Angiotensin Production by the Heart
A Quantitative Study in Pigs With the Use of Radiolabeled Angiotensin Infusions
Jorge P. van Kats, PhD; A.H. Jan Danser, PhD; Jan R. van Meegen, BSc;
Loes M.A. Sassen, MD, PhD; Pieter D. Verduw, PhD; Maarten A.D.H. Schalekamp, MD, PhD

Background—Beneficial effects of ACE inhibitors on the heart may be mediated by decreased cardiac angiotensin II (Ang II) production.

Methods and Results—To determine whether cardiac Ang I and Ang II are produced in situ or derived from the circulation, we infused 125I-labeled Ang I or II into pigs (25 to 30 kg) and measured 125I-Ang I and II as well as endogenous Ang I and II in cardiac tissue and blood plasma. In untreated pigs, the tissue Ang II concentration (per gram wet weight) in different parts of the heart was 5 times the concentration (per milliliter) in plasma, and the tissue Ang I concentration was 75% of the plasma Ang I concentration. Tissue 125I-Ang II during 125I-Ang II infusion was 75% of 125I-Ang II in arterial plasma, whereas tissue 125I-Ang I during 125I-Ang I infusion was <4% of 125I-Ang I in arterial plasma. After treatment with the ACE inhibitor captopril (25 mg twice daily), Ang II fell in plasma but not in tissue, and Ang I and renin rose both in plasma and tissue, whereas angiotensinogen did not change in plasma and fell in tissue. Tissue 125I-Ang II derived by conversion from arterially delivered 125I-Ang I fell from 23% to <2% of 125I-Ang I in arterial plasma.

Conclusions—Most of the cardiac Ang II appears to be produced at tissue sites by conversion of in situ–synthesized rather than blood-derived Ang I. Our study also indicates that under certain experimental conditions, the heart can maintain its Ang II production, whereas the production of circulating Ang II is effectively suppressed. (Circulation. 1998;98:73-81.)

Key Words: angiotensin ■ ACE inhibitors ■ renin

From experimental and clinical studies, there is evidence to suggest that the long-term beneficial effects of ACE inhibitor drugs on postinfarction cardiac failure and remodeling as well as on left ventricular hypertrophy are at least partly independent of their systemic effect on blood pressure.1,2 A reduction of Ang II production locally in the heart may explain the blood pressure–independent effects of ACE inhibition in cardiac patients.

Indeed, the heart contains all components required for Ang I and Ang II production, ie, renin, angiotensinogen, and ACE.3 Renin mRNA may be present in the heart in low concentrations,4–7 but observations on the effect of bilateral nephrectomy in pigs demonstrated that most, if not all, renin in the heart is derived from the kidney, at least under normal conditions.3 Angiotensinogen gene expression is also low in the normal heart,7,8 and experiments using the isolated perfused rat heart seem to indicate that the heart produces little Ang I and II when angiotensinogen is not added to the perfusion fluid.9 In contrast, the synthesis of ACE in the normal heart is an established fact.10–13 Angiotensinogen and ACE gene expression may be upregulated under pathological conditions.8,11,13

The study reported here focuses on the normal heart and is carried out in pigs. It addresses the following questions: (1) How much of the Ang I and II in cardiac tissue is derived from the circulation? (2) How much of the cardiac Ang II is synthesized locally by the conversion of blood-derived Ang I and how much by the conversion of Ang I that is formed locally in the heart? and (3) What is the effect of ACE inhibitor treatment on cardiac angiotensin production?

Methods

Animals
All experiments were performed under the regulations of the Animal Care Committee of the Erasmus University, Rotterdam, The Netherlands, in accordance with the “Guiding Principles in the Care and Use of Animals” as approved by the American Physiological Society. Twenty-four female pigs (crossbred Yorkshire×Landrace, Hedelse Varkens Combinatie, Hedel, The Netherlands) with a body weight of 25 to 30 kg were included in the study. Ten animals were pretreated with the ACE inhibitor captopril, 25 mg twice daily for 3
fibrillation while the 125 I-Ang I or II infusion was still running. The samples were centrifuged at 1000 g for 10 minutes at 4°C. Plasma had no Ang I–generating activity in the presence of inhibitors of angiotensinases and ACE.3 In short, 1 mL of homogenate was dialyzed for 48 hours at 4°C against 0.05 mol/L glycine buffer, pH 3.5, containing 0.095 mol/L NaCl. This was followed by dialysis at 4°C for 24 hours against 0.1 mol/L phosphate buffer, pH 7.4, containing 0.075 mol/L NaCl. The content of the dialysis bags was then collected, and the volume was adjusted to 1 mL with phosphate buffer.

