Hibernation during Hypoxia in Cardiomyocytes
ROLE OF MITOCHONDRIA AS THE O2 SENSOR*

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During myocardial hibernation, decreases in coronary perfusion elicit inhibition of contraction, suggesting that energy demand is attenuated. We previously found an inhibition of contraction and O2 consumption during hypoxia (3% O2; PO2 = 20 torr for >2 h) in cardiomyocytes, which was reversible after reoxygenation. This study sought to determine whether mitochondria function as cellular O2 sensors mediating this response. Embryonic cardiomyocytes were studied under controlled O2 conditions. Hypoxia produced no acute decrease in mitochondrial potential as assessed using tetramethylrhodamine ethylester (TMRE). Cellular [ATP] decrease in mitochondrial potential as assessed using tetramethylrhodamine ethylester; MgG, Magnesium Green; FCCP, carbonyl cyanide N,N,N,N'-tetramethyl-p-phenylenediamine; TMRE, tetramethylrhodamine ethylester; MgG, Magnesium Green; FCCP, carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone; BDM, 2,5-butanediene monoxime.

Regional decreases in myocardial oxygen delivery have been shown to result in decreased contractile activity and O2 consumption in a phenomenon termed hibernating myocardium. For example, Arai et al. (1) induced a 30% reduction in coronary blood flow in swine and observed an early depletion of ATP and phosphocreatine (PCr) and an increase in tissue lactate followed by a later recovery of PCr and lactate, despite continued hypoperfusion and impaired contraction. During progressive decreases in coronary blood flow, the same group found that regional decreases in contractile function occurred without a sustained decrease in the PCr to ATP ratio (2). Other investigators have also found evidence of myocardial hibernation in intact hearts (3–5). Collectively these results indicate that the myocardium can develop significant contractile inhibition during reductions in blood flow without apparent evidence of ischemia. This would appear to represent an adjustment in ATP demand in response to a decrease in regional O2 supply, which could protect the myocardium from ischemic injury in states where blood flow is reduced more severely (6). However, the mechanisms underlying this response are not fully known (5).

The inference that intact myocardium can down-regulate energy requirements and ATP demand during hypoxia suggests that cardiac myocytes may behave similarly. In contracting embryonic cardiomyocytes, we previously observed decreases in contractile motion and in the rate of O2 uptake during prolonged moderate hypoxia (PO2 = 20–40 torr for 1–2 h) (7). Moreover, this inhibition was reversible within 3 h after return to normoxia, which suggests that cardiac myocytes can detect moderate hypoxia and initiate a suppression of ATP utilization in response. Recently, Silverman et al. (8) found decreases in extent of shortening in rat cardiac myocytes after incubation under 1% O2 for 48 h, which is consistent with our observations and reveals that this response is not unique to embryonic cells. Collectively, these findings suggest that cardiomyocytes can respond to moderate hypoxia by reversibly decreasing contractile activity, at PO2 levels that should have been sufficient to sustain mitochondrial respiration. Although hibernating myocardium is a phenomenon of intact hearts by definition, studies of cellular responses to hypoxia may provide insight into the mechanisms involved in the intact ventricle.

A fundamental question in understanding the mechanism underlying the response to hypoxia relates to how cardiac myocytes detect changes in PO2. An ability to adjust cellular respiration in response to PO2 implies the existence of a sensor capable of detecting changes within the physiological range. It is conceivable that such an O2 sensor could then activate a signaling pathway leading to a down-regulation of contractile motion, energy utilization, and oxygen demand. Recent evidence points to the mitochondrial electron transport chain as a possible site of O2 transduction (9). In this regard, we previously found a reversible inhibition in cytochrome c oxidase Vmax during hypoxia, as evidenced by PO2-dependent decreases in TMPD-ascorbate respiration (7). Moreover, kinetic studies of isolated bovine heart cytochrome oxidase confirmed the existence of PO2-dependent alterations in Vmax (10). A PO2-dependent change in the kinetic activity of cytochrome oxidase could elicit changes in mitochondrial redox state, which could confer a sensitivity to PO2 and allow the mitochondria to act as the cellular O2 sensor. In the present study we assessed the effects of moderate hypoxia and reoxygenation on mitochondrial transmembrane potential to determine whether changes in the function of the oxidase were apparent in the intact myocyte, and whether under normoxic conditions the cytochrome c oxidase inhibitor sodium azide could mimic the reversible decrease in contraction observed during hypoxia.
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CARDIAC MYOCYTE ISOLATION—Myocytes were isolated using a method modified from Barry et al. (11) and previously described (7). Briefly, hearts of 10–11-day-old chick embryos were removed and placed in Hank’s balanced salt solution without magnesium and without calcium (Life Technologies, Inc.). The ventricles were minced, and the cells were dissociated using four to six cycles of trypsin (0.025%, Life Technologies, Inc.) degeneration at 37 °C with gentle agitation. Trypsin digestion was halted after 8 min by transferring dissociated cells to a trypsin inhibitor solution. After filtering (100 μm), the cells were centrifuged for 5 min at 1200 rpm at 4 °C and then resuspended in nutritive media (54% Barry’s CO2, 95% air at 37 °C) for 45 min to allow early adherence of fibroblasts.

