Active Downregulation of Myocardial Energy Requirements During Prolonged Moderate Ischemia in Swine

Andrew E. Arai, George A. Pantely, Cheryl G. Anselone, James Bristow, and J. David Bristow

We studied the effects of rapid atrial pacing during the final 10 minutes of a 70-minute, 31% reduction in coronary blood flow in anesthetized swine to understand the significance of apparent metabolic improvements during the initial 60 minutes of segmental ischemia. Within 5–10 minutes of ischemia, subendocardial phosphocreatine (PCr) and ATP were depleted to 47% and 63% of control, respectively; lactate accumulated within the subendocardium to 300% of control; and net arteriovenous lactate production occurred. Despite continued ischemia and no significant changes in the external determinants of myocardial oxygen consumption, by 60 minutes subendocardial PCr and lactate contents returned to near control levels and there was net arteriovenous lactate consumption. Ischemic left ventricular wall thickening and ATP levels remained depressed throughout the experiment. Atrial pacing during the final 10 minutes of ischemia again resulted in depletion of PCr and lactate production. Since the myocardium was capable of hydrolyzing PCr in response to atrial pacing at 60 minutes of ischemia, we conclude it was capable of hydrolyzing PCr during the period of constant ischemia when instead it was accumulating PCr. We propose the ischemic myocardium downregulates regional energy requirements below blood flow–limited rates of energy production during ischemia. This appears to be an active adaptation to ischemia and not a result of passive damage or cellular injury. (Circulation Research 1991;69:1458–1469)

Considerable clinical evidence suggests that myocardial hypoperfusion produces a chronic but reversible decrease in myocardial function.1,2 The cellular mechanisms regulating myocardial function under these conditions are not understood. Although the metabolic consequences of complete coronary occlusions and reperfusion are well documented, experimental conditions of total ischemia may exceed the limits to which the myocardium can adapt to reductions in coronary blood flow. A better understanding of the metabolic and functional response to prolonged incomplete coronary occlusion may help reveal regulatory processes overwhelmed by more severe ischemia.

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Two sets of experiments suggested to us that a prolonged moderate reduction in coronary blood flow triggers myocardial adaptations, resulting in a restoration of energy supply/demand balance despite ongoing ischemia. In contrast to the depletion of high energy phosphate (HEP) compounds and eventual onset of irreversible myocardial damage during sustained total coronary occlusion, we previously demonstrated that, after initial depletion, the myocardium regenerates phosphocreatine (PCr) during 1 hour of a constant partial reduction in coronary blood flow.3 Despite restoration of PCr content, regional myocardial function and ATP content remained depressed throughout the ischemic episode. These experiments suggested that, during constant subtotal ischemia, either impaired utilization of PCr occurs or myocardial energy supply/demand balance improves with time.

Also consistent with an adaptive process, myocardial lactate production does not remain constant during steady mild to moderate myocardial ischemia.4,5 It is not clear whether this reflects an improvement in myocardial energy balance or a pathological process such as glycogen depletion, gradually deteriorating myocardium, or other mechanism.4,6
The current experiments were designed to test the hypothesis that myocardial energy balance improves with time, allowing regeneration of PCr during prolonged moderate ischemia. If this is the case, then additional hemodynamic load at the end of a period of stable ischemia could upset the new balance between energy production and demand, once again causing PCr depletion or lactate production. If PCr regeneration and decreased lactate production resulted from pathological inability to use PCr and inability to produce lactate, further metabolic demand should have no further effect on these parameters. To differentiate between these possibilities, we studied the effects of increasing myocardial energy needs with rapid atrial pacing during the last 10 minutes of a 70-minute period of moderate ischemia in open-chest anesthetized pigs. In brief, our results support a pattern of improving energy balance during a period of constant moderate ischemia. These findings are not fully explainable by passive damage to the myocardium and thus seem to represent an active adaptation to ischemia.

**Materials and Methods**

Domestic swine of either sex that weighed between 42 and 51 kg were fasted overnight. After premedication with xylazine (2 mg/kg i.m.) and ketamine (10 mg/kg i.m.), anesthesia was induced with α-chloralose (100 mg/kg i.v.) and maintained with 50 mg/kg i.v. every 2 hours. All animals in protocol 1 also received a single dose of morphine sulfate (0.5–1.5 mg/kg i.v.) after completion of the surgical preparation. Acid–base status was maintained within normal limits (pH 7.35–7.45, PaCO₂ 35–45 mm Hg, and PaO₂ 100–150 mm Hg) by titration of mechanical ventilation and administration of intravenous bicarbonate. The arterial blood gas was always stable for at least 30 minutes before the experiment. Temperature was maintained with a heating pad as needed.

Catheters were placed in the aorta and inferior vena cava through femoral access. After median sternotomy, the heart was suspended in a pericardial cradle and the proximal left anterior descending coronary artery (LAD) was dissected. A 2.5-mm electromagnetic flow probe (Statham) and a polyvinyl hydraulic occluder were placed around the proximal LAD. Catheters (0.6 mm o.d., 0.3 mm i.d.) were placed below the occluder in the LAD and the anterior interventricular vein by the Herd-Barger technique. We previously documented the frequency response of the LAD pressure waveform measured with this system. Ultrasonic crystals (5 MHz) were sutured on the epicardial surface and inserted into the subendocardium to monitor the ischemic zone wall thickness. Systolic wall thickening was calculated as the end-systolic thickness minus end-diastolic thickness difference divided by end-diastolic thickness. Catheters were introduced into the left atrial appendage for radioactive microsphere injection (11.4 ± 0.1 μm) and the left ventricular (LV) apex for LV pressures (catheter tip manometer). Finally, epicardial pacemaker leads were attached to the appendage of the right atrium.

