Although ACE inhibitors are highly effective in treating hypertension-associated cardiac hypertrophy and congestive heart failure, studies with ACE inhibitors have raised the question whether ACE is the only enzyme involved in Ang II formation, since elevated plasma Ang II levels were found despite adequate and long-term ACE inhibition. Indeed, in a preliminary clinical study in patients with heart failure, the AT₁ receptor blocker losartan was more effective than the ACE inhibitor captopril.

The goal of the present study was to evaluate the local cardiac effects of human renin. To avoid confounding effects of endogenous renin produced by the rat, we used the isolated perfused hearts of rats harboring the human Aogen gene as a pharmacological model to study local Ang formation. Pharmacological concentrations of human renin were infused to facilitate the detection of local Ang formation from Aogen. This transgenic rat model allowed us a unique opportunity to study the effects of infused human renin and the actions of a human renin inhibitor. In this model, infusion of human renin induced persistent local Ang formation and coronary vasocon-
Selected Abbreviations and Acronyms

ACE = angiotensin-converting enzyme  
Ang = angiotensin  
AOGEN = angiotensinogen  
h-MCP = human heart chymase  
MCP = mast cell proteinase  
PTCA = percutaneous transluminal coronary angioplasty  
r-MCP 1 = rat MCP 1  
RIA = radioimmunoassay

striction in contrast to infused Ang I. In addition, locally formed Ang I was converted more efficiently than infused Ang I. These results are consistent with the notion that renin can be taken up by the cardiac tissue and that it exerts its action locally. Furthermore, the comparison of in vivo and in vitro Ang I conversion by ACE and chymase-like activity suggested that in vitro assays may underestimate the functional contribution of ACE to intracardiac Ang II formation.

Materials and Methods

Animals
Male heterozygous Sprague-Dawley rats harboring the complete human genomic AOGEN gene (TGR [hAOGEN] 1623) weighing 350 to 430 g were used for all experiments. The transgenic line was developed as outlined elsewhere. The rats were kept in rooms at 24±2°C and were fed a standard rat diet (No. C-1000, Altromin) containing 0.2% sodium by weight and were allowed free access to tap water. All procedures were performed according to guidelines from the American Physiological Society and were approved by local authorities (permit No. 95.00197).

Isolated Perfused Heart
Transgenic rats were previously heparinized and anesthetized with pentobarbital (150 mg/kg IP). Once the rat was deeply anesthetized, the heart was removed by sternectomy and placed in iced Krebs-Henseleit buffer. The heart was cannulated immediately via the aorta, and retrograde perfusion was performed in a Langendorff apparatus under constant pressure (90 mm Hg) with a modified Krebs-Henseleit solution of the following composition (mmol/L): NaCl 114.7, KCl 4.7, MgSO4 1.2, KH2PO4 1.5, NaHCO3 25, CaCl2 2.5, and glucose 11.1, along with 0.1% BSA. The solution was gassed with 95% O2/5% CO2 before,20 aiming at minimal handling in order not to lose ACE and/or chymase-like enzymes. Pieces of left ventricular myocardium were dissected, minced, and homogenized in 2.5 mL of 50 mmol/L potassium phosphate, pH 7.4, using a Polytron homogenizer (Heidolph). Finally, the pellets (1 g wet weight) were taken up in 2 mL of 0.5 mol/L sodium phosphate buffer, pH 7.4, including 150 mmol/L sodium chloride (NaCl); the protein concentration was measured by the method of Lowry at a52; and the aliquots were frozen at −80°C. A total of 25.6±1.9 mg protein per gram tissue wet weight was extracted.

