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 (PO_2) in the extracellular space. In addition, the intracellular radial gradients of PO_2 may not be ignored particularly when the cellular oxygen consumption is increased. These PO_2 gradients together result in a

quite low intracellular PO_2 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 PO_2 gradient in the myocardium are discussed. [Japanese Journal of Physiology, 48, 243–252, 1998]

Key Words: intracellular oxygen, diffusion, heart, PO_2 gradient, oxidative phosphorylation.

Oxygen transport from the capillary blood to intracellular mitochondria is a process governed by the law of diffusion. 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 diffusion path. Therefore the oxygen partial pressure (PO_2) 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 (S_{Mb}) in the normal beating heart addressed the presence of surprisingly steep PO_2 gradients in the extracellular space [1]. A consequence of this gradient is a very low intracellular PO_2 . Thus PO_2 gradients from the capillary blood to mitochondria are the major determinant of PO_2 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 PO_2 gradient in the heart will be discussed.

I. Extracellular PO_2 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 PO_2 between the surface of the red blood cell in the capillary and the sarcolemma is designated as the “extracellular PO_2 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 PO_2 gradients, despite a very short diffusion path length (usually assumed to be $<2\ \mu\text{m}$) [2–5].

1. Capillary blood PO_2 in the myocardium

Intramicrovascular blood PO_2 was first estimated from the perivascular PO_2 measured by a polarographic oxygen microelectrode [6]. This technique, however, turned out to be inappropriate because significant PO_2 gradients exist in the vicinity of the vascular wall [1, 7, 8]. Subsequently, the spectrophotometric determination of hemoglobin oxygen satura-

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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 PO_2 in the myocardium has not yet been accurately determined. Instead, coronary venous blood PO_2 was used to represent the mean end-capillary blood PO_2 ¹ [14]. In the beating heart at normal oxygen consumption, mean capillary blood PO_2 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 PO_2 , presumably of capillaries and venules in the epicardium 0.5–1 mm deep, ranged 18–26 Torr.

2. Sarcolemmal PO_2 estimated from average cytosolic PO_2

In the heart, a direct measurement of PO_2 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 PO_2 gradients was calculated by using the mean intracellular (cytosolic) PO_2 in cardiomyocytes. Rationales for the use of cytosolic PO_2 : (1) Accurate measurement of mean cytosolic PO_2 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 PO_2 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 PO_2 from the measurement of ^{14}CO binding to myoglobin (that competes with oxygen for myoglobin) in the biopsy specimens taken from the apex of canine

hearts. Mean cytosolic PO_2 thus determined in the normal beating heart *in vivo* was 4–6 Torr. Myoglobin oxygenation was quite stable to wide variations in arterial blood PO_2 , ranging ~ 30 to >400 Torr, which suggested a fine control of intracellular PO_2 *in vivo*.

Gayeski and Honig [1] conducted precise spectrophotometric measurements of S_{Mb} 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 S_{Mb} 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 PO_2 calculated from the measured myoglobin saturation (assuming $P_{50}=5.3$ 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, PO_2 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 PO_2 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 $\sim 45\%$), 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 $P_{50}=2.5$ Torr, cytosolic PO_2 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]. Summariz-

¹Coronary venous PO_2 is sometimes assumed to represent "tissue PO_2 ." This term is misleading because steep gradients of PO_2 exist within the myocardial tissue.

ing these data, the PO_2 value of ~ 5 Torr may be a reasonable estimation of the average intracellular (cytosolic) PO_2 in the *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/ μm in the heart. If the distance of $0.5\ \mu\text{m}$ [5] is used, the slope would be as steep as 30 Torr/ μm .

