Mitochondrial respiratory control can compensate for intracellular O₂ gradients in cardiomyocytes at low P O₂

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Takahashi, Eiji, and Koji Asano. Mitochondrial respiratory control can compensate for intracellular O₂ gradients in cardiomyocytes at low P O₂. Am J Physiol Heart Circ Physiol 283: H871–H878, 2002. First published May 16, 2002; 10.1152/ajpheart.00162.2002.—In isolated single cardiomyocytes with moderately elevated mitochondrial respiration, direct evidence for intracellular radial gradients of oxygen concentration was obtained by subcellular spectrophotometry of myoglobin (Mb). When oxygen consumption was increased by carbonyl cyanide m-chlorophenylhydrazone (CCCP) during superfusion of cells with 4% oxygen, P O₂ at the cell core dropped to 2.3 mmHg, whereas Mb near the plasma membrane was almost fully saturated with oxygen. Subcellular NADH fluorometry demonstrated corresponding intracellular heterogeneities of NADH, indicating suppression of mitochondrial oxidative metabolism due to relatively slow intracellular oxygen diffusion. When oxygen consumption was increased by electrical pacing in 2% oxygen, radial oxygen gradients of similar magnitude were demonstrated (cell core P O₂ = 2.6 mmHg). However, an increase in NADH fluorescence at the cell core was not detected. Because CCCP abolished mitochondrial respiratory control while it was intact in electrically paced cardiomyocytes, we conclude that mitochondria with intact respiratory control can sustain electron transfer with reduced oxygen supply. Thus mitochondrial intrinsic regulation can compensate for relatively slow oxygen diffusion within cardiomyocytes.

REGULATION OF OXIDATIVE PHOSPHORYLATION is crucial for the survival of cells under physiological and pathophysiological fluctuations of either oxygen delivery or demand. Extrapolating in vitro data, one might think that oxygen availability hardly affects mitochondrial oxidative metabolism in normal hearts because the cytochrome c oxidase Michaelis-Menten constant (Kₘ) for oxygen is <1 mmHg in isolated mitochondria (8, 31), whereas in vivo coronary venous P O₂ is ~20 mmHg (21, 33). Nevertheless, the oxygen transport from capillary to mitochondria in in vivo cardiac tissue is not simple. Besides coronary capillary P O₂ extracellular (from capillary blood to plasmalemma) and intracellular (from plasmalemma to mitochondrial inner membrane) P O₂ gradients determine P O₂ at mitochondria. Both gradients increase as cellular oxygen demand increases, thus lowering P O₂ at mitochondria. Even in normal hearts, these oxygen gradients are so steep that small decreases in coronary oxygen supply bring intracellular P O₂ to ranges in which mitochondrial metabolism is reset (23, 28, 35). In these regulatory P O₂ ranges, in situ myoglobin (Mb) is partially desaturated (7, 14, 35, also see Ref. 1).

In the extracellular space, oxygen diffusion is relatively slow because this compartment lacks an oxygen carrier (such as Mb in the cytosol). Thus large oxygen concentration gradients are produced despite a very short (<0.5–2 μm) diffusion distance (13). In the intracellular space of cardiomyocytes, oxygen diffusion may be significantly accelerated by Mb-facilitated oxygen diffusion (18, 33). However, a much longer (~10 μm in rat ventricular myocytes) diffusion distance from the plasma membrane to the center of cell may also produce significant radial oxygen concentration gradients. The mitochondria located near the plasma membrane should then be exposed to substantially higher P O₂ than those located near the center of the cell. This allows measurement of adaptive changes in mitochondrial oxidative phosphorylation to P O₂ reductions within single cardiomyocytes.

We demonstrated that in isolated single rat cardiomyocytes treated with carbonyl cyanide m-chlorophenylhydrazone (CCCP) and at low extracellular P O₂ (<15 mmHg), there are radial P O₂ gradients within the cell such that the core oxygen pressure is near zero (27), and, as a result, the NADH fluorescence is significantly increased (26). These experiments certainly demonstrated that intracellular oxygen diffusion may limit mitochondrial oxidative phosphorylation. The question remained, however, as to how cardiomyocytes deal with such relatively slow intracellular oxygen diffusion. Our previous studies did not shed light on the role of physiological regulatory mechanism (the respiratory control) that should interact with diffusional oxygen supply because we disrupted the regulation by an uncoupler of oxidative phosphorylation. Thus we undertook the present study to demonstrate that mitochondrial respiratory control can compensate for such insufficient intracellular oxygen diffusion so that electron transfer is sustained in mitochondria locating in the hypoxic cell core.

