Increased Ischemic Injury but Decreased Hypoxic Injury in Hypertrophied Rat Hearts

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The purpose of this study was to compare the degree of ischemic and hypoxic injury in normal versus hypertrophied rat hearts to investigate basic mechanisms responsible for irreversible myocardial ischemic injury. Hearts from rats with bands placed on the aortic arch at 23 days of age (BAND) and sham-operated rats (SHAM, 8 weeks postoperative) were isolated, perfused with Krebs buffer, and had a left ventricular balloon to measure developed pressure. Hearts were made globally ischemic until they developed peak ischemic contracture and were reperfused for 30 minutes. Additional hearts were perfused for 15 minutes with glucose-free hypoxic buffer followed by 20 minutes of oxygenated perfusion. There was an 87% increase in heart weight of BAND compared with SHAM (p<0.01). During ischemia, lactate levels increased faster in BAND compared with SHAM, ischemic contracture occurred earlier in BAND than in SHAM despite no difference in ATP levels, and postischemic recovery of left ventricular pressure was less in BAND (26.8±5.6% of control left ventricular pressure, mean±SEM) compared with SHAM (40±4.6%, p<0.05). During hypoxic perfusion, lactate release was greater in BAND than in SHAM (48.8±1.2 versus 26.6±0.97 µmol/g, p<0.01), and with reoxygenation, lactate dehydrogenase release was less in BAND than in SHAM (13.2±0.7 versus 19.5±0.2 IU/g, p<0.01). After hypoxia and reoxygenation, left ventricular pressure recovery was greater in BAND than in SHAM (93±8.4% versus 66±5.3%, p<0.01). Thus, this study suggests that hypertrophied hearts have a greater potential for glycolytic metabolism, resulting in an increased rate of by-product accumulation during ischemia, which may be responsible for the increased susceptibility of hypertrophied hearts to ischemic injury. (Circulation Research 1990;67:948–959)

The final outcome of persistent cardiac ischemia is myocardial necrosis. The mechanisms responsible for this transition from normal to irreversibly injured myocardium are of interest to both clinicians and basic scientists. The development of irreversible myocardial injury has been associated with decreased tissue ATP levels.1,2 Hearse et al1 noted that contracture developed when ATP levels decreased to approximately 12 µmol/g dry weight and ATP levels further decreased to 3–4 µmol/g dry wt when contracture was completed. These authors suggested that contracture was associated with the development of rigor complexes that formed because of decreased levels of ATP. Other investigators suggest that reduced ATP levels may not be the only factor responsible for ischemic damage to the myocardium.3–7 In studies of isolated perfused rat hearts, Neely and coworkers3,4 noted that the extent of ischemic damage did not correlate with ATP levels. Instead, recovery of function was inversely related to tissue lactate content. Perfusing hearts with lactate or depleting hearts of glycogen before ischemia resulted in increased or decreased ischemic myocardial injury, respectively. These investigators and others concluded that accumulation of by-products of anaerobic glycolytic metabolism (lactate, NADH, or H+), rather than loss of ATP, may be important in the pathogenesis of myocardial ischemic damage.3–5,7

Few experimental studies have evaluated the response of the hypertrophied heart to ischemia, and little is known about the role of ATP decline and lactate accumulation in ischemic hypertrophied hearts. The first descriptions of ischemic contracture or “stone heart” were noted in patients with left ventricular hypertrophy,8 suggesting that the hypertrophied myocardium may have an enhanced suscep-
tibility to ischemic injury. Peyton et al\textsuperscript{9} and Attarian et al\textsuperscript{10} found that ischemic contracture occurred earlier in hypertrophied rat and dog hearts compared with normal hearts. These investigators suggested that hypertrophied hearts were more susceptible to ischemic damage because of decreased subendocardial blood flow, decreased ATP levels, and depressed mitochondrial function. Studies from our laboratory\textsuperscript{11} and by Snoeckx et al\textsuperscript{12} have shown that hypertrophied hearts from spontaneously hypertensive rats develop ischemic contracture sooner and have decreased functional recovery after ischemic contracture and reperfusion when compared with normal hearts. Thus, in both clinical and experimental studies hypertrophied hearts are more susceptible to ischemic injury.

