Impact of Diffusional Oxygen Transport on Oxidative Metabolism in the Heart

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Summary: The resistance for the oxygen molecule to diffuse from the capillary blood to the cell surface produces remarkably large gradients of oxygen partial pressure (PO₂) in the extracellular space. In addition, the intracellular radial gradients of PO₂ may not be ignored particularly when the cellular oxygen consumption is increased. These PO₂ gradients together result in a quite low intracellular PO₂ in the cardiomyocyte in vivo. Thus, the cellular oxidative metabolism may be limited by diffusional transport of oxygen from the capillary blood to mitochondria. In this review, quantitative aspects and physiological relevances of the PO₂ gradient in the myocardium are discussed. [Japanese Journal of Physiology, 48, 243–252, 1998]

Key Words: intracellular oxygen, diffusion, heart, PO₂ gradient, oxidative phosphorylation.

Oxygen transport from the capillary blood to intracellular mitochondria is a process governed by the law of diffusivity. Despite being simple in its mathematical formulation, this physical law is of great physiological relevance in organs. A diffusional movement of oxygen molecules requires gradients of oxygen concentration along the diffusional path. Therefore, the oxygen partial pressure (PO₂) at the mitochondrial membrane should be substantially lower than in the capillary lumen. In fact, recent in vivo measurements of fractional oxygen saturation of cytosolic myoglobin (Smh) in the normal beating heart addressed the presence of surprisingly steep PO₂ gradients in the extracellular space [1]. A consequence of this gradient is a very low intracellular PO₂. Thus PO₂ gradients from the capillary blood to mitochondria are the major determinant of PO₂ at mitochondria and, presumably, oxidative phosphorylation.

Diffusional oxygen transport is especially significant in the myocardium because of the high oxygen demand and less reserve for oxygen deficiency of the organ. Also, the large size of the cardiomyocyte may hinder diffusional oxygen delivery to mitochondria, especially those in the core of the cell. In this review, the quantitative aspects and the physiological relevance of the PO₂ gradient in the heart will be discussed.

I. Extracellular PO₂ Gradients

Oxygen molecules released from the hemoglobin in the red blood cell diffuse out from the capillary lumen to the surface of the cardiomyocyte through a thin plasma layer, capillary wall, and a layer of interstitial fluid. The difference of PO₂ between the surface of the red blood cell in the capillary and the sarcolemma is designated as the “extracellular PO₂ gradient.” These extracellular spaces are distinct because they do not contain a specific carrier of oxygen molecule such as myoglobin in the cardiomyocyte and red skeletal muscle. A lack of facilitation of oxygen diffusion by the carrier molecule in this compartment appears to be the major cause of considerable PO₂ gradients, despite a very short diffusion path length (usually assumed to be <2 µm) [2–5].

1. Capillary blood PO₂ in the myocardium

Intravascular oxygen PO₂ was first estimated from the perivascular PO₂ measured by a polarographic oxygen microelectrode [6]. This technique, however, turned out to be inappropriate because significant PO₂ gradients exist in the vicinity of the vascular wall [1, 7, 8]. Subsequently, the spectrophotometric determination of hemoglobin oxygen satura-
tion in microvessels was made possible [9–13]. Applying this technique to the in vivo heart is extremely difficult, however, mainly because of the organ’s rapid movement, and capillary PO₂ in the myocardium has not yet been accurately determined. Instead, coronary venous blood PO₂ was used to represent the mean end-capillary blood PO₂[14]. In the beating heart at normal oxygen consumption, mean capillary blood PO₂ determined this way is ~20 Torr [5, 15, 16].

