Cardiac Angiotensin II Formation in the Clinical Course of Heart Failure and Its Relationship With Left Ventricular Function

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Abstract—In 76 patients with heart failure (HF) (New York Heart Association [NYHA] classes I through IV) and in 15 control subjects, cardiac angiotensin II (Ang II) generation and its relationship with left ventricular function were investigated by measuring aorta– coronary sinus concentration gradients of endogenous angiotensins and in a part of patients by studying 125I-labeled Ang I kinetics. Gene expression and cellular localization of the cardiac renin-angiotensin system components, the density of AT1 and AT2 on membranes and isolated myocytes, and the capacity of isolated myocytes for synthesizing the hypertrophying growth factors insulin-like growth factor-I (IGF-I) and endothelin (ET)-1 were also investigated on 22 HF explanted hearts (NYHA classes III and IV) and 7 nonfailing (NF) donor hearts. Ang II generation increased with progression of HF, and end-systolic wall stress was the only independent predictor of Ang II formation. Angiotensinogen and angiotensin-converting enzyme mRNA levels were elevated in HF hearts, whereas chymase levels were not, and mRNAs were almost exclusively expressed on nonmyocyte cells. Ang II was immunohistochemically detectable both on myocytes and interstitial cells. Binding studies showed that AT1 density on failing myocytes did not differ from that of NF myocytes, with preserved AT1/AT2 ratio. Conversely, AT1 density was lower in failing membranes than in NF ones. Ang II induced IGF-I and ET-1 synthesis by isolated NF myocytes, whereas failing myocytes were unable to respond to Ang II stimulation. This study demonstrates that (1) the clinical course of HF is associated with progressive increase in cardiac Ang II formation, (2) AT1 density does not change on failing myocytes, and (3) failing myocytes are unable to synthesize IGF-I and ET-1 in response to Ang II stimulation. (Circ Res. 2001;88:961-968.)

Key Words: angiotensins • congestive heart failure • AT receptors • failing myocytes • cardiomyopathy

Conclusive evidence has been presented that all of the components required for angiotensin II (Ang II) production are present in the human heart1 and that Ang II is continuously formed by cardiac tissues in healthy subjects.2 However, few studies have investigated the cardiac renin-angiotensin system (RAS) in human hypertrophy and heart failure (HF), as opposed to the large number of experimental studies performed in cell culture systems and experimental models (for studies, see Wollert and Drexler3). In human explanted failing hearts, levels of angiotensin-converting enzyme (ACE) mRNA,4 ACE activity, and ACE binding sites5 have been found to be increased as compared with control hearts. Levels of prorenin, renin, and ACE measured with enzyme kinetics are higher in failing than in control hearts.6 Although these studies provide important results, demonstrating that cardiac RAS is activated in explanted failing hearts, they do not provide information either about cardiac Ang II production during the clinical course of HF or about the relationship between Ang II and cardiac function. Moreover, these studies do not answer the critical question as to whether the enhanced cardiac expression of ACE actually promotes an increased production of Ang II, because several groups have challenged the notion that ACE is the major Ang II–forming enzyme in the human heart.7,8 A second open question is the identification of the cell types that actually express Ang II receptors in the failing myocardium and constitute the potential targets for Ang II receptor–mediated effects. Several groups have investigated the density of Ang II receptor subtypes in human failing hearts and have found a reduction in AT1,5,9–11 either without changes in AT210 or with an increase in them.5,11 However, these studies were only performed on myocardial homogenates, and no information is available on the regulation of Ang II receptor subtypes on myocytes and nonmyocyte cells. A last but no less
TABLE 1. Characteristics of Subjects Investigated

