Regulation of Angiotensin II Receptors on Ventricular Myocytes After Myocardial Infarction in Rats

Leonard G. Meggs, Joseph Coupet, Harer Huang, Wei Cheng, Peng Li, Joseph M. Capasso, Charles J. Homcy, and Piero Anversa

To determine the effects of acute myocardial infarction on the regulation of angiotensin II (Ang II) receptors and contractile performance of left and right ventricular myocytes, coronary artery ligation was surgically induced in rats, and Ang II receptor density and affinity and the mechanical properties of surviving muscle cells were examined 1 week later. Physiological determinations of cardiac pump function revealed the presence of ventricular failure, which was associated at the cellular level with a depression in the velocity of myocyte shortening and lengthening, a prolongation of time to peak shortening, and a reduction in the extent of cell shortening. These abnormalities in single-cell function were more prominent in left than in right ventricular myocytes. Cellular hypertrophy was documented by increases in cell length and width, which were also greater in the spared myocytes of the infarcted left ventricle. Reactive hypertrophy was accompanied by a 1.84- and 1.85-fold increase in the density of Ang II receptors on left and right myocytes, respectively. On the other hand, the affinity of Ang II receptors for the radiolabeled antagonist was not altered. However, Ang II-stimulated phosphoinositol turnover was enhanced by 3.7- and 2.5-fold in left and right myocytes, respectively, after infarction. Ventricular myocytes were found to possess the AT1 receptor subtype exclusively. In conclusion, myocardial infarction leads to impairment in the contractile behavior of the remaining cells and to the activation of Ang II receptors and effector pathway associated with these receptors, which may be involved in the reactive growth adaptation of the viable myocytes. (Circulation Research 1993;72:1149–1162)

KEY WORDS • myocyte growth • phosphoinositol turnover • ventricular loading • angiotensin II receptors

Studies investigating the mechanisms of acute and chronic myocyte growth after infarction have documented that hypertrophy in the spared left ventricular tissue results from increases in myocyte diameter and length associated with an augmentation in systolic and diastolic wall stress. At the subcellular level, the lateral expansion of myocytes is accomplished through the parallel addition of newly formed myofibrillar units, whereas myocyte lengthening is brought about by replication of sarcomeres in series with no change in sarcomere length. However, it remains to be determined whether the nature of the mechanical stimulus on myocytes selectively activates specific surface receptors, which may engender the addition in parallel of contractile elements, i.e., pressure hypertrophy, the in-series addition of sarcomeres (volume hypertrophy), or both. On the basis of observations showing that stimulation of surface α1-adrenoreceptors in vitro induces hypertrophy of neonatal and adult cardiac myocytes, increases the rate of myosin light chain-2 transcription, and mediates a transcriptional change in sarcomeric actin isoforms, transduction of signals via this receptor and its effector pathway were examined 1 week after myocardial infarction and left ventricular failure. The reactive hypertrophic growth adaptation of viable myocytes was found to be accompanied by an enhanced norepinephrine-stimulated phosphoinositol turnover and upregulation of the fetal sarcomeric actin isoform. These findings provided a construct for the hypothesis that α1-adrenoreceptors, singularly or in concert with other growth-promoting factors, may regulate myocyte hypertrophy. On the other hand, if the α1-adrenoreceptor controls only one component of the changes in myocyte length and diameter, other surface receptors may be implicated in the modulation of myocyte growth. Although the demonstration of angiotensin II (Ang II) as a mediator of cell growth in adult cardiac myocytes is lacking, observations of in vitro systems and of the effects of converting enzyme inhibition on pressure-overload hypertrophy strongly suggest that Ang II receptors may be implicated in the regulation of growth in these cells. Cardiac hypertrophy produced by renal and mechanical hypertension, which involves a lateral expansion of myocytes, may be fully prevented by the administration of angiotensin converting
enzyme inhibitors at doses that do not affect the magnitude of the afterload. In addition, a functional cardiac renin-angiotensin system appears to be a potential mediator of myocardial hypertrophy, and it seems to operate independent of circulating plasma Ang II levels. By inference, Ang II receptors may be responsible for the increases in myocyte diameter generated by the elevated afterload stress after infarction. Therefore, to characterize the role of these receptors in the setting of myocardial infarction, Ang II receptors were identified by radioligand binding, and signal transduction via these receptors was determined in left and right ventricular myocytes 1 week after coronary artery occlusion. This approach required the enzymatic dissociation of myocytes, which precluded the assessment of cardiac dimension of the intact heart. Such a limitation constrained the derivation of ventricular wall stress, which has been postulated to be an important determinant of myocyte growth. Thus, the evaluation of myocyte morphology was used in the present study as an index of the nature of the prior mechanical load on ventricular myocytes after infarction. The 7-day time interval was selected to expand the previous findings for α₁-adrenoreceptors in this animal model.

Materials and Methods

Myocardial Infarction

Male Sprague-Dawley rats weighing approximately 280 g were used for the surgical induction of myocardial infarction. Under ether anesthesia, by means of a procedure previously described, the thorax was opened, and the heart was exteriorized by applying a light pressure on the abdomen. The left coronary artery was ligated near its origin, the chest was closed, and the animals were allowed to recover. Sham-operated rats were similarly treated, with the exception that the ligature was not tied. In view of the objective of the present study in which ventricular failure was the end point, large myocardial infarcts had to be obtained, and this phenomenon was accompanied by nearly 60% mortality.

Ventricular Pressure Dynamics

One week after surgery and just before they were killed, the animals were anesthetized with chloral hydrate (300 mg/kg i.p.), and the right carotid artery was cannulated with a microtip pressure transducer catheter (model PR 249, Millar Instruments, Houston, Tex.). After monitoring arterial blood pressure, the transducer was advanced into the left ventricle for the evaluation of left ventricular pressures and dP/dt. An additional microtip pressure transducer with 120° curved tip was inserted in the right jugular vein and advanced through the superior vena cava and the right atrium into the right ventricular chamber for the measurements of right ventricular pressures and dP/dt.

Systemic Flow Measurements

After completion of ventricular pressure measurements, the rats were respirated with room air. A thoracotomy was performed, and a calibrated ultrasonic silastic flow probe (model DGF-110A-CP, 10 MHz, 2.0–2.5 mm i.d., Crystal Biotech, Hopkinton, Mass.) was placed around the ascending aorta. The flow probe was connected to a multichannel directional pulsed-Doppler device operating at 10 MHz. A high-speed pulsed-Doppler velocimeter (model HVPD-10) was used in conjunction with the flow probe to determine stroke volume and cardiac output. Bursts of eight cycles (0.4 μsec) were directed into the blood vessel by a piezoelectric transducer mounted in the probe at an angle of 45° to the vessel. The resulting echoes from the vessel walls and the blood cells were received by the same transducer and were amplified electronically. The returning echoes were separated in time according to distance, and time gating was used to select a portion of the returned echoes for processing. The Doppler difference frequency was obtained from the comparison of the phase shift between the echoes and the transmitting oscillator and the velocity sensed across the vessel. The product of the integration of the velocity profile across the vessel lumen and internal vessel cross-sectional area yielded average velocity. Under conditions of laminar flow with a parabolic velocity profile, the maximum velocity was calculated at twice the average velocity.