Recently, measurements of phosphorescence quenching of the metalloporphyrin/albumin complex by free oxygen have provided more accurate and detailed information regarding microvascular oxygenation [8, 17–20]. The technique is basically noninvasive and highly quantitative. Furthermore, the spatial resolution can be an order of 10⁻² mm, enabling generation of a two-dimensional map of intravascular oxygen in the tissue. Rumsey et al. [21] extended this method to the beating heart by synchronizing flash excitations of the phosphorescence oxygen probe and image acquisitions with the cardiac cycle. In the anesthetized piglet ventilated with room air, in vivo intravascular PO₂, presumably of capillaries and vessels in the epicardium 0.5–1 mm deep, ranged 18–26 Torr.

2. Sarcolemmal PO₂ estimated from average cytosolic PO₂

In the heart, a direct measurement of PO₂ at the sarcolemma has not been reported, although this might be possible from the fluorescence lifetime measurement of membrane soluble probes such as pyrene and mesoporphyrins [22–24]. Instead, the magnitude of extracellular PO₂ gradients was calculated by using the mean intracellular (cytosolic) PO₂ in cardiomyocytes. Rationales for the use of cytosolic PO₂: (1) Accurate measurement of mean cytosolic PO₂ is possible in the physiological range from the measurement of oxygen saturation of myoglobin, a heme protein occurring abundantly (0.2 mM) in the cytosol of cardiomyocytes [16]; and (2) Myoglobin-reported PO₂ appears uniform in the cytosolic space without appreciable intracellular gradients, at least at normal oxygen consumption [1, 5, 25].

The measurement of myoglobin oxygenation in the blood-perfused in vivo heart has been reported by two groups. Coburn et al. [26] estimated the mean cytosolic PO₂ from the measurement of ¹³CO binding to myoglobin (that competes with oxygen for myoglobin) in the biopsy specimens taken from the apex of canine hearts. Mean cytosolic PO₂ thus determined in the normal beating heart in vivo was 4–6 Torr. Myoglobin oxygenation was quite stable to wide variations in arterial blood PO₂, ranging ~30 to >400 Torr, which suggested a fine control of intracellular PO₂ in vivo.

Gayeski and Honig [1] conducted precise spectrophotometric measurements of SmB in the subepicardial myocytes in animals of various species. They quickly froze the ventricle of the beating heart in situ by using a copper heat sink that had been cooled in liquid nitrogen. The specimen was transferred to the cooled stage of the microscope, and SmB was determined spectrophotometrically. They demonstrated that myoglobin in the in vivo heart at normal oxygen consumption was almost half-saturated with oxygen. Thus median cytosolic PO₂ calculated from the measured myoglobin saturation (assuming P₅₀=5.3 Torr) was 4.3–7.0 Torr in 20 animals from 5 species.

Recently, Wang et al. [27] demonstrated that in vivo measurement of deoxymyoglobin is possible from the ¹H-NMR signal of the N-delta proton of the proximal histidine. In the heart, the ¹H-NMR measurement of intracellular oxygenation has been so far reported only in the isolated perfused preparation [28, 29], although it is potentially applicable to the in vivo heart. In the Langendorff perfused heart, PO₂ of the perfusion medium must be elevated to >400 Torr to maintain normal oxidative metabolism, because the oxygen-carrying capacity of the crystalloid is poor. Thus it is usually difficult to extrapolate the in vivo intracellular PO₂ from the data obtained in the crystalloid perfused heart. After compensation for the low convective oxygen delivery in the perfused heart by an addition of red blood cells to the perfusate (hematocrit ~45%), Jelicks and Wittenberg [28] conducted an ¹H-NMR spectroscopy of deoxymyoglobin in the isolated rat heart. The fractional oxygen saturation of cytosolic myoglobin volume averaged over a heart was 0.7 when the red cell containing perfusate was equilibrated with 20% oxygen gas. Assuming myoglobin P₅₀=2.5 Torr, cytosolic PO₂ was estimated to be 5.8 Torr.

