Transmural distribution of metabolic abnormalities and glycolytic activity during dobutamine-induced demand ischemia

Mohammad N. Jameel, Xiaohong Wang, Marcel H. J. Eijgelshoven, Abdul Mansoor, and Jianyi Zhang

Cardiovascular Division, Departments of Medicine, University of Minnesota Medical School, Minneapolis, Minnesota

Submitted 30 November 2007; accepted in final form 16 April 2008

Transmural distribution of metabolic abnormalities and glycolytic activity during dobutamine-induced demand ischemia. Am J Physiol Heart Circ Physiol 294: H2680–H2686, 2008. First published April 18, 2008; doi:10.1152/ajpheart.01383.2007.—The heterogeneity across the left ventricular wall is characterized by higher rates of oxygen consumption, systolic thickening fraction, myocardial perfusion, and lower energetic state in the subendocardial layers (ENDO). During dobutamine stimulation-induced demand ischemia, the transmural distribution of energy demand and metabolic markers of ischemia are not known. In this study, hemodynamics, transmural high-energy phosphate (HEP), 2-deoxyglucose-6-phosphate (2-DGP) levels, and myocardial blood flow (MBF) were determined under basal conditions, during dobutamine infusion (DOB: 20 μg·kg⁻¹·min⁻¹), and during coronary stenosis + DOB + 2-deoxyglucose (2-DG) infusion. DOB increased rate pressure products (RPP) and MBF significantly without affecting the subendocardial-to-subepicardial blood flow ratio (ENDO/EPI) or HEP levels. During coronary stenosis vs. maintaining contractile performance during demand ischemia. It is not known whether the glycolytically produced ATP is preferentially used to support cellular ion homeostasis (14, 27). However, the function of the glycolytic ATP production may be different in different forms of ischemia: maintaining cellular ion homeostasis during supply ischemia vs. maintaining contractile performance during demand ischemia. It is not known whether the myocardium perfused by a stenotic coronary artery in the setting of suppressed glycolysis can maintain its ion hemostasis after catecholamine stimulation. We hypothesized that the transmural pattern of demand ischemia is different from supply ischemia, and inhibition of glucose uptake can cause both metabolic changes and myocardial edema in the ischemic layer of the left ventricular (LV) wall has the highest level of energy expenditure. Catecholamine stimulation in the presence of coronary stenosis (demand induced ischemia in the setting of coronary stenosis) also produced similar transmural patterns of metabolic markers, with the subepicardial layer showing reduction in energetic states (3). These unexpected experimental observations lead to the postulation that transmural patterns of myocardial ischemia are significantly different between supply ischemia and demand ischemia.

Under normal conditions, a majority of the total cellular ATP is derived from the oxidation of fatty acids and pyruvate, with only a small portion coming from glycolysis (11, 13, 18, 28). Even during supply ischemia caused by a reduction of coronary blood flow by 30–60%, fatty acid oxidation continues to provide the majority (60–80%) of the ATP formed in the heart (11, 13, 18, 28). However, in supply ischemia, there is an increase in glucose extraction that maintains glucose uptake despite a decrease in myocardial blood flow (29). This is accompanied by an increase in net tissue glycogen breakdown and lactate accumulation, suggesting an increase in nonoxidative glycolysis under ischemic conditions (29). It is important to note that the accumulation of lactate during demand ischemia could be the limitation in glycolytic-reducing equivalents transfer to the mitochondria. The reducing equivalents that would otherwise be shuttled into the mitochondria for oxidative ATP synthesis remain in the cytosol because the malate aspartate shuttle exchange becomes the limiting factor for glucose oxidation due to the very high flux rates through the mitochondrial dehydrogenases (20). A similar increase in nonoxidative glycolysis is seen in demand-induced ischemia in the presence of coronary stenosis (2). Under basal conditions, cardiomyocyte contractile shortening uses ~60–80% of the ATP produced in the heart, and the rest is used to support ion pumps, especially the sarcoplasmic reticulum Ca²⁺-ATPase pump and the Na⁺/K⁺ exchanger (2, 30). It has been shown that the glycolytically produced ATP is preferentially used to support cellular ion homeostasis (14, 27). However, the function of the glycolytic ATP production may be different in different forms of ischemia: maintaining cellular ion homeostasis during supply ischemia vs. maintaining contractile performance during demand ischemia. It is not known whether the myocardium perfused by a stenotic coronary artery in the setting of suppressed glycolysis can maintain its ion hemostasis after catecholamine stimulation. We hypothesized that the transmural pattern of demand ischemia is different from supply ischemia, and inhibition of glucose uptake can cause both metabolic changes and myocardial edema in the ischemic...
region during catecholamine stimulation. Spatially localized $^{31}$P-nuclear magnetic resonance (NMR) spectroscopy was used to evaluate the transmural distributions of the HEP, $P_i$, and 2-deoxyglucose-6-phosphate (2-DGP) levels, which permitted transmurally differentiated evaluation of distributions of energy demand under these experimental conditions.