Criteria for Inclusion in the Study

Ventricular hemodynamic parameters were used to establish the presence of left-side failure, which was considered a prerequisite for inclusion in the study. Moreover, it has previously been shown that impairment in left ventricular function is associated with infarcts affecting nearly 50% of the myocyte population of this chamber. In essence, physiological measurements were used not only to document heart failure but also as an indirect estimation of infarct size. Under similar experimental conditions, infarct size has previously been shown to include nearly 50% of the left ventricular wall.

Myocyte Isolation

Calcium-tolerant myocytes were isolated according to a previously described procedure. In brief, rats were heparinized (500 units i.p.) and killed by decapitation. Hearts were excised and placed on a stainless-steel cannula for retrograde perfusion through the aorta. Each ventricle was separated and further processed to obtain muscle cells from each side of the heart.

The solutions were supplements of Joklik modified Eagle’s minimal essential medium (MEM) (DMC317, K.C. Biological). HEPES-MEM contained (mM) NaCl 117, KCl 5.7, NaHCO₃ 4.4, KH₂CO₃ 1.5, MgCl₂ 1.7, HEPES 21.1, glucose 11.7, and 1-glutamine 2, along with amino acids and vitamins and 21 units/ml insulin. The pH was adjusted to 7.2 with KOH. This solution is 292 mosm (isomolar with rat serum) and is the standard procedure contained no added calcium, although the measured calcium concentration was 5 μM. Resuspension medium was HEPES-MEM supplemented with 0.5% bovine serum albumin, 0.3 mM calcium chloride, and 10 mM taurine adjusted to 292 mosm. The cell isolation procedure consisted of three main steps: 1) low calcium perfusion, 2) mechanical tissue dissociation, and 3) separation of intact cells.

Low calcium perfusion. Blood washout and collagenase (selected Worthington type II) perfusion of the heart was carried out at 34°C with HEPES-MEM gassed with 85% O₂–15% N₂.

Mechanical tissue dissociation. After removing the heart from the cannula, the left ventricle inclusive of the
interventricular septum and the right ventricular free wall were separated and minced. Collagenase-perfused tissue was subsequently shaken in resuspension medium containing creatine, collagenase, and 1.2 mM CaCl₂. Supernatant cell suspensions were washed and placed in resuspension medium.

Separation of intact cells. Intact cardiac cells were enriched by centrifugation through Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden). Approximately 10⁶ cells were suspended in 10 ml isotonic Percoll (final concentrations, 41% in resuspension medium) and centrifuged for 15 minutes at 34g. Intact cells were recovered from the pellet, washed, and used as described below. By this methodology, the average yield of myocytes in infarcted hearts was approximately 7.0×10⁶ cells from the left ventricle and 4.0×10⁶ cells from the right ventricle. Corresponding values in sham-operated control hearts were 13×10⁶ cells and 4.0×10⁶ cells. Cell viability was assessed by Trypan blue exclusion and found to be nearly 90%.

Mechanical and Structural Properties of Myocytes

The isolated muscle cells were placed in an open perfusion microincubator cell bath (model PDMI-2, Medical Systems Corp., Greenvale, N.Y.) located on the stage of an inverted microscope (Axiovert 10, Zeiss, FRG). The composition of the buffer solution was (mM) NaCl 117, KCl 5.7, CaCl₂ 1.2, NaHCO₃ 4.4, KH₂CO₃ 1.5, MgCl₂ 1.7, HEPES 21.1, glucose 11.7, and t-glutamine 2, along with amino acids and vitamins and 21 units/ml insulin. The pH was adjusted to 7.2. Temperature was maintained at 30±0.2°C by a bipolar temperature controller (model TC-202, Medical Systems). External bath calcium was maintained at 1.2 mM, and myocytes were stimulated at 1.0 Hz by rectangular depolarizing pulses 5 msec in duration and twice diastolic threshold in intensity by platinum electrodes on either side of the cell and parallel with its long axis. Twenty myocytes from the left and right ventricles of each animal were evaluated mechanically as follows: A high-speed camera was attached to the eyepiece of the inverted microscope, and isolated myocytes were photographed at a final magnification of ×850 during contraction at a rate of 500 frames per second. This real image of the cell was obtained every 2 msec. This camera was synchronized with the stimulus pulse, and the unloaded velocity of cell shortening and lengthening and the time to peak shortening were obtained during still-frame playback by use of a computerized image analysis system.¹⁷,¹⁸ The entire apparatus necessary for measuring mechanics of isolated myocytes rests on an optical table, which is supported by four pneumastable isolators to eliminate extraneous horizontal and vertical vibration (model 13814, Oriel Corp., Stratford, Conn.).

After enzymatic isolation of myocytes, a drop of the solution containing the single contractile cells was placed in the chamber of the cell bath. Additional isolation solution was added to the bath to bring the volume to approximately 10 ml to maintain solution temperature at 30°C. Moreover, dilution of the isolated cells was necessary to prevent contact between cells during contraction and thus interference with estimations of mechanical and structural evaluations. Any cell chosen for inclusion in the present study was used for both mechanical and morphometric evaluations. Only cells that were contracting synchronously with stimulation and showed no evidence of extraneous contractile activity (i.e., aftercontractions, wavelike motions in the sarcomeres) were selected.¹⁷,¹⁸ In light of these criteria, the myocytes were then selected randomly by moving the muscle bath from right to left until 20 actively contracting cells were examined mechanically (four cells were selected from each of the four corners and the middle of the rectangular configuration outlined by the stimulating wires).

General measurements of myocyte geometric dimensions were accomplished through the aid of a computerized image analysis system (Morrell Instruments, Long Island City, N.Y.). The 20 myocytes from the left and right ventricles of each animal were measured to obtain length and width. Moreover, cell volume was derived from these geometric parameters (see below). The distribution of myocytes isolated from the ventricles was divided according to the fraction of cells in an established range of lengths. The range of lengths used in the present study was from 60 to 220 μm. Histogram buckets were then established with a size of 10 μm, and frequency distribution histograms were constructed by plotting the percentage of cells on the ordinate and cell length range on the abscissa. After construction of the cell-length histograms, a gaussian curve was fit to the data by calculation of the probability density function of each ordinate value, f(y), from the mean and standard deviation and plotting f(y) against the range of cell lengths.¹⁷,¹⁸ A similar approach was followed for the analysis of cell width, which corresponds to the major axis described below.

