Angiotensin II–Forming Pathways in Normal and Failing Human Hearts

Hidenori Urata, Bernadine Healy, Robert W. Stewart, F. Merlin Bumpus, and Ahsan Husain

Reduced preload and afterload to the heart are important effects of angiotensin converting enzyme (ACE) inhibitors in the treatment of congestive heart failure. However, since angiotensin II (Ang II) directly increases the strength of myocardial contraction, suppression of Ang II formation by ACE inhibitors could potentially reduce the beneficial effects of Ang II on the failing heart. To study how ACE inhibition suppresses cardiac Ang II formation in man, we characterized ACE-dependent and ACE-independent Ang II–forming pathways in eight normal and 24 failing human hearts obtained at cardiac transplantation. Ang II–forming activity in left ventricular (LV) membrane preparations was assessed by measuring the conversion of $^{[125]}$Iangiotensin I (Ang I) to $^{[125]}$IAng II. LV $^{[125]}$IAng II–forming activity in normal hearts (35.5 ± 2.7 fmol/min/mg, n = 8) was not different from that in hearts from patients with ischemic cardiomyopathy (25.5 ± 2.9 fmol/min/mg, n = 9) and was 48% lower ($p < 0.001$) in hearts from patients with idiopathic cardiomyopathy (18.5 ± 1.9 fmol/min/mg, n = 15). In LV tissue from normal hearts and in hearts from patients with idiopathic and ischemic cardiomyopathy, the ACE inhibitor captopril (1 mM) inhibited total $^{[125]}$IAng II–forming activity by 11 ± 4%, 11 ± 3%, and 4 ± 3%, respectively ($p < 0.05$ for each group), illustrating that ACE is not the major Ang II–forming enzyme in the LV. In LV tissue from normal hearts and in hearts from patients with idiopathic and ischemic cardiomyopathy, the serine proteinase inhibitor (soybean trypsin inhibitor, 100 μg/ml) inhibited total $^{[125]}$IAng II–forming activity by 80 ± 3% in all three groups ($p < 0.001$ for each group), further illustrating that the majority of $^{[125]}$IAng II–forming activity in the LV is due to one or more membrane-bound serine proteinases. In normal human serum (n = 5), $^{[125]}$IAng II formation was completely inhibited by 1 mM captopril and was not inhibited by 100 μg/ml soybean trypsin inhibitor. These in vitro studies suggest that chronic ACE inhibitor therapy may decrease angiotensinergic input to the vessels by inhibiting Ang II formation in blood. Since plasma Ang I levels are markedly elevated during chronic ACE inhibitor therapy and our studies show that the heart's major enzymatic pathway for Ang II formation is not blocked by ACE inhibitors, it seems likely that cardiac Ang II formation is not abolished during chronic therapy. The latter suggests sustained or even enhanced inotropic benefit of angiotensin in the heart in the face of circulating renin-angiotensin system blockade with ACE inhibitors. (Circulation Research 1990;66:883–890)

In addition to its vasoconstrictor effect and its stimulatory effects on aldosterone secretion, the octapeptide hormone angiotensin II (Ang II) has a positive inotropic and chronotropic effect on the heart. High affinity membrane Ang II receptor binding sites have been reported in several mammalian hearts, including the human heart. In human hearts, Ang II receptor binding sites have been localized to the myocardium and cardiac adrenergic nerves. In the isolated rabbit atria, human atrial trabeculae, and rabbit and cat papillary muscle, Ang II produces an increase in the rate of tension development. Since Ang II retains its positive inotropic activity in the denervated heart and in the presence of β-adrenoceptor blockade, a substantial component of the positive inotropic effect of Ang II on the heart is likely to be direct. Ang II facilitates stimulation-evoked release of [H]norepinephrine from rat and rabbit hearts and may additionally influence cardiac function by increasing adrenergic input to the heart.
In blood, Ang II is produced by sequential processing of angiotensinogen by renin and then by angiotensin converting enzyme (ACE). In congestive heart failure, when circulating renin and, thus, Ang II levels are elevated, the positive inotropic effect of Ang II is expected to be more important, especially since ventricular Ang II receptor levels are not diminished in the failing human heart. ACE inhibitors have been shown to be of great value in the treatment of congestive heart failure, presumably because of their vasodilator effects. It is not known, however, whether ACE inhibitors also block cardiac Ang II formation; presumably the effectiveness of these drugs on the failing heart would be diminished by such inhibition, but this information is not easy to obtain. Our knowledge of Ang II formation in the human heart is limited by the availability of fresh myocardial tissue suitable for biochemical study and the considerable species variability of the cardiac renin-angiotensin system, leaving in doubt which animal model most closely resembles the human heart. Furthermore, the absence of a good animal model of the failing human heart also limits studies on the therapeutic response of the failing heart to drug treatment.

