Inotropic Effects of Angiotensin II on Human Cardiac Muscle In Vitro

Christine Schomisch Moravec, PhD, Mark D. Schluchter, PhD, Lata Paranandi, MSHP, Barbara Czerska, MD, Robert W. Stewart, MD, Eliot Rosenkranz, MD, and Meredith Bond, PhD

The direct effects of angiotensin II (Ang II) on human cardiac muscle were investigated using isolated trabecular muscles from failing and functionally normal hearts. Atrial and ventricular trabeculae were studied. Results demonstrated a positive inotropic effect of Ang II on human cardiac muscle. Comparison of the effects of Ang II among groups indicated that the responsiveness tended to be greater in atrial and normal muscle compared with failing muscle. Results of this study also demonstrated heterogeneity in the responsiveness to Ang II among human muscles, which was not correlated with patient age, sex, diagnosis, prior treatment with angiotensin converting enzyme inhibitor, or heart function. A significant correlation between response to Ang II and response to isoproterenol was demonstrated in failing ventricular trabeculae, which may suggest that defects in β-adrenergic responsiveness in the failing human ventricle are accompanied by a loss of responsiveness to Ang II. Studies were extended to the Syrian cardiomyopathic hamster and its control. A dose-dependent inotropic response occurred in normal hamster ventricular muscle but was significantly diminished in cardiomyopathic muscle. Ang II did not shorten the timing of contraction, and pretreatment with adrenergic-blocking agents did not shift the dose-response curve, indicating that the response was not cyclic AMP mediated. This study demonstrates for the first time that Ang II can exert an inotropic effect directly on human cardiac muscle and confirms that there is a direct effect of Ang II on hamster cardiac muscle. The study further suggests, however, that the inotropic response to Ang II in cardiac muscle is heterogeneous and may be diminished by heart failure. (Circulation 1990;82:1973–1984)

Two developments in the field of cardiovascular physiology have renewed interest in the effects of angiotensin II (Ang II) on the myocardium. First, the observation that angiotensin converting enzyme inhibitors such as captopril are more efficacious in treating heart failure patients than direct-acting vasodilators suggests that the renin-angiotensin system or its perturbation may be involved in heart failure, although the mechanisms involved remain to be defined. Second, evidence exists in the myocardium that locally generated Ang II may act in an autocrine or paracrine fashion to directly modulate cardiac function by a mechanism independent of its peripheral effects.

Although it has long been recognized that Ang II can have an indirect effect on the heart due to the reflex response to its hemodynamic actions, there are at least two other mechanisms by which Ang II might directly influence cardiac output. Circulating Ang II may alter the inotropic state of the myocardium by activating myocardial Ang II receptors. Alternatively, Ang II produced locally within the myocardium may activate the receptors. The question of whether there is a direct action of Ang II on cardiac muscle and the relation between such an inotropic effect and indirect effects due to hemodynamic alterations have long been debated. Membrane receptors for Ang II have been demonstrated in the hearts of the rabbit, guinea pig, calf, chicken, hamster, neonatal rat, and most recently, normal and diseased human heart. However, Baker and

From the Departments of Heart and Hypertension Research (C.S.M., B.C., M.B.), Biostatistics and Epidemiology (M.D.S., L.P.), and Thoracic and Cardiovascular Surgery (E.R., R.W.S.), Cleveland Clinic Foundation, Cleveland, Ohio.
Supported in part by a Fellowship Award (C.S.M.) and a Grant-in-Aid (M.B.) from the American Heart Association, Northeast Ohio Affiliate.
Address for correspondence: Meredith Bond, PhD, Research Institute, FF1, Cleveland Clinic Foundation, One Clinic Center, Cleveland, OH 44195-5069.
Received December 18, 1989; revision accepted July 3, 1990.
Singer\textsuperscript{11} have shown that Ang II receptors in the guinea pig heart are not coupled to an inotropic response. Despite the apparent lack of an inotropic response to Ang II in the myocardium of the guinea pig\textsuperscript{11} and the adult rat,\textsuperscript{12} a positive inotropic response to Ang II has been demonstrated in cardiac muscle from the cat, rabbit, calf, and chicken and in isolated, perfused hearts from normal and cardiomyopathic hamsters.\textsuperscript{6–9,17,18,21} In isolated canine myocardial preparations, however, Kobayashi and coauthors\textsuperscript{10} reported a positive inotropic response to Ang II in some patients and no response or a negative inotropic response in others.

The variability and species specificity of the myocardial inotropic response to Ang II led us to investigate the nature of the response to Ang II in cardiac muscle isolated from human hearts. We report the results of our studies on 12 atrial trabeculae from the functionally normal hearts of eight human surgical patients as well as 16 atrial and 15 ventricular trabeculae from the failing hearts of 10 human cardiac transplant patients. The results directly demonstrate that Ang II has a positive inotropic effect on both failing and nonfailing human cardiac muscle and further suggest that the inotropic effect may be greater in atrial than in ventricular muscle. Due to the inability to procure ventricular tissue from functionally normal human hearts, we were unable to determine whether the relatively small inotropic response observed in ventricular muscle was unique to the failing heart. For this reason, we extended our studies to the ventricle of the Syrian cardiomyopathic hamster, a well-described animal model of heart failure, and its control. Although these studies confirmed that the inotropic response to Ang II is also relatively small in hamster ventricular tissue, they also demonstrated that the increase in tension produced by Ang II is significantly diminished in cardiac muscle from myopathic hearts compared with that from normal hearts.

The results support the hypothesis that Ang II, whether circulating or produced locally within the myocardium, can act as a positive inotropic agent to enhance the contractility of human cardiac muscle and suggest that the relative responsiveness to Ang II can be regulated by heart failure and other unidentified factors.

