Angiotensins and the Failing Heart

Enhanced Positive Inotropic Response to Angiotensin I in Cardiomyopathic Hamster Heart in the Presence of Captopril

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We examined the hypothesis that the positive inotropic effect of angiotensin I (Ang I) may be retained in the presence of angiotensin converting enzyme inhibitors so that it may have a direct beneficial effect on the heart. Accordingly, isolated perfused hearts (Langendorff preparation) of 300-day-old cardiomyopathic hamsters (a model of spontaneous cardiomyopathy) and age-matched normal hamsters (controls) were infused with Ang I in the presence of captopril; propranolol was added to the perfusing medium to block catecholamine-mediated effects of angiotensins on the heart. Left ventricular developed pressure and the rate of increase in left ventricular developed pressure increased significantly (p < 0.01) in both the cardiomyopathic and the normal hamster heart despite concomitant reduction in myocardial flow rate favoring a direct inotropic effect of Ang I in both normal and myopathic hearts; these changes were significantly higher by almost threefold in the cardiomyopathic than in the normal hamsters (p < 0.01) and were blocked by the angiotensin II (Ang II) antagonist [Sar1, Thr8] Ang II. Comparing dose–left ventricular contractility response curves for Ang I and Ang II, ED50 for responses was identical in both normal and myopathic hearts, whereas peak responses to Ang II were double those to Ang I in normal hearts but were almost identical in the myopathic hearts. Binding of [125I] Ang II in six cardiomyopathic and four normal hamster hearts was of high affinity, but there was no evidence for Ang I–saturable high-affinity binding sites. Therefore, we suggest that the positive inotropic effect of Ang I, in the presence of captopril, is not via a direct effect on either Ang I or Ang II receptors but most probably via conversion of Ang I to Ang II or another intermediate peptide that was mediated by an alternative converting enzyme. The positive inotropic effect of Ang I and, to a lesser extent, Ang II was accentuated in the myopathic compared with the normal hamster hearts; this finding suggests that Ang I conversion is increased in the diseased heart and that elevated levels of circulating Ang I during therapy with angiotensin converting enzyme inhibitors may be of direct benefit to the failing heart. (Circulation Research 1990;66:891–899)

Angiotensin converting enzyme (ACE) inhibitors are highly effective in the treatment of congestive heart failure. Long-term sustained improvement in hemodynamic indexes as well as clinical symptoms and improved quality of life have been reported by several investigators.1–11 This therapy has also been reported to cause significant improvement in exercise duration, a 45% reduction of ventricular premature beats, and reduction in frequency of hospitalization.2,4,10,11 However, the mechanism by which ACE inhibitors improve cardiac performance is still not well understood. The cardiac unloading effect of ACE inhibitors is shared by other vasodilators. Reduction in aldosterone release during treatment with ACE inhibitors was thought to mediate the lack of development of tolerance during therapy, but its direct relation to the long-term improvement of congestive heart failure has not been proven. Chronic reduction of angiotensin II (Ang II) concentration improves renal blood flow by reducing efferent glomerular arteriolar constriction12 and improves renal tubular excretion of sodium.13 In addition to these mechanisms, and in view of the reported positive inotropic effects of angiotensin peptides in isolated normal cat papillary muscle,14 there is yet another aspect to be considered: a direct cardiac positive inotropic effect of angiotensin I (Ang I), which is accumulated during inhibition of the Ang
I converting enzyme. However, this putative inotropic effect of Ang I was confounded by other reports showing a diminished Ang I inotropic action in the presence of an ACE inhibitor and a total loss of inotropic effect of Ang I in the presence of Ang II antagonists. These latter findings suggest that cardiac Ang I effects may be mediated in part via Ang II and Ang II receptors. We designed this study to 1) evaluate the mechanism of inotropic effects of Ang I during blockade of the known ACE and 2) determine if a differential inotropic effect of Ang I exists between cardiomyopathic hamster (CMH) and normal hamster (NH) hearts to explain a unique benefit in the setting of severe heart failure. We hypothesized that the positive inotropic effect of Ang I may be retained in the presence of captopril, so that it may produce a beneficial effect on the diseased myocardium. The Syrian hamster, which provides a model of spontaneous heart failure, was used to study the effects of Ang I on the compensated and failing heart. The isolated Langendorff method was chosen to allow examination of the effects of the peptide on the whole heart under constant perfusion pressure and heart rate without the influence of either circulating humoral factors or other factors such as respiration and autonomic reflexes.

