Angiotensin-(1-7) Formation in the Intact Human Heart
In Vivo Dependence on Angiotensin II as Substrate

Lawrence S. Zisman, MD; Glenn E. Meixell, PhD; Michael R. Bristow, MD, PhD; Charles C. Canver, MD

Background—Several enzymes that hydrolyze angiotensin I (Ang I) and Ang II to Ang-(1-7) have been identified, but their relative importance in the intact human heart is not known.

Methods and Results—Intracoronary (IC) 125I-Ang I was administered to 4 heart transplantation recipients. Arterial and coronary sinus (CS) samples were taken before and after coadministration of IC enalaprilat. 125I-Ang metabolites were separated by high-pressure liquid chromatography, and 125I-Ang-(1-7) and 125I-Ang II were quantified across the myocardial circulation. 125I-Ang II formation (as measured by fractional conversion) at steady state was 0.43±0.05 and was reduced to 0.042±0.02 after IC enalaprilat (P<0.01). The fractional conversion of 125I-Ang-(1-7) was 0.198±0.032 but was reduced to 0.06±0.01 during IC enalaprilat (P<0.01). Net Ang II production at steady state was 2720±704 pg/min. Ang-(1-7) production was 3489±768 pg/min. After IC enalaprilat, Ang II production fell to 436±66.8 pg/min (P<0.05 versus Ang II production). After suppression of Ang II production with enalaprilat, there was net uptake of Ang-(1-7): −289±144 pg/min (P<0.05).

Conclusions—Ang-(1-7) was formed in the intact human myocardial circulation and was decreased when Ang II formation was suppressed. These data indicate that the major pathway for Ang-(1-7) generation in the intact human heart was dependent on substrate availability of Ang II. Ang-(1-7)–forming enzymes that demonstrate substrate preference for Ang II are likely to play an important role in the regulation of Ang-(1-7) formation in the intact human heart.

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Key Words: angiotensin • enzymes • cardiomyopathy

Several enzymes that hydrolyze Ang I and Ang II to Ang-(1-7) have been identified, but their relative importance in the intact human heart is not well understood. Enzymes that have been shown to hydrolyze Ang I and/or II to Ang-(1-7) include neutral endopeptidase and the recently described homologue of angiotensin-converting enzyme (ACE), ACE2 (or ACEH). Because these angiotensinases have different substrate affinities, an understanding of the dependence of Ang-(1-7) formation on substrate availability of Ang II is required. Ang-(1-7)–forming enzymes that demonstrate substrate preference for Ang II are likely to play an important role in the regulation of Ang-(1-7) formation in the intact human heart.

Methods

Iodination of angiotensin I was performed at Golden Pharmaceuticals, Golden, Colo, as previously described. Enalaprilat was kindly provided by Merck Research Laboratories. The radioimmunoassay kit for Ang I was obtained from Peninsula Laboratories. The radioimmunoassay for Ang II was obtained from Nichols Institute Diagnostics. The antibody to Ang II cross-reacted with Ang-(1-7) and was used for the Ang-(1-7) radioimmunoassay after separation by high-pressure liquid chromatography (HPLC). Radioiodinations of Ang III, IV, 1-5, and 1-7 were performed by Dr Robert Speth (Washington State University Peptide Radioiodination Service, Pullman, Wash).

Patient Characteristics

Four orthotopic heart transplantation recipients with normal left ventricular function and coronary anatomy were recruited from a group of patients undergoing annual surveillance cardiac catheterization. All patients were receiving a standard regimen of cyclosporine, azathioprine, and diltiazem. Two patients were receiving aspirin, and 2 patients were receiving furosemide. No patients were receiving ACE inhibitor therapy. Three patients were male and 1 was female. Ages ranged from 34 to 59 years. A Multiple Institution Review Board approved this study, and all subjects gave written informed consent before the study.