Experimental Protocols
After an initial 20-minute baseline perfusion, perfusate for measurement of peptides was collected every 5 minutes for 30 minutes. All perfusate samples were collected over 20 to 20 seconds in the presence of an inhibitor cocktail containing the human specific renin inhibitor ciprokiren (2×10−5 mol/L), the ACE inhibitor cilazapril (2×10−5 mol/L), and 5% EDTA to prevent any Ang formation outside the hearts. Preliminary experiments (data not shown) demonstrated that this concentration of ciprokiren completely blocks renin activity during sample collection and handling. Renin measurements were performed without inhibitor cocktail in the presence of 500 μL BSA (Sigma). Purified human recombinant renin (Dr S. Mathews, Hoffmann-LaRoche, Basel, Switzerland) or Ang I was infused for 15 minutes. The Ang I dosage was chosen to achieve an equipotent concentration in contrast to infused Ang I. In addition, locally formed Ang I was converted more efficiently than infused Ang I. These results are consistent with the notion that renin can be taken up by the cardiac tissue and that it exerts its action locally. Furthermore, the comparison of in vivo and in vitro Ang I conversion by ACE and chymase-like activity suggested that in vitro assays may underestimate the functional contribution of ACE to intracardiac Ang II formation.

One sample was obtained after the washout period of each experiment to exclude contamination of the perfusion system with human renin or Ang peptides and to analyze the release of human AOGEN.

AOGEN Release
This protocol was conducted to examine the release of human AOGEN from the heart preparation. The effluent perfusate was collected at 20 minutes from transgenic rat hearts (n=5 to 9) of the different protocols without infusion of renin or other reagents.

Ang I and Ang II Release and Flow Effects After Human Renin or Ang I Infusion
We performed this protocol to determine the hemodynamic and biochemical differences between an infusion of human renin or Ang I. Human renin (50 ng/mL, n=9) or Ang I (300 pg/mL, n=6) was infused for 15 minutes. The effluent was collected every 5 minutes for 25 minutes. Effects on the coronary flow were registered according to the same protocol.

Rate of Ang I Conversion After Human Renin or Ang I Infusion
The rate of Ang I conversion was calculated as the ratio of the molar concentration of Ang II to the molar concentration of Ang I in the perfusate released from the perfused hearts.

ACE-Dependent and ACE-Independent Ang II Generation
We conducted this protocol to investigate a potential ACE-independent Ang II–generating pathway in the isolated perfused rat heart. After 20 minutes of baseline perfusion, either the ACE inhibitor captopril (n=7), the human specific renin inhibitor remikiren (n=6), or the AT1 receptor blocker EXP 3174 (n=6) was infused for 30 minutes. Five minutes after the addition of the inhibitors, a 15-minute human renin (50 ng/mL) infusion was begun. Perfusion samples for peptide measurements were obtained every 5 minutes, and the hemodynamic parameters were recorded.

In Vitro Measurement of Total Cardiac Ang II–Forming Activities
Membranes were prepared at 4°C in a manner similar to one described before,20 aiming at minimal handling in order not to lose ACE and/or chymase-like enzymes. Pieces of left ventricular myocardium were dissected, minced, and homogenized in 2.5 mL of 50 mmol/L potassium phosphate, pH 7.4, using a Polytron homogenizer (Heidolph). Finally, the pellets (1 g wet weight) were taken up in 2 mL of 0.5 mol/L sodium phosphate buffer, pH 7.4, including 150 mmol/L sodium chloride (NaCl); the protein concentration was measured by the method of Lowry at a21; and the aliquots were frozen at −80°C. A total of 25.6±1.9 mg protein per gram tissue wet weight was extracted.

The conversion of 125I-Ang I to 125I-Ang II was examined in the absence (maximal generation) and the presence of inhibitors of ACE or chymase-like enzymes (fractional conversion). The assay was as described earlier20 and was performed in 0.1 mol/L sodium phosphate, pH 7.4, including 150 mmol/L NaCl. To evaluate the conversion of 125I-Ang I, the incubation mixture was chromatographed on reverse-phase HPLC as described before,20 fractions were collected, and the radioactivity of each fraction was counted for quantification. Under our conditions, the Ang peptides could be well separated with the following retention times: 125I-Ang I fragments, 2 minutes; Ang II, 7 minutes; 125I-Ang II, 9 minutes; Ang I, 10 minutes; and 125I-Ang I, 12 minutes. The recovery for the chromatographed 125I-Ang I and 125I-Ang II was 94.7% and 87.5%, respectively.

