Exacerbation of Left Ventricular Ischemic Diastolic Dysfunction by Pressure-Overload Hypertrophy

Modification by Specific Inhibition of Cardiac Angiotensin Converting Enzyme

Franz R. Eberli, Carl S. Apstein, Souen Ngoy, and Beverly H. Lorell

Hearts with compensatory pressure-overload hypertrophy show an increased intracardiac activation of angiotensin II that may contribute to ischemic diastolic dysfunction. We studied whether pressure-overload hypertrophy in response to aortic banding would result in exaggerated diastolic dysfunction during low-flow ischemia and whether the specific inhibition of the cardiac angiotensin converting enzyme by enalaprilat could modify systolic and diastolic function during ischemia and reperfusion in either hypertrophied or nonhypertrophied hearts. Isolated, red blood cell-perfused isovolumic nonhypertrophied and hypertrophied rat hearts were subjected to enalaprilat (2.5 × 10⁻⁷ M final concentration) infusion during 20 minutes of baseline perfusion and during 30 minutes of low-flow ischemia and 30 minutes of reperfusion. Coronary flow per gram was similar in nonhypertrophied and hypertrophied hearts during baseline perfusion, ischemia, and reperfusion. At baseline, left ventricular developed pressure was higher in hypertrophied than nonhypertrophied hearts in untreated groups (224 ± 8 versus 150 ± 9 mm Hg; p < 0.01) and in enalaprilat-treated groups (223 ± 9 versus 145 ± 8 mm Hg; p < 0.01). During low-flow ischemia, left ventricular developed pressure was depressed but similar in all groups. All groups showed deterioration of diastolic function; however, left ventricular end-diastolic pressure increased to a significantly higher level in untreated hypertrophied than in nonhypertrophied hearts (65 ± 7 versus 33 ± 3 mm Hg; p < 0.001). Enalaprilat had no effect in nonhypertrophied hearts, but it significantly attenuated the greater increase in left ventricular end-diastolic pressure in hypertrophied hearts treated with enalaprilat compared with no drug (65 ± 7 versus 50 ± 5 mm Hg; p < 0.01). The beneficial effect could not be explained by differences in coronary blood flow per gram left ventricular weight, glycolytic flux as reported by lactate production, myocardial water content, oxygen consumption, and tissue levels of glycogen and high energy phosphate compounds. During reperfusion, all hearts showed a partial recovery of developed pressure to 70–74% of initial values. No effect of enalaprilat could be detected during reperfusion on systolic and diastolic function or restoration of tissue levels of high energy compounds. In conclusion, our experiments show that hypertrophied red blood cell-perfused hearts manifest a severe impairment of left ventricular diastolic relaxation in response to low-flow ischemia in comparison with control hearts. Further, our experiments support the hypothesis that the enhanced conversion of angiotensin I to angiotensin II in rats with pressure-overload hypertrophy contributes to the enhanced sensitivity of hypertrophied hearts to diastolic dysfunction during low-flow ischemia. (Circulation Research 1992;70:931–943)

**Key Words** • diastole • ischemia • cardiac hypertrophy • angiotensin II • angiotensin converting enzyme

Adaptation of the heart to chronic pressure-overload leads to concentric left ventricular hypertrophy in association with multiple changes in gene expression.1,2 In this regard there is increasing evidence for intrinsic tissue renin-angiotensin systems in multiple organs, including the heart,3-9 that may play a role in modulating cardiac hypertrophy.10-14 Schunkert and coworkers15 have recently shown an increased angiotensin converting enzyme activity and mRNA expression in rat hearts with pressure-overload hypertrophy in association with a higher angiotensin I to angiotensin II ratio.16-18 These observations have provided the rationale for the use of angiotensin converting enzyme inhibitors, such as enalaprilat, in the treatment of hypertension and congestive heart failure. Our study provides evidence that angiotensin converting enzyme inhibition can improve diastolic function during acute ischemia and reperfusion and that this effect is mediated by changes in tissue renin-angiotensin activity.

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angiotensin II conversion in isolated perfused hypertrophied hearts. The increased angiotensin II activation resulted in severe but reversible impairment of diastolic function in the hypertrophied heart.

Additional studies from our laboratory16–20 and by others21–29 have shown that hypertrophied hearts are distinguished by an enhanced susceptibility to ischemic and hypoxic contractile dysfunction in which impaired diastolic relaxation is prominent. It is not known if an increased capacity for local angiotensin II production contributes to the increased susceptibility to ischemic dysfunction in pressure-overload hypertrophy.

Specific inhibition of the cardiac renin-angiotensin system has the potential to protect against ischemic and reperfusion injury.30–33 However, the mechanism for the protective effect remains unclear and may include an abolishment of norepinephrine release on reperfusion,30,32,34 a reduction of bradykinin degradation,32 an anti–free radical effect by mechanisms related to the sulf/hydrol group of certain angiotensin converting enzyme inhibitors,35 an induction of prostaglandin synthesis,35,36 and the inhibition of circulating angiotensin or tissue angiotensin production.37 The potential protective effect of local inhibition of the angiotensin converting enzyme activity on ischemic dysfunction in hypertrophied and nonhypertrophied hearts has not been compared.

Using the isolated, isovolumic, working red blood cell–perfused heart preparation,17,18,36,37 we tested the hypotheses that rat hearts with compensatory left ventricular hypertrophy would show increased diastolic dysfunction during low-flow ischemia and reperfusion and that specific inhibition of the local cardiac angiotensin converting enzyme could attenuate increased ischemic diastolic dysfunction. Hypertrophied and nonhypertrophied hearts were subjected to 30 minutes of low-flow ischemia and 30 minutes of reperfusion and treated with either no drug or enalaprilat at a final concentration of 2.5×10⁻⁷ M. Enalaprilat was chosen because this angiotensin converting enzyme inhibitor lacks a sulf/hydrol group, allowing us to avoid the confounding effect on free oxygen radicals seen with some other inhibitors.38 Hypertrophied hearts showed a greater impairment of left ventricular diastolic function than nonhypertrophied hearts during low-flow ischemia. Enalaprilat markedly inhibited the development of ischemic diastolic dysfunction in hypertrophied hearts but had no effect in nonhypertrophied hearts. These findings support the hypothesis that the enhanced cardiac angiotensin converting enzyme activity that is characteristic of left ventricular hypertrophy may contribute to diastolic failure in hypertrophied hearts during ischemia. No effect of enalaprilat was found during reperfusion.