Despite considerable difficulties of the in vivo measurement in the heart, the data obtained from the different techniques indicate that cytosolic myoglobin in the blood-perfused heart beating at normal oxygen consumption is 30–50% deoxygenated. Partial deoxygenation of myoglobin in the in vivo heart is consistent with the proposed functions of myoglobin in facilitation of oxygen transport [4, 16, 30].

¹Coronary venous PO₂ is sometimes assumed to represent “tissue PO₂.” This term is misleading because steep gradients of PO₂ exist within the myocardial tissue.
ing these data, the \( PO_2 \) value of \( \sim 5 \) Torr may be a reasonable estimation of the average intracellular (cytosolic) \( PO_2 \) in the \emph{in vivo} heart at normal oxygen demand.

3. Magnitude of extracellular \( PO_2 \) gradients at normal oxygen flux

If the sarcolemmal \( PO_2 \) can be approximated by the average myoglobin-reported \( PO_2 \), the pressure head for moving oxygen molecules from the capillary to the cell surface would be about 15 Torr at the normal oxygen flux. If the typical distance from the capillary lumen to the sarcolemma of 2 \( \mu \text{m} \) [31] is used, the average slope of the extracellular \( PO_2 \) gradient would be 8 Torr/\( \mu \text{m} \) in the heart. If the distance of 0.5 \( \mu \text{m} \) [5] is used, the slope would be as steep as 30 Torr/\( \mu \text{m} \).

The \( PO_2 \) gradient is defined as the oxygen flux density (oxygen flux per unit area) divided by the oxygen conductance (diffusion coefficient \( \times \) solubility). The following factors account for the steep extracellular \( PO_2 \) gradients \emph{in vivo}. First, the effective oxygen conductivity of the extracellular space is small compared with that in the intracellular (cytosolic) space because of the lack of a specific oxygen carrier such as myoglobin in the cytosol. Honig and Gayeski [4] suggested the substantial role of such a carrier-free region in producing large oxygen diffusion resistance. Second, oxygen flux density at the capillary lumen is quite large because the capillary luminal surface area is about five times smaller than the surface area of the sarcolemma [5]. Consequently, large gradients of \( PO_2 \) are produced at the capillary lumen. Third, the unloading of oxygen from the capillary blood is neither spatially nor temporally uniform, since red blood cells in the capillary are separated by layers of plasma and flow in a discrete pattern [32]. Therefore, intermittent bursts of oxygen flux in accordance with the transit of red blood cells further increase the oxygen flux density and thus \( PO_2 \) gradients [5, 33–35]. It is generally believed that these factors together produce notably large \( PO_2 \) gradients in the extracellular space.

The validity of these interpretations was tested by using detailed mathematical models. In fact, the models of oxygen diffusion in the red skeletal muscle predict 10–20 Torr gradients of \( PO_2 \) in the extracellular space at maximal oxygen consumption [3, 36]. In contrast, mathematical models using known parameters for tissue oxygen diffusion univocally predict an extracellular \( PO_2 \) gradient in the resting red skeletal muscle of only 1–2 Torr [3]. The result does not seem to fit the experimental findings obtained in the beating heart that show remarkably large \( PO_2 \) gradients even at the normal oxygen consumption described above.

The reason for the inconsistency is not clear. The error may arise from the oxygen diffusion coefficient in the extracellular space, especially in the capillary wall [37, 38], and/or from the consumption of oxygen by the endothelial cell [39], which is neglected in most mathematical models of cellular oxygen transport. The assumption that the average cytosolic \( PO_2 \) is equivalent to the sarcolemmal \( PO_2 \) may be questioned because oxygen molecules, once entered into the intracellular space, rapidly bind to myoglobin; thus the concentration of free oxygen quickly drops [5]. This would significantly underestimate the \( PO_2 \) at the cell surface and give larger extracellular gradients (Section II-2).

