Effects of Brief Repetitive Ischemia on Contractility, Relaxation, and Coronary Flow

Exaggerated Postischemic Diastolic Dysfunction in Pressure-Overload Hypertrophy

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The recovery of systolic and diastolic function during unstable angina may be modified by the repetition of brief episodes of ischemia and by the presence of left ventricular hypertrophy (LVH). We studied the effects of six consecutive 5-minute cycles of no-flow ischemia and reperfusion followed by 25 minutes of recovery in isovolumic red blood cell–perfused hearts from aortic-banded rats with chronic LVH (n=8) and sham-operated control rats (n=8). At baseline (left ventricular end-diastolic pressure [LVEDP], 10 mm Hg), left ventricular developed pressure (123±5 versus 114±5 mm Hg/g) and coronary flow (2.5±0.3 versus 2.2±0.2 (mL/min)/g) were similar in LVH versus control rats. Repetitive ischemia was associated with progressive depression of postischemic recovery of left ventricular systolic function, and the recovery of left ventricular developed pressure after the final 25-minute reperfusion period was similar in LVH versus control rats (61±6% versus 72±4% of baseline, P=NS). Although there was no increase in isovolumic LVEDP during the initial cycle of transient ischemia, both groups showed a rapid and similar rise in LVEDP during subsequent ischemic cycles (∆A2±8 versus ∆A9±7 mm Hg/g in response to the final ischemia cycle for LVH versus control rats, respectively; P=NS). The control hearts showed complete restoration of LVEDP to baseline during final reperfusion, whereas the LVH hearts showed prolonged and severe postischemic diastolic dysfunction. The peak hyperemic coronary flow was also depressed in the LVH versus control group [2.2±0.3 versus 4.8±0.8 (mL/min)/g, P<.01]. These differences in postischemic diastolic dysfunction and coronary flow during reperfusion were not explained by any differences in glycolytic flux, myocardial ATP depletion, or edema between groups. Thus, hypertrophied hearts are susceptible to severe postischemic diastolic stunning during reperfusion after brief repetitive ischemia, which may be in part related to blunted reactive hyperemia and to persistent postischemic elevation of intracellular Na⁺ and Ca++. (Circulation Research 1993;73:550-558)

Key Words • left ventricular hypertrophy • stunning • ischemia • reperfusion

In the clinical setting of unstable angina, it is controversial whether brief repetitive periods of severe ischemia and reperfusion, which alone are not sufficient to induce myocardial necrosis, have an adaptive “conditioning” effect1-3 or a cumulative deleterious effect on the recovery of systolic and diastolic function and myocardial metabolism.4-6 It has been evident that even a short duration of ischemia without any myocardial necrosis may promote prolonged abnormalities of subcellular metabolism and mechanical function, in which diastolic dysfunction is prominent.7-10 In addition, many patients with coronary artery disease, chronic left ventricular hypertrophy due to pressure overload is a coexisting factor that may modify the response to repetitive ischemic episodes. Advanced cardiac hypertrophy is characterized by impaired relaxation and an increased susceptibility to severe diastolic dysfunction in response to prolonged ischemia.11-15

It is unclear if the presence of left ventricular hypertrophy (LVH) modifies the recovery of systolic and diastolic function and the hyperemic coronary flow response during reperfusion following brief repetitive episodes of ischemia, which as a single insult cause minimal impairment of postischemic contractile or lusitropic function. This study was performed to compare the recovery of left ventricular (LV) systolic versus diastolic function, coronary flow, and metabolism in response to repetitive brief 5-minute cycles of no-flow global ischemia and reperfusion in isovolumic and normothermic red blood cell–perfused hypertrophied and control rat hearts.

Materials and Methods

Preparation of Animals

Male Wistar rats were obtained from the Charles River Breeding Laboratories. Aortic stenosis was cre-
ated in weanling rats (body weight, 60 g; age, 3 to 4 weeks) by placing a stainless-steel clip of 0.6-mm internal diameter on the ascending aorta via a thoracic incision. The rats with aortic stenosis (LVH group, n=8) and the age-matched sham-operated control rats (control group, n=8) underwent a left thoracotomy under anesthesia with intraperitoneal sodium pentobarbital plus supplemental nasal oxygen. The rats were subsequently fed normal rat chow and water ad libitum and were used for experimentation 11 to 12 weeks after the operation.

