Angiotensin I and II Exert Inotropic Effects in Atrial But Not in Ventricular Human Myocardium
An In Vitro Study Under Physiological Experimental Conditions

Christian Holubarsch, MD; Gerd Hasenfuss, MD; Stephan Schmidt-Schweda, MD; Anke Knorr, MD; Burkert Pieske, MD; Thorsten Ruf, MD; Roland Fasol, MD; Hanjörg Just, MD

Background. The renin-angiotensin system with its renal-humoral and local myocardial components plays an important role in the development and progression of chronic heart failure. Whereas angiotensin receptors have been found in atrial and ventricular myocardium of different species including humans, its influence on myocardial contractility is not yet defined in human failing myocardium and especially in human nonfailing myocardium.

Methods and Results. We measured force development of right atrial and right and left ventricular myocardial preparations of patients with a variety of cardiac diseases. To evaluate the physiological effects of angiotensin, experimental temperature and stimulation rates were 37°C and 60 beats per minute, respectively. Angiotensin I and II increased peak developed force in atrial myocardial preparations obtained from patients without heart failure in a concentration-dependent manner. At optimal concentrations, peak developed force is increased from 10.2±1.8 to 12.3±1.9 mN/mm² by angiotensin I (P<.05) and from 15.4±2.1 to 20.5±3.3 mN/mm² by angiotensin II (P<.05). This effect was not influenced by pretreatment with propranolol (10⁻⁶ mol/L) and prazosin (10⁻⁷ mol/L) but was completely blocked by saralasin (10⁻⁸ mol/L). The positive inotropic effect of angiotensin I could be blocked by enalaprilate (10⁻⁵ mol/L). Neither angiotensin I nor angiotensin II had any effect in preparations of the left ventricle from patients with idiopathic dilated cardiomyopathy, mitral valve stenosis, and incompetence or in patients with no significant heart disease. Additionally, no effect could be seen when angiotensin II was applied to right ventricular preparations from infants undergoing reconstructive heart surgery for tetralogy of Fallot.

Conclusions. Angiotensin I and II exert positive inotropic effects via angiotensin receptors in atrial preparations but not in right or left ventricular preparations. Furthermore, the existence of a local myocardial angiotensin converting enzyme with functional importance is shown. (Circulation. 1993;88:1228-1237.)

Key Words • angiotensin • myocardium • inotropism • angiotensin converting enzyme

For three different reasons, the renin-angiotensin system has attracted much interest in cardiovascular physiology and pathophysiology during the last few years: (1) There is now clear evidence for the existence of angiotensin II receptors in cardiac tissues1-2 as well as for a localized cardiac renin-angiotensin system that is complete with respect to its different constituents and acts independently of the endocrine-humoral renin-angiotensin system.3,4 (2) Besides its potential role in short-term regulation of myocardial function, the renin-angiotensin system may play an important role as a growth factor by promoting the development of cardiac hypertrophy5-8 and especially interstitial fibrosis.9,10 (3) In a recently published clinical trial, it was demonstrated that angiotensin converting enzyme inhibition is superior to combined venous and arteriolar vasodilation in the treatment of chronic heart failure with respect to mortality.11 Therefore, the renin-angiotensin system must exert direct effects on cardiac muscle in addition to its influence on the peripheral vasculature.

Although angiotensin II receptors are very well characterized in avian12 and mammalian myocardium13-16 including human failing and nonfailing myocardium,2 the functional role of the angiotensin II receptors remains controversial: Whereas angiotensin II has been shown to exert positive inotropic effects in a variety of species,12,15-20 the existing angiotensin II receptors are not necessarily coupled to the regulation of myocardial con-
tractility, because no inotropic effect of angiotensin II was found in guinea pig myocardium; negative inotropic effects of angiotensin II were observed in cultured neonatal rat cardiomyocytes and different results were obtained in dog myocardium. Furthermore, only one study is available that deals with the inotropic effect of angiotensin in human preparations. Additionally, at present no data are available that are obtained from normal or nonfailing left ventricular human myocardium.

The question of whether human failing and nonfailing myocardia of atria and ventricles responds to angiotensin with an increase in contractility may be of some clinical importance: Angiotensin converting enzyme inhibition might prevent or abolish positive inotropism of the angiotensin II. Therefore, we investigated the effects of angiotensin I and II in human myocardium obtained from right atria and from failing and nonfailing left ventricles of adult patients. Because of the higher number of angiotensin II receptors in myocardium from young individuals, right ventricular preparations from infants with tetralogy of Fallot were also studied. Because positive inotropic effects are temperature and heart rate dependent, all experiments were conducted at physiological conditions (37°C experimental temperature; 60 beats per minute stimulation rate).