Penicillin (100 units/ml), and streptomycin (100 mg/ml)). These cells (Life Technologies, Inc.), 6% heat-inactivated fetal bovine serum and P

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minimize the leakage of ambient O2 into the perfusate. In some studies, to connect the equilibration column to the flow-through chamber to halted after 8 min by transferring dissociated cells to a trypsin inhibitor solution. After filtering (100 μm), the cells were centrifuged for 5 min at 1200 rpm at 4 °C and then resuspended in nutritive media (54% Barry’s solution (in mM: NaCl (117), KCl (1.3), NaHCO3 (22), MgSO4 (0.8), NaH2PO4 (1.0), CaCl2 (0.57), glucose (5.6)), 40% Mg199 with Earle’s salts (Life Technologies, Inc.), 6% heat-inactivated fetal bovine serum and penicillin (100 units/ml), and streptomycin (100 mg/ml)). These cells were then placed in a large Petri dish in a humidified incubator (5% CO2, 95% air at 37 °C) for 45 min to allow early adherence of fibroblasts. The nonadherent cells were then enumerated (hemacytometer), their viability confirmed at >85% (trypan blue) and between 0.6 and 1.5 × 106 cells were placed on glass coverslips (25 mm) in nutritive medium. Cell yield averaged 5–6 × 106 cells per embryo. Cells were maintained in a humidified incubator for 2–3 days at which point synchronous contractions of the monolayer were noted. All experiments were performed on spontaneously contracting cardia

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CO2 of the perfusate was supplied by a CO2 source to maintain the pH in the flow-through chamber. In some studies, the PO2 in the chamber was confirmed using an optical phosphorescence quenching method (Oxyspot, Medical Systems Inc.). In those studies, a porphyrin-based dye (20 μM) bound to albumin (5% w/v) was added to the perfusate (12, 13) and the fibroscopic light guide was positioned above the upper glass cover slip of the flow-through chamber. In those studies, the PO2 PO2 in the chamber was confirmed from the O2-dependent phosphorescence decay following a pulse of excitation light generated by a xenon flash lamp.

DATA ACQUISITION AND ANALYSIS—The inverted microscope was equipped for epifluorescent illumination and included a xenon light source (75 W), a cooled 12-bit digital CCD camera (Princeton Instruments), a shutter and filter wheel (Sutter), and appropriate excitation and emission filters. Data were acquired and analyzed using Metamorph software (Universal Imaging).

Contractile motion of cells was recorded at video rates on magnetic tape using a high resolution video camera (Hamamatsu), as described previously (7). Briefly, the cells were illuminated with visible light at low intensity using Hoffman modulation optics (Modulation Optics, Inc.). This optical system tended to accentuate the changes in surface topology that were apparent during contraction. Approximately 1 min of contractile motion was recorded at each stage for later analysis. Recorded sequential video frames were digitized and pixels were assigned an intensity value ranging from 0 to 255. For each pixel, the absolute change in intensity was summed over ~250 frames. These summed changes in intensity were summed for all pixels, providing a single measure of motion in the field that consistently described the motion that already was evident by inspection. The contraction analysis was carried out as a series of macroinstructions using the Metamorph software (Universal Imaging).