The following parameters were measured continuously and recorded on an eight-channel Mark 200 Brush recorder: mean LAD flow, phasic LAD flow, ischemic zone LV wall thickness, electrocardiogram, mean LAD pressure, phasic LAD pressure, LV pressure, and LV dp/dt. This allowed an observer to modify pressure in the hydraulic occluder and accurately maintain LAD blood flow or LAD mean pressure at selected levels. Any animals that developed hemodynamic instability during the experiment or erratic coronary flow signals were excluded from analysis.

Four sets of intermittent measurements were obtained from each animal. Obtained over 5 minutes, each set consisted of blood samples from the aorta and the anterior interventricular vein, microsphere blood flow determination, and a transmural LV drill biopsy (3 mm diameter). The biopsy was always the last sample in a set to avoid the brief (30–60 seconds) hemodynamic instability caused by this procedure. To minimize damage to the ischemic portion of the ventricle, control biopsies were usually obtained in the circumflex perfusion zone. Biopsies from the ischemic zone were obtained in a progression from distal to more proximal portions of the LV to avoid biopsy of an area with damaged vascular supply. Overall metabolic and hemodynamic stability of this preparation with serial biopsies has been reported, including comparison of circumflex and LAD zone biopsies.

The heart was removed after completion of the protocol. The right and circumflex coronary arteries were catheterized close to their origins, and the LAD was catheterized at the level of the occluder. Colored dyes were simultaneously injected at constant rate into each coronary artery to distinguish the ischemic myocardium from the nonischemic zone. This procedure verified that the ultrasonic crystals and experimental biopsies were all obtained from the ischemic zone.

The heart was fixed with formalin in preparation for microsphere blood flow determinations. The six zones analyzed included the subendocardial, midmyocardial, and subepicardial portions of the ischemic and nonischemic zones. Border zones between ischemic and nonischemic ventricle were excluded. The heart was weighed before and after dye injection and again after formalin fixation to compensate for a small (about 10%) but consistent weight change associated with dye injection and fixation.

**Protocol 1**

This group constitutes the primary focus of this paper, evaluating the functional availability of PCr that reaccumulates during ischemia. After control measurements were obtained (including an LV transmmyocardial drill biopsy, microsphere blood flow, and blood samples), transmural LAD blood flow was reduced 30% and held constant for 60 minutes. Inter-
Intermittent measurements were obtained at 5–10 minutes and 55–60 minutes of ischemia. After the 60-minute sample, atrial pacing was started at about 50 beats/min faster than the intrinsic heart rate and distal LAD pressure was held constant at the level maintained during ischemia by modifying pressure in the hydraulic occluder. This allowed LAD flow to stabilize at a new level. The increment in atrial pacing was reduced if aortic systolic pressure fell more than 20 mm Hg. Between about 65 and 70 minutes, the final intermittent measurements were obtained.

**Protocol 2**

This group was included to delineate the time course of lactate production between the 10- and 60-minute biopsies. Hemodynamic data and metabolic data other than lactate contents were previously reported. A complete set of extracts from myocardial biopsies was stored at −70°C for approximately 1 year (n=6). In these animals, transmural microsphere blood flow had been reduced 21% and maintained at that level throughout 1 hour of monitoring. Intermittent measurements were obtained at control, 5–10 minutes, 25–30 minutes, and 55–60 minutes. None of these animals received atrial pacing. The extracts were assayed for lactate content. Arterial and venous samples were not available.

**Protocol 3**

This protocol was designed to determine the percentage of collateral flow (non-LAD) contributing to blood collected from the anterior interventricular vein. This validation study is important in interpreting the arteriovenous differences of metabolites and calculating rates of ATP production. We previously documented the almost complete lack of collateral coronary arteries in normal swine but had not verified that this applied to the venous drainage, especially during ischemia.

Three animals were anesthetized and surgically prepared as previously outlined with the following modifications. A small Silastic catheter was introduced retrograde into the circumflex artery or the ramus intermedius artery within 5 mm of the left main coronary artery. This allowed infusion of fluorescein against the direction of blood flow to enhance mixing. Small Silastic catheters were placed in the anterior interventricular vein and a vein parallel to an obtuse marginal branch of the circumflex artery. The location of the anterior interventricular vein catheter and technique of insertion were identical to those used in protocols 1 and 2.

Simultaneous blood samples were obtained from the aorta, the anterior interventricular vein, and the vein in the circumflex distribution. Venous samples were obtained at a rate of 1 ml/min. After control samples were obtained, fluorescein (1 mg/ml at 1 ml/min) was infused continuously into the circumflex artery. Simultaneous blood samples were obtained after 1 minute of infusion and every 3 minutes thereafter for 13 minutes. The fluorescein infusion was then stopped. About 30 minutes later, the sampling procedure was repeated except that LAD flow was reduced by 40% compared with control levels as determined by the electromagnetic flow probe. After a stable functional state was reached (less than 2 minutes), the fluorescein infusion was restarted and blood samples were obtained for 13 minutes in the same sequence as previously indicated.