The concentration of renin in acid-pretreated cardiac tissue extract and in non–acid-pretreated plasma was determined by the enzyme-kinetic assay, which measures the rate of Ang I generation at pH 7.4 during incubation at 37°C with a saturating amount of porcine renin. Inhibition of porcine renin is virtually complete at concentrations of 10–7 mol/L remikiren. Any remaining Ang I–generating activity was assumed to be caused by enzymes other than renin. Plasma had no Ang I–generating activity in the presence of 10–7 mol/L remikiren.

The concentration of angiotensinogen was determined in non–acid-pretreated cardiac tissue extracts and plasma. It was measured as the maximum quantity of Ang I that was generated during incubation at 37°C with a saturating amount of porcine renin substrate, in the presence of inhibitors of angiotensinases, ACE, and serine proteases. The Ang I–generating activity of cardiac tissue extracts measured in the absence of the renin inhibitor remikiren minus the Ang I–generating activity in the presence of remikiren (final concentration, 10–7 mol/L) was taken as a measure of the concentration of renin. Inhibition of porcine renin is virtually complete at this concentration of remikiren. Any remaining Ang I–generating activity was assumed to be caused by enzymes other than renin. Plasma had no Ang I–generating activity in the presence of 10–7 mol/L remikiren.

Calculations

The possible sources of Ang I and II in cardiac tissue are shown in Figure 1 and summarized in Table 1. The level of arterially delivered
Ang II in cardiac tissue was calculated as

\[ [\text{Ang II}_{\text{tissue from Ang II}_\text{art}}] = R_1 \times [\text{Ang II}_\text{art}] \]

where \([\text{Ang II}_{\text{tissue from Ang II}_\text{art}}]\) is the Ang II concentration in cardiac tissue (fmol/g) derived from arterially delivered Ang II, \([\text{Ang II}_\text{art}]\) is the Ang II concentration in aortic blood plasma (fmol/mL), and \(R_1\) is given by

\[ R_1 = \frac{[\text{125I-Ang II}_\text{tissue}]}{[\text{125I-Ang II}_\text{art}]} \]

where \([\text{125I-Ang II}_\text{tissue}]\) and \([\text{125I-Ang II}_\text{art}]\) are the steady-state concentrations of 125I-Ang II in cardiac tissue (cpm/g) and aortic blood plasma (cpm/mL), respectively, both during the constant infusion of 125I-Ang II.

The level of arterially delivered Ang I in cardiac tissue was calculated as follows:

\[ [\text{Ang I}_{\text{tissue from Ang I}_\text{art}}] = R_2 \times [\text{Ang I}_\text{art}] \]

where \([\text{Ang I}_{\text{tissue from Ang I}_\text{art}}]\) is the Ang I concentration in cardiac tissue (fmol/g) derived from arterially delivered Ang I, \([\text{Ang I}_\text{art}]\) is the Ang I concentration in aortic blood plasma (fmol/mL), and \(R_2\) is given by

\[ R_2 = \frac{[\text{125I-Ang I}_\text{tissue}]}{[\text{125I-Ang I}_\text{art}]} \]

where \([\text{125I-Ang I}_\text{tissue}]\) and \([\text{125I-Ang I}_\text{art}]\) are the steady-state concentrations of 125I-Ang I in cardiac tissue (cpm/g) and aortic blood plasma (cpm/mL), respectively, both during the constant infusion of 125I-Ang I.