Cardiac Catheterization

An intracoronary (IC) infusion of 125I-Ang I (specific activity of 440 to 1890 μCi/μg) was infused into the left main coronary artery at 1 mL/min, 0.01 to 0.05 μg/min. The infusion was given for a period of 6 minutes, during which time blood sampling was performed from the femoral artery and the coronary sinus (CS). IC enalaprilat (0.01 mg/min) was then coadministered with the 125I-Ang I IC. Blood sampling was performed 5 minutes into the IC enalaprilat infusion. CS blood flow was measured by the technique of thermodilution.
Sample Analysis
Blood samples were immediately placed in inhibitor solution containing 0.01 mmol/L renin inhibitor (Ro 42-5892), 6.25 mmol/L EDTA, 1.25 mmol/L 1,10-phenanthroline, and 0.02 mmol/L enalaprilat. Plasma was extracted on C18 Sep-pak cartridges. The percent recovery from the Sep-pak extractions was 80.5±2.5%. Sample processing and HPLC separations were performed as previously described. This method resulted in complete separation of 123I-Ang II and 123I-Ang-(1-7). HPLC was performed with an Alltech C18 column. Mobile phase A consisted of 0.085% orthophosphoric acid/0.02% sodium azide; mobile phase B consisted of methanol. The method was isocratic, with A65%-B35% from 0 to 9 minutes followed by a gradient of mobile phase B to 55% over 9 minutes. Retention times were determined with 125I-Ang peptides and were 123I-Ang I, 19 minutes; 123I-Ang IV and 123I-Ang III, 15.5 minutes; 123I-Ang II, 11.5 minutes; 123I-Ang-(1-5), 8 minutes; and 123I-Ang-(1-7), 6 minutes. The fractional conversion of Ang-(1-7) was calculated by the formula:

\[
\text{Ang-(1-7) production} = \left[ \frac{\text{Ang-(1-7)(CS)} - \text{Ang-(1-7)(FA)}}{\text{CSBF}(1 - \text{Hct}/100)} \right] \times \text{CSBF} \times (1 - \text{Hct}/100).
\]

Statistical Analysis
One-way ANOVA followed by the Bonferroni correction was used to determine statistical significance (set at \( P < 0.05 \)). Data are presented as mean±SEM. The intra-assay coefficient of variation for the HPLC/radioimmunoassay was 1.88%, and the interassay coefficient of variation was 10.4%.

Results
Hemodynamics
The mean CSBF at baseline before the infusion of any study medication was 93.1±14.0 mL/min. The mean CSBF after the 6-minute IC infusion of 123I-Ang I was 106±8.0 mL/min, and the mean CSBF after the 5-minute combined IC 123I-Ang I/enalaprilat infusion was 106.1±7.43 mL/min.

Ang-(1-7) Formation
Figure 1 demonstrates a CS HPLC profile of 123I-Ang peptides before and after IC enalaprilat. The suppression of 123I-Ang II formation was associated with a decrease in the generation of 123I-Ang-(1-7). The fractional conversion of 123I-Ang I to 123I-Ang II in the coronary sinus at the 1-, 3-, and 6-minute time points of the IC 123I-Ang I infusion was 0.438±0.098, 0.456±0.057, and 0.432±0.054, respectively. Enalaprilat reduced the fractional conversion of 123I-Ang I to II to 0.042±0.026 (Figure 2A, \( P < 0.01 \) versus time points 1, 3, and 6). 123I-Ang-(1-7) formation across the myocardial

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vascular bed at the 1-, 3-, and 6-minute time points was 0.107±0.034, 0.171±0.019, and 0.198±0.032, respectively. After enalaprilat, Ang-(1-7) formation was reduced to 0.06±0.01 (Figure 2B, P<0.05 versus time point 3; P<0.01 versus time point 6).

Ang II production at the 3- and 6-minute time points was 2573±377 and 2720±704 pg/min, respectively. At the 11-minute time point (ie, after 5 minutes of IC enalaprilat), net Ang II production fell to 436±66.8 pg/min (P<0.05 versus net Ang II production at 3 and 6 minutes). Ang-(1-7) production at the 3-and 6-minute time points was 2718±483 and 3489±768 pg/min. At the 11-minute time point there was net uptake of Ang-(1-7) across the myocardial vascular bed: −289±144 pg/min (P<0.05 versus Ang-(1-7) production at 6 minutes).