If non-LAD blood was supplied to ischemic myocardium or to the anterior interventricular vein at the level of our venous catheter, then fluorescence of plasma from this zone would exceed levels that gradually accumulate in the systemic circulation. Intensity of fluorescence (exciting 487 nm, emission 515 nm, bandwidth 10 nm) from plasma samples was measured on a Hitachi spectrofluorometer (model F-2000). Standard curves in water and plasma demonstrated linearity over the range of concentrations collected (intensity versus concentration: r=0.997, n=7). Units reported reflect intensity detected by the photomultiplier.

**Chemical Analysis**

Transmyocardial drill biopsies (3 mm diameter) were frozen in liquid nitrogen within 1–2 seconds and stored at −70°C until extracted. The approximately 3×3×12-mm biopsy samples were divided into subendocardial, midmyocardial, and subepicardial segments on a stage submerged in liquid nitrogen. The subepicardium was distinguished from the subendocardium by a blue dye that had been painted on the epicardial surface of the heart before the biopsy was obtained. The frozen samples were extracted and neutralized as previously reported.

Arterial and venous blood samples were obtained anaerobically in cold syringes containing heparin fluoride to inhibit glycolysis. Samples were divided for oxygen and lactate content and stored on ice until processed immediately after the experiment. Oxygen content was measured in duplicate with an IL 382 hemoximeter (Instrumentation Laboratory, Lexington, Mass.). Plasma for lactate content was deproteinated with perchloric acid and neutralized with potassium hydroxide and imidazole buffer. Plasma samples were then frozen until enzymatic analysis.

Myocardial metabolite contents (ATP, PCR, and lactate) and plasma lactate concentrations were determined by the spectrofluorometric NAD/NADH enzyme-linked methods of Lowry and Passonneau. All samples were run in duplicate. The intra-assay coefficient of variation for ATP was ±2.4%, for PCR ±3.4%, and for lactate ±2.8%.

**Statistical Analysis**

Results are reported as mean±SD in parentheses or as error bars. One-way analysis of variance (ANOVA) with repeated measures followed by range testing (Tukey’s test) was performed for comparisons between groups. The tissue lactate content in the subendocardial and middle layers was analyzed by the Friedman test (nonparametric equivalent to
the one-way ANOVA with repeated measures) because the data exhibited severe inequality of variance between groups ($p<0.0005$, F test). Analysis of covariance was used to compare $x$-$y$ relations.

**Results**

Final results are reported for 18 of the 25 animals studied (protocol 1, $n=9$; protocol 2, $n=6$; and protocol 3, $n=3$) Since the purpose of protocol 1 was to evaluate the physiological significance of PCr regeneration, we excluded two animals that did not regenerate PCr to at least 67% of control by the 60-minute biopsy. Adding these two animals to the nine reported would alter average metabolite contents by less than 10% throughout the experiment. It is important, however, to exclude them because they do not meet the experimental criteria of interest. This also avoids a potential bias toward demonstrating lower average PCr content after pacing. Other animals were excluded for the following reasons: congenital ventricular septal defect discovered postmortem ($n=1$), abnormally low control ATP and PCr ($n=1$), hemodynamic instability ($n=1$), ventricular fibrillation after biopsy ($n=1$), and myocardial infarction of the nonischemic zone likely related to the circumflex zone biopsy ($n=1$).

**Protocol 1: Metabolic Effects of Partial Ischemia Before and During Atrial Pacing**

Radioactive microspheres validated the electromagnetic flow probe measurements at four time periods. After the control measurements, the blood flow through the LAD was decreased 30% by continuously monitoring the coronary blood flow and manually adjusting pressure in the hydraulic occluder. Ischemia resulted in a 31% reduction in transmural microsphere blood flow ($p<0.001$) as listed in Table 1. LAD blood flow was maintained at this level for 60 minutes, and transmural microsphere blood flow was essentially the same at 5 and 60 minutes. The subendocardium was more severely affected than the other layers, resulting in a 55% reduction in microsphere blood flow to this region ($p<0.001$) and significant reductions in the inner/outer blood flow ratio ($p<0.001$). Transmural distribution of blood flow did not change during the period of constant ischemia between 5 and 60 minutes (Table 1, Figure 1). There was a tendency for heart rate to increase and LV systolic blood pressure to fall during the period of constant ischemia (not significant versus control) (Table 2).

Atrial pacing during the final 10 minutes of ischemia increased heart rate by 42% and rate/pressure product by 35% compared with 60 minutes. Because mean LAD pressure was held constant during this time period, transmural myocardial microsphere flow was reduced 20% from the ischemic level (not significant versus 60 minutes). This resulted in statistically significant further reductions in LAD inner flow ($p<0.025$) and inner/outer flow ratio ($p<0.005$) compared with 60 minutes. Thus, after an hour of constant ischemia, atrial pacing imposed increased energy requirements in the face of a slight further decrease in oxygen supply (Tables 1 and 2).