In the present study, we examined the role of tissue ACE activity in the conversion of angiotensin I (Ang I) to Ang II in human hearts and found that the major enzymatic pathway for Ang II formation in the myocardium was not suppressed by ACE inhibitors.

Materials and Methods

Drugs, Hormones, and Reagents

Synthetic Ang I and Ang II were purchased from Bachem, Torrance, California. Sodium iodide I 125 was purchased from Amersham, Arlington Heights, Illinois. Ang I and Ang II were iodinated by the lactoperoxidase method, and the moniodinated peptides were purified by reverse-phase high-pressure liquid chromatography (HPLC); the specific activity of each peptide was 2,200 Ci/mmol. ACE radioassay kits were purchased from Ventrex Laboratories, Portland, Maine. Captopril was obtained from Squibb, Princeton, New Jersey. All other chemicals and reagents were obtained from Sigma Chemical, St. Louis, Missouri.

Human Tissue

Normal heart tissue was obtained from LifeBanc, Cleveland, Ohio. These hearts, obtained from seven men and one woman (aged 17-48 years), were unsuitable for donation. Diseased human hearts (n = 24), explanted from patients undergoing cardiac transplantation at the Cleveland Clinic Foundation, were the source of diseased ventricular tissue. The latter patients all had end-stage congestive heart failure with 1) underlying coronary artery disease (ischemic, n = 9) or 2) dilated cardiomyopathy, with or without active myocarditis, and patent coronary arteries (idiopathic, n = 15). All heart tissues were kept in cold cardioplegia solution from the time of removal and were frozen within 6 hours (usually within 2 hours). Blood samples were obtained from normal volunteers and were the source of normal serum.

Preparation of Human Cardiac Membranes

Hearts were dissected to yield 0.2-2.0-g pieces. To compare ACE and Ang II-forming activities in both normal and diseased hearts, midventricular portions of the ventricles were used. Pieces of heart tissue were trimmed clean of fat and epicardium, placed in 20 mM NaH2PO4 buffer, pH 7.1 at 4°C (5 ml/0.5 g), and homogenized with a Polytron homogenizer (Brinkman Instruments, Fullerton, California) at 9,500 rpm for 15 seconds. The homogenate was centrifuged at 40,000g for 20 minutes at 4°C. The supernatant was discarded, and the pellet was resuspended in 20 mM NaH2PO4 buffer, pH 7.1, containing 100 mM NaCl and 10 mM MgCl2; a hand-driven glass/glass homogenizer was used for this procedure. Membrane protein was determined by the method of Lowry et al.

Measurement of ACE Activity in Cardiac Membranes

Membrane ACE (dipeptidyl carboxypeptidase I) activity was measured using the ACE activity radioassay system (Ventrex Laboratories) as described by the manufacturer. This assay measures [125I]hippuric acid released from the ACE substrate [125I]hippuryl-Gly-Gly. Cardiac membrane preparations (200-500 µg protein in 50 µl) were incubated in assay buffer with 40 mM [125I]hippuryl-Gly-Gly for 30 minutes at 37°C. At the end of the incubation, the reaction mixture was acidified by adding 50 µl of 0.5 M HCl, and generated [125I]hippuric acid was then extracted into 1.5 ml scintillation cocktail #1 (Ventrex Laboratories). Radioactivity in the organic phase was then determined in a liquid scintillation counter (model LS 3801, Beckman Instruments, Fullerton, California). The interassay and intra-assay coefficients of variation of this assay were 8.6% and 5.1%, respectively. To characterize the specificity of the ACE activity in heart membranes, ACE assays were performed in the presence of selective peptidase inhibitors including 1 mM captopril, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM N-ethylmaleimide (NEM), 100 µg/ml soybean trypsin inhibitor (SBTI), and 1 mM phenylmethylsulfonyl fluoride (PMSF).