**Experimental Preparation**

We used an isolated isovolumic red blood cell–perfused heart preparation that was developed by Marshall and Zhang and modified in our laboratory. Rats were injected intraperitoneally with 1.5 to 2.0 mL sodium pentobarbital (15 mg/mL), and the thorax was rapidly opened. After thoracotomy, the heart was isolated, a perfusion cannula was inserted into the ascending aortic stump and positioned immediately above the aortic valve, and the coronary arteries were perfused via the aortic root with the red blood cell–containing perfusate. The interval between opening the thorax and initiation of coronary perfusion was less than 20 seconds in all experiments.

The bovine red blood cell perfusate was prepared from fresh whole bovine blood containing 15 000 U sodium heparin per liter, which was spun in a refrigerated centrifuge at 5°C with a rotor speed of 3000 rpm for 15 minutes. The supernate was aspirated, and 100 mL of the resulting packed cells were mixed with 100 mL of Krebs-Henseleit buffer, washed three times, and centrifuged at 3000 rpm for 15 minutes at 5°C. The resulting packed cells were thus essentially white blood cell and platelet free. The packed cells were stored at 4°C, washed daily, and used within 1 week of preparation.

The red blood cells were suspended at a final hematocrit of 40% in Krebs-Henseleit buffer containing (mmol/L) 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 (No. P-9767, as a source for free fatty acid, Sigma Chemical Co, St Louis, Mo); along with 4% free fatty acid–free bovine serum albumin (No. A-7030, Sigma). The buffer and red blood cell suspension were 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 20% O₂–3% CO₂–77% N₂ to achieve a PO₂ of 100 to 140 mm Hg and pH of 7.35 to 7.45 in all experiments.

After initiation of coronary perfusion, an apical drainage cannula was inserted in the LV apex to vent the thebesian drainage. The pulmonary artery and venae cavae were ligated, and a cannula was inserted through the right atrium into the right ventricle to continuously collect the coronary venous effluent and empty the right ventricle completely. A pacing electrode connected to an electric stimulator (model S6, Grass Instrument Co, Quincy, Mass) and a thermistor (model 43TA, Yellow Springs Instrument Co, Yellow Springs, Ohio) were inserted into the right ventricle via the right atrium. Heart rate was maintained at a paced physiological rate of 4 Hz, and myocardial temperature was maintained at 37°C. A collapsed thin-walled latex balloon was placed in the LV via the left atrium. The balloon was large enough so that no measurable pressure was generated by the balloon itself over the range of LV volume used in this experiment. The heart was then placed in a water-jacketed constant-temperature chamber.

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 arterial reservoir was pressurized, and coronary perfusion pressure was controlled by a valve that adjusted the pressure of the reservoir. Coronary blood flow was allowed to vary and thus depended on coronary vasomotor autoregulation.

**Hemodynamic Measurements**

Coronary perfusion pressure, LV pressure, and its first derivative were recorded continuously on a multichannel physiological recorder (Gould, Inc, Cleveland, Ohio). Coronary perfusion pressure was monitored via a side arm of the aortic cannula connected to a Gould-Statham P23Db pressure transducer. LV pressure was measured with short, stiff, fluid-filled polyethylene tubing attached to a Gould-Statham P23Db pressure transducer. The frequency response and damping characteristics of this system have been described in a previous study from this laboratory and satisfy the requirements shown by Falsetti et al for accurate measurements of LV pressure and its first derivative. LV dia- stolic chamber distensibility should ideally be assessed from a plot of the relation between diastolic pressure and volume over a wide range. In the present study, the rapid changes in diastolic function during ischemia and reperfusion precluded serial measurements of diastolic pressure–volume points. In the isolated perfused heart model, we have previously shown that an upward shift in isovolumic diastolic pressure closely correlates with an upward shift in the diastolic pressure–volume relation measured over a wide range of diastolic volumes. Thus, it is our interpretation that an increase in isovolumic LV end-diastolic pressure (LVEDP) in the presence of constant LV balloon volume signifies a decrease in LV diastolic chamber distensibility.