Experimental evidence obtained in the normal \emph{in vivo} heart demonstrated large extracellular \( PO_2 \) gradients that consequently result in low intracellular \( PO_2 \). The mitochondrial respiratory enzyme (cyt aa3) is half saturated with oxygen at extremely low \( PO_2 \) (\( K_m = 0.05–0.1 \) Torr) [40]. In the heart, therefore, average cytosolic \( PO_2 \) \emph{in vivo} is still several Torr higher than the critical \( PO_2 \) of mitochondria. Therefore, whether diffusional oxygen transport affects the mitochondrial respiration depends on the \( PO_2 \) gradient from the sarcolemma to the mitochondrial inner membrane, the "intracellular \( PO_2 \) gradient."

II. Intracellular \( PO_2 \) Gradients

Because average cytosolic (sarcolemmal) \( PO_2 \) of the \emph{in vivo} heart is low, intracellular \( PO_2 \) gradients may be highlighted as a critical determinant of \( PO_2 \) at mitochondrial cyt aa3 and thus oxidative phosphorylation. It should be emphasized here that intracellular \( PO_2 \) gradients have two distinct meanings, separately discussed below. One refers to the difference of \( PO_2 \) between mitochondrial membrane and the adjacent cytosol at any point within a cell ("perimitochondrial \( PO_2 \) gradient"). The other means gradual changes in intracellular \( PO_2 \) along the diffusion path of oxygen. Ideally, this is equivalent to changes in intracellular \( PO_2 \), measured perpendicular to the capillary direction (or long axis of the cell), from the sarcolemma to the center of the cell ("radial \( PO_2 \) gradient").

1. Perimitochondrial \( PO_2 \) gradient

The consumption of oxygen in a mitochondrion should produce gradients of oxygen concentration around this intracellular oxygen sink. The magnitude of the perimitochondrial \( PO_2 \) gradient is indeed a matter of serious controversies.

Tamura \emph{et al.} [41] first demonstrated in the isolated perfused rat heart that graded reductions of perfusate \( PO_2 \) resulted in concomitant reductions of cytochrome
aa₃ oxidation and myoglobin oxygenation. PO₂ at half maximal reduction of cytochrome aa₃ was 0.21 μM (0.15 Torr) in state 3 isolated pigeon heart mitochondria [42] and P₅₀ of myoglobin was 2.4 Torr at 25°C. Therefore the parallel changes in myoglobin oxygenation and cyt aa₃ oxidation were interpreted to imply significant (>2 Torr) gradients of PO₂ between cytosol and mitochondrial inner membrane. These concurrent changes in the two oxygen indicators with different oxygen affinities, later termed the gradient coherence [43], were considered to be the evidence for significant perimitochondrial PO₂ gradients [41, 44, 45].

Dependency of the gradient coherence on oxygen flux was demonstrated in the isolated perfused rat heart working under an elevated load, being consistent with the proposed origin of the coherency [46]. In the recent study of Chung and Jue [2], myoglobin oxygenation in the perfused rat heart was determined by the ¹H-NMR technique, while myocardial oxygen consumption was regarded to represent oxygen availability at mitochondria. The slope of the relationship between myoglobin oxygenation (ranging 0–100%) and myocardial oxygen consumption was unity with the null intercept, thus demonstrating a strict gradient coherence. Kennedy and Jones [45] showed such a gradient coherence in single cardiomyocytes isolated from the adult rat. Interestingly, the relationship between Sₘₕ and cyt aa₃ oxidation was not affected by changes in oxygen flux induced by using an uncoupler of oxidative phosphorylation (FCCP) or a respiratory inhibitor (antimycin A).