Measurement of [125I]Ang II Formation

The formation of Ang II from Ang I in the presence or absence of captopril was examined to assess the contribution of membrane ACE activity and serum ACE activity in total Ang II formation. Comparisons of conversion of [125I]Ang I to [125I]Ang II by normal and diseased human heart tissue were made by using membrane preparations of left ventricles. To determine total [125I]Ang II formation in left ventricular tissue and in serum, 200 fmol [125I]Ang I was incubated with either ventricular membranes
(200 µg protein) or 50 µl serum, respectively, in 10 mM NaH₂PO₄ buffer, pH 7.1, containing 100 mM NaCl in a total incubation volume of 250 µl. Membranes were incubated for 20 minutes at 37° C, and serum samples were incubated for 5 minutes at 37° C. To define captopril-blockable [¹²⁵I]Ang II formation and SBTI-blockable [¹²⁵I]Ang II formation, the incubation buffer contained 1 mM captopril and 100 µg/ml SBTI, respectively. Reactions were terminated by additions of 2 ml ice-cold ethanol. The precipitated proteins were removed by centrifugation at 12,000 g for 10 minutes, and the supernatants containing [¹²⁵I]Ang I, [¹²⁵I]Ang II, and their metabolites were evaporated to dryness. The residues were resuspended in 100 µl of 25 mM triethylammonium phosphate (TEAP) buffer, pH 3.0, and applied to a C₁₈ Nova Pak HPLC column (Waters Associates, Milford, Massachusetts) with a 12-minute linear acetonitrile gradient (10–35%) in 25 mM TEAP, pH 3.0, at a flow rate of 1 ml/min. Radioactivity in the column eluent was detected by a continuous-flow radioactivity detector (model 170, Beckman Instruments). Retention times of [¹²⁵I]Ang II and [¹²⁵I]Ang I were 9.8 and 11.2 minutes, respectively. [¹²⁵I]Ang II formation by tissue samples was expressed as femtomoles [¹²⁵I]Ang II formed per minute per milligram membrane protein. [¹²⁵I]Ang II formation in serum was expressed as femtomoles [¹²⁵I]Ang II formed per minute per milliliter serum. The intra-assay coefficient of variation of this assay was 2% (n=8).

Statistical Methods

The results are expressed as mean±SEM unless otherwise indicated. Statistical analyses were performed, where indicated, by the paired or unpaired Student’s t test or the Bonferroni test after analysis of variance indicated significant differences. Pearson’s correlation was used to test for association. When either age or handling time was found to be significantly correlated with the response, it was adjusted by using analysis of covariance; the Bonferroni test was used to compare the adjusted means. Comparisons were considered significant at p<0.05.

Results

Specificity of ACE Activity Assay in Myocardial Tissue

The specificity of the ACE activity assay was assessed by using membranes from normal human left ventricles incubated in the presence of selective proteinase inhibitors. ACE activity, measured by the timed hydrolysis of [³H]hippuryl-Gly-Gly, was completely inhibited by 1 mM captopril and inhibited by over 80% by 1 mM EDTA (Figure 1). The latter, by chelating metal ions, should inhibit ACE, which is a metal ion–requiring dipeptidyl carboxypeptidase. The generation of [³H]hippuric acid by cleavage of the [³H]hippuryl-Gly bond was not blocked by inhibitors of serine proteinases (1 mM PMSF or 100 µg/ml SBTI) or sulfhydryl proteinases (1 mM NEM) (Figure 1); indeed, SBTI, NEM, and PMSF produced small but significant increases in the cardiac membrane–dependent hydrolysis of [³H]hippuryl-Gly-Gly. The mechanism of activation of ACE activity was not further studied.

