Myocardial oxygenation and high-energy phosphate levels during graded coronary hypoperfusion

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Zhang, Jianyi, Kamil Ugurbil, Arthur H. L. From, and Robert J. Bache. Myocardial oxygenation and high-energy phosphate levels during graded coronary hypoperfusion. Am J Physiol Heart Circ Physiol 280: H318–H326, 2001.—This study was performed to determine the myocyte PO2 required to sustain normal high-energy phosphate (HEP) levels in the in vivo heart. In 10 normal dogs, myocyte PO2 values were calculated from the myocardial deoxymyoglobin resonance (Mb-δ) intensity determined with 1H-NMR spectroscopy during sequential flow reductions produced by a hydraulic occluder that decreased coronary perfusion pressure to ~60, 50, and 40 mmHg and, finally, during total occlusion. Myocardial blood flow was measured with microspheres, and HEP levels were determined with 31P magnetic resonance spectroscopy. During control conditions, Mb-δ was undetectable. Myocardial blood flow was 1.11 ± 0.06 ml·min⁻¹·g⁻¹ during basal conditions and decreased with sequential graded occlusions to 0.78 ± 0.05, 0.58 ± 0.03, and 0.38 ± 0.04 ml·min⁻¹·g⁻¹, respectively; blood flow during total occlusion was 0.07 ± 0.02 ml·min⁻¹·g⁻¹. Reductions of blood flow caused progressive increases of Mb-δ, which were associated with decreases of phosphocreatine (PCr), ATP, and the PCr-to-ATP ratio, as well as progressive increases of the P₂-to-PCr ratio. There was a strong linear correlation between normalized blood flow and Mb-δ (R² = 0.89, P < 0.01). Reductions of HEP and PO2 were also highly correlated (although nonlinearly); with the assumption that myoglobin was 90% saturated with O2 during basal conditions and 5% saturated during total coronary occlusion, the intracellular PO2 values for 20% reductions of PCr and ATP were ~4.4 and ~0.9 mmHg, respectively. The data indicate that O2 availability plays an increasing role in regulation of oxidative phosphorylation when mean intracellular PO2 values fall below 5 mmHg in the in vivo heart.

myocardium; blood flow; myoglobin oxygen saturation; ischemia

BOTH THERMODYNAMIC AND KINETIC models of oxidative phosphorylation regulation in the heart incorporate regulatory roles for myocardial ADP, P_i, NADH, and O2 (3, 8). O2 extraction by the heart is greater than in most other organs, with resting coronary venous PO2 commonly as low as 20–25 mmHg in the normal in vivo heart (32). Because the very high level of myocardial O2 extraction during basal conditions limits the ability to increase O2 uptake by augmenting extraction, increases of O2 demands during exercise or other stress must be met by parallel increases of coronary blood flow (32). Furthermore, reductions of coronary blood flow by as little as 10–20% in the intact in vivo heart cause decreases of contractile performance, suggesting that even modest reductions of myocardial perfusion can result in O2 insufficiency (31). Kreutzer and Jue (18) examined the critical O2 level at which O2 availability failed to meet cellular energy demands in isolated perfused rat hearts working at 25°C. Using 1H-NMR spectroscopy to assess the degree of myocardial myoglobin desaturation and to calculate intracellular PO2 during progressive decreases of O2 in Krebs-Henseleit perfusion buffer, they observed that the phosphocreatine (PCr) level began to decrease at an intracellular PO2 of ~2 mmHg and fell to 80% of the basal value at an intracellular PO2 of 1.8 mmHg (18). Further reductions of perfusate oxygenation resulted in sharp further decreases of PCr. Since these data were obtained in isolated hearts perfused with non-Hb-containing buffer at 25°C, it is unclear whether a similar relationship would exist in the intact heart in vivo. Consequently, the present study was carried out to examine the effect of decreasing myocyte oxygenation on myocardial high-energy phosphate (HEP) content in the intact heart in vivo. Graded reductions of coronary blood flow were produced to document the threshold level of myocyte oxygenation at which perceptible reductions of the PCr-to-ATP ratio (PCr/ATP; corresponding to an increase of myocardial free ADP) occurred and to examine the effect of progressive reductions of myocyte oxygenation on myocardial HEP content.