Several studies are, however, in contradiction to significant perimitochondrial PO₂ gradients. Using a suspension of isolated cardiomyocytes of the rat and the spectrophotometric determination of Sₘₕ and cyt aa₃ oxidation, Wittenberg and Wittenberg [25] reached the contrasting conclusion. They demonstrated that the respiratory function of mitochondria was not altered until myoglobin was largely deoxygenated. Thus PO₂ difference between cytosol and mitochondrial inner membrane was <0.2 Torr even when mitochondrial oxygen consumption was increased 3.5-fold by an uncoupler of oxidative phosphorylation. Using the oxygen quenching of phosphorescence of water-soluble and lipid-soluble probes, Vanderkooi et al. [24] directly quantitated the PO₂ drop at the mitochondrial surface. The oxygen concentration drop was less than the error of measurement (<1 μM or 0.7 Torr). These results are consistent with predictions by mathematical models of oxygen diffusion near the isolated mitochondria [47, 48]. Extremely small perimitochondrial PO₂ gradient is inferred simply from the very low oxygen flux density at mitochondrial membrane, because the surface area of the mitochondrial membrane is 50–200 times larger than that of the capillary lumen [5].

As outlined above, agreement on the magnitude and physiological significance of the perimitochondrial gradients has not been obtained yet. Theoretically, diffusional oxygen concentration gradients between cytosol and mitochondria of as small as a few Torr may account for the gradient coherence [41, 45], although the direct measurement of PO₂ near the surface of mitochondria and theoretical analyses do not seem to favor such concentration gradients. Instead, these facts seem to suggest the possibility that the gradient coherence may not always imply diffusional concentration gradients of oxygen at the mitochondrial surface. Chance [43] proposed that the gradient coherence can be demonstrated in the tissue volume that consists partly of normoxic populations and partly of anoxic populations of mitochondria. These situations are likely to arise from microscopic intercapillary gradients of oxygen concentration at relatively high oxygen flux [43, 49]. Then radial PO₂ gradients produced within an individual cardiomyocyte would be highlighted as a factor that determines cyt aa₃ oxidation, rather than the perimitochondrial gradients.

2. Radial PO₂ gradient

Myoglobin significantly facilitates diffusion of oxygen in the in vivo heart [16]. The rate of facilitation partly depends on the PO₂ at which myoglobin is exposed, in such a way that facilitation of oxygen diffusion increases as myoglobin is deoxygenated. The maximum facilitation occurs when myoglobin is almost deoxygenated where the effective oxygen conductivety would increase by a factor of approximately 6 [3]. Thus the facilitated diffusion of oxygen is believed to account for shallow intracellular PO₂ gradients. On the other hand, oxygen molecules must diffuse a much longer distance (8.5 μm) [50], compared with the extracellular diffusion path length (<2 μm), until they reach the core of the myocyte from the sarcolemma. This leads to speculation that significant gradients of PO₂ may be generated between the sarcolemma and the core of a cardiomyocyte (radial PO₂ gradients), especially when oxygen flux is elevated.

In the cardiomyocyte, mitochondria (oxygen sink) run parallel with the myofibrils without any condensation within a cardiomyocyte. Therefore to explore the radial PO₂ gradients arising from mitochondrial oxygen consumption, intracellular PO₂ should be measured in a single individual cardiomyocyte with a spatial resolution of the size of mitochondria (~1 μm). Gayeski and Honig [1] attempted to resolve the intracellular distribution of oxygenated myoglobin in an
Fig. 1. Representative data demonstrating the radial changes in intracellular oxygenation in an individual single cardiac myocyte isolated from the rat ventricle. Oxygen consumption of the cell was significantly elevated by using an uncoupler of oxidative phosphorylation, 1 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP). A: Fractional myoglobin oxygen saturation determined by a microspectrophotometric technique at extracellular PO₂ of 15.2, 22.8, and 30.4 Torr. The data are from the identical cell. B: Profile of radial intracellular PO₂ gradients calculated from the actually measured Sₘ₉₁ in (A) and the separately determined calibration curve. Intracellular PO₂ profile was not calculated for extracellular PO₂=30.4 Torr. The width of this particular cell was 23.9 μm. Measurements were conducted at 27°C. See Takahashi et al. [52] for detail.

