Krogh’s diffusion coefficient for oxygen in isolated *Xenopus* skeletal muscle fibers and rat myocardial trabeculae at maximum rates of oxygen consumption

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Van der Laarse, Willem J., Ariane L. des Tombe, Brechje J. van Beek-Harmsen, Marleen B. E. Lee-de Groot, and Richard T. Jaspers. Krogh’s diffusion coefficient for oxygen in isolated *Xenopus* skeletal muscle fibers and rat myocardial trabeculae at maximum rates of oxygen consumption. *J Appl Physiol* 99: 2173–2180, 2005. First published July 28, 2005; doi:10.1152/japplphysiol.00470.2005.—The value of the diffusion coefficient for oxygen in muscle is uncertain. The diffusion coefficient is important because it is a determinant of the extracellular oxygen tension at which the core of muscle fibers becomes anoxic (PO2crit). Anoxic cores in muscle fibers impair muscle function and may limit adaptation of muscle cells to increased load and/or activity. We used Hill’s diffusion equations to determine Krogh’s diffusion coefficient (Da) for oxygen in single skeletal muscle fibers from *Xenopus laevis* at 20°C (n = 6) and in myocardial trabeculae from the rat at 37°C (n = 9). The trabeculae were dissected from the right ventricular myocardium of control (n = 4) and monocrotaline-treated, pulmonary hypertensive rats (n = 5). The cross-sectional area of the preparations, the maximum rate of oxygen consumption (VO2 max), and PO2crit were determined.

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the value of $PO_{2\text{crit}}$ is inversely related to $D\alpha$, which is usually determined in whole muscle at rest, not inside contracting muscle cells at $V\dot{O}_2\max$. $D\alpha$ may depend on fat content, extracellular space, and unknown factors (2, 9, 19).

The aim of the present experiments was to validate Eq. 1 and to determine $D\alpha$ at $V\dot{O}_2\max$ under conditions in which myoglobin-facilitated oxygen diffusion is negligible and in muscle preparations of different composition. This was done by determination of $PO_{2\text{crit}}$, $V\dot{O}_2\max$, and the CSA of isolated single skeletal muscle fibers of Xenopus and of control and hypertrophied rat myocardial trabeculae in which extracellular space varied.

**MATERIALS AND METHODS**

**Animals and preparations.** The local Animal Experimental Commission approved the experiments, which were conducted according to the guidelines stipulated by the American Physiological Society on the use of experimental animals. *Xenopus laevis* females (body length ~10 cm) were cooled on ice and killed by decapitation. The heads were immediately frozen in liquid nitrogen to end electrical activity in the brain and freeze-dried before they were discarded. The iliofibularis muscle was dissected and placed in oxygenated Ringer solution (in mM: 116.5 NaCl, 2.0 KCl, 1.9 CaCl$_2$, 2 Na$_2$HPO$_4$, 0.1 EGTA; pH 7.2). After recovery, the muscle was transferred to a dissection trough, and single, high-oxidative type 2 or 3 fibers were isolated by using small forceps and scissors under dark-field illumination (25). Small rings, made from 50-μm-diameter platinum wire, were tied to the tendons using 20-μm nylon thread. The fibers were dissected 1 day before the experiment and were kept overnight at 7°C.

Male Wistar rats, body weight 200–300 g, were used. Five rats were injected scubutanously with 40 mg monocrystaline/kg body wt, when body weight was 170–190 g, to induce pulmonary hypertension and myocardial fibrosis (18, 26). The rats were used 3–4 wk after the injection, when body weight was decreasing by ~2%/day. The rats were anesthetized with ether, and the hearts were excised and perfused with Tyrode solution (in mM: 120 NaCl, 5 KCl, 1.2 MgSO$_4$, 2 Na$_2$HPO$_4$, 27 NaHCO$_3$, 1 CaCl$_2$, 10 glucose, and 20 butanedione monoxime, equilibrated with 5% CO$_2$–95% O$_2$, pH 7.2). After recovery, the muscle was transferred to a dissection trough, and single, high-oxidative type 2 or 3 fibers were isolated by using small forceps and scissors under dark-field illumination (25). Small rings, made from 50-μm-diameter platinum wire, were tied to the tendons using 20-μm nylon thread. The fibers were dissected 1 day before the experiment and were kept overnight at 7°C.