**Methods**

**Tissue Preparation**

One group of human myocardial trabeculae was obtained from the explanted hearts of patients with cardiac failure undergoing transplantation at the Cleveland Clinic Foundation, as described previously.\textsuperscript{22} The time from surgical excision of the heart until its arrival in the laboratory ranged from 20 to 45 minutes. During transport to the laboratory, the heart was maintained in ice-cold, oxygenated cardioplegia solution of the following composition (mM): NaCl 147.16, MgCl\textsubscript{2} 16.00, KCl 20.0, NaHCO\textsubscript{3} 10.0, CaCl\textsubscript{2} 2.25, and procaine 1.0. Once in the laboratory, the heart was placed in cold (8–10°C), oxygenated (95% O\textsubscript{2}-5% CO\textsubscript{2}) Krebs-Henseleit buffer [composition (mM): NaCl 100.0, KCl 4.0, CaCl\textsubscript{2} 2.5, MgSO\textsubscript{4} 1.5, NaHCO\textsubscript{3} 20.0, NaH\textsubscript{2}PO\textsubscript{4} 1.5, Na\textsubscript{2}C\textsubscript{2}H\textsubscript{3}O\textsubscript{2} 20.0, glucose 10.0, and ascorbic acid 0.1]. Long, thin trabeculae were carefully dissected from the right or left ventricle or right atrial appendage. Average time between delivery of the explanted heart to the laboratory and mounting of the isolated trabeculae in the baths was 11 ± 4 (±SD) minutes.

A second group of human atrial trabeculae was dissected from pieces of right atrium removed during cardiac surgery for the purpose of improving access to the operative site or suture line. This tissue was obtained directly from the operating room, and since it was not part of a transplantation procedure, it was placed immediately into cold, oxygenated Krebs-Henseleit buffer for transport to the laboratory rather than being placed into cardioplegia. Although this resulted in a small difference in the handling procedure for tissue from normal hearts and that from explanted diseased hearts, the explanted hearts remained in cardioplegia for the minimal time necessary for transport to the laboratory, and washout of the cardioplegia solution was complete before the experiment began. Once in the laboratory, trabeculae were carefully dissected from the piece of tissue removed during surgery.

Hamster left ventricular papillary muscles were obtained from Syrian cardiomyopathic hamsters of the Bio 14.6 strain and normal golden hamsters (Canadian Hybrid Farms, Nova Scotia). Both groups of hamsters ranged in age from 300 to 320 days. Average body weight was 140.8 ± 15.9 (±SD) for normal hamsters (n = 8) and 114.4 ± 17.5 for myopathic (n = 9) hamsters. Hamsters received 2,000 units/kg body wt i.p. heparin 30 minutes before death to prevent coagulation of blood in the heart. Hamsters were killed by decapitation, which was performed in accordance with procedures approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic Foundation. Upon removal, the heart was placed in cold (8–10°C), oxygenated Krebs-Henseleit buffer, and the papillary muscle was carefully dissected from the left ventricle.

Isometric contractions were recorded from each muscle as previously described.\textsuperscript{22,23} Spring clips were attached to each end, and the muscle was mounted between a Grass FT03 force transducer (Grass Instruments, Quincy, Mass.) and a stationary hook in a water-jacketed muscle bath containing Krebs-Henseleit buffer. The bath was maintained at 28–29°C and bubbled with 95% O\textsubscript{2}-5% CO\textsubscript{2} at a constant flow rate of 103.9 ml/min. The muscle was allowed to stabilize at minimal resting tension (0.1–0.2 g) with no stimulation for 40–50 minutes, during which time the solution in the bath was replaced at 10-minute intervals to wash out the cardioplegia solution in the explanted human tissues as well as to reestablish normal ion gradients after incubation in
the cold. At the end of this period, the muscle was prestretched to a resting tension of 0.5–1.0 g/mm². Stimulation was initiated through two parallel platinum electrodes that came into contact with the muscle. Stimulation frequency was set at 0.2 Hz, duration at 5 msec, and voltage at 20% above threshold (usually 8–10 V for human tissue and 1.0–1.5 V for hamster tissue). The response to stimulation was allowed to stabilize for 30 minutes. The muscle length was then increased in increments of 0.1 mm until Lmax (length associated with maximal developed tension) was reached. At Lmax, the response was again allowed to stabilize for 20–30 minutes, by which time stress relaxation was complete. Contractile parameters recorded from each muscle included the resting tension (RT; tension produced by the muscle in an unstimulated state as a function of its length), developed tension (DT; tension produced by the muscle when stimulated), time to peak tension (TPT; time from the beginning of the contraction to the peak response), and time to half relaxation (T1/2R; time from the peak of the response to the halfway point of relaxation).

Once the response was stable at Lmax, the dose-response curve to Ang II was initiated. Doses of Ang II were added cumulatively, from 10⁻¹⁰ to 10⁻⁵ M. The maximal response to each dose of Ang II was recorded 5 minutes after the dose was administered, at the plateau of the inotropic response. Six minutes elapsed between doses of Ang II. After the dose-response curve to Ang II was complete, 10⁻⁶ M isoproterenol was administered to determine the maximal inotropic response. The muscle was not washed between the Ang II dose-response curve and the dose of isoproterenol. At the end of each experiment, the muscle length at Lmax was measured using Vernier calipers. The muscle was weighed, and the cross-sectional area was calculated by dividing muscle weight by length times density, assuming a cylindrical shape and a density of 1.0 mg/mm². To minimize differences resulting from muscle size alone, the muscle cross-sectional area was used to normalize the values of RT and DT for all human trabeculae and hamster papillary muscles.

One group of hamster papillary muscles was preincubated with 10⁻⁵ M propranolol in combination with 10⁻⁵ M phentolamine before the Ang II dose-response curve. Once the muscle was stable at Lmax, phentolamine and propranolol were added directly to the muscle bath, and the response of the muscle was then allowed to further stabilize for 20 minutes before beginning the Ang II dose-response curve.

**Drugs**

Human Ang II (Bachem Inc., Torrence, Calif.) was used for all studies. A stock solution was prepared in phosphate-buffered saline at pH 6.0, and aliquots of the solution were frozen at −20°C. Each aliquot was thawed at time of use and not refrozen. Isoproterenol hydrochloride was prepared by Elkins-Sinn Inc. (Cherry Hill, N.J.). Ascorbic acid (10⁻⁴ M) was added to the isoproterenol to prevent oxidation. DL-Propranolol (Sigma Chemical, St. Louis, Mo.) was dissolved in deionized water. Phentolamine mesylate (CIBA Pharmaceutical Company, Summit, N.J.) was reconstituted with deionized water immediately before use.