3. Effects of intracellular PO₂ gradients on oxidative phosphorylation

The apparent Kₘ of cyt aa₃ determined in isolated mitochondria can be used to predict the oxygen dependency of cardiac energy metabolism with the knowledge of intracellular (cytosolic) PO₂. For example, Hoshi et al. [40] reported that Kₘ for isolated rabbit heart mitochondria in state 3 is 0.16 μM (0.11 Torr). When Michaelis-Menten kinetics are assumed, mitochondrial oxidative phosphorylation would be partially (20%) inhibited at the PO₂ of 0.44 Torr. If the highest Kₘ value recently reported for isolated state 3 heart mitochondria of 1.5 μM (1.1 Torr) [55] is used, such critical intracellular PO₂ would elevate to about 4.4 Torr. Values for P₅₀ of cardiac myoglobin at 37°C determined in vitro are 2.1 Torr [16], 2.3 Torr [5], 2.4 Torr [56], and 5.3 Torr [1]. Because cytosolic myoglobin is partially deoxygenated in the in vivo heart, it is predictable that intracellular PO₂ gradients of a small magnitude definitely affect cyt aa₃ oxidation [57].

This conclusion is supported from the in vitro studies of oxygen dependencies of cellular oxidative metabolism [31, 58, 59]. For example, Kreutzer and Jue [31] demonstrated in the isolated perfused heart that cardiac work was reduced by 20% with a concomitant
increase in the rate of lactate formation when cytosolic $P_O_2$ was lowered to 4 Torr. In the isolated single cardiomyocytes, Smolenksi et al. [59] detected tissue hypoxia by the release of intracellular adenosine at $P_O_2=3$ Torr. These results seem to suggest that at normal oxygen demand, $P_O_2$ usually found at cytosolic space would be in the regulatory range of oxidative phosphorylation in the presence of moderate intracellular $P_O_2$ gradients.

At elevated oxygen flux, Takahashi et al. [60] directly demonstrated the effect of intracellular radial $P_O_2$ gradients on oxidative metabolism in a single cardiomyocyte where mitochondrial reduced nicotinamide adenine dinucleotide (NADH) fluorescence was used as an endogenous indicator of oxygen deficiency. In a single cardiomyocyte treated with an uncoupler of oxidative phosphorylation, significant heterogeneities of NADH fluorescence were demonstrated that were the mirror image of the spatial pattern of $S_{Mb}$ (Fig. 2). In contrast, no significant heterogeneity was found in the NADH fluorescence of untreated individual single cardiomyocytes. These results strongly suggest that steep radial gradients of intracellular $P_O_2$ are the major limiting factor of oxidative phosphorylation at elevated oxygen demand.

It is concluded that the intracellular $P_O_2$ gradient, although its absolute magnitude may be small, affects the oxidative metabolism in the in vivo heart at normal oxygen consumption. A limitation of cellular oxidative metabolism imposed by the diffusional intracellular oxygen delivery is most profound at increased oxygen demand where the radial $P_O_2$ gradient is significantly increased.

III. Regulations of Intracellular Oxygen Supply and Oxidative Phosphorylation

Because of the low mitochondrial $P_O_2$ in the normal heart resulting from significant extracellular and intracellular $P_O_2$ gradients, mitochondrial oxidative phosphorylation and thus mechanical functions of the normoxic heart are in part determined by diffusional oxygen delivery to mitochondria. Dependency of the cardiac oxidative metabolism on intracellular oxygen diffusion is most evident when oxygenation of mitochondria is compromised either by hypoxemia or by elevated myocardial oxygen demand.

Because of the effect of diffusional oxygen supply on the cardiac metabolism, oxygen supply to mitochondria and oxidative phosphorylation should be under precise regulation in the in vivo heart. It has been known that coronary venous $P_O_2$ is maintained relatively constant under wide variations of arterial oxygen supply [15]. The tight linkage between cardiac work and myocardial oxygen consumption implies the precise matching of the mitochondrial oxygen demand and diffusional supply of oxygen under remarkably wide changes in oxygen flux.