RIA and Enzyme Kinetic Determinations
The polyclonal antibody for Ang II RIA was raised in rabbits (ICN, 5.5 mol/L assay tube). It was extremely specific for Ang II, with no essentially cross-reactivities against other Ang peptides and fragments.
100% for Ang II; 0.37±0.1% for Ang I; and <0.02% for Ang III, Ang (2–10), Ang (3–8), Ang (4–8), and Ang (5–8). The cross-reactivity of the anti–Ang I antibody (IC_{50}, 2.4 fmol/assay tube) was <0.01% to Ang II, <0.01% to (Val5)Ang II, 100% to (des-Asp1)Ang I, 0.03% to Ang I/II (1–7), and 0.02% to Ang III. Immunoactive Ang I and Ang II concentrations were determined by direct RIA. Mean intra-assay and interassay variabilities of Ang RIA measurements ranged between 10% and 16%. There was no interference of remikiren, EXP 3174, and captopril in these immunoassays.

Human AOGEN concentrations in the perfusate were determined by an in vitro enzyme kinetic assay. Human AOGEN was completely exhausted by cleavage by using an excess of human recombinant renin for 1 hour at 37°C, as described before. Ang I was measured by direct RIA, and human AOGEN concentrations were expressed as pmol/ml on the basis of an equimolar production of Ang I from AOGEN.

Human renin concentration was determined by direct and by enzyme-kinetic assays. Direct renin measurements were performed with a IRMA Pasteur kit (Sanofi Pasteur) according to the manufacturer’s description. For the enzyme kinetic assay, perfusate was incubated together with an excess of human AOGEN at pH 5.7 and 37°C for 1 hour, as described before. Rat AOGEN was not cleaved during incubation because of the absence of any detectable rat renin in the perfusate. Ang I generated during the enzyme kinetic assays was measured by direct RIA, and human renin concentration was expressed as pmol Ang I · ml⁻¹ · h⁻¹.

Data Analysis
The Ang I conversion is expressed as the ratio of the molar concentration of Ang II to the molar concentration of Ang I measured in the hindquarter effluent. For comparison of Ang release after renin and Ang I infusion or coinfusion of the inhibitors, Ang levels are corrected for coronary blood flow and expressed as fmol/min. Data are expressed as mean±SEM. Repeated-measures ANOVA was used to study the influence of renin and Ang I infusion and the time of perfusion on Ang release and the rate of conversion; ANOVA was followed by unpaired Student’s t test as a post hoc test. Statistical significance between protocols with coinfusion of inhibitors was estimated by one-way ANOVA and Scheffe’s test. A value of P<.05 was accepted as significant.

Results
To compare the effects of renin infusion with the effects of exogenous Ang I administration, we infused the compounds into the perfusate of Langendorff preparations and measured Ang I and Ang II concentrations in the effluent. Ang I and Ang II were not detectable before the experimental protocols were started. The infusion of renin or Ang I promptly resulted in Ang II formation. Despite significantly higher Ang I levels during Ang I infusion compared with renin infusion (171±13 versus 61±15 fmol/ml, P<.001, 15 minutes) (Fig 1, top panel), Ang II formation was not different between the two groups during the infusion (4.5±0.6 versus 6.4±1.7 fmol/ml, 15 minutes) but significantly increased after the infusions were stopped (Fig 1, bottom panel). After cessation of the infusion, the release of Ang II induced by renin continued, whereas after Ang I infusion, Ang II disappeared.

The rate of Ang I conversion was significantly higher after renin infusion (0.109±0.027 versus 0.026±0.003, 15 minutes, P=.017) compared with infused Ang I (Fig 2). The rate of Ang I conversion increased over time during the renin infusion. This increase continued further after renin was stopped. In contrast, after cessation of Ang I, the rate of Ang I conversion could no longer be determined because the levels of one or both compounds were below the assay detection limit (Fig 2). The time course of renin and Ang II concentration after cessation of the infusion provided evidence for uptake of renin in cardiac or coronary vascular tissue. Renin in the effluent
decreased to undetectable levels within 5 minutes after the infusion was stopped; however, Ang II formation still continued and remained present in appreciable concentrations even after 25 minutes (Fig 3).