When placed in physiological medium, isolated cells assume a cross-sectional area that resembles a flattened ellipse. On average, the minor axis of this ellipse was one-fourth that of the major axis. The ratio between the major and minor axes was determined by focusing on the edge of the myocyte in the cell bath. Cell volume (Vc) was calculated on the basis of an elliptical cross section with a major axis that was equivalent to cell width and a minor axis that was one-fourth of the major axis, whereas cell length (L) was measured directly. Thus,

\[ V_c = \pi a b L \]

where a is the major axis and b is the minor axis. Ten sham-operated and 10 infarcted rats were used in this part of the investigation. These animals were subgroups of those used for the determination of Ang II–stimulated phosphoinositol turnover.

Identification and Characterization of Ang II Receptors on Ventricular Myocytes

Myocytes isolated from unoperated rats were used first to detect the presence of Ang II binding sites and subsequently Ang II receptor subtypes. Competition curves with peptide analogues of Ang II and nonpeptide Ang II receptor–selective antagonists were performed by use of membranes prepared from isolated ventricular myocytes (see below). Scatchard transformation of binding isotherms was used to determine the affinity constant (Kc) and the maximal number of binding sites (Bmax) for the Ang II receptor ligand ¹²⁵I-[Sar¹,Ile⁸]Ang...
II in these membranes. Thirty unoperated adult rats were used in this part of the investigation.

**Ang II Receptor Assay**

Purified myocytes were collected by centrifugation and homogenized with a Polytron (setting 8, twice for 15 seconds each, 5.0×10⁶ cells, Brinkmann Instruments, Inc., Westbury, N.Y.). This procedure was accomplished in ice-cold 0.25 M sucrose containing 0.03 M histidine, 1 mM EDTA, and 0.1 M phenylmethylsulfonyl fluoride (PMSF). The homogenate was centrifuged at 14,000g for 20 minutes, and the pellet was discarded. Subsequently, the supernatant was centrifuged at 45,000g, and the resulting pellet was saved. This pellet was resuspended in 50 mM Tris-HCl containing 10 mM MgCl₂, 1 mM EDTA, and 0.1 M PMSF to give a protein concentration of 1–2 mg/ml. Protein concentration was determined by the method of Lowry et al. All preparatory procedures were performed at 4°C. Membranes were immediately used for the radioligand binding assay.

Membrane proteins (140–180 μg) were incubated in 50 mM Tris-HCl buffer, pH 7.4, containing 0.1 mM PMSF and 0.2% heat-inactivated bovine serum albumin, with increasing concentrations of the radiolabeled Ang II antagonist [125I]-[Sar²,Ile⁶]Ang II (specific activity, 2,200 Ci/mmol, New England Nuclear, Boston). The reaction was initiated by the addition of membrane protein and continued for 60 minutes at 22°C. Total incubation volume was 200 μl. Nonspecific binding was measured in the presence of 1.0 μM unlabeled [Sar²,Ile⁶]Ang II. The binding assay was terminated by rapid vacuum filtration over glass-fiber filters (Gelman Sciences Inc., Ann Arbor, Mich.). The filters were washed three times with 4 ml of 0.9% NaCl containing 0.02% bovine serum albumin. Bound radioactivity was determined by an automatic gamma counter at a counting efficiency of 80%. Saturation isotherms were performed with increasing concentrations of [125I]-[Sar²,Ile⁶]Ang II between 0.01 and 2 nM. Binding assays were done in duplicate or triplicate. Specific binding was defined as the portion of total counts displaced by 1 μM [Sar²,Ile⁶]Ang II. Similar inhibition was seen with Ang II or with nonpeptide antagonists. At ligand concentrations equivalent to the Kᵅ, specific binding averaged 85%. Values in figures and tables refer to specific binding. In displacement experiments, competing unlabeled peptide and nonpeptide antagonists were used over a wide concentration range. Twenty-four sham-operated and 24 infarcted rats were used in this part of the investigation.

**Ang II Receptors and Phosphoinositol Turnover**

In preliminary studies, the ability of Ang II to stimulate phosphoinositol turnover in ventricular myocytes was established. This was accomplished by incubating myocytes prelabeled with [³H]myoinositol with Ang II. The specificity of this response was demonstrated by the ability of the potent nonpeptide AT₁ receptor subtype antagonist 5,7-dimethyl-2-ethyl-3-[2’-(1H-tetrazol-5-yl)[1,1’-biphenyl]-4-yl]methyl]-1H-imidazo[4,5-b]pyridine monohydrate (L-158809) to inhibit the increase in phosphoinositol metabolism in the presence of Ang II. Fifteen unoperated adult rats were used in this part of the investigation.

**Ang II–Stimulated Phosphoinositol Turnover**

Ventricular myocytes isolated from the left and right ventricles were suspended in 37°C modified Krebs-Henseleit buffer containing (mM) NaCl 118, KCl 4.7, KH₂PO₄ 1.2, CaCl₂ 0.8, MgSO₄ 1.2, NaHCO₃ 20.6, and glucose 11, pregassed with 95% O₂–5% CO₂, and allowed to equilibrate for 1 hour with adjustments of pH to 7.2 and three changes of buffer. Subsequently, myocytes were labeled with 20 μCi myo-[²²H(N)]inositol (specific activity, 15 Ci/mmol, American Radiochemical Co., St. Louis, Mo.) in 2.5 ml fresh buffer. After 60 minutes, cells were washed three times with 20 ml warm buffer as described above but containing 111 mM NaCl and 7 mM LiCl to remove unincorporated [³H]myoinositol and soluble proteases. Myocytes were then gravity-packed and distributed in 30-μl aliquots among flat-bottom vials in the same buffer to which 100 μM Ang II was added. Vials were gassed, and incubation was carried out for 60 minutes. Vials for basal activity did not contain Ang II. Each determination was performed with 125,000 myocytes. The reaction was terminated by adding 900 μl chloroform/methanol/12 M HCl (100:200:1 [vol/vol/vol]), vortexing for 30 seconds, and storing overnight at 4°C. Subsequently, total labeled inositol phosphates formed were measured by the method described by Berridge et al. Briefly, chloroform (300 μl) and H₂O (300 μl) were added to separate the aqueous and organic phases. The mixture was vortexed and centrifuged at 1,500g for 2 minutes. The upper aqueous phase (800 μl) was removed and diluted with 2 ml water to reduce the ionic strength. The mixture was kept in hot (40–50°C) water for 15 minutes to expel traces of chloroform. The aqueous fluid was applied onto a column made of a 2-ml slurry of Dowex (50% [vol/vol], AG 1×8, 100–200 mesh, formate form, Bio-Rad Laboratories, Richmond, Calif.). The columns were first washed with 20 ml distilled water to remove free [³H]inositol and then with 12 ml of 60 mM sodium formate/5 mM disodium tetraborate to remove [³H]glycerophosphoinositol. Total [³H]inositol phosphate was eluted two times with 3 ml of 200 mM ammonium formate in 100 mM formic acid. Three milliliters of the eluate was added to 18 ml aqueous counting scintillant (Amersham Corp., Arlington Heights, Ill.) for liquid scintillation counting. For determination of the [³H]inositol incorporated into lipid, the remaining upper phase and interface were aspirated, and 50-μl aliquots of the chloroform layer were evaporated to dryness and counted in 10 ml organic counting scintillant (Amersham). The results were expressed as the fraction of the total radioactivity incorporated, which was converted to [³H]inositol phosphates. Eight sham-operated and 12 infarcted rats were used in this part of the investigation.