Increases in myocardial oxygen extraction were unable to maintain oxygen consumption at control levels with this degree of ischemia (Table 3). Regional systolic LV wall thickening decreased to a new plateau during ischemia, usually within 30 seconds

![Figure 1. Distribution of myocardial blood flow. Left anterior descending coronary artery blood flow was manually reduced 30% for 60 minutes. The degree of ischemia and distribution of microsphere blood flow remained constant until rapid atrial pacing imposed added demand and further reduced subendocardial blood flow.](http://circres.ahajournals.org/Downloaded from http://circres.ahajournals.org/)
(data between 0 and 30 seconds not shown). Percent wall thickening remained stable throughout the period of constant ischemia and decreased further with the demands of rapid atrial pacing (Table 2).

Ischemia resulted in a graded metabolic response across the ventricular wall with the most prominent effects in the subendocardium (Table 3). Subendoocardial ATP content fell to 63% of control during the first 10 minutes of ischemia and to 49% of control by 60 minutes (Figure 2A, both p<0.001 versus control). Atrial pacing did not further change ATP levels.

Subendocardial PCr was transiently depleted to 47% of control during the first 10 minutes of ischemia (p<0.001) but, during the last 50 minutes of constant ischemia, was regenerated to 96% of preischemic levels (Figure 2B, p<0.005 versus 10 minutes). That the ischemic myocardium can use this regenerated store of PCr was demonstrated by the subsequent depletion in response to atrial pacing. The fall in PCr content was of similar magnitude to the initial fall after onset of ischemia.

The subendocardial lactate content increased to approximately 300% of control levels by 5–10 minutes after the onset of ischemia (Figure 3A). This was a transient phenomenon, and myocardial lactate levels were not significantly different from control at 55–60 minutes. Similarly, plasma samples across the ischemic LAD vascular bed demonstrated the transition from a control lactate-consuming state to a lactate-producing state at 5–10 minutes (Figure 3B). By 60 minutes the ischemic myocardium returned to a net lactate-consuming state that was not statistically different from control. Atrial pacing caused subendocardial lactate accumulation (p<0.001 versus control) and net arteriovenous lactate production (p<0.01 versus control). This indicates the myocardium was capable of producing substantial amounts of lactate at the end of an hour of ischemia at a time when there was virtually no net flux through anaerobic glycolysis.

### Table 2. Hemodynamic Effects of Partial Ischemia Before and During Atrial Pacing

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>5–10 Minutes</th>
<th>55–60 Minutes</th>
<th>65–70 Minutes (paced)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>102 (13)</td>
<td>103 (16)*</td>
<td>106 (19)*</td>
<td>150 (14)*</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>118 (9)</td>
<td>111 (12)*</td>
<td>107 (17)*</td>
<td>103 (16)‡</td>
</tr>
<tr>
<td>HR×LVSP (×10³)</td>
<td>12.1 (1.4)</td>
<td>11.5 (1.9)*</td>
<td>11.4 (2.6)*</td>
<td>15.5 (3.0)†</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>9 (1)</td>
<td>12 (3)*</td>
<td>12 (4)*</td>
<td>16 (3)§</td>
</tr>
<tr>
<td>LAD mean pressure (mm Hg)</td>
<td>95 (10)</td>
<td>46 (4)†</td>
<td>48 (3)†</td>
<td>48 (4)†</td>
</tr>
<tr>
<td>LV wall thickening</td>
<td>28% (5)</td>
<td>15% (7)†</td>
<td>15% (9)†</td>
<td>7% (7)†</td>
</tr>
</tbody>
</table>

Results are listed as means. Numbers in parentheses indicate standard deviation. HR, heart rate; LVSP, left ventricular (LV) systolic pressure; LVEDP, LV end-diastolic pressure; LAD, left anterior descending coronary artery.

*NS, †p<0.001, ‡p<0.01, §p<0.005, vs. control.