**METHODS**

**Surgical preparation.** Ten adult mongrel dogs weighing 18–23 kg were anesthetized with pentobarbital sodium (30 mg/kg and 4 mg/kg $^{-1}$ h $^{-1}$ iv). The animals were intubated and ventilated with a respirator using room air supplemented with oxygen. A heparin-filled polyvinyl chloride catheter (3.0 mm OD) was inserted in the left femoral artery and advanced in the ascending aorta. A left thoracotomy was performed through the fourth intercostal space. The pericardium was opened, and the heart was suspended in a pericardial cradle. Heparin-filled catheters were inserted in the left ventricle through the apical dimple and in the left atrium through the atrial appendage and secured with purse string sutures. A 1.5- to 2.0-cm segment of the proximal left anterior descending coronary artery (LAD) was dissected free, and a hydraulic occluder constructed of polyvinyl chloride tubing (2.7 mm OD) was placed around the artery, proximal to the first major arterial branch. A silicone elastomer catheter (0.75 mm ID) was placed in the LAD distal to the occluder by the method of Gwirtz (4). A Doppler flow probe was placed on the LAD proximal to the occluder.

The region of the left ventricle that became cyanotic upon inflation of the arterial occluder was determined by visual inspection, and a 28-mm-diameter NMR surface coil was sutured on the pericardium overlying the ischemic area. The pericardial cradle was then released, and the heart was allowed to assume its normal position. The surface coil leads were connected to a balanced, tuning circuit, and the animals were placed within the magnet.

All experimental procedures followed in accordance with the animal use guidelines of the University of Minnesota, and the experimental protocol was approved by the University of Minnesota Animal Resources Committee. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No 85-23, revised 1985).

**NMR methods.** Measurements were performed in a 40-cm bore 4.7-T magnet interfaced with a SISCO (Spectroscopy Imaging Systems, Fremont, CA) console. The LV pressure signal was used to gate NMR data acquisition to the cardiac cycle, whereas respiratory gating was achieved by triggering the ventilator to the cardiac cycle between data acquisitions (6, 12, 25, 35). $^{31}$P- and $^1$H-NMR frequencies were 81 and 200 MHz, respectively. Spectra were recorded in late diastole with a pulse repetition time of 6–7 s. This repetition time allowed full relaxation for ATP and inorganic phosphate (Pi) resonances, and 90% relaxation for the creatine phosphate (PCr) resonance. The 90° pulse length for the phosphonoacetic acid reference positioned between the ATP and PCR resonance, off resonance effects on these peaks were virtually nonexistent. However, ATPβ suffers from off-resonance phenomenon, and this effect varies with proximity to the surface coil due to the inhomogeneity of the magnetic field generated by the surface coil (6, 12). Consequently, ATPβ is not suitable for evaluating the relative ATP contents or the PCr-to-ATP ratio. The numerical values for PCR, ATP, and 2-DGP in each voxel were expressed as ratios of PCR/ATP and 2-DGP/PCr. Pi levels were measured as changes from baseline values, using integrals obtained in the region covering the Pi resonance. The change of Pi level was expressed as the $\Delta$P$_i$/PCR ratio. Data are reported for the subepicardial (EPI), midmyocardial (MID), and subendocardial (ENDO) voxels.