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>NF Hearts</th>
<th>NYHA Class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=15</td>
<td>n=7</td>
<td>I n=15</td>
</tr>
<tr>
<td>Age, years</td>
<td>60±10</td>
<td>44±5*</td>
<td>51±17</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>13/2</td>
<td>5/2</td>
<td>10/5</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.89±0.10</td>
<td>1.86±0.18</td>
<td>1.88±0.19</td>
</tr>
<tr>
<td>Treatments, No.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Digitalis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Furosemide</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Etiology (ICM/DCM)</td>
<td>...</td>
<td>...</td>
<td>8/7</td>
</tr>
<tr>
<td>Left ventricular end-diastolic diameter, mm/m²</td>
<td>26.5±1.3</td>
<td>26.4±3.1</td>
<td>32.2±2.8†</td>
</tr>
<tr>
<td>Left ventricular mass index, g/m²</td>
<td>109±13</td>
<td>104±18</td>
<td>175±39†</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>65±6</td>
<td>61±3.9</td>
<td>54±11†</td>
</tr>
<tr>
<td>Velocity of circumferential fiber shortening, s⁻¹</td>
<td>1.16±0.17</td>
<td>1.09±0.23</td>
<td>1.00±0.26†</td>
</tr>
<tr>
<td>End-systolic stress, kdyn/cm²</td>
<td>70±9</td>
<td>...</td>
<td>99±18†</td>
</tr>
<tr>
<td>End-diastolic stress, kdyn/cm²</td>
<td>14±3</td>
<td>...</td>
<td>22±5†</td>
</tr>
<tr>
<td>Cardiac index, L/m²·min⁻¹</td>
<td>3.23±0.49</td>
<td>...</td>
<td>2.80±0.26*</td>
</tr>
<tr>
<td>Left ventricular end-diastolic pressure, mm Hg</td>
<td>8.3±1.5</td>
<td>...</td>
<td>13.6±4.1†</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure, mm Hg</td>
<td>11±3</td>
<td>...</td>
<td>14±4</td>
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<tr>
<td>Pulmonary capillary wedge pressure, mm Hg</td>
<td>7±3</td>
<td>...</td>
<td>8±4</td>
</tr>
<tr>
<td>PRA, ng/mL·hour⁻¹</td>
<td>0.82±0.28</td>
<td>...</td>
<td>1.09±0.81</td>
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</tbody>
</table>

*P<0.05, †P<0.01 vs control subjects.

important question is the functional significance of cardiac Ang II formation in myocardial hypertrophy and HF. Although Ang II has been most frequently associated with both experimental and human hypertrophy,12–14 the precise role of Ang II in cardiac hypertrophy remains elusive.15,16 because cardiac hypertrophy may develop in the presence of Ang II generation inhibitors or AT₁ blockade.17,18 Moreover, human compensatory hypertrophy is associated with increased cardiac formation of insulin-like growth factor-I (IGF-I) in volume overload and endothelin (ET)-1 and IGF-I in pressure overload, but not with cardiac Ang II.14

Thus, the functional significance of ACE mRNA and increased ACE activity found in expanded hearts remains to be clarified. Therefore, the present study was planned with the following objectives: (1) to investigate whether and when cardiac Ang II formation increases during the clinical course of HF and its relationship with left ventricular function, (2) to identify and examine the distribution of Ang II receptor subtypes on myocytes and nonmyocyte cells from failing and nonfailing (NF) myocardium, and (3) to study the relationship between Ang II and the capacity of myocytes for producing the hypertrophying growth factors IGF-I and ET-1.

Materials and Methods
We investigated 76 patients with HF due to dilated cardiomyopathy (DCM, n=36) or to ischemic cardiomyopathy (ICM, n=40). Diagnosis of DCM or ICM was based on clinical and echocardiographic examination, cardiac catheterization, and coronary angiography. HF patients were classified into functional groups according to the New York Heart Association (NYHA) classification (Table 1).

Patients with a recent history (<6 months) of myocardial infarction, a history of hypertension, echocardiographic evidence of valve or congenital heart disease, or an inability to suspend ACE inhibitors and diuretic treatment for 3 days were not considered for the study. The control group was made up of 15 normotensive patients with atypical chest pain in whom angiography and routine diagnostic procedures did not reveal any abnormalities.

Cardiac specimens were obtained from 9 ICM and 13 DCM patients in NYHA classes III (n=10) and IV (n=12), who underwent cardiac transplantation, and 7 deceased donors with no history or signs of heart disease, whose hearts could not be transplanted because of surgical reasons or blood group incompatibility (NF hearts) (Table 1).