### Table 3. Metabolic Effects of Partial Ischemia Before and During Atrial Pacing

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>5–10 Minutes</th>
<th>55–60 Minutes</th>
<th>65–70 Minutes (paced)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (µmol/g wet wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subendocardium</td>
<td>3.91 (0.31)</td>
<td>2.48 (1.10)*</td>
<td>1.91 (0.90)*</td>
<td>1.74 (0.85)*</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>4.43 (0.42)</td>
<td>3.09 (1.19)†</td>
<td>2.70 (0.88)*</td>
<td>2.92 (1.01)‡</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>3.75 (0.58)</td>
<td>3.73 (0.51)§</td>
<td>3.73 (0.54)§</td>
<td>3.61 (0.83)§</td>
</tr>
<tr>
<td>Phosphocreatine (µmol/g wet wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subendocardium</td>
<td>6.78 (0.68)</td>
<td>3.20 (1.76)*</td>
<td>6.54 (2.01)§</td>
<td>3.63 (2.11)‡</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>7.90 (0.78)</td>
<td>4.75 (2.37)‡</td>
<td>7.45 (1.60)§</td>
<td>5.65 (1.72)§</td>
</tr>
<tr>
<td>Subepicardium</td>
<td>7.09 (1.25)</td>
<td>6.79 (1.38)§</td>
<td>7.92 (1.12)§</td>
<td>6.48 (1.75)§</td>
</tr>
<tr>
<td>Lactate (µmol/g wet wt)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subendocardium</td>
<td>2.97 (1.12)</td>
<td>9.60 (5.04)*</td>
<td>4.88 (1.73)§</td>
<td>8.48 (5.10)*</td>
</tr>
<tr>
<td>Midmyocardium</td>
<td>2.83 (1.11)</td>
<td>7.08 (5.81)†</td>
<td>3.90 (2.21)§</td>
<td>5.82 (3.29)§</td>
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<tr>
<td>Subepicardium</td>
<td>3.40 (1.19)</td>
<td>3.61 (1.10)§</td>
<td>3.97 (3.24)§</td>
<td>3.46 (1.15)§</td>
</tr>
<tr>
<td>Lactate consumption (µmol/100 g/min)</td>
<td>115 (86)</td>
<td>−136 (81)*</td>
<td>35 (42)§</td>
<td>−15 (41)†</td>
</tr>
<tr>
<td>Oxygen consumption (ml/100 g/min)</td>
<td>11.67 (3.69)</td>
<td>8.97 (1.89)*</td>
<td>9.00 (1.78)§</td>
<td>7.09 (2.50)*</td>
</tr>
<tr>
<td>ATP production (µmol/100 g/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>2,330 (690)</td>
<td>1,791 (354)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Negligible</td>
<td>155 (93)</td>
<td>2 (5)</td>
<td>46 (47)</td>
</tr>
<tr>
<td>Total</td>
<td>2,330 (690)</td>
<td>1,945 (406)§</td>
<td>1,798 (332)§</td>
<td>1,462 (440)*</td>
</tr>
</tbody>
</table>

Results are listed as mean. Numbers in parentheses indicate standard deviation.

*p<0.001, †p<0.01, ‡p<0.005, §NS, ‖p<0.05, vs. control.
Protocol 2: Time Course of Metabolic Changes During Partial Ischemia

This group of pigs was studied during 1 hour of a steady 21% reduction in transmural LAD blood flow. The functional and nonlactate metabolic data for these animals were reported previously. These animals demonstrated similar hemodynamics and myocardial blood flow distributions to the animals presented in protocol 1. Ischemic zone systolic wall thickening was reduced 50% during ischemia and remained stable. Although absolute lactate content should be interpreted with some caution considering these extracts had been frozen for one year, this should not preclude a qualitative assessment of the

time course of lactate accumulation and normalization. Lactate accumulated in the subendocardium during early ischemia to levels comparable to those observed in protocol 1 (from control, 2.23±0.97, to 8.95±4.31 μmol/g wet wt, p<0.005). PCR and lactate followed reciprocal courses. Lactate levels appear to plateau between 30 and 60 minutes (4.30±1.74 and 4.20±1.59 μmol/g wet wt, respectively; neither was significantly different from control). Thus, anaerobic glycolysis provides minimal energy production in late ischemia under these conditions.

Combined Results From Protocols 1 and 2

The relation between subendocardial PCR content and regional LV wall thickening may provide clues concerning potential mechanisms regulating myocardial function during ischemia (Figure 4). There is a linear relation between subendocardial PCR content

Figure 2. Myocardial high energy phosphate contents. Panel A: Four transmyocardial drill biopsies were obtained from each animal. Ischemia produced a gradient of ATP depletion across the myocardium. Subendocardial ATP contents fell to 63% of control by 10 minutes and only slightly farther between 10 and 60 minutes. Panel B: In contrast to the persistent derangement observed for ATP contents, phosphocreatine (PCR) was regenerated to near control levels by 60 minutes. The myocardium was capable of using PCR stores during late ischemia (when PCR was instead accumulating) as evidenced by the decreased levels after atrial pacing (65–70 minutes).

Figure 3. Myocardial lactate metabolism. The myocardium was consuming lactate at control. During early ischemia, the myocardium produces lactate resulting in both intramyocardial accumulation of lactate (panel A) and net arteriovenous production (panel B). These changes are transient despite continuous ischemia. The myocardium was capable of producing lactate at 60 minutes as evidenced by the response to atrial pacing.
and LV wall thickening as has been reported by Schaefer et al. Since PCr regenerates during ischemia without a significant improvement in regional function, there is a significant shift in the relation between these variables when comparing early ischemia (control and 5–10-minute data) with late ischemia (55–60-minute data only), there is a time-dependent shift in the relation between subendocardial PCr content and regional function (p<0.0001 by analysis of covariance). Animals from protocol 1 that do not fully regenerate PCr (next to data point) appear to fall along the same relation as the other animals during late ischemia. This suggests PCr regeneration during ischemia is a continuous function, not an “all or none” phenomenon.