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Starting in the pulmonary artery, the right ventricle was carefully opened, and a 1.5- to 3-mm long trabecula without side branches was isolated. Small hooks with rings were tied to the ends as described above.

$V\dot{O}_2$ and force measurements. The preparation was transferred to a glass chamber, volume 170 µl, as described in detail previously (11). One end of the preparation is hooked to the pivot of a spinner, located at the bottom of the chamber, and the other end is hooked to a tungsten wire, which leaves the chamber through a capillary and is suspended from a force transducer (AE 801, SensoNor, Horten, Norway). The spinner circulates the solution in the chamber; the response time (95%) of the system is ~4 s. After mounting of the preparations in the chamber, the Ringer solution used for dissection of Xenopus fibers was replaced by bicarbonate-buffered Ringer solution (in mM: 100 NaCl, 20 NaHCO$_3$, 2.0 KCl, 1.9 CaCl$_2$, 0.1 EGTA, equilibrated with mixtures of 5% CO$_2$ in N$_2$ and 5% CO$_2$ in O$_2$, to adjust $PO_2$ in the chamber and keep CO$_2$ at 5%; pH 7.2). The Tyrode solution used for dissection of myocardial trabeculae was replaced by the Tyrode solution given above without butanedione monoxime, but with 2 mM CaCl$_2$, and equilibrated with the same gas mixture described above to adjust the $PO_2$ in the chamber. Equilibration with different gas mixtures was done at the experimental temperatures: 20°C for *Xenopus* and 37°C for rat trabeculae. The solutions were exchanged between each series of contractions using a syringe pump to flush the chamber. $PO_2$ in the chamber was determined with a polarographic oxygen electrode, which was constructed following the design of Kimmich and Kreuzer (21). The sensitivity of the oxygen electrode was determined by pumping Ringer or Tyrode solution with known oxygen concentrations through the chamber. Solubilities of oxygen in Ringer solution at 20°C of 30.15 ml/l and in Tyrode solution at 37°C of 22.73 ml/l were used in the calculations (3). The dark current of the oxygen electrode was determined after the experiment in 5% CO$_2$ in N$_2$. The preparations were stimulated by end-to-end stimulation with square-wave pulses, with duration 0.4 ms and 30% above threshold. After mounting, the preparations were stimulated at 0.2 Hz and were stretched to optimum length (i.e., the length at which twitch force was maximal). Then three or four experiments were conducted at high $PO_2$ in the chamber to determine the twitch frequency at which the *Xenopus* fibers reach $V\dot{O}_2\max$ and to determine the highest $PO_2$ in the chamber at which $V\dot{O}_2\max$ could not be reached anymore. Stimulus trains lasted 4 min to prevent fatigue (35, 45). Starting $PO_2$ values were lowered by 10–30 Torr for each following a series of twitches. $PO_{2\text{crit}}$ was calculated as the mean of the lowest $PO_2$ at which $V\dot{O}_2\max$ was reached and the highest $PO_2$ at which $V\dot{O}_2\max$ could not be reached.

Rat trabeculae were stimulated at 10 Hz, which corresponds to the maximum heart rate in rats (23) and is usually the highest rate possible without activation failure in vitro. $PO_{2\text{crit}}$ was determined in the same way as for *Xenopus* muscle fibers, but starting $PO_2$ values were reduced by ~100 Torr for the next series.

Time between stimulus trains was 20–30 min in all cases. After the last series of hypoxic contractions, $PO_2$ was raised to the initial hyperoxic value, to check the stability of the preparation. Force and $PO_2$ signals were analog-to-digital converted and sampled by a computer at 1,000 and 1 Hz, respectively.