ACE Activity in Human Hearts

Preliminary studies. For one normal study heart, from a 17-year-old previously healthy man, the entire specimen was analyzed to obtain a map of ACE activity, region by region, to guide future studies. In that heart, ACE activity was measured from 16 separate anatomical sites within the heart, including atria. As shown in Table 1, ACE activity varied more than 10-fold in different regions of the heart. The lowest levels of activity occurred at the apex of the left ventricle, and highest levels were present in the right atrium. There was a more than threefold variation in ACE distribution within the ventricles; the base of left and right ventricles, including intraventricular septum, contained higher levels of ACE activity than the apex. Since these differences in ventricular ACE activity were greater than the intraassay and interassay coefficients of variation of the ACE assay, in subsequent studies, comparisons of ACE and Ang II–forming enzyme activities in normal and failing hearts were made by using midventricular regions.

Since there were differences in the average handling time (i.e., time between removal of heart and freezing of heart tissue) for donor hearts compared with hearts obtained from patients with ischemic or idiopathic cardiomyopathy (Table 2), we examined whether enzyme activity changed during the initial storage of hearts in the cold cardioplegia solution; accordingly, we studied the relation between ventricular ACE activity and the initial tissue handling time.
ACE activity in the midventricular portion of normal and failing hearts is summarized in Table 2. ACE activity levels in the left ventricle of patients with ischemic or idiopathic cardiomyopathy were not significantly different from those of the normal left ventricle. ACE activity levels in the right ventricle of patients with idiopathic cardiomyopathy, but not ischemic cardiomyopathy, were 41% lower from those of the normal left ventricle ($p<0.05$). Comparison of ACE activity levels between the left and right ventricles of normal hearts in and from patients with ischemic or idiopathic cardiomyopathy showed that ACE activity was 1.4- to 2.4-fold higher in the right ventricle compared with the left ($p<0.05$ for each group).

A relation between ventricular ACE activity and sex of the patient was examined in hearts obtained from patients with idiopathic cardiomyopathy since five of the 15 hearts obtained from patients with idiopathic cardiomyopathy were female, whereas all patients with ischemic cardiomyopathy that we studied were male and seven of the eight normal hearts were from male donors. Table 3 shows that the ACE activity in the left ventricle was 67% higher in females compared with males ($p<0.05$). A significant difference was not observed in right ventricular ACE activity between females and males.

**Effect of chronic ACE inhibitor therapy.** The effect of chronic ACE inhibitor therapy on ventricular ACE activity was examined in hearts obtained from male patients with idiopathic cardiomyopathy. This homogeneous subgroup of patients was chosen to eliminate the impact of variables of the type of disease, age, tissue handling time, and sex. Table 4 shows that chronic ACE inhibitor therapy did not significantly alter left or right ventricular ACE activity levels.

Conversion of $[^{125}I]$Ang I to $[^{125}I]$Ang II by Myocardial Membranes From Midventricular Regions of the Left Ventricle and by Serum

Enzymes other than ACE have been reported to form Ang II from Ang I. Therefore, we examined conversion of $[^{125}I]$Ang I to $[^{125}I]$Ang II by cardiac membranes from normal left ventricular tissue in the presence of the ACE inhibitors captopril and EDTA as well as serine proteinase inhibitors SBTI and
TABLE 3. Sex Differences in Angiotensin Converting Enzyme Activity and [125I]Angiotensin II–Forming Activity in the Hearts of Patients With Idiopathic Cardiomyopathy