Protocol 3: Specificity of Anterior Interventricular Blood Samples in the Swine

Before infusion of fluorescein, no fluorescent emission was detected in the aorta, anterior interventricular vein, or circumflex vein. Infusion of fluorescein into the circumflex artery resulted in strong fluorescent emission in the circumflex vein (1.583±372 units with basal LAD blood flow and 1.313±251 units during a 40% reduction in LAD blood flow). Fluorescein also stained a sharply demarcated zone on the surface of the heart that demonstrated the area perfused by the circumflex artery. Fluorescent activity in the anterior interventricular vein slowly increased over time but on average was less than 10% of intensities detected in the circumflex vein (Figure 5A). Aortic fluorescence resulting from recirculation of dye accounted for 93.4% and 95.4% of the fluorescence detected in the anterior interventricular vein during basal LAD blood flow (r=0.966) and 40% reduced LAD blood flow (r=0.977), respectively (Figure 5B). Collateral blood flow from the circumflex artery contributed less than 2% of anterior interventricular vein blood flow (95% confidence limits, 1.8% to

-1.9% during basal flow and 1.4% to -1.9% during reduced LAD flow). Thus, blood samples obtained from the anterior interventricular vein represent drainage from the ischemic myocardium with virtually no contamination from nonischemic zones.

Calculations

Net rate of ATP production (μmol/100 g/min) at a given time is the sum of the rates of aerobic ATP production and anaerobic ATP production. Aerobic ATP production (μmol/100 g/min) is derived from the oxygen consumption data and assumes a phos-
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phosphorus/oxygen (P:O) ratio of 2.4.\textsuperscript{15} Although radioisotopic tracer studies have demonstrated that a small amount of lactate production can occur at a time of net lactate consumption,\textsuperscript{16,17} we assume for these calculations that this contributes a negligible amount of ATP during the control state.

\[
\text{Net ATP production} = \text{aerobic ATP production} + \text{anaerobic ATP production} \quad (1)
\]

\[
\text{Anaerobic production} = \frac{1 \text{ mol ATP}}{1 \text{ mol lactate}} \times (\text{arteriovenous lactate production} + \text{lactate accumulation}) \quad (2)
\]

Anaerobic ATP production (\(\mu\text{mol/100 g/min}\)) is calculated from arteriovenous lactate production and transmural accumulation of lactate. Between 1.0 and 1.5 moles of ATP are produced per minute for each mole of lactate produced or accumulated per minute, depending on the relative proportion of glucose and glycogen serving as substrate. To avoid overestimating anaerobic ATP production and in keeping with the fact that even the subendocardium maintains approximately 50% of basal myocardial blood flow in these experiments, we assume glucose is the primary substrate for anaerobic glycolysis and 1.0 mole of ATP is produced per mole of lactate.\textsuperscript{17}

Because of the time delays inherent to each measurement, blood samples for oxygen and lactate were obtained at 5, 55, and 65 minutes of ischemia, and LV biopsies were obtained at 10, 60, and 70 minutes. If one assumes a linear accumulation of lactate between 0 and 10 minutes, the rate of lactate accumulation at 5 minutes can be calculated from the difference in lactate contents between control and 10 minutes and dividing by 10 minutes. Similar calculations provide ATP production at 55 and 65 minutes except that lactate accumulation is negligible between 30 and 60 minutes (protocol 2).

Calculated rates of aerobic and anaerobic ATP production from the eight animals in protocol 1 with complete oxygen consumption data are shown in Figure 6 and Table 3. Control rates of ATP production indicate the total HEP pool turns over about twice per minute at this workload. During early ischemia, anaerobic lactate production contributes 7.8% of total ATP production. Although total ATP production during early ischemia is not statistically different from control, this is likely a Type II statistical error since anaerobic ATP production accounts for less than 30% of the decrement in aerobic ATP production between control and 5 minutes. The increase in anaerobic ATP production is significantly less than the decrease in aerobic ATP production between control and 5 minutes (two-tailed, paired \(t\) test, \(p<0.05\)). By 55 minutes of ischemia, anaerobic ATP production accounts for 0.1% of total ATP production. Thus, the new homeostasis, characterized by persistently reduced wall thickening and regenerated PCr, occurs at a time when the myocardium produces 23% less ATP per minute than at control (\(p<0.025\)) and no longer requires anaerobic glycolysis.

**Discussion**

We have previously reported that the myocardium regenerates PCr during 60 minutes of moderate hypoperfusion.\textsuperscript{3} These data, along with evidence for a decrease in arteriovenous lactate production,\textsuperscript{4,5} are consistent with an improvement in the myocardial metabolic state during prolonged partial ischemia. However, these results could also be compatible with pathological abnormalities as noted in each of these articles. Subcellular compartmentation may have allowed accumulation of PCr in a location inaccessible to use by the myofibrils. Alternatively, PCr regeneration might have been due to selective inhibition of cytosolic creatine kinase. Either situation would limit the availability or rate of utilization of PCr and could lead to paradoxical accumulation of PCr during ischemia. Similarly, inhibition of anaerobic glycolysis caused by intracellular acidosis or lack of substrate, such as glycogen, might explain decreasing arteriovenous lactate production during late ischemia.

The current experiments were designed to differentiate whether these dynamic metabolic changes represent energetic improvements during ischemia or progressive deterioration. Our current results confirm our earlier findings of PCr regeneration in the majority of animals undergoing 60 minutes of moderate myocardial ischemia. We add several important observations suggesting the metabolic changes during 60-minute reductions in coronary blood flow represent overall improve-
ments in myocardial energetics despite ongoing ischemia. The decrease in arteriogenous lactate production parallels decreased subendocardial lactate content. That the subendocardium is capable of producing lactate in response to the added stress of atrial pacing demonstrates that substrate availability does not limit lactate production during late ischemia. Since the myocardium is capable of using PCr stores in response to atrial pacing, we conclude the myocardium is capable of using these stores during late ischemia when it instead reaccumulates the high energy compound. Taken one step further, we can now conclude PCr regeneration represents accumulation of useful high energy stores during ischemia. Thus, the metabolic response to atrial pacing is critical to understanding the importance of PCr regeneration during the period of stable ischemia. This logic forms the crux of our thesis that myocardial energy balance is improving during moderate ischemia.