Materials and Methods

Animals

Male Syrian CMHs (BIO 14.6; 300–330 days old) from the Canadian Hybrid Farm, Halls Harbour, Nova Scotia, Canada, and random-bred NHs of the same age were used. All were fed a regular diet of Purina Rodent Laboratory Chow 5001 (ICN Biochemicals, Cleveland, Ohio), which contains 23.4% protein, 4.5% fat, 4.25 kcal/g gross energy, 3.0 mg/g sodium, 11.0 mg/g potassium, 12.0 mg/g calcium, 2.1 mg/g magnesium, 5.8 mg/g chloride, and 8.6 mg/g phosphorus, with ad lib intake of water in a climate-controlled room for at least 1 week before the experiment. The animals were killed by excision of the heart under pentobarbital anesthesia as explained below. Baseline cardiac function was assessed in 49 CMHs and 39 NHs. Of these, 29 hamsters were used to test cardiac responses to angiotensins, comprising 15 CMHs and 14 NHs. Each category included two subgroups, one to test the dose-response curve to Ang I peptide (seven CMHs and seven NHs) and the other to test the dose-response curve to Ang II peptide (eight CMHs and seven NHs). In addition, 10 hamsters were used to assess cardiac angiotensin receptor density and sensitivity (six CMHs and four NHs), and six others (three CMHs and three NHs) were used to assess the effect of blockade of Ang II receptors by [Sar\(^{1}\),Thr\(^{3}\)]Ang II on the cardiac responses to Ang I (plus captopril), as explained below. Therefore, a total of 104 hamsters are reported in this study.

Receptor Studies

Preparation of hamster cardiac membranes. Hearts were dissected to separate the left ventricle (LV) from the rest of the cardiac tissues. Ang II receptor binding site densities were determined in normal and cardiomyopathic LVs. Pieces of LV tissue were trimmed clean of fat and epicardium, placed in 20 mM NaH\(_2\)PO\(_4\) buffer, pH 7.1, at 4°C (5 ml/0.5 g), and homogenized with a polystyrene homogenizer (Brinkman Instruments, Fullerton, California) at setting 8 for 15 seconds. The homogenate was centrifuged at 40,000g for 20 minutes at 4°C and then rehomogenized and centrifuged as above. The tissue pellet was resuspended in 50 mM NaH\(_2\)PO\(_4\) buffer, pH 7.1, containing 100 mM NaCl and 10 mM MgCl\(_2\); a hand-driven glass/glass homogenizer was used for this procedure. Membrane protein was determined by the method of Lowry et al. These isolated membranes are composed of membranes from myocytes, fibroblasts, nerves, and vessels of the heart. By use of this method, the membrane yield was 31 ± 2 mg membrane protein/g LV wet wt (mean ± SEM) in six CMH hearts and 33 ± 1 mg membrane protein/g LV wet wt (mean ± SEM) in six NH hearts (all 300 days old); these averages were not statistically different.

Ang I and Ang II receptor binding site analysis. Ang I and Ang II receptor binding sites were assayed as described by Daud et al. The assay buffer was 50 mM NaH\(_2\)PO\(_4\), pH 7.2, containing 100 mM NaCl, 1 mM EGTA, 10 mM MgCl\(_2\), 0.2% bovine serum albumin (BSA), and 10 μg/ml bacitracin. [\(^{125}\)I]Ang II or [\(^{125}\)I]Ang I (200–2,000 pM, final concentration) was incubated at 22°C for 60 minutes in assay buffer with 50 μl membrane suspension in a final volume of 250 μl. At the end of all incubations, 5 ml of 50 mM NaH\(_2\)PO\(_4\) buffer, pH 7.2, at 4°C was added to the incubation media, and the mixtures were immediately filtered through glass-fiber filters presoaked with 0.1% BSA. The filters were washed twice with 5 ml of 50 mM NaH\(_2\)PO\(_4\) buffer at pH 7.1. Radioactivity retained on the filter was assayed by γ-scintillation counting at an efficiency of 78%. Non-specific binding for Ang II or Ang I receptors (determined in the presence of 1 μM Ang II or Ang I, respectively) was subtracted from total binding to derive specific radioligand binding to hamster heart membranes. For each radioligand concentration, binding measurements were from duplicate assays. The Scatchard equation (Figure 1) was used to calculate K\(_d\) and B\(_{max}\).

