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Modelling diffusive O_2 supply to isolated preparations of mammalian skeletal and cardiac muscle

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Abstract

The purpose of this study was to use A. V. Hill's equation describing diffusion of O_2 into cylindrical muscles to assess the adequacy of O_2 supply for commonly used isolated preparations of mammalian cardiac and skeletal muscles. The diffusion equation was solved numerically to give the maximum, steady state O_2 diffusion distances (i.e. the distance from the surface of the muscle to the radial location where P_{O_2} is 0) for both resting and contracting muscles and for a range of temperatures. Non-steady state solutions for the rest-to-work transition were also determined to estimate how long contractile activity could be continued before anoxia develops at the muscle centre. The influence on muscle oxygenation of myoglobin-facilitated O_2 diffusion was also assessed. The analysis was performed for typical sized, whole muscles from adult rats and mice, for frog sartorius muscle and for a range of temperatures. Muscle O_2 consumption rates were taken from the literature. The results indicated that (1) diffusive O_2 supply would be adequate to support resting metabolism of soleus and EDL muscles of rat and mouse but may not be adequate to support the transient high resting metabolic rate of papillary muscles shortly after dissection, (2) during steady contractile activity of soleus and EDL muscles, particularly those from the rat, over a reasonable range of duty cycles, adequate O_2 supply could only be ensured if the radii of preparations was substantially smaller than those of whole muscles and (3) for cardiac muscles, diffusive O_2 supply could only support steady-state metabolism at twitch frequencies <1 Hz for whole papillary muscles from rat and <3 Hz for those from mouse. Reducing experimental temperature markedly enhances O_2 supply to skeletal, but not cardiac, muscle. O_2 supply from myoglobin had only minimal effects on oxygenation under typical isolated muscle conditions.

Introduction

An isolated muscle preparation is a muscle, bundle of muscle fibres or single fibre removed from an animal to be studied *in vitro*. If kept in an appropriate saline solution and supplied with O_2, such preparations remain viable for many hours. Isolated preparations of skeletal and cardiac muscle have contributed to knowledge of contraction mechanisms (Hill, 1964; Ford *et al.*, 1977), the bases of muscle fatigue (Spande and Schottelius, 1970; Segal *et al.*, 1986; Dawson *et al.*, 1978; Cummins *et al.*, 1989; Barclay, 1992, 1995; Lodder and de Haan, 1992; Pedersen *et al.*, 2003; Kristensen *et al.*, 2005), the relationship between energy supply and demand (Crow and Kushmerick, 1982; Barclay *et al.*, 1995) and, more recently, have been used to determine the physiological consequences of muscle-focussed genetic modifications (e.g. Bluhm *et al.*, 2000; Dahlstedt *et al.*, 2000).

In the absence of vascular perfusion, the metabolic needs of isolated muscle preparations must be met by diffusion of O_2 from the preparation's outer surface. Whereas the distances O_2 must travel from blood vessels to the centre of muscle cells *in vivo* is no more than a few tens of micrometers, the distances O_2 must diffuse into isolated preparations are typically 10- to 100-fold greater. Consequently, the adequacy of diffusive O_2 supply to meet the metabolic needs of an isolated muscle cannot be taken for granted. However, it is apparent from some recent papers that good or even adequate appreciation of the possible limitations of diffusive O_2 supply is far from universal.

O_2 is consumed as it diffuses through an isolated muscle. If the rate of O_2 consumption is high relative to the rate of diffusive supply, an anoxic region may develop in the centre of the muscle (Hill, 1928). High-energy phosphate stores in an anoxic region will become depleted and the cells in that region will most likely will go into rigor, cease to contribute to active force generation, and cease consuming energy (e.g. Holubarsch *et al.*, 1982). Consequently, mass specific energy consumption and active force generation will decrease. If fatigue, recovery from fatigue or the consequences of ischaemia or hypoxia were being studied, then unrecognised development of anoxia would confound interpretation of mechanical and energetic data.