The prolonged release of Ang II led to long-lasting coronary vasoconstriction (Fig 4). Coronary blood flow decreased with both Ang I and renin infusion. When the Ang I infusion was stopped, coronary blood flow returned to basal levels within 5 minutes. In contrast, when the renin infusion was stopped, coronary blood flow remained at low levels throughout the rest of the observation period. This finding suggests that the continued Ang II generation resulted in the constriction of the coronary circulation.

We next compared the effects of renin inhibition, ACE inhibition, and AT1 receptor blockade on the renin infusions. Table 1 shows human renin and human AOGEN in the perfusate after human renin administration alone or with captopril, remikiren, or the AT1 receptor blocker. Remikiren decreased the human renin values to almost undetectable levels. High amounts of human AOGEN were released from isolated perfused transgenic rat hearts harboring the human AOGEN gene. AOGEN release was not different between the various experimental groups.

To test the hypothesis that rat cardiac tissue may use additional enzymes other than ACE in converting Ang I to Ang II, we prepared homogenates of rat hearts and administered 125I-labeled Ang I. These incubation studies clearly showed a chymase-like activity. Total cardiac Ang II–forming activity in heart homogenates was partially inhibited by chymostatin (2.6±0.3 fmol Ang II·min⁻¹·mg⁻¹) and by cilazaprilat (4.1±0.1 fmol Ang II·min⁻¹·mg⁻¹) but almost completely by both drugs (0.9±0.1 fmol Ang II·min⁻¹·mg⁻¹) versus controls (5.6±0.4 fmol Ang II·min⁻¹·mg⁻¹) (Fig 5).

The infusion of the renin inhibitor during renin infusion led to significantly decreased Ang I (Fig 6, top panel) and Ang II levels (Fig 6, bottom panel). Captopril only suppressed Ang II (Fig 6, bottom panel), whereas the AT1 receptor blocker EXP 3174 did not affect peptide generation. All the drugs prevented a coronary blood flow decrease induced by renin (Fig 7). Table 2 shows the comparison of Ang I and Ang II release expressed as absolute values (fmol/mL) and Ang levels corrected for coronary blood flow (fmol/min).

**Discussion**

The present study shows that human renin can be taken up from the circulation by the heart and remains active much longer than previously described.

**TABLE 1. Human Renin and Human AOGEN Measurements**

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Renin</th>
<th>Renin+ Captopril</th>
<th>Renin+ Remikiren</th>
<th>Renin+ AT1 Receptor Blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunoreactive human renin, ng/mL</td>
<td>58±5</td>
<td>35±3</td>
<td>48±6</td>
<td>34±1</td>
</tr>
<tr>
<td>Human PRC, nmol Ang I·mL⁻¹·h⁻¹</td>
<td>17.0±1.8</td>
<td>11.8±2.4</td>
<td>0.005±0.0006*</td>
<td>13.2±2.2</td>
</tr>
<tr>
<td>-5 Min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human AOGEN, pmol/mL</td>
<td>7.7±0.9</td>
<td>10.3±2.1</td>
<td>7.7±0.6</td>
<td>9.3±1.5</td>
</tr>
</tbody>
</table>

PRC indicates plasma renin concentration. Values are mean±SEM. *P<.05 for renin+ inhibitors or Ang I vs renin.
longer than its presence in the circulation would explain. Ang II formation and its vasoconstriction also continue after cessation of renin infusion. In contrast, Ang I infusion results in a short-lasting decrease of coronary blood flow. Furthermore, the Ang I conversion caused by renin was much more efficient compared with exogenously infused Ang I.

In the second part of the study, we examined tissue Ang II generation further. We analyzed the existence of non-ACE Ang II–forming pathways in the rat heart. We measured total cardiac Ang II–forming activities in cardiac homogenates, which were only partially inhibited by cilazaprilat and inhibited to a larger extent by chymostatin. Administration of both compounds terminated Ang II formation almost completely. These results show that rat heart homogenates exhibit a chymase-like activity. Despite the existence of this non-ACE activity in homogenates, isolated perfused hearts showed an almost complete biochemical and hemodynamic inhibition by captopril and remikiren. Thus, acutely chymase-like activity does not seem to play a significant role in this preparation.