**Hemodynamic measurements.** Aortic, LV, and mean distal LAD pressures were measured using Spectromed TNF-R pressure transducers positioned at midstich level. All data were recorded on an eight-channel Coulbourne R14–28 direct-writing recorder.

**Myocardial blood flow measurements.** Transmural myocardial blood flow was measured using radionuclide-labeled microspheres, 15 µm in diameter, suspended in low-molecular-weight dextran. Microspheres labeled with four different radioisotopes ($^{51}$Cr, $^{85}$Sr, $^{95}$Nb and, $^{46}$Sc) were agitated in an ultrasonic mixer for 10 min before injection. The microsphere suspension containing $2 \times 10^6$ microspheres was injected through the left atrial catheter and flushed with 10 ml of normal saline. A reference sample of arterial blood was withdrawn from the aortic catheter at a rate of 15 ml/min beginning 5 s before microsphere injection and continuing for 120 s. Radioactivity in the myocardial and blood reference specimens was determined using a gamma spectrometer with multichannel analyzer (model 5912; Packard Instrument, Downers Grove, IL) at window settings chosen for the combination of radioisotopes used during the study. Activity in each energy window, background activity, and sample weight were entered in a digital computer programmed to correct for overlap between isotopes and for background activity, and to compute the corrected counts per minute per gram of myocardium. Knowing the rate of withdrawal of the reference blood specimen (Qr) and the radioactivity of the reference specimen (Cr), myocardial radioactivity (Cm) was used to compute myocardial blood flow (Qm) as: Qm = Qr (Cm/Cr).

Blood flow was expressed as milliliters per gram of myocardium per minute.
Oxygen consumption measurement. Blood specimens were withdrawn aerobically in iced syringes from the aortic and coronary sinus catheters (34). PO2 and PCO2 were measured with a blood gas analyzer (model 1304; Instrumentation Laboratory, Lexington, MA) calibrated with known gas mixtures. Hemoglobin content was determined by the cyanmethemoglobin method. Blood oxygen content was calculated as hemoglobin \( \times 1.34 \times O_2 \) saturation + (0.0031 \( \times \) PO2), using the oxygen dissociation curve for dog blood. Myocardial oxygen consumption (MV\( \text{O}_2 \)) was then computed as the arterial-venous oxygen content difference multiplied by the myocardial blood flow.

Myocardial specimen weight measurements. Tissue pieces were deblotted and weighed when obtained (before they dehydrate at all). This was termed the wet weight. Myocardial tissue (5 g) was placed overnight in the oven at 100°C, which caused it to fully dehydrate. Dry weight was measured immediately after removal from the oven to prevent rehydration.

Study protocol. Ventilation rate, volume, and inspired oxygen content were adjusted (on the basis of arterial blood gas and pH measurements) as required to maintain physiological values. Aortic, LV, and mean LAD (distal to occluder) pressures were monitored continuously throughout the study. During each intervention, myocardial contractile function, blood flow, and hemodynamic measurements were collected simultaneously with the acquisition of transmural \(^{31}\text{P}-\text{NMR}\) spectra.

Following control observations, animals received 20 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) iv dobutamine. After waiting for ~5 min to allow systemic hemodynamics to stabilize, all measurements were repeated. The occluder was then slowly inflated with a micrometer-driven syringe to decrease the mean coronary flow, as indicated by the Doppler signal, to the basal level and was maintained constant thereafter. The distal coronary perfusion pressure of this mean flow was recorded. The 2-deoxyglucose (2-DG) infusion was then started at a rate of 15 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) iv. All measurements, except myocardial blood flow measurements, were repeated every 15 min in the presence of the coronary stenosis with dobutamine and 2-DG infusion continued. Microspheres were injected only at 45 min after the start of 2-DG infusion.

