Myoglobin facilitates oxygen diffusion

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ABSTRACT

In this study, the hemodynamic effects of acute myoglobin (Mb) inhibition with CO on isolated hearts of wild-type (WT) mice were examined at different degrees of oxygenation. Hearts from myoglobin knockout (myo−/−) mice served as appropriate controls. The intracellular MbO₂ dissociation curve, as measured by ¹H NMR, was determined by systematically lowering the O₂ content of the perfusion medium. At 100% MbO₂ saturation (buffer O₂: 75%), complete inhibition of Mb with 20% CO did not alter left ventricular developed pressure (LVDP) or coronary venous PO₂ (PvO₂) and thus myocardial O₂ consumption. At 87% MbO₂ saturation (buffer O₂: 65%), CO applied to WT hearts significantly decreased LVDP by 12% and increased PvO₂ by 30% (both P<0.005) respectively, whereas no effects were observed in myo−/− hearts. Cell width in isolated myo−/− as compared with WT cardiomyocytes was reduced (4.8 µm vs. 5.4 µm, P<0.001), whereas cell length did not differ. At ambient PO₂ of 8 mm Hg, oxygen consumption of stimulated myo−/− cardiomyocytes was only 60% that of WT controls (P<0.001). Our results do not support Mb-mediated oxidative phosphorylation in the beating mouse heart. However, we find conclusive evidence that Mb is important in facilitating O₂ diffusion from the vasculature to mitochondrial cytochromes and that the oxygen reservoir of myoglobin is of functional relevance in the beating mammalian heart.

Key words: facilitated diffusion • ischemia • magnetic resonance spectroscopy • myoglobin knockout mouse • oxidative phosphorylation

Myoglobin (Mb) is generally considered an important intracellular O₂ binding hemoprotein found in the cytoplasm of vertebrate type I and IIa skeletal and cardiac muscle tissue (1). In mammals, O₂ half saturation of Mb is achieved at intracellular O₂ partial pressure as low as 2 mmHg (horse heart Mb, 35°C, pH 7.0 (2)), which suggests a predominance of oxygenated Mb under basal conditions. In exercising skeletal muscle and in the beating heart, Mb is therefore thought to serve as a short-term O₂ reservoir, tiding the muscle over from one contraction to the next (3). In diving mammals, Mb concentrations exceed those in terrestrial mammals up to 10-fold and may serve as an O₂ store contributing to the extension of diving time (4, 5). Similarly, Mb is expressed in high concentrations in skeletal muscle of mammals and humans adapted to high altitudes (6, 7).
Tissue hemoproteins have also been identified as biochemical catalysts serving various functions. Studies conducted in mammalian isolated mitochondria and cardiomyocytes have suggested that Mb can augment mitochondrial oxidative phosphorylation directly (8–10). However, the precise mechanism remains elusive and attempts to demonstrate this effect in perfused myocardium have led to inconsistent findings (11–13).

Mb has further been proposed to facilitate intracellular O₂ diffusion (4), positioning Mb as critical link between capillary O₂ supply and the O₂-consuming cytochromes located in mitochondria. Most likely there is a parallel flux of Mb-bound O₂ (facilitated O₂ diffusion) and dissolved O₂. Facilitated O₂ diffusion has been demonstrated unambiguously in concentrated Mb and hemoglobin solutions (14). Experiments aimed at investigating this function of Mb in isolated cells, papillary muscle, and on the whole organ-level have yielded conflicting results (13, 15–20). Likewise, model calculations have both supported and refuted a contribution of Mb-bound O₂ to total O₂ flux (21–23). In addition, the specificity of inhibitors used in some of the above studies (most commonly carbon monoxide and nitrite) has been a matter of intense debate.

The recent, independent generation of Mb knockout (myo⁻/⁻) mice by Garry et al. (24) and our laboratory (25) has provided the foundation for a new experimental approach to study the proposed diverse functions of Mb. We have recently demonstrated that potent compensatory mechanisms, all aimed to steepen the intracellular PO₂ gradient, are activated in myo⁻/⁻ mice (25). These mechanisms include a higher capillary density, increase in coronary flow and flow reserve, as well as an elevated hematocrit. These findings were taken as indication that Mb may play an important role for the delivery of O₂ by facilitated diffusion.