<table>
<thead>
<tr>
<th></th>
<th>Male (n=10)</th>
<th>Female (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>34.5±4.3</td>
<td>33.4±6.3</td>
</tr>
<tr>
<td>Handling time (hr)</td>
<td>1.1±0.1</td>
<td>1.7±0.2*</td>
</tr>
<tr>
<td>LV ACE activity (units/mg)</td>
<td>0.45±0.07</td>
<td>0.74±0.09</td>
</tr>
<tr>
<td>RV ACE activity (units/mg)</td>
<td>0.66±0.09</td>
<td>0.92±0.30</td>
</tr>
<tr>
<td>LV [125I]Ang II–forming activity (fmol/min/mg)</td>
<td>15.6±1.4</td>
<td>20.8±2.6</td>
</tr>
<tr>
<td>Captopril blockable</td>
<td>0.9±0.3</td>
<td>3.2±1.4</td>
</tr>
<tr>
<td>SBTI blockable</td>
<td>13.1±1.1</td>
<td>15.4±2.8</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
LV, left ventricular; ACE, angiotensin converting enzyme; RV, right ventricular; Ang II, angiotensin II; SBTI, soybean trypsin inhibitor.

* p<0.01 compared with the male group by unpaired Student’s t test.
† p<0.05 compared with the male group by unpaired Student’s t test.

PMSF and the sulfhydryl proteinase inhibitor NEM. In two experiments (a representative experiment is shown in Figure 2), conversion of [125I]Ang I to [125I]Ang II by left ventricular membranes was reduced by incubation with SBTI and PMSF in an effect more potent than that of captopril or NEM. Therefore, we determined total, captopril-blockable, and SBTI-blockable [125I]Ang II–forming activity in membrane preparations from mid–left ventricular regions of normal hearts and in hearts from patients with idiopathic and ischemic cardiomyopathy. These hearts were the same 32 hearts that were used to determine ACE activity described above.

Ang II–forming activity in membrane preparations from mid–left ventricular regions of normal hearts and in hearts from patients with idiopathic and ischemic cardiomyopathy is summarized in Table 5. [125I]Ang II–forming activity in the left ventricular tissue was positively correlated with patient age (y=0.25x+15.0, r²=0.13, p<0.05) but not with initial tissue handling time (p=0.07).

To study the effect of disease on [125I]Ang II–forming activity in the left ventricular tissue, we compared data normalized for differences in patient age. Conversion of [125I]Ang I to [125I]Ang II by cardiac membranes from normal left ventricular tissue (35.5±2.7 fmol/min/mg) was similar to the levels observed in left ventricular tissue from ischemic hearts (25.5±2.9 fmol/min/mg), but [125I]Ang II–forming activity was 48% lower (p<0.001) in left ventricular tissue from patients with idiopathic cardiomyopathy (18.5±1.9 fmol/min/mg).

Effect of captopril. In left ventricular tissue from normal hearts and in hearts from patients with idiopathic and ischemic cardiomyopathy, 1 mM captopril inhibited total [125I]Ang II–forming activity by only 11±4%, 11±3%, and 4±3%, respectively (p<0.05 for each group). Thus, most [125I]Ang II formation by these membranes occurred by an enzyme activity other than ACE. Captopril-blockable [125I]Ang II–forming activity in the left ventricular tissue was positively correlated with initial tissue handling time (y=1.0x−0.06, r²=0.38, p<0.001) but not with patient age (p=0.50). To study the effect of disease on captopril-blockable [125I]Ang II–forming

FIGURE 2. Effect of proteinase inhibitors on the conversion of [125I]angiotensin I to [125I]angiotensin II by myocardial membranes from normal ventricular tissue. [125I]Angiotensin I (200 fmol) was incubated with ventricular membranes in 10 mM NaH2PO4 buffer, pH 7.1, containing 100 mM NaCl in a total volume of 250 μl for 20 minutes at 37°C. Separation of the resulting radioactive angiotensin I fragments by high-performance liquid chromatography was performed as described in "Materials and Methods." Incubations were carried out in the presence of either no inhibitor (panel A), 1 mM captopril (panel B), 1 mM ethylenediaminetetraacetic acid (EDTA; panel C), 1 mM N-ethylmaleimide (NEM; panel D), 100 μg/ml soybean trypsin inhibitor (SBTI; panel E), or 1 mM phenylmethylsulfonyl fluoride (PMSF; panel F).

Radioactivity peaks occurring in the elution positions of [125I]angiotensin II and [125I]angiotensin I are identified by arrows labeled AII and AI, respectively.