Langendorff Preparation

The Langendorff preparation used in our laboratory has been described in detail previously. LV developed pressure was measured with a 5F transducer-tipped catheter (Mikro-tip, Millar Instrument, Houston, Texas) introduced in the LV via the mitral valve. Because of the small size of the hamster heart, we could not use an LV balloon.
The hamster was given 1,000 units i.p. heparin 30 minutes before anesthesia. Under pentobarbital anesthesia (30 mg/kg/body wt i.p.) and adequate ventilation with positive end-expiratory pressure via tracheostomy (rodent respirator, Harvard Apparatus, Millis, Massachusetts), the heart was rapidly dissected out and placed in a cold Krebs-Henseleit bicarbonate buffer solution saturated with oxygen; pericardial and lung tissues were gently trimmed away, and the aorta was then securely attached to the plastic groove-tipped cannula of the Langendorff apparatus via the aortic stump. The heart was then immediately perfused retrogradely at 37°C with an oxygenated modified Krebs-Henseleit bicarbonate solution at a constant perfusion pressure of 50 mm Hg, which was monitored with a pressure transducer (model MP-150, Micron Instruments, Los Angeles, California) situated at the level of the aortic valve. The perfusate contained the following (mM): NaCl 117, KCl 4.7, CaCl₂ 2.5 plus 0.5 to balance EDTA, KH₂PO₄ 1.2, NaHCO₃ 25, Na₃EDTA 0.5, and dextrose 11.0. pH of the buffer was 7.4 when oxygenated with 95% O₂-5% CO₂ at 37°C.

Immediately after the start of perfusion, the base of the pulmonary artery was incised to allow efficient drainage of the fluid accumulating from the coronary sinus and Thebesian vessels into the right ventricle. The time at which regular spontaneous beating of the heart resumed (usually below 10 seconds after starting perfusion) was noted as time zero of the experiment. Five minutes after the beginning of the spontaneous heart beating, a tipped-transducer catheter (Millar Instrument) was inserted gently into the LV via the mitral valve. The atrioventricular node was then crushed by small forceps, and the heart was immediately paced at a constant rate of 240 beats/min to avoid the effects of different or varying heart rate on indexes of cardiac performance. Pacing was achieved by a stimulator (Model S-9, Grass Instruments, Quincy, Massachusetts); the duration of the stimulus was 5 msec at 2–3 V.

Twenty-five to 30 minutes was allowed for equilibration. Data obtained included LV systolic and diastolic pressure and its first derivative, LV +dP/dt, monitored continuously and recorded on a Brush recorder (Gould, Cleveland, Ohio) at a paper speed of 0.05 mm/sec. At the end of the equilibration period, the paper speed was increased to 50 mm/sec to obtain baseline readings. Myocardial flow was measured by collecting the effluent for 5-minute periods. Myocardial flow rate was calculated in milliliters per gram of left ventricular weight per minute.

At the end of the experiment, heart, brain, and liver weights were obtained with a precision balance (model PC440, Mettler Instrument, Hightstown, New Jersey). LV weight was normalized both by body weight and by brain weight, since brain weight was reported to be constant irrespective of physiological or nutritional stimuli. In some cases, liver, lung, and kidney were examined histologically after fixation with buffered formalin for evidence of congestion, and the LV specimen was examined for histological evidence of myocardial disease.