METHODS

Studies were performed in 10 adult mongrel dogs of either gender weighing 20–27 kg. All experimental procedures were approved by the University of Minnesota Animal Resources Committee. The investigation conformed to the Guide for the...
Experimental preparation. The dogs were anesthetized with pentobarbital sodium (30–35 mg/kg bolus followed by 4 mg·kg⁻¹·h⁻¹ iv), intubated, and ventilated with a respirator with supplemental O₂ to maintain arterial blood gases within the physiological range. A heparin-filled 3.0-mm-OD polyvinyl chloride catheter was introduced into the right femoral artery and advanced into the ascending aorta. A left thoracotomy was performed through the fourth intercostal space, and the heart was suspended in a pericardial cradle. A heparin-filled 3.0-mm-OD catheter was introduced into the left ventricle (LV) through the apical dimple and secured with a purse-string suture. A similar catheter was inserted into the left atrium through the atrial appendage. A 1.5- to 2.0-cm segment of the proximal left anterior descending (LAD) coronary artery was dissected free, and a hydraulic occluder constructed of 2.7-mm-OD polyvinyl chloride tubing was placed around the artery. A silicone-elastomer catheter (0.75 mm ID) was placed into the LAD distal to the occluder by the method of Gwirtz (11). The region of the LV that became cyanotic on inflation of the occluder was determined by visual inspection, and a 28-mm-diameter NMR surface coil was sutured onto the pericardium overlying the ischemic area. The pericardial cradle was then released, the heart was allowed to assume its normal position, and the surface coil leads were connected to a balanced tuned circuit. Animals were placed on a water-filled thermal jacket connected to a circulating water bath to maintain rectal temperature at 37–38°C and placed into the magnet.

NMR general methods. Measurements were performed in a 400-cm-bore 4.7-Tesla 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, while respiratory gating was achieved by the LV pressure signal was used to gate NMR data acquisition to the cardiac cycle between data the cardiac cycle. Figure 1 illustrates a typical sensitivity profile from a canine heart with an ~1-cm-thick LV wall; the arrow indicates the position of the surface coil. These data demonstrate that the Mb-δ signal originates from all layers across the LV wall with a somewhat higher contribution from the mid-to-inner layers of the LV.

Spatially localized ³¹P-NMR technique. ³¹P-NMR spectra were acquired in late diastole with a pulse repetition time of 6–7 s. This repetition time allowed full relaxation for ATP and Pᵢ resonances and ~90% relaxation for the PCr resonance. PCr resonance intensities were corrected for this minor saturation. RF transmission and signal detection were performed with a 28-mm-diameter surface coil. A capillary containing 15 μl of 3 M phosphonoacetic acid was placed at the coil center to serve as a reference. The proton signal of the water resonance was used to homogenize the magnetic field and to adjust the position of the animal in the magnet so that the coil was at or near the magnet and gradient isocenter. This was accomplished using a spin-echo experiment with a readout profile. The information gathered in this step was also utilized to determine the spatial coordinates for spectroscopic localization. Chemical shifts were measured relative to PCr, which was assigned a chemical shift of ~2.55 ppm relative to 85% phosphoric acid at 0 ppm. Spatial localization of the signal detected with NMR is of importance. The relative contributions from the different layers of the wall of the ventricle will depend on the radio-frequency (RF) power setting, so that only for relatively low RF powers (i.e., powers that achieve much less than 90° rotation at the coil center) will the outer layer dominate the Mb-δ signal. This condition was avoided by adjusting the RF pulse so that the water signal detected was maximized. With such a power setting, signal contributions will tend to penetrate deeper into the LV wall. To examine the detection sensitivity profile for Mb-δ along the γ-axis (perpendicular to the LV wall), the γ-axis gradient echo was switched on using gradient-echo-image sequence to obtain the one-dimensional profile of the water proton along the γ-axis using the identical RF pulse, pulse sequence and RF power that was used for the Mb-δ measurements. Figure 1 illustrates a typical sensitivity profile from a canine heart with an ~1-cm-thick LV wall; the arrow indicates the position of the surface coil. These data demonstrate that the Mb-δ signal originates from all layers across the LV wall with a somewhat higher contribution from the mid-to-inner layers of the LV.
across the LV wall was performed with the RAPP-ISIS/FSW method. The technical details of this method, including voxel profiles, voxel volume, and the accuracy of spatial localization obtained in phantom studies and in vivo, have been published elsewhere (12, 24, 26). Briefly, signal origin was restricted by using B2 gradients and adiabatic inversion pulses to an 18 mm × 18 mm column coaxial with the surface coil and perpendicular to the LV wall. Within this volume, the signal was further localized by using the B1 gradient to five voxels spanning the LV wall from epicardium to endocardium. Each set of spatially localized transmural spectra consisted of a total of 96 scans accumulated in a 10-min block. Resonance intensities were quantified by using integration routines provided by SISCO software. The values for PCr and ATP in each voxel were normalized to those present in the basal state, and the PCr-to-ATP ratio (PCr/ATP) was determined for each voxel. Pi resonances were also measured, and the ratio of Pi to PCr (Pi/PCr) was calculated.