Assessment of Mitochondrial Transmembrane Potential—The mitochondrial transmembrane potential was measured using the cationic dye tetramethylrhodamine ethyl ester (TMRE, Molecular Probes). This fluorophore enters the cells and is accumulated in mitochondria according to the Nernst equation (14, 15) and has been used previously to assess relative changes in mitochondrial potential. Unlike with rhodamine 123, we did not observe quenching of cellular fluorescence with TMRE unless excessive dye was loaded into the cells. Excitation (535 nm), dichroic (565 nm), and emission (610 nm) filters were used (Chroma Technology). To minimize photobleaching of the dye, the excitation intensity was attenuated with a neutral density filter, and exposure times were limited to 100 ms. Coverslips with spontaneously contracting cells were loaded for 1 h with TMRE (100 nM) in a humidified incubator at 37 °C. The cells were then placed in the flow-through chamber and continuously perfused with the buffered salt solution containing TMRE (10 nM). After allowing 60–90 min for equilibration, a digital image was obtained of a field of ~40 cells using a 40× oil immersion objective lens. Using the data acquisition software, individual cells or clusters of several (<10) cells were identified as areas of intense background identified as an area without cells or with minimal cellular fluorescence. Subsequently, sequential digital images were obtained every 1–3 min, and the average fluorescence intensity for all of the cell regions and background was recorded for later analysis. TMRE fluorescence intensity is reported as the average of the fluorescence of all identified cell regions, less background, for each coverslip.

Assessment of Cellular ATP Hydrolysis—ATP has a greater affinity for Mg++ than does ADP, and the cytosolic ionized magnesium concentration increases during ATP hydrolysis. The increases in cytosolic [Mg++] can be assessed using the intracellular fluorescent indicator Magnesium Green (MgG), and the behavior of this dye in cardiomyocytes has been studied in detail by Leyssens et al. (16). We measured MgG fluorescence (excitation 480 nm, emission 535 nm) in contracting cardiomyocytes during moderate hypoxia and reoxygenation. To assess ATP hydrolysis, cells on coverslips were loaded with MgG in the acetoxymethyl ester form (5 μM, Molecular Probes) for 30 min at 37 °C in a humidified incubator. Subsequently, the cells were transferred to the flow-through chamber and perfused with buffered salt solution under controlled O2 and CO2 conditions. To abolish the capacity for anaerobic glycolysis, 2-deoxyglucose (20 mM) was added to the perfusate, along with pyruvate (5 mM) to support mitochondrial respiration. Cells continued to contract spontaneously under these conditions. Fluorescent images were collected for multiple regions of interest every 60 s, using 100-ms exposure times. Average fluorescence was then calculated for each coverslip and analyzed as a percentage of initial values after subtraction of background fluorescence. At the end of the study, the mitochondrial uncoupler FCCP was added (5 μM) to induce ATP hydrolysis to confirm activity of the probe.

RESULTS

Effects of Prolonged Moderate Hypoxia in Contracting Cardiomyocytes—The contractile response to moderate hypoxia and reoxygenation was studied in cultured cardiomyocytes (Fig. 1). After 2 h under normoxic conditions (PO2 = 100 torr), the perfusate PO2 was reduced to ~20 torr (3% O2) for 3 h. No immediate effect on contraction was noted, but within 1–2 h a significant decrease in motion was consistently observed. When the perfusate O2 tension was returned to 100 torr, no immediate effect on contractile motion was noted. However, a progressive return of contraction developed over 2–3 h, and no significant difference from control levels was apparent at 3 h. These reversible decreases in contraction were similar to those noted previously (7).

Effect of Prolonged Moderate Hypoxia on [ATP] in Contracting Cardiomyocytes—Previous studies of noncontracting cardiomyocytes in suspension indicated that prolonged moderate hypoxia was associated with a decrease in oxygen consumption rate without a decrease in cellular ATP or phosphocreatine concentrations (7). To determine whether ATP depletion occurs during prolonged hypoxia in contracting cells, MgG fluorescence was used to assess ATP hydrolysis (16). To determine the capability of this dye to detect ATP hydrolysis, cells loaded with...
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MgG were imaged every 60 s during continuous normoxia (Fig. 2). After 4 min, the mitochondrial uncoupler FCCP was administered to limit ATP synthesis, and an abrupt increase in fluorescence was noted, consistent with a rise in intracellular Mg2+. In other cells loaded identically, the ATP synthase inhibitor oligomycin (10 μg/ml) produced a similar abrupt increase in fluorescence, as predicted (Fig. 3). To determine the effects of hypoxia on ATP hydrolysis, cells loaded with MgG were imaged every 5 min during 2.5 h of incubation at PO2 = 20 torr or 100 torr. As shown in Fig. 4, a gradual loss of fluorescence was noted in both groups, which appeared to be a consequence of the progressive photobleaching behavior of the MgG probe. However, no difference was noted between control and hypoxic cells. Moreover, the absence of an increase in fluorescence during hypoxia suggested that cytosolic [Mg2+] did not increase as a result of increased ATP hydrolysis. However, when ATP synthesis was halted by FCCP (5 μM) at the end of the study, a marked increase in fluorescence indicating an increase in [Mg2+] was noted.