Materials and Methods

Preparation of Animals

Male Wistar rats were obtained from the Charles River Breeding Laboratories. Aortic stenosis was created in weanling rats (body weight, 60 g; age, 3–4 weeks) by placing a stainless steel clip of 0.6 mm internal diameter on the ascending aorta via a thoracic incision. Both the rats with aortic stenosis and the age-matched controls underwent a left thoracotomy under anesthesia with intraperitoneal sodium pentobarbital with supplemental nasal oxygen. The rats were subsequently fed normal rat chow and water ad libitum and were used for experimentation 11–12 weeks after the operation. The body weights were recorded before the animals were killed.

Perfusion Technique

We used an isolated, isovolumic, working red blood cell–perfused heart preparation that was developed by Marshall and Zhang38 and in our laboratory.17,18,36,37 Rats were injected intraperitoneally with 1.0–1.5 ml sodium pentobarbital (15 mg/ml), and the thorax was rapidly opened. A short perfusion cannula was inserted into the aortic root just below the level of the aortic clip, and the coronary arteries were perfused via the aortic root with red blood cell–containing perfusate. Less than 20 seconds elapsed between opening the thorax and the initiation of coronary perfusion in all experiments.

The perfusion system consisted of a “venous” reservoir, a variable flow pump, an oxygenator, a water-jacketed “arterial” reservoir, and a filter of 20 µm pore size. In this system, the perfusion pressure was controlled by a valve that adjusted the pressure of the arterial reservoir. Coronary blood flow was allowed to vary and thus depended on coronary vasomotor autoregulation.

A red blood cell perfusate as previously described was used, consisting of bovine red blood cells at a final hematocrit of 40% in Krebs-Henseleit buffer.37,38 Krebs-Henseleit buffer contained (millimoles per liter) NaCl 118, KCl 4.7, CaCl₂ 2.0, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 1.2, glucose 5.5, lactate 1.0, and palmitic acid 0.4 (as a source for free fatty acid) as well as 4% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). Essentially free fatty acid–free bovine serum albumin (Sigma) was first dissolved in Krebs-Henseleit buffer. Palmitic acid (Sigma) was then added to this mixture. The buffer was prepared fresh daily. Gentamicin (0.2 mg/dl) was added to the red blood cell perfusate to retard bacterial growth. The perfusate was equilibrated with 5% O₂–5% CO₂–77% N₂ to achieve a PO₂ of 120–140 mm Hg and a pH of 7.38–7.42 throughout all experiments.

Perfusion pressure was monitored via a side arm of the aortic cannula connected to a pressure transducer (Gould-Statham P23Db, Gould Inc., Oxnard, Calif.). A small apical drain was inserted into the left ventricular apex to drain the thebesian circulation. The pulmonary artery was ligated. A second cannula was inserted through the right atrium into the right ventricular apical region to completely collect coronary venous effluent and to empty the right ventricle. A pacing wire (model 59, Grass Instrument Co., Quincy, Mass.) and a thermistor (model 400, Yellow Springs Instruments, Boulder, Colo.) were inserted into the right ventricle via the superior vena cava and right atrium. A collapsed thin-walled latex balloon was placed in the left ventricle via the left atrium and secured in place. The balloon was connected to a pressure transducer (Gould-Statham P23Db) to measure left ventricular pressure and its first derivative. The fidelity of this pressure recording system satisfies the criteria required for accurate measurement of left ventricular pressure and its first derivative and has been documented in this laboratory.40 The heart
was then placed in a water-jacketed constant temperature chamber and maintained at 37°C.

Coronary perfusion pressure, left ventricular pressure, and its first derivative were recorded continuously on a multichannel physiological recorder (Gould Inc., Cleveland, Ohio). Coronary blood flow was measured by a timed collection of the venous effluent. In the subgroup of hearts undergoing reperfusion (see below), arterial and venous blood gas analysis (blood gas analyzer, Allied Instrumentation Laboratory, Decatur, Ga.) was performed every 10 minutes throughout the protocol. Myocardial oxygen consumption was calculated from the arteriovenous oxygen content difference. Oxygen content of arterial and venous samples was derived from oxygen saturation curves for the resuspended Krebs buffer over the experimental range of pH and PO₂ values.

Lactate analysis of arterial and venous samples was done using a specific enzymatic method. Measurements are expressed as micromoles per gram left ventricular dry weight.

**Study Groups**

Four groups were studied. The first group (CTL) consisted of 13 nonhypertrophied hearts that received saline carrier (NaCl 0.9%) infusion. The second group (CTL/ENA) comprised 13 nonhypertrophied treated hearts, for which enalaprilat (Enalaprilat, Vasotec Inc., Merck and Co., Inc., West Point, Pa.) at a final concentration of 2.5×10⁻⁷ M was added to the perfusate. This dose of enalaprilat was chosen because prior studies in buffer-perfused hearts showed that this concentration inhibited the intracardiac conversion of angiotensin I to angiotensin II by greater than 80%. This observation has been confirmed in additional experiments in the same model of pressure-overload hypertrophy (unpublished data). The third group (LVH) consisted of 14 hypertrophied untreated hearts obtained from rats with aortic banding. The fourth group (LVH/ENA) was composed of 14 hypertrophied hearts perfused with enalaprilat at a final concentration of 2.5×10⁻⁷ M.

**Experimental Protocol**

*Stabilization period and enalaprilat infusion.* The hearts were perfused for a stabilization period of 20 minutes at a paced heart rate of 4 Hz, which was continued throughout the experiment. Coronary perfusion pressure was set at 75 mm Hg in sham-operated control hearts (CTL and CTL/ENA) and at 100 mm Hg in the hypertrophied hearts (LVH and LVH/ENA). These differing levels of coronary perfusion pressure were selected in recognition of the difference between the in vivo mean coronary perfusion pressures to which the control and LVH groups were chronically exposed. Adequate preischemic perfusion pressures are of critical importance for the postischemic functional recovery in hypertrophied hearts and a prerequisite for comparison of recovery in nonhypertrophied hearts. Prior studies showed that the chosen perfusion pressures would achieve comparable myocardial flow rates per gram left ventricular weight. Left ventricular balloon volume was initially adjusted so that left ventricular end-diastolic pressure was 10 mm Hg in all groups, and this balloon volume was kept constant so that an increase in left ventricular end-diastolic pressure signified a decrease in diastolic chamber distensibility. This initial level of left ventricular end-diastolic pressure was chosen because it allowed the study of the nonhypertrophied and hypertrophied hearts at an operational point of the left ventricular pressure-volume curves, where volumes were comparable (0.22±0.02 and 0.23±0.02 ml [CTL and CTL/ENA] versus 0.17±0.02 and 0.19±0.01 ml [LVH and LVH/ENA]; p=NS).