Although suggestive, previous data do not exclude the possibility that the effect observed as a result of chronic loss of Mb might be only indirectly related to the loss of Mb in myo⁻/⁻ mice. The aim of the present study therefore was to investigate the effect of acute inactivation of Mb in wild-type (WT) mice in which no compensatory adaptation took place. For Mb inactivation, we used carbon monoxide (CO) with myo⁻/⁻ mice serving as appropriate controls.

This approach enabled us to: a) address the question of CO specificity as inhibitor of Mb; b) quantify the role of Mb as oxygen store in the beating mammalian heart; c) test the hypothesis of Mb-mediated oxidative phosphorylation; and d) demonstrate the functional relevance of Mb in intracellular O₂ supply by facilitating O₂ diffusion.

MATERIAL AND METHODS

Animals

Myo⁻/⁻-mice were generated by deleting the essential exon-2 via homologous recombination in embryonic stem cells as described previously (25). A total of 38 and 70 male mice were used in the myo⁻/⁻ and WT group, respectively. Measurement of Mb concentrations was performed in four WT mouse hearts by densitometric scanning of cardiac protein extracts separated by SDS/PAGE. Body weight ranged from 28 to 38 g and heart weight from 180 to 250 mg, with no significant differences between the two groups.
Langendorff experimental setup

We prepared murine hearts and retrograde perfusion at 100 mmHg constant pressure with modified Krebs-Henseleit buffer—gassed at 95% O₂/5% CO₂ (carbogen), resulting in a pH of 7.4 and heated to 35°C—essentially as described (26), by using an isolated heart apparatus (Hugo Sachs Elektronik, March-Hugstetten, Germany). Perfusion pressure, coronary flow, left ventricular developed pressure (LVDP), and coronary venous PO₂ (ṖO₂) were measured continuously, the latter via a Clark-type electrode. Signals were recorded using a PC with dedicated software (EMKA Technologies, Paris, France). All hearts were paced at 400 bpm and allowed to stabilize for 30 min with carbogen buffer at 100 mmHg constant perfusion pressure prior to data acquisition.

H NMR protocol

For NMR measurements, hearts were placed inside a 10 mm NMR tube and transferred into a heated (35°C) 10 mm ¹H/¹⁹F dual probe inside a Bruker AMX 400 NMR spectrometer, as previously described (26). Mouse hearts (4 myo⁻/⁻, 36 WT) were then switched to constant flow perfusion. Following the switch to constant flow, baseline ¹H NMR spectra were acquired for all hearts. The WT hearts were subsequently perfused with buffer equilibrated with either 75%, 65%, 58%, 51%, 44%, 38%, 32%, 25%, or 12% O₂ (n = 4 hearts per O₂ level) and ¹H NMR spectra were acquired. WT hearts were reperfused with carbogen buffer and recovery ¹H NMR spectra were obtained. All hearts were subjected to buffer containing 20% CO, 75% O₂, and 5% CO₂ with final spectra acquisition to determine the background noise of the MbO₂ signal.

Functional decline during ischemia

Hearts of myo⁻/⁻ and WT mice (n = 10 each) were perfused with buffer equilibrated with 20% N₂, 75% O₂, and 5% CO₂. Hearts were switched to constant flow perfusion, while the flow rate at which they had stabilized was maintained. Baseline values were acquired as above and hearts subjected to 15 s of no-flow ischemia followed by 10 min reperfusion. Hereafter, perfusion was switched to buffer equilibrated with 20% CO, 75% O₂, and 5% CO₂. After 10 min of stabilization, baseline values were once more acquired and hearts were subjected to 15 s of ischemia and 10 min reperfusion as above.

Effect of CO on O₂ diffusion

Twenty-four myo⁻/⁻ and 24 WT mouse hearts were switched to constant flow and perfused either with 75%, 65%, 58%, or 51% O₂ (n = 6 + 6 each) buffer. After 12 min, stable functional parameters and ṖO₂ values were obtained. Hearts were then switched to buffer containing 20% CO, keeping the respective O₂ content constant. Stable functional parameters and ṖO₂ values were acquired 12 min later.