Protocol of the Study

Angiotensin dose–LV performance response curve was obtained for Ang I and II in cardiomyopathic and control hearts. The effects of Ang I and Ang II were compared. In all experiments, captopril (150 μg/ml) was dissolved in the perfusate to block the intracardiac captopril-sensitive converting enzyme. Since Ang I and II may enhance the release of catecholamines from the nerve endings, all experiments were carried out in the presence of β-blockade (100 nM propranolol in the perfusate); this dose of propranolol was found to completely block the maximum inotropic effect of isoproterenol (6.9 × 10⁻² μg/min) in the mammalian heart.

Ang I and Ang II were prepared by M. Khosla, PhD, of The Cleveland Clinic Foundation. Captopril was offered by Research Chemical Distribution, E.R. Squibb & Sons, Princeton, New Jersey. Propranolol hydrochloride (Inderal) was obtained from Ayerst Laboratories, New York, New York.

Peptides and Dosages

The solutions of Ang I or II (10⁻⁴ M for both) were prepared anew just before the experiment by dissolving the peptide in the oxygenated Krebs-Henseleit bicarbonate buffer. After recording the baseline data, Ang I or II solution was infused continuously via a Harvard pump into the cannula just above the aortic stump in five graded infusion rates (0.005, 0.0103, 0.0206, 0.051, and 0.103 ml/min), each for 5 minutes. The doses of the drug infused were calculated as 0.66, 1.33, 2.67, 6.61, and 13.36 μg/min for Ang I and 0.53, 1.08, 2.16, 5.34, and 10.78 μg/min for Ang II. These doses were then normalized for either
myocardial flow rate (in nanomoles per milliliter) or LV weight (in nanomoles per minute per gram LV). The LV pressure data were recorded continuously at a slow paper speed (0.05 mm/sec) throughout the study, but at the end of each infusion period, the paper speed was increased to 50 mm/sec to minimize reading errors. The myocardial flow was collected throughout each infusion period (5 minutes) and then normalized by LV weight as milliliters per gram of left ventricular weight per minute.

Assessment of Ang I and Ang II in the Effluent

Ang I and Ang II were labeled with $^{125}$I by the lactoperoxidase method, and the iodinated products were purified by reverse-phase high-performance liquid chromatography (HPLC) according to the method of Urata et al. The specific activity of each iodinated angiotensin was 2,200 Ci/mmol. To determine Ang I conversion to Ang II in the hamster heart, 2.5 pmol of $[^{125}$I]Ang I was injected into the coronary artery of the hamster heart prepared in a Langendorff apparatus as described above. Twenty-five milliliters of the effluent from the hamster heart was collected into 100 ml ice-cold ethanol, well-vortexed, and evaporated to dryness by a stream of nitrogen gas. The resulting residue was resuspended into 200 µl of 25 mM triethylammonium phosphate buffer (TEAP), pH 3, and analyzed on a reverse-phase HPLC column (model C4, Vydac, Heperia, California) preequilibrated with 25 mM TEAP, pH 3, containing 10% acetonitrile. The column was developed with a linear acetonitrile gradient (from 10% to 32% acetonitrile in 12 minutes) at a flow rate of 1 ml/min. The column was calibrated with $[^{125}$I]Ang I and $[^{125}$I]Ang II.

Effect of Blockade of Ang II Receptors

To clarify whether the positive inotropic response to Ang I in the presence of captopril was via an effect on Ang II receptors, the cardiac response to the peptide (dose-response curve) was tested before and during blockade of the receptor by [Sar$^1$,Thr$^8$]Ang II. Captopril and propranolol were added to the perfusate throughout the experiment in the same doses as described above. Three myopathic and three normal hamster hearts were subjected to this protocol. After the initial cardiac-Ang I dose-response curve was obtained, a recovery period was allowed. Then the Ang II antagonist (4.9 µg/min [Sar$^1$,Thr$^8$]Ang II) was infused solely for 5 minutes to occupy the Ang II receptors. Then the cardiac-Ang I dose-response curve was repeated during continuous infusion of [Sar$^1$,Thr$^8$]Ang II by using a double-infusion pump simultaneously with Ang I infusion and at the same infusion rate. Six graded infusion rates of Ang I peptide were administered consecutively, each for 5 minutes (0.0051, 0.0103, 0.0206, 0.051, 0.103, and 0.206 ml/min). The doses of the peptide infused were calculated as 0.66, 1.33, 2.67, 6.61, 13.36, and 26.72 µg/min for Ang I and 4.9, 9.8, 19.7, 48.7, 98.4, and 196.9 µg/min for Ang II antagonist.