TABLE 4. Effect of Chronic Captopril Therapy on Angiotensin Converting Enzyme Activity and [125I]Angiotensin II–Forming Activity in the Hearts of Male Patients With Idiopathic Cardiomyopathy

<table>
<thead>
<tr>
<th>Chronic captopril therapy</th>
<th>Yes (n=6)</th>
<th>No (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>33.8±6.9</td>
<td>35.5±4.7</td>
</tr>
<tr>
<td>Handling time (hr)</td>
<td>1.0±0.2</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>LV ACE activity (units/mg)</td>
<td>0.40±0.10</td>
<td>0.53±0.09</td>
</tr>
<tr>
<td>RV ACE activity (units/mg)</td>
<td>0.60±0.15</td>
<td>0.75±0.05</td>
</tr>
<tr>
<td>LV [125I]Ang II–forming activity (fmol/min/mg)</td>
<td>15.7±1.9</td>
<td>15.5±2.6</td>
</tr>
<tr>
<td>Captopril blockable</td>
<td>1.0±0.4</td>
<td>0.8±0.5</td>
</tr>
<tr>
<td>SBTI blockable</td>
<td>13.5±1.8</td>
<td>12.5±2.0</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
LV, left ventricular; ACE, angiotensin converting enzyme; RV, right ventricular; Ang II, angiotensin II; SBTI, soybean trypsin inhibitor.
activity in the left ventricular tissue, we compared data normalized for differences in tissue handling time. No significant differences in captopril-blockable \[^{125}\text{I}]\text{Ang II}
forming activity in the left ventricular tissue were observed between normal hearts and in hearts from patients with ischemic or idiopathic cardiomyopathy (Table 5). After normalization, the adjusted means were 2.0±0.7 fmol/min/mg for normal, idiopathic, and ischemic hearts, respectively.

| Pathological diagnosis          | Age (yr) | Sex (M/F) | Handling time (hr) | \[^{125}\text{I}]\text{Ang II}
formation (fmol/min/mg) | Total | Captopril blockable | SBTI blockable |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>29.9±4.2</td>
<td>7/1</td>
<td>3.2±0.7</td>
<td>33.0±3.5 (3.4±1.0</td>
<td>26.1±3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic cardiomyopathy</td>
<td>34.1±3.4</td>
<td>10/5</td>
<td>1.3±0.1*</td>
<td>17.2±1.4 (1.7±0.5</td>
<td>13.9±1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic cardiomyopathy</td>
<td>52.7±2.2</td>
<td>9/0</td>
<td>2.1±0.4</td>
<td>29.8±3.0 (1.1±0.5</td>
<td>23.7±2.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data in parentheses or square brackets are mean±SEM normalized for either age or handling time, respectively. Data were normalized for age or handling time if a significant correlation was observed between \[^{125}\text{I}]\text{Ang II}
formation activity and these variables.

\*p<0.05 compared with normal group by unpaired Student’s t test.

\*p<0.001 compared with normal group by analysis of variance followed by pairwise comparison with the Bonferroni test.

The effect of chronic ACE inhibitor therapy on left ventricular \[^{125}\text{I}]\text{Ang II}
forming activity was examined in hearts obtained from male patients with idiopathic cardiomyopathy. Table 4 shows that chronic ACE inhibitor therapy did not significantly alter total, captopril-blockable, or SBTI-blockable \[^{125}\text{I}]\text{Ang II}
forming activity in the left ventricle.

Conversion of \[^{125}\text{I}]\text{Ang I}
 to \[^{125}\text{I}]\text{Ang II}
by serum from normal volunteers (490±122 fmol/min/mg) was inhibited by 100% in the presence of 1 mM captopril but was not at all inhibited by 100 µg/ml SBTI. Serum ACE activity level in these normal volunteers was 8.4±1.1 units/ml.