The Mb-δ resonance detected using the present methodology represents an unweighted average of the Mb-δ content across the entire LV wall. A comparable whole wall measurement of HEP levels does not exist, because the sensitivity for detection of the phosphorus resonance decreases as a function of distance from the surface coil. Therefore, average whole wall values of HEP were obtained by averaging the HEP contents in the subepicardial, midwall, and subendocardial voxels that were acquired with our spatially localized spectroscopy technique (26). This technique corrects voxel HEP content measurements for the decreased sensitivity that occurs with increasing distance from the surface coil, thereby yielding true average whole wall PCr and ATP contents.

Myocardial blood flow measurements. Myocardial blood flow was measured using 15-μm-diameter microspheres labeled with 51Cr, 85Sr, 99mTc, and 46Sc. Microsphere suspension containing 2 × 10⁶ microspheres was injected through the left atrial catheter, while a reference sample of arterial blood was withdrawn from the aortic catheter at a rate of 15 ml/min beginning 5 s before the microsphere injection and continuing for 120 s. Radioactivity in the myocardial and blood reference specimens was determined using a gamma spectrometer (Packard Instruments, Downers Grove, IL) at window settings chosen for the combination of radioisotopes of 80%, although a value this low is unlikely, since we have found that the present technique can detect ~10% fractional content of Mb-δ (unpublished observations).

Study protocol. Aortic and LV pressures were measured with fluid-filled pressure transducers positioned at midsternum level and recorded on an eight-channel direct-writing recorder (Coulbourne Instrument, Lehigh Valley, PA). LV pressure was recorded at normal and high gain for measurement of end-diastolic pressure. Hemodynamic measurements and 31P- and 1H-NMR spectra were first obtained under baseline conditions. Midway through the 20-min data acquisition period, a microsphere injection was performed for determination of myocardial blood flow. After completion of baseline measurements, the hydraulic coronary occluder was slowly inflated with a micrometer-driven syringe to reduce distal LAD pressure to ~60 mmHg (stenosis I). When a stable coronary pressure had been maintained for 5 min, 31P and 1H spectra were obtained, and a microsphere injection was performed. The occluder was then further inflated to decrease distal coronary pressure to ~50 mmHg (stenosis II), and all measurements were repeated. Measurements were then obtained at a coronary pressure of ~40 mmHg (stenosis III). Finally, the LAD was completely occluded (total occlusion), and all measurements were repeated. In three animals, PO2, and (PO2)50 is the PO2 at which myoglobin is half-saturated with O2 (2.38 mmHg at 37°C) (27). Therefore, if the integral of the Mb-δ resonance intensity measured during complete coronary occlusion represents total myoglobin content (in the Mb-δ form), then the resonance integrals determined during other experimental conditions can be normalized relative to this value to result in Mb-δ expressed as a fractional value of total myoglobin content. The fractional content of myoglobin O2 during each experimental period can be calculated from the relationship Mb-O2 = 1 – Mb-δ. The fractional contents of oxymyoglobin and Mb-δ and the temperature-corrected (PO2)50 can then be employed to calculate intracellular PO2 using Eq. 1. Because of continuing collateral blood flow, it is unlikely that myoglobin would be completely deoxygenated during coronary occlusion. Consequently, calculations were performed assuming that the Mb-δ resonance measured during coronary occlusion represented 90 or 95% of the total myoglobin, and the Mb-δ resonances obtained during the study were normalized in relation to that value. Similarly, the fractional myoglobin O2 content in the basal state (i.e., at a time when no Mb-δ resonance was detected) could not be directly determined (5).

On the basis of a recent report using reflectance spectroscopy in open-chest swine which demonstrated that myoglobin O2 saturation is >92% under basal conditions, we calculated intramyocardial PO2 values assuming that the fractional Mb-δ content in the basal state was 90 or 95%. We also carried out calculations assuming a basal Mb-δ O2 saturation of 80%, although a value this low is unlikely, since we have found that the present technique can detect ~10% fractional content of Mb-δ (unpublished observations).