Tachyphylaxis to repeated administration of Ang I was tested by repeated infusion of Ang I peptide in the same six graded infusion rates and doses as above, each for 5 minutes in two NH hearts. After completion of the first dose-response curve, Ang I infusion was discontinued, and recovery time was allowed to wash out Ang I, as denoted by restabilization of LV pressure. Ang I was infused again in the same graded doses in the same hamster heart.

Statistical Analysis

All statistical analyses were done by PROPHET, a national computer resource supported in part by Biotechnology Resource Program, Division of Research Resources, National Institutes of Health. Comparison between the groups was evaluated by BMDP2V (analysis of variance and covariance including repeated measures). A two-way analysis of variance for repeated measures was used to compare different groups and the responses to different doses of Ang I or Ang II. If the interaction was significant, unpaired $t$ test (for two groups) or one-way analysis of variance (for more than two groups) followed by Newman-Keuls multiple-range test was done. The changes in the parameters in each group were analyzed by one-way analysis of variance followed by Newman-Keuls multiple range test.

Results

Baseline Data

Basal characteristics of the hamsters were analyzed in 49 CMHs and 39 NHs including those 29 used for the Langendorff study. Body weight, brain weight, and liver weight were significantly smaller in

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>Brain (mg)</th>
<th>Liver (mg)</th>
<th>LV (mg)</th>
<th>LV/BW (mg/g)</th>
<th>LV/brain (mg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMH</td>
<td>116±3*</td>
<td>1.017±10*</td>
<td>3.720±117*</td>
<td>3.39±0.07*</td>
<td>0.38±0.01†</td>
<td></td>
</tr>
<tr>
<td>NH</td>
<td>130±2</td>
<td>1.078±17</td>
<td>4.489±103</td>
<td>3.53±7</td>
<td>2.72±0.05</td>
<td>0.34±0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM. BW, body weight; LV, left ventricle; CMH, 300-day-old cardiomyopathic hamsters; NH, 300-day-old normal hamsters; n, number of animals.

*p<0.01 vs. NH.
†p<0.05 vs. NH.
CMHs compared with NHs (Table 1). LV weight was significantly higher in CMHs compared with NHs ($p<0.01$). LV weight normalized by body weight (LV/BW in Table 1) or brain weight (LV/Brain in Table 1) was also significantly higher in CMHs compared with NHs ($p<0.05$). Baseline cardiac indexes in the four groups are summarized in Table 2. LV developed pressure and LV +dP/dt were significantly lower in CMHs compared with NHs. Also, baseline myocaridal flow rate was smaller in CMHs compared with NHs.

Angiotensin Receptors in Hamster Hearts

Specific $[^{125}]$IAng II binding sites were determined by Scatchard analysis; Figure 1 shows a typical Scatchard analysis. Figure 2 shows that Ang II receptor density in the LV of 300-day-old NHs and CMHs was not significantly different. LV Ang II receptor affinity was 35% higher in the CMH compared with the NH ($p<0.02$). Ang I receptor binding sites in 300-day-old NH and CMH LVs were of low specificity (>90% of the binding was nonspecific) and were not saturable. Thus, there was no evidence for the presence of Ang I receptor in NH or CMH LVs.

Cardiac Responses to Ang I and Ang II

For evaluation of the LV +dP/dt response to the peptide, only stable preparations with no decay of baseline LV pressure after crushing the atrioventricular node were used. Based on this criterion, a total of six unstable hearts were not included in this report, one CMH heart and one NH heart in the Ang I experiments and two CMH hearts and two NH hearts in the Ang II experiments.