**Measurement of Heart Function In Vivo**

Percent fractional shortening of the left ventricular wall [%FSH(LV)] for each of the patients from whom tissue was obtained was measured from the last echocardiogram obtained before transplant or cardiac surgery. Standard M-mode and two-dimensional echocardiograms were obtained using Hewlett-Packard or Irex-Meriden instruments with a 2.5-MHz transducer. M-mode measurements were performed according to the recommendations of the committee of the American Society of Echocardiography. [%FSH(LV)] was determined using the following calculation:

\[
\text{%FSH}(LV) = \frac{(LV \text{ EDD} - LV \text{ ESD})}{LV \text{ EDD}} \times 100
\]

where LV EDD is left ventricular end-diastolic diameter, and LV ESD is left ventricular end-systolic diameter.

**Analysis of Data**

**Human tissue.** All data from human hearts were analyzed according to the following procedures. All muscles with cross-sectional areas of more than 1.5 mm² were excluded from the study. Although a diameter of less than 1.1 mm² (or a cross-sectional area of less than 0.94 mm²) is considered optimal for tissue oxygenation at 28–29°C and a stimulation rate of 0.2 Hz, human trabecular muscles frequently exceed this size limitation. We therefore verified that DT produced by muscles in each group with a cross-sectional area between 1.1 and 1.5 mm² did not differ from that produced by muscles with a cross-sectional area of less than 1.1 mm² (p=0.9 for atrial muscles from functionally normal hearts, p=0.09 for atrial muscles from failing hearts, and p=0.3 for ventricular muscles from failing hearts; Kruskal-Wallis analysis). Additionally, muscles that showed a negative inotropic response to 10⁻⁶ M isoproterenol, (i.e., DT after 10⁻⁶ M isoproterenol less than the baseline value) were excluded from the study. Baseline contractile parameters (RT, DT, TPT, and T1/2R) were compared among the three groups of muscles using a Kruskal-Wallis analysis.

In response to cumulative doses of Ang II (10⁻¹⁰ to 10⁻⁷ M), changes in RT, DT, TPT, and T1/2R were recorded. Each of these responses was transformed by the relation log₂ (Ang II response/baseline response) to normalize the distribution of responses. Responses to Ang II were analyzed in two ways. A repeated-measures analysis of variance (ANOVA) was performed for each of the four contractile parameters within each group of muscles to determine whether there was a dose-dependent effect.
The repeated-measures ANOVA takes into consideration the fact that all doses were administered to the same muscle, so responses are not independent of one another. Second, because of the large degree of variability among muscles, DT was analyzed separately for each trabecular muscle by computing the slope of the dose-response curve to Ang II. If the slope was significantly greater than zero (p<0.05), the muscle was considered to be responsive to Ang II. This analysis allowed determination of the relative percentage of responsive muscles within each group.

An attempt was also made to correlate the Ang II response with muscle and patient characteristics. Spearman correlations were used to compare the Ang II response (maximum response to Ang II) with the muscle characteristics of cross-sectional area, baseline RT, baseline DT, baseline TPT, baseline T1/2R, and change in DT in response to 10−6 M isoproterenol. Spearman correlations were also performed between Ang II response and numerical values for patient age and %FSH(LV). Nonparametric Kruskal-Wallis tests were used to compare the median slope of the Ang II dose-response curve with the patient's sex and presence or absence of capotrolip in the pretransplant drug regimen.

A repeated-measures ANOVA was used to compare the responses of atrial and ventricular muscles from explanted hearts, and an ANOVA was also used to compare the mean responses at each dose. The responses of atrial muscles from explanted hearts and ventricular muscles from explanted hearts were each compared with those of atrial muscles from surgical patients using a nested ANOVA, which nested hearts within groups and muscles within hearts. Unless otherwise noted, all values are given as mean±SEM.

Hamster tissue. Data obtained from hamster cardiac muscle were analyzed as follows. Dose-response curves to Ang II for each of the contractile parameters were compared for papillary muscles from myopathic hearts and from normal hearts using a repeated-measures ANOVA. Responses of control papillary muscles and those treated with phentolamine and propranolol were compared using the same analysis.

### Results

#### Human Trabecular Muscles Used

A total of 26 right atrial and 23 ventricular (20 right and three left) trabecular muscles were obtained from 13 cardiac transplant patients. Ten atrial and four ventricular muscles were eliminated from this group because of cross-sectional areas of more than 1.5 mm². Four additional ventricular muscles were eliminated because their function declined over the course of the experiment. This left a total of 16 atrial and 15 ventricular muscles from 10 patients to be studied (Table 3). A total of 16 atrial trabecular muscles were obtained from nine cardiac surgical patients. Four trabecular muscles were eliminated from this group because of cross-sectional areas of more than 1.5 mm², leaving a total of 12 muscles from eight patients to be studied (Table 4).

Baseline contractile data for human trabecular muscles are presented in Table 1. Contractile parameters recorded from these muscles are comparable to those we have previously reported. Nonparametric Kruskal-Wallis tests were used to compare the median slope of the Ang II dose-response curve with the patient’s sex and presence or absence of capotrolip in the pretransplant drug regimen.