Data analysis. HEP and P, measurements were obtained as described above. Hemodynamic data were determined from the strip chart recordings. Transmural blood flow distribution was determined from the microsphere measurements. Data were analyzed with one-way ANOVA for repeated measurements. A value of \( P < 0.05 \) was considered significant. When significant results were found, individual comparisons were made using the Bonferroni correction (26).

For comparing regional myocardial blood flow data or NMR data of the PCR-to-ATP ratios, there are two factors being compared with experimental conditions and the myocardial region of interest between EPI, midwall, and ENDO layers. Therefore, a two-way ANOVA analysis is applied (26).

RESULTS

Hemodynamic measurements. Hemodynamic data are shown in Table 1. Heart rate and LV systolic pressure increased significantly during dobutamine infusion. Rate pressure product (RPP) reached 30,000 mmHg/min, a level that has been shown not to cause demand ischemia (34). Following application of the stenosis, distal coronary pressure decreased to 48 mmHg. RPP also decreased significantly, which was mainly caused by a decrease in LV systolic pressure, since heart rate did not change during this intervention. LV end diastolic pressure did not change significantly during any of the experimental conditions.

Myocardial blood flow. Myocardial blood flow data are summarized in Table 2. In response to dobutamine stimulation, blood flow increased significantly parallel across the LV wall compared with baseline (\( P < 0.05 \), Table 2). Coronary stenosis (with continued dobutamine infusion) induced a significant decrease in myocardial blood flow based on the Doppler flow probe (data not shown). During dobutamine (20 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) iv) + coronary stenosis + 2-DG infusion, myocardial flow in the posterior wall remained significantly increased compared with baseline (Table 2). The anterior wall myocardial blood flow was successfully restricted to the baseline level (Table 2) by observing the Doppler flow and adjusting the hydraulic occluder.

Myocardial HEP levels. Figure 1 shows an example of transmural set of \(^{31}\text{P}-\text{NMR}\) spectra from one heart. Figure 1A displays the baseline spectra for five layers spanning the LV chamber. However, only five voxels centered about any phase angle and consequently any “depth,” including the LV chamber. However, only five voxels centered around the phase angles specified were displayed in the current study. In these five voxel transmural sets of spectra, every other voxel is virtually nonoverlapping; however, adjacent voxels contain some overlap (5, 24). The spectrum at the bottom of the transmural stack (voxel 1) is nearest to the surface coil and accordingly is labeled “EPI.” Spectra from the midwall and the subendocardium are labeled as “MID” and “ENDO,” respectively. Under the basal conditions, myocardial PCR and ATP resonances are well observed, whereas the Pi signal is too low to be detected across the wall (Fig. 1A). In response to catecholamine stimulation, this heart increased RPP from 18,750 mmHg \( \times \) beats/min to 44,000 mmHg \( \times \) beats/min.

Under this work state, there is a clear increase of myocardial Pi level with a tendency toward more pronounced changes in the