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METHODS

Isolation of single cardiomyocytes. Prior approval for the experiment was obtained from the Animal Research Committee, Yamagata University School of Medicine. Single cardiomyocytes were isolated from adult male Sprague-Dawley rats using type 2 collagenase (Worthington) and suspended in a HEPES buffer solution containing (in mM) 130 NaCl, 6 HEPES, 10 glucose, 5.4 KCl, 1.2 KH2PO4, 1.2 MgSO4, 2.5 pyruvate, 10 taurine, and 2 glutamate, supplemented with 0.1% bovine serum albumin. All the reagents used in this study were of the highest grade.

Increasing oxygen consumption. To address the effects of radial intracellular gradients of diffusional oxygen delivery on mitochondrial metabolism, it is important to significantly increase mitochondrial respiration because the magnitude of radial oxygen gradients is proportional to intracellular flux of oxygen. This was done by two distinct techniques. In the first group, mitochondrial oxygen consumption (VO2) was elevated with the use of 1 μM CCCP, an uncoupler of oxidative phosphorylation, in Ca2+-free HEPES solution at room temperature. In the second group, mitochondrial respiration was stimulated by electrical pacing of cardiomyocytes at 300 beats/min for 10 min in the presence of 2 μM norepinephrine and 1 mM Ca2+ at 36°C. For this purpose, a pair of 6 × 0.2-mm ø-platinum wires was placed on the bottom quartz glass in the measuring cuvette and connected to an electrical stimulator (model SEN-3301, Nihon Kohden).

Measurement of Mb oxygen saturation with subcellular spatial resolution. Intracellular heterogeneities of oxygen concentration were assessed by the spectrophotometric measurement of fractional oxygen saturation of Mb (Smb) with a subcellular spatial resolution. Reconstruction of intracellular oxygenation consists of the following steps. First, an aliquot of 6-μl cell suspension was placed on the airtight measuring cuvette on the stage of the inverted microscope (model IX-70, Olympus) and was superfused with humidified mixed gas of a given PO2 (balance N2). Transmitted light image of a single cell at 435.2 nm (an absorption peak of deoxy Mb) was acquired exactly 5 s after termination of a 10-min electrical pacing. Second, the transmitted light image of electrically paced cells was acquired exactly 5 s after termination of a 10-min electrical pacing. However, at this time point, cardiomyocytes were usually still hypercontracted. Thereafter, cardiomyocytes slowly restored their original shapes. These morphological changes might have affected the focus and light path length. These artifacts were compensated for by subtracting the cellular image at an isosbestic wavelength (424.5 nm) taken immediately after each 435.2-nm image acquisition. Therefore, Smb in the electrically paced cells was determined by

\[ Smb = \frac{(OD_{hypoxic} - OD_{anoxic})}{(OD_{hypoxic} - OD_{anoxic})} \] (1)

All of the image arithmetic was carried out with IPLab software (Scanalytics).

Determination of radial Smb gradients in intact electrically paced single cardiomyocytes requires a special care. First, because mitochondrial respiratory control is intact, the redox level of mitochondrial cytochromes, another important intracellular chromophore, varies according to oxygen and energy levels. These changes are associated with changes in cytochrome light absorption, thus affecting measurement of Mb light absorption. In the present high-spatial resolution spectrophotometry, we could distinguish in the transmitted light image of a cell, regions mainly involving cytochrome light absorption (Fig. 1). Thus Smb was determined only in regions with the least contamination of cytochromes light absorption. This was carried out by calculating Smb only from trough values in the radial profile of cellular OD image (Fig. 3). Second, the transmitted light image of electrically paced cells was acquired exactly 5 s after termination of a 10-min electrical pacing. Therefore, Smb in the electrically paced cells was determined by

\[ Smb = \frac{(OD'_{hypoxic} - OD'_{anoxic})}{(OD'_{hypoxic} - OD'_{anoxic})} \] (2)

where OD' denotes transmitted image at the isosbestic wavelength. Because Smb was determined only at troughs in the radial OD profiles (Fig. 3A), the nadir of Smb was interpolated from these measured Smb by using a hyperbolic function (Fig. 3B).

