Myoglobin facilitates oxygen diffusion

Marc W. Merx, Ulrich Flögel, Thomas Stumpe, Axel Gödecke, Ulrich K. M. Decking, and Jürgen Schrader

Institut für Herz-und Kreislaufphysiologie, Heinrich-Heine-Universität Düsseldorf, Germany

Corresponding author: Jürgen Schrader, Institut für Herz-und Kreislaufphysiologie, Heinrich-Heine-Universität Düsseldorf, Postfach 101007, 40001 Düsseldorf. E-mail: schrader@uni-duesseldorf.de

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 (P_v O₂) 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 P_v O₂ values were obtained. Hearts were then switched to buffer containing 20% CO, keeping the respective O₂ content constant. Stable functional parameters and P_v O₂ 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 133¹T 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 133¹T 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
chronic lack of Mb in hearts from myo−/− mice (acute inhibition of Mb by CO was identical to the effect of ischemia-induced decrease in cardiac function in WT hearts after 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/dtmax (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 PO2 in the perfusion medium by equilibrating the perfusion medium with O2 ranging from 95% to 12%. At the same time the extent of Mb oxygenation was measured by 1H NMR spectroscopy. This measurement enabled us to determine the intracellular MbO2 dissociation curve within the beating heart (Fig. 3A). To exclude effects of changes of coronary flow and thus O2 delivery, all experiments were carried out in the constant volume mode. Mb desaturation first appeared at 65% arterial O2 when Mb was desaturated by 13 ± 3%. Equilibrating the medium with 51% O2 decreased MbO2 saturation to 46 ± 8%, the lowest MbO2 saturation of 18.6 ± 5% was reached at 12% arterial O2. Upon reperfusion with carbogen, hearts showed an almost complete recovery. The relationship between arterial O2 and intracellular PO2 is depicted in Figure 3B. We calculated the intracellular PO2 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% O2 (432 mmHg), the observed 13% reduction of MbO2 saturation corresponds to an intracellular PO2 of ~16 mmHg (Fig. 3B). Further decrease in the O2 content of the medium steeply reduced MbO2 saturation and intracellular PO2 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 O2 utilization in myo−/− and WT mice further. As shown in Figure 4, myo−/− and WT hearts perfused at an arterial O2 of 65% exhibited opposite responses when subjected to 20% CO. WT hearts revealed a highly significant increase in coronary venous PO2 (P<0.001) accompanied by a decrease in LVDP of 11% (P<0.001). In contrast, myo−/− hearts displayed a slight decrease in P2O (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 P2O (Fig 5B) and LVDP (Fig 5C) at the different levels of MbO2, ranging from 100% to 51%. When Mb was fully O2 saturated (arterial medium equilibrated with 75% O2), CO induced no significant change in P2O 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 P2O and a decrease in LVDP. This effect was most pronounced at 87% MbO2 saturation (arterial O2 at 65%). In contrast, myo−/− hearts, when subjected to CO, could always sustain LVDP with unaltered, or even slightly decreasing, P2O.

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 6A, 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 O$_2$ storage capacity (1 mol O$_2$/mol Mb) and VO$_2$ are known, the maximum period during which O$_2$ 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 O$_2$ uptake of resting isolated rat CMs. As underlying mechanism, a preferred uptake of Mb-bound O$_2$ by mitochondria and/or the acceptance of electrons by sarcoplasmic Mb with concomitant reduction of heme iron ligated O$_2$ to H$_2$O were suggested. According to this hypothesis, one would expect myocardial VO$_2$ 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 O$_2$ in the saline-perfused mouse heart (see Figs. 1 and 3) had no effect on myocardial VO$_2$ and contractile parameters—LVDP, dP/dt$_{max}$, and rate-pressure-product (RPP)—(Table 1); although the perfusion-medium was of sufficiently high PO$_2$ 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 VO$_2$ 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 VO$_2$ at any given ambient PO$_2$ below 15 mmHg (Fig. 6), which clearly indicates an increase in the apparent diffusive resistance to O$_2$. 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 O$_2$. Together these findings demonstrate that Mb is essential for the delivery of O$_2$ from the sarcolemma to the mitochondria by way of Mb-facilitated O$_2$ diffusion.