Table 1. Hemodynamic data

<table>
<thead>
<tr>
<th>Intervention</th>
<th>n</th>
<th>Heart Rate, beats/min</th>
<th>MAP, mmHg</th>
<th>LVSP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>Coronary Pressure, mmHg</th>
<th>RPP, 10^3 mmHg ( \times ) beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10</td>
<td>151 ± 8</td>
<td>95 ± 5</td>
<td>117 ± 7</td>
<td>7.1 ± 0.7</td>
<td>95 ± 5</td>
<td>17.2 ± 1.2</td>
</tr>
<tr>
<td>Db20</td>
<td>10</td>
<td>203 ± 11*</td>
<td>109 ± 7</td>
<td>194 ± 15*</td>
<td>7.0 ± 0.8</td>
<td>96 ± 5</td>
<td>29.8 ± 2.2*</td>
</tr>
<tr>
<td>Db20 + coronary stenosis + 2-DG</td>
<td>10</td>
<td>203 ± 11*</td>
<td>88 ± 7</td>
<td>167 ± 16*</td>
<td>7.5 ± 1.5</td>
<td>48 ± 2**</td>
<td>24.6 ± 2.6**</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of dogs. MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; RPP, rate pressure product; Db20, dobutamine at 20 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \); 2-DG, 2-deoxyglucose at 15 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). *\( P < 0.05 \) compared with baseline (*) and compared with Db20 (†).
outer layers of the LV wall (Fig. 1B). When coronary stenosis was applied to reduce the mean coronary flow (measured by Doppler flow probe) to the basal level, combined with continued infusion of dobutamine (same rate as before) and additional infusion of 2-DG (15 μmol·kg body wt⁻¹·min⁻¹ iv), the Pi level further increased and 2-DGP accumulation was clearly seen (Fig. 1C; spectra acquired between 30 and 40 min after 2-DG infusion started). Again these changes are most prominent in EPI layers of the LV wall (Fig. 1C).

The summarized PCr-to-ATP, ΔP to PCr, and 2-DGP-to-PCr ratios are shown in Table 3. PCr/ATP did not change significantly in response to catecholamine stimulation. Two of the eight animals studied showed an increase of Pi in the outer layers during dobutamine stimulation, but the mean value of the group did not reach a significant change (Table 3). During stenosis plus dobutamine stimulation together with intracoronary infusion of 2-DG, myocardial PCr/ATP decreased significantly (Table 3). ΔP/PCr increased significantly across the wall uniformly. Because during this experimental condition the levels of PCr were not significantly different across the transmural different layers (Table 3), the levels of accumulation of 2-DGP across the LV wall were expressed as the 2-DGP-to-PCr ratio. This ratio is not subject to variability in PCr across the LV wall. 2-DGP accumulated markedly, and the 2-DGP-to-PCr ratio was significantly higher in the EPI region than in ENDO (Table 3).

Table 2. Myocardial blood flow data

<table>
<thead>
<tr>
<th></th>
<th>Anterior Wall (Ischemic)</th>
<th>Posterior Wall (Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>EPI</td>
</tr>
<tr>
<td>Baseline</td>
<td>8</td>
<td>0.61±0.04</td>
</tr>
<tr>
<td>Db20</td>
<td>8</td>
<td>1.52±0.13*</td>
</tr>
<tr>
<td>Db20 + coronary stenosis + 2-DG</td>
<td>7</td>
<td>0.95±0.11†§</td>
</tr>
</tbody>
</table>

Values (in ml·min⁻¹·g wet wt⁻¹) are means ± SE; n, no. of dogs. EPI, subepicardium; MID, midmyocardium; ENDO, subendocardium. P < 0.05 compared with baseline (*), compared with Db20 (†), compared with EPI (‡), and vs. posterior wall (§).
**H2684** TRANSMURAL DISTRIBUTIONS OF HEP LEVELS DURING DEMAND ISCHEMIA