At the end of the stabilization period, baseline measurements of left ventricular and coronary flow parameters and sampling of arterial and coronary venous effluent for lactate and oxygen content analysis were made. Perfusion with enalaprilat was begun in 13 of the control hearts (CTL/ENA) and 14 of the hypertrophied hearts (LVH/ENA) and subsequently continued throughout the protocol. The red blood cell perfusate was not recirculated. All hemodynamic and metabolic measurements were repeated at the end of a 20-minute saline or enalaprilat infusion period before the start of ischemia.

*Low-flow ischemia and reperfusion.* After the 20-minute stabilization period and the subsequent 20-minute period of enalaprilat (CTL/ENA and LVH/ENA) or drug-free perfusion (CTL and LVH) under oxygenated conditions, low-flow ischemia was imposed by reducing coronary perfusion pressure to 12 mm Hg for 30 minutes in all groups. Repeated measurements of hemodynamic parameters, coronary flow, and lactate production were made every 5 minutes, and blood gas analyses were obtained every 10 minutes for calculation of oxygen consumption in the subgroup of hearts undergoing reperfusion (n=6 in each group).

When ventricular fibrillation occurred and was sustained for more than 15 seconds, hearts were electrically converted. This occurred in one to four hearts of each group (p=NS). Hearts that could not be promptly converted were discarded. Because of sustained ventricular fibrillation during ischemia, the following hearts were excluded (and not further reported): two each in the CTL and CTL/ENA groups, three in LVH, and one in LVH/ENA (p=NS).

At the end of the ischemic period, eight hearts (minus atrial tissue) from each group were freeze clamped for determination of myocardial tissue levels of ATP, creatine phosphate (CP), lactate, and glycogen.

*Reperfusion.* Additional hearts (n=6 in each of the four groups) were subjected to reperfusion at preischemic coronary perfusion pressures for 30 minutes. Left ventricular pressure and its first derivative were measured every minute during the first 5 minutes of reperfusion and every 5 minutes thereafter. Coronary blood flow and arterial and venous blood samples were also obtained every minute during the first 5 minutes and at minutes 10, 20, and 30 of reperfusion. During reperfusion, most of the hearts elicited a few beat runs of ventricular fibrillation or tachycardia, but no heart had to be excluded because of sustained ventricular fibrillation. At the end of the reperfusion period, all hearts were freeze clamped for measurement of tissue metabolites as described above.

*Oxygenated perfusion.* To determine the stability of contractile and metabolic function over the time course
of the experimental protocols, we also perfused additional control hearts (n=5) at a coronary perfusion pressure of 75 mm Hg and hypertrophied hearts (n=5) at a coronary perfusion pressure of 100 mm Hg for 100 minutes. The left ventricular/body weight ratios of the control and hypertrophied groups that underwent oxygenated perfusion were not significantly different from those of the control and hypertrophied groups subjected to low-flow ischemia. Left ventricular developed pressure was significantly higher in these additional hypertrophied hearts compared with the additional controls (197±6 versus 133±9 mm Hg; p<0.001) and declined by 9.0±3.1% in the hypertrophied and 19.5±4.7% in the control hearts (p<0.05) over 100 minutes. Coronary blood flow per gram left ventricular weight was similar in the two groups (1.96±0.31 versus 1.56±0.18 ml/min/g; p=NS), as was lactate extraction. There was no difference in oxygen consumption per gram per millimeter of mercury developed pressure between the groups. At the end of the nonischemic perfusion period, hearts were freeze clamped for measurement of tissue metabolites for comparison with the ischemic-reperfused groups. In these additional nonhypertrophied and hypertrophied control hearts, glycogen content was 198±25 and 187±16 μmol glucose equivalent per gram dry weight, respectively (p=NS), at the end of the nonischemic perfusion. Thus, these results indicate functional and metabolic stability of the control and LVH hearts under well-oxygenated perfusion conditions for the time period of our experimental protocol.

Statistical Analysis

Data are reported as the mean±SEM. Data acquired by repeated sequential measurements during ischemia and during reperfusion in individual hearts were tested by analysis of variance for repeated measures. Comparison between two experimental groups was performed by two-way analysis of variance. A difference of a single metabolic measurement between hypertrophied and nonhypertrophied treated and untreated groups was tested by two-factor factorial analysis of variance. If overall analysis of variance indicated a significant difference of groups, trials, or interaction, values at specific time points were examined by the method of least significant differences.46 A value of p<0.05 was considered significant.

Table 1. Baseline Characteristics of Hypertrophied and Nonhypertrophied Rat Hearts

<table>
<thead>
<tr>
<th></th>
<th>Body wt (g)</th>
<th>Heart wt (g)</th>
<th>LV wet wt (g)</th>
<th>LV/body wt (g/kg)</th>
<th>LV dry wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL (n=13)</td>
<td>429±10</td>
<td>1.51±0.07</td>
<td>1.20±0.06</td>
<td>2.80±0.14</td>
<td>0.27±0.05</td>
</tr>
<tr>
<td>LVH (n=14)</td>
<td>385±18</td>
<td>1.78±0.09*</td>
<td>1.43±0.07*</td>
<td>3.73±0.12*</td>
<td>0.32±0.05*</td>
</tr>
<tr>
<td>CTL/ENA (n=13)</td>
<td>404±14</td>
<td>1.46±0.07</td>
<td>1.15±0.06</td>
<td>2.88±0.15</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>LVH/ENA (n=14)</td>
<td>401±9</td>
<td>1.85±0.08*</td>
<td>1.49±0.06*</td>
<td>3.73±0.17*</td>
<td>0.33±0.05*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LV, left ventricular; CTL, nonhypertrophied hearts; LVH, hypertrophied hearts; CTL/ENA, nonhypertrophied hearts treated with enalaprilat; LVH/ENA, hypertrophied hearts treated with enalaprilat.

*p<0.01 LVH vs. CTL, or LVH/ENA vs. CTL/ENA.

Results

Baseline characteristics of all experimental groups are shown in Table 1. Body weight was comparable in all groups. Heart weight, left ventricular wet and dry weights, and left ventricle/body weight ratios were higher in hypertrophied than nonhypertrophied hearts.

Baseline and Enalaprilat Infusion

After the 20-minute equilibration period, hypertrophied hearts showed higher values of left ventricular developed pressure and left ventricular +dP/dt than nonhypertrophied hearts did (Table 2). After the 20-minute enalaprilat infusion under baseline well-oxygenated conditions, there was no significant effect of enalaprilat on baseline function in either LVH or control groups.