Measurement of mitochondrial oxidative metabolism with subcellular spatial resolution. Dependency of mitochondrial oxidative metabolism on diffusional oxygen delivery was assessed by the high spatial resolution imaging of mitochondrial NAD(P)H (26). We assumed that local increases in NADH indicate limitation of electron transfer in the respiratory chain due to insufficient oxygen supply in that particular region. Mitochondrial NADH was assessed from autofluorescence on ultraviolet (UV) excitation (9, 30).

In both CCCP-treated and electrically paced cardiomyocytes, protocols for the NADH measurement were similar to those of the Smb measurement except that hyperoxic super-
fusion was omitted. Autofluorescence image of cardiomyocytes on UV excitation (330–385 nm) was captured by the CCD camera during hypoxic superfusion. To quantify NADH fluorescence, this image was normalized to that taken while NADH was fully reduced by superfusion with anoxic gas (>99.999% N2). Background nonspecific fluorescence was subtracted. Photobleaching was determined separately for which anoxic NADH fluorescence was compensated. These were conducted with the same spatial resolution as in the S\textsubscript{Sm} measurement.

Data for each experiment were collected from at least six individual cardiomyocytes and represent means ± SD. Differences in the mean values taken from different regions within a cell were judged by repeated-measure analysis of variance, followed by Scheffé’s test, where \( P < 0.05 \) was considered significant.

**RESULTS AND DISCUSSION.** In a suspension of cardiomyocytes containing \(-2 \times 10^4 \text{ cells/ml, } \dot{V}_{O_2}\) was determined by the conventional \(P_{O_2}\) electrode method (27). At room temperature (25°C) in the absence of extracellular \(Ca^{2+}\), 1 \(\mu\text{M CCCP}\)-stimulated mitochondrial respiration from 46 ± 19 to 334 ± 108 nmol \(O_2\)·min\(^{-1}\)·10\(^6\) cells\(^{-1}\) (\(n = 12\)). Normal hearts can increase steady-state oxygen utilization 20-fold (34). A 7.3-fold increase in \(\dot{V}_{O_2}\) in the present CCCP-treated quiescent cardiomyocytes may then represent a moderate increase.

In the second group, electrical pacing was conducted at 36°C with 1 mM \(Ca^{2+}\) in the suspension medium. Without electrical pacing, the \(\dot{V}_{O_2}\) of these cells was 141 ± 29 nmol \(O_2\)·min\(^{-1}\)·10\(^6\) cells\(^{-1}\) (\(n = 12\)), being substantially lower than the CCCP-treated cells. Rose et al. (20) reported a linear relationship between pacing frequency from 0 to 6 Hz and \(\dot{V}_{O_2}\) in isolated single cardiomyocytes in suspension containing 1 \(\mu\text{M isoprenaline}\) and 1.8 mM \(Ca^{2+}\). On the basis of this study, electrical pacing at 300 beats/min (5 Hz) would have increased \(\dot{V}_{O_2}\) by \(-3\) times. If so, \(\dot{V}_{O_2}\) of the present electrically paced cardiomyocytes would match that of the CCCP-treated cardiomyocytes. However, we did not measure \(\dot{V}_{O_2}\) in electrically paced cardiomyocytes in suspension because the magnitude of contraction to an electrical pulse differed considerably from cell to cell, whereas the optical measurements of intracellular oxygenation and mitochondrial metabolism were conducted only in most vigorously contracting rod-shaped single cardiomyocytes with the least deterioration. Thus conventional \(\dot{V}_{O_2}\) measurement in cell suspension was expected to severely underestimate \(\dot{V}_{O_2}\). Instead, we directly measured intracellular deoxygenation in individual cardiomyocytes to determine whether electrical pacing produced a “moderate” hypoxic load as in the CCCP-treated cells.

**Radial S\textsubscript{Sm} gradients.** In 1 \(\mu\text{M CCCP}\)-treated cardiomyocytes, the transition between full oxygenation and deoxygenation of Mb produced ~3% changes in transmitted light intensity (incident light intensity = 100%, Fig. 2B). When the same cell was superfused with hypoxic gas, radial profiles of transmitted light intensity near plasma membranes were similar to those in hyperoxia, whereas those near the center were close to those in anoxia (Fig. 2B). Thus radial gradients of S\textsubscript{Sm} were demonstrated in a single cardiomyocyte (Fig. 2C). In the electrically paced cardiomyocytes, there were again significant parallel shift of OD profiles between hyperoxia and anoxia (Fig. 3A). Although the hypoxic profile was not completely analogous to those of hyperoxia and anoxia with respect to the absolute position of troughs (due to hypercontracture), calculation of S\textsubscript{Sm}...
using corresponding trough values successfully demonstrated significant radial gradients of $S_{Mb}$ (Fig. 3B).