**Table 3. Transmural myocardial energetics data**

<table>
<thead>
<tr>
<th></th>
<th>PCr/ATP</th>
<th>2-DGP/PCr</th>
<th>ΔPc/PCr</th>
<th>PCr, %</th>
<th>ATP, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.30±0.14</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EPI</td>
<td>2.06±0.13</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MID</td>
<td>2.04±0.11</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ENDO</td>
<td>2.40±0.27</td>
<td>0.05±0.03</td>
<td>0.98±0.01</td>
<td>0.95±0.03</td>
<td></td>
</tr>
<tr>
<td>Db20</td>
<td>2.16±0.25</td>
<td>0.05±0.03</td>
<td>0.93±0.04</td>
<td>0.91±0.03</td>
<td></td>
</tr>
<tr>
<td>ENDO</td>
<td>2.09±0.12</td>
<td>0</td>
<td>0.98±0.05</td>
<td>0.98±0.05</td>
<td></td>
</tr>
<tr>
<td>Db20 + coronary stenosis + 2-DG</td>
<td>1.77±0.12*†</td>
<td>0.55±0.12*†</td>
<td>0.54±0.08*†</td>
<td>0.51±0.07*†</td>
<td>0.62±0.05*†</td>
</tr>
<tr>
<td>EPI</td>
<td>1.70±0.11*†</td>
<td>0.52±0.10*†</td>
<td>0.50±0.06*†</td>
<td>0.47±0.05*†</td>
<td>0.56±0.06*†</td>
</tr>
<tr>
<td>MID</td>
<td>1.72±0.12*†</td>
<td>0.37±0.08*†</td>
<td>0.48±0.07*†</td>
<td>0.57±0.07*†</td>
<td>0.69±0.07*†</td>
</tr>
</tbody>
</table>

Values are means ± SE. PCr, phosphocreatine; ΔPc, change in inorganic phosphate from baseline; 2-DGP, 2-deoxyglucose-6-phosphate. *P < 0.05 compared with baseline (*). †P < 0.05 compared with Db20 (†), and compared with EPI (‡).

**MV**\textsubscript{O}2 and lactate metabolism. MV O\textsubscript{2} of the LAD-perfused area increased significantly in response to dobutamine infusion (Table 4). When stenosis was applied with continuous infusion of dobutamine and 2-DG, the MV O\textsubscript{2} in the ischemic region returned to baseline values, despite the fact that RPP was still significantly higher compared with the control situation. Net lactate uptake also increased with dobutamine and showed a similar decrease with combined dobutamine and 2-DG infusion in the presence of stenosis (Table 4).

**Myocardial specimen data.** In the ischemic zone, the myocardial wet weight-to-dry weight ratio was significantly increased compared with the normal zone (5.9 ± 0.5 vs. 4.4 ± 0.4; P < 0.05).

**DISCUSSION**

The results of the present study have further elucidated the metabolic abnormalities occurring in demand-induced ischemia caused by dobutamine infusion in the setting of coronary flow restriction. First, the transmural energetic abnormalities in demand-induced ischemia are discordant with the transmural changes in myocardial blood flow, such that the EPI layer has the most pronounced energetic changes despite having higher blood flow in this experimental setting. Second, it has been shown that 31P-NMR-detectable 2-DGP accumulates in the in vivo canine hearts in the setting of demand-induced ischemia. Moreover, there is a transmural gradient to the accumulation of 2-DGP such that the concentration of 2-DGP is highest in the subepicardium. Third, glycolytic inhibition as a result of 2-DGP accumulation in hearts undergoing demand-induced ischemia leads to pronounced metabolic abnormalities and consequently myocardial edema.