The findings were unexpected in as far as atrial preparations showed constant effects of angiotensin on isometric force development, whereas a positive inotropic response was not observed in the preparations from failing and nonfailing left ventricles. The results are of clinical relevance because they support the view that angiotensin converting enzyme inhibition reduces force development of the atria and thereby filling pressure of the ventricles in chronic heart failure but leaves contractility of the left ventricle unaltered.

Methods

Human Cardiac Tissues

Myocardium from human hearts in the present study was obtained from four different sources. First, small pieces of the right atrium were received from 44 patients (38 men, 6 women; 40 to 78 years old) undergoing routine aortocoronary bypass surgery because of two- or three-vessel disease without manifestation of congestive heart failure. Mean left ventricular ejection fraction was 57±4.0%, and all patients were in sinus rhythm. During transportation, the cardiac tissue was stored in Krebs-Ringer solution that contained 30 mmol/L 2,3-butanedione monoxime (BDM, see below) at room temperature. The solution was constantly bubbled with a gas mixture of 95% O2 and 5% CO2. The maximum time that passed between excision of the tissue and the end of preparation was 30 minutes. From each specimen up to four myocardial strips were prepared. Second, parts of the left ventricular papillary muscle were taken from patients undergoing routine mitral valve replacement because of mitral valve stenosis or mitral valve regurgitation. Four of these patients had mitral valve stenosis (two men, two women) and two had mitral valve regurgitation (one woman, one man). The time for transportation and preparation was 30 minutes. These six patients had a mean age of 63 years, and their left ventricular ejection fraction was 53±7%. Third, myocardium from the left ventricle was obtained from 9 explanted hearts of patients with dilated cardiomyopathy being in endstage heart failure. The mean age was 57, and all of the patients were in New York Heart Association class III or IV. Left ventricular ejection fraction was 16±1%. All of the patients were treated with digitalis (digoxin or digitoxin) and angiotensin converting enzyme inhibitors (captopril, enalapril). Three patients had intravenous low-dose dopamine. Also, one normal donor heart was received that could not be used for transplantation for technical reasons. After explantation, papillary muscle and parts of the left ventricular wall were dissected and immediately submerged into Krebs-Ringer solution at room temperature containing 30 mmol/L BDM. Transportation plus preparation time was 30 minutes in four preparations, 1 hour in two preparations, and 4 hours for all other cases. Fourth, small pieces of the right ventricle of four infants were received. The age of the children was between 8 and 42 months: these children underwent reconstructive heart surgery for tetralogy of Fallot. Right ventricular muscle tissue was immediately submerged into room-temperature, BDM-containing Krebs-Ringer solution, which was bubbled with a 95% O2 and 5% CO2 gas mixture. The time of the myocardium being in BDM-containing solution was 2 hours and 30 minutes for all cases.

To exclude the possibility that the time during which the myocardium was kept in BDM-containing solution had any significant influence on the functional integrity of angiotensin II receptors and on the viability of these preparations, additional animal studies were performed. Because the hamster myocardium is especially sensitive to angiotensin II, this species was chosen. Ten Syrian hamsters (strain Han: AURA) weighing between 100 and 140 g were anesthetized and killed. In six preparations, experiments were performed immediately after cardiotomy so that the time in BDM-containing solution was less than 30 minutes. In the other eight preparations, experiments were carried out after an exposure to BDM-containing solution for a period of 6 hours. In the first group, peak developed isometric tension was 7.1±0.9 mN/mm2 and in the second group, 6.9±0.7 mN/mm2 (NS). These preparations were exposed to increasing concentrations of angiotensin II (Fig 1). Between the two groups, no significant differences could be found regarding the dose-response curves. At 10−6 mol/L angiotensin II, isometric tension increased to 226±19% in the first group (30 minutes in BDM) and to 204±7% in the second group (6 hours in BDM). The responsiveness of hamster myocardium to angiotensin II was not significantly different between the two groups. From these data, two conclusions can be drawn: First, the function of the angiotensin II receptors appear to be independent on BDM pretreatment and on the time of the treatment. Second, the data demonstrated unchanged viability of these preparations that were exposed to BDM over a period of 6 hours.