The cardiac tissue level of Ang II that is derived by conversion from arterially delivered Ang I was calculated as

\[ [\text{Ang II}_{\text{tissue from Ang I}_\text{art}}] = R_3 \times [\text{Ang I}_\text{art}] \]

where \([\text{Ang II}_{\text{tissue from Ang I}_\text{art}}]\) is the Ang II concentration in cardiac tissue (fmol/g) derived from arterially delivered Ang I, \([\text{Ang I}_\text{art}]\) is the Ang I concentration in aortic blood plasma (fmol/mL), and \(R_3\) is given by

\[ R_3 = \frac{[\text{125I-Ang II}_\text{tissue}]}{[\text{125I-Ang I}_\text{art}]} \]

\[ - (R_2 \times [\text{Ang II}_{\text{tissue from Ang II}_\text{art}}]) \]

**TABLE 1. Sources of Ang I and II in Cardiac Tissue**

<table>
<thead>
<tr>
<th>Ang I Source</th>
<th>Ang II Source</th>
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<tbody>
<tr>
<td>Ang I in coronary artery</td>
<td>Ang II in coronary artery</td>
</tr>
<tr>
<td>Ang I produced by the fluid-phase reaction of renin with angiotensinogen in the coronary circulation</td>
<td>Ang I produced by the fluid-phase reaction of renin with angiotensinogen in the coronary circulation</td>
</tr>
<tr>
<td>Ang I synthesized in situ in cardiac tissue</td>
<td>Ang I synthesized in situ in cardiac tissue</td>
</tr>
</tbody>
</table>

where \([\text{125I-Ang II}_\text{tissue}]\) and \([\text{125I-Ang II}_\text{art}]\) are the steady-state concentrations of 125I-Ang II in cardiac tissue (cpm/g) and aortic blood plasma (cpm/mL), respectively, and \([\text{125I-Ang I}_\text{art}]\) is the steady-state concentration of 125I-Ang I in aortic blood plasma (cpm/mL), all during the constant infusion of 125I-Ang I.

Some of the Ang I and II in cardiac tissue may originate from Ang I and II that is generated by the action of circulating renin with circulating angiotensinogen during the passage of blood from the arterial to the venous end of the coronary circulation. The Ang I–generating capacity of blood plasma was calculated as

\[ \text{AGC}_{\text{plasma}} = [\text{Ren}] 	imes [\text{Aog}] \times (K_m + [\text{Aog}]) \]

where \(\text{AGC}_{\text{plasma}}\) is the Ang I–generating capacity of plasma (fmol Ang I · mL\(^{-1}\) · min\(^{-1}\)), \([\text{Ren}]\) is the renin concentration of plasma (fmol Ang I · mL\(^{-1}\) · min\(^{-1}\)), \([\text{Aog}]\) is the angiotensinogen concentration of plasma (pmol/mL), and \(K_m\) is the Michaelis-Menten constant for the reaction of porcine renin with porcine angiotensinogen, which equals 420 pmol/mL.

The cardiac tissue level of Ang I that originates from the Ang I–generating capacity of plasma was calculated as

\[ [\text{Ang I}_{\text{tissue from AGC}_{\text{plasma}}}] = R'_2 \times \text{AGC}_{\text{plasma}} \times t \]

where \([\text{Ang I}_{\text{tissue from AGC}_{\text{plasma}}}]\) is the Ang I concentration in cardiac tissue (fmol/g) originating from the Ang I–generating capacity of plasma, \(t\) is the coronary blood transit time, which equals 0.1 minute, and \(R'_2\) is given by Equation 4, and \(R'_2\) is given by

\[ R'_2 = \frac{[\text{125I-Ang I}_\text{tissue}]}{[\text{125I-Ang I}_\text{art}]} \]

where \([\text{125I-Ang I}_\text{tissue}]\) and \([\text{125I-Ang I}_\text{art}]\) are the steady-state concentrations (cpm/mL) of 125I-Ang I in coronary venous and aortic blood plasma, respectively, both during the constant infusion of 125I-Ang I.

The cardiac tissue level of Ang II that originates from Ang I that originates from the Ang I–generating capacity of plasma was calculated as

\[ [\text{Ang II}_{\text{tissue from AGC}_{\text{plasma}}}] = (R'_2 \times R'_3) \times \text{AGC}_{\text{plasma}} \times t \]

where \(R'_3\) is given by

\[ R'_3 = \frac{[\text{125I-Ang II}_\text{tissue}]}{[\text{125I-Ang II}_\text{art}]} \]

\[ - (R'_2 \times [\text{Ang II}_{\text{tissue from Ang II}_\text{art}}]) \]