Studies from our laboratory\textsuperscript{13,14} and by others\textsuperscript{15} have noted decreased rates of O\textsubscript{2} consumption per gram of tissue in hypertrophied rat hearts. This is consistent with other studies in which increased glycolytic enzyme levels and shifts in myosin, creatine kinase, and lactate dehydrogenase isoenzyme patterns have been reported.\textsuperscript{15–20} These isoenzyme shifts result in more anaerobic ("fetal")-type isoenzymes, with hypertrophied hearts having decreased rates of oxidative metabolism and increased anaerobic metabolism. In these studies the proportion of different isoenzymes and their messenger RNAs varies with the developmental or physiological state of the heart.\textsuperscript{16,18,21–24} Fetal forms of the isoenzymes, including LDH-M,\textsuperscript{16,25} CK-BB,\textsuperscript{18,26} and the V\textsubscript{3} isofrom of myosin,\textsuperscript{23,24,27} are increased in hearts with chronic pressure overload–induced hypertrophy. Myosin V\textsubscript{3} has been associated with slower contraction and more efficient use of ATP.\textsuperscript{23,28} These results suggest that the hypertrophied heart has an increased glycolytic potential.

The present study was designed to test the hypothesis that hypertrophied hearts have an increased glycolytic potential compared with nonhypertrophied hearts and that there will be an enhanced production and accumulation of glycolytic catabolites during ischemia. This increased accumulation of glycolytic by-products during ischemia may then be responsible for increased ischemic injury in hypertrophied hearts. If, on the other hand, normal and hypertrophied hearts are subjected to continuous hypoxic perfusion, glycolytic by-products will be washed out during the hypoxic insult. In this case, normal and hypertrophied hearts would have similar glycolytic by-product levels, and we would expect that the degree of hypoxic injury would be less in hypertrophied hearts because of the increased ATP production via glycolytic pathways during hypoxia. Thus, the purpose of this study was to compare the degree of ischemic and hypoxic injury in normal versus hypertrophied rat hearts to investigate the basic mechanisms responsible for irreversible myocardial ischemic injury.

Materials and Methods

Experimental Preparation

Male Sprague-Dawley rats (21 days old) were obtained from the Charles River Breeding Labora-
tory, Inc., Kingston, N.Y. Animals were maintained and handled in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 85–23). The animals were housed in temperature-controlled rooms on a 12-hour light-dark cycle and were fed rat diet and water ad libitum. Rats (23 days old, approximately 50 g body wt) were anesthetized with methohexital sodium (0.5 mg/100 g i.p.; Brevital, Eli Lilly and Co., Indianapolis), the thorax was opened, and a 0.6-mm band was placed around the ascending aorta. Control animals underwent a similar operative procedure without placement of an aortic band. Studies were conducted on the aortic-banded rats (BAND) and the sham-operated controls (SHAM) 8 weeks after the banding operation. Animals were given 300 IU heparin i.p. and 15 minutes later were anesthetized with sodium pentobarbital (5 mg/100 g i.p.). The hearts were quickly extirpated and immersed in ice-cold buffer; after extracardial tissue was trimmed away, total heart weight was determined. The aorta was cannulated, and the coronary arteries were perfused with oxygenated buffer by retrograde perfusion of the aortic stump.

Experimental Protocol

The modified Langendorff isolated perfused rat heart model used in these experiments has been described.\textsuperscript{11,13,14} All hearts were perfused at a constant pressure of 100 mm Hg with Krebs-Henseleit buffer gassed with 95% O\textsubscript{2} and 5% CO\textsubscript{2} to produce a pH of 7.4. High potassium buffer, used to arrest the hearts, had a potassium concentration of 30 mM with sodium lowered correspondingly to maintain normal osmolality. A fluid-filled latex balloon was inserted into the left ventricle via the left atrium to monitor left ventricular pressure. The pulmonary artery was cannulated to anaerobically collect coronary sinus effluent. All hearts were paced at 300 beats/min, and a needle thermistor probe was inserted into the right ventricle to monitor the temperature of the heart, which was immersed in nonaerated buffer surrounded by a water-jacketed warming chamber and kept at 37\degree C.

The volume of the left ventricular balloon was increased until the heart produced 60 mm Hg pressure while maintaining less than 4 mm Hg end-diastolic pressure. Our preliminary studies showed that at this low left ventricular pressure, mean wall stress was not statistically different in BAND compared with SHAM. After a 15-minute equilibration period, a control pressure-volume curve was generated by inflating the intraventricular balloon in four 0.01-ml increments and recording the developed and end-diastolic pressure at each volume increment. Hearts with abnormal pressure-volume responses were rejected. The balloon was then deflated back to the original volume. The coronary flow rate was determined by timed measurements of total effluent from the whole heart and was expressed in milliliters per minute per gram wet heart weight. Myocardial
oxygen consumption (MV0₂) was determined while hearts were paced at 300 beats/min and were producing 60 mm Hg left ventricular pressure by measuring the difference in oxygen concentration between the perfusate and the coronary sinus effluent (pH/Blood Gas Analyzer 813, Instrumentation Laboratory, Boston). Oxygen consumption was expressed as micromoles O₂ per minute per gram dry heart weight. 13,14 Hearts were then arrested by perfusion with high potassium buffer, and the electrical pacers were turned off. The coronary flow rate and MV0₂ in the arrested hearts were measured.