Cardiomyocytes

Cardiomyocytes (CMs) were prepared from 4 myo⁻/⁻ and 4 WT mice (29–35 g) essentially as described (27). To measure “intactness” and cell geometry, rod-shaped CMs (47 ± 7% n = 8)
were fixed in glutaraldehyde (0.2%). For functional analysis, the cell suspension was injected into the oxystat system (27). This system enables the incubation of electrically stimulated CMs (9 Hz, 37°C) at a precisely defined ambient PO₂ while measuring O₂ consumption (VO₂). Ambient PO₂ was consecutively reduced from 40 to 30, 20, 15, 10, 8, 5, 2, 1, and 0.5 mmHg. At each ambient PO₂, VO₂ was measured under steady-state conditions for 5–10 min. The protein content was determined at 40 (start), 10, and 0.5 mmHg (end) as described previously (27).

NMR method

For selective excitation of the MbO₂ and MbCO Val E11 resonance (28) at –2 to –3 ppm, the standard 133T pulse sequence of the Bruker library was used. The delay for binomial H₂O suppression was set to 166 µs, which resulted in maximal excitation of the region of interest. A 45° pulse (12.5 µs; estimated from the H₂O signal) was applied, and 16K transients were averaged for a typical 1H NMR spectrum that required 15 min of signal accumulation (acquisition time 42 ms, sweep width 12195 Hz, data size 1K, zero filling to 2K, exponential weighting resulting in a 40 Hz line broadening; chemical shifts were referenced to the H₂O resonance at 4.7 ppm). Excitation with the 133T sequence led to large phase dispersion, which resulted in positive lipid signals and negative signals for the MbO₂ and MbCO Val E11 resonance (Fig. 1, for a theoretical explanation see (29, 30)). Relative peak areas were obtained by integration after baseline correction.

Statistical analysis

We compared changes within groups by using multivariate ANOVA for repeated measurements. Differences between groups were analyzed by multivariate ANOVA followed by Bonferroni’s post-hoc test (all statistical analysis calculated with SPSS 8.0, SPSS, Chicago). A P value <0.05 was taken to indicate a statistically significant difference.

RESULTS

Myoglobin as oxygen reservoir

Cardiac Mb content in WT hearts was determined after SDS/PAGE to be 0.19 ± 0.03 mmol of Mb/kg wet weight (n = 4) and found to be absent in myo⁻/- hearts. 1H NMR experiments were undertaken to identify the MbO₂ signal and to verify its absence in myo⁻/- hearts. As illustrated in Figure 1, the MbO₂ signal was found in WT hearts at –2.8 ppm with excellent signal-to-noise ratio (15 min. accumulation time). Perfusion with the CO-containing medium (20% CO) resulted in the appearance of the MbCO signal at –2.3 ppm, whereas the MbO₂ signal at –2.8 ppm was no longer detectable (Fig. 1), which demonstrated complete inhibition of the oxygen binding capacity of Mb. Both Mb signals were absent in myo⁻/- hearts. As is apparent from Table 1, neither WT nor myo⁻/- hearts displayed a functional response to CO under these conditions.

To assess the functional relevance of the MbO₂ buffer in the mammalian heart, Langendorff perfused hearts from myo⁻/- and WT mice were subjected to brief no-flow ischemia. Figure 2 compares the decrease in dP/dtₘₐₓ observed after the onset of ischemia (baseline parameters given in Table 1). As can be seen, cardiac function decreased more steeply in myo⁻/- hearts,
especially during the first seconds of ischemia. Respective values after 2 sec of ischemia were 9.4 ± 2.8% in the knockout versus 5.4 ± 2.1% in the WT (P<0.05). Thereafter, a more parallel pattern of functional decline develops. Significant differences of similar magnitude were also seen for LVDP and dP/dt\text{min} (P<0.05; data not shown). When beat-to-beat analysis was used, differences in the rate of functional decline were observed for 18 ± 3 heartbeats (P<0.05, data not shown). The ischemia-induced decrease in cardiac function in WT hearts after acute inhibition of Mb by CO was identical to the effect of chronic lack of Mb in hearts from myo-/- mice (Fig. 2). Baseline function was regained within 10 min of reperfusion (data not shown).