In Equation 11, \([\text{125I-Ang II}_\text{tissue}]\) and \([\text{125I-Ang II}_\text{art}]\) are the steady-state concentrations (cpm/mL) of 125I-Ang II in coronary venous and aortic
blood plasma, respectively, and $[^{125}\text{I}-\text{Ang I}]_a$ is the steady-state concentration (cpm/mL) of $^{125}\text{I}$-Ang I in aortic blood plasma, all during the constant infusion of $^{125}\text{I}$-Ang I. R is given by

$$R = \frac{[^{125}\text{I}-\text{Ang II}]_a}{[^{125}\text{I}-\text{Ang I}]_a}$$

where $[^{125}\text{I}-\text{Ang II}]_a$ and $[^{125}\text{I}-\text{Ang II}]_a$ are the steady-state concentrations (cpm/mL) of $^{125}\text{I}$-Ang II in coronary venous and aortic blood plasma, respectively, both during the constant infusion of $^{125}\text{I}$-Ang II.

Equation 10 gives a maximum value of $[\text{Ang II tissue from AGC plasma}]$, $[^{125}\text{I}-\text{Ang II ven}] / [^{125}\text{I}-\text{Ang II art}]$ (12)

$$[^{125}\text{I}-\text{Ang II ven}] / [^{125}\text{I}-\text{Ang II art}]$$

concentrations of Ang II in cardiac left ventricular tissue and coronary venous plasma, respectively, derived from arterially delivered Ang I and expressed as a fraction of the concentration of Ang I in aortic plasma. R3 represent the concentrations of Ang I in cardiac left ventricular tissue and coronary venous plasma, respectively, derived from arterially delivered Ang I and expressed as a fraction of the concentration of Ang I in aortic plasma. Data are mean±SD or means and ranges.

$^*P<0.01$ for difference from results in control animals receiving $^{125}\text{I}$-Ang I infusion (unpaired Student’s t test).

**Results**

**Hemodynamic Effects of $^{125}\text{I}$-Labeled Angiotensins**

Baseline heart rate, cardiac output, and mean arterial pressure did not differ in untreated and captopril-treated pigs (Table 2). Infusions of either $^{125}\text{I}$-Ang I or $^{125}\text{I}$-Ang II did not affect any of these parameters (data not shown), which is in agreement with previous studies. 14,16,17

**$^{125}\text{I}$-Labeled Angiotensins in Cardiac Tissue and Blood Plasma**

The steady-state levels of $^{125}\text{I}$-Ang I and II in aortic plasma during constant infusion of $^{125}\text{I}$-Ang I or II are shown in Table 2. $^{125}\text{I}$-Ang II in plasma was lower and $^{125}\text{I}$-Ang I was higher in the captopril-treated pigs than in the controls. The ratio of $^{125}\text{I}$-Ang II to I in plasma, which is a measure of the degree of ACE inhibition, fell from 0.71 to 0.11 after captopril.

Table 3 gives the cardiac tissue and coronary venous plasma concentrations of Ang I and II derived from arterially delivered Ang I or II, expressed as a fraction, R, of the Ang I or II concentration in arterial blood plasma. The R values were calculated from the steady-state $^{125}\text{I}$-Ang I and II levels in cardiac tissue (cpm/g), coronary venous plasma (cpm/mL), and aortic plasma (cpm/mL) during $^{125}\text{I}$-Ang I or II infusions.

After captopril treatment, the coronary venous plasma concentration of $^{125}\text{I}$-Ang II that is derived by conversion from arterially delivered $^{125}\text{I}$-Ang I fell from 32% to 4% of the $^{125}\text{I}$-Ang I concentration in arterial plasma. This demonstrates effective ACE inhibition in the coronary vascular bed.

**Table 3. Cardiac Tissue and Coronary Venous Plasma Concentrations of Ang I and II Derived From Arterially Delivered Ang I or II, Expressed as a Fraction, R, of the Ang I or II Concentration in Arterial Plasma**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Mean Arterial Pressure, mm Hg</th>
<th>Heart Rate, bpm</th>
<th>Cardiac Output, L/min</th>
<th>$^{125}\text{I}$-Ang I Concentration, cpm/mL</th>
<th>$^{125}\text{I}$-Ang II Concentration, cpm/mL</th>
<th>Ratio of $^{125}\text{I}$-Ang II to I</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{125}\text{I}$-Ang I infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>86 ± 12</td>
<td>123 ± 12</td>
<td>2.5 ± 0.5</td>
<td>1350 ± 380</td>
<td>960 ± 390</td>
<td>0.71 ± 0.22</td>
</tr>
<tr>
<td>Captopril (n=10)</td>
<td>85 ± 4</td>
<td>112 ± 15</td>
<td>2.7 ± 0.6</td>
<td>1960 ± 720*</td>
<td>240 ± 210*</td>
<td>0.11 ± 0.04*</td>
</tr>
<tr>
<td>$^{125}\text{I}$-Ang II infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=4)</td>
<td>78 ± 13</td>
<td>110 ± 15</td>
<td>2.3 ± 0.7</td>
<td>-</td>
<td>1960 ± 530</td>
<td>...</td>
</tr>
</tbody>
</table>

*P<0.01 for difference from results in control animals (unpaired Student’s t test).