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 *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 unequivocally 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 *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 *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 *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 *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. PO_2 at half maximal reduction of cytochrome aa₃ was 0.21 μ M (0.15 Torr) in state 3 isolated pigeon heart mitochondria [42] and P_{50} 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 PO_2 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 PO_2 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 S_{Mb} 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 PO_2 gradients. Using a suspension of isolated cardiomyocytes of the rat and the spectrophotometric determination of S_{Mb} 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 PO_2 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 PO_2 drop at the mitochondrial surface. The oxygen concentration drop was less than the error of measurement ($<1 \mu$ M 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 PO_2 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 PO_2 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 PO_2 gradients produced within an individual cardiomyocyte would be highlighted as a factor that determines cyt aa₃ oxidation, rather than the perimitochondrial gradients.

2. Radial PO_2 gradient

Myoglobin significantly facilitates diffusion of oxygen in the *in vivo* heart [16]. The rate of facilitation partly depends on the PO_2 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 conductivity would increase by a factor of approximately 6 [3]. Thus the facilitated diffusion of oxygen is believed to account for shallow intracellular PO_2 gradients. On the other hand, oxygen molecules must diffuse a much longer distance (8.5 μ m) [50], compared with the extracellular diffusion path length ($<2 \mu$ m), until they reach the core of the myocyte from the sarcolemma. This leads to speculation that significant gradients of PO_2 may be generated between the sarcolemma and the core of a cardiomyocyte (radial PO_2 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 PO_2 gradients arising from mitochondrial oxygen consumption, intracellular PO_2 should be measured in a single individual cardiomyocyte with a spatial resolution of the size of mitochondria ($\sim 1 \mu$ m). 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 P_{O_2} of

15.2, 22.8, and 30.4 Torr. The data are from the identical cell. **B:** Profile of radial intracellular P_{O_2} gradients calculated from the actually measured S_{Mb} (in A) and the separately determined calibration curve. Intracellular P_{O_2} profile was not calculated for extracellular P_{O_2} =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.

individual cardiomyocyte of the *in vivo* heart by using the cryospectrophotometry. Using a measuring diaphragm of $2 \times 3 \mu\text{m}$, they compared S_{Mb} of the regions near the sarcolemma and the cell core. In a single cardiomyocyte 16 μm in diameter, the difference of S_{Mb} measured within 2 μm of the sarcolemma and at the center of the cell cross-section were identical. Thus the radial P_{O_2} gradient in the cardiomyocyte at normal oxygen flux seems negligible.

Isolated single cardiomyocytes offer an opportunity to examine heterogeneities of intracellular P_{O_2} without the effects of heterogeneous extracellular oxygen supply by capillary vessels. Takahashi and Doi [51] demonstrated that average intracellular oxygen level can be determined in a single individual cardiomyocyte by using myoglobin light absorption. The method was further extended to generate a S_{Mb} map of a single individual cardiomyocyte with a subcellular spatial resolution [52]. Then the magnitude of the radial P_{O_2} gradient was determined at an average S_{Mb} of 0.4–0.7 that is similar to the *in vivo* heart [1, 5, 26]. Radial P_{O_2} gradients were clearly detected within a single cell when the oxygen flux was increased approximately 8-fold by an uncoupler of oxidative phosphorylation (Fig. 1). The calculation of intracellular P_{O_2} from the measured S_{Mb} revealed the presence of a quite steep drop of intracellular P_{O_2} near the sarcolemma, and changes in P_{O_2} were significantly attenuated near the cell core (Fig. 1). The profile of intracellular P_{O_2} is in good agreement with that predicted

by the model of maximally working red muscle [3, 53, 54]. No significant change in S_{Mb} was found in a quiescent single cardiomyocyte.