**Myocardial Infarction and Ang II Receptor Antagonist**

Five infarcted rats were exposed to a dose of 2 mg/kg body wt L-158809 in the drinking water immediately after surgery and throughout the 7-day experimental period. Subsequently, the hemodynamic characteristics were established and, after myocyte isolation, average cell length and width were determined as described above.

**Statistical Analysis**

Values are reported as mean±SD. Comparisons between values were performed by a two-tailed unpaired test.
TABLE 1. Effects of Myocardial Infarction on Ventricular Performance

<table>
<thead>
<tr>
<th></th>
<th>Sham-operated rats</th>
<th>Infarcted rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>373.5±34.9</td>
<td>440.0±53.2*</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>113.3±6.2</td>
<td>91.7±10.7*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>79.9±10.0</td>
<td>67.6±12.7*</td>
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<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>91.1±7.7</td>
<td>75.6±11.7*</td>
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<tr>
<td>LVEDP (mm Hg)</td>
<td>5.9±2.4</td>
<td>23.2±11.4*</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>114.1±6.7</td>
<td>91.9±10.7*</td>
</tr>
<tr>
<td>LVDP (mm Hg)</td>
<td>108.3±6.2</td>
<td>68.6±13.6*</td>
</tr>
<tr>
<td>LV +dP/dt max (mm Hg/sec)</td>
<td>12,107±1,421</td>
<td>7,779±806*</td>
</tr>
<tr>
<td>LV −dP/dt max (mm Hg/sec)</td>
<td>9,981±1,315</td>
<td>6,662±952*</td>
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<tr>
<td>RVEDP (mm Hg)</td>
<td>1.6±0.8</td>
<td>7.0±2.5*</td>
</tr>
<tr>
<td>RVSP (mm Hg)</td>
<td>26.5±1.9</td>
<td>31.1±3.4*</td>
</tr>
<tr>
<td>RVDP (mm Hg)</td>
<td>24.9±1.9</td>
<td>24.1±2.5</td>
</tr>
<tr>
<td>RV +dP/dt max (mm Hg/sec)</td>
<td>3,000±363</td>
<td>2,531±322*</td>
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<tr>
<td>RV −dP/dt max (mm Hg/sec)</td>
<td>2,250±351</td>
<td>2,057±343</td>
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<tr>
<td>CVP (mm Hg)</td>
<td>1.7±0.9</td>
<td>5.6±0.9*</td>
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<tr>
<td>Cardiac output (ml/min)</td>
<td>84.6±7.4</td>
<td>50.3±10.1*</td>
</tr>
<tr>
<td>Cardiac index (ml·min⁻¹·kg⁻¹)</td>
<td>270.1±23.6</td>
<td>182.8±36.9*</td>
</tr>
<tr>
<td>Stroke volume (µl)</td>
<td>218.4±21.9</td>
<td>119.9±23.5*</td>
</tr>
<tr>
<td>Stroke volume index (µl/kg)</td>
<td>697±65</td>
<td>436±89*</td>
</tr>
</tbody>
</table>

bpm, Beats per minute; LVEDP, left ventricular (LV) end-diastolic pressure; LVSP, LV systolic pressure; LVDP, LV developed pressure; RVEDP, right ventricular (RV) end-diastolic pressure; RVSP, RV systolic pressure; RVDP, RV developed pressure; CVP, central venous pressure. Values are mean±SD. Measurements of cardiac output were collected from 11 sham-operated and 11 infarcted rats. All other parameters were obtained from 32 sham-operated and 36 infarcted rats. *p<0.05 vs. control value.

Results

Ventricular Pressure Dynamics

Measurements of systemic arterial blood pressure in sham-operated and infarcted rats indicated that a fall in systolic, diastolic, and mean arterial pressures developed 7 days after myocardial infarction (Table 1). In contrast, heart rate was significantly increased. The alterations in left ventricular pump dynamics are also listed in Table 1. Left ventricular end-diastolic pressure increased 3.9-fold, from 5.9 to 23.2 mm Hg. However, a 19% reduction in left ventricular peak systolic pressure occurred in experimental animals in association with a 37% decrease in left ventricular developed pressure. Moreover, a substantial depression in +dP/dt (36%) and −dP/dt (33%) was evident in infarcted hearts. When +dP/dt was normalized by dividing the measured peak rate of pressure rise by the instantaneous ventricular pressure (Pi), a 24% reduction in this parameter was found (control hearts, +dP/dt/Pi=152±23 mm Hg·sec⁻¹·mm Hg⁻¹; experimental hearts, +dP/dt/Pi=115±23 mm Hg·sec⁻¹·mm Hg⁻¹; p<0.0001).

The pumping ability of the right side of the heart after infarction is also shown in Table 1. In a manner comparable to that observed in the left ventricle, right ventricular end-diastolic pressure was markedly increased (4.4-fold) in experimental animals. Compared with the left ventricle, peak systolic pressure in the right ventricle was found to be elevated in infarcted hearts (17%). The combination of these changes led to a 3% reduction in right ventricular developed pressure, which, however, was not statistically significant. Moreover, the right ventricular peak rate of pressure rise was decreased 16%, whereas the peak rate of pressure decay was unaffected in rats exposed to coronary artery occlusion. In contrast, central venous pressure was elevated with infarction.

Table 1 lists last in vivo estimations of myocardial pump performance in control and experimental rats. Myocardial infarction resulted in a marked decrease in cardiac output (41%) and stroke volume (45%). Both these changes were statistically significant. In summary, myocardial infarction resulted in global cardiac failure.

Heart Weight

The necessity of isolating myocytes from the left and right ventricular myocardium precluded the separation of the left and right ventricles for individual weight determinations. Heart weight was 888±87 mg in sham-operated control animals and 884±95 mg in experimental animals. Since body weight was reduced by 15% in infarcted rats (control rats, 313±16 g; experimental rats, 267±15 g), the heart weight/body weight ratio increased...
after infarction (control rats, 2.8±0.3 mg/g; experimental rats, 3.3±0.4 mg/g). The 18% change in this ratio was statistically significant. In summary, cardiac hypertrophy appeared to develop 7 days after myocardial infarction.