Discussion

To our knowledge, this study constitutes the first report of Ang-(1-7) formation in the intact human heart. When Ang II formation was suppressed by the administration of enalaprilat, the generation of Ang-(1-7) was markedly diminished. Although there was excess [125I]-Ang I available as substrate, [125I]-Ang-(1-7) formation was relatively low when [125I]-Ang II formation was inhibited. Analysis of net Ang II and Ang-(1-7) production confirmed that a decrease in Ang II production across the myocardial circulation was associated with a decrease in Ang-(1-7) production. Indeed, when Ang II production was suppressed by enalaprilat, there was net uptake of Ang-(1-7). This negative value could be explained by a greater rate of degradation of Ang-(1-7) relative to its formation and/or uptake of Ang-(1-7) either by specific or nonspecific binding.

ACE has been reported to hydrolyze Ang-(1-7) to Ang-(1-5).4 As shown in Figure 1, enalaprilat appeared to decrease [125I]-Ang-(1-5) formation. This effect of enalaprilat would function to limit degradation of Ang-(1-7), but whether it would be expected to increase the concentration of Ang-(1-7) would depend on the relative rate of formation of Ang-(1-7). It is unlikely that enalapril itself inhibited the enzyme responsible for Ang-(1-7) formation from Ang II in vivo. Therefore, it is reasonable to conclude that the major pathway for Ang-(1-7) formation in the intact human heart showed significant substrate preference for Ang II compared with Ang I.

The recently described homologue of ACE, ACE2, is a carboxypeptidase that hydrolyzes Ang I to Ang-(1-9) and Ang II to Ang-(1-7) and is not inhibited by selective ACE inhibitors. ACE2 shows much more favorable kinetics for the hydrolysis of Ang II compared with Ang I.5 Furthermore, in human heart ventricular membrane preparations, ACE2 mediated virtually all Ang-(1-7) formation from Ang II.6 In these preparations, inhibitors of ACE, neutral endopeptidase, endothelin-converting enzyme, prolylendopeptidase, carboxypeptidase A, and angiotensinase C had no effect on the hydrolysis of Ang II to Ang-(1-7).6 The in vivo dependence of Ang-(1-7) formation on Ang II substrate availability is consistent with these in vitro data and suggests that ACE2 is a major pathway for Ang-(1-7) formation in the intact human heart.

We have previously shown that IC enalaprilat, as a highly selective ACE inhibitor, suppressed >85% of Ang II formation across the myocardial circulation in the intact human heart.3 The reported differences in the relative contribution of ACE and chymase to Ang II formation may be related to different methods used to prepare human heart tissue before assay.7 Regardless of these differences, the decreased formation of [125I]-Ang II in the study reported herein probably was the main reason for the decreased formation of [125I]-Ang-(1-7) during the administration of enalaprilat.

Limitations

This study was not able to positively identify which enzyme or enzymes were responsible for the conversion of Ang II to Ang-(1-7). Nevertheless, the in vitro demonstration of ACE2 as the major pathway for hydrolysis of Ang II to Ang-(1-7) in human heart and the fact that there was a significant decrease in [125I]-Ang-(1-7) formation when [125I]-Ang II availability as substrate was suppressed, taken together, point to an important role for ACE2 in the intact human heart.

Another important limitation of this study is that we did not directly measure angiotensin metabolites in the interstitial space. Wei et al8 demonstrated that an interstitial infusion of Ang I increased interstitial Ang II 100-fold. An Ang II gradient between the intravascular and interstitial compartments could result either from increased Ang II generation in the interstitial space and/or increased degradation in the intravascular space. Our data are consistent with the latter possibility: namely, that ACE2-mediated Ang-(1-7) formation in the intravascular space contributes to the maintenance of this gradient.

Conclusions

Ang-(1-7) was formed in the intact human myocardial circulation and was markedly decreased when Ang II formation was suppressed. Ang-(1-7)–forming enzymes that demonstrate substrate preference for Ang II compared with Ang I, such as ACE2, are likely to play an important role in regulating Ang-(1-7) formation in the intact human heart.

Acknowledgments

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References

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