**Myoglobin-facilitated oxygen diffusion**

**Isolated heart**

To approximate conditions that might mimic the in vivo situation with respect to Mb, we progressively lowered the PO\text{2} in the perfusion medium by equilibrating the perfusion medium with O\text{2} ranging from 95% to 12%. At the same time the extent of Mb oxygenation was measured by \textsuperscript{1}H NMR spectroscopy. This measurement enabled us to determine the intracellular MbO\text{2} dissociation curve within the beating heart (Fig. 3A). To exclude effects of changes of coronary flow and thus O\text{2} delivery, all experiments were carried out in the constant volume mode. Mb desaturation first appeared at 65% arterial O\text{2} when Mb was desaturated by 13 ± 3%. Equilibrating the medium with 51% O\text{2} decreased MbO\text{2} saturation to 46 ± 8%, the lowest MbO\text{2} saturation of 18.6 ± 5% was reached at 12% arterial O\text{2}. Upon reperfusion with carbogen, hearts showed an almost complete recovery. The relationship between arterial O\text{2} and intracellular PO\text{2} is depicted in Figure 3B. We calculated the intracellular PO\text{2} by using the data on Mb saturation and assuming Mb to be half-saturated at 2 mmHg (2). When perfusion medium was equilibrated with 65% O\text{2} (432 mmHg), the observed 13% reduction of MbO\text{2} saturation corresponds to an intracellular PO\text{2} of ~16 mmHg (Fig. 3B). Further decrease in the O\text{2} content of the medium steeply reduced MbO\text{2} saturation and intracellular PO\text{2} to values <2 mmHg.

Having defined the range at which Mb desaturation occurs in the perfused beating heart, we analyzed the effect of CO on cardiac performance and O\text{2} utilization in myo-/- and WT mice further. As shown in Figure 4, myo-/- and WT hearts perfused at an arterial O\text{2} of 65% exhibited opposite responses when subjected to 20% CO. WT hearts revealed a highly significant increase in coronary venous PO\text{2} (P, 29 ± 6 => 36.7 ± 5.6 mmHg, P<0.001) accompanied by a decrease in LVDP of 11% (P<0.001). In contrast, myo-/- hearts displayed a slight decrease in P, 37 ± 5 => 35 ± 5 mmHg, P<0.05) and could maintain LVDP.

Figure 5 summarizes the influence of CO (20% in equilibrating gas phase) on P, (Fig. 5B) and LVDP (Fig. 5C) at the different levels of MbO\text{2}, ranging from 100% to 51%. When Mb was fully O\text{2} saturated (arterial medium equilibrated with 75% O\text{2}), CO induced no significant change in P, or functional parameters of WT and myo-/- hearts. Under conditions resulting in partially saturated Mb, however, WT hearts responded to CO with an increase in P, and a decrease in LVDP. This effect was most pronounced at 87% MbO\text{2} saturation (arterial O\text{2} at 65%). In contrast, myo-/- hearts, when subjected to CO, could always sustain LVDP with unaltered, or even slightly decreasing, P,.
Cardiomyocytes