By study design, left ventricular end-diastolic pressure was set to a similar level at baseline in all groups (Table 2). During the 20-minute infusion of enalaprilat, left ventricular end-diastolic pressure decreased slightly by about 2 mm Hg in all nonhypertrophied (CTL/ENA) and hypertrophied (LVH/ENA) hearts. No decrease of left ventricular end-diastolic pressure was detected in untreated hearts over this time interval. This difference between treated and untreated hearts was not statistically significant.

At baseline, coronary blood flow per gram left ventricular weight was comparable in nonhypertrophied and hypertrophied hearts (Table 2). Enalaprilat did not increase coronary blood flow in either the hypertrophied (LVH/ENA) group versus untreated (LVH) group or the nonhypertrophied (CTL/ENA) group versus the untreated (CTL) group. At baseline, oxygen consumption per gram left ventricular weight was higher in untreated hypertrophied hearts than in untreated nonhypertrophied hearts (Table 3). After 20 minutes of enalaprilat or drug-free perfusion, preischemic oxygen consumption per gram left ventricular weight was similar in the hypertrophied groups compared with the control groups (Table 3). The relation between oxygen consumption and mechanical function reflects overall metabolic mechanical efficiency. In these experiments, because contraction was isovolumic and heart rate was held constant, a depression of the ratio of developed pressure to oxygen consumption can serve as an index of depression of myocardial efficiency. Oxygen consumption per millimeter of mercury developed pressure...
TABLE 2. Hemodynamics and Coronary Flow in the Presence or Absence of Angiotensin Converting Enzyme Inhibition

<table>
<thead>
<tr>
<th></th>
<th>LV Dev P (mm Hg)</th>
<th>LVEDP (mm Hg)</th>
<th>LV +dP/dt (+mm Hg/sec)</th>
<th>LV −dP/dt (−mm Hg/sec)</th>
<th>CBF/g (ml/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL (n=13)</td>
<td>150±9</td>
<td>10±0.3</td>
<td>3,898±367</td>
<td>2,051±125</td>
<td>1.7±0.16</td>
</tr>
<tr>
<td>LVH (n=14)</td>
<td>224±8*</td>
<td>10±0.4</td>
<td>6,047±341*</td>
<td>3,021±109*</td>
<td>2.0±0.17</td>
</tr>
<tr>
<td>CTL/ENA (n=13)</td>
<td>145±8</td>
<td>11±0.3</td>
<td>3,501±239</td>
<td>2,074±112</td>
<td>1.7±0.14</td>
</tr>
<tr>
<td>LVH/ENA (n=14)</td>
<td>223±9*</td>
<td>11±0.4</td>
<td>5,857±542*</td>
<td>2,890±115*</td>
<td>2.0±0.16</td>
</tr>
<tr>
<td><strong>After 20 min enalapril or no drug treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>138±8</td>
<td>9±0.3</td>
<td>3,511±314</td>
<td>1,828±102</td>
<td>1.6±0.16</td>
</tr>
<tr>
<td>LVH</td>
<td>210±6*</td>
<td>10±0.4</td>
<td>5,710±289*</td>
<td>2,955±95*</td>
<td>2.1±0.19</td>
</tr>
<tr>
<td>CTL/ENA</td>
<td>128±7</td>
<td>9±0.6</td>
<td>3,015±179</td>
<td>1,875±105</td>
<td>1.7±0.15</td>
</tr>
<tr>
<td>LVH/ENA</td>
<td>213±9*</td>
<td>9±0.6</td>
<td>5,407±419*</td>
<td>2,784±102*</td>
<td>2.2±0.23</td>
</tr>
<tr>
<td><strong>After 30 min low-flow ischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>22±4</td>
<td>33±3</td>
<td>645±122</td>
<td>329±66</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>LVH</td>
<td>10±2</td>
<td>65±7*</td>
<td>326±67*†</td>
<td>155±33</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>CTL/ENA</td>
<td>17±3</td>
<td>36±4</td>
<td>499±107</td>
<td>259±56</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>LVH/ENA</td>
<td>11±3</td>
<td>50±5*‡</td>
<td>379±93</td>
<td>205±55</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td><strong>After 30 min reperfusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>95±8</td>
<td>14±3</td>
<td>2,205±212</td>
<td>1,326±163</td>
<td>1.3±0.30</td>
</tr>
<tr>
<td>LVH</td>
<td>158±9*</td>
<td>18±2</td>
<td>3,779±230*</td>
<td>2,133±95†</td>
<td>1.9±0.34</td>
</tr>
<tr>
<td>CTL/ENA</td>
<td>97±7</td>
<td>13±2</td>
<td>2,375±165</td>
<td>1,508±108</td>
<td>1.6±0.26</td>
</tr>
<tr>
<td>LVH/ENA</td>
<td>158±12*</td>
<td>15±1</td>
<td>3,575±321†</td>
<td>2,075±155†</td>
<td>1.7±0.17</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LV Dev P, left ventricular (LV) developed pressure; LVEDP, LV end-diastolic pressure; LV +dP/dt, LV maximal positive dP/dt; LV −dP/dt, LV maximal negative dP/dt; CBF/g, coronary blood flow per gram left ventricular weight; CTL, nonhypertrophied hearts; LVH, hypertrophied hearts; CTL/ENA, nonhypertrophied hearts treated with enalaprilat; LVH/ENA, hypertrophied hearts treated with enalapril.

*p<0.01, †p<0.05 LVH vs. CTL, or LVH/ENA vs. CTL/ENA. ¥p<0.01 LVH vs. LVH/ENA.

Effect of Low-Flow Ischemia

At the onset of ischemia, coronary flow per gram left ventricular weight decreased in nonhypertrophied controls to 0.30±0.05 and 0.32±0.04 ml/min/g (CTL and CTL/ENA, respectively; p=NS) and in hypertrophied hearts to 0.23±0.03 and 0.24±0.03 ml/min/g (LVH and LVH/ENA, respectively; p=NS) (Figure 1). Although absolute coronary flow per gram was not different among the groups, it is recognized that reduction of coronary flow expressed as a percentage of the preischemic baseline value was greater in untreated hypertrophied versus nonhypertrophied hearts (11.3±0.9% versus 17.4±2.3% of preischemic flow [LVH versus CTL]; p<0.01), as well as in treated hypertrophied versus nonhypertrophied hearts (11.2±1.0% versus 19.2±1.7% of preischemic flow [LVH/ENA versus CTL/ENA]; p<0.01). During the 30-minute ischemic period, at a