Our results are limited in several respects and cannot be directly extrapolated to the clinical effects of drugs interfering with ACE or the AT1 receptor. We investigated only short-term hemodynamic effects but not long-term trophic effects of local Ang II formation. In spite of our attempts to study the human renin-angiotensin system in transgenic animals, Ang II–forming pathways in humans could be different from the pathways observed in the present study. Notwithstanding these limitations, our results challenge several widely accepted notions regarding chymase-like Ang II–forming activity. First, we readily detected a chymostatin-sensitive Ang II–forming activity in rat heart homogenate. We did not further characterize the enzyme responsible for this activity; however, we believe that the conclusions drawn by others regarding the absence of chymase-like Ang II–forming activity in the rat are not justified.20,23–25 Second, the generation of Ang II in the perfused intact heart was totally dependent on ACE, despite the predominance of chymase-like activity in homogenized cardiac tissue. A similar relationship was found in dog heart.26 These data emphasize the need to study intact organs rather than tissue homogenates, which contain enzymes from all cell compartments.

Although our knowledge of the tissue renin-angiotensin system has increased greatly in the last several years, the question of tissue Ang formation and the functional significance of local Ang peptides remains imperfectly defined.27,28 Local Ang formation in the heart may contribute to the

Figure 5. The bar graph shows the effects of ACE and protease inhibitors on the processing of 125I-Ang I. The membrane preparations were incubated with either saline (control), cilazaprilat (100 μmol/L), chymostatin (100 μmol/L), or a combination of inhibitors. Data are mean±SEM. *P<.05.

Figure 6. Top, The bar graph shows the effect of various inhibitors of the renin-angiotensin system on Ang I release at 10 minutes. Renin and the coinfusion with captopril and EXP 3174 led to Ang I formation. In contrast, Ang I formation was blocked after renin inhibition. Bottom, Bar graph shows the Ang II release. Only the inhibitors captopril and remikiren suppressed Ang II formation. Data are mean±SEM. *P<.05.

Figure 7. The bar graph shows the effect of various inhibitors of the renin-angiotensin system on coronary blood flow (CBF) after renin infusion (minute 10). Renin (n=9) alone decreased the flow by 23±4%. The coinfusion of the ACE inhibitor captopril (n=6, increase of 2±3%), the human specific renin inhibitor remikiren (n=7, no change [0±2%]), and the AT1 receptor blocker EXP 3174 (n=6, increase of 6±3%) abolished the CBF decrease. Data are mean±SEM. *P<.05.
pathogenesis of cardiac hypertrophy,\textsuperscript{29,30} congestive heart failure,\textsuperscript{31} and tissue remodeling.\textsuperscript{31} Many studies addressing the cardiac effects of Ang II have been performed by infusing Ang I or Ang II into the coronary circulation. However, the standard rat models have important limitations. Pure rat renin is currently not available in sufficient amounts for biochemical and hemodynamic studies. To avoid confounding effects of endogenous renin produced by the rat, we used the isolated perfused hearts of rats harboring the human AOGEN gene as a pharmacological model to study local Ang formation. Human AOGEN is not cleaved by rat renin in any appreciable amounts.\textsuperscript{19,33} Similarly, human renin is cleaving rat AOGEN 16-fold slower than human substrate.\textsuperscript{33} Previously, we have demonstrated that high amounts of human renin do not induce vascular Ang formation in nontransgenic Sprague-Dawley rats.\textsuperscript{32} Pharmacological concentrations of human renin were infused to facilitate the detection of local Ang formation from AOGEN. Our approach also gave us the opportunity to study the human renin-angiotensin system in an animal model. This feature also permitted us to study renin inhibition by remikiren. This human renin inhibitor does not inhibit rat renin and therefore cannot otherwise be applied in rat studies.\textsuperscript{34}