To investigate whether the observed differences in the rate of VO₂ can also be determined at the cellular level, isolated cardiomyocytes (CM) from WT and myo⁻/⁻ hearts were analyzed at defined PO₂ by using an Oxystat system (27). VO₂ of freshly isolated, stimulated mouse CMs was found to be identical in WT and myo⁻/⁻ CMs at 14.5 ± 1.5 (n = 4) and 14.6 ± 1.0 nmol-min⁻¹·mg protein⁻¹ (n = 3), respectively. As illustrated in Figure 6, reduction in ambient PO₂ from 40 mmHg to 8 mmHg did not change VO₂ of WT myocytes, whereas PO₂ values below 8 mmHg led to a rapid decline of VO₂. In myo⁻/⁻ CMs, the onset of VO₂ decrease was observed at a higher PO₂ and VO₂ reduction was more pronounced. Thus, VO₂ was decreased to a significantly greater extent at low ambient PO₂ in myo⁻/⁻ compared with WT CMs (i.e., at 10 mmHg ambient PO₂: 57 ± 15 versus 89 ± 10% of baseline, respectively). Cell morphology revealed the width of myo⁻/⁻ CMs to be smaller than that from WT CMs (4.8 ± 2.1 vs. 5.4 ± 2.6 µm, n = 133 and 163 respectively, p<0.001), whereas length did not differ (74 ± 21 vs. 71 ± 18 µm, p = n.s.).

DISCUSSION

The major finding of this study is that acute inhibition of Mb under conditions of minor Mb deoxygenation leads to significant impairment of left ventricular contractility and reduced myocardial O₂ consumption. In conjunction with our studies on the O₂ uptake of stimulated cardiomyocytes, these findings show conclusively that Mb facilitates the diffusion of O₂ from the vasculature to mitochondrial cytochromes in the beating heart. We also quantified the role of Mb as O₂ reservoir and tested the hypothesis of Mb-mediated oxidative phosphorylation. Although Mb appears important during brief periods of ischemia, our data do not support a direct Mb effect on oxidative phosphorylation in the mouse heart.

Myoglobin as oxygen reservoir

Our data demonstrate that lack of Mb significantly enhances the ischemia-induced decline in left ventricular force development. This effect was most pronounced within the first 2 s of ischemia. Because myocardial Mb content (0.19 mmol of Mb/kg wet weight), its O₂ storage capacity (1 mol O₂/mol Mb) and VO₂ are known, the maximum period during which O₂ released from Mb can support myocardial oxidative metabolism is calculated to be 2.8 seconds. This value is consistent with our experimental findings. In addition to tiding the heart over from contraction to contraction, the advantage of such a delay in functional decline may be relevant during short periods of cardiac arrhythmia with resulting decrease in coronary perfusion.

Myoglobin-mediated oxidative phosphorylation

Wittenberg and Wittenberg (8) were the first to propose that Mb supports ATP generation by cardiac cells under conditions of fully oxygenated Mb. In their study, a CO blockade of intracellular Mb function was shown to abolish about one-third of the O₂ uptake of resting isolated rat CMs. As underlying mechanism, a preferred uptake of Mb-bound O₂ by mitochondria and/or the acceptance of electrons by sarcoplasmic Mb with concomitant reduction of heme iron ligated O₂ to H₂O were suggested. According to this hypothesis, one would expect myocardial VO₂ to be decreased in hearts lacking Mb or following acute blockade of Mb.
However, we found no significant functional differences between isolated myo⁻/⁻ and WT hearts (Table 1). Furthermore, acute inhibition of Mb by CO at 75% arterial O₂ in the saline-perfused mouse heart (see Figs. 1 and 3) had no effect on myocardial VO₂ and contractile parameters—LVDP, dP/dt max, and rate-pressure-product (RPP)—(Table 1); although the perfusion-medium was of sufficiently high PO₂ to fully oxygenate sarcoplasmic Mb (see Fig. 3). Thus, we found no evidence for Mb-mediated oxidative phosphorylation in the mouse heart.

Myoglobin-facilitated oxygen diffusion

This study establishes that acute inhibition of Mb with CO under conditions of partially deoxygenated Mb results in a significant decrease in VO₂ and contractility (Fig. 4 and Fig. 5). This effect is specific for Mb, because no functional changes were observed in myo⁻/⁻ hearts under otherwise identical conditions. In addition, contracting CMs from myo⁻/⁻ hearts was characterized by lower VO₂ at any given ambient PO₂ below 15 mmHg (Fig. 6), which clearly indicates an increase in the apparent diffusive resistance to O₂. This increase in resistance was observed although cell width in myo⁻/⁻ CMs was significantly reduced (4.7 versus 5.2 µm), which decreases diffusion distances for O₂. Together these findings demonstrate that Mb is essential for the delivery of O₂ from the sarcolemma to the mitochondria by way of Mb-facilitated O₂ diffusion.