**Structural Myocyte Characteristics**

Two hundred myocytes from the left and right ventricles of sham-operated and infarcted animals were categorized according to cell length and cell width. As illustrated in Figure 1, the distribution of myocyte length from control left ventricles (panel A) differed from that obtained from myocytes isolated from infarcted left ventricles (panel C). In the former case, most cell lengths were clustered around the mean value, although some scatter was apparent. After myocardial infarction, cell length in the surviving left ventricular myocytes was more widely dispersed. In addition, the peak of the curve was shifted toward higher values. When these data were combined to yield average cell length dimension, this parameter was found to be 121±12 μm in sham-operated rats and 139±13 μm in experimental rats. The 14.4% increase with infarction was statistically significant (p<0.005).

A comparable analysis performed on right ventricular myocytes from control (Figure 1B) and experimental (Figure 1D) rats demonstrated that myocardial infarction minimally affected cell length on this side of the heart. This is reflected by no apparent shift in the distribution of myocyte lengths and in their clustering pattern. Mean myocyte length was 120±14 and 128±10 μm in control and infarcted hearts, respectively. The modest 7% increase with infarction was not statistically significant.

Figure 2 illustrates the effects of myocardial infarction on myocyte width distribution. In comparison with control animals, this cellular dimension increased after infarction, leading to a shift to the right of the gaussian curve for the left ventricle. This change was less apparent for right ventricular myocytes. The differential rightward shift in the two ventricles with infarction was reflected by a smaller change in the average increase in myocyte diameter in right ventricular muscle cells. Myocyte width was 26.4±3.2 and 30.2±4.1 μm in left ventricular myocytes of sham-operated and infarcted rats, respectively. The 14.3% widening detected in the latter group was statistically significant (p<0.03). Corresponding values for right ventricular myocytes were 22±3 and 24±2.8 μm. The 8.8% increase found in these cells was not statistically significant.

Cell-volume changes after infarction were calculated by assuming an elliptical cross-sectional area with a minor radius one fourth of the major radius. By this approach, it could be seen that myocyte cell volume increased 49%.
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panel B sham-operated (panels C and D) rats. The gaussian distribution curve of LV myocytes after infarction is shifted to the right (compare panel A with panel C), whereas a less apparent change is noted for RV myocytes (compare panel B with panel D). For control studies, n=10 rats and 200 myocytes; for experimental studies, n=10 rats and 200 myocytes.

Figure 2. Distribution of left ventricular (LV) and right ventricular (RV) myocytes plotted as a function of cell width in sham-operated (panels A and B) and infarcted (panels C and D) rats. The gaussian distribution curve of LV myocytes after infarction is shifted to the right (compare panel A with panel C), whereas a less apparent change is noted for RV myocytes (compare panel B with panel D). For control studies, n=10 rats and 200 myocytes; for experimental studies, n=10 rats and 200 myocytes.

Figure 3 shows the changes in myocyte cell shortening between sham-operated and infarcted rats. Since differences in cell length were found after infarction (Figure 1), myocyte shortening was expressed as a percent of overall myocyte length. By this approach, it was determined that cell shortening was decreased 44% (p<0.0001) in left ventricular myocytes of infarcted hearts (Figure 3A). The corresponding change in right ventricular myocytes was 10%, but this depression in cell shortening did not attain statistical significance (Figure 3B). These alterations in cell shortening were accompanied by an increase in time to peak shortening, which was more pronounced in myocytes obtained from the infarcted left ventricle (46%, p<0.0001) than in myocytes isolated from the noninfarcted right ventricle (9%, p<0.004) (Figures 3C and 3D).

Figure 4 documents how the unloaded velocity of muscle cell shortening was affected after myocardial infarction. This cellular property was decreased by 33% (p<0.0001) in left myocytes (panel A) and 6% (p=NS) in right myocytes (panel B). Moreover, the velocity of myocyte relengthening was reduced by 42% (p<0.0001) in left myocytes (panel C) and 14% (p<0.01) in right myocytes (panel D). In summary, myocyte mechanical behavior was impaired biventricularly after infarction.

Myocyte Mechanical Characteristics

Figure 3 shows the changes in myocyte cell shortening between sham-operated and infarcted rats. Since differences in cell length were found after infarction (Figure 1), myocyte shortening was expressed as a percent of overall myocyte length. By this approach, it was determined that cell shortening was decreased 44% (p<0.0001) in left ventricular myocytes of infarcted hearts (Figure 3A). The corresponding change in right ventricular myocytes was 10%, but this depression in cell shortening did not attain statistical significance (Figure 3B). These alterations in cell shortening were accompanied by an increase in time to peak shortening, which was more pronounced in myocytes obtained from the infarcted left ventricle (46%, p<0.0001) than in myocytes isolated from the noninfarcted right ventricle (9%, p<0.004) (Figures 3C and 3D).

Figure 4 documents how the unloaded velocity of muscle cell shortening was affected after myocardial infarction. This cellular property was decreased by 33% (p<0.0001) in left myocytes (panel A) and 6% (p=NS) in right myocytes (panel B). Moreover, the velocity of myocyte relengthening was reduced by 42% (p<0.0001) in left myocytes (panel C) and 14% (p<0.01) in right myocytes (panel D). In summary, myocyte mechanical behavior was impaired biventricularly after infarction.

Ang II Receptors on Myocytes

The specificity and selectivity of the radioligand $^{125}$I-[Sar$^1$,Ile$^1$]Ang II to label Ang II receptors on ventricular myocytes were demonstrated by performing a series of competition curves with peptide and nonpeptide competitors of Ang II. The nonpeptide receptor subtype antagonists Dup 753 and PD 123319 were used to identify AT$_1$ and AT$_2$ receptor subtypes. The determinations illustrated in Figure 5 were all done in duplicate, and two or three separate assays were performed in each case.
The results illustrated in Figure 5 indicate that myocytes possess the AT₁ receptor subtype exclusively. The Hill coefficient for displacement of the radioligand by Dup 753 was 0.98, consistent with the recognition of a uniform set of binding sites (panel A). The calculated $K_i$ for Dup 753 was 0.62 nM (panel B). In contrast, PD 123319 failed to displace the radioligand, and the Hill coefficient (0.16) indicated the absence of recognizable AT₂ binding sites (panel C). In addition, $K_i$ was greater than 1,000 nM (panel D). On the other hand, the nonselective peptide analogue [Sar¹, Ile⁸]Ang II displaced the radioligand as expected. The Hill coefficient and calculated $K_i$ for this partial agonist were 0.73 and 0.1 nM, respectively (panels E and F).