Lindpaintner et al\textsuperscript{6} provided evidence of a cardiac renin-angiotensin system with local Ang formation. Our data confirm and extend these findings. We showed that renin was taken up by cardiac tissue and induced a long-lasting Ang II formation with coronary vasoconstriction, which continued after cessation of the infusion. In contrast, Ang I infusion resulted in a similar degree of vasoconstriction, which promptly disappeared after Ang I was discontinued. The long-lasting Ang II formation was caused by the renin, which, because of a different cardiac hemodynamic effect, was taken up by the tissue and not secondary. Renin and AT\textsubscript{1} receptor blocker coinfusion also showed a long-lasting Ang II formation, whereas coronary blood flow was, like after Ang I infusion, unchanged. Renin infusion led to 4-fold higher Ang I conversion, expressed as ratio of molar concentration of released Ang II to released Ang I, compared with infused Ang I. Danser et al\textsuperscript{15} also reported that the cardiac Ang I conversion rate (ratio of cardiac Ang II to Ang I) was 2 to 3 times higher than in plasma. They measured tissue Ang levels in the heart, whereas we calculated the ratio from the release of Ang peptides from the heart into the perfusate. During renin infusion in our perfused hearts, Ang formation was dependent on the interaction of renin and AOGEN in the lumens and on the local Ang formation in the tissue. After cessation of renin infusion, the ratio reflects tissue Ang formation because renin was already absent from the perfusate. Locally formed Ang peptides may be degraded on the vascular surface and in the interstitial fluid by peptidases, as suggested by De Lannoy et al.\textsuperscript{36} Since our experiments with captopril mainly inhibited vascular ACE and prevented local Ang II formation induced by vascular wall–bound renin, we cannot rule out the possibility that chymase-like activity may have contributed to local Ang I metabolism in the interstitial fluid. Such a contribution would be underestimated by our peptide measurements. Nevertheless, the fact that captopril totally prevented Ang II generation following renin infusion, as well as Ang II–related vascular effects, casts doubt on the notion that acutely chymase-like activity may play a major role in Ang II–induced hemodynamic effects.

The notion that plasma-derived renin may be retained in the vessel wall was first reported by Loudon et al\textsuperscript{3} and Thurston et al.\textsuperscript{3} Okamura et al\textsuperscript{37} demonstrated the existence of renin in the endothelium of human arteries and showed that renin activity within vessels with endothelium was higher than in those without endothelium. They concluded that the endothelium plays an important role in the control of vascular tone through local Ang II generation. In addition, different renin binding proteins have been described by several investigators.\textsuperscript{38–42} Campbell and Valentijn\textsuperscript{46} reported that renin binds to vascular membranes. However, in their study they could not find any renin binding in the heart. This finding is in contrast to the affinity binding site studies in various tissues, including the heart, described by Sealey et al.\textsuperscript{49} We have previously shown

\begin{table}[ht]
\centering
\caption{Angiotensin I and II Release Expressed in Absolute Values and Corrected for CBF}
\begin{tabular}{lcccc}
\hline
\textbf{Experimental Conditions} & \textbf{Ang I Alone} & \textbf{Renin Alone} & \textbf{Renin+ Captopril} & \textbf{Renin+ Remikiren} & \textbf{Renin+ AT\textsubscript{1} Receptor Blocker} \\
\hline
\multicolumn{5}{l}{15 Min} \\
Ang I, fmol/mL & 171±13\textsuperscript{*} & 61±15 & 103±29 & 0±0\textsuperscript{*} & 52±9 \\
Ang I, fmol/min & 2917±329\textsuperscript{*} & 741±147 & 1243±205 & 0±0\textsuperscript{*} & 798±116 \\
\multicolumn{5}{l}{25 Min} \\
Ang I, fmol/mL & 6±3 & 14±5 & 17±9 & 0±0\textsuperscript{*} & 12±2 \\
Ang I, fmol/min & 113±58 & 169±51 & 182±93 & 0±0\textsuperscript{*} & 190±29 \\
\multicolumn{5}{l}{15 Min} \\
Ang II, fmol/mL & 4±0.6 & 6.4±1.7 & 11±0.2\textsuperscript{*} & 0.3±0.2\textsuperscript{*} & 5.4±1.3 \\
Ang II, fmol/min & 74±10 & 84±22 & 16±4\textsuperscript{†} & 6±3\textsuperscript{†} & 82±17 \\
\multicolumn{5}{l}{25 Min} \\
Ang II, fmol/mL & 0±0 & 2.1±0.6 & 0.6±0.2\textsuperscript{*} & 0.1±0.1\textsuperscript{*} & 1.7±0.3 \\
Ang II, fmol/min & 0±0\textsuperscript{*} & 31±9 & 9.3±4.7 & 1.6±1.1\textsuperscript{*} & 29±5 \\
\hline
\end{tabular}
\footnotesize{CBF indicates coronary blood flow. Values are mean±SEM. \\
\textsuperscript{*}P<.05 and †P=.051 for renin+ inhibitors or Ang I vs renin infusion.}
\end{table}
that renin is taken up to the vascular wall in an isolated perfused hindquarter preparation.\textsuperscript{22} Thus, additional studies are necessary to elucidate the nature of renin binding in tissues.