Downregulation of Energy Requirements During Ischemia

We propose that the myocardium actively downregulates regional energy requirements during ischemia, allowing reaccumulation of PCr during ongoing ischemia. This hypothesis may be an extension of the observation that the normally perfused myocardium must precisely balance energy production to meet increased energy requirements. It is widely accepted that ATP production must increase to meet the demand of higher myocardial workloads under normal conditions. This has been demonstrated in the isolated perfused rat heart by direct assessment of the creatine kinase reaction rate. Furthermore, the well-perfused myocardium rapidly increases ATP production to meet the demands of increased workload without depleting PCr or ATP.

Ischemia clearly interferes with the balance between energy utilization and production and results in an ATP and PCr depleting phase. This has been well described under experimental conditions of anoxia, hypoxia, no blood flow ischemia, and partial reductions in coronary blood flow. If the absolute difference between the rates of ATP utilization and production remained static, the rate of depletion would remain constant and eventually the total HEP pool would be depleted. Quantification of the rate at which the total adenine nucleotide pool is depleted demonstrates that the rate of depletion slows after the rapid phase during early total ischemia. During constant partial reductions in coronary flow, ATP content eventually stabilizes, suggesting that a new balance between utilization and production has been established.

Studying the relation between regional function and coronary blood flow per beat, Gallagher et al. concluded myocardial energy requirements and function might be gradually decreased during ischemia until a "new equilibrium between (limited) supply and (reduced) demand" was established.

Despite these observations, the concept of downregulation of myocardial energy requirements during ischemia is not well accepted. To further illustrate that myocardial energy utilization must be downregulated during ischemia, the metabolic consequences of three different models of myocardial downregulation will be discussed. In each of these examples, the time course of ATP production is extrapolated from the four determinations made in protocol 1 and the fact that subendocardial lactate levels were close to control levels by 30 minutes in protocol 2. Three different time courses of ATP utilization are compared with the same interpolated ATP production curve. The difference between the integral of ATP production and ATP utilization determines the net accumulation or depletion of HEP contents.

The rate of ATP utilization for an ischemic myocardium with no capacity for downregulating regional energy requirements should remain at baseline levels while ATP production falls during ischemia. Because ATP utilization exceeds production at all times during the ischemic episode, the myocardium should continuously deplete HEP stores. With a 25% decrease in myocardial oxygen consumption and an HEP turnover rate of two to four times per minute, total depletion of ATP and PCr should occur between 1 and 2 minutes. This degree of depletion does not even occur with complete occlusions of much longer duration. Thus, a model stating that regional myocardial energy requirements are determined largely by external factors and are not locally regulated is incompatible with virtually every study measuring HEP contents during ischemia.

Broadly defined, passive downregulation of local myocardial energy requirements could encompass conditions ranging from metabolite depletion and undetectable structural damage to myocardial infarction. In each case the primary mechanism altering regional energy requirements involves injury to the energy-utilizing machinery. Because of this unspecified damage, energy requirements gradually fall until a new balance is reached between ischemic ATP production and utilization (Figure 7A). Since energy production falls more rapidly, there is a transient depletion of HEP compounds. Later ischemia is characterized by a new homeostasis, and HEP contents could remain stable indefinitely if the pathological process that initiated these changes was not progressive. Whether or not the myocardium continues to produce lactate during late ischemia may depend on the severity of the blood flow reduction. This model is consistent with the findings of Neill et al. during moderate ischemia and Gallagher et al. Because the rate of ATP utilization never falls below the rate of ATP production, this model does not predict an energy-accumulating state during late ischemia as demonstrated in our experiments.

If regional myocardial energy requirements could be actively downregulated during ischemia, then ATP utilization could fall with time without necessarily resulting in myocardial damage. HEP depletion would occur only during the initial phase of ischemia until utilization equals production. The important
ATP Production During Ischemia

There are several possible mechanisms to explain why the rate of ATP production appears to decrease slightly between 10 and 60 minutes of stable ischemia. A gradual reduction in energy requirements between 5 and 60 minutes, as postulated in our model of actively downregulated energy requirements during ischemia, could account for decreased anaerobic ATP production during late ischemia. The transition back to a lactate-consuming state might therefore be a marker for the transition to a PCR-accumulating state or vice versa. Additional possibilities are considered in the following paragraphs.

The free energy change for ATP hydrolysis (dG/de) represents the maximal amount of chemical work available from hydrolysis of the ATP pool. As an exergonic reaction, dG/de is a negative quantity, indicating hydrolysis of ATP tends to occur spontaneously and can supply chemical energy to drive other reactions. The concentrations of ATP, free ADP, and Pi influence dG/de as shown in Equation 3, where \( \Delta G^\circ \) is the standard free energy change of the reaction, R is the gas constant, and T is the absolute temperature:

\[
dG/de = \Delta G^\circ - RT \ln \left( \frac{[ATP]}{[ADP][Pi]} \right)
\] (3)

Although the absolute value of dG/de likely does not decrease sufficiently to explain the observed decrease in regional wall thickening, these changes may, however, be large enough to account for why ATP production is higher during early ischemia than late ischemia.