To further document whether Ang II receptor subtypes in myocytes are exclusively AT₁, membranes were incubated with $^{125}$I-[Sar¹, Ile⁸]Ang II and 5 mM dithiothreitol. This assay was done in duplicate and performed two times. The latter compound inactivates AT₁ binding sites and, as expected, prevented the interaction between the radioligand and the ligand recognition unit of the receptor. The presence of dithiothreitol fully interfered with the identification of binding sites (data not shown).

Figure 3. Bar graphs showing effects of acute myocardial infarction on the mechanical properties of ventricular myocytes. Cell shortening is decreased in left ventricular myocytes (panel A) and is unchanged in right ventricular myocytes (panel B). Time to peak shortening (TPS) is prolonged in both left (panel C) and right (panel D) ventricular myocytes after infarction. Results are presented as mean±SD. *Statistically significant at $p<0.05$ (n=10 each for sham-operated and infarcted rats).

Figure 4. Effects of acute myocardial infarction on the mechanical properties of ventricular myocytes. Velocity of myocyte shortening ($V_s$) is reduced in left ventricular myocytes (panel A) and is unchanged in right ventricular myocytes (panel B). Velocity of myocyte relengthening ($V_r$) is reduced in both left (panel C) and right (panel D) ventricular myocytes after infarction. Results are presented as mean±SD. *Statistically significant at $p<0.05$ (n=10 each for sham-operated and infarcted rats).
Figure 6 shows a binding isotherm and Scatchard transformation of the radioligand $^{125}$I-[Sar$^1$,Ile$^8$]angiotensin II binding to myocyte membranes obtained from hearts of unoperated rats. This analysis demonstrated a homogeneous set of binding sites with high affinity. Since the density of binding sites ($B_{max}$) was 13.4 fmol/mg membrane protein, the number of receptors per myocyte was calculated to be 1,493.$^{25}$ The affinity of the receptor for the radioligand ($K_d$) was 0.20 nM.

It was indicated in “Materials and Methods” that the membrane preparations were obtained by discarding the 14,000g spin to eliminate cellular debris and nuclear particles. However, the possibility had to be raised that binding sites could have been lost in this fraction. Therefore, binding isotherms were performed on the resuspended pellet to determine whether the low binding capacity stated above was affected by this protocol. No specific binding was detected in the 14,000g fraction, confirming the validity of the procedure. This analysis was repeated three times (data not shown). In summary, ventricular myocytes possess a finite number of Ang II receptors, which are restricted to the AT$_2$ subtype.
Ang II Receptors on Myocytes and Myocardial Infarction

Figure 7 illustrates the effects of myocardial infarction on the density of Ang II receptor binding sites in the surviving myocytes of the left ventricle and the noninfarcted right ventricle. This parameter was found to be increased in experimental animals by 1.84-fold in left myocytes and by 1.85-fold in right myocytes. Ang II receptor density in left and right myocytes was comparable in sham-operated and infarcted rats. When the results obtained from the membrane preparations were expressed on a cellular basis, it could be calculated that left and right ventricular myocytes of control hearts possessed 1,297±167 and 1,151±252 binding sites, respectively. Corresponding values after infarction were 2,384±140 and 2,126±612 binding sites.

The affinity of Ang II receptors for the radiolabeled antagonist was not altered by myocardial infarction. $K_d$ values of 0.37±0.19 and 0.37±0.11 nM were found in left and right ventricular myocytes, respectively, of sham-operated rats, and $K_d$ values of 0.28±0.12 and 0.30±0.12 nM were found in left and right ventricular myocytes, respectively, of infarcted rats. Corresponding values of $n$ for these determinations were 4, 3, 4, and 3. In summary, the density of surface AT$_1$ receptors on myocytes was upregulated biventricularly after infarction without alteration in the affinity of the receptor for the radioligand antagonist.

Ang II Receptors and Phosphoinositol Turnover

To establish whether the identified AT$_1$ receptors on ventricular myocytes were coupled with phospholipase C and subsequent hydrolysis of phosphoinositides, muscle cells isolated from unoperated rats were labeled with $[^{3}H]$myoinositol and incubated with Ang II. The generation of total inositol phosphates was then determined 60 minutes later. The specificity of this response was also demonstrated by the addition in the incubation mixture of the nonpeptide-selective AT$_1$ receptor subtype antagonist L-158809. Ang II was capable of stimulating the generation of inositol phosphates in enzymatically dissociated adult rat myocytes. Since practically identical results in the formation of inositol phosphates were obtained from myocytes isolated from sham-operated control rats, these data are not shown. In view of the fact that pharmacological doses of Ang II (100 μM) were necessary to stimulate phosphoinositol turnover in myocytes in vitro, a dose–response curve with the AT$_1$ receptor antagonist L-158809 was performed to demonstrate the specificity of this reaction. As illustrated in Figure 8, L-158809 at equimolar concentration completely abolished the generation of total inositol phosphates produced by Ang II. In summary, ventricular myocytes possess a functional AT$_1$ receptor subtype.

Ang II–Stimulated Phosphoinositol Turnover and Myocardial Infarction

Figure 9 illustrates the effects of myocardial infarction on the generation of total inositol phosphates in left and right ventricular myocytes. Ang II was found to stimulate phosphoinositol turnover by 14% in left myocytes of sham-operated rats and by 52% in the corresponding muscle cells of experimental rats. This difference implies a 3.7-fold increase in the magnitude of formation of inositol phosphate after infarction. An identical analysis for right ventricular myocytes showed that the percent increments in phosphoinositol turnover were 20% and 49% in control and infarcted hearts, respectively. Thus, in the right side, myocardial infarction was associated with a 2.5-fold augmentation in the generation of inositol phosphates. When the changes in phosphoinositol turnover between the ventricles after infarction were examined, the accumulation of inositol phosphates in left and right myocytes was found to be comparable.

The absolute values obtained during the calculations of the percent changes depicted in Figure 9 were as follows: for sham-operated rats, left myocytes=292±48 (basal) and 331±45 (stimulated) and right myo-
cutes = 234 ± 32 (basal) and 282 ± 53 (stimulated); for the corresponding values in infarcted rats, left myocytes = 240 ± 28 (basal) and 364 ± 65 (stimulated). These determinations are all based on 2 x 10⁶ myocytes, and the unit of expression is disintegrations per minute [³²P]inositol phosphates formed per 1,000 disintegrations per minute inositol lipid incorporated. Values of n are eight each for left and right ventricular myocytes of sham-operated and infarcted rats. In summary, Ang II–stimulated phosphoinositol turnover increased biventricularly after infarction.