The different effects on the coronary blood flow after renin and Ang I infusion provide further evidence for local Ang formation by the heart independent of the circulation. Renin caused a long-lasting vasoconstriction, whereas after Ang I infusion both Ang I and Ang II and coronary blood flow returned to basal levels. We cannot determine whether renin was taken up by a specific mechanism or whether renin was merely “trapped” in the vessel wall as a result of physical and chemical properties. Whatever mechanism may promote renin uptake, the enzyme is retained in the tissue and contributes to local Ang II formation, which may have important pathophysiological implications.

The major pathway for Ang II formation in the human heart may involve more than ACE-related Ang I conversion. A dual pathway for Ang II formation in human hearts in vitro was reported by Utz et al\textsuperscript{19} and recently by Wolny et al.\textsuperscript{20} They found that a majority of the total Ang II formation was due to a hitherto-unknown serine proteinase, whereas ACE mediated Ang II production to a far lesser extent. On the basis of these in vitro studies, they proposed that the enzyme h-MCP is primarily responsible for cardiac Ang II formation.\textsuperscript{21} We examined whether ACE was the only enzyme involved in Ang II formation in our rat hearts. Our incubation studies of heart homogenates clearly showed the presence of Ang II-forming activity. The homogenates also showed a chymostatin-sensitive Ang fragment formation, which could be a result of the hydrolysis by r-MCP 1. Recently, Ide et al\textsuperscript{22} described a novel Ang II–forming α-chymase, rat MCP 3. Its amino acid sequence was very similar to that of mouse MCP 5 (95.1%) or h-MCP, two other α-chymases.

The fact that the heart can produce Ang II without ACE should perhaps come as no surprise. Fetal cardiomyocytes in culture can release Ang II into the surrounding medium when they are stimulated by stretch.\textsuperscript{23} Stretch activates the A0GEN gene in these cells. Precisely which enzymes are responsible for generating Ang II under these circumstances is unclear. Sadoshima et al\textsuperscript{24} found no evidence that ACE, or renin for that matter, was responsible. Cathepsin and chymase could be candidates. The possibility remains that chymase-like activity may be of some importance to Ang II production in disease states, such as after myocardial infarction.

In the present study, a cell homogenate exhibited Ang I–converting activity. Intracellular enzymes were likely to have been responsible. Both captopril and remikiren blocked the cardiac Ang II formation, despite the fact that renin had been taken up and acted at sites within the tissue in our study. Thus, the acute hemodynamic effects induced by renin are not influenced by chymase-like activity. However, non-ACE Ang II–forming activity, which we observed in our in vitro experiments, could be of importance in pathological long-term changes such as fibrosis, cardiac hypertrophy, and remodeling.

In conclusion, renin was taken up into cardiac or coronary vascular tissue and induced a long-lasting local Ang II generation with coronary vasoconstriction. Locally formed Ang I was apparently converted much more effectively than infused Ang I. Despite the existence of non-ACE activity in homogenates, Ang II release of isolated perfused hearts and its acute hemodynamic effects were not influenced by non-ACE pathways. However, a potential physiological role for heart chymase is not ruled out in the rat. Further studies are needed to elucidate this issue and to evaluate the mechanism for the persistent effects seen after renin infusion.

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