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Hill (1928, 1965: pp. 208–241) derived mathematical expressions to describe O_2 diffusion into muscles of various geometries and used these to assess adequacy of oxygenation of frog muscle at the low experimental temperatures typically used for those muscles (e.g. 0°C). Hill's expression for calculating the maximum radius to which O_2 could diffuse into a cylindrical muscle (i.e. the "critical" radius) for a steady rate of O_2 consumption has been used to estimate the adequacy of oxygenation of isolated muscles. However, in most experimental protocols used with isolated muscles, rate of O_2 consumption is unlikely to be steady. For example, a common contraction protocol is a short series of contractions, lasting 5–20 s. During such a protocol, rate of O_2 consumption is initially that of the resting muscle and rises towards an eventual steady value with a time course that is approximately exponential. To assess the adequacy of oxygenation during such non-steady-state situations, Hill derived expressions, and their analytical solutions, describing temporal and spatial dependencies of P_{O_2} (Hill, 1965: pp. 222–238). However, the mathematics required to use Hill's analytical solutions for cylindrical muscles or to solve the partial differential equations renders these approaches to assessing adequacy of oxygenation intractable to all but the highly mathematically competent. Hill provided illustrations of the solutions for specified conditions but these applied largely to whole frog muscle at low temperatures. In contrast, isolated muscle preparations used now are typically smaller than frog muscles, are from small mammals and are studied at higher temperatures (25–37°C). Using preparations with smaller radii facilitates diffusive O_2 supply but using higher experimental temperatures hinders O_2 supply because metabolic rate is more temperature-sensitive than O_2 diffusivity (the index of rate of O_2 diffusion through tissue) (Mahler *et al.*, 1985).

The purpose of this study was to update AV Hill's work on amphibian muscles (Hill, 1928, 1965: pp. 208–241) by illustrating solutions to the diffusion equation for mammalian muscles of approximately cylindrical shape, using reported metabolic rates for mammalian muscles and for a practical range of contraction duty cycles. The results are presented so that experimenters can readily select experimental conditions, contraction protocols and preparation size that will ensure adequate oxygenation of isolated preparations.

Methods

Mathematical analysis

Simple diffusion of O_2 into an isolated muscle

Isolated muscles were assumed to be of uniform circular cross-section and to be long relative to their radius so that diffusion of O_2 into the ends of the cylinder could be ignored. Equation (1) describes the temporal and spatial dependencies of P_{O_2} in a cylindrical muscle (Hill, 1965: p. 229).

$$\frac{\delta P_{O_2}}{\delta t} = K\left(\frac{\delta^2 P_{O_2}}{\delta r^2} + \frac{1}{r}\cdot\frac{\delta P_{O_2}}{\delta r}\right) - A(t) \tag{1}$$

where t is time, r is the radial distance from the muscle's centre, $A(t)$ is the rate of O_2 consumption, which may be time-dependent, and K is diffusivity of O_2 through muscle (i.e. product of O_2 solubility and the diffusion coefficient). K was taken to be 2.37×10^{-5} ml cm^{-1} min^{-1} atm^{-1} at 22.8°C and to have a Q_{10} (change in rate for a 10°C increase in temperature) of 1.06 in the temperature range of 20–37°C (Mahler *et al.*, 1985). The steady-state version of this equation is as follows (Hill, 1965: p. 217).

$$\frac{d^2 P_{O_2}}{dr^2} + \frac{1}{r}\cdot\frac{\delta P_{O_2}}{\delta r} = \frac{A(t)}{K} \tag{2}$$

The solution to Eq. (2) for the case in which $P_{O_2} = 0$ at the muscle centre gives the maximum radius (r_c) to which O_2 can diffuse for a given metabolic rate.

$$r_c = 2\sqrt{\frac{Ky_0}{A_S}} \tag{3}$$

where y_0 is the P_{O_2} at the muscle surface and A_S is the steady-state rate of O_2 consumption.

For non-steady-state solutions to Eq. (1), it was assumed that the time course of increase in metabolic rate during the transition from rest to steady activity could be well-described by a single exponential function (Mahler, 1978b, 1985; Paul, 1983; Barclay and Weber, 2004):

$$A(t) = A_R + A_S\cdot(1 - e^{-t/\tau}) \tag{4}$$

where A_R is the resting rate of O_2 consumption and τ is the time constant for the increase in rate of O_2 consumption during the transition from rest to steady activity. $A(t)$ is also P_{O_2}-dependent such that $A(t)$ is reduced at low values (Wittenberg and Wittenberg, 1985; Schenkman, 2001). It was assumed that the relationship between $A(t)$ and P_{O_2} was described by a sigmoid so that:

$$A(t, P_{O_2}) = \left(A_R + A_S\cdot(1 - e^{-t/\tau})\right)\cdot\frac{P_{O_2}^n}{P_{50}^n + P_{O_2}^n} \tag{5}$$

where n determines the steepness of the relationship and P_{50} is the P_{O_2} at which $A = 0.5(A_S + A_R)$. P_{50} was taken to be 1.5 mm Hg and n to be 1.5 (Wittenberg and Wittenberg, 1985; Schenkman, 2001).