Myocyte oxygenation. Myocyte oxygenation was estimated from the myoglobin O2 saturation-Po2 relationship as follows:

\[ \text{Po2} = \frac{\text{Mb-O2}(\text{Po2})50}{\text{Mb-δ}} \quad (I) \]

where Mb-O2 and Mb-δ are fractional contents of oxymyoglobin and deoxymyoglobin, respectively. Po2 is the intramyocyte Po2, and (Po2)50 is the Po2 at which myoglobin is half-saturated with O2 (2.38 mmHg at 37°C) (27). Therefore, if the integral of the Mb-δ resonance intensity measured during complete coronary occlusion represents total myoglobin content (in the Mb-δ form), then the resonance integrals determined during other experimental conditions can be normalized relative to this value to result in Mb-δ expressed as a fractional value of total myoglobin content. The fractional content of myoglobin O2 during each experimental period can be calculated from the relationship Mb-O2 = 1 – Mb-δ. The fractional contents of oxymyoglobin and Mb-δ and the temperature-corrected (Po2)50 can then be employed to calculate intracellular Po2 using Eq. 1. Because of continuing collateral blood flow, it is unlikely that myoglobin would be completely deoxygenated during coronary occlusion. Consequently, calculations were performed assuming that the Mb-δ resonance measured during coronary occlusion represented 90 or 95% of the total myoglobin, and the Mb-δ resonances obtained during the study were normalized in relation to that value. Similarly, the fractional myoglobin O2 content in the basal state (i.e., at a time when no Mb-δ resonance was detected) could not be directly determined (5).

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<table>
<thead>
<tr>
<th>Heart Rate</th>
<th>LV Systolic Pressure</th>
<th>LV Endo Diastolic Pressure</th>
<th>Mean Aortic Pressure</th>
<th>Coronary Pressure</th>
<th>Myocardial Blood Flow, ml·min⁻¹·g⁻¹</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Epi</td>
</tr>
<tr>
<td>Baseline</td>
<td>136 ± 5</td>
<td>122 ± 5</td>
<td>3 ± 1</td>
<td>93 ± 4</td>
<td>96 ± 5</td>
</tr>
<tr>
<td>Stenosis I</td>
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<td>119 ± 5</td>
<td>6 ± 2</td>
<td>91 ± 4</td>
<td>59 ± 2‡</td>
</tr>
<tr>
<td>Stenosis II</td>
<td>148 ± 7</td>
<td>111 ± 4</td>
<td>7 ± 2</td>
<td>86 ± 3</td>
<td>50 ± 2‡</td>
</tr>
<tr>
<td>Stenosis III</td>
<td>152 ± 6</td>
<td>105 ± 4</td>
<td>8 ± 2</td>
<td>81 ± 2</td>
<td>39 ± 2‡</td>
</tr>
<tr>
<td>Total occlusion</td>
<td>152 ± 5</td>
<td>100 ± 3†</td>
<td>9 ± 2</td>
<td>79 ± 2</td>
<td>20 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 dogs. LV, left ventricular; Epi, epicardial; Endo, endocardial; Mid, midmyocardial. *P < 0.05 vs. baseline; †P < 0.05 vs. preceding experimental condition; ‡P < 0.01 vs. preceding experimental condition.
hearts were stained with triphenyltetrazolium chloride to ensure that the experimental protocol did not result in myocardial necrosis. In these animals, coronary reperfusion was allowed for 2 h after completion of the protocol before the hearts were harvested. The hearts were then sectioned into 1-cm-thick layers parallel to the mitral valve annulus and incubated for 15 min in 4 g of triphenyltetrazolium chloride dissolved in 400 ml of Sörenson’s buffer at 37°C. No evidence of infarct was observed in any of these hearts.

Data analysis. Hemodynamic data were measured from the chart recordings. 31P spectra were analyzed as described above. Transmural blood flow distribution was determined from the microsphere measurements. Data were analyzed with one-way ANOVA for repeated measures. 
P, 0.05 was considered significant. When a significant result was found, individual comparisons were made using the method of Scheffé.

RESULTS

Hemodynamic and blood flow data. Hemodynamic and myocardial blood flow measurements during each experimental condition are shown in Table 1. Heart rate tended to increase and mean aortic pressure tended to decrease with increasing stenosis severity, although these changes did not achieve statistical significance. LV systolic pressure decreased significantly during stenosis II and III, as well as during total occlusion (each P < 0.05). LV end-diastolic pressure increased significantly with application of stenosis I and underwent a further significant increase during total occlusion (P < 0.05). Coronary perfusion pressure progressively decreased as the degree of stenosis was increased.

As shown in Table 1, myocardial blood flow decreased progressively in response to increasing stenosis severity, with the decrease most severe in the subendocardial layer, as indicated by a progressive decrease in the endocardial-to-epicardial blood flow ratio. During complete LAD occlusion, the residual myocardial blood flow (representing collateral flow) accounted for an average of 6% of the baseline blood flow and was directed preferentially toward the subepicardial layers.