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Cardiac Tissue</th>
<th>Coronary Venous Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_1$</td>
<td>$R_2$</td>
</tr>
<tr>
<td>Control</td>
<td>0.75 (0.71–0.81)</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td></td>
<td>(n=3)</td>
<td>(n=3)</td>
</tr>
<tr>
<td>Captopril</td>
<td>ND</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>(n=3)</td>
<td>(n=3)</td>
</tr>
</tbody>
</table>

ND indicates not done. R through $R_3$ are given by Equations 2, 4, and 6 and $R_3$ through $R_3$ are given by Equations 9, 11, and 12. The R and $R_3$ values were calculated from the $^{125}\text{I}$-Ang I and II levels in cardiac left ventricular tissue, coronary venous blood plasma, and aortic blood plasma during $^{125}\text{I}$-Ang I or II infusions. $R_1$ and $R_2$ represent the concentrations of Ang II in cardiac left ventricular tissue and coronary venous plasma, respectively, derived from arterially delivered Ang II and expressed as a fraction of the concentration of Ang II in aortic plasma. $R_3$ and $R_3$ represent the concentrations of Ang I in cardiac left ventricular tissue and coronary venous plasma, respectively, derived from arterially delivered Ang I and expressed as a fraction of the concentration of Ang I in aortic plasma. Data are mean±SD or means and ranges.

$^*P<0.01$ for difference from results in control animals (unpaired Student’s t test).
In the control group, the $^{125}$I-Ang II concentration in cardiac tissue during $^{125}$I-Ang II infusion was 75% of its concentration in arterial plasma. In contrast, the cardiac tissue concentration of $^{125}$I-Ang I during $^{125}$I-Ang I infusion was 4% of its concentration in arterial plasma. The tissue concentration of $^{125}$I-Ang II that was derived by conversion from arterially delivered $^{125}$I-Ang I was 23% of the $^{125}$I-Ang I concentration in arterial plasma. This percentage was much lower, <2%, in the captopril-treated group, which is again an indication of effective blockade of conversion of Ang I to II.

Endogenous Angiotensins in Cardiac Tissue and Plasma

The levels of endogenous Ang I and II in cardiac tissue and blood plasma are shown in Figures 2 and 3. Plasma Ang I and II in untreated pigs were within the normal range. No significant differences were observed between aortic and coronary venous plasma.

The tissue levels in the various parts of the heart were not significantly different in either the captopril-treated or untreated pigs, but there were marked differences between tissue and plasma. The tissue concentration of Ang II in the untreated group was $\approx 5$ times the plasma concentration of Ang II. In addition, the tissue concentration of Ang II was $>5$ times the concentration of Ang I, whereas in plasma, Ang II was lower than Ang I.

Ang I rose after captopril both in tissue and in plasma. Ang II in tissue did not change after captopril, whereas in plasma, Ang II fell to values close to the detection limit of the assay.

Routinely, as described in the “Methods” section, Ang I and II were measured in cardiac tissue that was frozen within 15 seconds after the heart had been stopped and removed from the body. When cardiac tissue was kept at 37°C for 1 hour after the heart had been removed from the body, the tissue levels of Ang I and II were virtually constant during this period, and they were similar to the routinely measured levels (Figure 4).

Renin and Angiotensinogen in Cardiac Tissue and Blood Plasma

The tissue levels of renin and angiotensinogen in the various parts of the heart were not significantly different in either the captopril-treated animals or in controls (Table 4). The tissue concentration of renin (expressed per gram tissue) was similar to the plasma concentration (expressed per milliliter). This suggests that the presence of renin in tissue is not restricted to the extracellular fluid compartment. In contrast, the tissue concentration of angiotensinogen (expressed per gram tissue) was 10% to 30% of the plasma concentration (expressed per milliliter).