Coronary blood flow was measured by timed collections of coronary venous effluent. Arterial blood gas analysis (Blood Gas Analyzer, Allied Instrumentation Laboratory, Lexington, Mass) was performed every 10 minutes throughout the protocol. Arterial and coronary venous lactate content was measured by the specific enzymatic method of Apstein et al.

**Experimental Protocol**

All hearts were perfused for a 30-minute warm-up period. At baseline, coronary perfusion pressure was set at 80 mm Hg in the control group and at 100 mm Hg in the LVH group. This strategy was selected because previous studies in our laboratory and have shown that comparable coronary flow per unit LV mass could be consistently obtained by this approach. In both groups, LV balloon volume was initially adjusted to achieve an LVEDP of 10 mm Hg under baseline conditions, and this balloon volume was kept constant throughout the experiment. At this operational point on the diastolic pressure–volume relations of the LVH and control hearts, LV balloon volumes were similar in both groups.
After baseline measurements of LV pressure, its first derivative, and coronary flow, both groups were subjected to six repetitive cycles of “ischemia/reperfusion.” Each ischemia/reperfusion cycle consisted of 5 minutes of no-flow ischemia and 5 minutes of reperfusion at the baseline coronary perfusion pressure, and coronary flow was determined by autoregulation. After the last ischemia/reperfusion cycle, all hearts were subjected to a 25-minute final period of reperfusion. The LV pressure and its first derivative were measured at the end of each cycle of 5-minutes of ischemia (cycles 11 through 61), at the end of each cycle of 5 minutes of reperfusion (cycles 1R through 6R), and at the end of the experiment (final 25 minutes of reperfusion). The protocol is illustrated in Fig 1. During each reperfusion period, we measured coronary blood flow every minute and considered the peak flow rate as an index of reactive hyperemia. Samples of the arterial and coronary venous effluent were obtained for determination of lactate content at baseline and continuously during each 5-minute reperfusion cycle. The samples were collected in trichloroacetic acid (final concentration, 5%) and stored at 4°C before processing.

At the end of the experiment, the total heart and LV wet weight were measured. The LV was oven-dried for 2 weeks for calculation of the LV wet to dry weight ratio.

**Analysis of Myocardial ATP Content**

Myocardial ATP content at the end of the experiment was determined in four of eight hearts in each group. At the end of the final 25 minutes of reperfusion, the heart was quickly trimmed of atria and right ventricular free wall, and the LV was rapidly frozen with Wollenberger aluminum clamps cooled with liquid nitrogen. Each frozen sample was rapidly weighed and pulverized in a mortar under liquid nitrogen. An aliquot of the frozen powder was weighed and then heated (37°C) in an oven for 48 hours to determine the frozen to dry weight ratio. The remainder of the sample was mixed with 0.6N perchloric acid, homogenized, centrifuged, and neutralized with 5 mol/L potassium carbonate. The aliquot of neutralized homogenate was placed in preweighed reagent vials and analyzed for ATP by methods of Adams. Measurements are expressed as micromoles per gram LV dry weight. In our laboratory, basal values of ATP for control and LVH hearts measured after oxygenated red blood cell perfusion for the duration of this experiment were as follows: control hearts, 16.6±1.5 μmol/g LV dry weight; LVH hearts, 15.0±1.6 μmol/g LV dry weight (P=NS). To obtain the LV wet weight, part of each frozen sample was quickly defrosted at room temperature. LV wet weight was calculated by the wet to frozen weight ratio.

**Statistical Analysis**

Data are presented as mean±SEM. Statistical comparisons between groups in response to six repeated cycles of ischemia and in response to the six repeated cycles of reperfusion were performed using analyses of variance for repeated measures. Comparisons between the control and LVH groups for baseline and end-experiment hemodynamic data were tested by Student’s t test for unpaired data with the Bonferroni correction, and metabolic measurements between two groups were tested by Student’s t test for unpaired data. A value of P<0.05 was accepted as the level of significance.