**FIG. 1. Effect of hypoxia on total motion of spontaneously contracting cardiomyocytes during perfusion with buffered salt solutions under controlled O2 conditions.** Hypoxia (PO2 = 20 torr; 3% O2) was initiated after base-line measurements. Recovery to PO2 = 100 torr; (14% O2) was initiated at 180 min, but restoration of contractile motion required ≥3 h to reach base-line values (n = 4, p < 0.01).

**FIG. 2. Effects of mitochondrial uncoupling on MgG fluorescence in spontaneously contracting cardiomyocytes.** Cells loaded with MgG were perfused under normoxic conditions (PO2 = 100 torr; 14% O2) and administered FCCP (5 μM) to inhibit oxidative phosphorylation. The marked increase in fluorescence suggests an increase in Mg2+ released as a result of ATP hydrolysis.

**FIG. 3. Effects of mitochondrial ATP synthase inhibition on MgG fluorescence in spontaneously contracting cardiomyocytes.** Cells loaded with MgG were perfused under normoxic conditions (PO2 = 100 torr; 14% O2) and administered oligomycin (10 μg/ml) to inhibit oxidative phosphorylation. The marked increase in fluorescence suggests an increase in Mg2+ release as a result of ATP hydrolysis.

**FIG. 4. Effects of hypoxia on MgG fluorescence in spontaneously contracting cardiomyocytes.** Under base-line conditions, cells loaded with MgG were perfused with normoxic (PO2 = 100 torr; 14% O2) solution. At t = 30 min, perfusate PO2 was reduced to 20 torr (3% O2) in the hypoxia group while controls remained normoxic. At t = 180 min, the mitochondrial uncoupler FCCP (5 μM) was administered to elicit ATP hydrolysis. No statistical difference between groups was detected (n = 3).
should produce hyperpolarization of the mitochondrial potential. To confirm this, BDM (30 mM) was added to the perfusate of contracting cardiac myocytes at $t = 50$ (Fig. 7). As an inhibitor of Ca$^{2+}$-dependent actin-myosin interaction, BDM produced an immediate and significant increase in TMRE fluorescence, which was accompanied by an immediate cessation of contraction. Collectively, these results show that TMRE serves as an appropriate qualitative measurement of mitochondrial membrane potential.

**Effect of Hypoxia and Reoxygenation on Mitochondrial Membrane Potential**—Mitochondrial membrane potential was assessed during hypoxia under two different experimental protocols. In the first, TMRE fluorescence in contracting cells was measured in images obtained as the perfusate O$_2$ tension was decreased from 100 to 25 torr within 10 min (acute hypoxia). As shown in Fig. 8, acute hypoxia produced no acute change in TMRE fluorescence, which suggests that membrane potential was maintained. In the second, mitochondrial potential was recorded in contracting cells that had been perfused for 2 h at $P_{O2} = 25$ torr, as the $P_{O2}$ was rapidly increased from 25 to 100 torr (acute reoxygenation). Fig. 9a shows that TMRE fluorescence increased significantly during reoxygenation, which suggests that an increase in membrane potential had occurred. This relative hyperpolarization is consistent with an increase in the rate of electron transport during acute reoxygenation, compared with the rate of ATP utilization. Interestingly, the change in membrane potential did not occur until $t = 4$ min after the equilibration mixture was switched to normoxia. In separate studies, the $P_{O2}$ within the cell chamber was assessed under identical conditions of reoxygenation using a phosphorescence-quenching method (12). Those measurements revealed that the $P_{O2}$ within the chamber reached a value of $>60$ torr at 4 min, which suggested that the delay in response was caused by the slow response in reoxygenating the system (Fig. 9b).