As expected, the plasma and tissue levels of renin were higher in the captopril group than in controls. The plasma levels of angiotensinogen were not different between the two
groups, but the tissue levels of angiotensinogen were lower in the captopril group, which is an indication of increased substrate consumption due to elevated renin.

Contributions of Blood-Derived Angiotensins to the Angiotensin Levels in Cardiac Tissue

Figures 5 and 6 show the results for Ang I and II in left ventricular free wall tissue both in the captopril-treated pigs and in controls. It appears that >90% of the Ang I in tissue is synthesized in the tissue itself and is not derived from the circulation. Most of the Ang II in tissue is also synthesized in the tissue, and its source is Ang I synthesized in situ rather than Ang I from the circulation. The contribution of Ang I from the circulation to the cardiac tissue level of Ang II was small in the control group and fell to nearly zero after captopril because of the blockade of Ang I–to–II conversion.

Discussion

In the present study, $^{125}$I-Ang I and $^{125}$I-Ang II were infused into the left cardiac ventricle to determine how much of the Ang I and II in cardiac tissue is derived from the circulation and how much is generated in situ. The results indicate that most, if not all, Ang I we measured in cardiac tissue was synthesized in situ and that most of the cardiac Ang II was produced by the conversion of this in situ–synthesized Ang I.

We used tracer doses of $^{125}$I-Ang II to minimize the chance of a physiological effect. No hemodynamic response was detectable. The steady-state plasma level of $^{125}$I-Ang II was $\approx 2000$ cpm/mL (Table 2), which corresponded with a plasma concentration of $\approx 0.55$ fmol/mL. The plasma level of endogenous Ang II was 2 fmol/mL. Thus, the $^{125}$I-Ang II infusion caused an increase of the plasma Ang II concentration by only 25%. With the $^{125}$I-Ang I infusions, the increase of plasma Ang II was even less. An important effect on AT₁ receptor density is therefore unlikely.

In our calculations, it was assumed that the fate of the radiolabeled angiotensins in cardiac tissue and the coronary circulation is comparable to that of arterially delivered nonlabeled angiotensins. Previous studies in pigs and humans demonstrated that the $^{125}$I-Ang I–to–II conversion rate, both in vitro and in vivo, is about two times the Ang I–to–II conversion rate and that the rates at which $^{125}$I-Ang I and Ang I are degraded into peptides other than $^{125}$I-Ang II and Ang II are not different. The $^{125}$I-Ang II and Ang II degradation rates are also not different.

![Figure 5. Contributions of various sources of Ang I to cardiac tissue (left ventricular free wall) level of this peptide in untreated and captopril-treated pigs. Values (means and ranges of 3 experiments) are percentage of total tissue level of Ang I. A, Arterially delivered Ang I; B, Ang I produced by Ang I–generating capacity of plasma in coronary circulation; C, Ang I synthesized in situ in cardiac tissue.](http://circ.ahajournals.org/)

### Table 4. Concentrations of Renin, Angiotensinogen, and the Ratio of Ang II to I Concentration in Cardiac Tissue and Arterial Blood Plasma in Untreated (n=14) and Captopril-Treated (n=10) Pigs

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Aortic Plasma</th>
<th>Left Atrium</th>
<th>Right Atrium</th>
<th>Left Ventricle</th>
<th>Right Ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin, fmol Ang I · mL$^{-1}$ · min$^{-1}$ or fmol Ang I · g$^{-1}$ · min$^{-1}$</td>
<td>26±34</td>
<td>38±25</td>
<td>40±17</td>
<td>31±16</td>
<td>25±13</td>
</tr>
<tr>
<td>Captopril</td>
<td>78±39*</td>
<td>114±61‡</td>
<td>101±43‡</td>
<td>107±52‡</td>
<td>85±39‡</td>
</tr>
<tr>
<td>Angiotensinogen, pmol/mL or pmol/g</td>
<td>340±72</td>
<td>62±47</td>
<td>47±17</td>
<td>98±39</td>
<td>73±45</td>
</tr>
<tr>
<td>Captopril</td>
<td>339±103</td>
<td>41±25‡</td>
<td>30±6‡</td>
<td>60±42‡</td>
<td>44±27‡</td>
</tr>
<tr>
<td>Ang I–generating capacity, fmol Ang I · min$^{-1}$ · mL</td>
<td>Control 12±16</td>
<td>29±14*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captopril</td>
<td>29±14*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of Ang II to I</td>
<td>Control 0.67±0.35</td>
<td>15.9±6.4</td>
<td>8.4±5.0</td>
<td>5.2±2.2</td>
<td>9.4±9.3</td>
</tr>
<tr>
<td>Captopril</td>
<td>0.06±0.07†</td>
<td>6.2±5.2‡</td>
<td>2.3±2.1‡</td>
<td>2.8±1.8‡</td>
<td>5.0±7.7‡</td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.01 for difference from control animals (unpaired Student’s t test).