**Discussion**

ACE, through its effects on the formation of Ang II, has been proposed to be a modulator of tissue function, including that of the heart.\(^{23,24}\) Our studies demonstrate the presence of an ACE-dependent and an ACE-independent Ang II-forming enzymatic pathway in the human heart and provide the biochemical evidence that the ACE-independent pathway of Ang II formation in the heart that is not blocked by ACE inhibitors is dominant over Ang II formation that occurs by ACE. Further, since these pathways are present in the failing heart, cardiac Ang II formation may participate in preserving the function of the failing heart by providing inotropic support.

**ACE Activity in the Human Heart**

In the left ventricle, ACE activity levels in normal donor hearts were similar to those in hearts from patients with idiopathic or ischemic cardiomyopathy. Plasma renin activity levels and circulating Ang I levels are elevated in patients with heart failure,\(^{17}\) and left ventricular ACE activity is maintained in the failing human heart; these findings suggest that ACE localized to the left ventricle may be important in heart failure via enhanced cardiac Ang II formation. We were concerned that the in vitro ACE assay could underestimate ACE activity in tissues obtained from patients on chronic captopril therapy. Since most study patients with ischemic and idiopathic
cardiomyopathy were being treated with captopril and the normal hearts were not exposed to ACE inhibitors, we examined the effect of chronic ACE inhibitor therapy on ventricular ACE activity. To avoid problems associated with other complicating factors such as nature of disease and patient sex, we made the comparison by using data obtained from male patients with idiopathic cardiomyopathy. Surprisingly, no differences were observed in left ventricular ACE activity of patients receiving ACE inhibitors compared with those patients not receiving ACE inhibitors. It is unclear, at present, whether this lack of difference in ventricular ACE activity occurs because ACE inhibitor, putatively coupled to ACE in vivo, dislodges from the membrane enzyme during tissue preparation or because cardiac ACE is incompletely inhibited during chronic ACE inhibitor therapy.

A consistent observation in normal and in failing hearts was that ACE activity in the right ventricle was about twofold higher than activity in the left ventricle, suggesting more ACE-dependent Ang II formation in the right ventricle. Because high levels of ACE occur in the lung, it is likely that blood entering the left ventricle from the lung contains higher Ang II levels than blood entering the right ventricle. Higher Ang II levels in the left ventricular blood may, by negative feedback, influence the level of left ventricular ACE activity.

**Dual Pathway for Ang II Formation by the Human Heart**

Although cardiac ACE activity, measured by use of \([{}^3H\)]hippuryl-Gly-Gly as substrate, was specific for the metal ion–dependent dipeptidyl carboxypeptidase activity designated as ACE (EC 3.4.15.1), we examined what proportion of total Ang I conversion to Ang II was due to ACE in normal and diseased left ventricular membranes and whether alternate conversion pathways were present. Surprisingly, \([{}^{125}I\)]Ang I conversion to \([{}^{125}I\)]Ang II by myocardial membranes from normal and diseased left ventricles was not markedly inhibited by the ACE inhibitor captopril. However, conversion of \([{}^{125}I\)]Ang I to \([{}^{125}I\)]Ang II in the presence of captopril accounted for approximately 90% of the total \([{}^{125}I\)]Ang II formation by myocardial membranes obtained from normal and diseased left ventricles. The majority (> 80%) of this alternate \([{}^{125}I\)]Ang II–forming enzyme activity appeared to be due to one or more serine proteinases, since this activity was inhibited by the serine proteinase inhibitors SBTI and PMSF but not by inhibitors of sulphydryl or metal ion–requiring proteinases. In normal human serum, \([{}^{125}I\)]Ang I conversion to \([{}^{125}I\)]Ang II was completely suppressed by captopril and not inhibited by SBTI. Thus, unlike the preeminent role of ACE in the conversion of \([{}^{125}I\)]Ang I to \([{}^{125}I\)]Ang II in serum, ACE appeared to be minimally involved in the conversion of \([{}^{125}I\)]Ang I to \([{}^{125}I\)]Ang II in the left ventricle. This biochemical finding may explain the observation that the positive inotropic effect of Ang I on isolated intact hearts7,25 and on isolated papillary muscle preparations26 is only marginally reduced by ACE inhibitors.