TABLE 3. Oxygen Consumption at Baseline, After 20 Minutes of Treatment, and During Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>MVO2/g LV wt (μmol/min/g)</th>
<th>MVO2/g LV wt/mm Hg LV Dev P (μmol/min/g/mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 20 Min treatment</td>
<td>Baseline 20 Min treatment</td>
</tr>
<tr>
<td>CTL</td>
<td>26.2±4.2</td>
<td>25.1±3.8</td>
</tr>
<tr>
<td>LVH</td>
<td>41.9±4.8*</td>
<td>36.7±4.2†</td>
</tr>
<tr>
<td>CTL/ENA</td>
<td>38.8±4.4</td>
<td>32.3±5.0†</td>
</tr>
<tr>
<td>LVH/ENA</td>
<td>40.5±4.0</td>
<td>40.2±4.3</td>
</tr>
<tr>
<td><strong>10 Min reperfusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>28.2±8.7</td>
<td>23.0±5.3</td>
</tr>
<tr>
<td>LVH</td>
<td>33.8±6.0†</td>
<td>29.8±5.9†</td>
</tr>
<tr>
<td>CTL/ENA</td>
<td>35.4±4.4</td>
<td>26.1±3.2†‡</td>
</tr>
<tr>
<td>LVH/ENA</td>
<td>39.1±4.0</td>
<td>28.1±2.3†‡</td>
</tr>
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<td><strong>30 Min reperfusion</strong></td>
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<tr>
<td>CTL</td>
<td>28.2±8.7</td>
<td>23.0±5.3</td>
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<tr>
<td>LVH</td>
<td>33.8±6.0†</td>
<td>29.8±5.9†</td>
</tr>
<tr>
<td>CTL/ENA</td>
<td>35.4±4.4</td>
<td>26.1±3.2†‡</td>
</tr>
<tr>
<td>LVH/ENA</td>
<td>39.1±4.0</td>
<td>28.1±2.3†‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=6 in each group. MVO2/g LV wt, oxygen consumption per gram left ventricular dry weight; LV Dev P, left ventricular developed pressure; CTL, nonhypertrophied hearts; LVH, hypertrophied hearts; CTL/ENA, nonhypertrophied hearts treated with enalaprilat; LVH/ENA, hypertrophied hearts treated with enalaprilat.

*p<0.05 *compared with CTL, †compared with baseline, or ‡compared with 10 minutes of reperfusion.
Constant coronary perfusion pressure of 12 mm Hg, there was a further slight decline of coronary blood flow in all groups ($p<0.001$). Whereas absolute coronary flow per gram was again similar between the groups, the percentage of ischemic flow relative to baseline was higher in untreated nonhypertrophied versus hypertrophied hearts ($7.8\pm1.4\%$ versus $3.2\pm0.7\%$ [CTL versus LVH]; $p<0.05$) as well as in treated nonhypertrophied versus hypertrophied hearts ($7.6\pm1.7\%$ versus $3.7\pm0.6\%$ of preischemic flow [CTL/ENA versus LVH/ENA]; $p<0.05$).

This decrease indicates an increase in coronary vascular resistance that may be due to an increase in intramural compressive forces or increased left ventricular end-diastolic pressure as ischemic contracture is developing. Enalaprilat had no effect on coronary flow during the 30 minutes of low-flow ischemia in either the hypertrophied (LVH/ENA) or nonhypertrophied (CTL/ENA) group.

During low-flow ischemia, left ventricular systolic function as assessed by left ventricular developed pressure was similar in all groups. Left ventricular developed pressure declined immediately to 12–23% of initial values and then diminished slightly further in all groups (Figure 2). This pattern differs from experiments with no-flow ischemia, in which deterioration of systolic function is precipitous and followed by complete loss of contractile function. There was no effect of enalaprilat on systolic function in hypertrophied or nonhypertrophied hearts compared with untreated hearts.

Immediately after the onset of ischemia, there was a gradual rise in diastolic pressure ($0.5–1.5$ mm Hg) in all groups, consistent with a loss of coronary turgor. After a delay of approximately 10 minutes of low-flow ischemia, there was a gradual rise in left ventricular end-diastolic pressure to a significantly higher level in hypertrophied than nonhypertrophied hearts in both the untreated groups ($65\pm7$ versus $33\pm3$ mm Hg [LVH versus CTL]; $p<0.01$) and the enalaprilat-treated groups ($50\pm5$ versus $36\pm4$ mm Hg [LVH/ENA versus CTL/ENA]; $p<0.01$) (Figure 3). Enalaprilat had no effect on diastolic function in nonhypertrophied hearts. In contrast, enalaprilat attenuated the marked rise in left ventricular isovolumic diastolic pressure that was observed in untreated hypertrophied hearts ($65\pm7$ versus $50\pm5$ mm Hg [LVH versus LVH/ENA]; $p<0.01$).

After onset of low-flow ischemia, oxygen consumption decreased to 11–20% of baseline values in all
At the end of ischemia, tissue levels for glycogen, ATP, and CP were all depleted compared with the hypertrophied and nonhypertrophied subsets of hearts perfused for 100 minutes without ischemia \( (p<0.01) \). Tissue glycogen levels were 68±11 and 56±13 \( \mu \text{mol} \) glucose equivalent per gram dry weight in nonhypertrophied hearts (CTL and CTL/ENA, respectively; \( p=NS \)) and 44±14 and 46±14 \( \mu \text{mol} \) glucose equivalent per gram dry weight in hypertrophied hearts (LVH and LVH/ENA, respectively; \( p=NS \)). Similarly, total myocardial ATP levels at the end of ischemia were comparable in all groups (Figure 5). Myocardial CP tissue levels were lower in hypertrophied than nonhypertrophied hearts, but there was no difference in hearts treated without or with enalaprilat (Figure 5).

Myocardial water content was assessed by wet weight/dry weight ratio. Enalaprilat had no effect on myocardial water content in nonhypertrophied hearts \( (4.29±0.09 \text{ versus } 4.27±0.14 \text{ [CTL versus CTL/ENA]; } p=NS) \) and hypertrophied hearts \( (4.34±0.03 \text{ versus } 4.27±0.07 \text{ [LVH versus LVH/ENA]; } p=NS) \).

Subgroups of Hearts With Similar Percent Reduction of Coronary Blood Flow During Low-Flow Ischemia

Low-flow ischemia was imposed by reduction of coronary perfusion pressure to 12 mm Hg in both groups,
and residual coronary flow was controlled by autoregulation. Ischemic coronary flow showed a considerable variation in all groups. Although coronary flow was not different among the groups, the reduction of coronary flow assessed as percentage of preischemic flow was greater in untreated and treated hypertrophied hearts than in nonhypertrophied hearts (see above). Even though the difference in residual coronary flow was small (maximally 0.08 ml/min/g; i.e., 8% of preischemic flow), it may nevertheless have contributed to differences between nonhypertrophied and hypertrophied hearts in systolic and diastolic function and lactate production during low-flow ischemia. Therefore, to definitively test the hypothesis of an increased susceptibility of hypertrophied hearts to ischemic injury, we also analyzed our data including only the subsets of hearts with an initial decline to >9% and <20% of preischemic flow.