To assess the relative contribution of Mb-facilitated O$_2$ diffusion versus the diffusion of physically dissolved oxygen to intracellular O$_2$ 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 O$_2$ diffusion depends primarily on the intracellular concentrations of O$_2$ and MbO$_2$, and on the in vivo diffusion coefficients of O$_2$ and MbO$_2$, respectively. The average intracellular concentrations of O$_2$ and MbO$_2$ differ by at least 1 order of magnitude. Assuming an intracellular PO$_2$ of 10 mmHg, the concentration of physically dissolved O$_2$ 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 O$_2$ and MbO$_2$, which favors Mb-facilitated diffusion, is counterbalanced by the diffusion coefficient of physically dissolved O$_2$ (DO$_2$), which is up to 100-fold higher than the diffusion coefficient of Mb (DMb) (~2 x 10^-7 cm$^2$/s for DO$_2$ (33) vs. 2 – 23 x 10^-7 cm$^2$/s for DMb (21, 34)). Unfortunately, precise in vivo data for both DO$_2$ 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/DO$_2$ would result in a higher fraction of O$_2$ supply by Mb-facilitated O$_2$ diffusion and vice versa.

Intracellular O$_2$ diffusion is governed not by the average concentration of O$_2$ or MbO$_2$, but by the respective concentration differences between the site of O$_2$ supply (the sarcolemma) and the site of O$_2$ consumption (the mitochondria). Therefore, the concentration gradients for O$_2$ and MbO$_2$ have to be considered. Under conditions of a fully oxygenated Mb; that is, in absence of an MbO$_2$ concentration gradient at a high intracellular PO$_2$, theory predicts that Mb-facilitated O$_2$ diffusion does not contribute to the intracellular O$_2$ flux to any significant extent. In line with this prediction, inactivating Mb with CO at a high arterial PO$_2$ was without functional effects on the saline perfused heart (see Table 1). Under these conditions, apparently O$_2$ flux was solely dependent on the diffusion of physically dissolved O$_2$. In contrast, when arterial O$_2$ supply was lowered and intracellular Mb partially deoxygenated, blocking Mb by CO significantly reduced VO$_2$, which demonstrated the importance of Mb-facilitated O$_2$ diffusion in presence of a MbO$_2$ gradient within the CM. Direct experimental proof for the intracellular radial MbO$_2$ gradient has been demonstrated recently in single CMs, with high MbO$_2$ 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 O$_2$ 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 O$_2$ 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 O$_2$ diffusion.

**Functional implications**
In the present study, Mb-facilitated diffusion clearly played a substantial role in intracellular \( O_2 \) transport when ambient \( P_{O_2} \) was low (8 mmHg as seen in isolated cardiomyocytes) and myoglobin was partially deoxygenated (13% Mb vs. 87% MbO\(_2\), as shown in the saline perfused heart). Whether similar conditions exist \textit{in vivo} has been a matter of debate for decades.

In striated muscle of exercising humans, \(^1\text{H} \) NMR spectroscopy enabled the study of MbO\(_2\) saturation (35–37). These studies unequivocally demonstrated that MbO\(_2\) desaturates with exercise; that is, that cellular \( P_{O_2} \) is low enough for unloading of \( O_2 \) from MbO\(_2\). Thus parallel diffusion of physically dissolved \( O_2 \) and Mb-bound \( O_2 \) is likely to occur.

Several groups have also addressed the question of cardiac MbO\(_2\) saturation on the whole organ level \textit{in vivo}. Based on data obtained by scanning reflectance spectroscopy, average MbO\(_2\) 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 \(^1\text{H} \) NMR spectroscopy. However, the apparent contrast, Zhang et al. (39) were unable to detect deoxy-Mb imposes a relatively low sensitivity of \(^1\text{H} \) NMR to deoxy-Mb.

REFERENCES

32. Armstrong, R. B., Essen-Gustavson, B., Hoppeler, H., Jones,


Received August 22, 2000; revised November 28, 2000.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>WT, n=10</th>
<th>myo⁻ /⁻, n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>+CO</td>
</tr>
<tr>
<td>MVO₂</td>
<td>6.5±0.7</td>
<td>6.4±0.8</td>
</tr>
<tr>
<td>LVDP</td>
<td>76.3±9.3</td>
<td>77.7±8.5</td>
</tr>
<tr>
<td>dP/dt max</td>
<td>4327±512</td>
<td>4367±534</td>
</tr>
<tr>
<td>RPP</td>
<td>30520±3720</td>
<td>31080±3400</td>
</tr>
</tbody>
</table>

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_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 PO$_2$ (P$_v$O$_2$) (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 MbO$_2$ saturation as depicted in (A).
Figure 6. Effect of ambient $\text{PO}_2$ on cellular $\text{O}_2$ consumption ($\text{VO}_2$) 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.