Solutions, Instruments, and Study Protocol

The solution used in this study contained (mmol/L): Na+ 152, K+ 3.6, Cl− 135, HCO3− 25, Mg2+ 0.6, HPO42− 1.3, SO42− 0.6, Ca2+ 2.5, glucose 11.2, insulin 10 IU/L. This solution was constantly bubbled with a gas mixture of 5% CO2 and 95% O2. Solutions that were used for transportation and dissection purposes additionally con-
two parallel platinum electrodes being located on both sides of the muscle and connected to a stimulation unit (Hugo Sachs Elektronik Type 215/I). Stimulation duration was 5 milliseconds, and voltage was set to 25% above threshold. Threshold voltage ranged between 5 and 8 V. Force was measured by force transducer F30 type 372 (Hugo Sachs Elektronik, resonance frequency 450 Hz) and recorded on a Linearcorder Mark VII Graphotec. After steady-state conditions were observed at short length of muscle, the preparations were carefully stretched to Lmax, the optimum length at which maximum force is developed, by 0.10- and 0.05-mm stretches. Bath temperature was regulated to 37°C by means of an electronic feedback system.

In 50% of all experiments, the quality of the preparation was tested at the end of the experimental protocol according to Paradise et al.28 Developed tension never decreased more than 10% in any of the preparations when the Krebs-Ringer solution was bubbled with 15% N2/5% CO2/80% O2. This suggests that no hypoxia was present in the preparations under the experimental conditions used.

Microscopic Investigations

When experiments were performed in preparations after mechanical removal of the endocardium, routine histological investigations were performed at the end of the protocol. In all preparations, complete removal of the endocardium was shown.

Materials and Concentrations

Materials and concentrations used were 2,3-butanedi-one-monoxime (Sigma Chemical Co), 30 mmol/L; angiotensin I (Sigma), 10^-5, 10^-4, 10^-3, 10^-2 mol/L; angiotensin II (Sigma), 10^-3, 10^-2, 10^-1, 10^-0 mol/L; prazosin (Sigma), 10^-5 mol/L; enalaprilate (Merck Sharp & Dohme Research Laboratories), 10^-3 mol/L; and (Sar1, Val5, Ala8)-angiotensin II (Sigma), 10^-2 mol/L.

Statistics

Values are given as mean±SEM in text and tables. In the figures, values were normalized for baseline conditions; mean±SE is indicated. When comparisons were made within one group of experiments with repeated measurements, the paired t test was applied using the Bonferroni-Holm procedure.29,30 Comparison between different groups of experiments was accomplished using ANOVA and the Student-Newman-Keuls test29,30

Results

Myocardial Preparations From Right Atria

In this study, 75 trabecular preparations from right atria obtained from 49 patients were investigated. Average cross-sectional area, average muscle length, and average peak twitch tension under control conditions were 0.48±0.052 mm², 3.4±0.22 mm, and 12.7±2.1 mN/mm², respectively (Table 1). In each of the investigated preparations, there was a significant positive inotropic effect on force development after angiotensin I and II application except after pretreatment with saralasin and enalaprilate, respectively (see below). After application of angiotensin II, there was a trend toward positive inotropic effects at a concentration of

![Graph showing dose-response curves for angiotensin II in left ventricular preparations of hamsters. In one group, preparations and experiments were performed immediately after cardiomyectomy. The hamster myocardium responded to angiotensin II (10^-5 mol/L) with an increase in peak twitch tension from 100% to 226±19% (n=6). In the second group, preparations were stored in BDM-containing solution at room temperature over a period of 6 hours before preparation and experimental protocol (n=8). The dose-response curves for angiotensin II are not significantly different between the two groups. I indicates initial values; P, propranolol; •—•, immediately performed experiments; ×—×, 6 hours in BDM-containing solution.