Ischemic Contracture

After the 5-minute potassium arrest, coronary flow was stopped to produce global ischemia. Left ventricular pressure was monitored throughout the ischemic interval, and the onset of ischemic contracture was recorded. After development of peak ischemic contracture, coronary flow was re instituted with oxygenated normokalemic Krebs-Henseleit buffer, and hearts were reperfused for 30 minutes. A postischemic pressure-volume curve was generated as previously described, and the left ventricular balloon was removed. The hearts used for morphological examination were then perfusion-fixed with 15 ml of 2% phosphate buffered glutaraldehyde at 100 mm Hg pressure. Another group of hearts were made ischemic until contracture, were reperfused for 30 minutes with oxygenated buffer, and after the left ventricular balloon was removed, were perfused with Monastral blue dye (Sigma Chemical Co., St. Louis) to delineate the area of no-reflow. A constant-pressure (100 mm Hg) system was used to infuse the Monastral dye into the aortic cannula to assure reproducible controlled injections. The hearts were then immersion-fixed in 10% buffered formalin and cut into six equal transverse slices. These slices were photographed and weighed. Monastral dye delineated the areas of flow within the myocardium. Photographs of all heart slices were traced around perfused and non perfused areas with a soni digitizing system interfaced to a computer; by using the weight of each tissue slice, the percent of non perfused myocardium was quantitated.

To characterize the degree of ischemic injury, the preischemic and posts ischemic end-dias tolic pressures were compared. At each balloon volume increment, the end-diastolic and developed left ventricular pressures were recorded. To determine the percent recovery of left ventricular function, the posts ischemic left ventricular developed pressure of each heart at the 20-μl balloon volume increment was divided by the preischemic left ventricular pressure at the same balloon volume. Another method used to characterize posts ischemic myocardial injury was to compare the end-diastolic pressure at each balloon volume increment before and after ischemia. To do this, the posts ischemic end-diastolic pressure was subtracted from the preischemic end-diastolic pressure at each balloon volume. This difference in preischemic and posts ischemic end-diastolic pressures was then plotted versus balloon volume, and the slope of this line was determined by linear regression analysis. The steepness of this slope is an indicator of myocardial stiffness and thus can be equated to the degree of myocardial injury. 29 This myocardial stiffness constant was used to compare the degree of myocardial injury in BAND and SHAM hearts.

Hypoxic Perfusion

After 5 minutes of potassium arrest, the coronary perfusate was switched to Krebs-Henseleit buffer gassed with 95% N₂ and 5% CO₂ in which glucose had been replaced by an equimolar amount of mannitol. During the 15-minute hypoxic perfusion, total coronary effluent was collected, and samples were analyzed at 1-minute intervals for the first 5 minutes and again at 10 and 15 minutes. After 15 minutes of hypoxia, hearts were reoxygenated with oxygenated normokalemic buffer, and coronary effluent samples were again collected at 1-minute intervals for the first 5 minutes and again at 10, 15, and 20 minutes of reoxygenation. A posthypoxic pressure-volume curve was generated to compare with the prehypoxic curve, and the percent recovery of left ventricular pressure as well as the myocardial stiffness constant were determined as described above for the ischemic perfusion protocol. The left ventricular balloon was removed, and hearts were perfusion fixed with 15 ml of 2% phosphate buffered glutaraldehyde at 100 mm Hg pressure.