At the onset of ischemia, in these subgroups coronary flow per gram left ventricular weight decreased in untreated nonhypertrophied hearts (CTL, n=9) to 0.24±0.05 ml/min/g and in untreated hypertrophied hearts (LVH, n=11) to 0.26±0.03 ml/min/g (13.2±1.8% versus 12.4±0.9% of preischemic flow [CTL versus LVH]; p=NS). Coronary flow per gram decreased in treated nonhypertrophied hearts (CTL/ENA, n=8) to 0.28±0.05 ml/min/g and in treated hypertrophied hearts (LVH/ENA, n=11) to 0.27±0.03 ml/min/g (15.2±1.2% versus 12.3±1.0% of preischemic flow [CTL/ENA versus LVH/ENA]; p=NS). During the 30-minute ischemic period, coronary flow decreased further in untreated control versus hypertrophied hearts to 0.11±0.03 versus 0.08±0.02 ml/min/g (6.5±1.6% versus 4.0±0.7% of preischemic flow [CTL versus LVH]; p=NS) and in the enalaprilat-treated hearts to 0.11±0.04 and 0.10±0.02 ml/min/g (5.4±1.8% versus 4.4±0.6% of preischemic flow [CTL/ENA versus LVH/ENA]; p=NS).

At the onset of ischemia, left ventricular developed pressure was similar in untreated subgroups (22±5 mm Hg [19.1±2.6% of baseline value] versus 30±2

<table>
<thead>
<tr>
<th>TABLE 4. Total Lactate Production</th>
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<tr>
<td><strong>Washout during ischemia</strong></td>
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<tr>
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<tr>
<td>CTL (n=13)</td>
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<tr>
<td>LVH (n=14)</td>
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<tr>
<td>CTL/ENA (n=13)</td>
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<td>LVH/ENA (n=14)</td>
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Values are mean±SEM measured in micromoles per gram dry weight. CTL, nonhypertrophied hearts; LVH, hypertrophied hearts; CTL/ENA, nonhypertrophied hearts treated with enalaprilat; LVH/ENA, hypertrophied hearts treated with enalaprilat.

*p<0.05 LVH vs. CTL, or LVH/ENA vs. CTL/ENA.

**FIGURE 5.** Bar graphs showing tissue levels of ATP (left panels) and creatine phosphate (CP) (right panels) at end ischemia (top panels), at the end of 30 minutes of reperfusion (middle panels), and after 60 minutes of continuous perfusion in the absence of ischemia in additional control hearts (bottom panels). At the end of the ischemic period, tissue levels of ATP were similar in all groups, and CP levels in untreated and enalaprilat-treated hypertrophied hearts (LVH and LVH/ENA) were lower compared with untreated and enalaprilat-treated nonhypertrophied hearts (CTL and CTL/ENA). During 30 minutes of reperfusion, ATP and CP were restored to a similar level in all groups.
mm Hg [13.6±0.8% of baseline]; CTL versus LVH; p=NS) and in treated subgroups (23±5 mm Hg [15.5±2.1% of baseline] versus 28±4 mm Hg [12.0±1.3% of baseline]; CTL/ENA versus LVH/ENA; p=NS). During the 30-minute ischemic period, left ventricular developed pressure declined to a similar extent in untreated (14±4 mm Hg [10.3±2.7% of baseline] versus 13±2 mm Hg [5.6±0.9% of baseline]; CTL versus LVH; p=NS) and treated subgroups (13±2 mm Hg [8.1±2.9% of baseline] versus 14±3 mm Hg [5.8±1.5% of baseline]; CTL/ENA versus LVH/ENA; p=NS).

Comparison of these subgroups with comparable reduction in coronary blood flow relative to baseline reaffirmed the increased susceptibility of hypertrophied hearts to diastolic dysfunction relative to nonhypertrophied hearts. Left ventricular end-diastolic pressure increased to a significantly higher level in untreated hypertrophied hearts of the subgroup compared with nonhypertrophied hearts of the subgroup (58±8 versus 36±4 mm Hg [LVH versus CTL]; p<0.01). In enalaprilat-treated subgroups, the greater increase of left ventricular end-diastolic pressure during ischemia in the hypertrophied hearts was no longer observed (44±4 versus 40±5 mm Hg [LVH/ENA versus CTL/ENA]; p=NS).

Total lactate production was increased in hypertrophied hearts of the subgroups (100±42 and 126±33 μmol/g dry wt [LVH and LVH/ENA]; p=NS) compared with nonhypertrophied hearts (95±39 and 92±24 μmol/g dry wt [CTL and CTL/ENA]; p=0.052).

Similar to the initial analysis, enalaprilat had no effect on coronary blood flow, systolic function, or lactate production in these subgroups during ischemia. Therefore, the analysis of these subgroups with comparable degree of ischemic hypoperfusion relative to baseline confirmed that hypertrophied hearts manifest a severe impairment of left ventricular diastolic function in response to low-flow ischemia in comparison with control hearts and that this difference in ischemic diastolic function between the hypertrophied and nonhypertrophied hearts was abolished by enalaprilat infusion.

Effect of Reperfusion

The initial hyperemic response during reperfusion and the subsequent levels of coronary flow per gram left ventricular weight were similar in all groups, and no effect of enalaprilat could be detected (Table 2). Left ventricular developed pressure recovered to 70–74% of baseline values in all groups, and there was no effect of enalaprilat on recovery of systolic function parameters of left ventricular developed pressure and +dP/dt in hypertrophied and nonhypertrophied hearts. The time course of restoration of left ventricular end-diastolic pressure (Figure 6) and −dP/dt was somewhat slower than systolic function but similar in all groups. At the end of reperfusion in all groups, the elevated left ventricular end-diastolic pressure had returned toward baseline but remained slightly increased compared with preischemic values (p<0.05 for CTL and CTL/ENA; p<0.01 for LVH and LVH/ENA).

During early reperfusion, when left ventricular developed pressure was 70–74% of baseline, oxygen consumption was similar to baseline but then declined significantly during 30 minutes of reperfusion (Table 3). Concomitantly, metabolic mechanical efficiency as assessed by the ratio of oxygen consumption to developed pressure was slightly impaired in all groups during early reperfusion but recovered after 30 minutes. There was no difference between the groups.