Figure 4 summarizes the hypoxic core in single cardiomyocytes with moderately increased $V_{O_2}$. Regardless of the method for stimulating respiration (1 μM CCCP or electrical pacing), Mb in regions near the center of cell was significantly desaturated compared with those locating near plasma membranes as oxygen concentration of superfusion gas was lowered.

In red muscles, intracellular diffusion of oxygen may be significantly facilitated by Mb that abundantly occurs in the cytoplasm (33). In the heart, Gayeski and Honig (10) demonstrated by the cryospectrophotometry of in vivo $S_{Mb}$ that average intracellular $P_{O_2}$ was very low (4.3–7.0 mmHg) and uniform among cells even when metabolic oxygen demand and/or coronary oxygen supply were significantly altered. In the heart and working skeletal muscle, intracellular radial $S_{Mb}$ gradients were very shallow and within the error of saturation determination (10, 11). They interpreted that such low but uniform intracellular $P_{O_2}$ reflects physiological roles of Mb, thus discounting physiological significance of intracellular oxygen gradients. Recently, the radial diffusion coefficient of Mb in cardiac tissue has been determined by using two different techniques (19). The value was surprisingly low (~1/10) compared with that in diluted protein solution that was frequently used for mathematical simulation of intracellular oxygen diffusion. Model studies (15, 17) of intramuscular oxygen diffusion at near maximal performance using the revised Mb diffusion constant predicted significant intracellular radial $P_{O_2}$ gradients. Furthermore, spatial resolution of the optical measurement of Gayeski and Honig has been seriously ques-
tioned (15, 29). Thus the conclusion that “very shallow intracellular oxygen concentration gradients” needs substantial reconsideration. We had predicted that due to much longer oxygen diffusion distance compared with the extracellular space and relatively low diffusivity of in situ Mb, intracellular radial gradients of oxygen concentration must have much more impact than what was expected, particularly in muscle cells with increased activity.

Recently, $^1$H nuclear magnetic resonance (6, 28) or reflectance spectrophotometry (1) was utilized to determine Mb deoxygenation in in vivo hearts. Although applicable to in vivo hearts, low spatial resolution of these techniques would not allow detection of intracellular oxygen gradients and the hypoxic core. We felt that direct imaging of Mb light absorption with subcellular spatial resolution in a single cardiomyocyte, if possible, would be the most straightforward way to prove intracellular radial oxygen gradients. Spectrophotometry in single cardiomyocyte was only possible with the use of low noise and wide dynamic range (16 bits) CCD camera, because 1% change in $S_{Mb}$ was equivalent to only 3/10,000 of incident light intensity. With a subcellular spatial resolution, we have clearly demonstrated that intracellular diffusion of oxygen may produce a oxygen-depleted core within a cell although Mb near the cell surface is almost fully saturated with oxygen (Figs. 2–4). Such radial gradients were demonstrated irrespective to the method of stimulating oxygen consumption (CCCP or pacing). Therefore, the present findings indicate that for moderately stimulated mitochondria, intracellular radial diffusion of oxygen may not be fast enough to catch up with the rate of consumption. The local hypoxic core thus produced may well affect mitochondrial metabolism.

$P_{O2}$ in the hypoxic core. Before we compare mitochondrial metabolism in different intracellular oxygen supply, we should determine the level of hypoxia in the present study. The CCCP experiment was conducted at 25°C, whereas the pacing experiment was done at

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**Fig. 5.** Representative data demonstrating reconstruction of the radial NADH profile in a 1 μM CCCP-treated single cardiomyocyte. A: as in the $S_{Mb}$ measurement, a rectangular ROI (5.2 μm wide) was defined and autofluorescence intensity was measured along the short axis of the cell. B: NADH fluorescence was normalized to that for the fully reduced state. au, Arbitrary units.