Myocyte Size and AT₁ Receptor Antagonist

The treatment of infarcted rats with L-158809 for 7 days resulted in a reduction in systemic arterial blood pressure and left ventricular systolic and end-diastolic pressures. In particular, systolic, diastolic, and mean arterial pressures were 76 ± 5, 44 ± 8, and 55 ± 6 mm Hg, respectively. Thus, in comparison with nontreated infarcted rats (Table 1), these parameters were reduced 18% (p < 0.01), 35% (p < 0.008), and 27% (p < 0.01). Similarly, left ventricular systolic pressure after L-158809 administration was 78 ± 5 mm Hg, which indicated a 15% (p < 0.03) decrease with respect to untreated infarcted animals. Finally, left ventricular end-diastolic pressure (14 ± 2 mm Hg) was 39% lower than in the corresponding experimental group, but this change did not reach statistical significance (p = 0.1).

Measurements of left ventricular myocyte length and width in treated infarcted rats were 124 ± 13 and 27 ± 4 μm, respectively. The corresponding cellular properties in right ventricular myocytes were 121 ± 18 and 25 ± 4 μm. Such cellular dimensions were consistently smaller than those measured in nontreated infarcted rats (see “Structural Myocyte Characteristics”). When an analysis of variance was performed among control, untreated, and treated rats, the changes in left and right myocyte dimensions after infarction in the presence of L-158809 were found not to be different from control values. A similar lack of statistical difference was noted with respect to untreated infarcted hearts. In summary, L-158809 decreased the loading state on the myocardium and tended to reduce myocyte size after infarction.

Discussion

Findings in this study indicate that myocardial infarction resulted in global cardiac failure, which also involved alterations in the mechanical properties of individual myocytes of both ventricles. Myocyte performance was depressed, as evidenced by a decrease in the velocity of cell shortening and lengthening and a prolongation of time to peak shortening. Moreover, peak shortening was reduced. Structurally, myocyte hypertrophy occurred in left and right ventricular muscle cells through increases in myocyte diameter and length. These functional and morphological adaptations at the cellular level were accompanied by an upregulation of surface Ang II receptors and by an enhanced phosphoinositol turnover in myocytes biventricularly. Thus, acute myocardial infarction leads to abnormalities in cell contractility and changes in myocyte size and shape that are consistent, at least in part, with pressure-overload reactive hypertrophy. These configurational and mechanical characteristics appear to be associated with activation of Ang II receptors and their effector pathway, which may be implicated in the modulation of the growth response of the remaining viable cells after infarction. However, a relation between mechanical stresses and regulatory modifications of Ang II receptors on myocytes after infarction cannot be claimed at this time.

Myocardial Infarction, Ventricular Loading, and Myocyte Hypertrophy

The current results demonstrate that, 1 week after coronary artery ligation, left ventricular pump function was severely compromised and right ventricular hemodynamic performance was also depressed. These observations are consistent with previous findings in which the hemodynamic profile of the infarcted left ventricle has been shown to be characterized by a marked elevation in diastolic and systolic stress on the surviving myocytes 7 days after infarction. Although limited information is available concerning the loading state of the right ventricle shortly after myocardial infarction, indirect evidence points to an increased pressure and volume load on this chamber. The elevated right ventricular systolic pressure and depressed +dP/dt found here strongly suggest that left-side failure may have resulted in an immediate increase in right ventricular systolic pressure, leading to the initiation of right ventricular dysfunction and diastolic abnormalities.

The analysis of the structural and mechanical properties of single myocytes performed in this investigation has allowed a characterization of the nature of the overload on the remaining viable cells as well as a description of the effects of myocardial infarction on muscle cell function. Since left ventricular myocytes increased 14.4% in length and 14.3% in diameter, the suggestion can be made that these cells were exposed to an elevated systolic and diastolic wall stress. Similarly, right ventricular myocytes were expanded 9% in diameter and 7% in length, most likely as a reflection of augmentations in systolic and diastolic cellular loads. These cellular modifications are consistent with configurational changes described in different models of car-

FIGURE 9. Bar graph showing percent increase in the generation of total inositol phosphates in left ventricular (LV) and right ventricular (RV) myocytes after exposure to 100 μM angiotensin II. The formation of inositol phosphates is increased in LV and RV myocytes after infarction. Results are presented as mean ± SD. *Statistically significant at p < 0.05 (n = 8 for both LV and RV myocytes of sham-operated and infarcted rats).
diac hypertrophy and failure.1-3,17,26 The present results also demonstrate that myocardial infarction led to alterations in cell contractile function, which involved the viable myocytes of the left ventricle and, to a lesser extent, right ventricular myocytes. Although a depression in the mechanical performance of left myocytes after infarction has recently been documented,18 the abnormalities of right myocytes have not previously been described. The greater impact of myocardial infarction on the mechanics of left myocytes may be accounted for by the variability in loading between the left and right ventricle under this setting.1-3 The isotonic properties of single myocytes appear to be influenced by conditions of overload in various experimental systems,17,18,26 raising the possibility that acute and chronic changes in wall stress may impinge on the mechanical characteristics of left and right ventricular myocytes. A similar modification in ventricular hemodynamics has been encountered in the present study after infarction, and this may have affected the contractile behavior of the surviving cells biventricularly. On the other hand, the etiology of such a depression in myocyte contractility remains to be determined.

Impairment of myocyte performance has been found to be coupled with increases in myocyte diameter,18 which constitute the morphological modification of pressure-overload hypertrophy.3 Moreover, reductions in the velocity of cell shortening and lengthening have been repeatedly observed in studies of muscle mechanics in vitro of models of increased afterload.27,28 Thus, the alterations in mechanical behavior observed here strongly support the contention that left and right ventricular myocytes after infarction are exposed to an elevation in systolic stress, which may represent the major determinant of depressed muscle cell function.

Ang II Receptors and Ventricular Myocytes

A prerequisite of the current study was the demonstration that adult rat cardiac myocytes possess a class of pharmacologically distinct and functionally active surface Ang II receptors. The accumulated data provide the first identification and characterization of Ang II binding sites on these cells. Cardiocytes exhibit the AT1 receptor subtype exclusively. It should be emphasized that these results were obtained by using membranes prepared from purified isolated ventricular myocytes and by using radiolabeled antagonist [125I]-[Sar1,Ile8]Ang II to detect binding sites. This approach was found to be advantageous for two reasons: 1) the elimination of the contaminating effect of other cell types, such as vascular smooth muscle,28 and 2) the use of a radiolabeled Ang II receptor antagonist, which circumvented the potential difficulty encountered by the use of radiolabeled agonists. Agonist binding may recognize multiple receptor affinities, complicating Scatchard transformation.30,31