After the $V\dot{O}_2$ measurements, the length of the preparation in the experimental chamber and the diameters of the preparation were measured under a microscope using an ocular scale. The smaller and larger diameters were measured at ×100 magnification at three different places along the length by rotating the preparation along the longitudinal axis. The CSA and the volume of the preparation were calculated, assuming an elliptical cross section. Force was normalized by the CSA and $V\dot{O}_2$ by the volume of the preparation. $V\dot{O}_2$ of the preparation was calculated from the decrease of the oxygen tension in the chamber. A second-order polynomial function was used to correct for oxygen loss from the chamber when the preparation was not stimulated. The parameters of the equation of the baseline were determined from oxygen decay in the chamber before the stimulation period and after the recovery period (11). After correction of oxygen loss from the chamber, $V\dot{O}_2$ of the preparation was determined from the slope of the curve determined by linear regression on measurements taken during the last 2 min of the 4-min stimulation period. Oxygen loss from the chamber without a preparation for $PO_2$ between 300 and 700 Torr was 30.7 ± 3.0 (SE)·$PO_2$ ml/s at 37°C, where SE denotes standard error ($r = 0.85$, $P < 0.0001$). At lower $PO_2$ values, oxygen disappears from the chamber mainly due to consumption by the oxygen electrode (0.6–0.9 ml/s at 121 Torr and 20°C). At subatmospheric $PO_2$ values required for experiments with *Xenopus* muscle fibers, oxygen could leak into the chamber. In such cases, the mean of the change in oxygen tension before and after the stimulation period was used to correct $V\dot{O}_2$.

Distribution of mitochondria and extracellular space. After the $V\dot{O}_2$ measurements, the preparations were embedded in 15% gelatin in dissection solution, pH 7.2, and frozen in liquid N$_2$. Cryostat sections were cut and incubated for succinate dehydrogenase activity in a medium consisting of 37.5 mM sodium phosphate buffer, pH 7.6, 75 mM sodium succinate, 0.4 mM tetramitroblue tetrazolium, and 5 mM Na$_3$N$_x$N$_y$N, as described previously (*Xenopus*: 16-μm-thick sections for 45 min at 20°C, Ref. 45; rat trabeculae: 5-μm-thick sections for 7 min at 37°C, Ref. 8). The relationship between $V\dot{O}_2$ at 10 Hz and the succinate dehydrogenase activity of the control trabeculae has been described elsewhere (8). The preparations were used to determine the mitochondrial distribution in the muscle fibers and to determine extracellular space in the trabeculae. Extracellular space was deter-
mained by using a microscope densitometer (26) and the threshold option in NIH Image to produce a binary image of the preparation. The mean gray value of the image is used to calculated extracellular space. This rapid procedure yields the same results as manual image editing used previously (compare with Fig. 3, A, C, F, and H in Ref. 8). Extracellular space included endothelial cells, fibroblasts, and leukocytes in the trabeculae.

Calculation of Do. Do for oxygen was calculated by using an equation similar to the one in the Introduction, derived for preparations with an elliptical cross section (16):

\[ PO_{2\text{crit}} = \frac{V_{O2\text{max}}}{(2D\alpha)}(4/l^2 + 4/m^2) \]

(2)

where \( l \) and \( m \) are the larger and the smaller diameter of the preparation, respectively. For clarity, we define a shape factor \( b \) as the ratio of larger and smaller diameter, \( b = l/m \), and use \( \text{CSA} = \pi lm/4 = \pi bm^2/4 \), where \( \text{CSA} \) is the area of the ellipsoid. Substituting \( l^2 = b^2m^2 \) and \( m^2 = 4\text{CSA}/\pi b \) into equation 2, and rearranging, gives:

\[ D\alpha = bV_{O2\text{max}}\text{CSA}/2(1 + b^2)P_{O2\text{crit}} \]

(5)

This equation is the same as Eq. 1 when the cross section of the preparation is a circle, i.e., when \( b = 1 \).

Statistics. Unless stated otherwise, values are given as means with standard deviation (SD). Two-sided \( t \)-tests with unequal variances were used to determine differences between means. SPSS 9.0 (SPSS, Chicago, IL) was used for multivariate linear regression analysis. \( P < 0.05 \) was considered significant.