#### Effects of Angiotensin II on Human Tissue

Ang II produced a dose-dependent increase in DT in all groups of human muscles (Figures 1A–1C). Considering all muscles in each of the groups, the dose-response relation was statistically significant in all three groups of muscles (p=0.05 for atrial trabeculae from failing hearts, p=0.02 for ventricular trabeculae from failing hearts, and p=0.04 for atrial trabeculae from functionally normal hearts). The increase in DT was accompanied by a significant decrease in RT in all three groups (p=0.01 for atrial trabeculae from functionally normal hearts, p=0.0003 for atrial trabeculae from failing hearts, and p=0.0001 for ventricular trabeculae from failing hearts). Neither TPT nor T1/2R changed in response to Ang II in ventricular muscles from failing hearts. Ang II caused a significant increase in T1/2R with no

### Table 1. Baseline Contractile Data From All Human Trabeculae Used for Angiotensin II Studies

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Resting tension (g/mm²)</th>
<th>Developed tension (g/mm²)</th>
<th>Time to peak tension (msec)</th>
<th>Time to half relaxation (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial trabeculae from transplant hearts</td>
<td>16</td>
<td>1.31±0.14</td>
<td>0.85±0.19</td>
<td>219.69±17.94</td>
<td>162.50±18.98</td>
</tr>
<tr>
<td>Atrial trabeculae from surgical patients</td>
<td>12</td>
<td>1.21±0.20</td>
<td>0.62±0.13</td>
<td>211.25±15.15</td>
<td>136.25±12.82</td>
</tr>
<tr>
<td>Ventricular trabeculae from transplant patients</td>
<td>15</td>
<td>1.73±0.26</td>
<td>1.70±0.27*</td>
<td>438.00±17.96</td>
<td>314.67±15.25*</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM.

*p<0.001 compared with each group of atrial muscles.
change in TPT in atrial muscles from both failing (p=0.05) and functionally normal hearts (p=0.016).

The inotropic response to Ang II varied considerably between atrial and ventricular muscles from the same hearts (Table 2). The average maximal increase in DT produced by Ang II tended to be greater in atrial muscles than in ventricular muscles from failing hearts (Table 2). The greatest percent increase in DT in response to Ang II was observed in atrial muscles from functionally normal hearts, in which Ang II caused DT to increase by an average of 113.9±47.3% in responsive muscles (Table 2). The threshold dose for an inotropic response to Ang II in ventricular muscles was 10⁻¹⁰ M, and a maximal response was reached at 10⁻⁶ M. In atrial muscles from failing hearts, the threshold dose was also 10⁻¹⁰ M, whereas atrial muscles from functionally normal hearts did not exhibit a response until a dose of 10⁻⁸ M. In both groups of atrial muscles, a significant increase in the response was still observed at a dose of 10⁻⁵ M Ang II.

Although Ang II increased DT in the three groups of human muscles, it did not produce a maximal inotropic effect, as evidenced by the ability of a single dose of 10⁻⁶ M i.p. administered at the end of the Ang II dose-response curve to cause an additional increase in DT (+169.5±78.1%, +150.4±82.5%, and +246.4±125.9% in ventricular muscle from failing hearts, atrial muscle from failing hearts, and atrial muscle from normal hearts, respectively). The single dose of isoproterenol after the Ang II dose-response curve also caused an additional decrease in RT in all groups of muscles as well as a decrease in both TPT and T₁/₂R.

**Heterogeneity of Angiotensin II Response in Human Muscle**

Analysis of the average dose-response curves to Ang II in human muscles revealed significant variability within each group. This was due to the fact that some muscles showed a dose-dependent increase in DT in response to Ang II, whereas other muscles did not respond. Analysis of dose-response curves for each muscle revealed that seven of 12 atrial muscles from functionally normal patients (58%) as well as five of 15 atrial muscles (33%) and eight of 16 ventricular muscles from transplant patients (50%) responded to Ang II (Table 2). Average percent increase in DT in response to Ang II is summarized in Table 5 for all muscles within each group as well as for the subset of responsive muscles. Some hearts were characterized by one or more muscles that showed a positive response and others that failed to respond (Tables 3 and 4). In attempting to correlate Ang II responsiveness with patient characteristics (Tables 3 and 4), there was no relation between response to Ang II and patient age, sex, diagnosis, or pretreatment with captopril or with %FSH(LV) used as an index of function of the heart. There were also no significant correlations between individual muscle characteristics, such as baseline contractile parameters or cross-sectional area, and Ang II response in atrial muscle. In ventricular
TABLE 2. Inotropic Responsiveness to Angiotensin II in Human Trabeculae

<table>
<thead>
<tr>
<th>All responses</th>
<th>Atrial trabeculae (transplant)</th>
<th>Ventricular trabeculae (transplant)</th>
<th>Atrial trabeculae (surgical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responsive (%)</td>
<td>50</td>
<td>33</td>
<td>58</td>
</tr>
<tr>
<td>Nonresponsive (%)</td>
<td>50</td>
<td>67</td>
<td>42</td>
</tr>
<tr>
<td>Threshold dose* (M)</td>
<td>$10^{-10}$</td>
<td>$10^{-10}$</td>
<td>$10^{-8}$</td>
</tr>
<tr>
<td>Maximum dose* (M)</td>
<td>$10^{-5}$</td>
<td>$10^{-5}$</td>
<td>$10^{-5}$</td>
</tr>
<tr>
<td>Maximum change in DT* (%)</td>
<td>+88.0±26.7</td>
<td>+52.3±24.0</td>
<td>+113.9±47.3</td>
</tr>
<tr>
<td>Maximum change in RT* (%)</td>
<td>-19.0±6.3</td>
<td>-19.0±5.3</td>
<td>-11.1±6.2</td>
</tr>
<tr>
<td>Maximum change in TPT*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Maximum change in T↓2R* (%)</td>
<td>+10.1±5.3</td>
<td>NS</td>
<td>+22.8±11.6</td>
</tr>
</tbody>
</table>

Threshold dose, first dose at which a significant positive inotropic effect occurred; maximum dose, dose at which average maximum inotropic effect occurred; DT, developed tension; RT, resting tension; TPT, time to peak tension; T↓2R, time to half relaxation.

*Values are given as mean±SEM for muscles that showed a significant response to angiotensin II (eight of 16 atrial muscles from failing hearts, five of 15 ventricular muscles from failing hearts, and seven of 12 atrial muscles from normal hearts).