Direct measurements of intracellular (cytosolic) $P_O_2$ in the heart are supportive for such homeostasis. Gayeski and Honig [1] demonstrated that $S_{Mb}$ in the left ventricle of the in vivo canine heart was affected neither by changes in arterial blood $P_O_2$ ranging from 57 to 326 Torr, nor by a 5-time increase in the cardiac work. Similarly, Coburn et al. [26] pointed out a constancy of $S_{Mb}$ in the beating heart under wide changes in arterial $P_O_2$ unless it was below 30–35 Torr. In the red cell perfused isolated heart, Jellicks and Wittenberg [28] showed that an 8-fold increase in the heart rate resulted in no change in the $^1$H-NMR-determined deoxymyoglobin signal.

From the standpoint of diffusional oxygen transport, the stability of intracellular $P_O_2$ and protection against impaired coronary oxygen supply and/or increased cellular oxygen demand in the in vivo heart may be explained, according to the extent of perturbations, as follows.

1. Regulation of oxygen supply by changes in the coronary capillary density

In the normal myocardium, only 60–80% of the
more than 2,000 capillaries per mm\(^2\) tissue are functioning [50]. A deficiency of oxygen supply, irrespective of the cause (hypoxemia or elevated metabolic oxygen demand), significantly increases the number of functioning capillaries by the recruitment of closed capillary vessels. An increase in the capillary density produces at least two beneficial effects on the diffusional oxygen delivery. First, it augments the convective oxygen supply that maintains oxygenation of the capillary blood, thus sustaining extracellular PO\(_2\). Second, it decreases the diffusion distance for oxygen [61], for example, to 5.5 \(\mu\)m, from the normal 8.5 \(\mu\)m, during prolonged anoxia [50]. A reduction of the distance from the capillary to the cell core reduces the intracellular radial PO\(_2\) gradients, thus sustaining intracellular PO\(_2\). Although the signal and its transduction mechanism are not fully understood, the regulation of coronary circulation in intracellular hypoxia serves as the most important adaptive mechanism of diffusional intracellular oxygen supply in vivo.

2. Maintenance of oxidative metabolism by a mitochondrial intrinsic mechanism

The synthesis of ATP coupled with the mitochondrial respiration is regulated directly or indirectly by numerous signals arising from cytosol and mitochondria, including PO\(_2\) at cyt aa\(_3\), cytosolic phosphorylation potential (\([\text{ATP}]/[\text{ADP}][\text{P}])\), mitochondrial redox potential (\([\text{NAD}^+]/[\text{NADH}]\)), cytosolic phosphocreatine concentration, intracellular free calcium (Ca\(^{2+}\)) concentration, and even cytosolic regulatory protein such as IF\(_1\) [62–65]. Among these, the interaction of cyt aa\(_3\) and oxygen appears to be an important regulatory factor, especially in the in vivo heart.

Oxygen affinity of the mitochondrial cyt aa\(_3\) may change in such a way that the apparent \(K_a\) of cyt aa\(_3\) decreases when the cellular energy state defined by the phosphorylation potential and mitochondrial redox potential is low [40, 55, 66–68]. Thus cyt aa\(_3\) provides an intrinsic mechanism by which mitochondria are able to sustain oxygen consumption and ATP production even in severe hypoxia. This mechanism may operate after augmentation of the coronary capillary oxygen supply can no longer sustain intracellular oxygenation.

3. Restoration of mitochondrial oxygen supply by reduction of oxygen flux, the final defense mechanism

In mild intracellular hypoxia, the mechanisms described above may effectively maintain oxidative phosphorylation in the in vivo heart. In prolonged severe hypoxia/anoxia, these mechanisms are not sufficient to maintain cellular energy production, and glycolysis is significantly stimulated with concomitant downregulation of cardiac contractions.