The protocol of this study complies with the principles of the Helsinki declaration. All patients gave their informed written consent to participate and to have their heart and blood samples used for the study. Echocardiographic and hemodynamic measurements were performed prospectively, as previously described.14

Cardiac formation of endogenous angiotensins was estimated as the aorta-coronary sinus gradient.14 Ang I and Ang II plasma concentrations were measured by RIA after HPLC separation as previously described.14 In 16 patients and 5 control subjects, the study of [¹²⁵I]-labeled Ang I ([¹²⁵I]-Ang I) kinetics was simultaneously performed.14

Cardiomyocytes were isolated and membrane suspension was prepared from a noninfarcted portion of left ventricle free wall.19 All
mRNA expression and peptide release by myocytes in vitro (100 000 nmol/L) on ET-1, IGF-I, and platelet-derived growth factor (PDGF) (Peninsula Laboratory, Inc). The stimulating effects of Ang II (10

chemical method, using anti-Ang II rabbit antiserum (RIN 7002, ence of Ang II in myocardium was detected with the immunohisto-

probes. 14 Myocardial cell types were identified by immunohisto-

performed using specific cDNA photobiotin-labeled (Vector) probes. 14 The in situ hybridization procedure was obtained in NF hearts. 14 The in situ hybridization procedure was performed on freshly isolated cells. Binding studies were performed at equilibrium using 125 I-Ang II (100 pmol/L, 2000 Ci/mmol, Amersham) and selective AT1, (Valsartan, a kind gift of Dr V. Abbruscato, Novartis, Italy) or AT2 (PD-123319) antagonists. mRNAs for renin, chymase, angiotensinogen (AGTN), ACE, AT1, and AT2 were quantified by reverse transcriptase–polymerase chain reaction (RT-PCR) using specific primers with GAPDH as internal standard14 and were expressed as the percentage of the values obtained in NF hearts. 14 The in situ hybridization procedure was performed using specific cDNA photobiotin-labeled (Vector) probes. 14 Myocardial cell types were identified by immunohistochemical methods using specific monoclonal antibodies. The pres-

cence of Ang II in myocardium was detected with the immunohisto-

chemical method, using anti–Ang II rabbit antisera (RIN 7002, Peninsula Laboratory, Inc). The stimulating effects of Ang II (10 nmol/L) on ET-1, IGF-I, and platelet-derived growth factor (PDGF) mRNA expression and peptide release by myocytes in vitro (100 000

myocytes/mL) were investigated in myocytes isolated from NF (n=4) and HF hearts (5 DCM and 4 ICM).

Data are mean±SD. Comparisons between groups were per-
formed using 1-way ANOVA. For multivariate reevaluation of univariate correlations, considering cardiac Ang II formation as a dependent variable: left ventricular end-diastolic diameter index, relative wall thickness, left ventricular mass index, left ventricular end-systolic volume index (LVESVI), left ventricular end-diastolic volume index (LVEDVI), left ventricular ejection fraction (LVEF), mean midwall velocity of circumferen-
tial fiber shortening (Vcf), end-systolic stress (ESS), end-diastolic stress, left ventricular end-diastolic pressure, Vcf/ESS ratio, Vcf/ 

LVEDVI ratio, ESS/LVESVI ratio, and plasma renin activity (PRA). An expanded Materials and Methods section can be found in an online data supplement available at http://www.circresaha.org.

Results

Cardiac Ang II Formation and Ventricular Function

Cardiac production of Ang II, as expressed by the mean aorta-coronary sinus concentration gradient, was already mildly but significantly increased in NYHA class I patients and further increased with the worsening of the functional class, with no differences being found between DCM and ICM patients (Figure 1A).

The 125I-Ang I kinetics parameters are reported in Table 2. Neither extraction of both radiolabeled angiotensins nor the 125I-Ang I to 125I-Ang II conversion rate differed between patients and control subjects (Table 2). Both Ang I and Ang II formation by cardiac tissues were on average significantly (P<0.01) higher in patients with mild HF (NYHA classes I and II) than in control subjects and further increased in patients with severe HF (NYHA classes III and IV; P<0.01). The increased Ang I de novo formation in patients with mild HF was mainly attributable to cardiac tissues, given that Ang I formed by PRA during the transcardiac passage of blood was not significantly different from that of control subjects (Table 2). In patients with severe HF, the total amount of Ang I formed was further increased versus mild HF with an increase in the contribution also by PRA (Table 2). Cardiac Ang II forma-

![Figure 1. A, Ang II aorta–coronary sinus gradient indexed by coronary blood flow and cardiac mass (cardiac Ang II formation) in control subjects (●) and HF patients (○) indicates ICM; ○, DCM). Values under columns are mean±SD. *P<0.05 vs control subjects. B, Relationship between cardiac Ang II formation and ESS in HF patients.](http://circres.ahajournals.org/)

![Table 2. 125I-Ang I and 125I-Ang II Concentrations at Steady State During 125I-Ang I Infusion and Kinetics Parameters](http://circres.ahajournals.org/)
Ang II formation measured by $^{125}$I-Ang I kinetics was highly correlated with the aorta-coronary sinus gradient ($r=0.89$, $P<0.001$).