To assess the relative contribution of Mb-facilitated O₂ diffusion versus the diffusion of physically dissolved oxygen to intracellular O₂ flux, we have to consider several factors affecting these two parallel pathways. Mass flux via diffusion in general is governed by a) the concentration difference (‘gradient’), b) the diffusion coefficient, and c) the diffusion distance (for review see ref 23). It follows that the quantitative extent of Mb-mediated O₂ diffusion depends primarily on the intracellular concentrations of O₂ and MbO₂ and on the in vivo diffusion coefficients of O₂ and MbO₂, respectively.

The average intracellular concentrations of O₂ and MbO₂ differ by at least 1 order of magnitude. Assuming an intracellular PO₂ of 10 mmHg, the concentration of physically dissolved O₂ is about 13 µM (31). This finding is comparable with a myoglobin concentration of 190 µM determined in the mouse heart in the present study, which is similar to that of larger mammals (e.g., 250 µM Mb in horse heart (32)). In striated muscle, Mb is in the mM range (32)); under well-oxygenated conditions, most of the myoglobin will be oxygenated (see below).

The difference in concentration of O₂ and MbO₂, which favors Mb-facilitated diffusion, is counterbalanced by the diffusion coefficient of physically dissolved O₂ (DO₂), which is up to 100-fold higher than the diffusion coefficient of Mb (DMb) (~2 x 10⁻⁵ cm²/s for DO₂ (33) vs. 2 – 23 x 10⁻⁷ cm²/s for DMb (21, 34)). Unfortunately, precise in vivo data for both DO₂ and DMb are difficult to obtain and a matter of intense debate. Moreover, there seem to be differences depending on muscle type; for example, Conley et al. reported finding higher DMb values in cardiac muscle than in striated muscle (15). An increase in the ratio DMb/DO₂ would result in a higher fraction of O₂ supply by Mb-facilitated O₂ diffusion and vice versa.

Intracellular O₂ diffusion is governed not by the average concentration of O₂ or MbO₂, but by the respective concentration differences between the site of O₂ supply (the sarcolemma) and the site
of O₂ consumption (the mitochondria). Therefore, the concentration gradients for O₂ and MbO₂ have to be considered. Under conditions of a fully oxygenated Mb; that is, in absence of an MbO₂ concentration gradient at a high intracellular PO₂, theory predicts that Mb-facilitated O₂ diffusion does not contribute to the intracellular O₂ flux to any significant extent. In line with this prediction, inactivating Mb with CO at a high arterial PO₂ was without functional effects on the saline perfused heart (see Table 1). Under these conditions, apparently O₂ flux was solely dependent on the diffusion of physically dissolved O₂. In contrast, when arterial O₂ supply was lowered and intracellular Mb partially deoxygenated, blocking Mb by CO significantly reduced VO₂, which demonstrated the importance of Mb-facilitated O₂ diffusion in presence of a MbO₂ gradient within the CM. Direct experimental proof for the intracellular radial MbO₂ gradient has been demonstrated recently in single CMs, with high MbO₂ saturation measured close to the sarcolemma and the nadir located at the cell center (18).

When comparing WT and myo⁻⁻ hearts, it is important to note that the diffusion distance is not identical. We have shown previously that the capillary distance is reduced in myo⁻⁻ hearts (25). The present study demonstrates a reduced width of cardiomyocytes, thereby complementing this finding. It emphasizes that, in the case of Mb deficiency, nature has optimized diffusion distances to boost O₂ delivery by simple diffusion. In line with this interpretation, the highest Mb content has been reported in muscle fibers that exhibit the greatest diffusion distances (15). In this context it is remarkable that O₂ consumption was lower in myo⁻⁻ CMs despite shorter diffusion distance (Fig. 6), which suggests that the reduction in cell width is not sufficient to compensate for the loss of Mb-facilitated O₂ diffusion.