% Force

log M Angiotensin II

1 2 3 4 5 6

100 150 200 250
TABLE 1. Atrial Preparations

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>EF (%)</th>
<th>CS (mm²)</th>
<th>ML (mm)</th>
<th>PTT (mN/mm²)</th>
<th>Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT II</td>
<td>10</td>
<td>61±5</td>
<td>0.46±0.09</td>
<td>3.4±0.4</td>
<td>15.4±2.1</td>
<td>5</td>
</tr>
<tr>
<td>AT II 6h BDM</td>
<td>7</td>
<td>62±5</td>
<td>0.37±0.04</td>
<td>3.0±0.2</td>
<td>13.6±3.0</td>
<td>4</td>
</tr>
<tr>
<td>AT I</td>
<td>10</td>
<td>56±4</td>
<td>0.60±0.06</td>
<td>3.2±0.2</td>
<td>10.2±1.7</td>
<td>7</td>
</tr>
<tr>
<td>AT II PP</td>
<td>10</td>
<td>55±3</td>
<td>0.49±0.08</td>
<td>3.8±0.2</td>
<td>12.9±3.5</td>
<td>5</td>
</tr>
<tr>
<td>AT II SARA</td>
<td>8</td>
<td>63±3</td>
<td>0.46±0.06</td>
<td>3.5±0.4</td>
<td>11.8±2.6</td>
<td>6</td>
</tr>
<tr>
<td>AT I ENA</td>
<td>10</td>
<td>55±5</td>
<td>0.42±0.05</td>
<td>3.4±0.2</td>
<td>16.9±1.4</td>
<td>6</td>
</tr>
<tr>
<td>AT I SARA</td>
<td>10</td>
<td>56±4</td>
<td>0.57±0.07</td>
<td>3.6±0.2</td>
<td>12.0±1.1</td>
<td>6</td>
</tr>
<tr>
<td>AT I ER</td>
<td>11</td>
<td>52±3</td>
<td>0.47±0.06</td>
<td>3.0±0.2</td>
<td>8.9±1.3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>49</td>
</tr>
</tbody>
</table>

Mean±SEM

EF indicates ejection fraction; CS, cross-sectional area; ML, muscle length; PTT, peak twitch tension; AT, angiotensin; AT II 6h BDM, dose-response curve in 7 preparations exposed for a period of 6 hours before preparation and experiment; AT II PP, dose-response curve after application of propranolol and prazosin; AT II SARA, dose-response curve after application of saralasin; AT I ENA, dose-response curve after application of enalaprilat; AT I ER, endocardium mechanically removed.

10⁻⁹ mol/L and were significant effects at 10⁻⁸ mol/L (Figs 2 and 3). Systolic force increased maximally from 15.4±2.1 to 20.5±3.3 mN/mm² at 10⁻⁷ mol/L angiotensin II. About the same increments of force development were seen with angiotensin I (from 10.2±1.6 to 12.3±1.9 mN/mm² at 10⁻⁶ mol/L) except at 10-fold-higher concentrations (Figs 2 and 3).

To study the question of whether the increase in force development was mediated by a facilitated release of norepinephrine and consecutive stimulation of α- or β-adrenoceptors, experiments were conducted under α- and β-adrenoceptor blockade. Propranolol (10⁻⁶ mol/L) and prazosin (10⁻⁷ mol/L) were applied before addition of angiotensin II. This treatment decreased myocardial peak force development from 14.5±3.6 to 12.9±3.5 mN/mm². When angiotensin II was applied, force increased to 17.2±4.7 mN/mm² (P<.01, Fig 4). To study the specificity of angiotensin II effect, preparations were pretreated with 10⁻⁵ mol/L saralasin before addition of angiotensin II. Under these conditions, the angiotensin II effect was completely eliminated (Fig 4).

The effect of angiotensin I could be completely blocked by pretreatment of either saralasin (10⁻⁵ mol/L) or enalaprilat (10⁻⁶ mol/L) (Fig 5). These experiments demonstrate that angiotensin I is converted to angiotensin II in vitro, which acts via angiotensin II receptors in a positive inotropic way.

We also studied the responsiveness of atrial myocardium to angiotensin I after mechanical removal of the endocardium. This procedure had no effect on the quantity and quality of the influence of angiotensin I on myocardial force of atrial preparations (from 8.9±1.3 to 11.8±1.5 mN/mm² at 10⁻⁵ mol/L; P<.05; Table 1 and

FIG 2. Typical inotropic response of atrial human preparations to angiotensin II (10⁻⁷ mol/L, lower panel) and angiotensin I (10⁻⁶ mol/L, upper panel). The preparations contracted isometrically, stimulation rate was 60 beats per minute, and experimental temperature was 37°C.

FIG 3. Dose-response curves for angiotensin I and II in atrial human preparations. At maximum concentrations, both angiotensins are equally effective with respect to myocardial stimulation; however, the dose-response curve for angiotensin I is shifted to higher concentration by a factor of 10 when compared with angiotensin II. ■ Indicates angiotensin II; ○, angiotensin I; I, initial values. *P<.05 compared with initial values.
tetralogy of Fallot. Again, no inotropic response of angiotensin II was observed in any of the investigated preparations, whereas all of these preparations responded to isoproterenol (Table 4).

Discussion

Background of the Study

Three important mechanisms are well known to regulate myocardial contractility: (1) According to Starling,31 preload defines maximum developed force. (2) Force development depends on heart rate32: In the nonfailing human myocardium, force development increases with higher heart rates.33-35 These two mechanisms are inherent to the myocardium and present a kind of autoregulation of the heart as a pump. (3) In addition, the sympathetic nervous system modulates myocardial contractility via release of catecholamines, which activate β1- and β2-adrenoceptors.36 The latter system displays a feedback control system between peripheral circulation and the pump and couples myocardial function to the central nervous system.