‡Tissue concentrations of renin and angiotensinogen and the ratio of Ang II to I tissue concentration in the two groups of animals are significantly different (P<0.01, MANOVA).

$g$ indicates gram tissue wet weight. Data are mean±SD.

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conversion rate is somewhat higher than the Ang I–to–II conversion rate may have led us to overestimate the amount of Ang II in cardiac tissue that is derived from Ang I in the circulation. Therefore, this does not invalidate our conclusion that most of the Ang II in cardiac tissue is generated from in situ–synthesized rather than blood-derived Ang I.

An important methodological aspect of this study relates to the question of whether the radiolabeled and endogenous angiotensin levels measured in cardiac tissue are representative of the levels in vivo. The finding that the ex vivo cardiac tissue levels of Ang I and II remained practically constant at 37°C lends support to the assumption that the measured levels are close to the in vivo levels. The ex vivo half life of 125I-Ang II in cardiac tissue at 37°C is 30 to 40 minutes. Thus, the 125I-Ang II level we measured in cardiac tissue that was frozen within 15 seconds after the 125I-Ang I and II infusions had been stopped and the heart had been removed from the body is probably also close to the 125I-Ang II level in vivo. Ex vivo production of Ang II may explain why endogenous cardiac Ang II, as opposed to radiolabeled Ang II, remained constant when cardiac tissue was kept at 37°C.

The tissue level of 125I-Ang I was too low to determine the ex vivo half life of 125I-Ang I. The 125I-Ang I level we measured in cardiac tissue that was frozen within 15 seconds after 125I-Ang I infusion had been stopped and the heart had been removed from the body was <4% of the plasma level in the aorta. The fact that the tissue level of 125I-Ang I is probably only a small fraction of the level in arterial plasma in vivo as well is supported by the following considerations: (1) the rate of Ang I production in tissue ex vivo is probably not higher than in vivo, (2) the tissue concentration of Ang I we measured is close to the level in vivo, and (3) Ang I delivery by the aorta contributes to the tissue level of Ang I in vivo but not ex vivo. The third consideration implies that if the two other considerations are correct, arterially delivered Ang I will contribute little to its level in tissue.

Our finding that the tissue-to-plasma ratio of 125I-Ang II during 125I-Ang II infusion was much higher than the tissue-to-plasma ratio of 125I-Ang I during 125I-Ang I infusion may suggest that the two peptides are located in different tissue compartments. An earlier study in which we infused 125I-Ang I and 125I-Ang II into the left cardiac ventricle of pigs provided evidence that most of the 125I-Ang II in cardiac tissue had been accumulated by the cells via an angiotensin AT1 type receptor–mediated process. 125I-Ang I does not bind to the AT1 receptor and does not enter the cells via this receptor, so the location of 125I-Ang I in the tissue may be restricted to the extracellular compartment.

Studies with a modified rat Langendorff heart model, which allowed us to collect interstitial fluid transudate separately from the coronary effluent, showed that the Ang I concentration in interstitial fluid during perfusion of the heart with Ang I was only 10% to 20% of the Ang I concentration of the inflowing perfusion fluid. When this also holds for the 125I-Ang I we infused into the pigs in the present experiments and when, as discussed above, 125I-Ang I in cardiac tissue is restricted to the extracellular compartment, it is easy to understand why the cardiac tissue level of 125I-Ang I we measured in the present study was so low.

The conclusion that 125I-Ang I and therefore also the arterially delivered nonlabeled Ang I are localized in the extracellular compartment and that 125I-Ang II and the arterially delivered nonlabeled Ang II are accumulated in the cells via binding to AT1 receptors may also hold for Ang I that is synthesized at tissue sites and for Ang II that originates from this in situ–synthesized Ang I. Further studies are needed to settle this issue.