At all doses of Ang I (in the presence of 150 μg/ml captopril and 100 nM propranolol in the perfusate), LV developed pressure and LV +dP/dt were significantly lower ($p<0.05$) in CMH hearts ($n=7$) compared with NH hearts ($n=7$), as illustrated in Figure 3. However, the increase in both LV pressure and LV +dP/dt from corresponding baseline values was significantly greater (approximately threefold) in CMH compared with NH hearts (Figure 4, top panel). The peak increase in LV pressure in response to Ang I was 30±5 mm Hg in CMH hearts and 11±4 mm Hg in NH hearts ($p<0.01$), while the peak rise in LV +dP/dt was 348±45 mm/sec in CMH hearts and 99±37 mm Hg/sec in NH hearts ($p<0.01$).

Myocardial flow rate tended to decrease in both CMH and NH hearts during Ang I infusion (Figure 4, bottom panel); however, these changes did not attain statistical significance in CMH hearts but were significant in NH hearts. LV pressure and LV +dP/dt responses to graded Ang II infusion were obtained in eight CMH hearts and seven NH hearts. These cardiac function indexes

**TABLE 2. Baseline Cardiac Indexes**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MFR (ml/g LV/min)</th>
<th>LVP (mm Hg)</th>
<th>LV +dP/dt (mm Hg/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Ang I infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMH</td>
<td>7</td>
<td>14.0±1.4</td>
<td>36.0±2.4</td>
<td>438±54*</td>
</tr>
<tr>
<td>NH</td>
<td>7</td>
<td>17.8±1.5</td>
<td>72.0±6.4</td>
<td>990±104</td>
</tr>
<tr>
<td>Pre-Ang II infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMH</td>
<td>8</td>
<td>13.6±1.9</td>
<td>35.4±8.1</td>
<td>483±81*</td>
</tr>
<tr>
<td>NH</td>
<td>7</td>
<td>16.0±1.3</td>
<td>70.0±2.0</td>
<td>998±25</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, number of animals; MFR, myocardial flow rate; LVP, left ventricular developed pressure; LV +dP/dt, change in LVP from baseline; CMH, 300-day-old cardiomyopathic hamsters; NH, 300-day-old normal hamsters.

*p<0.01 vs. NH.

**FIGURE 2.** Bar graphs showing angiotensin II (Ang II) receptor density in the left ventricles of 300-day-old hamster. Whereas Ang II receptor density was similar in cardiomyopathic (CMH) and normal (NH) hamster left ventricles (panel A), receptor affinity was significantly higher (Kₐ lower) in the CMH model (panel B). *p<0.05 CMH vs. NH.
increased in both groups with increasing doses of Ang II. The dose-response curve to Ang II remained parallel in the two groups of hamsters; the CMH group was lower than the NH group at baseline and remained lower throughout all doses. The changes from baseline in LV pressure and LV +dP/dt were also higher in the CMH compared with the NH group, but differences in responses between the two groups did not attain statistical significance (Figure 5, top panel). Myocardial flow rate decreased in both CMH and NH hearts, but this reduction did not reach statistical significance in the CMH group (Figure 5, lower panel).

The two peptides, Ang I and Ang II, had almost the same potency when compared at the same doses in the CMH hearts, but the LV dP/dt responses in the NH hearts to Ang II were more than double the responses to Ang I.

These results were concordant whether peptide doses were expressed as nanomoles per milliliter for myocardial flow rate or nanomoles per minute per gram for LV weight.

**Ang I and Ang II in Effluent**

Conversion of [125I]Ang I to [125I]Ang II or other fragments was not observed in the effluent from NH and CMH hearts. There was no difference in the recovery of radioactivity in the effluent after perfusion; it was 70±3% and 63±7% for NH and CMH hearts, respectively (n=3 in each group). Possible sequestration before or after metabolism of [125I]Ang I by the heart tissue was not examined.