Under certain circumstances, a fourth system may come into play and interact with the mentioned mechanisms, the renin-angiotensin system. The renin-angiotensin system is activated under pathological conditions, ie, loss of salt and water and in bleeding and cardiogenic shock, and plays a fundamental role in the development and progression of chronic heart failure.3,37,38 Although the importance of the renin-angiotensin system in heart failure patients is now very well recognized and the benefit of angiotensin converting enzyme inhibitors has been shown and led to a worldwide use of these compounds, the effect of angiotensin on myocardial function is still discussed controversially. The positive inotropic effect of angiotensin II seems to be differently pronounced between species. Koch-Weser was the first to demonstrate clear positive inotropic effects in ventricular myocardium.17,18 In contrast, angiotensin exerted only small effects in atrial preparations of cats. Furthermore, the quantity of the positive inotropic effect was shown to depend on physical factors, ie, heart rate, experimental temperature, and calcium concentration.18 Positive inotropic effects of angiotensin II were also observed in the myocardiun of rabbits, calves, chickens, and hamsters.25 In contrast, findings in canine hearts were different: Only in three out of five preparations, slight positive inotropic responses were observed.24 Furthermore, in guinea pig myocardium, no positive inotropic effect was found at all, although angiotensin II receptors as well as activation of the inositol phosphate system were shown to be present in these preparations.13 Even negative inotropic effects of angiotensin II were observed in cultured neonatal rat cardiomyocytes.23 Therefore, angiotensin II receptors might exist in the myocardium without coupling to mechanical function, and experimental physical factors might additionally modify the quantity of such an inotropic effect. Recently, positive inotropic responses were reported in myocardial preparations from human atrial and left ventricular myocardium.25 However, these responses were inconsistent: 50% of the atrial preparations and only 33% of the ventricular preparations responded with significant increases in force development. No report is available on normal or nonfailing

Myocardial Preparations From Left Ventricles

Altogether, 32 left ventricular myocardial preparations obtained from 16 individuals were studied. On the average, muscle cross-sectional area, muscle length, and peak twitch tension were 0.63±0.11 mm², 4.6±0.2 mm, and 7.5±0.9 mN/mm², respectively. Neither angiotensin I nor angiotensin II showed significant effects in any of the preparations (Tables 2 and 3). Additional treatment with isoproterenol at a concentration of 10⁻⁷ mol/L increased development of force significantly by 58.9±9.4%, indicating sufficient contractile force reserve (Table 2). Additional treatment with saralasin at a concentration of 10⁻⁵ mol/L had no significant effect.

When preparations are separated into those obtained from failing left ventricles (dilated cardiomyopathy), from left ventricles with mitral valve stenosis, or incompetence and from the normal donor heart, no significant effects of angiotensin II could be evaluated (Table 2).

Myocardial Preparations From Right Ventricles of Infants

In addition to the preparations obtained from ventricles of adult patients, we investigated 10 preparations that were obtained during heart surgery in four infants (ages between 8 months and 5 years) suffering from
myocardium of the left ventricle. Data on normal left ventricular myocardium, however, are important because uncoupling between the angiotensin II receptors and myocardial function may have occurred because of heart failure.

We therefore studied the positive inotropic effects of angiotensin I and angiotensin II on failing left and right ventricular human myocardium as well as on nonfailing atrial and left ventricular human myocardium. Because it is known that experimental physical factors have a strong influence on the myocardial responsiveness to angiotensin, we conducted all experiments at a physiological temperature of 37°C and a physiological heart rate of 60 beats per minute, whereas calcium concentration was chosen to be 2.5 mmol/L. Therefore, our data more closely simulate in vivo conditions.

**Right Atrial Myocardium**

The results obtained in the present study were unexpected in several ways. In atrial preparations, angiotensin I and II gave very consistent results: In all of the muscles except of those pretreated with enalaprilate or saralasin, positive inotropic responses were recorded. On the average, force increased by 32% with angiotensin II and by 27% with angiotensin I (Figs 2 and 3). These data are in contrast to the findings of Moravec et al.25 in two different aspects. First, Moravec et al measured much higher maximum response to angiotensin in those preparations that were responders to angiotensin. This discrepancy may be explained by the different experimental conditions used in the two studies. As shown by Koch-Weser,18 increase in force development caused by application of angiotensin is much smaller at 37°C than at lower experimental temperature.25 Differences in heart rate may also explain why maximum response to angiotensin is only about 30% in this study and up to 100% in the other.25 Second and more important, Moravec et al25 had to separate between responders and nonresponders, whereas in the present study, all of the atrial preparations responded significantly to angiotensin by increases in developed force.