TABLE 3. Transplant Patients From Whom Tissue Was Obtained

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Pretransplant captopril</th>
<th>%FSH(LV)</th>
<th>Muscles used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>M</td>
<td>DCM</td>
<td>Yes</td>
<td>31.0</td>
<td>2RV,* 1LV</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>F</td>
<td>DCM</td>
<td>Yes</td>
<td>17.0</td>
<td>1RV</td>
</tr>
<tr>
<td>3</td>
<td>47</td>
<td>M</td>
<td>DCM</td>
<td>Yes</td>
<td>8.0</td>
<td>1RA*</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>M</td>
<td>DCM</td>
<td>No</td>
<td>10.0</td>
<td>1RA*</td>
</tr>
<tr>
<td>5</td>
<td>35</td>
<td>M</td>
<td>DCM</td>
<td>No</td>
<td>16.0</td>
<td>2RV*</td>
</tr>
<tr>
<td>6</td>
<td>43</td>
<td>M</td>
<td>IsHD</td>
<td>No</td>
<td>15.0</td>
<td>3RV, 2RA</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>F</td>
<td>DCM</td>
<td>No</td>
<td>26.0</td>
<td>1RV, 3RA***</td>
</tr>
<tr>
<td>8</td>
<td>51</td>
<td>M</td>
<td>IsHD</td>
<td>Yes</td>
<td>11.0</td>
<td>2RV,* 4RA*</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>F</td>
<td>DCM</td>
<td>No</td>
<td>13.0</td>
<td>1RV, 3RA</td>
</tr>
<tr>
<td>10</td>
<td>56</td>
<td>M</td>
<td>IsHD</td>
<td>Yes</td>
<td>20.0</td>
<td>2RV,* 2RA*</td>
</tr>
</tbody>
</table>

%FSH(LV), percent fractional shortening of left ventricle wall; DCM, dilated cardiomyopathy; IsHD, ischemic heart disease; RV, right ventricle; LV, left ventricle; RA, right atrium.

*Significant positive inotropic response to angiotensin II (number of * indicates number of responders if more than one muscle was used).

TABLE 4. Surgical Patients From Whom Tissue Was Obtained

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>%FSH(LV)</th>
<th>Preoperative captopril</th>
<th>Muscles used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>M</td>
<td>Transposition of great arteries</td>
<td>43.0</td>
<td>No</td>
<td>1RA*</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>F</td>
<td>Tetralogy of Fallot</td>
<td>37.0</td>
<td>No</td>
<td>2RA*</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>F</td>
<td>Atrial septal defect</td>
<td>42.0</td>
<td>No</td>
<td>2RA</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>M</td>
<td>Congenital heart disease</td>
<td>46.0</td>
<td>No</td>
<td>2RA</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>F</td>
<td>Atrial septal defect</td>
<td>35.0</td>
<td>No</td>
<td>1RA*</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>F</td>
<td>Atrial septal defect</td>
<td>42.0</td>
<td>No</td>
<td>1RA*</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>F</td>
<td>Atrioventricular septal defect</td>
<td>45.0</td>
<td>No</td>
<td>1RA*</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>M</td>
<td>Atrioventricular septal defect</td>
<td>38.0</td>
<td>No</td>
<td>2RA**</td>
</tr>
</tbody>
</table>

%FSH(LV), percent fractional shortening of left ventricle wall; RA, right atrium.

*Significant positive inotropic response to angiotensin II (number of * indicates number of responders if more than one muscle was used).
Our baseline values for muscles from normal hamster hearts are comparable to those obtained by other investigators.\textsuperscript{32} In response to successive doses of Ang II, ventricular muscle from normal hamster hearts produced an increase in DT. Although an increase in DT was also observed in ventricular muscle from myopathic hamster hearts, the increase was significantly less than that observed in muscle from the normal hamster hearts (\textit{p}<0.04, Figure 2). In both groups of hamster papillary muscles, the increase in DT was accompanied by a decrease in RT and no change in TPT or T\textsubscript{1/2}R. The dose/response curve to Ang II in normal papillary muscle was no different in the presence of the \(\beta\)-adrenergic receptor antagonist propranolol and the \(\alpha\)-adrenergic receptor antagonist phentolamine (Figure 3).

### Table 5. Inotropic Effects of Angiotensin II in All Muscles Versus Responders Only

<table>
<thead>
<tr>
<th>Muscles</th>
<th>Dose (M)</th>
<th>(10^{-10})</th>
<th>(10^{-9})</th>
<th>(10^{-8})</th>
<th>(10^{-7})</th>
<th>(10^{-6})</th>
<th>(10^{-5})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal atria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>12</td>
<td>-5.8±3.0</td>
<td>-3.4±3.7</td>
<td>+9.3±7.6</td>
<td>+26.3±13.0</td>
<td>+52.5±22.8</td>
<td>+69.1±34.8</td>
</tr>
<tr>
<td>Resp</td>
<td>7</td>
<td>-8.3±4.6</td>
<td>-3.3±5.2</td>
<td>+21.8±9.0</td>
<td>+53.4±13.9</td>
<td>+91.0±29.1</td>
<td>113.9±47.3</td>
</tr>
<tr>
<td>Failing atria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>16</td>
<td>+1.7±2.8</td>
<td>+3.6±3.8</td>
<td>+20.2±11.9</td>
<td>+28.0±14.2</td>
<td>+34.8±17.4</td>
<td>+47.6±17.5</td>
</tr>
<tr>
<td>Resp</td>
<td>8</td>
<td>+8.2±3.2</td>
<td>+12.5±3.9</td>
<td>+48.0±18.7</td>
<td>+64.3±21.1</td>
<td>+80.6±24.9</td>
<td>+88.0±26.7</td>
</tr>
<tr>
<td>Failing ventricle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>15</td>
<td>+5.3±2.3</td>
<td>+9.2±3.7</td>
<td>+19.8±8.6</td>
<td>+20.2±9.3</td>
<td>+19.7±10.0</td>
<td>+18.3±9.2</td>
</tr>
<tr>
<td>Resp</td>
<td>5</td>
<td>+8.9±3.6</td>
<td>+15.3±7.6</td>
<td>+44.1±20.4</td>
<td>+48.8±22.3</td>
<td>+52.3±24.0</td>
<td>+50.8±20.7</td>
</tr>
</tbody>
</table>

All, all muscles within a group; Resp, those muscles that showed a significant positive dose-response to angiotensin II. Results are presented as percent change from baseline in developed tension.