**Effects of Blockade of Ang II Receptors**

There was no evidence of tachyphylaxis by repeated infusion of Ang I (under cover of captopril) in the hamster heart. In one of the two hamster hearts subjected to this procedure, the increase in LV developed pressure was 18 mm Hg during the first infusion of Ang I and 15 mm Hg during the second infusion of Ang I. In the second hamster heart, the increases in LV developed pressure were 67 mm Hg and 50 mm Hg, respectively. On the other hand, the cardiac responses (LV developed pressure and LV dP/dt) to Ang I (under cover of captopril) were blunted in both NH and CMH hearts in the presence of the Ang II antagonist [Sar^1, Thr^9]Ang II, as illustrated in Table 3.

**Discussion**

A positive inotropic effect of Ang I has been reported in isolated normal myocardial preparations, including perfused cat and rabbit heart and bathed guinea pig atrium, but not in the myopathic or failing hearts. The novelty of our findings include 1) demonstration of a positive inotropic response to Ang I in the presence of captopril and 2) a greater increment in cardiac performance induced by Ang I in the CMH compared with the NH hearts. These findings are of potential clinical significance since captopril is widely used in the treatment of congestive heart failure and has been reported to exhibit more prolonged control of the disease compared with other vasodilators.

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**Figure 4.** Graphs showing the effects of angiotensin I infusion on change in left ventricular developed pressure (Δ+dp/dt) and on myocardial flow rate (MFR) in isolated perfused hearts (50 mm Hg, 240 beats/min) of 300-day-old Syrian hamsters. Top panel: The angiotensin I dose–LV+dp/dt response curve shows an accentuated response in cardiomyopathic hamster (CMH) hearts compared with normal hamster (NH) hearts. ED50 was identical in CMH and NH hearts. Bottom panel: In contrast, MFR decreased in both CMH and NH hearts in response to angiotensin I administration.
We used the 300-day-old Syrian hamster model of cardiomyopathy as a unique model of end-stage dilated congestive heart failure with some resemblance to human cardiac dysfunction in its response to treatment with digitalis and diuretics. Moreover, this animal model offers the advantage of spontaneous development of normotensive cardiac dysfunction without the need of exposing the animal to surgery or to administration of myocardial depressant agents. The Langendorff technique was used in this study to assess the direct effects of Ang I and Ang II on the heart under constant perfusion and heart rate without the confounding effects of in vivo influences such as respiration, autonomic nervous system, loading conditions, and humoral and volume factors.

At baseline, CMH hearts were unable to develop pressures comparable with NH hearts. Although there are no reports of cardiac performance in the isolated perfused hearts of 300-day-old (or older) hamsters, our data are similar to those of Sievers et al. and Wikman-Coffelt et al. who studied 240–250-day-old hamsters, by use of the Langendorff preparation, with a perfusion pressure of 90–100 mm Hg. We have used a perfusion pressure of 55 mm Hg in our studies to limit cardiac tissue edema during perfusion throughout the duration of our experiments. The same perfusion pressure was used in both normal and myopathic hearts. Under these conditions, we identified a depressed cardiac performance in the CMHs. Baseline myocardial flow rate tended to be lower in the CMH compared with the NH, but differences did not achieve statistical significance, as also seen by Sievers et al.

Ang I, in the presence of captopril, exerted a positive inotropic response in both CMH and NH hearts, but the absolute values of LV developed pressure and LV +dP/dt remained lower in CMH hearts compared with NH hearts at all levels of peptide stimulation. This peak positive inotropic effect occurred despite a reduction in myocardial flow rate. The occurrence of positive inotropic responses to Ang I, despite concomitant reduction in myocardial flow rate, favors a direct effect of the compound on the myocardium. Moreover, the observed positive inotropic effects of Ang I, despite the presence of propranolol in the perfusion medium, indicate that these effects of Ang I cannot be mediated via increased catecholamine release.

Ang I was reported to have 9–26% of the potency of Ang II to produce a positive inotropic action in normal atrial tissues. However, the mechanism of this inotropic effect is not clear. Trachte and Lefer reported that the positive inotropic effects of Ang I on cat papillary muscle were not significantly altered by captopril, suggesting a direct action of Ang I itself (i.e., without conversion of Ang II) on ventricular contractility.