High-Pressure Liquid Chromatography and Biochemical Determinations

Additional BAND and SHAM hearts were isolated and either quickly frozen with liquid nitrogen-cooled Wollenberger clamps after 15 minutes of control perfusion or subjected to ischemic or hypoxic perfusion protocols as described above and freeze-clamped at specific time points. Sections of left ventricular tissue from these frozen hearts were used for high-pressure liquid chromatography (HPLC), lactate, glycogen, and water content determinations. For HPLC and lactate measurements, tissues were weighed, pulverized under liquid nitrogen in a mortar and pestle, and mixed with ice-cold 6% perchloric acid; after extraction, samples were neutralized with 5 M potassium carbonate to a pH of 7.4±0.3 and centrifuged at 4° C. These tissue extracts were used for HPLC analysis (described below) and for lactate determination with standard enzymatic spectrophotometric techniques (Sigma). Myocardial glycogen was determined by treatment of tissues with 30% KOH, followed by acid hydrolysis of the glycogen, and glucose was measured spectrophotometrically. Tissue glycogen levels are expressed as glucose equivalents per gram of dry heart weight. Lactate and lactate dehydrogenase (LDH) levels were determined in coronary effluent samples by standard enzymatic spectrophotometric techniques (Sigma).
control animals for both the ischemic and hypoxic perfusion protocols were combined to form a single control group (see Tables 2 and 3, Control).

The energetic status of the hearts was determined by HPLC analysis of ATP and phosphocreatine (PCr). These levels were determined by a reverse-phase ion-paired HPLC system (Waters Corp., Milford, Mass.). Tetraethylammonium hydroxide (TBAH) was used as the ion-pair agent. The two-solvent mobile phase consisted of solution A (100 mM KH₂PO₄, 2.5 mM TBAH, pH 4.4) and solution B (100 mM KH₂PO₄, 15 mM TBAH, 30% methanol, pH 5.9). A gradient control with an initial flow ratio of 1:0 (A:B) ran for 3 minutes followed by a 12-minute ramp to a flow ratio of 1:9. This ratio was maintained for 1.4 minutes followed by a 2.5-minute ramp back down to a ratio of 1:0, then a 15.1-minute reequilibration. The samples were stored in a refrigerated (1°C) autosampler until they were analyzed. The column was maintained at 30°C. The system was calibrated with known amounts of pure standards at the beginning of each run, and standards were interspersed throughout the unknown samples in each run.

Morphological Evaluation
Ten transmural tissue samples from the left and right ventricles of each perfusion-fixed heart were embedded in Spurr epoxy resin. Serial 1-µm sections from each block were affixed to glass slides and stained with toluidine blue for light microscopic examination. From these sections, representative areas from the endomycocardium, midwall, and epimycocardium were identified. The tissue blocks were trimmed, and ultrathin sections were cut for examination by transmission electron microscopy.

Data Analysis
All data are expressed as mean±SEM, except where otherwise noted. Statistical evaluation between groups was done by Student’s t test.

Results
The model of pressure-overload cardiac hypertrophy that was used in these experiments involves placing a mildly occlusive band on the ascending aorta of 23-day-old rats. As the rat grows from 50 g at 23 days of age to approximately 350 g at 8 weeks postbanding, the aortic coarctation becomes relatively more constricting. Aortic banding produced significant cardiac hypertrophy in BAND compared with SHAM at 8 weeks postbanding (Table 1, Figure 1). There was a modest reduction in body weight gain in BAND; however, absolute heart weights were increased by 87%. This model of cardiac hypertrophy produced significant increases in the weights of the left ventricle plus septum, the right ventricle, and the atria. Early signs of congestive heart failure developed in some rats by 8 weeks, with pleural fluid accumulation, mild evidence of pulmonary congestion and edema, and hepatic congestion.

Basal coronary flow rates in these isolated perfused hearts, expressed as milliliters per minute per gram wet weight, were lower in BAND (9.7±0.52) compared with SHAM (14.2±0.75, p<0.01). MVo₂ was measured while the isolated buffer-perfused hearts were paced at 300 beats/min and the intraventricular balloon was inflated such that the heart was producing 60 mm Hg developed left ventricular pressure. The MVo₂ of beating hearts was decreased in BAND (16.7±0.79 µmol O₂/g dry wt/min) compared with SHAM (22.6±0.83, p<0.05),

![Figure 1. Gross photograph of hearts from an aortic-banded rat (left) and a sham-operated control rat (right) 8 weeks after the banding procedure. The band is visible on the ascending aorta of the banded animal (arrow). Scale is in millimeters.](http://circres.ahajournals.org/DownloadedFrom)
and after cardiac arrest with 30 mM potassium buffer, MVo2 of BAND hearts was again less than SHAM (5.9±0.65 versus 8.6±0.98, p<0.05).