Myocyte oxygenation and HEP levels. Typical 1H and 31P spectra are shown in Figs. 2 and 3. To take into account differences in absolute blood flow between animals (reflecting variations in the rate of basal state metabolism), blood flow for each individual animal was normalized to the basal state value. As shown in Fig. 4, reductions of myocardial blood flow produced by progressive increases in stenosis severity resulted in parallel stepwise decreases of myoglobin O2 saturation. The relationship between myoglobin saturation and blood flow was well described by the linear regression.
equation shown in Fig. 4A ($R^2 = 0.89, P < 0.01$). When intracellular $P_O2$ was calculated from the Mb-d measurements, an exponential relationship was observed between myocardial blood flow and intracellular $P_O2$ (Fig. 4B).

Increasing stenosis severity resulted in progressive decreases of PCr and ATP (Fig. 2) with decreases of PCr/ATP and increases of Pi/PCr (Table 2). The HEP abnormalities were most prominent in the subendocardium, reflecting the pattern of hypoperfusion, which was most severe in the deeper myocardial layers. Additionally, PCr/ATP decreased and Pi/PCr increased (Table 2) during ischemia, and the transmural gradient of blood flow was reversed (epicardial $>$ endocardial; Table 1). Consistent with these findings, PCr/ATP reductions and Pi/ATP increments were greatest in the subendocardium (Table 2).

The relationships of PCr and ATP with intracellular PO2 are shown in Fig. 5. This plot was made assuming that the Mb-d resonance measuring total coronary occlusion represented 95% of the total myoglobin and that the fractional myoglobin O2 content in the basal state was 90%.

Fig. 4. Myoglobin percent saturation (A) and calculated myocyte PO2 (B) plotted against myocardial blood flow (MBF) normalized to the prestenosis control value. It was assumed that the Mb-d resonance measuring total coronary occlusion represented 95% of the total myoglobin and that the fractional myoglobin O2 content in the basal state was 90%.

Fig. 5. PCr (A) and ATP (B) normalized to the prestenosis control value plotted against myocyte PO2. PCr decreased to 80% of the control value when intracellular PO2 had decreased to 4.4 mmHg, while ATP was reduced to 80% of the control value at PO2 of 1.1 mmHg. It was assumed that the Mb-d resonance measuring total coronary occlusion represented 95% of the total myoglobin and that the fractional myoglobin O2 content in the basal state was 90%.

Table 2. $3^1P$-NMR measurements

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<th>PCr/ATP</th>
<th>P/PCr</th>
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<tr>
<td></td>
<td>Epi</td>
<td>Endo</td>
</tr>
<tr>
<td>Baseline</td>
<td>2.16 ± 0.06</td>
<td>2.02 ± 0.05</td>
</tr>
<tr>
<td>Stenosis I</td>
<td>2.05 ± 0.04</td>
<td>1.70 ± 0.04‡</td>
</tr>
<tr>
<td>Stenosis II</td>
<td>1.78 ± 0.04‡</td>
<td>1.50 ± 0.04‡</td>
</tr>
<tr>
<td>Stenosis III</td>
<td>1.45 ± 0.05‡</td>
<td>1.02 ± 0.10‡</td>
</tr>
<tr>
<td>Total occlusion</td>
<td>0.75 ± 0.07‡</td>
<td>0.34 ± 0.09‡</td>
</tr>
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</table>

Values are means ± SE; $n = 10$ dogs. PCr/ATP, phosphocreatine-to-ATP ratio; P/PCr, Pi-to-PCr ratio; Mb-d, deoxymyoglobin intensity normalized to the total occlusion value, which is assumed to reflect 95% of total myoglobin content; the baseline Mb-d value was assumed to be 0.10. *$P$, concentration was too low to be NMR visible. †$P < 0.05$ vs. preceding experimental condition; ‡$P < 0.01$ vs. preceding experimental condition.
reflect the presence of $O_2$ limitation of oxidative phosphorylation (18). As shown in Table 3, the $P_{O_2}$ values at which PCr was reduced to 80% ($[P_{O_2}]_{80}$) were 4.3–4.5 mmHg when myoglobin $O_2$ saturation was assumed to be 90 or 95%; computed values were similar whether myoglobin $O_2$ saturation during total coronary occlusion was assumed to be 5 or 10%. However, if myoglobin $O_2$ saturation during basal conditions was assumed to be 80%, a much lower ($P_{O_2}$)$_{80}$ was obtained at 2.0–2.2 mmHg. Values at which PCr was reduced to 50% ($[P_{O_2}]_{50}$) of control were 1.0–1.2 when myoglobin was assumed to be 90 or 95% saturated during basal conditions and 5 or 10% saturated during total coronary occlusion. Substantially lower values for ($P_{O_2}$)$_{50}$ were calculated when myoglobin was assumed to be only 80% saturated during basal conditions (Table 3). The relationship between ATP and $P_{O_2}$ is shown in Fig. 5; this plot was obtained assuming that myoglobin was 90% saturated during basal conditions and 5% saturated during total coronary occlusion. The ($P_{O_2}$)$_{50}$ for ATP was 0.9–1.0 mmHg when myoglobin was assumed to be 90 or 95% saturated during basal conditions but fell to 0.4–0.5 mmHg when myoglobin was assumed to be 80% saturated during basal conditions. A ($P_{O_2}$)$_{50}$ value for ATP could not be determined, because this degree of ATP reduction was not achieved. The lower critical $P_{O_2}$ for ATP than for PCr is not surprising, because the fall of ATP during ischemia is buffered by PCr through the creatine kinase reaction.