**Fig. 6.** Radial NADH gradients in single cardiomyocytes with moderately increased respiration. NADH fluorescence intensities near plasma membranes and the center of cell are shown, whereas oxygen concentration of superfusion gas was changed. Peripheries 1 and 2 indicate regions ~4 μm inside from plasma membranes. In 1 μM CCCP-treated (A) and electrically paced cardiomyocytes (B), the NADH level at nonlimiting $P_{O2}$ was 44 ± 5% and 63 ± 6% of full NADH reduction, respectively. *$P < 0.05$. 

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36°C. The calculation of cell core PO\textsubscript{2} from S\textsubscript{Mb} then needs adjustments for the temperature dependency of Mb oxygen binding. In the CCCP-treated cardiomyocytes, cell core S\textsubscript{Mb} during superfusion with 2% and 4% oxygen were 0.32 and 0.75, respectively (Fig. 4). Because PO\textsubscript{2} at half-maximal saturation of Mb oxygen binding is 0.78 mmHg at 25°C (24), corresponding cell core PO\textsubscript{2} were 0.4 and 2.3 mmHg, respectively. In the electrically paced cardiomyocytes, the nadir S\textsubscript{Mb} was 0.56 and 0.76 for 2% and 4% oxygen-superserved gases, respectively (Fig. 4), which corresponds to 2.6 and 6.4 mmHg with an assumption that PO\textsubscript{2} at half-maximal saturation is 2.03 mmHg at 36°C (24). These differences in the nadir intracellular PO\textsubscript{2} suggest that VO\textsubscript{2} of the electrically paced cardiomyocytes might not have reached the level attained by CCCP. In isolated rat cardiomyocytes, Stumpe and Schrader (25) demonstrated a reversible downregulation of VO\textsubscript{2} and contractile activity at a low PO\textsubscript{2} (6 mmHg) without any effect on cellular energy state. Thus an alternative explanation is that in electrically paced cardiomyocytes, mitochondria locating in the oxygen-depleted core might have downregulated their VO\textsubscript{2}, thus reducing radial PO\textsubscript{2} drop. In either case, we could produce a similar deoxygenation level in the cell core in cardiomyocytes with intact and abolished respiratory control (2% oxygen-superserved electrically paced cells and 4% oxygen-superserved CCCP-treated cells, respectively).

Radial gradients of mitochondrial oxidative metabolism in CCCP-treated cardiomyocytes. In cardiomyocytes, autofluorescence under UV epi-illumination mainly represents mitochondrial NADH (9). Thus the magnitude of NADH oxidation can be continuously assessed in single cardiomyocytes. At least three factors affect the level of NADH in mitochondria: activities of mitochondrial dehydrogenases, cytosolic energy state (phosphorylation potential, [ATP]/[ADP][Pi]), and cytochrome c oxidation ([c\textsuperscript{2-}]/[c\textsuperscript{3-}]) (31). The last one takes place at the complex IV of the respiratory chain and is quite sensitive to oxygen availability at mitochondria (5, 9). To assess oxygen dependency of mitochondrial metabolism, we compared NADH fluorescence in different regions within one cardiomyocyte where mitochondria were exposed to different PO\textsubscript{2}.

Figure 5 demonstrates reconstruction of NADH heterogeneities within a single cardiomyocyte. In CCCP-treated cells superfused with 4% O\textsubscript{2}, NADH fluorescence was significantly increased in the cell core compared with regions near the plasma membrane (Fig. 6A). The radial profile of NADH fluorescence was just a mirror image of the radial S\textsubscript{Mb} gradients (Figs. 2 and 5). With abundant oxygen supply, such heterogeneities were eliminated (Fig. 6A). These results imply that intracellular radial gradients of oxygen supply...
may inhibit electron transfer in mitochondria located at the cell core. Thus a moderate increase in $V_{02}$ may produce the anoxic core (metabolic inhibition) in CCCP-treated single cardiomyocytes.