Ang II formation expressed by the aorta coronary sinus gradient was usually increased in patients with reduced LVEF, but it was found to be enhanced in a proportion of patients (18 of 76, 23%) with only mildly reduced LVEF ($\approx 40\%$), provided that ESS was increased. Indeed, univariate regression analysis showed that Ang II formation was negatively correlated with left ventricular ejection fraction (LVEF, $r=20.77$, $P<0.001$) and with the various indexes of ventricular contractility (Vcf, $r=-20.72$, $P<0.001$; Vcf/ESS, $r=-20.75$, $P<0.001$; Vcf/LVEDVI, $r=-20.72$, $P<0.001$; and ESS/LVESVI, $r=-0.49$, $P<0.001$). Conversely, it was positively correlated with ventricular systolic volume (LVESVI, $r=20.74$, $P<0.001$) and diastolic volume (LVEDVI, $r=20.62$, $P<0.001$). The multivariate stepwise analysis revealed that ESS was the most predictive independent variable for Ang II formation ($r=0.85$, $P<0.001$) (Table 3, Figure 1B).

### mRNA Expression of the Cardiac RAS Components

#### RT-PCR Assay
AGTN, ACE, and chymase genes were expressed in the NF hearts, whereas renin mRNA was not detectable (Figure 2). AGTN and ACE mRNA levels were higher in failing than in NF hearts, without any significant differences between ICM and DCM hearts (Figure 2). Conversely, chymase mRNA expression did not significantly differ in failing and NF hearts (Figure 2).

Messengers for AT$_1$ and AT$_2$ were expressed in all myocardial specimens. The AT$_1$ mRNA levels were lower in failing than in NF hearts ($P<0.01$) (Figure 2).

### Immunohistochemical Localization of Ang II
In NF hearts no immunostaining for Ang II was detected. In HF hearts immunostaining for Ang II was detected in both myocytes and interstitial cells (Figure 3).

### Ang II Binding in NF and Failing Hearts
The density, affinity, and relative proportion of AT$_1$ to AT$_2$ subtypes in membranes and isolated myocytes are reported in Table 4.

In membranes from NF hearts, the proportion of AT$_1$ to AT$_2$ subtypes was 62:38 (Table 4). Total Ang II receptor and AT$_1$ subtype densities were significantly reduced in failing hearts as compared with NF hearts, with no significant differences between ICM and DCM hearts (Table 4).

### Table 3. Univariate and Stepwise Multiple Regression Between Ang II Cardiac Formation and Hemodynamic and Echocardiographic Parameters

<table>
<thead>
<tr>
<th></th>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESS, kdyn/cm$^2$</td>
<td>0.85$^*$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>-0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vcf/ESS</td>
<td>-0.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVESVI, mm/m$^2$</td>
<td>0.74</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vcf, s$^{-1}$</td>
<td>-0.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Relative wall thickness, %</td>
<td>-0.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vcf/LVEDVI</td>
<td>-0.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EDS, kdyn/cm$^2$</td>
<td>0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDDI, mm/m$^2$</td>
<td>0.65</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PRA, ng/mL × hour$^{-1}$</td>
<td>0.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDVI, mm/m$^2$</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI, g/m$^2$</td>
<td>0.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESS/LVESVI</td>
<td>-0.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>0.37</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Vcf indicates velocity of circumferential fiber shortening; LVEDDI, left ventricular end-diastolic diameter index; LVMI, left ventricular mass index; and LVEDP, left ventricular end-diastolic pressure.

*Independent variable at stepwise regression.
Myocytes isolated from the left ventricle of NF hearts showed a prevalence of the AT₁ subtype (AT₁:AT₂ = 79:21). AT₁ and AT₂ density and affinity were unchanged in myocytes from failing hearts as compared with NF hearts (Table 4).

**Effects of Ang II Stimulation on PreproET-1 (ppET-1), IGF-I, and PDGF Synthesis**

Ventricular cardiomyocytes from NF hearts expressed very low levels of ppET-1, IGF-I, and PDGF mRNAs at quiescent state (Figure 4). Stimulation of myocytes with Ang II (10 nmol/L) did not change GAPDH gene expression (Figure 4) but induced a marked increase in ppET-1, IGF-I, PDGF-A, and PDGF-B mRNAs (Figure 4).