**Ischemic Contracture**

Coronary flow to the heart was stopped to produce complete global ischemia, and the hearts were immersed in 37°C buffer. After an ischemic interval in which no change in left ventricular pressure was noted, the left ventricular pressure began a gradual increase as the heart developed ischemic contracture (Figure 2). The initial rise in left ventricular pressure was recorded as the time to onset of ischemic contracture. Hypertrophied hearts (n=37) developed ischemic contracture in 16.4±0.42 minutes, while SHAM (n=36) did not develop ischemic contracture until 20.1±0.53 minutes (p<0.05). Maximum contracture was reached in 4–8 minutes, and the hearts were reperfused with oxygenated buffer. Hypertrophied hearts started contracture earlier but took longer to reach maximal contracture than did controls. The mean time to peak contracture in BAND was 23.7±0.60 minutes, with a range of 21–26 minutes. SHAM hearts reached peak contracture at 24.2±0.48 minutes, with a range of 21–27 minutes. Because each heart was reperfused at peak contracture, the mean total ischemic time was similar in BAND and SHAM. The developed left ventricular pressure and the heart rate were variable during the initial reperfusion period (Figure 2). After 15 minutes of reperfusion, the hearts were paced for the remainder of the reperfusion period. The percent recovery of developed left ventricular pressure after ischemic contracture and 30 minutes of reperfusion was lower in BAND (23.8±5.6%) than in SHAM hearts (45.6±4.6%, p<0.05). Another way to characterize ischemic injury is to determine the difference in preischemic and postischemic myocardial stiffness, as described above (“Materials and Methods”). The myocardial stiffness constant was greater in BAND (4.8±1.31 mm Hg/µl balloon volume) compared with SHAM (1.3±0.57, p<0.01), suggesting increased myocardial damage in BAND.

To further characterize the degree of myocardial injury after ischemic contracture and reperfusion, the percent of myocardium that was not reperfused was determined from Monastral blue dye-injected hearts. We have previously shown that the no-reflow area corresponds to irreversibly injured myocardium.11 The no-reflow area of hypertrophied hearts was 52.3±3.08% (n=6), while in SHAM only 26.4±3.78% of the left ventricle was not reperfused (n=5, p<0.05).

In another set of experiments, the time course of myocardial ATP, PCr, and glycogen decline as well as myocardial lactate accumulation was determined. The perfusion protocol was as described above. At specific time points the hearts were frozen and assayed (Table 2). The control levels of ATP and PCr were significantly lower in hearts from BAND compared with SHAM. Lactate and glycogen were not different between groups. After 5 minutes of potas-
sium arrest, ATP and PCr levels increased in the BAND hearts, although PCr was still significantly lower in BAND than in SHAM. Eight minutes of ischemia resulted in a significant decrease in high-energy phosphates and glycogen. Myocardial lactate levels were significantly higher in BAND. At the onset of ischemic contracture and at peak contracture, the ATP, PCr, and glycogen levels were similar in BAND and SHAM. Lactate levels were higher in BAND at the onset of contracture, but by peak contracture there was no difference. After 30 minutes of reperfusion, there was a significant increase in PCr, which was most pronounced in SHAM hearts. ATP levels recovered to approximately 15% of control values in both groups, and there were no differences in lactate or glycogen levels between BAND and SHAM.

**Hypoxic Perfusion**

The hypoxic perfusion protocol was not intended to mimic or equal the degree of myocardial injury observed during our ischemic contracture protocol. Instead, preliminary studies were performed to characterize a hypoxic perfusion time that would cause myocardial injury but would not impair myocardial function to such an extent that posthypoxic functional measurements could not be made. From these preliminary studies, we developed the hypoxic perfusion protocol used in these studies.

The hypoxic buffer, with mannitol replacing glucose, was bubbled vigorously with 95% N₂ and 5% CO₂ and had a PO₂ of 19–34 mm Hg at a pH range of 7.34–7.45. After the 5-minute potassium arrest, the isolated hearts were perfused with hypoxic buffer at 100 mm Hg. The potassium-arrested hearts began beating immediately after perfusion with hypoxic buffer (electrical pacer was off), but the left ventricular developed pressure quickly fell (Figure 3). By 3–4 minutes the hearts stopped beating, and the left ventricular end-diastolic pressure began to rise. This hypoxic contracture curve was steeper than the ischemic contracture curve, and contracture was complete in both BAND and SHAM within 2 minutes. During hypoxic perfusion, coronary flow increased slightly in both BAND and SHAM, but this change was not statistically significant. After 15 minutes of hypoxia, hearts were reoxygenated with oxygenated buffer containing glucose. End-diastolic pressure decreased significantly after reoxygenation and continued to decrease during the reoxygenation period, but did not reach prehypoxic levels. Left ventricular developed pressure increased throughout the reoxygenation period. The percent recovery of peak left ventricular pressure after hypoxia and 20 minutes of reoxygenation was significantly greater in BAND (93.4±6.4%) compared with SHAM (66.2±7.2%, p<0.05). The myocardial stiffness constant was less in BAND (1.85±0.65 mm Hg/μl balloon volume) than in SHAM (2.99±0.81, p<0.05), suggesting less myocardial injury in BAND.