RESULTS

Figure 1 shows \( V_O2 \) and twitch force production of a Xenopus muscle fiber. Experiments started at high \( P_{O2} \) in the chamber (i.e., 21% oxygen, \( P_{O2} \)) in the Xenopus chamber (i.e., 21% oxygen, \( P_{O2} \)). Experiments started at high \( P_{O2} \) in the chamber (i.e., 21% oxygen, \( P_{O2} \)). Extracellular space included endothelial cells, fibroblasts, and leukocytes in the trabeculae.

Comparison of Fig. 1, A and B, and 1C shows that the \( V_O2 \) increase at the onset of the stimulus train was insensitive to hypoxia. Similar results were found for the other Xenopus fibers. These results are in agreement with Kindig et al. (22), who measured intracellular \( P_{O2} \) changes in Xenopus fibers as a function of extracellular \( P_{O2} \).

Figure 2 shows results of a control rat trabecula. Passive force of control trabeculae increased during 10-Hz stimulation under hypoxic conditions (Fig. 2F). The contracture recovered during reoxygenation of the preparation, but, after reoxygenation, active force production of the trabeculae was depressed, whereas \( V_O2 \) at 10 Hz was not changed. \( V_O2 \) at 10 Hz was 0.58 mM/s (SD 0.01) in four control trabeculae when oxygen was not rate limiting.

Figure 3 shows results of a hypertrophied trabecula. \( V_O2 \) at 10 Hz was 0.74 mM/s (SD 0.20) in five hypertrophied trabeculae when oxygen was not rate limiting. Hypertrophied trabeculae did not develop a contracture, despite stimulation-induced hypoxia. \( V_O2 \) at 10 Hz of hypertrophied trabecula was not different from control.

\( V_O2 \) of Xenopus muscle fibers and control trabeculae at rest were below the detection limit, i.e., indistinguishable from the rate of oxygen loss from the chamber without a preparation, but \( V_O2 \) of hypertrophied trabeculae at rest was considerable:
0.43 mM/s (SD 0.17). \( V\dot{O}_2 \) at rest by hypertrophied trabeculae requires further study.

\( P_{O2\text{crit}} \) of the preparations was calculated as the mean of the lowest oxygen tension at which the \( V\dot{O}_2 \) max was reached and the highest oxygen tension at which the maximum rate could not be reached. \( P_{O2\text{crit}} \) as a function of \( bV\dot{O}_2 \max \frac{CSA}{2}/H \) is shown in Fig. 4. Equation 3 predicts that this relationship is proportional with slope \( 1/D\alpha \). For *Xenopus* and control trabeculae similar, proportional relationships are found, indicating that Hill’s diffusion model applies to these preparations. However, hypertrophied trabeculae deviate from these relationships. \( D\alpha \) increased in the order: *Xenopus* muscle fibers \( D\alpha = 1.23 \text{ nM} \cdot \text{mm}^2 \cdot \text{mmHg}^{-1} \cdot \text{s}^{-1} \) (SD 0.12), control rat trabeculae \( D\alpha = 2.29 \text{ nM} \cdot \text{mm}^2 \cdot \text{mmHg}^{-1} \cdot \text{s}^{-1} \) (SD 0.24) (\( P =

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Fig. 2. Paired determinations of \( V\dot{O}_2 \) (A–C) and force production (D–F) of a control rat trabecula at 10 Hz and 37°C. A and D, and B and E: experiments under hyperoxic conditions at different starting \( P_{O2} \). C and F: experiment in hypoxia. Note the increase in passive force induced by hypoxia and the decrease of \( V\dot{O}_2 \) during the stimulus train in hypoxia. See legend to Fig. 1 for details.