This study confirms our previous finding that, in myocardium perfused by a stenotic coronary artery, dobutamine stimulation resulted in a distinct transmural ischemic pattern, with the most prominent changes appearing in the outer layers of the LV wall (3). In contrast, it is well established that, during the basal work state in response to a partial or total occlusion of a coronary artery, the myocardial metabolic ischemic markers occur predominantly in the inner myocardial layers (21, 37). In the present study, we used a model of demand ischemia induced by imposing a high workload in a condition of fixed coronary stenotic flow in which the decrease of PCr/ATP and increase of ΔPc/PCr and 2-DG/ATP were either uniform across the wall or most prominent in the outer layers (Table 3). These data are thereby different from results usually obtained during supply ischemia, with the changes of metabolic ischemic markers being most pronounced in the inner layers of the LV wall. This suggests that the two types of ischemia have distinct metabolic and functional characteristics. In supply ischemia induced by a decrease of blood flow delivery, the metabolic ischemic markers were concordant with the pattern of myocardial hypoperfusion, with the largest changes occurring in the subendocardium (21, 37). In contrast, when coronary stenosis is combined with dobutamine stimulation, the metabolic markers of ischemia were transmurally uniform or greater in the subepicardium and did not correspond to the pattern of myocardial hypoperfusion. In this regard, it is important to note that one important mechanism for the accumulation of lactate during demand ischemia is a limitation in glycolytic-reducing equivalents transfer into the mitochondria (10, 20). The reducing equivalents, which would otherwise be shuttled into mitochondria for oxidative ATP synthesis, remain in the cytosol because the malate aspartate shuttle exchange becomes the limiting factor due to the high flux rates through the mitochondrial dehydrogenases. This mechanism could also contribute to the observation that the metabolic changes are dissociated from the transmural blood perfusion pattern. This pattern has also been observed in hearts without partial stenosis.

**Table 4. MV\textsubscript{O}2 and lactate metabolism data**

<table>
<thead>
<tr>
<th></th>
<th>Oxygen Consumption</th>
<th>Lactate Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a-v, ml O2/100 ml</td>
<td>MV O2, ml·min(^{-1})·100 g(^{-1})</td>
</tr>
<tr>
<td>Baseline</td>
<td>8.16±0.99</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Db20</td>
<td>8.26±1.23</td>
<td>0.11±0.01*</td>
</tr>
<tr>
<td>Db20 + coronary stenosis + 2-DG</td>
<td>8.04±1.28</td>
<td>0.06±0.01†</td>
</tr>
</tbody>
</table>

Values are means ± SE. a-v, Arteriovenous; MV O\textsubscript{2}, myocardial O\textsubscript{2} consumption. *P < 0.05 compared with baseline (*). †P < 0.05 compared with Db20 (†).
where demand ischemia occurred after cardiac workload had been increased to a very high level (34). During a medium-level dobutamine infusion, RPP increased significantly to ~30,000 mmHg/min. At this work state, myocardial ATP synthetic rate can keep up with energy demand and consequently the PCr-to-ATP and ΔP/PCr ratios did not change (Table 3). This is consistent with the previous studies (34). However, when stenosis was applied to reduce the mean flow to approximately 30% of baseline conditions, PCr/ATP decreased, ΔP/PCr increased, and 2-DGP accumulated significantly. These changes were either uniform across the LV wall or more pronounced in the subepicardium (Table 3). Taken together with the significantly higher blood flow in this region (Table 2), these data suggest that, under this experimental condition, energy utilization is significantly higher in the outer layers of the LV wall, assuming that mitochondrial density and ATP synthetic capacity are similar in the inner and outer layers of the myocardium. The higher blood flow in the outer layer and therefore higher 2-DG delivery could also contribute to the higher 2-DGP accumulation. However, the myocardial blood flow was not ceased in the inner layers during the 2-DG and dobutamine infusion. In fact, the inner layer perfusion is similar to the basal level (Table 2), suggesting the higher 2-DGP-to-PCr ratio in the outer layer more likely reflects a higher glycolytic activity and a limitation in glycolytic-reducing equivalents transfer in the mitochondria in the outer layers under this experimental condition. However, it is not clear why the workload is distributed more to the outer layers. It is likely that, when inner layers experienced underperfusion as a consequence of stenosis, the workload is redistributed to the outer layers where flow is higher. If dobutamine receptors are not completely saturated under this condition and their distribution is uniform across the wall, then higher blood flow in the outer layer means that more dobutamine is delivered to this region, which may result in more energy utilization. During partial coronary occlusion combined with dobutamine and 2-DG infusion, RPP was significantly lower compared with dobutamine stimulation alone (Table 1). Whether this was caused by partial glycolytic inhibition by 2-DGP is not certain. However, Yoshiyama et al. (33) have shown that 2-DG infusion during baseline conditions and during hyperperfusion did not affect hemodynamics. Furthermore, because both LV end diastolic pressure (Table 1) and the duration of the diastole (data not shown) did not differ during dobutamine infusion alone or during stenosis plus dobutamine and 2-DG infusion, it is unlikely that decreased glycolytic ATP production due to inhibition by 2-DGP is the cause of the decreased RPP. The fact that RPP is still elevated during stenosis together with dobutamine and glycolytic inhibition by 2-DGP is probably caused by the continued increased pressure development of the posterior part of the LV wall. Posterior region blood flow, and thereby oxygen and substrate supply, increased even more after the LAD had been partially occluded (Table 2). Work and blood flow of the posterior region increased in parallel, thereby maintaining the balance between demand and supply in this region of the heart (Table 2).