**Results**

**Baseline Characteristics**

The magnitude of hypertrophy and baseline hemodynamics for the LVH and control groups are shown in the Table. The LVH group was characterized by a 47% increase in LV weight and 55% increase in the LV to body weight ratio relative to the control group. Both groups showed similar levels of coronary flow per gram LV mass, aerobic myocardial lactate extraction, and LV developed pressure (per gram LV weight) under baseline conditions. Because the LV (balloon) volumes were

### Baseline Characteristics of Pressure-Load Hypertrophy and Left Ventricular Hemodynamic Parameters

<table>
<thead>
<tr>
<th>C</th>
<th>LVH</th>
<th>P (C vs LVH)</th>
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</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>423±18</td>
<td>396±7</td>
</tr>
<tr>
<td>LVW (g)</td>
<td>1.03±0.02</td>
<td>1.51±0.05</td>
</tr>
<tr>
<td>LVW/BW (g/kg)</td>
<td>2.46±0.11</td>
<td>3.81±0.12</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>127±5</td>
<td>194±5</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>10.0±0.3</td>
<td>10.9±0.2</td>
</tr>
<tr>
<td>LV dev P (mm Hg)</td>
<td>117±5</td>
<td>184±5</td>
</tr>
<tr>
<td>LV dev P (g mm Hg/g)</td>
<td>114±5</td>
<td>123±5</td>
</tr>
<tr>
<td>LV +dP/dt (mm Hg/s)</td>
<td>3169±185</td>
<td>4981±277</td>
</tr>
<tr>
<td>LV −dP/dt (mm Hg/s)</td>
<td>2197±114</td>
<td>3400±101</td>
</tr>
<tr>
<td>CF/g [(mL/min)/g]</td>
<td>2.2±0.2</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>Lac Ext [(μmol/g)/min]</td>
<td>0.33±0.19</td>
<td>0.25±0.20</td>
</tr>
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C, control rats; LVH, rats with left ventricular (LV) hypertrophy; BW, body weight; LVW, LV weight; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure; LV dev P, LV developed pressure (absolute values and per gram LV wet weight); LV +dP/dt, peak positive LV dP/dt; LV −dP/dt, peak negative LV dP/dt; CF, coronary flow per gram LV weight; Lac Ext, lactate extraction under baseline nonischemic conditions; NS, not significant. Values are mean±SEM.
comparable in both groups (LVH group, 0.18±0.01 mL; control group, 0.22±0.02 mL; P=NS), LV developed pressure per unit of LV mass can be utilized as an approximation of the LV wall stress developed under our experimental conditions, as described previously.24 LV peak systolic pressure, LV +dP/dt, and LV –dP/dt were significantly higher in the LVH group than the control group.

LV Systolic Function in Response to Repetitive Cycles of Ischemia and Reperfusion

Immediately after the onset of no-flow ischemia, LV developed pressure declined to less than 50% of baseline within 1 minute and fell to a level of zero within 3 minutes of ischemia. Fig 2 shows the recovery of LV developed pressure normalized per gram LV weight during each reperfusion cycle. During repetitive cycles of reperfusion, the magnitude of the recovery of LV developed pressure per gram progressively declined in both the LVH and control groups. In contrast to LV diastolic function as described below, there was no difference in the recovery of LV developed pressure per gram between the groups in response to the six repetitive 5-minute cycles of ischemia and reperfusion, and there was also no significant difference in the recovery of LV developed pressure per gram at the end of the final 25-minute reperfusion between the LVH and control groups (72±4% of baseline versus 61±6% of baseline, P=NS).