Fig. 3. Paired determinations of \( V\dot{O}_2 \) (A–C) and force production (D–F) of a hypertrophied rat trabecula at 10 Hz and 37°C. A and D, and B and E: experiments under hyperoxic conditions at different starting \( P_{O2} \). C and F: experiment in hypoxia. This trabecula was also stimulated at a starting \( P_{O2} \) of 300 Torr and reached critical \( P_{O2} \) (\( P_{O2\text{crit}} \)) during the stimulus train, indicated by a decrease of \( V\dot{O}_2 \) during the last minute of the stimulus train (not shown). The trabecula started to contract spontaneously (at 3 Hz) when \( P_{O2} \) in the chamber decreased <204 Torr. See legend to Fig. 1 for details.
0.0012 vs. *Xenopus*), and hypertrophied rat trabeculae $D\alpha = 6.0 \text{ nM} \cdot \text{mm}^{-2} \cdot \text{mm Hg}^{-1} \cdot \text{s}^{-1}$ (SD 2.8) ($P = 0.039$ vs. control rat trabeculae). Note that $D\alpha$ varied considerably in hypertrophied trabeculae.

**Effect of extracellular space on $D\alpha$.** After the $\dot{V}O_2$ measurements, cross sections were cut from the preparations and incubated for succinate dehydrogenase activity to determine mitochondrial distribution and extracellular space. The images and examples of the analyses are shown in Fig. 5. Succinate dehydrogenase activity was distributed fairly homogeneously in the type 2 and 3 *Xenopus* fibers selected for the experiments. Extracellular space in control trabeculae is 17.5% (SD 2.7) of the CSA and is significantly ($P = 0.04$) larger in hypertrophied trabeculae, 30% (SD 10), which confirms fibrosis. Figure 6 shows the relationship between $D\alpha$ and extracellular space in the preparation. Spearman’s rank correlation coefficient = 0.92 is significant ($P < 0.001$) also when $D\alpha$ in *Xenopus* muscle fibers is corrected for the difference in experimental temperature (see **DISCUSSION**).

Multivariate linear regression analysis of the nine trabeculae detects two significant, independent determinants of $PO_{2\text{crit}}$ ($R^2 = 0.78$, $P = 0.011$):

$$PO_{2\text{crit}} = 245(\text{SE 98}) - 8.4(\text{SE 3.0})\text{extracellular space} + 11,702(\text{SE 846})\text{CSA}$$

where SE denotes standard error. CSA of the preparations was 0.026 mm$^2$ (SD 0.011; range 0.015–0.046 mm$^2$). $\dot{V}O_2$ of the preparations was 0.67 mM/s (SD 0.16), and the ratio of larger and smaller diameter ($b$ in Eq. 3) was 1.54 (SD 0.40). The variation of the latter two factors was relatively small, and their regression coefficients were not significant.

**DISCUSSION**

We have tested a simple model for oxygen diffusion in muscle preparations and used the model to determine values for $D\alpha$ at $\dot{V}O_2$ max. The results on single muscle fibers and myocardial trabeculae indicate that the model is valid when
extracellular space in the preparation is 20% of the volume of the preparation or less.

\( V_{\text{O}_2} \text{ max} \) of *Xenopus* type 2 and 3 muscle fibers was similar to the values reported before in phosphate-buffered Ringer solution (45), and \( V_{\text{O}_2} \) at 10 Hz of myocardial trabeculae of the rat under hyperoxic conditions is similar to the rate expected on the basis of mitochondrial enzyme activity (33), when differences between volume densities among dog, pig, and rat are taken into account (1, 4).

For *Xenopus*, the predicted proportionality of \( P_{\text{O}_2 \text{crit}} \) and \( V_{\text{O}_2 \text{ max}} \cdot \text{CSA} \) is found (the correction factor for deviations from a circular CSA is ignored in this discussion for simplicity), indicating that Hill’s diffusion equation predicts \( P_{\text{O}_2 \text{crit}} \) in these cells accurately. \( D_\alpha \) for *Xenopus* muscle fibers is 45% smaller than the value for whole frog sartorius muscle at 20°C reported by Mahler et al. (28). This difference can possibly be explained by the effect of extracellular space on \( D_\alpha \), which might have played a role in the experiments of Mahler et al., or other experimental differences, like muscle fiber type (see below). \( D_\alpha \) for *Xenopus* fibers is close to the original value for frog’s abdominal wall reported by Krogh (24), which corresponds to 1.37 nM·mm\(^{-2}\)·mmHg\(^{-1}\)·s\(^{-1}\).