In cardiomyocytes from NF, ICM, or DCM hearts, Ang II failed to induce ppET-1 and IGF-I gene expression, whereas PDGF-B and PDGF-A gene expression was comparable among groups (Figure 4).

**TABLE 4. Characterization of Ang II Receptors on Cardiomyocytes and Cardiac Membranes**

<table>
<thead>
<tr>
<th></th>
<th>NF</th>
<th>ICM</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiomyocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bₘₐₓ total, fmol/mg</td>
<td>0.57±0.21</td>
<td>0.55±0.10</td>
<td>0.60±0.11</td>
</tr>
<tr>
<td>Kᵦ, nmol/L</td>
<td>0.19±0.08</td>
<td>0.21±0.06</td>
<td>0.23±0.10</td>
</tr>
<tr>
<td>AT₁:AT₂</td>
<td>79:21</td>
<td>76:24</td>
<td>73:27</td>
</tr>
<tr>
<td>Bₘₐₓ AT₁, fmol/mg</td>
<td>0.45±0.17</td>
<td>0.41±0.07</td>
<td>0.44±0.08</td>
</tr>
<tr>
<td>Bₘₐₓ AT₂, fmol/mg</td>
<td>0.12±0.04</td>
<td>0.14±0.04</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td><strong>Cardiac membranes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bₘₐₓ total, fmol/mg</td>
<td>5.1±0.7</td>
<td>3.6±1.1*</td>
<td>3.4±0.9*</td>
</tr>
<tr>
<td>Kᵦ, nmol/L</td>
<td>0.27±0.08</td>
<td>0.26±0.05</td>
<td>0.25±0.6</td>
</tr>
<tr>
<td>AT₁:AT₂</td>
<td>62:38</td>
<td>58:42</td>
<td>47:53</td>
</tr>
<tr>
<td>Bₘₐₓ AT₁, fmol/mg</td>
<td>3.1±0.5</td>
<td>2.1±0.5*</td>
<td>1.6±0.5*</td>
</tr>
<tr>
<td>Bₘₐₓ AT₂, fmol/mg</td>
<td>1.9±0.4</td>
<td>1.5±0.5</td>
<td>1.8±0.4</td>
</tr>
</tbody>
</table>

*P<0.05 vs control subjects.

The assay of big ET-1, IGF-I, and PDGF-AB in the conditioned media from stimulated cardiomyocytes confirmed at the peptide level the selective impairment of ET-1 and IGF-I response to Ang II stimulation (Figure 4).

**Discussion**

The major findings of this study may be summarized as follows: (1) the progression of HF is associated with a progressive increase in cardiac Ang II formation, regardless of the etiology of the HF and with a strong correlation with the increasing ESS; (2) the density and relative proportion of AT₁ and AT₂ on isolated HF myocytes are not significantly different from those on NF myocytes; and (3) Ang II stimulation does not induce HF myocytes to synthesize ET-1 and IGF-I.

**Increased Ang II Formation and Cardiac Function**

Although the gene overexpression of ACE in dilated cardiomyopathy was previously reported, the finding of a high aorta–coronary sinus concentration gradient of both endogenous angiotensins and the kinetics study of ¹²⁵I-Ang I in the present work demonstrate an increased cardiac Ang II formation in HF. Upregulation of ACE and AGTN mRNAs and the immunohistochemical demonstration of Ang II in failing hearts corroborate this finding. It is worth stressing that the increased Ang II generation is independent of the HF etiology. In failing hearts, Ang II was detectable at immunohistochemistry both in myocytes and interstitial cells, whereas AGTN and ACE mRNAs were expressed predominantly in nonmyocytes. These findings may not be conflictual because in situ hybridization may be unable to reveal a low increase in mRNA expression and Ang II is uptaken by AT₁ and via endocytosis rapidly accumulated in myocytes. The enhanced Ang II generation is mainly due to the de novo Ang I formation by cardiac tissues, despite the fact that RT-PCR did not reveal any expression of renin mRNA in either the NF
or the failing hearts (Figure 2). However, the presence of renin mRNA and its overexpression in cardiac tissues from HF patients is not indispensable for Ang I formation, because there is wide evidence that renin may be uptaken from plasma by various renin binding proteins and bound to subendothelium and endothelial cells. There is also evidence that cardiac-bound renin may use the plasma AGTN present in cardiac extracellular fluid in addition to locally synthesized cardiac-bound renin to generate Ang II.6 Therefore, the finding of increased cardiac Ang I does not in any way contradict the finding that renin mRNA was not detectable in the HF hearts.