**DISCUSSION**

In a previous study using $^1$H-NMR, we found that no Mb-δ was visible in in vivo canine or swine myocardium operating at basal or elevated work states, despite the observation that HEP levels fell during high work states (34). This suggested that, even at high work states, $O_2$ availability was nonlimiting and indicated that HEP changes that occur at high workload cannot be ascribed to $O_2$ limitation. The present study was carried out to define the relationships between myocardial blood flow, myocyte $P_{O_2}$, and HEP levels in in vivo myocardium under conditions where limited $O_2$ availability does limit the rate of oxidative phosphorylation.

The effect of decreased $O_2$ availability on myocardial HEP has been reported previously in isolated working rat hearts perfused with Krebs-Henseleit buffer at 25°C. Using progressive reductions of the perfusate flow rate while intracellular $P_{O_2}$ was determined from the degree of myoglobin oxygenation using $^1$H-NMR spectroscopy, Kreutzer and Jue (19) observed that when the intracellular $P_{O_2}$ fell to ~4 mmHg, contractile performance decreased and myocardial lactate production occurred. At this time, myocardial $O_2$ consumption and HEP remained normal. Not until intracellular $P_{O_2}$ had fallen to 2 mmHg was there a perceptible decrease in myocardial PCr and $O_2$ consumption. The findings suggested that an increase in myocardial NADH was able to compensate for the decreased $P_{O_2}$ at $P_{O_2}$ values between 4 and 2 mmHg, so that the PCr concentration and rate of $O_2$ consumption were maintained. When using $^1$H-NMR measurements of the Mb-δ resonance for calculation of intracellular $P_{O_2}$, assumptions must be made concerning the degree of myocardial $O_2$ saturation during basal conditions as well as the fraction of myoglobin that is deoxygenated during total coronary occlusion. In the dog, a small amount of collateral inflow continues during total arterial occlusion, so that complete myoglobin desaturation is unlikely. Within reasonable limits, the degree of myoglobin $O_2$ saturation assumed during total coronary occlusion had a very small effect on the calculated $P_{O_2}$ values at which PCr fell to 80 or 50% of the basal level. However, assumptions concerning the degree of $O_2$ saturation during basal conditions did influence the calculated critical $P_{O_2}$ values for PCr and ATP. Thus, if myoglobin was assumed to be 90 or 95% saturated during basal conditions, ($P_{O_2}$)$_{50}$ values for PCr were 4.3–4.5 mmHg. However, if myoglobin was assumed to be only 80% saturated during basal conditions, then calculated ($P_{O_2}$)$_{50}$ values were decreased by one-half. We believe that the values obtained when myoglobin saturation during basal conditions is assumed to be 90–95% saturated are more likely to be correct for three reasons. First, the $^1$H-NMR technique utilized in this study is sufficiently sensitive to detect a proton resonance when myoglobin is >10% desaturated. Second, using data obtained with in vivo reflectance spectroscopy, Arai et al. (1) determined that myoglobin $O_2$ saturation during basal conditions, ($P_{O_2}$)$_{50}$ values for PCr were 4.3–4.5 mmHg. However, if myoglobin was assumed to be only 80% saturated during basal conditions, then calculated ($P_{O_2}$)$_{50}$ values were decreased by one-half.

**Table 3. Critical $P_{O_2}$ values for PCr and ATP**

<table>
<thead>
<tr>
<th>Assumptions</th>
<th>PCr</th>
<th>ATP</th>
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<tr>
<td>Mb-$O_{max}$</td>
<td>Mb-$O_{min}$</td>
<td>$P_{O_2}$</td>
</tr>
<tr>
<td>%</td>
<td>%</td>
<td>(mmHg)</td>
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<tr>
<td>95</td>
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<td>4.4</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
<td>2.4</td>
</tr>
</tbody>
</table>

 Mb-$O_{max}$ and Mb-$O_{min}$, maximum and minimum myoglobin $O_2$ saturation, respectively; ($P_{O_2}$)$_{50}$ and ($P_{O_2}$)$_{80}$ = $P_{O_2}$ at which PCr or ATP are at 80 and 50% of control values, respectively.
ate Po2; in this situation, the persistently high coronary flow rate would result in washout of lactate and prevent acidosis (18). With this experimental model, slightly higher critical Po2 values were obtained; PCr began to decrease at an intracellular Po2 of 2 mmHg, with a (Po2)so of 1.8 mmHg. Nevertheless, the intracellular Po2 values calculated to produce reductions of PCR were higher in the in vivo canine heart in the present study than in the isolated perfused rat hearts. It is possible that reduced metabolic rates of rat hearts perfused at 25°C, compared with normothermic in vivo conditions, could contribute to a lower critical Po2 for PCR in the perfused hearts.