In blood, trypsinlike enzymes are inhibited by the circulating high molecular weight proteins \(\alpha_1\)-antitrypsin and \(\alpha_2\)-macroglobulin. Thus, it is not surprising that SBTI does not have an effect on serum conversion of \([{}^{125}I\)]Ang I to \([{}^{125}I\)]Ang II. The penetration of high molecular weight proteins across the capillary endothelium is known to be restricted22; therefore, levels of \(\alpha_1\)-antitrypsin and \(\alpha_2\)-macroglobulin are likely to be considerably reduced in the interstitial fluid surrounding cells. Thus, membrane-bound serine proteinase in the human left ventricle that forms Ang II may be functional at the level of the myocyte in vivo.

SBTI-blockable Ang II–forming activity in the left cardiac ventricle of normal hearts was similar to that in ischemic hearts but was 47% lower in hearts from patients with idiopathic cardiomyopathy. Due to the special problems associated with the procurement of human heart tissue, there were significant differences between tissue handling time and patient age between normal and diseased heart groups. However, these differences do not appear to affect the conclusion that SBTI-blockable Ang II–forming activity is selectively altered in left ventricular tissue in patients with idiopathic cardiomyopathy, since similar findings were observed by using the raw data or the data normalized, when necessary, for these variables. Although it remains to be established if the change in SBTI-blockable Ang II–forming activity in the left ventricle of patients with idiopathic cardiomyopathy is causally related to the disease process, our data would suggest that cardiac Ang II formation may be compromised in end-stage heart failure of idiopathic origin.

**Clinical Implications**

Captopril therapy has proven to be highly effective in the treatment of congestive heart failure and to be of considerable benefit beyond that afforded by vasodilator treatment with other agents.18,19,28,29 Our study provides a partial explanation for the seeming paradox of ACE-inhibitor benefit in which preload and afterload are reduced, but at the presumed cost of a reduced inotropic benefit afforded by Ang II. The presence of a dual pathway for Ang II formation in the human heart presents a potential escape from complete inhibition of cardiac Ang II formation by ACE inhibitors. The recent report30 that ACE inhibitor infused acutely into the coronary circulation of myopathic human hearts produces a mild negative inotropic response is not inconsistent with our hypothesis. We show that captopril-blockable conversion of \([{}^{125}I\)]Ang I to \([{}^{125}I\)]Ang II by cardiac membranes represents approximately 10% of total \([{}^{125}I\)]Ang II formation. Since plasma Ang I levels are elevated more than 300% during chronic ACE inhibitor therapy,17 the reduction in ACE-dependent cardiac Ang II formation in ACE inhibitor–treated patients is likely to be small compared with the increase in cardiac Ang II formation by the alternate pathway, which would result from a greater delivery
of Ang I to the heart. Captopril therapy may in part improve heart function by decreasing angiotensinergic input to vessels, thereby decreasing preload and afterload to the heart, but this decrease occurs without compromising and possibly by enhancing angiotensinergic input to the diseased myocardium. The finding that levels of ventricular Ang II receptors are not reduced in the failing human heart further supports this hypothesis.

Acknowledgments

We thank members of the heart transplant team of The Cleveland Clinic Foundation for supplying human heart tissue, Dr. Mark D. Schluchter and Ms. Geri Locker, Department of Biostatistics and Epidemiology, Cleveland Clinic, for advice and help with the statistical analysis, and Mr. Dennis Wilk for efficient technical help.

References

25. Diebold H, Moreau D, Chardigny JM, Rochett L: Direct and cardiac effects of converting enzyme inhibitors (IEC) and angiotensin I (AI) and II (AII) on the isolated rat heart. Arch Mal Coeur 1988;81(suppl HTA):29–32

Key Words • angiotensin converting enzyme • human heart • angiotensin II-forming enzymes • angiotensin II • angiotensin I
Angiotensin II-forming pathways in normal and failing human hearts.
H Urata, B Healy, R W Stewart, F M Bumpus and A Husain

doi: 10.1161/01.RES.66.4.883

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/66/4/883

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/