During hypoxic perfusion, total coronary effluent was collected and analyzed for lactate and LDH. Preliminary studies showed that perfusion with Monastral blue dye after hypoxia and reoxygenation can delineate areas of perfusion deficits. In these

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**Table 2. High-Pressure Liquid Chromatography and Enzymatic Analysis of Hearts From Aortic-Banded and Sham-Operated Rats Subjected to Ischemia and Reperfusion**

<table>
<thead>
<tr>
<th></th>
<th>PCr (μmol/g dry)</th>
<th>ATP (μmol/g dry)</th>
<th>Lactate (μmol/g dry)</th>
<th>Glycogen (μmol glucose/g dry)</th>
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<tbody>
<tr>
<td></td>
<td>S</td>
<td>B</td>
<td>S</td>
<td>B</td>
</tr>
<tr>
<td>Control</td>
<td>27.4</td>
<td>20.4</td>
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<td>(S=17, B=15)</td>
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<td>(S=10, B=10)</td>
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<td>±5.31†</td>
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<td>(S=10, B=11)</td>
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<td>±0.22</td>
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<td>contracture</td>
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<tr>
<td>contracture</td>
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<td>±4.35</td>
<td>±5.52†</td>
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Values are mean±SEM. Numbers of rats are given in parentheses. PCr, phosphocreatine; S, sham-operated rats; B, aortic-banded rats.

*p<0.01.
†p<0.05.
hearts, areas of no-reflow were not seen; thus, collection of coronary effluent gives an accurate assessment of interstitial metabolite and enzyme levels. Lactate release into the coronary effluent increased sharply in both groups during hypoxia, peaked after 2–3 minutes in SHAM, but continued to increase in BAND (Figure 4). Total lactate release during the entire 15-minute hypoxic period was significantly higher in BAND compared with SHAM. Lactate levels in the coronary effluent were insignificant during reoxygenation. LDH release during hypoxia was no different from control values (Figure 5). However, immediately after reoxygenation, LDH levels increased significantly in both groups. The amount and duration of LDH release was less in BAND than in SHAM, indicating less myocyte injury in BAND.

The myocardial ATP, PCr, and glycogen contents in hypoxic perfused hearts are shown in Table 3. After 8 minutes of hypoxic reperfusion, PCr levels were significantly decreased compared with prehypoxic control values in both groups. ATP levels were also markedly decreased compared with control, but ATP was higher in BAND compared with SHAM.

FIGURE 3. Representative left ventricular pressure tracing from the hypoxic perfusion protocol demonstrating the prehypoxic pressure-volume (PV) curve, hypoxia and reoxygenation, and posthypoxic PV curve.

FIGURE 4. Panel A: Time course of lactate release in the coronary effluent during hypoxic perfusion and reoxygenation. Lactate mean±SEM is expressed as micromoles lactate per gram dry heart weight per minute. Panel B: Total lactate release during the entire 15-minute hypoxic perfusion period. *p<0.01.
hearts. Glycogen levels tended to be lower in BAND than SHAM, but this difference was not statistically significant. At the end of hypoxic perfusion, all values were markedly reduced compared with controls, but there were no differences between BAND and SHAM. After 20 minutes of reoxygenation, the PCr levels were improved but were lower in BAND than SHAM. There was only modest recovery of ATP and glycogen in both groups.

Morphology of Ischemic and Hypoxic Injury

Nonischemic and nonhypoxic hearts from BAND and SHAM rats were examined by light and electron microscopy. There was a mild increase in fibrous connective tissue in BAND, predominantly in the subendocardial region. There was no evidence of ongoing inflammation or necrosis in any heart from either group. The morphological changes of ischemic contracture and reperfusion in the BAND and SHAM hearts from this study are similar to the changes described in our previous studies with hearts from normal and spontaneously hypertensive rats. During ischemic contracture there were significant morphological changes consistent with ischemic injury, but no changes that would definitively demonstrate lethal myocyte injury, that is, sarcolemmal disruption or contraction band necrosis. After ischemic contracture and reperfusion there was a distinct zone of contraction band change at the flow/no-reflow border (Figure 6). The morphological changes in BAND and SHAM hearts were identical, except that the area of no-reflow was greater in BAND. The myocardial injury in hearts from both hypoxic groups was less severe than the changes seen after ischemic injury, and no differences were noted between BAND and SHAM. There were no grossly visible areas of no-reflow in hearts perfused with Monastral dye after hypoxia and reoxygenation. The time course of morphological changes was consistent with the time course of LDH release in that only minimal LDH was present in the coronary effluent during hypoxia, but with reoxygenation there was a substantial release of LDH. There were multifocal areas of contraction band change throughout the myocardium (Figure 7). These lesions occurred throughout all regions of the myocardium, with no apparent predilection for endomyocardium, midwall, or epimyocardium. Ultrastructural changes in myocytes from both hearts.