The response of myocardial HEP levels to decreased coronary perfusion could be altered by changes of myocardial O2 demands; an active decrease of energy demands in response to O2 limitation would allow the heart to accommodate to the decreased O2 availability and delay the development of HEP changes. Gregg (10) demonstrated that myocardial O2 consumption can be influenced by coronary flow and perfusion pressure. Pressure in the coronary arterial system has been proposed to act to distend the ventricle, thereby augmenting contractility and O2 consumption by increasing sarcomere length (2). In addition, Kitakaze and Marban (17) observed that, in perfused ferret hearts, changes of coronary pressure and flow resulted in parallel changes in the calcium transient, implying a preload-independent mechanism for perfusion-related alterations of contractile force and O2 demands. Zündorf and colleagues (35), using isolated rat hearts in which graded hypoxia was produced by decreasing the Po2 of the perfusate buffer, found that reduced cytochrome aa3 was not detected until contractile performance had fallen to 25% of control. These findings imply that decreased O2 availability (despite constant coronary flow) also caused a decrease of contractile performance that allowed the heart to accommodate to the decreased O2 availability and maintain myocardial energy balance.

Differences in the rate and magnitude of downregulation of myocardial energy demands in response to reductions of coronary perfusion might explain different critical Po2 values for PCR between in vitro perfused hearts and in vivo hearts. There is evidence that such flow-mediated alterations of contractile performance are more prominent in isolated perfused hearts. Thus, Marshall (20) observed that when coronary flow was decreased in isolated rabbit hearts, tissue lactate did not increase until O2 consumption and developed pressure had decreased by nearly one-half, while PCr and ATP did not decrease until O2 consumption and systolic function had decreased to 20–30% of the control level. Similarly, Keller and Cannon (15), using 31P-NMR to measure myocardial HEP content in perfused rat hearts, found that modest reductions of coronary flow resulted in proportionate decreases of O2 consumption and contractile performance without significant reductions of PCr. Adaptations of energy demands in response to reduced coronary perfusion have also been observed in intact animals, but these appear to be of lesser magnitude and to require a longer period of adjustment. Thus, in open-chest swine or dogs, 30–40% reductions of coronary blood flow initially caused a decrease of myocardial PCr, but this was followed by recovery to near-control values within 60 min, despite persistent hypoperfusion (7, 22). This response has been termed the early phase of myocardial hibernation. It is possible that a difference in response to decreased O2 delivery could explain the difference in curves relating intramyocyte Po2 to coronary perfusion between perfused rodent hearts and the in vivo hearts in the present study. This is supported by the finding of Kreutzer and Jue (19) in the isolated rat heart that not until coronary flow was decreased from 11 to <1 ml/min was there appearance of Mb-δ or detectable loss of PCr. In contrast, in the present study a 30% reduction of coronary blood flow caused a perceptible loss of PCr.

The rapid ability of the isolated perfused rodent heart to decrease energy demands in response to reduced perfusion could explain a difference in critical Po2 for PCr from the in vivo heart. An additional concern is whether deoxyhemoglobin present in the in vivo heart might interfere with 1H-NMR spectroscopic measurements of Mb-δ. In a previous study from this laboratory, we demonstrated that because of the short T2 relaxation times of the α- and β-subunits of deoxyhemoglobin, they are not NMR visible in canine myocardium with the 1-ms excitation pulse used in the present study (5).