Myoglobin-facilitated diffusion

Slow-twitch skeletal muscle and cardiac muscles contain the intracellular O_2 binding molecule myoglobin. The physiological role of myoglobin remains the sub-

ject of debate; it may transport O_2 when intracellular P_{O_2} is low (for a review, see Wittenberg and Wittenberg, 2003) or simply act as an intracellular O_2 store (for a review, see Jurgens *et al.*, 2000). Myoglobin-facilitated O_2 diffusion can be incorporated into the diffusion equation (Eq. 1) as described by Loiselle (1987).

$$\frac{\delta P_{O_2}}{\delta t} = K\left(\frac{\delta^2 P_{O_2}}{\delta r^2} + \frac{1}{r}\cdot\frac{\delta P_{O_2}}{\delta r}\right) + c_{Mb} D_{Mb}\left(\frac{\delta^2 S}{\delta r^2} + \frac{1}{r}\cdot\frac{\delta S}{\delta r}\right) - A(t) \quad (6)$$

where c_{Mb} is the concentration of myoglobin (400 μmol l^{-1}, Jurgens *et al.*, 2000), D_{Mb} the diffusion coefficient of myoglobin in muscle (1.25×10^{-7} cm^2 s^{-1}, $Q_{10} = 1.46$, Jurgens *et al.*, 2000) and S is the fraction of myoglobin that is saturated with O_2. The relationship between myoglobin saturation and P_{O_2} was assumed to be described by a sigmoid, with half-maximal saturation when $P_{O_2} = 2.2$ mm Hg and slope of 1.5 (Schenkman, 2001). This curve must lie to the right of that describing the dependence of A on P_{O_2} (Eq. 5) (Loiselle, 1987). It was assumed that the kinetics of O_2 dissociation from myoglobin did not limit the rate of O_2 supply from this source.

Initial and boundary conditions for non-steady-state solutions

Changes in intramuscular P_{O_2} with time during the transition from rest to steady activity were determined by first calculating the P_{O_2} profile through the resting muscle (Eq. 2). Equation (2) was treated as a two-point boundary value problem with a constant P_{O_2} at the muscle surface and the rate of change in P_{O_2} with radial distance equal to 0 at the muscle centre (i.e. $dP_{O_2}/dr = 0$ when $r = 0$). Numerical solutions of Eq. (2) were obtained using the shooting method (Press *et al.*, 1998: pp. 757–759). The resting P_{O_2} profile was used as the initial condition for solving Eq. (1) with a time-varying rate of O_2 uptake (Eq. 5). The boundary conditions were the same as used for solving Eq. (2). Solutions to Eqs. (1) and (6) were calculated using the "pdesolve" function of Mathcad (v. 12, Mathsoft Inc. Cambridge, MA, USA). This function uses the numerical method of lines, which is appropriate for solving parabolic partial differential equations.

Metabolic data

The metabolic data used for the simulations are presented in Table 1. Rates of oxygen consumption of resting and contracting muscles, the temperature sensitivities of those rates and the time constant for increases in active metabolism during the rest-to-work

Table 1. Metabolic data used for modelling diffusive O_2 supply

Muscle	Resting O_2 consumption			Active O_2 consumption					r (mm)
	Rate (μl s^{-1} g^{-1})	Q_{10}^R	T (°C)	Rate (μl s^{-1} g^{-1})	Q_{10}^A	τ (s)	Q_{10}^τ	T (°C)	
Skeletal muscle									
Mouse soleus	0.05[a]	2[b]	20	4[c]	2[g]	24[d]	1.8[d]	25	0.35
Mouse EDL	0.05[a]	2[b]	20	13.5[c]	2[g]	25[d]	2.0[d]	25	0.40
Rat soleus	0.11[e]	2[b]	27	2.5[f]	2[g]	25[f]	2[g]	27	0.7–1.0
Rat EDL	0.11[e]	2[b]	27	12.7[e]	2[g]	28[f]	2[g]	27	0.7–1.0
Frog sartorius	0.014[b]	4/2[h]	23	4.6[i]	2[h]	540[j]	2.1[k]	0	0.9
Cardiac muscle									
Mouse papillary	0.4–1.25[l]	1.3[m]	27	0.25[n]	0.6[o]	12[n]	2[g]	27	0.3
Rat papillary	0.2–1[m]	1.3[m]	27	0.35[p]	0.6[o]	11[q]	2[g]	27	0.5