LV Diastolic Function in Response to Repetitive Cycles of Ischemia and Reperfusion

Fig 3 shows the isovolumic LVEDP and the change in LVEDP normalized per unit of LV myocardium (ΔLVEDP per gram) at the end of each cycle of 5 minutes of no-flow ischemia. At baseline, initial LVEDP was set at 10 mm Hg in both groups. During the first cycle of no-flow ischemia, there was no increase in isovolumic LV diastolic pressure in either group. However, during subsequent repeated cycles of 5 minutes of no-flow ischemia, there was a rapid and progressive increase of isovolumic LVEDP. During the ischemic cycles, the elevation of LVEDP was similar in the control and LVH groups.

Fig 4 shows the reversal of ischemic LV diastolic contracture at the end of each of the repeated cycles of reperfusion. In both groups, there was a slight and transient further increase in LVEDP (<5 mm Hg) during the initial 90 seconds of reperfusion. In the control group, the ischemic increase in LVEDP was almost completely reversed to baseline within each 5-minute cycle of reperfusion. In contrast, in the LVH group, the abatement of ischemic diastolic contracture in response to reperfusion was incomplete and became progressively more impaired during each reperfusion cycle. The elevation of LVEDP at the end of each successive 5-minute reperfusion cycle was significantly higher in the LVH group than the control group. Recovery of LVEDP after the sixth cycle of ischemia/reperfusion during the final 25-minute period of reperfusion is illustrated in Fig 5. Recovery of LVEDP was also incomplete after the final 25-minute period of reperfusion in the LVH versus control group (28.6±3.9 versus 11.9±0.8 mm Hg, P<.05). In the control group, there was complete recovery to the baseline level of LVEDP. This difference of the recovery in LVEDP was still evident when the change of LVEDP was corrected by LV mass (Fig 4, bottom).

Coronary Flow, Myocardial Lactate Production, and Tissue Water Content

Fig 6 shows the peak value of coronary flow during each repetitive cycle of reperfusion and at the end of the experiment. Peak coronary flow (peak reactive hyperemia) occurred between 60 and 120 seconds in each
FIG 4. Top, Graph showing left ventricular end-diastolic pressure (LVEDP) at baseline (B), at the end of each reperfusion (R) cycle, and at the end of 25 minutes of final reperfusion. C, control rats; LVH, rats with left ventricular hypertrophy; NS, not significant. Bottom, Graph showing ΔLVEDP per gram left ventricular wet weight at the end of each R cycle and 25 minutes of final reperfusion. In comparison with the control group, the recovery of LVEDP in each R cycle was more incomplete and progressively deteriorated in the LVH group.

reperfusion cycle. The control group showed reactive hyperemia to a level of 210±19% of baseline during the initial cycle of reperfusion, which was preserved during subsequent repetitive cycles of reperfusion. However, in the LVH group, there was minimal reactive hyperemia (121±10% of baseline) in response to the initial cycle of reperfusion, and the magnitude of reactive hyperemia fell during subsequent reperfusion cycles. At the end of the experiment in response to the final 25-minute reperfusion period, the LVH and control groups showed similar levels of coronary flow [1.8±0.3 versus 2.2±0.3 (mL/min)/g, P=NS]. Total myocardial lactate washout was maximal during the initial cycle of reperfusion and gradually declined during subsequent reperfusion cycles in both groups. The magnitude of total

FIG 5. Tracings showing the time course of recovery of left ventricular end-diastolic pressure (LVP) after the sixth cycle of no-flow ischemia and the subsequent final 25-minute period of reperfusion in a control heart (top) and in a heart with left ventricular hypertrophy (LVH, bottom). During the final 25-minute period of reperfusion, the control heart showed complete recovery of LVP to the baseline level of 10 mm Hg, whereas recovery of LVP was incomplete in the LVH heart, consistent with prolonged postischemic diastolic stunning.

FIG 6. Graph showing coronary blood flow (CBF, per gram left ventricular wet weight) at baseline (B), peak reactive hyperemia in each reperfusion (R) cycle, and 25 minutes of final reperfusion. C, control rats; LVH, rats with left ventricular hypertrophy; NS, not significant. The LVH group showed minimal reactive hyperemia, and its magnitude was gradually decreased compared with normal reactive hyperemia in the control group.
myocardial lactate washout during each 5-minute cycle of reperfusion (Fig 7) was comparable in both groups. The LV wet to dry weight ratio at the end of reperfusion was similar in the LVH and control groups (4.44±0.05 versus 4.38±0.02, P=NS).