In severe hypoxia/anoxia, an insufficiency of oxygen supply to mitochondria automatically reduces mitochondrial oxygen consumption according to the Michaelis-Menten kinetics. This decrease of oxygen flux also decreases PO\(_2\) gradients from the capillary blood to mitochondria because the magnitude of PO\(_2\) gradients is proportional to the oxygen flux. This reduction of PO\(_2\) gradients during hypoxia was demonstrated in a single cardiomyocyte by Takahashi and Doi [69]. Namely, PO\(_2\) gradients from the extracellular medium to cytosolic space was 2.1 Torr for extracellular PO\(_2\) of 4.4 Torr, which was reduced to 1.6 Torr when the extracellular PO\(_2\) was lowered to 2.4 Torr. The PO\(_2\) gradient was negligible at extracellular PO\(_2\) of 0.6 Torr.

A reduction of oxygen flux because of severe hypoxia/anoxia thus partially restores the oxygen supply and finally sustains respiration at a lower level. In other words, at low intracellular PO\(_2\) (<2 Torr), PO\(_2\) at mitochondria is at the equilibrium point determined from (1) PO\(_2\)-dependent respiration of mitochondria (the Michaelis-Menten relationship) and (2) respiration-dependent PO\(_2\) at mitochondria (Fick's first law of diffusion) (Fig. 3).

In the anoxic cardiomyocyte, cell death is preceded by a release of loads of Ca\(^{2+}\) accumulated in mitochondria. This may occur immediately following the collapse of mitochondrial membrane potential [70]. During severe hypoxia/anoxia, a deficiency of mitochondrial oxygen supply decreases mitochondrial respiration according to the Michaelis-Menten kinetics. A low rate of oxidative phosphorylation in this situation would be unable to produce ATP sufficient to sustain cardiac contractions, while a minimum PO\(_2\) at the respiratory chain, just enough to prevent disruption of mitochondrial membrane potential, may be possible [64] by significantly reduced PO\(_2\) gradients. Therefore this mechanism may serve as the final defense mechanism of diffusional oxygen transport for the prevention of irreversible cell damage.

IV. Conclusion

In the beating heart, a relatively high oxygen flux produces steep gradients of PO\(_2\) in the extracellular space. Consequently, intracellular PO\(_2\) in the normal heart is low and myoglobin is thus partially deoxygenated. Although oxygen affinity of mitochondrial cyt aa\(_3\) is remarkably high, the low intracellular PO\(_2\) affects the redox state of the respiratory enzyme in the presence of intracellular PO\(_2\) gradients of a small
Fig. 3. A diagram illustrating the effect of the depression of mitochondrial oxygen consumption (VO₂) on restoration of PO₂ at mitochondria (PM) in hypoxia.

Lines a and b represent Fick's law of diffusion, which determines PM as a function of VO₂. VO₂ = (PCap - PM) / R where PCap and R denote capillary blood PO₂ and oxygen diffusion resistance from the capillary lumen to the mitochondrial inner membrane, respectively. Curve c defines the relationship between PM and VO₂ (Michaelis-Menten relationship). The interception of line a and curve c determines PM (A) and VO₂ in normoxic myocardium. In hypoxia, a decrease in PCap shifts line a to line b. If mitochondrial oxygen consumption is not altered (dashed line) despite a reduction of PCap (line b), mitochondrial PO₂ would be greatly decreased to point C. However, a concomitant decrease in VO₂ restores PM to point B.

magnitude. The limitation of oxidative metabolism by the diffusional transport of oxygen is most significant in hypoxemia and elevated oxygen consumption. Especially in the latter, radial intracellular PO₂ gradients could produce a significant oxygen-depleted core in the intracellular space of individual myocytes. Therefore the oxidative metabolism in the in vivo heart depends on diffusional oxygen transport.

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