\( D_\alpha \) in myocardial trabeculae is larger than \( D_\alpha \) in *Xenopus* muscle fibers. There are several known factors that could explain this difference: myoglobin concentration, experimental temperature, and composition of the preparation, e.g., differences in extracellular space or fat content (6, 9). These factors are discussed briefly below.

**Myoglobin-facilitated diffusion.** Myoglobin is undetectable in *Xenopus* muscle fibers and in *Xenopus* heart (unpublished observation), whereas it is \(~0.25\) mM in right ventricular cardiomyocytes of control and monocrotaline-treated, pulmonary hypertensive rats (8). According to a Hill-type diffusion model, which does take myoglobin-facilitated oxygen diffusion into account (32, 34), this concentration can reduce \( P_{\text{O}_2 \text{crit}} \) of individual control and hypertrophied cardiomyocytes by 25 and 30%, respectively (42). Using the same model and values for the diffusion coefficients of myoglobin, oxygen dissociation constant of myoglobin used by van Beek-Harmsen et al. (42), and the mean parameter values for the present preparations given above, we find for control and hypertrophied trabeculae that myoglobin-facilitated diffusion would reduce \( P_{\text{O}_2 \text{crit}} \) by <1%. This indicates that myoglobin-facilitated diffusion is negligible in isolated myocardial trabeculae, even when myoglobin could diffuse freely.

According to the model, the cause of the difference between the effect of myoglobin on \( P_{\text{O}_2 \text{crit}} \) of trabeculae on the one hand and cardiomyocytes on the other is the difference in size. Because \( P_{\text{O}_2 \text{crit}} \) of trabeculae is high compared with \( P_{\text{O}_2 \text{crit}} \) of individual cardiomyocytes, myoglobin in trabeculae is saturated with oxygen, except in cardiomyocytes in the core of the trabecula at \( P_{\text{O}_2 \text{crit}} \). This agrees with similar conclusions of Loiselle (27) and Dutta et al. (10). We conclude that myoglobin-facilitated diffusion cannot explain the difference between \( D_\alpha \) of *Xenopus* muscle fibers and rat trabeculae.

**Temperature.** The effect of temperature on \( D_\alpha \) is believed to be small, \(~1\%)°C, because the \( D \) increases with temperature and the \( \alpha \) decreases with temperature (16). The present experiments were carried out at physiological temperature, 20 and 37°C, for *Xenopus* (29) and rat, respectively. Using the temperature dependency of \( D_\alpha \) in frog sartorius muscle (28), the value for \( D_\alpha \) of *Xenopus* at 37°C would be \(~10\%) larger than at 20°C, i.e., 1.35 nM·mm\(^{-2}\)·mmHg\(^{-1}\)·s\(^{-1}\) (SD 0.13), which is still smaller than \( D_\alpha \) in control (\( P = 0.0017 \)) and in hypertrophied trabeculae (\( P = 0.02 \)). Because the fat content in *Xenopus* type 2 and 3 fibers is probably larger than the fat content of the *Rana* sartorius muscle (38), it may be that the temperature dependency of \( D_\alpha \) in *Rana* determined by Mahler et al. (28) does not apply to *Xenopus* type 2 and 3 fibers (for discussion see Ref. 10). Using the temperature dependency of \( D_\alpha \) for hamster retractor muscle, 2.6%/°C (6) \( D_\alpha \) for *Xenopus* type 2 and 3 fibers would be 1.97 nM·mm\(^{-2}\)·mmHg\(^{-1}\)·s\(^{-1}\) (SD 0.19) at 37°C, which is not significantly different from \( D_\alpha \) in control trabeculae. Volume density of mitochondria in the hamster retractor muscle, which may be related to the solubility of oxygen in muscle, is 11.4% (41), similar to the volume density of mitochondria in *Xenopus* type 2 and 3 fibers, 11.1 and 17.4%, respectively (32). The temperature dependency of \( D_\alpha \) in *Xenopus* muscle remains to be determined.