In stunned myocardium, it has been shown that 2-DG uptake is most pronounced in the inner layers of the myocardium (33). Yoshiyama et al. (33) also showed that this gradient was flow independent. It has further been shown that a concentration gradient of glycolytic enzymes and glycogen exists, with the highest levels being present in the ENDO layers (7, 15). In the present study, during demand ischemia, 2-DG uptake is higher in the outer layers of the heart, suggesting that the two types of ischemia have different metabolic characteristics. The greater uptake of 2-DG in the outer myocardial layers in the current study may be attributed to the increased glycolytic ATP production in that layer to support the cardiac performance.

Using a swine model of demand-induced ischemia in the setting of coronary stenosis, Chandler et al. (2) recently reported an increase in net glycogen breakdown, glucose uptake, and lactate production, suggesting an increase of nonoxidative glycolysis. 2-DG is a glucose analog that is transported in the cell by the sarcolemmal glucose transporter and is subsequently phosphorylated by hexokinase (8). Clearance of phosphorylated 2-DG from the heart is very slow because it cannot undergo further glycolytic metabolism, and it is very slowly dephosphorylated (8, 23). In the present study, the partial inhibition of glycolysis by 2-DGP accumulation caused a myocardial edema, as evidenced by the significant increase of the wet weight-to-dry weight ratio. As a result of the accumulation of 2-DGP, glucose uptake is significantly inhibited (23).

Although under normal circumstances glycolysis provides only a small fraction of total myocardial ATP production, there is increasing evidence that glycolytically produced ATP may be preferentially used to support cellular ion homeostasis. Indeed, glycolysis has been proposed to provide ATP for preferential use by the sarcolemmal Ca" pump (19, 22) and the Na"-K" pump (1, 16). The reason for the apparent dependence of cellular ion homeostasis on glycolytically produced ATP is not clear but may be related to the functional coupling of glycolytic enzymes with membrane channels and ion pumps. The function of glycolysis is in the same compartments as the ion pumps (31, 32). Because of the functional compartmentation of metabolic processes, possible damage to the sarcolemma during ischemia or hypoxia is delayed or even prevented by glycolytic ATP production. In the present study, the wet-to-dry weight ratio in the ischemic (stenotic) 2-DG perfused area of the LV is significantly higher than in the control area, which is not observed in ischemic (stenotic) hearts without 2-DG perfusion (21). This suggests that the edema present in the ischemic area is caused by the 2-DGP accumulation associated glycolytic inhibition that consequently failed to maintain the ion homeostasis.

In summary, the present study demonstrates that the EPI layer shows greater metabolic abnormalities and accumulation of 2-deoxyglucose despite higher blood flow during dobutamine-induced demand ischemia in the setting of a stenosed coronary artery. These data suggests that energy expenditure is greater in the EPI than in the endocardial layers during dobutamine stimulation-induced demand ischemia.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-50470, HL-61353, and HL-67828.

REFERENCES


2. Chandler MP, Huang H, McElfresh TA, Stanley WC. Increased nonoxidative glycolysis despite continued fatty acid uptake during demand-