### Discussion

**Human Muscle Response to Angiotensin II**

Direct inotropic effects of Ang II on the myocardium may be species-specific. Positive inotropic responses to Ang II have been demonstrated in cardiac muscle from the cat,\textsuperscript{6,7} rabbit,\textsuperscript{8,9} calf,\textsuperscript{21} and chicken\textsuperscript{17} and isolated hamster hearts,\textsuperscript{18} but not the hearts of the adult rat\textsuperscript{12} or guinea pig.\textsuperscript{11} In the dog, Kobayashi et al\textsuperscript{10} demonstrated heterogeneity in the response of the myocardium to Ang II. Results of the present study indicate that the inotropic effects of Ang II extend to the human myocardium, consistent with the recent demonstration of Ang II receptors in the human heart.\textsuperscript{20} A significant positive dose-response relation to Ang II was demonstrated in atrial muscle from functionally normal hearts [%FSH(LV) within normal range\textsuperscript{33,34}] of cardiac muscle.

**Figure 2.** Plots of comparison of dose-response curves for developed tension in papillary muscles from 300-day normal (\(n=8\)) and cardiomyopathic (\(n=9\)) hamsters. Responses are shown as percent increase over baseline developed tension. Values are given as mean±SEM. Dose-response curves are significantly different from each other at \(p<0.04\).

**Figure 3.** Plots of comparison of dose-response curves for developed tension in hamster papillary muscles pretreated with \(10^{-3}\) M propranolol and \(10^{-3}\) M phentolamine with untreated controls. Responses are indicated as the percent increase over baseline developed tension. Values are given as mean±SEM.
surgical patients as well as in atrial and ventricular muscles from explanted hearts.

In all groups of human and hamster muscles studied, a dose-dependent decrease in RT accompanied the increase in DT in response to Ang II (Table 2). This implies that in the presence of Ang II, cytosolic Ca\(^{2+}\) is decreased below normal levels during relaxation. This may suggest that like catecholamines, Ang II has an effect on the rate of uptake of Ca\(^{2+}\) by the sarcoplasmic reticulum at the end of myocardial contraction or that Ang II stimulation results in a decrease in affinity of the regulatory calcium binding site on troponin C.

In ventricular muscle from failing hearts, the increase in DT in response to Ang II was not accompanied by any change in the timing parameters of contraction, TPT or T\(_{1/2}\)R (Table 2). These data argue against a cyclic AMP–mediated mechanism for the effects of Ang II since such pathways would result in increased rates of release and reuptake of Ca\(^{2+}\) by the sarcoplasmic reticulum, producing decreases in both TPT and T\(_{1/2}\)R.\(^{29,35-37}\) The failure of Ang II to have an effect on the timing parameters in human ventricular muscle was also observed in the hamster muscles used in this study and agrees with earlier studies on the effects of Ang II on ventricular muscle from animal models.\(^6,7\) Catecholamines, which elevate intracellular cyclic AMP, also shorten both TPT and T\(_{1/2}\)R.\(^{29}\) A single dose of 10\(^{-6}\) M isoproterenol administered to the same human ventricular muscles at the end of the Ang II dose-response curve decreased both TPT and T\(_{1/2}\)R in human and hamster ventricular muscles.

In human atrial muscles, although Ang II caused no significant change in TPT, a significant, dose-dependent increase in T\(_{1/2}\)R (\(p=0.02\) and 0.05 for atrial muscles from functionally normal hearts and failing hearts, respectively) was observed. The meaning of this increase in T\(_{1/2}\)R is unclear. Although this pattern of responsiveness (increased DT, no change in TPT, and increased T\(_{1/2}\)R) is different from other inotropic agents whose mechanism of action is known,\(^{29}\) the actions of most inotropic agents have been studied on ventricular tissue, and their effects on atrial tissue are less well characterized. Despite the fact that the mechanism of action of Ang II has not been definitely established, at least one recent study has suggested that its actions on the myocardium may be mediated by the activation of protein kinase C.\(^{38}\) Protein kinase C has been shown to phosphorylate phospholamban in the sarcoplasmic reticulum membrane,\(^{39}\) which could have an effect on sarcoplasmic reticulum Ca\(^{2+}\) uptake and, therefore, on the rate of relaxation, but this mechanism of action might be expected to shorten rather than lengthen T\(_{1/2}\)R. Alternatively, protein kinase C may act via an undefined mechanism to decrease the rate of uptake of Ca\(^{2+}\) by the sarcoplasmic reticulum, or the Ang II–mediated inotropic effect may involve the action of second messengers other than protein kinase C. The fact that T\(_{1/2}\)R increased in response to Ang II in both groups of atrial muscle but not in ventricular muscle may relate to the differences in intracellular calcium handling between the two types of muscle.\(^{40}\)