**Extracellular space.** The relative volume occupied by cardiomyocytes in hypertrophied trabeculae is smaller than in control trabeculae. Assuming that the diffusion coefficients for oxygen inside normal and hypertrophied cardiomyocytes are the same, the results indicate that extracellular space facilitates oxygen transport in contracting muscle preparations. An increase of \( D_\alpha \) with extracellular space can be due to a larger \( D_\alpha \) in the extracellular space compared with sarcoplasm: \( D_\alpha \) in water is 2.4 times and in 15% gelatin 2 times larger than in muscle (24). It is also a possibility that the extracellular fluid is mixed by the contraction-relaxation cycles. Sarcomeres in trabeculae with fixed ends shorten by \(~20\%) due to series elasticity (7). Because the volume of muscle cells is independent of muscle length, muscle shortening must distort extracellular space. This will cause at least some mixing of extracellular fluid and seems to be the simplest explanation for the relationship between \( D_\alpha \) and extracellular space in trabeculae.

Mixing of the extracellular fluid will reduce angular variations of intercellular oxygen tension due to discrete capillary sources in vivo. Federspiel (13) calculated that these angular variations do not penetrate deeply into myoglobin-containing fibers. This complication in the application of Hill’s equations for oxygen diffusion may, therefore, be negligible in working...
muscle. To what extent $D\alpha$ depends on muscle shortening remains to be investigated. In the meantime, Eq. 4 can be useful in the design of experiments on myocardial trabeculae.

In conclusion, the results indicate that Hill’s model for oxygen diffusion is valid for single muscle fibers without myoglobin and a fairly homogeneous mitochondrial distribution consuming oxygen at the maximum rate. PO$_{2\text{crit}}$ at V$\dot{O}_2$ max of these cells is close to arterial PO$_2$ (12), suggesting that V$\dot{O}_2$ max of Xenopus muscle fibers cannot be reached in vivo.

Extracellular space in multicellular, contracting myocardial trabeculae facilitates oxygen diffusion. Because control trabeculae have the smallest amount of extracellular space, the value for $D\alpha$ in these preparations provides an upper limit for $D\alpha$ for oxygen inside cardiomyocytes consuming oxygen at the maximum rate: 2.29 nM$\cdot$mm$^2$·mmHg$^{-1}$·s$^{-1}$ (SD 0.24). $D\alpha$ in single Xenopus muscle fibers, adjusted to 37°C, may be a lower limit for $D\alpha$ in cardiomyocytes: 1.35 nM$\cdot$mm$^2$·mmHg$^{-1}$·s$^{-1}$ (SD 0.13) or 1.97 nM$\cdot$mm$^2$·mmHg$^{-1}$·s$^{-1}$ (SD 0.19), depending on temperature dependency.

Taking $D\alpha = 2$ nM$\cdot$mm$^2$·mmHg$^{-1}$·s$^{-1}$ (corresponding to 2·10$^{-5}$ ml O$_2$·cm$^{-1}$·atm$^{-1}$·min$^{-1}$), mean V$\dot{O}_2$ at 10 Hz corrected for extracellular space given above and a CSA of 120 and 380 $\mu$m$^2$ for control and hypertrophied cardiomyocytes (8), PO$_{2\text{crit}}$ according to Eq. 1 (without myoglobin) is 3.1 and 16.2 Torr in control and hypertrophied cardiomyocytes, respectively. The latter value is close to the predicted critical end-capillary PO$_2$ (30) and will lead to hypoxia in hypertrophied cardiomyocytes. Cytochrome c is released from mitochondria in all cardiomyocytes with CSA larger than 600 $\mu$m$^2$ in the right ventricular wall of rats with monocrotaline-induced pulmonary hypertension (43). The present results indicate that cytochrome c release in these cells may be triggered by hypoxia.

GRANTS

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REFERENCES