3. Effects of intracellular P_{O_2} gradients on oxidative phosphorylation

The apparent K_m 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) P_{O_2} . For example, Hoshi *et al.* [40] reported that K_m 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 P_{O_2} of 0.44 Torr. If the highest K_m value recently reported for isolated state 3 heart mitochondria of 1.5 μM (1.1 Torr) [55] is used, such critical intracellular P_{O_2} would elevate to about 4.4 Torr. Values for P_{50} 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 P_{O_2} 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

Fig. 2. Representative data demonstrating intracellular heterogeneities of oxidative metabolism as assessed by the mitochondrial NADH fluorescence. The intensity of NADH fluorescence is normalized and indicated in pseudo colors (calibration bar on the right). The cell was treated with $1\ \mu\text{M}$ CCCP at 27°C . Extracellular PO_2 was 22.8 Torr. The increase in the NADH fluorescence near the cell core suggests a suppression of oxidative phosphorylation, presumably a result of deficiency of diffusional oxygen supply to this region. Scale bar = $10\ \mu\text{m}$. See Takahashi *et al.* [60] for details.

increase in the rate of lactate formation when cytosolic PO_2 was lowered to 4 Torr. In the isolated single cardiomyocytes, Smolenski *et al.* [59] detected tissue hypoxia by the release of intracellular adenosine at $PO_2 = 3$ Torr. These results seem to suggest that at normal oxygen demand, PO_2 usually found at cytosolic space would be in the regulatory range of oxidative phosphorylation in the presence of moderate intracellular PO_2 gradients.

At elevated oxygen flux, Takahashi *et al.* [60] directly demonstrated the effect of intracellular radial PO_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 PO_2 are the major limiting factor of oxidative phosphorylation at elevated oxygen demand.

It is concluded that the intracellular PO_2 gradient, although its absolute magnitude may be small, affects the oxidative metabolism in the *in vivo* heart at nor-

mal 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 PO_2 gradient is significantly increased.

III. Regulations of Intracellular Oxygen Supply and Oxidative Phosphorylation

Because of the low mitochondrial PO_2 in the normal heart resulting from significant extracellular and intracellular PO_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 PO_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) PO_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 PO_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 PO_2 unless it was below 30–35 Torr. In the red cell perfused isolated heart, Jelicks and Wittenberg [28] showed that an 8-fold increase in the heart rate resulted in no change in the ^1H -NMR-determined deoxymyoglobin signal.

From the standpoint of diffusional oxygen transport, the stability of intracellular PO_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 mm³ 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 μm , from the normal 8.5 μm , 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 aa₃, cytosolic phosphorylation potential ($[\text{ATP}]/[\text{ADP}][\text{P}_i]$), mitochondrial redox potential ($[\text{NAD}^+]/[\text{NADH}]$), cytosolic phosphocreatine concentration, intracellular free calcium (Ca^{2+}) concentration, and even cytosolic regulatory protein such as IF₁ [62–65]. Among these, the interaction of cyt aa₃ and oxygen appears to be an important regulatory factor, especially in the *in vivo* heart.

Oxygen affinity of the mitochondrial cyt aa₃ may change in such a way that the apparent K_m of cyt aa₃ decreases when the cellular energy state defined by the phosphorylation potential and mitochondrial redox potential is low [40, 55, 66–68]. Thus cyt aa₃ 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 suffi-

cient 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 aa₃ 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 (VO_2) on restoration of PO_2 at mitochondria (P_{Mt}) in hypoxia. Lines a and b represent Fick's law of diffusion, which determines P_{Mt} as a function of VO_2 ; $VO_2 = (P_{cap} - P_{Mt})/R$ where P_{cap} and R denote capillary blood PO_2 and oxygen diffusion resistance from the capillary lumen to the mitochondrial inner membrane, respectively. Curve c defines the relationship between P_{Mt} and VO_2 (Michaelis-Menten relationship). The interception of line a and curve c determines P_{Mt} (A) and VO_2 in normoxic myocardium. In hypoxia, a decrease in P_{cap} shifts line a to line b. If mitochondrial oxygen consumption is not altered (dashed line) despite a reduction of P_{cap} (line b), mitochondrial PO_2 would be greatly decreased to point C. However, a concomitant decrease in VO_2 restores P_{Mt} 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 PO_2 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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