In addition to the effects of Ang II on T\(_{1/2}\)R, a second difference between atrial and ventricular muscle was the dose-response relation. An inotropic response was first observed in atrial muscle from functionally normal hearts at a dose of 10\(^{-8}\) M Ang II and in atrial muscle from failing hearts at 10\(^{-10}\) M Ang II. Both groups of atrial muscles continued to exhibit an increase in their inotropic response to Ang II at the final dose, 10\(^{-5}\) M Ang II. In ventricular trabeculae, however, an inotropic response was first observed at 10\(^{-10}\) M Ang II, and a plateau was reached at 10\(^{-6}\) M Ang II (Table 2). These data suggest that muscle from failing hearts may be more sensitive to the inotropic effects of Ang II than is muscle from functionally normal hearts. This finding may be related to the demonstration of two classes of Ang II binding sites with different affinities in the guinea pig atrium\(^{11}\) and the rabbit heart.\(^{13}\) The half-maximal contractile response in human ventricular muscle in this study occurred at 5 \(\times\) 10\(^{-8}\) M Ang II, which is close to the K\(_D\) for the high-affinity Ang II binding site identified in the normal human left ventricle\(^{20}\) and the guinea pig\(^{11}\) and rabbit\(^{13}\) hearts. Assuming a maximal or close-to-maximal response had been reached in atrial trabeculae at 10\(^{-5}\) M Ang II, the half-maximal response to Ang II can be calculated as \(\geq\)10\(^{-8}\) M Ang II (atrial trabeculae from explanted hearts) or \(\geq\)10\(^{-7}\) M Ang II (atrial trabeculae from surgical patients). The K\(_D\) for the low-affinity Ang II binding site was shown to be 3 \(\times\) 10\(^{-7}\) M in guinea pig atrium\(^{11}\) and 1 \(\times\) 10\(^{-5}\) M in rabbit heart.\(^{13}\) Our data suggest that the human heart may also possess more than one type of binding site for Ang II and that the two classes of binding sites may exist in different chambers of the heart or may be regulated differentially during heart failure.

The inotropic response to Ang II appeared to be larger in both groups of atrial trabeculae than in ventricular trabeculae (Figure 1), although the difference reached significance only when comparing atrial trabeculae from surgical patients with ventricular trabeculae, most likely due to the large variability in responsiveness in atrial trabeculae from failing hearts. The reason for the difference in responsiveness in atrial and ventricular muscle is unclear. The simplest explanation would be a difference in the number of Ang II receptors between the chambers of the heart. An increased number of Ang II receptors in the right atrium compared with both ventricles has been demonstrated in the normal human heart,\(^{29}\) but it remains to be established whether this pattern holds true in the diseased human heart. Alternatively, the increased inotropic response to Ang II in atrial muscle may result from intrinsic differences between the two types of muscle. The fact that Ang II causes a positive inotropic effect on cardiac muscle suggests that it elevates intracellular Ca\(^{2+}\). Several
established differences between atrial and ventricular muscle imply that their mechanism of handling intracellular Ca\(^{2+}\) may be different. Calcium channels in the atria are predominantly of the T type, whereas those in the ventricles are mainly L-type channels. This difference may be particularly relevant in light of the recent suggestion that Ang II can increase the current through the T-type calcium channels. Additionally, it has been demonstrated that atrial muscle is more sensitive than ventricular muscle to calcium influx from outside the cell. It might also be argued that atrial muscle from failing hearts is not as diseased as the ventricular muscle and that the inotropic response is therefore preserved. The data obtained from normal and myopathic hamsters in this study indicate that normal ventricular muscle exhibits a greater inotropic response to Ang II than does diseased ventricular muscle, but hamster atria still demonstrated a greater inotropic response to Ang II than did either group of ventricular muscles. We have previously demonstrated that the inotropic effect of endothelin is also greater in atrial muscle than in ventricular muscle from either human or rat hearts. The greater inotropic response to Ang II in atrial muscle, therefore, may not be unique to Ang II but may be characteristic of many inotropic agents. Inotropic support to the atrium, especially in the case of a failing ventricle, may have physiological significance since increased contractility during atrial systole can augment ventricular diastolic filling.

**Heterogeneity of Response of Human Trabeculae to Angiotensin II**

Although the inotropic response to Ang II, averaged over all human muscles in each group, is not large compared with the inotropic response to isoproterenol, the averaging of the data from all muscles may not be the most accurate representation of the cardiac muscle response to Ang II because of the demonstrated heterogeneity in responsiveness. Averaging the responses from only those muscles that showed a significant positive response to Ang II demonstrates the potential for a substantial positive inotropic response mediated by Ang II (Figures 1A–1C). The reason for heterogeneity in Ang II responsiveness among muscles remains to be established. In some cases, responsive and nonresponsive muscles came from the same heart, which may relate to the finding of heterogeneity in receptor distribution throughout the human myocardium. A similar pattern of responsiveness to Ang II was observed by Kobayashi and coworkers in the canine myocardium, in which a positive inotropic response to Ang II was demonstrated in five of nine canine cardiac muscle strips, a negative response was seen in one strip, and no response was observed in three strips. In the human heart, Bruckner and coworkers described heterogeneity of responsiveness to the \(\alpha\)-adrenergic agonist phenylephrine with positive inotropic responses in 14 left ventricular muscle preparations. They were unable to explain this phenomenon on the basis of any known differences in muscles or patients. Since several studies have indicated that the intracellular actions of Ang II and \(\alpha\)-adrenergic agonists in cardiac muscle may both involve second-messenger pathways related to the breakdown of membrane phospholipids, the data from this study taken with those of Bruckner et al’s study could suggest that a component of the phosphatidyl inositol signalling pathway may be disrupted during human heart disease.

Among individual muscle characteristics that might explain the observed heterogeneity in response, the fact that there was no relation between Ang II response and cross-sectional area of the muscles indicates that Ang II responsiveness was unrelated to the degree of oxygenation of the muscle preparation in vitro. We investigated a possible relation between muscle cross-sectional area and DT since larger muscles may not be as well oxygenated in vitro and also since it has been suggested that Ang II or its analogues may actually be more effective inotropic agents during hypoxia. However, this analysis revealed that Ang II responsiveness was not related to muscle size. There was also no relation between baseline RT, DT, or T\(_{1/2}\)R and Ang II responsiveness in any of the groups of muscles, indicating that heterogeneity of responsiveness to Ang II cannot be attributed to a difference in baseline performance or to random damage during dissection. In atrial trabeculae, there was also no correlation between baseline TPT and response to Ang II, but a relation was demonstrated between these two variables in ventricular trabeculae (i.e., muscles that required a longer time to reach maximal tension responded better to inotropic stimulation by Ang II). Several investigators have demonstrated that ventricular muscle from failing hearts is characterized by increased values of both TPT and T\(_{1/2}\)R compared with normal muscle and that this may be indicative of altered Ca\(^{2+}\) cycling within the myocardial cell. Our demonstration that increased baseline TPT is correlated with an increased response to Ang II may, again, indicate that the different response to Ang II seen in failing ventricular muscle is related to changes in sarcoplasmic reticulum Ca\(^{2+}\) cycling.