Compared with the hypertrophied and nonhypertrophied hearts perfused for 100 minutes without ischemia, high energy phosphates (ATP+CP) were substantially restored in all groups during reperfusion (Figure 5). In hypertrophied hearts treated with enalaprilat and without drug, CP tissue levels were restored to the level measured after the identical period of oxygenated control perfusion in hypertrophied hearts (Figure 5). In contrast, myocardial glycogen levels were still significantly depleted at the end of reperfusion (p<0.05). Tissue glycogen levels in nonhypertrophied hearts were 109±26 and 103±18 μmol glucose equivalent per gram dry weight (CTL and CTL/ENA, respectively; p=NS) and in hypertrophied hearts 78±15 and 86±7 μmol glucose equivalent per gram dry weight (LVH and LVH/ENA, respectively; p=NS).

At the end of reperfusion, myocardial water content in all groups was increased significantly compared with values at the end of ischemia, but myocardial water content was not affected by enalaprilat treatment in either the nonhypertrophied hearts (4.65±0.13 versus
Discussion

Pressure-overload cardiac hypertrophy is characterized by left ventricular diastolic dysfunction, which is especially prominent during energy deprivation. Our purpose was to test the hypothesis that the specific inhibition of cardiac angiotensin converting enzyme with enalaprilat in isolated red blood cell–perfused hypertrophied and nonhypertrophied rat hearts exerted a protective effect on systolic and/or diastolic function in nonhypertrophied and hypertrophied rat hearts during low-flow ischemia and reperfusion.

During baseline perfusion, we found no inotropic effect of enalaprilat in the nonhypertrophied and hypertrophied rat hearts. This is consistent with reports that angiotensin II does not exert a positive inotropic effect in rats and guinea pigs, different from some other species and possibly from humans.

The current study resulted in two major observations. First, during low-flow ischemia, hypertrophied hearts developed greater diastolic dysfunction than nonhypertrophied hearts did. This observation was confirmed in a separate analysis of subsets of nonhypertrophied and hypertrophied hearts with a similar percent reduction in coronary flow per gram left ventricular weight and similar depression of developed pressure during low-flow ischemia relative to baseline. Previous studies have reported that hypertrophied hearts also show greater diastolic dysfunction during hypoxia and pacing-induced ischemia and develop diastolic contracture earlier during zero-flow ischemia, especially when hypertrophy is associated with failure. In this experiment, the greater susceptibility of the hypertrophied hearts to ischemic diastolic dysfunction was found in the presence of comparable coronary flows during baseline and low-flow ischemia, comparable depression of contractile function, and a preserved lactate washout. Therefore, in our model, preischemic underperfusion and greater lactate accumulation during ischemia were avoided and were excluded as factors suggested by others as responsible for the increased susceptibility of hypertrophied hearts to ischemic dysfunction.

In this study using a physiological red blood cell–containing perfusate and incorporating free fatty acid as additional substrate, the overall total lactate production was greater in hypertrophied than nonhypertrophied hearts. This observation differs from our previous observation in the deoxycorticosterone acetate–salt model of hypertensive hypertrophy that glycolytic ATP production during hypoxia was attenuated in rats with hypertensive left ventricular hypertrophy and that impaired recruitment of anaerobic glycolysis contributed to hypoxic diastolic dysfunction in this model. Lactate production during ischemia has also been shown to be impaired in canine failing hypertrophied hearts. Thus, critical assessment of glycolytic ATP production is important, since glycolytic ATP production appears to be functionally important in the prevention of diastolic contracture during partial ischemia or hypoxia.

The observations in the present study are in accordance with a report by Anderson et al., who showed an increased capacity for lactate production in a similar model of aortic-banded rat hearts during ischemia. Thus, in the present study, a depressed capacity for glycolytic ATP production cannot account for the exaggerated diastolic dysfunction during ischemia in the hypertrophied hearts.

A second major finding of this study was that perfusion with enalaprilat attenuated the deterioration of left ventricular diastolic chamber distensibility during ischemia in hypertrophied but not in nonhypertrophied hearts. This effect could not be explained by differences in ischemic coronary flow, levels of contractile work, myocardial water content, oxygen consumption, total lactate production, and tissue levels of high energy phosphate compounds at end ischemia between treated and untreated hearts.

The possible contribution of the intrinsic cardiac renin-angiotensin system in the hypertrophied heart during ischemia and reperfusion has not been examined. In this regard, others have observed a beneficial effect of angiotensin converting enzyme inhibition in nonhypertrophied rat hearts on ischemia/reperfusion injury. In those studies, pretreatment or perfusion with an angiotensin converting enzyme inhibitor attenuated or prevented reperfusion arrhythmias, which was associated with a better recovery of left ventricular function and preservation of high energy compounds.

In these studies, the reduction of reperfusion arrhythmias and hemodynamic dysfunction was attributed to several possible mechanisms including a reduced degradation of bradykinin, an abolition of norepinephrine overflow, an induction of prostaglandin synthesis, and an anti–free radical effect of the angiotensin converting enzyme inhibitor (captopril and ramiprilat). Since in all these studies the untreated group showed considerably longer episodes of ventricular fibrillation, the better contractile and metabolic recovery of the hearts treated with angiotensin converting enzyme inhibition may have been mediated by avoiding energy–costly prolonged fibrillation and not by a direct effect of an inhibition of the locally formed angiotensin II on the myocardium. Our experimental design attempted to avoid the confounding effect of prolonged ventricular fibrillation by immediately electrically converting the hearts or excluding them from analysis if conversion was not possible within 15 seconds. Under these conditions in which the confounding effects of prolonged ventricular fibrillation and sulfhydryl–group free radical interaction were avoided, enalaprilat had a marked protective effect on diastolic dysfunction in the hypertrophied hearts during low-flow ischemia but exerted no protective effect during ischemia on systolic contractile function or anaerobic metabolism in the hypertrophied and nonhypertrophied hearts.