Insignificant anoxic core in electrically paced cardiomyocytes. Figure 7 illustrates the reconstruction of radial NADH profile in an electrically paced single cardiomyocyte. To assess the roles of mitochondrial respiratory control under insufficient mitochondrial oxygen supply, we compared NADH heterogeneities in the electrically paced cells and the CCCP-treated cells at similar cell core PO$_2$. In both 2% oxygen-supersaturated paced cells and 4% oxygen-supersaturated CCCP-treated cells, Mb near plasma membranes was almost fully saturated with oxygen (SMb = 0.93 ± 0.10 and 0.95 ± 0.05, respectively; see Fig. 4). Consequently, NADH in these regions was almost fully oxidized (11 ± 24% and 12 ± 10% reductions from the aerobic level, respectively; Fig. 6), indicating that mitochondrial oxygen metabolism in these regions was never limited by oxygen supply. In contrast, at the cell core, level of NADH reduction was significantly higher in the CCCP-treated cells (50 ± 14% reduction from the aerobic level; Fig. 6A) than in the electrically paced cells (9 ± 13% reduction from the aerobic level) at similar PO$_2$ (2.3 and 2.6 mmHg, respectively; Fig. 6B). These results indicate that electrically paced single cardiomyocytes can suppress accumulation of NADH caused by insufficient oxygen supply to mitochondria. Thus mitochondria with intact respiratory control seem to maintain almost normal electron transfer in the hypoxic core. These NADH heterogeneities were further studied in more hypoxic cardiomyocytes (Fig. 8). Maximum radial NADH fluorescence gradient (20 ± 17% reduction from the aerobic level) was found in the electrically paced cardiomyocytes superfused with 1% oxygen, but was far smaller than NADH augmentation in the core of CCCP-treated cells.

Regulation of mitochondrial oxidative metabolism in oxygen depletion. In CCCP-treated and electrically paced single cardiomyocytes, mitochondria in relatively uniform biochemical environment but exposed to substantially different PO$_2$ behaved quite differently. Mitochondrial respiration and electron transfer in electrically paced cells are regulated by the mitochondrial respiratory control mechanism (i.e., dependency on energy state), whereas it is abolished in CCCP-treated cells. We conclude that mitochondria with intact respiratory control can continue electron transfer in the respiratory chain when diffusive oxygen supply is limited. This mechanism may maintain the electrochemical gradient across the mitochondrial membrane, the key parameter for mitochondrial functions. Thus mitochondrial physiological regulatory mechanism can compensate for the relatively slow oxygen diffusion within cardiomyocytes.

Several mechanisms may be involved. First, the near-equilibrium hypothesis of oxidative phosphorylation predicts that oxidation of NADH ([NADH]/[NAD$^+$]) is closely linked with phosphorylation potential and cytochrome c oxidation as follows

$$\frac{[NADH]}{[NAD^+]^2} = \frac{([ATP]/[ADP][Pi])^2}{K_{eq} \times ([e^2]/[e^3])^2}$$

where $K_{eq}$ is mass action ratio (31). Thus increase in $[e^2]/[e^3]$ caused by hypoxic inhibition of cytochrome c oxidation can be counteracted by concomitant decreases in phosphorylation potential, leaving [NADH]/[NAD$^+$] almost unchanged. Second, the oxygen affinity of cytochrome c oxidase is not constant but is affected by energy state. In isolated mitochondria, the apparent oxygen dissociation constants for respiration and cytochrome c oxidase (heme a + a$_3$) are lowered when phosphorylation potential becomes low (8, 22, 32). Therefore, at the hypoxic cell core where the energy level might be lower, it is possible that electron transfer is sustained with a lower PO$_2$, and NADH oxidation continues. These two hypotheses predict the presence of heterogeneities of energy state within one cardiomyocyte. In the same line, Gnaiger et al. (12) proposed that at low PO$_2$, phosphorylation efficiency for ATP synthesis is high due to suppression of uncoupled respiration. Third, because nitric oxide (NO) is a potent competitive inhibitor of cytochrome c oxidase (4), Brown postulated that endogenous NO, by shifting the $K_m$ into the physiological oxygen range, makes mitochondrial respiration sensitive to physiological variations of oxygen supply (2, 3). NO is synthesized from molecular oxygen and arginine by NO synthase (NOS). The apparent $K_m$ of NOS for oxygen is significantly higher than that of cytochrome c oxidase. In the case of hypoxia, NO production may then be decreased before cytochrome c oxidation is compromised. If so, NOS can function as an oxygen sensor. Recently, NOS was identified in the mitochondria (mtNOS) of the mouse heart (16). It is then presumable that lower PO$_2$ at the cell core inhibits NO production by mtNOS and increases oxygen affinity of cytochrome c oxidase located in this region. Thus NADH oxidation and electron transfer might be sustained in the hypoxic cell core. Large anoxic core demonstrated in the present CCCP-treated cardiomyocytes is compatible with this hypothesis because proton ionophore appears to inhibit NO production by mtNOS (16).

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REFERENCES