Finally, a positive correlation was demonstrated between Ang II response and the response to isoproterenol in ventricular muscles from failing hearts. Although it has previously been established that \(\beta\)-adrenergic responsiveness is diminished in failing human hearts, results of this study suggest that this alteration is accompanied by a decrease in the inotropic response to Ang II. Although the second messenger through which Ang II exerts its inotropic effects on the heart has not been established, evidence from this study and others suggests that the second messenger is not cyclic AMP. The simultaneous decrease in responsiveness to both agents, therefore, suggests a defect that is common to both inotropic pathways, possibly distal to their second-
messenger systems. Both the β-adrenergic pathway and the Ang II pathway, along with other inotropic agents, presumably cause their inotropic responses by increasing Ca\(^{2+}\) release from the sarcoplasmic reticulum. A defect in sarcoplasmic reticulum calcium release in failing hearts\(^{30,51,50}\) could therefore have an effect on both cyclic AMP–dependent and –independent pathways. The well-described defects in the β-adrenergic signalling pathway in ventricular muscle during heart failure\(^{48,49}\) could thus coincide with a decrease in responsiveness to Ang II and/or other inotropic agents.

In further support of the fact that cardiac failure may modify the inotropic response to Ang II, there was a larger percentage of “responsive” muscles in the group of atrial trabeculae from surgical patients than in either of the other two groups of muscles (Table 2). Additionally, the maximal increase in DT attained in response to Ang II was greatest in atrial trabeculae from functionally normal hearts. It is unlikely that muscle from explanted hearts was more damaged as a result of the transplant procurement procedure than muscle obtained from surgery as there was no significant difference between the baseline parameters of contraction measured in atrial trabeculae from either source (Table 1). The fact that a larger percentage of muscles from surgical patients was responsive to Ang II may imply that although heterogeneity of responsiveness to Ang II does exist in the normal myocardium, the process of cardiac disease has an additional effect on the inotropic response. This contention is supported by the data from the hamster hearts, in which cardiac muscle from normal hearts showed a significantly greater inotropic response to Ang II than did cardiac muscle from the failing hearts of myopathic hamsters. Since Ang II receptor numbers in the human heart\(^{20}\) and cardiomyopathic hamster hearts\(^{18}\) have been shown to be unchanged by heart failure, this decreased responsiveness could be the result of an alteration distal to the receptor. The decreased response could be due to alterations in the uptake of Ca\(^{2+}\) by the sarcoplasmic reticulum in heart failure\(^{30,51,50}\) and an inability to influence the rate of uptake. Alternatively, a defect in another component of the signalling pathway, similar to the increase in G\(_{\beta}\) that is believed to be partially responsible for decreased responsiveness to β-adrenergic agonists in cardiac muscle from heart failure patients,\(^{51}\) could result in diminished responsiveness to Ang II in failing hearts.

The reason for the heterogeneity of Ang II responsiveness in the human myocardium (which was not observed in the hamster heart) has not been established by these studies and will need further investigation. Possible reasons that have not been addressed here might include the level of sodium in the diet, which has been shown to modulate inotropic responsiveness to Ang II,\(^{5} \) and the stimulation of prostaglandin release by Ang II,\(^{52-55}\) which could modify the effects of Ang II on contractile force.\(^{56-58}\)

The physiological significance of an inotropic response to Ang II remains to be established. In light of the discovery of a local renin-angiotensin system within the myocardium,\(^{3,4}\) it is reasonable to hypothesize that fairly high concentrations of Ang II could be achieved locally in the heart without the deleterious vasoconstrictor effects of circulating Ang II. That is, sites of Ang II action in the heart may be compartmentalized. Additionally, the demonstration that Ang II formation in the heart may occur via an alternative converting enzyme that is insensitive to captopril\(^{59}\) implies that local levels of Ang II in the heart could remain high despite therapeutic intervention with Ang II converting enzyme inhibitors.

While some may argue that deleterious effects on the coronary vasculature would arise from such locally high concentrations of Ang II, the question of the effects of Ang II on the coronary arteries in vivo has not been fully resolved. In particular, there is some evidence to support the hypothesis that Ang II releases prostaglandin E\(_2\) and prostacyclin from the endothelial lining of coronary blood vessels\(^{50-52}\) and that these prostaglandins, although they have only minimal vasodilatory actions under baseline conditions, may be potent vasodilators in the case of prior vasoconstriction.\(^{60}\) The balance between the vasoconstrictor actions of Ang II and the vasodilator actions of the prostaglandins would then determine the degree of coronary blood flow. Some experimental evidence supports Ang II–dependent vasodilation\(^{60,61}\) or only minimal vasoconstriction\(^{62}\) in the coronary vasculature and shows that pretreatment with indomethacin significantly increases the Ang II vasoconstrictor effects on coronary arteries.\(^{61}\) The balance of vasoconstrictor and vasodilator effects may change as heart failure progresses and vasodilator mechanisms become limited,\(^{60}\) but coronary vasoconstriction may not be a limitation of Ang II–mediated inotropic support to the heart, at least in the early stages of heart failure.

In summary, our demonstration of a direct inotropic action of Ang II on human myocardium in both diseased and healthy hearts may suggest an important cardiac action of this naturally occurring peptide under both physiological and pathological conditions in addition to and independent of its vascular and hemodynamic effects.

References


51. Feldman AM, Cates AE, Veazey WB, Hershberger RE, Bristow MR, Baughman KL, Baumgartner WA, Van Dop C:


KEY WORDS • angiotensin II • cardiac muscle • Syrian cardiomyopathic hamster • cardiomyopathy
Inotropic effects of angiotensin II on human cardiac muscle in vitro.
C S Moravec, M D Schluchter, L Paranandi, B Czerska, R W Stewart, E Rosenkranz and M Bond

doi: 10.1161/01.CIR.82.6.1973

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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/82/6/1973

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/