**TABLE 2. Left Ventricular Myocardium, Angiotensin II**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Diagnosis</th>
<th>EF (%)</th>
<th>Time (h)</th>
<th>Base</th>
<th>10⁻⁷ AT II</th>
<th>10⁻⁸ AT II</th>
<th>ISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>DCM</td>
<td>20</td>
<td>4.5</td>
<td>9.6</td>
<td>9.5</td>
<td>9.5</td>
<td>11.8</td>
</tr>
<tr>
<td>2</td>
<td>57</td>
<td>DCM</td>
<td>20</td>
<td>1</td>
<td>5.3</td>
<td>4.8</td>
<td>4.8</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>DCM</td>
<td>10</td>
<td>4.5</td>
<td>6.2</td>
<td>5.8</td>
<td>5.8</td>
<td>10.6</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>DCM</td>
<td>21</td>
<td>4.5</td>
<td>1.9</td>
<td>1.4</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>DCM</td>
<td>16</td>
<td>4.5</td>
<td>2.1</td>
<td>1.7</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>57</td>
<td>16</td>
<td>5.9</td>
<td>5.9</td>
<td>5.2</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>1.1</td>
<td></td>
<td>0.9</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>23</td>
<td>DH</td>
<td>*</td>
<td>0.5</td>
<td>6.7</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>MS</td>
<td>50</td>
<td>0.5</td>
<td>6.3</td>
<td>5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>69</td>
<td>MS</td>
<td>50</td>
<td>0.5</td>
<td>1.7</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>MS</td>
<td>70</td>
<td>0.5</td>
<td>7.8</td>
<td>7.2</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>72</td>
<td>MI/MS</td>
<td>60</td>
<td>0.5</td>
<td>12.8</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>72</td>
<td>MI</td>
<td>30</td>
<td>0.5</td>
<td>8.1</td>
<td>7.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>63</td>
<td>53</td>
<td>7.6</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>7</td>
<td></td>
<td>2</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>60</td>
<td>32</td>
<td>6.5</td>
<td>5.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>5</td>
<td></td>
<td>1.4</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EF indicates ejection fraction; Time, time in BDM-containing solution before start of experiment; Base, baseline value of peak developed tension before angiotensin II (AT II); ISO, peak twitch tension after exposure to 10⁻⁷ mol/L isoproterenol; DCM, dilated cardiomyopathy; DH, donor heart; MS, mitral valve stenosis; MI, mitral valve incompetence; *left ventricular function defined echocardiographically to be normal.
may be present in human myocardium obtained from patients with cardiac hypertrophy and/or failure. Moravec and coworkers obtained right atrial muscle tissue from a group of patients who were transplanted because of severe heart failure and another group of patients who underwent reconstructive heart surgery because of congenital heart disease including transposition of the great arteries, tetralogy of Fallot, atrial septal defects, and atroventricular septal defects. In both groups of patients, pressure and volume overload of the right atrium might have led to myocardial hypertrophy and even failure. In the present study, right atrial tissue was exclusively obtained from patients with coronary artery disease who had no signs of heart failure clinically or left ventricular dysfunction angiocardiographically. Therefore, in contrast to the study of Moravec et al., the present study normal right atrial myocardium was used exclusively, which may explain the constancy of the response to angiotensin among the individual preparations.

**Nature of the Positive Inotropic Effects of Angiotensin I and II**

To obtain more information about the molecular mechanisms of the positive inotropic effects of angiotensin I and II in atrial preparations, more experiments were performed. Inotropic effects of angiotensin may be mediated by facilitation of the release of endogenous catecholamines from their intramyocardial stores. To rule out such a mechanism, preparations were pretreated by the \( \alpha \)-blocker prazosin and the \( \beta \)-blocker propranolol. Neither pretreatment prevented the inotropic responses of atrial preparations to angiotensin I and II, either qualitatively or quantitatively (Fig 4). Therefore, the inotropic effect of angiotensin I and II appears to be directly mediated by angiotensin II receptors. This is shown by application of saralasin, which completely inhibited the inotropic effects of angiotensin I and II at all concentrations studied (Figs 4 and 5). On the other hand, pretreatment with enalaprilate prevents