**Tissue ATP at the End of Reperfusion**

LV myocardial ATP content was determined at the end of the 25 minutes of final reperfusion. In comparison with baseline values, myocardial ATP was depleted to 43% of baseline in the LVH group and 40% of baseline in the control group (LVH versus control, P=NS). There was no significant difference in ATP content between the LVH and control groups (6.5±0.4 versus 5.8±0.9 µmol/g dry weight, P=NS). The LV frozen to dry weight ratio was also similar in the LVH and control groups (4.67±0.08 versus 4.82±0.10, P=NS).

**Discussion**

In normal red blood cell–perfused rat hearts, six repetitive 5-minute cycles of no-flow ischemia and reperfusion are associated with progressive impairment of the recovery of LV systolic function, the preservation of postischemic reactive hyperemia, and near-complete restoration of the ischemic rise of LV diastolic pressure to baseline during reperfusion. A novel finding of this study is the presence of profound and prolonged diastolic dysfunction and blunted reactive hyperemia in hearts with chronic pressure-overload hypertrophy, whereas the recovery of systolic function and myocardial ATP levels during reperfusion is similar to that in control hearts.

**Cumulative Effects of Repetitive Ischemia**

There is controversy regarding the cumulative effect of repetitive brief periods of ischemia, in which a single cycle alone is not severe enough to induce myocardial necrosis and is followed by minimal depression of contractile function. In canine models of transient coronary occlusion with the potential for collateral flow, the direct effects of ischemia and reperfusion per se may be obscured by the confounding effects of heterogeneity in the extent and magnitude of ischemia, stretch of the ischemic region by adjacent nonischemic muscle, and pericardial–right ventricular constraint.1–5 In the present study, the isolated and isovolumic normothermic red blood cell–perfused model has the advantages of the imposition of homogenous global no-flow ischemia as well as the lack of changes in loading factors extrinsic to the LV.

In this study, the observation that isovolumic developed pressure fell to greater than 50% of baseline within the first minute of ischemia and reached zero in less than 5 minutes in the nonhypertrophied and hypertrophied hearts is similar to the studies of Koretsune et al.,25 who reported a similar time course of early ischemic failure in isolated ferret hearts. This rapid time course of the decline of pressure development was attributed to collapse of the vasculature with a lesser contribution of proton and inorganic phosphate accumulation.25 During reperfusion, the control and the hypertrophied hearts showed a similar cumulative depressant effect of repetitive brief episodes of global ischemia and reperfusion on recovery of systolic contractile function and depletion of myocardial high-energy phosphate stores. This observation differs from that of Reimer et al.,3 who reported no cumulative ATP depletion in a canine model after four brief periods of repetitive ischemia. Similar to our findings, Asimakis et al6 showed that comparable repetitive cycles of global ischemia and reperfusion produced cumulative ATP loss and depletion of mitochondrial adenine nucleotides in an isolated buffer-perfused rat heart model. Our finding that recovery of developed pressure was similar in hypertrophied and normal hearts corroborates our previous observation of similar recovery of systolic function in normal and hypertrophied red blood cell–perfused hearts after 30 minutes of severe low-flow ischemia.11 This finding differs from that of Buser et al.,14 who observed impaired posts ischemic recovery of systolic function in hypertrophied buffer-perfused rat hearts exposed to 30 minutes of continuous no-flow ischemia. These discrepancies may be related to the effects of continuous versus intermittent ischemia or depressed myocardial perfusion rates in the hypertrophied hearts at baseline and recovery.