**Effect of Azide on Contraction and Mitochondrial Potential**—To test whether a partial inhibition of cytochrome oxidase function could elicit the same response as moderate hypoxia, sodium azide (1 mM) was added to the perfusate in cells maintained under normoxic conditions. In preliminary studies this concentration of azide was found to be sufficient to reduce the $V_{max}$ of cytochrome oxidase, but too low to limit respiration in cells. When azide was added, no immediate effect was seen but a progressive suppression in contractile motion was developed over 2 h (Fig. 10). Washout of the azide required $>10$ min and was associated with a progressive return of contractile motion that mimicked the response to hypoxia. To assess the effects of azide (1 mM) on mitochondrial potential, cells loaded with TMRE were imaged every minute for 5 min, at which point sodium azide (1 mM) was added to the perfusate (Fig. 11). No acute...
A decrease in potential was observed, suggesting that membrane potential was preserved. In other cells loaded with TMRE and incubated with azide for 2 h, washout of the azide was associated with an acute increase in membrane potential, suggesting that a relative hyperpolarization of the membrane had developed. These responses were similar to those seen during onset and recovery from moderate hypoxia.

**DISCUSSION**

Myocardial hibernation is a chronic abnormality of contractile function associated with coronary hypoperfusion (17). This dysfunction is reversed if coronary blood flow is restored, which suggests that the abnormality is not a consequence of ischemic cellular damage. Studies using 31P NMR spectroscopy suggest inorganic phosphate levels are not increased during hibernation, which supports the view that the attenuation of contraction is not a consequence of a reduction in energy stores (5). However, the mechanisms underlying myocardial hibernation are not fully understood.

We previously reported that spontaneously contracting cardiomyocytes down-regulate contractile motion and O2 consumption during prolonged (1–2 h) moderate hypoxia (PO2 = 25 torr) (7). Cells kept under those conditions for 24 h showed no decrement in viability, suggesting that the inhibition was not a consequence of cell damage. Similar findings of decreased contractile motion with sustained viability have recently been reported in rat cardiomyocytes cultured under 1% O2 for 48 h (8). That study indicates that our results are not unique to embryonic cells. Moreover, it extends our previous work by demonstrating that hibernating cardiac myocytes were more tolerant to acute severe hypoxic challenge, in terms of the time required to elicit ATP-depletion. These studies suggest that a phenomenon similar to myocardial hibernation develops in cardiac myocytes during prolonged hypoxia, which affords protection from anoxic stress and is reversible within hours after recovery to normoxic culture conditions.

**Hypoxia Reversibly Decreases Contraction—**In this study,
cardiomyocytes superfused at $P_{O_2} = 20$ torr showed progressive decreases in contractile motion that reached 40% of control levels within $-2$ h. Recovery to $P_{O_2} = 100$ torr was associated with a recovery of contraction, although several hours were required to reach baseline levels. We previously observed that cardiomyocytes maintained under normoxic conditions for $-6$ h showed no significant changes in contractile motion, so the observed response to hypoxia cannot be explained by the effects of time alone. During brief exposures to hypoxia, Rumsey et al. (18) found that adult rat cardiac myocytes could maintain normal rates of $O_2$ consumption until the extracellular $P_{O_2}$ fell to less than $-7$ torr (18). Moreover, the observations that cells continued to function normally during the first hour of hypoxia and that the decreases in contraction were reversible suggest that the effects of hypoxia we found could not be explained by an $O_2$ supply limitation of mitochondrial ATP synthesis.

Nevertheless, to test whether hypoxia elicited ATP supply limitation, we used Magnesium Green to assess ATP hydrolysis in contracting cells during moderate hypoxia. The fluorescence of this dye increases with the intracellular $Mg^{2+}$ concentration, which increases during ATP hydrolysis because ATP has a higher affinity for $Mg^{2+}$ than does ADP (16). Indeed, marked increases in fluorescence were seen in normoxic cells treated with the mitochondrial uncoupler FCCP or with the ATP synthase inhibitor oligomycin, both of which should inhibit oxidative phosphorylation and cause ATP depletion. However, no increase in MgG fluorescence was seen during prolonged exposure to $P_{O_2} = 20$ torr, suggesting that ATP hydrolysis was minimal. We previously measured [ATP] and [PCr] in quiescent cardiomyocytes and found that cellular levels were preserved during prolonged moderate hypoxia. The present study extends these results by showing that ATP levels are similarly preserved in contracting cells during prolonged hypoxia.