**Table 3. Ventricular Preparations, Angiotensin I**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Diagnosis</th>
<th>EF (%)</th>
<th>Time (h)</th>
<th>Base</th>
<th>10(^{-7}) AT I</th>
<th>10(^{-6}) AT I</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>MS</td>
<td>65</td>
<td>0.5</td>
<td>15.1</td>
<td>7.9</td>
<td>6.6</td>
</tr>
<tr>
<td>2</td>
<td>53</td>
<td>DCM</td>
<td>11</td>
<td>4.5</td>
<td>8.8</td>
<td>6.5</td>
<td>6.2</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>DCM</td>
<td>23</td>
<td>4.5</td>
<td>6.6</td>
<td>5.2</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>DCM</td>
<td>25</td>
<td>0.5</td>
<td>4.5</td>
<td>5.8</td>
<td>5.4</td>
<td>5.0</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>DCM</td>
<td>21</td>
<td>4.5</td>
<td>2.1</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Mean</td>
<td>55</td>
<td></td>
<td>20</td>
<td></td>
<td>9.2</td>
<td>7.3</td>
<td>6.9</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>2.6</td>
<td>2.2</td>
<td>1.9</td>
</tr>
<tr>
<td>All</td>
<td>56</td>
<td></td>
<td>29</td>
<td></td>
<td>9.7</td>
<td>7.1</td>
<td>6.7</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
<td>2.1</td>
<td>1.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

EF indicates ejection fraction; Time, time in BDM-containing solution before start of experiment; Base, baseline value of peak developed tension before angiotensin I (AT I); MS, mitral valve stenosis; DCM, dilated cardiomyopathy.

**Table 4. Right Ventricular Myocardium From Infants**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (mo)</th>
<th>Time (h)</th>
<th>Base</th>
<th>10(^{-7}) AT II</th>
<th>10(^{-6}) AT II</th>
<th>ISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>2.5</td>
<td>3.5</td>
<td>3.1</td>
<td>2.8</td>
<td>4.9</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>2.5</td>
<td>2.5</td>
<td>4.2</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>2.5</td>
<td>7.3</td>
<td>5.8</td>
<td>5.3</td>
<td>6.3</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>2.5</td>
<td>2.5</td>
<td>3.6</td>
<td>3.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Mean</td>
<td>24</td>
<td></td>
<td>2.5</td>
<td>10.0</td>
<td>9.6</td>
<td>9.4</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>2.5</td>
<td>1.4</td>
<td>1.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Time, indicates time in BDM-containing solution before start of experiment; Base, baseline value of peak developed tension before angiotensin II (AT II); ISO, peak twitch tension after exposure to 10\(^{-7}\) mol/L isoproterenol.
FIG 5. Dose-response curves for angiotensin I in atrial preparations: The inotropic response of atrial preparations to angiotensin I is completely inhibited by pretreatment with the angiotensin II receptor antagonist saralasin (10^{-9} mol/L) and enalaprilat (10^{-5} mol/L, angiotensin converting enzyme inhibitor). S indicates saralasin (D--D); E, enalaprilat (○--○); angiotensin I, ●--●; I, initial values; B, baseline. *P<.05 compared with baseline and compared with S and E.

FIG 6. Dose-response curves for angiotensin I in preparations with and without endocardium. Endocardium present is indicated by ●--●; endocardium removed, ○--○; I, initial values. *P<.05 compared with I.

Left and Right Ventricular Myocardium

Myocardium obtained and prepared from the left ventricular wall did not show any significant positive inotropic effect when treated with angiotensin I or II (Tables 2 and 3). Even when looking at the individual preparations, no preparation out of 42 could be called a responder. It is important to note that neither preparations of failing ventricles nor those of ventricles with mitral valve stenosis or incompetence nor those of normal ventricles responded to angiotensin II. Because in two patients significant mitral valve incompetence was present—and this may result in left ventricular failure—myocardium from those patients cannot be considered to exhibit normal myocardial function. In contrast, left ventricular myocardium obtained from patients with mitral valve stenosis was not exposed to chronic pressure or volume overload. Even two preparations from the normal donor heart showed no effect to angiotensin II. Again, this is in contrast to the data reported by Moravec et al, who demonstrated positive inotropic effects in 33% of their left ventricular preparations. One could argue that the quality of preparations obtained from the left ventricle may be different between these two studies, especially because in the present study preparations were cut from thick pieces of tissue, whereas Moravec et al used only thin trabeculae. Indeed, peak developed tension was 17.0 mN/mm² in the cited study and only 7.4 mN/mm² in our study. However, in the present study, experimental conditions were chosen to be physiological. Because force decreases with increasing temperature and increasing heart rate in myocardium from failing left ventri-
the lower average value of peak developed force is not unexpected and is sufficiently explained by physical factors. Second, contractile reserve may be reduced or even abolished in preparations from the left ventricle in the present study. However, in left ventricular preparations that did not respond to angiotensin, isoproterenol was very effective in increasing peak developed force (see "Results"). Third, one could argue that the use of BDM in the present study may have specific influence on angiotensin receptors and myocardial function. This argument can be ruled out because atrial muscle preparations were treated in the same way as left ventricular muscles and yielded constant positive inotropic responses to angiotensin (Fig 7). At present, two hypotheses may explain the differences between the results of Moravec et al and the present findings: Angiotensin may exert its positive inotropic effects only at lower temperatures and stimulation rates and not at 37°C and physiological heart rates in the human left ventricular myocardium. On the other hand, with respect to inotropic response to angiotensin, thin left ventricular trabeculae of human hearts as used by Moravec et al may behave differently from myocardium cut from the left ventricular free wall of human hearts.