**Postischemic Diastolic Dysfunction**

The effects of brief or repeated cycles of ischemia and reperfusion on diastolic function in stunned myocardium have received little attention. In canine and human models of transient regional ischemia, the time course of recovery of diastolic function has generally paralleled the recovery of systolic thickening of regionally stunned myocardium.7,10 In this study, the observation that the normal hearts showed no increase in isovolumic LVEDP during the first 5-minute period of no-flow ischemia is consistent with prior studies of diastolic function during the initial minutes of no-flow or low-flow ischemia.11,13,17,18,20,25 Recent observations by Kihara et al26 using the Ca$^{2+}$ indicator aequorin show that a single brief episode of global ischemia is characterized by an immediate increase in diastolic levels of free Ca$^{2+}$. In the first few minutes of ischemia, the simultaneous development of intracellular acidosis and coronary artery depressurization initially mitigate against the immediate development of a Ca$^{2+}$–activated increase in diastolic tone.18,26–39

In this study, subsequent 5-minute periods of global ischemia resulted in the development of progressive and
severe diastolic contracture suggestive of impairment of the extent of myocardial relaxation and residual diastolic crossbridge interaction. Despite the marked elevation of LVEDP that developed during repetitive ischemia, the normal hearts showed brisk recovery of LVEDP to near baseline levels during each 5-minute reperfusion cycle and complete recovery during the final 25-minute reperfusion period. These observations suggest that normal hearts can rapidly restore normal diastolic crossbridge dissociation during reperfusion. Thus, for the normal nonhypertrophied heart, there is a dissociation between the cumulative development of persistent postischemic systolic dysfunction and accompanying depletion of myocardial ATP and the rapid recovery of postischemic diastolic dysfunction during reperfusion after brief repetitive ischemia.

**Effects of Repetitive Ischemia and Reperfusion on Coronary Flow**

By study design, coronary flow per gram LV mass was adjusted at baseline to similar levels in both groups by utilization of different levels of coronary perfusion pressure, which suggested higher coronary resistance in the hypertrophied heart before ischemia.11,14 The control group maintained a stable level of reactive hyperemia during each reperfusion cycle, whereas in the hypertrophied hearts, reactive hyperemia was depressed during the first reperfusion cycle and fell during successive reperfusion cycles. These results are consistent with previous studies suggesting that hypertrophied hearts are characterized by an impaired coronary reserve in response to coronary vasodilators and transient ischemia.14,15,29,30 In the present study, a cause-and-effect relation in the LVH hearts between postischemic diastolic dysfunction and impaired reactive hyperemia is unclear since the postischemic elevation in LV diastolic pressure may itself have limited coronary perfusion. In this regard, preliminary studies of impaired subendocardial coronary reserve in dogs with heart failure suggest that this impairment may be directly related to the elevation of LVEDP and perivascular compressive forces.31

**Potential Mechanisms of Postischemic Diastolic Dysfunction in Hypertrophied Hearts**

In the hypertrophied hearts, the observation that ischemic elevation of diastolic pressure failed to recover during reperfusion is consistent with a failure of hypertrophied hearts to rapidly and completely restore diastolic crossbridge dissociation to normal during reperfusion, despite similar recovery of systolic function compared with the control hearts. An enhanced coronary turgor effect on diastolic distensibility20,27 cannot explain the difference in the recovery of LV diastolic function between the groups. The magnitude of reactive hyperemia in the LVH group was more depressed during each 5-minute reperfusion cycle, and coronary flow was similar after the final 25 minutes of reperfusion compared with the control group. Furthermore, the wet to dry weight ratios were similar in both groups. Hence, the potential contribution of coronary turgor and myocardial edema to diastolic dysfunction was not exaggerated in the hypertrophied hearts during reperfusion. Freeze clamping of hearts in this study for assessment of ATP levels precluded simultaneous histological assessment of myocardial necrosis. However, there is a close correlation between recovery of systolic function and the limitation of myocardial necrosis. Thus, the similar recovery of systolic function in both groups argues against exaggerated myocardial necrosis as the cause of prolonged diastolic dysfunction during reperfusion in the hypertrophied hearts.