Postischemic functional and metabolic recovery was similar in all groups. The degree of recovery indicated a partially reversible rather than an irreversible injury. In addition to a comparable recovery of untreated and enalaprilat–treated hypertrophied hearts, hypertrophied hearts exhibited a similar recovery at 30 minutes of systolic and diastolic contractile function and metabolism compared with nonhypertrophied hearts. This is in accordance with other studies that showed a comparable recovery after zero–flow ischemia in hearts with...
compensated hypertrophy compared with nonhypertrophied hearts\textsuperscript{26} and when coronary perfusion conditions were matched in hypertrophied and nonhypertrophied hearts.\textsuperscript{24,27}

Limitations of the Study

We postulate that the beneficial effect of enalaprilat infusion on ischemic diastolic dysfunction in the hypertrophied hearts was related to an inhibition of the local formation of angiotensin II. We did not directly measure angiotensin converting enzyme activity and angiotensin II in these experiments in which there is a technical limitation of very rapid degradation of angiotensin by nonspecific peptidases in blood-perfused preparations.\textsuperscript{56,57} However, using a buffer-perfused preparation in our laboratory, Schunkert et al.\textsuperscript{15} have shown an increased angiotensin converting enzyme activity as well as an increased conversion of angiotensin I to angiotensin II in the same isolated heart model of left ventricular hypertrophy caused by aortic banding used in this study.

Only one concentration of enalaprilat was used, and a direct nonspecific effect cannot be ruled out. However, in prior experiments we have confirmed that enalaprilat at this particular concentration was highly effective in inhibiting the increased conversion of angiotensin I to angiotensin II in this model of pressure-overload hypertrophy.\textsuperscript{15} Therefore a direct, nonspecific effect of enalaprilat seems unlikely as a main factor for the observed effect of enalaprilat on ischemic diastolic dysfunction in the hypertrophied hearts.

In the intact animal, angiotensin II may act by facilitation of sympathetic activation on the heart,\textsuperscript{9,24} and the inhibition of the formation of angiotensin II could be protective by indirectly diminishing local nor-epinephrine release during ischemia and reperfusion. We have not ruled this out as a contributing factor to the observed protective effect of enalaprilat on diastolic dysfunction during low-flow ischemia. However, this seems highly unlikely, since we have previously shown that mildly inotropic levels of $\beta$-adrenergic stimulation are protective of both systolic and diastolic function in this blood-perfused model of low-flow ischemia.\textsuperscript{58} Further, others have shown that the action of angiotensin II is independent of endogenous catecholamine stores and cannot be influenced by adrenergic blocking agents.\textsuperscript{49,50} and the cardiac effects of angiotensin II do not appear to be mediated by adenylate cyclase activation and cAMP.\textsuperscript{48,52} In addition, the exact sites where enalaprilat inhibits angiotensin converting activity in the heart remain to be defined: both the coronary vasculature and the cardiac myocyte must be considered. Determination of the subcellular localization of various components of the renin-angiotensin system in normal and hypertrophied hearts may allow clarification of this important issue.

Effects of Angiotensin Converting Enzyme Inhibition on Diastolic Function

The exaggerated sensitivity of the hypertrophied heart to develop diastolic dysfunction during ischemia is postulated to be related to the rise in intracellular free $\text{Ca}^{2+}$ that occurs immediately during ischemia\textsuperscript{59,60} in the presence of the intrinsic prolongation of $\text{Ca}^{2+}$-activated force in hypertrophied myocardium.\textsuperscript{2,61} We speculate that the protective effect of enalaprilat on diastolic dysfunction during low-flow ischemia may be related to the effects of angiotensin II on phosphoinositide second messengers, which may exacerbate the severe impairment of $\text{Ca}^{2+}$ handling in the hypertrophied heart during ischemia. The subcellular transduction of angiotensin II appears to be associated with the activation of phosphoinositide second messengers,\textsuperscript{53,62,63} and can be simulated by phorbol ester activation of protein kinase C.\textsuperscript{64} There is evidence that inositol 1,4,5-trisphosphate may promote $\text{Ca}^{2+}$ release from the sarcoplasmic reticulum, whereas protein kinase C activation may depress $\text{Ca}^{2+}$ transport by the sarcoplasmic reticulum and phosphorylate the Na$^+$/H$^+$ antiporter, resulting in intracellular alkalosis that increases myofilament $\text{Ca}^{2+}$-activated force.\textsuperscript{12,63-66} Furthermore, recent studies suggest that inositol 1,4,5-trisphosphate–induced sarcoplasmic reticulum $\text{Ca}^{2+}$ release is enhanced in hypertrophied hearts.\textsuperscript{67} These effects would tend to further amplify the increase in $[\text{Ca}^{2+}]_i$, and impairment of diastolic force inactivation in the ischemic hypertrophied heart in which the $[\text{Ca}^{2+}]_i$ transient is intrinsically prolonged. Thus, it is possible that during the immediate intracellular $\text{Ca}^{2+}$ overload that occurs during ischemia, an enhanced angiotensin II production in hypertrophied hearts further impairs $\text{Ca}^{2+}$ homeostasis and contributes to the observed greater diastolic dysfunction in hypertrophied versus control hearts. Future studies are needed to elucidate subcellular action of angiotensin II in hypertrophied myocardium and to test this hypothesis that angiotensin converting enzyme inhibition may preserve diastolic function in hypertrophied hearts by modifying the effects of phosphoinositide metabolites on the $\text{Ca}^{2+}$-dependent force in diastole.

Clinical Implications

The effect of inhibition of angiotensin converting enzyme by enalaprilat in patients with cardiac hypertrophy associated with dilated cardiomyopathy has been investigated by Foul et al.\textsuperscript{52} After intracoronary infusion of enalaprilat, they found a reduction in elevated left ventricular diastolic pressure without change in end-diastolic volume despite a slight depression of systolic pump function.\textsuperscript{52} In patients with chronic ischemic heart disease, Rousseau et al.\textsuperscript{48} found an improved left ventricular diastolic distensibility and reduced diastolic wall stress after intravenous administration of the angiotensin converting enzyme inhibitor benzaeprilat. These clinical studies support our observation that intracardiac activation of angiotensin II may contribute to abnormal diastolic function in the hypertrophied heart and that its specific inhibition may be especially protective during ischemic stress. However, thus far, this hypothesis has not been studied in the human heart with pressure-overload hypertrophy at baseline or during transient ischemia.

In conclusion, we showed that isolated red blood cell–perfused hearts with compensatory pressure-overload hypertrophy show severe left ventricular diastolic dysfunction compared with nonhypertrophied hearts during low-flow ischemia, and this prominent ischemic diastolic dysfunction is favorably modified by the specific angiotensin converting enzyme inhibitor enalapril-
lat. We have previously shown that there is enhanced activation of the cardiac renin-angiotensin system in chronic pressure-overload hypertrophy. This study suggests that the activation of the “primed” renin-angiotensin system in hypertrophied hearts during ischemia has a deleterious effect on diastolic function that can be beneficially influenced by specific inhibition of cardiac angiotensin converting enzyme.

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Exacerbation of left ventricular ischemic diastolic dysfunction by pressure-overload hypertrophy. Modification by specific inhibition of cardiac angiotensin converting enzyme.

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