Certainly, the quantity of a positive inotropic effect may depend on the number of the angiotensin II receptors present in the specific tissue. Because it is known that the number of angiotensin II receptors is relatively low in left ventricular myocardium of adult men, the influence of angiotensin II was also tested in right ventricular myocardium obtained from infants, in whom a greater amount of angiotensin II receptors has been described. However, even in right ventricular myocardium from infants, no positive inotropic effect could be observed. This finding further supports the hypothesis that two types of angiotensin II receptors may exist, one type that modulates contractility and another type that is not functionally coupled but rather may be involved in stimulation of cell growth.

**Pathophysiological and Clinical Implications of the Results**

The significant positive inotropic effect of angiotensin on atrial cardiac tissue is consistent with other properties of the renin-angiotensin system. During volume depletion and hypotension, angiotensin not only provides the contraction of the vascular smooth muscles of the venous and arterial vessels and conserves sodium and water via stimulation of aldosterone secretion but also stimulates the atria and thereby provides a better filling of the ventricles. Furthermore, a positive inotropic effect of angiotensin on left ventricular myocardium would not be helpful in a situation where ventricular contractility is normal and volume depletion has led to insufficient filling of the ventricles.

The situation is different in heart failure, however: When the renin-angiotensin system is activated, angiotensin II stimulates atrial tissue and thereby increases atrial pressure significantly during end diastole and provides further increase in left ventricular pressure and volume and therefore diastolic sarcomere stretch. Treatment with angiotensin converting enzyme inhibitors, which lower increased angiotensin II levels, may diminish atrial pressure and thereby end-diastolic filling pressure. This effect is unique for angiotensin converting enzyme inhibitors and is not present when pure vasodilators are applied to individuals with heart failure. This may explain why angiotensin converting enzyme inhibitors, which also dilate the venous and arterial vascular bed, are superior to pure vasodilation as shown clinically and by mortality trial. However, this pathophysiological concept may not be valid for all groups of patients because an increased filling pressure may be important for left ventricles in which diastolic compliance disturbances cause heart failure symptoms. Nevertheless, treatment with angiotensin converting enzyme inhibitors cannot have negative effects on left ventricular contractility because angiotensin receptors are not coupled to myocardial function in either failing or nonfailing left ventricular myocardium. This finding may explain why treatment with angiotensin converting enzyme inhibitors is safe with respect to cardiac function and may explain why hemodynamic deterioration is rarely observed when starting angiotensin converting enzyme inhibition therapy even in patients with end-stage heart failure.

Furthermore, the results may provide the basis for explaining the described protection and repair of the myocardium in heart failure induced by angiotensin converting enzyme inhibition: Reduction of atrial muscle contractility prevents or ameliorates end-diastolic stretch of left ventricular sarcomeres in patients with systolic dysfunction and may have beneficial influence.
on further structural dilation and progression of heart failure.

Acknowledgment

This study was supported in part by the Deutsche Forschungsgemeinschaft (Ho 915/4-1/1489/2).

References

Angiotensin I and II exert inotropic effects in atrial but not in ventricular human myocardium.
An in vitro study under physiological experimental conditions.
C Holubarsch, G Hasenfuss, S Schmidt-Schweda, A Knorr, B Pieske, T Ruf, R Fasol and H Just

Circulation. 1993;88:1228-1237
doi: 10.1161/01.CIR.88.3.1228

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/88/3/1228

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at:
http://circ.ahajournals.org/subscriptions/