Our observations in nephrectomized pigs demonstrated that at least in the healthy heart, most if not all of the cardiac Ang I and II is generated by blood-derived renin. The captopril-induced parallel increments in plasma renin and cardiac Ang I, as shown in the present study, support the assumption that also during captopril treatment, the cardiac production of Ang I and II depends on blood-derived renin. This, together with the evidence that most of the Ang I and II in cardiac tissue does not originate from the circulation but rather from local production, confirms the concept, already proposed by Loudon et al, that the principal functions of renin secretion by the kidney is to ensure the delivery of this enzyme to vascular tissues for the production of angiotensins in these tissues.

Recent evidence seems to indicate that binding sites for renin are present in the cell membrane fractions of rat cells from different organs, including the heart, blood vessels, and kidney. Also in the porcine heart, renin was found to be membrane bound. Cell membrane binding would be a mechanism by which renin from the circulation is accumulated at certain tissue sites, resulting in higher renin concentrations at these sites than in the circulating blood.

In view of the possibility that the beneficial effects of ACE inhibitor drugs on cardiac function and structure depend on their effect on cardiac Ang II production rather than on a decrease in circulating Ang II, it is of interest to note that in
our experiments, the cardiac tissue level of Ang II, as opposed to its level in plasma, did not fall after ACE inhibition by captopril. Other investigators, using various ACE inhibitor drugs in rats, observed a reduction in cardiac Ang II. However, these studies also indicated differences between the effects of ACE inhibitor treatment on Ang II production in the circulation and in tissues. Perindopril, for instance, caused a dose-dependent decrease in the Ang II–to–I concentration ratio in plasma, but at each dose, the effect on this ratio was greater in plasma than in cardiac tissue. Quinapril lowered cardiac Ang II in rats with volume overload–induced cardiac hypertrophy but not in normal rats, whereas plasma Ang II was suppressed in both groups. Results may therefore differ depending on the type and dose of ACE inhibitor and on whether the animals are studied under normal or pathological conditions.

In our experiments, the compensatory increase in Ang I production in the heart might have overcome the blockade of ACE. The increased tissue concentration of Ang I and the decreased tissue concentration of angiotensinogen, as observed in our study, are indications that the cardiac production of Ang I was indeed increased. It is also possible that captopril does not reach some of the tissue sites of Ang II production. Furthermore, enzymes other than ACE may be involved in the Ang I–to–II conversion in cardiac tissue, eg, chymase. However, the role of enzymes other than ACE in cardiac Ang II production remains questionable.

The results of the present study, together with our recent observations on the AT₁ receptor–mediated cardiac uptake of Ang II and its long intracellular half life, also raise the interesting possibility that AT₄ receptor antagonism and ACE inhibition have different effects on the distribution of locally produced Ang II over the intracellular and extracellular cardiac tissue compartments. Both treatment modalities tend to reduce the number of AT₄ receptors that are occupied with Ang II, and in both cases this is counteracted by a compensatory response of stimulated renin and Ang I production. However, AT₄ receptor–mediated endocytosis of Ang II protects this peptide against the enzymes that degrade extracellular Ang II, so that during AT₄ receptor blockade a larger proportion of tissue Ang II is exposed to these enzymes. This is also supported by the finding that the ratio of cardiac Ang II to I concentration was decreased in rats by treatment with the AT₄ receptor antagonist losartan, which had no effect on cardiac ACE.

It is therefore possible that, for a given increment of Ang I, the tissue level of Ang II is more reduced by AT₄ receptor antagonist drugs than by ACE inhibitors. This may have clinical consequences, in light of growing evidence that the physiological responses to Ang II not only are mediated by signal transduction from cell surface–bound Ang II receptors but that intracellular Ang II also contributes to these responses.

Further studies of the effects of ACE inhibitors, AT₄ receptor antagonists, and renin inhibitors on the local production of Ang I and II in cardiac and vascular tissues along the lines of the present study will clarify the pathophysiological significance of Ang II production in these tissues and may help to better define the place of these drugs in the management of heart failure and hypertension.

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References


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Jorge P. van Kats, A. H. Jan Danser, Jan R. van Meegen, Loes M. A. Sassen, Pieter D. Verdouw and Maarten A. D. H. Schalekamp

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