To further test whether hypoxia elicited ATP supply limitation, we assessed mitochondrial potential using the dye TMRE. Mitochondrial membrane potential provides the driving force for ATP synthesis. The potential is generated by the supply of NADH through the matrix dehydrogenases and electron flux through electron transport chain. The ATP synthase uses the energy from mitochondrial membrane potential to synthesize ATP from ADP plus inorganic phosphate. In steady state, the mitochondrial membrane potential therefore reflects a balance between the rate of electron transport and the rate of ATP utilization by the cell. An inhibition within the electron transport chain during hypoxia would therefore result in a decrease in electron flux and a depolarization of the membrane, while a sudden inhibition of ATP utilization or an inhibition of the ATP synthase should produce a relative hyperpolarization. Indeed, these responses were observed when electron transport was inhibited with myxothiazol or when the ATP synthase was inhibited with oligomycin. Therefore, if hypoxia limited ATP synthesis by restricting mitochondrial electron transport, then a profound decrease in membrane potential should have been evident at the start of hypoxia in the TMRE studies. But mitochondrial potential was maintained during acute hypoxia, which suggests that ATP utilization and ATP synthesis remained closely matched. This conclusion is consistent with the absence of ATP hydrolysis indicated by the MgG data. Collectively, these findings suggest that hypoxia decreases respiration in cardiomyocytes by activating a signaling pathway that causes a reduction in contraction and ATP demand, rather than by limiting ATP supply.

**Hypoxia Causes a Partial Mitochondrial Inhibition**—Although mitochondrial potential was not depleted during hypoxia, small increases in potential were detected at reoxygenation. This suggests that there was a rapid increase in electron transport without a corresponding increase in ATP utilization. Such an increase would be predicted if a partial inhibition of cytochrome oxidase were rapidly removed at reoxygenation. In previous studies of the oxidase, we have observed an immediate increase in $V_{max}$ upon reoxygenation after prolonged hypoxia (9, 10, 19). We therefore conclude that the changes in mitochondrial potential at reoxygenation reflect functional changes in the kinetics of the oxidase that develop during prolonged hypoxia. These observations indicate that mitochondria can respond to changes in $O_2$ tension within the physiological range, which could allow them to function as an $O_2$ sensor.

**Cytochrome c Oxidase Serves as an $O_2$ Sensor**—Sodium azide is a noncompetitive inhibitor of cytochrome c oxidase (20). At high concentrations (5–10 mM) it can throttle respiration by limiting $O_2$ consumption, resulting in an immediate cessation of contraction and depletion of mitochondrial potential. By contrast, low concentrations of azide could mimic the effects of hypoxia on the enzyme. Our observation that azide during normoxia produced the same changes in contractile function and mitochondrial potential seen during moderate hypoxia implicates cytochrome oxidase as the oxygen sensor underlying the functional response to hypoxia.

The observation that cells respond to physiological levels of $O_2$ suggests the existence of a signal transduction pathway linking the sensor to the functional response. Some investigators have argued that cytochrome oxidase is ill-suited to function as an $O_2$ sensor because its apparent $K_{m}$ for $O_2$ is $<1$ μM (8, 21). Therefore, at physiological $O_2$ concentrations the velocity of such an enzyme system would remain independent of $O_2$ until near-anoxic conditions were reached, limiting its ability to signal changes in the physiological range. However, changes in the $V_{max}$ of the oxidase produced with hypoxia or azide (1 mM) should increase the reduction state of mitochondrial electron carriers upstream from the oxidase (19). It is conceivable that subsequent redox-linked activation of second messenger systems (22, 23) could then lead to an inhibition of ATP utilization within the cell. Although not yet confirmed, the above sequence would be consistent with (a) the previously observed changes in cytochrome oxidase $V_{max}$ during hypoxia, (b) the observed changes in mitochondrial potential during hypoxia and reoxygenation, (c) the possible delay between onset of hypoxia or reoxygenation and the changes in cell motion and ATP demand, and (d) the sustained coupling between cellular respiration and ATP demand as indicated by membrane potential and MgG. However, further study will be required to fully identify the signaling cascade(s) mediating the functional response.

**Significance for Intact Tissues**—A number of similarities between the response to hypoxia in cardiomyocytes and that seen in hibernating myocardium should be noted. First, both appear to involve a matching between ATP utilization and $O_2$ supply without evidence of ischemia. Second, both conditions are fully reversible upon restoration of base-line conditions, without loss of cell viability or necrotic damage. One notable difference is that contractile function in hibernating myocardium recovers immediately upon restoration of flow (24), whereas recovery of cardiomyocytes required 2–3 h after restoration of normoxic conditions. Although the present study provides insight into the mechanisms contributing to the response to hypoxia in cardiomyocytes, further work will be required to clarify the relationship of these findings to the events in the intact hibernating myocardium.
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