An important question is why any O2 limitation should occur at intramyocyte Po2 values that far exceed the apparent Michaelis-Menten constant of cytochrome oxidase with respect to O2. Chance (4) observed in isolated myocardial mitochondria that the critical Po2 at which oxidative phosphorylation began to decrease was ~0.01 mmHg. The finding in the present study that PCr levels began to decrease when intramyocyte Po2 fell below 5 mmHg indicates that additional factors must influence oxidative phosphorylation in the intact heart. One possibility is that the high critical Po2 in the intact heart might result, in least in part, from inhomogeneities of O2 availability; the observed NMR measurements could represent an average of some areas of myocardium that are adequately perfused with some areas that are profoundly ischemic. Decreases of coronary blood flow are known to result in increased spatial heterogeneity of perfusion (23). A flow-limiting arterial stenosis causes redistribution of perfusion away from the subendocardium so that blood flow, HEP, and presumably myoglobin saturation are lowest in the subendocardium (25). This transmural redistribution occurs during hypoperfusion in in vivo hearts and isolated perfused rat hearts (33), so that differences in perfusion pattern could not account for differences in the flow-Po2 relationship between the two experimental models. Furthermore, the sensitivity profile for detection of Mb-δ using the present 1H-NMR spectroscopy system is essentially uniform across the LV wall, so that differences in the myocardial regions sampled between 31P- and 1H-NMR spectra could not account for differences in the critical Po2 values for PCr.
and ATP between the present in vivo and the perfused rat hearts.

Inhomogeneities of O\textsubscript{2} availability can also occur at the cellular level. Large Po\textsubscript{2} gradients exist between the erythrocyte and cytosol as the result of diffusional resistance to O\textsubscript{2} transport in the extracellular carrier-free region (13). Furthermore, high-resolution spectrophotometric measurements performed on single cardiac myocytes have demonstrated a steep spatial Po\textsubscript{2} gradient near the sarcolemma, while Po\textsubscript{2} values toward the center of the cell are nearly flat (30). Takahashi et al. (29) observed that as the extracellular Po\textsubscript{2} was decreased, NAD(P)H autofluorescence and myoglobin desaturation became heterogeneous, with greatest fluorescence remote from the sarcolemma in a pattern that was a mirror image to the local intracellular Po\textsubscript{2}. A significant Po\textsubscript{2} gradient between the cytosol and the inner mitochondrial membrane could explain the difference between the Michaelis-Menten constant of cytochrome oxidase for O\textsubscript{2} in isolated mitochondrial preparations and the mean cytosolic Po\textsubscript{2} values that are associated with HEP alterations in the intact heart. However, taking into account the low O\textsubscript{2} flux density at the mitochondrial membrane, mathematical models of O\textsubscript{2} diffusion suggest very low perimitochondrial O\textsubscript{2} gradients (6). This is difficult to reconcile with reports that graded reductions of perfusate Po\textsubscript{2} in isolated rat cardiomyocytes resulted in parallel reductions of cytochrome aa\textsubscript{3} oxidation and myoglobin oxidation (coherence) (4, 14). Chance (4) and Ito et al. (14) postulated that since the mitochondrial membrane is impermeable to myoglobin, a significant Po\textsubscript{2} gradient can occur because O\textsubscript{2} must diffuse passively through a myoglobin free volume before it can react with cytochrome oxidase. Using \textsuperscript{31}P-NMR measurements of HEPs, they found that the phosphorylation potential began to decrease when myoglobin oxygenation fell to 80% of control (14). These results are remarkably similar to the present findings in the in vivo heart showing a linear relationship between myocyte oxygenation and PCr.

Ischemia can cause several changes that have opposing effects on the degree of heterogeneity of Po\textsubscript{2}. Although perfusion heterogeneity increases when coronary flow is decreased (23), capillary recruitment occurs which can decrease the O\textsubscript{2} diffusion distance and reduce O\textsubscript{2} flux density (21). Since the red cell capillary transit time is inversely proportional to the blood flow rate, it is likely that the extracellular O\textsubscript{2} gradient would decrease as flow rates are reduced by the coronary stenosis. Furthermore, simulated ischemia caused a decrease in the magnitude of the intracellular Po\textsubscript{2} gradient in isolated cardiomyocytes, both as the result of a decrease in the rate of O\textsubscript{2} flux into the cell and a reduction of the rate of O\textsubscript{2} consumption (28). Myoglobin is able to facilitate O\textsubscript{2} diffusion in the cytosol based on a gradient of oxymyoglobin from the sarcolemma to the core of the cell. In the present study and in the report of Arai et al. (1), Mb-6 was not detectable during control conditions, indicating that myoglobin facilitation is not required to maintain O\textsubscript{2} availability for mitochondrial respiration. However, at the low Po\textsubscript{2} values that exist when flow is limited by an arterial stenosis, myoglobin-facilitated O\textsubscript{2} transport is likely to become increasingly important.

Summary. During graded reductions of coronary blood flow, perceptible loss of PCr first occurred at a mean intracellular Po\textsubscript{2} of \textasciitilde 5 mmHg. Further reductions of coronary blood flow resulted in a precipitous fall of PCr and PCr/ATP as well as progressive increases of Pi. The data indicate that, in the normal in vivo heart, O\textsubscript{2} availability plays an increasing role in regulation of oxidative phosphorylation when mean intracellular Po\textsubscript{2} values fall below 5 mmHg.

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