In essence, if the magnitude of dG/de decreases transiently, less chemical work could be derived from the ATP pool during early ischemia compared with 60 minutes. Thus, if mechanical function and other determinants of ischemic zone energy requirements remained stable throughout the first hour of ischemia, a transient decrease in the magnitude of dG/de would require a transient increase in ATP turnover, which is consistent with our observations.

Transient decreases in the efficiency of mitochondrial oxygen utilization could also explain why ATP production appears higher in early ischemia than at 60 minutes. Partial uncoupling of oxidative phosphorylation would mean our estimate of aerobic ATP production is falsely high since we assume a constant P:O ratio of 2.4. If partial uncoupling of oxidative phosphorylation occurred for a limited time, this could also explain the brief need for lactate production. A decrease in the P:O ratio associated with a change in mitochondrial substrate would have similar effects.

Conversely, increases in the efficiency of mitochondrial function during ischemia could mean our estimates of ATP production are falsely low. An increase in the P:O ratio from 2.4 to 3.0 would be sufficient to balance ATP production to control levels with the degree of ischemia studied. When analyzed by layers
across the LV wall, the P:O ratio in the subendocardium would need to increase even further to account for the 55% decrease in subendocardial blood flow. To date, there is no experimental work to suggest such a mechanism is possible. We feel that such a mechanism is less likely than local downregulation of energy requirements in light of the marked impairment in regional wall thickening throughout ischemia.

With the exception of an increase in mitochondrial efficiency during ischemia, none of the mechanisms described eliminates the conceptual need for active local downregulation of energy requirements during ischemia as an explanation for the regeneration of PCr during ischemia.

Limitations

The greatest limitations of these methods are inherent to tissue extraction and enzymatic analysis. The inability to measure free [ADP] is not unique to chemical determinations. In experiments by nuclear magnetic resonance spectroscopy, free [ADP] is generally calculated from measurements of other reactants in the creatine kinase equilibrium. Similarly, our inability to measure intracellular concentrations of inorganic phosphorus, free intracellular calcium (Ca\(^{2+}\)), and pH requires circumstantial analysis.

The relation between regional function and myocardial PCr content shifts between early ischemia and late ischemia (Figure 4). These relations may also shift during early reperfusion but in the opposite direction. The mechanisms responsible for the time-dependent modification in the PCr versus function relation cannot be determined by our data. However, assuming intracellular pH improves on a similar time course to the myocardial lactate content, normalization of pH would have been predicted to improve contractile function for a given energetic state, opposite the trend in Figure 4. Similarly, intracellular Pi should have decreased as PCr is regenerated, which should improve function during late ischemia. Thus, we hypothesize that Pi and pH cannot be the sole regulators of myocardial function during ischemia as is also suggested by From et al. Alterations in Ca\(^{2+}\) transient size or altered myofibril–Ca\(^{2+}\) interactions may play an important role during late ischemia.

The experimental model used to evaluate these systems may be important. Our in situ model of regional ischemia functions under relatively low workloads with enough nonischemic ventricle to support global ventricular function. The myocardium may be able to adapt to much more severe ischemic insults than we have studied but at the cost of markedly depressed function that would not be tolerated in situ and at the cost of continuous myocardial lactate production.

Although the majority of animals regenerate PCr during continuous moderate ischemia, two animals from protocol 1 and three animals from protocol 2 did not regenerate PCr to at least 67% of control levels by 60 minutes. These animals may provide clues to the mechanisms allowing accumulation of PCr despite ongoing ischemia. The animals failing to regenerate PCr by 60 minutes appeared to follow the same PCr versus function relation as the other animals during late ischemia (Figure 4). So, although these animals appear to have downregulated regional wall thickening during late ischemia, this was not a sufficient adaptation to allow PCr reaccumulation. Factors such as size of the ischemic zone or baseline oxygen consumption or factors we cannot yet identify may determine the limits to which the myocardium can actively downregulate regional energy requirements and the rate of PCr regeneration during ischemia. Resolution of these issues will require further study.

Implications and Conclusions

Despite certain limitations, these studies provide considerable insight into the dynamic changes that occur during the course of an hour of myocardial ischemia. This work indicates the ischemic myocardium actively downregulates myocardial energy utilization beyond that necessary to achieve energy balance. This allows the myocardium to accumulate PCr and eliminate anaerobic glycolysis despite ongoing ischemia. Although reduced function and energy requirements during prolonged subtotal ischemia are important characteristics of the hibernating myocardium, extrapolation from our observations to the clinically described situation remains indirect. Studies of anoxia or complete coronary occlusion may exceed the capability of these regulatory mechanisms. Further study of the relation between myocardial function and metabolism during prolonged partial ischemia may provide important insight into the mechanisms that regulate myocardial function and into the common clinical correlates to these problems.

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Myocardial Energy Requirements During Ischemia


KEY WORDS • ATP • phosphocreatine • lactate • metabolism • hibernation
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