ACE mRNA levels in myocardium from failing hearts were higher than in NF hearts. In contrast, mRNA for chymase, a chymostatin-sensitive serine-proteinase, was expressed only in traces, without any significant differences between NF and failing hearts. Thus, the present results suggest that in failing hearts Ang II generation mainly occurs through an ACE-dependent pathway.

Cardiac renin and plasma renin have been found to be in a diffusional steady state in rats, and a positive correlation has been reported between renin plasma levels and the renin concentration in end-stage hearts from patients with DCM.6 In this study as well, cardiac Ang II formation was correlated with PRA values in patients with severe HF (NYHA classes III and IV; $r=0.47, P<0.001$) but not in those with mild HF (NYHA classes I and II; $r=0.32, NS$), the majority of whom had increased Ang II formation by cardiac tissues, although the PRA did not differ from that of control subjects. Likewise, multiple-regression analysis did not select PRA as a predictive independent variable for Ang II formation. These findings suggest that local cardiac factor(s) are preeminent in the activation of cardiac RAS in HF, even if important elevation in PRA contributes to the cardiac Ang II formation. Independently of the adjunctive role of PRA, the cardiac generation of both angiotensins progressively increases in relation with the impairment of cardiac function. The stepwise regression analysis revealed the ESS as the only variable independently correlated with Ang II formation ($r=0.85$). That the increase in ESS may play a major role in the upregulation of cardiac RAS is also supported by several studies that have shown that stretch induces Ang II formation both by isolated myocytes and by beating hearts. In patients with cardiac hypotrophy due to aortic valve disease, cardiac Ang II formation was again enhanced only in the patients with high ESS (>90 kdyn/cm²), regardless of the type of overload (pressure or volume). The close relationship between increased cardiac Ang II formation and high ESS values is not surprising, because ESS is a sensitive indicator of decapsulation of dilated cardiomyopathy. Thus, clinical and experimental results suggest that ESS is a causative factor for the increase in Ang II formation in human hearts, probably stimulating the upregulation of both ACE and AGTN genes and facilitating the diffusion of renin from plasma to cardiac tissues. Cardiac Ang II production was negatively correlated with myocardial contractility as expressed not only by Vcf but also by the ESS/LVESVI ratio, which is a sensitive contractility index independent of ventricular size. However, ESS was the strongest predictor of Ang II production, thus indicating that a mechanical factor rather than the loss of contractility affects cardiac Ang II production.

**Myocyte and Membrane Receptor Binding Sites**

Although the presence of AT1 was demonstrated using in situ RT-PCR in both myocytes and interstitial cells, the density of Ang II binding sites both in failing and NF hearts has been studied only on cardiac homogenates. The results indicate a general tendency of AT1 to decrease in failing hearts, with no changes in AT2. In contrast with these studies, Tsutsumi et al reported that AT1 binding sites and mRNA expression were increased in patients with dilated cardiomyopathy. In the present study, binding sites for Ang II receptors were separately investigated in isolated myocytes and in myocardial membranes. The results showed notable differences between myocytes and membranes from NF hearts in the distribution and relative proportion of the Ang II receptor subtypes. Ang II binding sites on myocytes from NF hearts were ~10% of the binding sites detected on ventricular membranes, with a clear predominance of AT1, which were ~4 times more numerous than AT2. Most importantly, whereas binding site density for AT1 was lower on heart membranes from failing than from NF hearts, the density of both AT1 and AT2 on isolated myocytes from HF hearts did
not significantly differ from that of NF hearts. Thus, for the first time we demonstrated that AT₁ density is decreased on cardiac membranes but not on myocytes from failing hearts, and the myocytes may therefore remain in end-stage failing hearts as potential targets for Ang II–mediated effects. The downregulation of Ang II receptors on nonmyocyte cells appears to be related to the HF and not to a disease-specific mechanism, because no differences between DCM and ICM hearts were found in the receptor density on either myocytes or membranes. Thus, the pattern of Ang II receptors does differ from that of ET-1 binding sites, which were increased in ICM hearts but not in DCM ones, with a proportional increase in ET₁ and ET₂ receptors both on myocytes and membranes.¹⁹