TABLE 3. High-Pressure Liquid Chromatography and Enzymatic Analysis of Hearts From Aortic-Banded and Sham-Operated Rats Subjected to Hypoxic Perfusion and Reoxygenation

<table>
<thead>
<tr>
<th></th>
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<th>Glycogen (µmol glucose/g dry)</th>
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<tr>
<td>Control</td>
<td>27.4, ±2.19</td>
<td>20.4 ±3.09*</td>
<td>21.0, ±2.29</td>
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<tr>
<td>(S=17, B=15)</td>
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<tr>
<td>8-min Hypoxia</td>
<td>1.62 ±0.38</td>
<td>1.81 ±0.49</td>
<td>1.29 ±0.33</td>
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<td>(S=7, B=8)</td>
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<tr>
<td>15-min Hypoxia</td>
<td>0.93 ±0.06</td>
<td>1.08 ±0.22</td>
<td>1.18 ±0.32</td>
</tr>
<tr>
<td>(S=11, B=13)</td>
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<tr>
<td>Hypoxia+reoxygenation</td>
<td>25.3 ±2.18</td>
<td>17.9 ±2.33*</td>
<td>8.43 ±0.47</td>
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Values are mean±SEM. Numbers of rats are given in parentheses. PCr, phosphocreatine; S, sham-operated rats; B, aortic-banded rats.
P<0.01.
P<0.05.
FIGURE 6. Light micrograph of a toluidine blue-stained 1-μm plastic-embedded heart tissue from aortic-banded rats after ischemic contracture and reperfusion. Note extensive areas of contraction band change in this section from the left ventricular midwall region (arrows). Magnification, ×383.

FIGURE 7. Light micrograph of a toluidine blue-stained 1-μm plastic-embedded heart tissue from aortic-banded rats after hypoxia and reoxygenation. Multifocal areas of contraction band change are seen throughout the myocardium (arrows). Magnification, ×383.
BAND and SHAM consisted of loss of glycogen granules, mitochondrial swelling, increased interstitial and intracellular space, and central chromatolysis of nuclei with peripheral clumping of chromatin. In the multifocal areas of contraction band change, myocytes contained dense accumulations of myofibrillar material and areas of disrupted sarcoplasm with no myofilaments (Figure 8). These changes are characteristic of the ultrastructural features of contraction band change in this isolated perfused rat heart model.8

**Discussion**

The results of this study demonstrate that hypertrophied BAND hearts are more susceptible to ischemic injury than are normal SHAM hearts. However, if hearts are subjected to continuous hypoxic perfusion, hypertrophied hearts are less sensitive to hypoxic myocardial damage than normal hearts. Taken together, these data suggest that in this model of pressure-overload hypertrophy, the increased rate of glycolytic metabolism results in a more rapid accumulation of glycolytic by-products during ischemia, which contribute to the increased susceptibility of hypertrophied hearts to ischemic injury.

The increased susceptibility of hypertrophied hearts to ischemic injury has been known for many years, but the pathogenetic mechanisms are not well understood.8-12,32-34 Neely and colleagues3,4 have shown that by-products of glycolytic metabolism, especially lactate, NADH, and H⁺, may play an important role in the pathogenesis of ischemic myocardial injury. In their studies of isolated working rat hearts, the extent of myocardial injury was greater in hearts that were perfused with lactate before ischemia. Conversely, if hearts were depleted of glycogen, thereby decreasing the amount of lactate produced during ischemia, the degree of ischemic myocardial injury was markedly decreased. Changes in myocardial ATP levels during these studies did not correlate with the degree of myocardial injury. Thus, these studies show that lactate levels and not ATP may be important in ischemic myocardial injury.