On the other hand, Ackerly et al. reported a diminished Ang I action in the presence of an ACE inhibitor or a total abolishment with Ang II antagonists in rabbit atria; these findings suggest that the inotropic effect is mediated solely via Ang II and Ang II receptors. To explain our results, several possibilities should be considered. In our study, there
was no evidence for Ang I-saturable high-affinity binding sites in the hamster heart ventricular cardiac tissue as determined by Scatchard analysis. Therefore, we suggest that the effects of Ang I peptide on the hamster hearts were not mediated by a direct interaction between the Ang I itself and receptor sites. On the other hand, our findings that the positive inotropic response to Ang I (plus captopril) in our experiments was blocked or blunted by the Ang II antagonist [Sar\(^1\),Thr\(^8\)]Ang II suggest that Ang I may be converted to Ang II within the heart and that the newly formed Ang II acted on Ang II receptors to produce the positive inotropic responses. Indeed, Ang II receptors were present in the hamster cardiac ventricular tissue; their presence has been previously demonstrated in human and several other mammalian hearts.33–37 Because all our studies were performed in the presence of captopril, conversion of Ang I to Ang II could only occur via an alternative noncaptopril-sensitive enzyme system. Moreover, because we could not detect \([^{125}I]\)Ang II peptide in the effluent (after infusion of \([^{125}I]\)Ang I), we postulate that this putative conversion of Ang I to Ang II did not occur in the lumen of the coronary vessels. However, we cannot exclude the tissue generation of Ang II after diffusion of Ang I from vascular lumen to extraluminal sites. Indeed, several studies38,39 have demonstrated local tissue renin-angiotensin systems.38–40 It is also known that besides the well-known Ang I converting enzyme, other enzymes exist in vascular tissues and lungs41 that generate Ang II from Ang I. Ikeda et al42 have shown that a toxin-inhibitor complex could quantitatively hydrolyze Ang I and synthetic tripeptide renin substrate to form Ang II. Alternatively, one may consider the possibility that the generated Ang II has been rapidly degraded locally or that intracardiac conversion of Ang I to an intermediate active peptide other than Ang II, which also acts on Ang II receptors, might have occurred.

The observation that the positive inotropic effect of Ang I, and to a lesser extent Ang II, was accentuated in the CMH heart raises many questions about the physiological differences between the normal and diseased heart. One possible explanation of the accentuated response is an increased conversion of Ang I to Ang II in the CMH heart. This possibility is partly supported by the similar potency of Ang I and Ang II on the inotropic responses of CMH hearts compared with the lower potency of Ang I versus Ang II in NH hearts.

In conclusion, the present study demonstrates that the positive inotropic response to Ang I occurred in both CMH and NH hearts despite the presence of captopril in the perfusion medium and that the positive inotropic effect of Ang I was accentuated in the CMH compared with the NH heart. Although the mechanisms mediating these effects are still not clear, one may postulate that the concomitant reduction in myocardial flow rate and increased ventricular contractility favors a direct inotropic effect of angiotensins and suggests a differential in sensitivity to angiotensins between coronary vessels and myocardium in both NH and CMH hearts. The positive inotropic effect of Ang I in the presence of captopril, the absence of saturable Ang I binding sites, and the blockade of cardiac inotropic effect of Ang I (plus captopril) by \([\text{Sar}^1,\text{Thr}^8]\)Ang II may denote that Ang II has been generated and acted on Ang II receptors and that this conversion of Ang I to Ang II has been mediated via an alternate enzyme other than the captopril-sensitive one. Also, the lack of \([^{125}I]\)Ang II in the cardiac effluent after infusion of \([^{125}I]\)Ang I suggests tissue rather than luminal conversion of Ang I to Ang II or even an intermediate active peptide (other than Ang II) that will still act via Ang II receptors. Therefore, we suggest that, similar to exogenously administered Ang I, endogenously accumulated Ang I (during treatment of chronic heart failure with ACE inhibitors) might be converted in the heart to Ang II (or a fragment thereof), which will increase cardiac performance by direct effect on myocyte Ang II receptors. This chain of events might represent another mechanism by which ACE inhibitors mediate sustained control of congestive heart failure.

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