The mechanisms responsible for the lack of downregulation of AT₁ on myocytes in comparison with nonmyocyte cells, notwithstanding the increased Ang II, have not yet been clarified. Mechanical stretching increases AGTN mRNA levels and upregulates the number of AT₁ s and AT₂ s in neonatal cultured cardiac myocytes,²⁸–³⁰ and this effect is potentiated by Ang II.²⁹ Moreover, Ang II levels were found to be directly related to AT₁ promoter activity in pressure-overloaded myocytes.³¹ The combination of stretching and Ang II might thus keep the receptor density on failing heart myocytes similar to that of control subjects; conversely, the lower stretching effect on the nonmyocyte cells in comparison with myocytes might result in a predominance of the downregulating activity by the enhanced Ang II. Another possibility is that the different regulation of AT₁ and AT₂ may depend on differences in the regulation mechanism(s). Indeed, a different molecular mechanism has been found to regulate Ang II receptor subtype expression in the rat heart in a cell- and subtype-specific manner.³² A similar mechanism might differently regulate the gene expression of AT₁ subtype on myocytes and on nonmyocyte cells. However, specifically addressed studies are needed to investigate the different expression of Ang II receptor subtypes in the failing human heart.

Incacity of the Failing Myocytes to Synthesize IGF-I and ET-1 and Role of Ang II in HF

The third major result in this study is the demonstration that Ang II induces IGF-I, ET-1, and PDGF-AB synthesis by human isolated NF myocytes and that failing myocytes are selectively unable to produce appreciable amounts of IGF-I and ET-1 in response to Ang II stimulation, notwithstanding the similar density and binding capacity of Ang II receptor subtypes. The preserved PDGF-AB generation by failing myocytes after Ang II stimulation suggests a specific impairment of the pathways leading to IGF-I and ET-1 formation rather than a general aspecific desensitization of the Ang II receptors. The mechanisms responsible for the incapacity of failing myocytes to produce IGF-I and ET-1 after Ang II stimulation remain to be investigated. Multiple intracellular signal transduction pathways or in transcription factors. Specific studies are, however, necessary to investigate this problem.

Cardiac IGF-I and ET-1 play a critical role in supporting cardiac adaptive response to hemodynamic overload. Human compensatory hypertrophy due to aortic valve disease is associated with an increased cardiac myocyte formation of IGF-I in volume overload and IGF-I and ET-1 in pressure overload.¹⁴ Cardiac production of these growth factors is positively related to myocardial contractility.¹⁴ The depression of contractility, the increase in ESS, and the progression toward noncompensatory hypertrophy and HF are associated with the decrease in IGF-I and ET-1 myocyte formation and with the increase in Ang II generation.¹⁴ In experimental pressure or volume overload in pigs, aortic banding or the creation of an aorta-cava shunt are immediately followed by an increased cardiac Ang II formation (within 3 hours), leading to enhanced ET-1 and IGF-I generation by myocytes with recovery of contractility, ESS normalization, and return of Ang II formation to resting values within 12 hours.³⁴ It is important to highlight that Ang II, ET-1, and IGF-I are synthesized by myocytes, in contrast with the incapacity of human overloaded¹⁴ or failing myocytes to synthesize not only ET-1 and IGF-I, but also Ang II itself, which appears to be essentially formed by nonmyocyte cells (Reference 14 and the present study). Thus, the increased cardiac Ang II formation triggered by the increase in the ESS operates as an inducing factor for the formation of IGF-I and ET-1 (and perhaps other growth factors) by myocytes. In noncompensatory hypertrophy and HF, myocytes are unable to respond to the stimulating activity of Ang II. Because AT₁ s on myocytes are not downregulated in failing hearts, the long-lasting excessive Ang II formation can mediate detrimental effects on overloaded or failing myocytes, including depression of contractility or impaired relaxation.³⁵ AT₁ s also remain well represented on fibroblasts from failing hearts with consequent interstitial extracellular matrix accumulation.¹¹ Moreover, experimental evidence suggests that local Ang II through AT₁ activates the transcription factor nuclear factor-kB,³⁷ thereby promoting overexpression of numerous genes, including various cytokines and adhesion molecules.

In conclusion, the present study demonstrates that early in the clinical course of HF, cardiac Ang II formation is increased, myocyte AT₁ s are not downregulated, and myocytes are unable to synthesize IGF-I and ET-1 in response to Ang II stimulation.

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