In the present study with BAND hearts, hypertrophied hearts were more susceptible to ischemic injury than were normal hearts. The hypertrophied hearts in this study also had higher lactate levels during ischemia than did controls, although lactate levels at peak contracture were not different. This suggests that hypertrophied hearts have an increased glycolytic potential, which resulted in an increased accumulation of glycolytic by-products during ischemia. PCr and ATP levels were lower in hypertrophied hearts before ischemia, but after 5 minutes of potassium arrest these differences were partially mollified. During the ischemic insult, there were no differences in PCr or ATP levels between hypertrophied and normal hearts, despite the significant differences in lactate levels and functional recovery. With hypoxic perfusion, lactate production was again increased in hypertrophied hearts; however, the continuous perfusion washed the lactate out of the tissue. During the hypoxic period there were no significant differ-

**FIGURE 8.** Electron micrograph from area of contraction band change in aortic-banded rats after hypoxia and reoxygenation. Areas of contracted myofilaments (arrows) and disrupted sarcoplasm are shown. Bar, 2 μm.
ences in PCr or ATP in the hypertrophied and normal hearts. After posthypoxic reoxygenation, the hypertrophied hearts had less myocardial injury as evidenced by lower LDH release and better functional recovery than controls. ATP levels were similar in both groups after 20 minutes of reoxygenation; however, PCr was decreased in the hypertrophied hearts. These findings suggest that the increased glycolytic potential of hypertrophied hearts has a protective effect during hypoxia, when glycolytic by-products are not allowed to accumulate in the myocardial tissue.

Previous studies have shown that hypertrophied hearts have increased glycolytic enzyme levels and shifts in myosin, creatine kinase, and LDH isoenzyme patterns. These isoenzyme shifts result in more anaerobic (fetal)-type isoenzymes, with hypertrophied hearts having decreased rates of oxidative metabolism and increased anaerobic metabolism. This is consistent with our previous studies, which noted decreased rates of O2 consumption per gram of tissue in hypertrophied rat hearts, again suggesting decreased dependence on aerobic metabolism. The hypertrophied hearts used in the present studies also had decreased rates of O2 consumption per gram of tissue, both while beating and during potassium arrest. Additionally, lactate accumulation during ischemia was increased, providing further support for an increased glycolytic potential in hypertrophied hearts.

In contrast to the findings in this study, Cunningham et al recently reported decreased lactate release from hypertrophied hearts of deoxycorticosterone-salt hypertensive rats during hypoxic perfusion. These investigators used a different model of cardiac hypertrophy than the model used for this study, and in contrast to our study, they included glucose and lactate in the perfusate during the 15-minute hypoxic perfusion. These differences in the experimental protocol may account for the differences seen in their study compared with the present study.

Many studies of hearts from neonates have also demonstrated an enhanced glycolytic potential. In studies of neonatal hearts subjected to continuous hypoxic perfusion, tissue ATP levels did not fall as fast or as far as in adult hearts, and functional recovery was greater in the neonatal hearts, suggesting a protective effect of increased glycolysis. In ischemic injury studies with rat, rabbit, dog, and pig hearts, there was a decreased time to develop ischemic contracture, an increased lactate accumulation during ischemia, and decreased postischemic functional recovery in neonatal hearts compared with adult hearts. Thus, glycolytic by-product accumulation during ischemia appears to have an important detrimental effect on the ischemic neonatal myocardium. However, other studies have shown that neonatal hearts are in fact less sensitive to ischemic injury than adult hearts. Thus, controversy does exist in this area, and further work needs to be done to resolve these issues.

The studies described in this report, with the isolated buffer-perfusate rat heart, do not attempt to recapitulate regional ischemia during myocardial infarction or intraoperative cardioplegic arrest in humans. Instead, this model system allows us to manipulate the experimental conditions and, with proper experimental design, answer specific questions about the basic mechanisms of ischemic myocardial injury. The results of this study suggest that in the hypertrophied heart, compensatory changes resulting in an increased rate of glycolytic metabolism may ultimately make the heart more susceptible to ischemic injury. Specifically, the increased glycolytic potential in hypertrophied hearts leads to increased accumulation of glycolytic by-products during ischemia and results in increased ischemic myocardial damage. Conversely, when glycolytic by-products are removed, as in hypoxic perfusion and perhaps in ischemic conditions with collateral flow, the increased rate of glycolytic metabolism of the hypertrophied heart provides some protection against injury. The exact mechanisms involved in this process and the role of other factors involved in the development of irreversible ischemic injury remain to be elucidated.

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References


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Increased ischemic injury but decreased hypoxic injury in hypertrophied rat hearts.

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