**Glycolytic Flux**

The impaired reactive hyperemia observed in the LVH hearts could potentially promote an impaired washout of myocardial acidosis and the depression of anaerobic glycolytic ATP production. ATP production from glycolysis has been shown to regulate the onset and magnitude of ischemic contracture that is dissociated from total myocardial ATP levels.17,32 In both groups, myocardial lactate production and, by stoichiometric inference, glycolytic ATP production were maximal during the first ischemic episode and then progressively declined in response to subsequent ischemic episodes. Recent observations by Owen et al12 lend support to the proposition that this decline of glycolytic ATP production directly contributed to the similar onset and magnitude of transient diastolic contracture in both groups during repetitive ischemia. However, depressed glycolytic ATP production is unlikely to account for exaggerated postischemic diastolic dysfunction in the hypertrophied hearts, since total myocardial lactate production and the recovery of systolic function were similar in the groups.

**Potential Effects of Ischemia and Reperfusion on Ca""-Sensitive Diastolic Force**

Hypertrophied myocardium is characterized by a reduced density and capacity of sarcoplasmic reticulum Ca""-ATPase pumps as well as alterations in sarcoplasmic cation pumps that regulate the Na"+ gradient.33-36 During transient global ischemia, severe intracellular acidosis develops within minutes and may partially inhibit Na"+-Ca"+ exchange.25,27,28,37 Recent nuclear magnetic resonance studies of the effects of 20 minutes of no-flow ischemia in isolated rat hearts have shown that the rise in [Ca"+], precedes the rise in [Na"+], indicating that Na"+-Ca"+ exchange cannot be the sole mechanism responsible for an ischemic rise in Ca"+ and diastolic tone.28 Thus, during repetitive global ischemia in the present experiment, the progressive development of ischemic contracture may be predominantly determined by the complex interplay of cumulative myocardial ATP depression and diastolic Ca"+ accumulation secondary to depressed sarcoplasmic reticulum function and the opposing effects of loss of turgor and acidosis.18,25-28 A limitation of the present experiment is that direct measurements of [Na"+] and [Ca"+] are technically difficult in red blood cell–perfused hearts and were not performed. Nonetheless, the observation that repetitive global ischemia promoted a similar magnitude of ischemic diastolic contracture in the control and hypertrophied hearts does support the speculation of a similar magnitude of diastolic Ca"+ overload and/or ATP depletion during ischemic cycles.

In the present study, the observation that the magnitude and time course of the abatement of ischemic diastolic contracture was impaired in the hypertrophied hearts raises the hypothesis that reperfusion causes the
ischemic elevation of diastolic Ca$^{2+}$ to fall differently in hypertrophied than in normal hearts. During ischemia, prior studies have shown that [Na$^{+}$] increases because of Na$^{-}$H$^{+}$ exchange driven by the increased proton load, with the potential during reperfusion for enhanced Na$^{-}$Ca$^{2+}$ exchange when acidosis is relieved, particularly if Na$^{+}$,K$^{-}$-ATPase activity is depressed.\textsuperscript{38,36-40} In nonhypertrophied rat and ferret hearts, recent\textsuperscript{20} because of intrinsic alterations in membrane pumps that regulate Ca$^{2+}$ and Na$^{+}$ homeostasis,\textsuperscript{35-36} the hypertrophied heart may be handicapped by a depressed reserve for rapidly restoring diastolic Na$^{+}$ and, secondarily, diastolic Ca$^{2+}$ to normal diastolic levels during reperfusion.

In conclusion, brief repetitive cycles of ischemia and reperfusion in red blood cell–perfused rat hearts result in equivalent depression of the recovery of systolic function, glycolytic flux, and myocardial ATP depletion in normal and hypertrophied hearts. In normal hearts, there is near-complete reversal of ischemic diastolic contracture with reperfusion, indicative of a dissociation between the magnitude and time course of postischemic recovery of systolic and diastolic function. In contrast, the hypertrophied hearts exhibit profound and prolonged postischemic diastolic dysfunction during reperfusion compared with control hearts. The postischemic diastolic stunning in the hypertrophied hearts cannot be attributed to differences in coronary turgo, glycolytic flux, or myocardial ATP levels, and we speculate that responsible mechanisms include depressed postischemic coronary reserve and impaired restoration of diastolic Na$^{+}$ and Ca$^{2+}$ levels.

Acknowledgments

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