The binding capacity of ventricular myocytes for Ang II reported in the present study in the adult heart is significantly lower than that found in cultured neonatal rat cardiac myocytes,32 raising the question of whether measured differences reflect the biological impact of maturation on Ang II receptor density or technical limitations in the procedure. One potential problem, which was considered in the present work, concerns the loss of binding sites during the preparation of myocyte membranes. However, the observation that no specific binding could be detected in the discarded 14,000g membrane fraction argues against this possibility. Thus, the high density of Ang II receptors on myocytes shortly after birth may correspond to the rapid and intense growth phase of these cells, whereas the relatively low number of Ang II receptors in adult heart may be consistent with the attenuation of this growth process later in life. Species differences also exist in Ang II receptor number and in the capacity of Ang II to influence physiological responses at the cellular level.33-35

The current findings indicate that the AT1 receptor subtype on myocytes is coupled to phospholipase C, leading to the generation of inositol phosphates. However, a high percentage of receptors may have to be occupied by the agonist to elicit this response. Although the AT1 receptor antagonist Dup 753 was used for the binding experiments, it was difficult to obtain a consistent inhibitory effect in the inositol phosphate studies. On the other hand, the availability of the nonpeptide Ang II receptor antagonist L-15880920 has made these studies possible. Moreover, L-158809 has been found to be 10 times more potent than Dup 753.36 Since an excessive concentration of Ang II was required for the in vitro assay of phosphoinositide turnover, it is difficult to extrapolate these results to the in vivo ligand receptor interaction. However, a relation appears to exist between Ang II receptor density and the quantities of Ang II necessary to induce the accumulation of inositol phosphate in multiple cell systems.29,34 Importantly, it should be recognized that results in this investigation do not document that a causal relation exists between the activation of Ang II receptor on myocytes and the magnitude of cellular hypertrophy after infarction. Similarly, the impossibility of establishing the extent of loading on the left and right ventricles under this setting leaves unanswered the question of whether a correlation is present between mechanical stress and Ang II receptors on myocytes.

It has recently been documented that the rat myocardium possesses both AT1 and AT2 receptor subtypes.37 In addition, each receptor subtype has been shown to account for 50% of the specific binding. Thus, these observations appear to be in contrast with the result of the current study in which myocytes have been demonstrated to exhibit exclusively the AT1 receptor subtype. This apparent contradiction may be explained by the fact that the cited work was performed on the entire ventricular tissue without separating the myocyte compartment from the vascular and interstitial components of the myocardium. However, it cannot be excluded that the myocyte isolation procedure may have altered receptor density. On the other hand, it would seem unlikely that the AT1 receptor subtype would be affected more than the AT1 receptor subtype.

Myocardial Infarction, Ang II Receptors, and Signal Transduction

An upregulation of Ang II receptors occurred in the viable myocytes of the left and right ventricles 1 week after coronary occlusion. In view of the postulated role of Ang II as a growth factor for several cell systems, including cardiac myocytes,9,9,38,39 the enhanced expression of Ang II surface receptors may facilitate the transduction of signals via these receptors, influencing
the metabolic and growth properties of surviving myocytes. In addition, stimulation of Ang II receptors by ambient and/or circulating Ang II may exert a positive inotropic effect on myocytes by increasing transmembrane calcium conductance. Ang II may also affect voltage-dependent sodium channels, enhancing the contractile ability of myocytes through an increase in the rate of tension development of these cells. It should also be acknowledged that Ang II may exert its action on the myocardium indirectly by increasing the release of neurotransmitters from adrenergic cardiac presynaptic nerve terminals. On the other hand, a negative inotropic effect of Ang II has also been documented in adult rat myocytes.

The multiple biological actions of Ang II in myocytes render the distinction of each property very complex in an in vivo system and in pathological states in particular. The alterations in myocyte cell function and the initiation of reactive hypertrophy in the surviving muscle cells complicate the recognition of whether the increased expression of Ang II receptors is involved in growth mechanisms, the mechanical support of contractility, or both. However, the Ang II-mediated inotropic property remains in question and does not appear to be dependent on intermediates of the phosphoinositol pathway. Therefore, to provide supportive evidence for the role of Ang II receptors on myocytes after infarction, the generation of intracellular second messengers via agonist-stimulated hydrolysis of membrane-bound phosphoinositides was measured. The present results indicate that inositol phosphate formation in the presence of Ang II was increased biventricularly in myocytes after infarction. It should be recognized that the documentation in vitro of elevated phosphoinositide turnover does not necessarily imply that a similar phenomenon may be operative in vivo. Moreover, the increase in agonist-stimulated inositol phosphate generation after myocardial infarction simply indicates that Ang II receptors on myocytes are functional and may respond to local or circulating levels of Ang II. These findings cannot be interpreted as evidence for a trophic effect of Ang II on myocyte growth. On the other hand, this contention appears to be supported by observations demonstrating the ability of Ang II to induce early growth response gene 1 and to activate the nuclear proto-oncogene c-fos in myocytes. In addition, an increase in angiotensinogen gene expression and converting enzyme activity have been documented in the surviving myocardium after acute occlusion of the coronary artery. Although the treatment with the AT2 receptor antagonist L-158809 tended to reduce the magnitude of myocyte size after infarction, which would favor a role for this receptor subtype on cellular hypertrophy, the concomitant alteration in the loading state on the myocardium complicates the interpretation of these results. Therefore, we cannot establish whether the impact of this agent on cellular morphology depends on a direct action on the viable cells or is mediated indirectly by the interference with the systemic and ventricular hemodynamic characteristics of the heart after infarction.

It has repeatedly been shown that left ventricular hypertrophy in the infarcted heart is accomplished by a prevailing increase in myocyte length, whereas right ventricular hypertrophy is generated by the lateral expansion of myocytes only. These differential adaptations have been claimed to reflect variations in ventricular loading. Although a direct relation between the characteristics of the overload and the stimulation of the cardiac renin-angiotensin system cannot be established at present, the possibility may be advanced that systolic wall stress abnormalities may be associated with the activation of Ang II receptors on the surface of the cells that modulate the synthesis of new myofibrils in parallel and the expansion in the transverse diameter of myocytes. This notion finds support in the capability of angiotensin converting enzyme inhibitors to prevent cardiac hypertrophy induced by pressure overload without altering the magnitude of the afterload.

Conversely, in the presence of large infarcts in rats, these agents cannot abrogate the extent of ventricular dilation that occurs chronically, through the progressive increase in myocyte length. As documented in the present study, increases in myocyte cross-sectional area involved both left and right ventricular myocytes, whereas the expansion in myocyte length appeared to be restricted to left myocytes. Since myocyte hypertrophy in the infarcted left ventricle has been linked, at least in part, to the activation of surface a1-adrenergic receptors, it seems reasonable to infer that Ang II and a1-adrenergic receptors may modulate the growth response of left myocytes, whereas Ang II receptors may condition right myocyte hypertrophy.

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