Functional implications

In the present study, Mb-facilitated diffusion clearly played a substantial role in intracellular O₂ transport when ambient PO₂ was low (8 mmHg as seen in isolated cardiomyocytes) and myoglobin was partially deoxygenated (13% Mb vs. 87% MbO₂, as shown in the saline perfused heart). Whether similar conditions exist in vivo has been a matter of debate for decades.

In striated muscle of exercising humans, ¹H NMR spectroscopy enabled the study of MbO₂ saturation (35–37). These studies unequivocally demonstrated that MbO₂ desaturates with exercise; that is, that cellular PO₂ is low enough for unloading of O₂ from MbO₂. Thus parallel diffusion of physically dissolved O₂ and Mb-bound O₂ is likely to occur.

Several groups have also addressed the question of cardiac MbO₂ saturation on the whole organ level in vivo. Based on data obtained by scanning reflectance spectroscopy, average MbO₂ saturation was calculated to be in the order of 92% (38). In apparent contrast, Zhang et al. (39) were unable to detect deoxy-Mb by surface coil ¹H NMR spectroscopy. However, the relatively low sensitivity of ¹H NMR to deoxy-Mb imposes a limit of detection ~10% (39, 40). Consequently, it is very likely that deoxygenated Mb (and thus a MbO₂ concentration gradient) do exist in the blood-perfused heart. Because the present study demonstrated Mb-mediated facilitated diffusion of O₂ in the presence of minor Mb deoxygenation, this effect appears to be functionally relevant also under in vivo conditions. On the basis of the results reported here, we would expect the delivery of oxygen via Mb-facilitated diffusion to become even more important
under conditions of reduced O$_2$ supply (e.g., coronary artery disease) or extended diffusion distance (e.g., cardiac hypertrophy).

REFERENCES


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Table 1.

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Table 1. Hemodynamic data and myocardial O₂ consumption (VO₂) of isolated WT and myo⁻/⁻ hearts perfused under control conditions (medium equilibrated with 75% O₂, 20% N₂, and 5% CO₂) and after replacement of N₂ by 20% CO in the arterial buffer. LVDP, left ventricular developed pressure; dP/dt_max, rate of maximal pressure development; RPP, rate-pressure product.
Figure 1. $^1$H NMR spectra of WT and myo$^{-/-}$ hearts. The MbO$_2$ signal is detected at –2.8 ppm and the MbCO signal at –2.3 ppm. The shift of the NMR signal reflects the CO-induced change in the chemical and electronic environment of the planar heme group, taking effect on the neighboring Val 68 methyl group protons. No Mb signals are detected for myo$^{-/-}$ hearts. Part of the lipid signal is depicted on the left border of all spectra. Data on myoglobin structure were taken from X-ray studies on MbO$_2$ and MbCO (41, 42).
Figure 2. Decline of cardiac function during no-flow ischemia. $dP/dt_{\text{max}}$, rate of maximal pressure development.
Figure 3. Relationship between arterial buffer oxygenation, MbO$_2$ saturation (A) and calculated intracellular PO$_2$ (B) as determined by $^1$H NMR spectroscopy.
Figure 4. Effect of CO (20% in equilibrating gas phase) on venous PO$_2$ (P$_O$$_2$) (A) and left ventricular developed pressure (LVDP) (B) in the WT and myo$^{-/-}$ group (n = 6, each), 65% arterial O$_2$. The chosen degree of oxygenation resulted in 87% MbO$_2$ saturation (see Fig. 3).
Figure 5. Synopsis of changes in venous PO₂ (P_vO₂) (B) and left ventricular developed pressure (LVDP) (C) induced by CO (20% in equilibrating gas phase) in WT and myo⁻/⁻ hearts (each bar, n = 6) at different oxygen levels, correlated to degrees of MbO₂ saturation as depicted in (A).
Figure 6. Effect of ambient PO$_2$ on cellular O$_2$ consumption (VO$_2$) of isolated mouse cardiomyocytes (CM) in an open system. Where standard deviation is given, value is mean of 4–6 CM preparations. (**: P<0.001, n = 6). Lines are a sigmoidal curve fit of the respective data.