Active O_2 consumption: O_2 consumed in excess of that consumed by resting muscle; T: temperature; Q_{10}^R, Q_{10}^A, Q_{10}^τ: temperature sensitivities of resting rate of O_2 consumption, active rate of O_2 consumption and of the time constant for increase in rate of O_2 consumption during rest-to-work transition; τ: time constant for increase in rate of O_2 consumption during rest-to-work transition; T: temperature for quoted values of rate of O_2 consumption and τ; r: typical average radius of whole muscles; calculated from mass (m) and length (l) of preparations, $r = \sqrt{m/(\pi \cdot l \cdot \rho)}$ where ρ is muscle density (1.06 kg l^{-1}).

[a]Crow and Kushmerick (1982).
[b]Mahler (1978a), assumed Q_{10} for frog muscle resting metabolism applies to mammalian muscle.
[c]Barclay (1996).
[d]Barclay, unpublished values.
[e]Cox and Gibbs (1997).
[f]Gibbs and Gibson (1972), Wendt and Gibbs (1973, 1976).
[g]Assumed value.
[h]$Q_{10} = 4$ between 0 and 10°C and 2 between 10 and 23°C, Mahler (1978a).
[i]Hill and Woledge (1962).
[j]Hill (1940).
[k]From comparison of data from Hill (1940) at 0°C and Mahler (1985) at 20°C.
[l]Widén and Barclay (2005), resting metabolic rate varies with time.
[m]Gibbs and Loiselle (2001), assumed value for rat papillary muscle applicable to mouse.
[n]Widén and Barclay, unpublished observations.
[o]Buckberg *et al.* (1977), from *in situ* dog hearts.
[p]Baxi *et al.* (2000).
[q]Mellors *et al.*, unpublished observations.

transition were obtained from the literature and from previously unpublished work from the author's laboratory. Q_{10} values were used to quantify temperature sensitivity. It was assumed that oxidative processes were the sole mechanism of regenerating high-energy phosphates. Although EDL and frog sartorius are typically described as having a high capacity for glycolytic energy supply, the quantitative contribution of this mechanism to total energy supply in the presence of oxygen, even during prolonged tetanic contraction, is minimal (see Figure 1 in Crow and Kushmerick, 1982).

The time courses of increase and decrease in rate of oxidative metabolism at the start and end of contractile activity are well described by a single exponential function (Hill, 1940; Mahler, 1985; Mast *et al.*, 1990; Barclay *et al.*, 2003). The time course of increase in rate of O_2 consumption during the rest-to-work transition was quantified by the time constant (τ). Values of τ were obtained from published time courses of O_2 consumption during the rest-to-work transition or from the decline in either rate of O_2 consumption or recovery heat production following cessation of contractile activity. In the latter cases, it was assumed that τ for the rise in rate of O_2 consumption at the start of activity equals that for the decline in rate after a series of contractions.

Rates of O_2 consumption in Table 1 are expressed as $\mu l\ g^{-1}\ s^{-1}$ for skeletal muscles and $\mu l\ g^{-1}$ twitch^{-1} for cardiac muscles. Steady-state rates at different contraction duty cycles (contraction duration/interval between start of successive contractions) were then estimated on the basis that in an energetic steady state a quantity of O_2 equal to that required to fully reverse the initial biochemical change is consumed in the interval between the start of successive contractions; this is the definition of an energetic steady state (Paul, 1983). This procedure was validated by comparing estimated rates of O_2 uptake with experimental measurements of steady state rates of recovery metabolism for mouse soleus and EDL muscles (Barclay *et al.*, 1995) and for frog sartorius muscles (Paul, 1983). The estimated and measured values agreed to within $\pm 5\%$. Where published metabolic rates were given as rates of enthalpy output, these were converted to rates of O_2 consumption using an energetic equivalent of 20 mJ μl^{-1}. Complete data sets are available for the slow-twitch soleus, fast-twitch EDL and cardiac papillary muscles of both the rat and mouse and for frog sartorius muscle (Table 1).

Results

O_2 diffusion into resting isolated muscle

The maximum distance to which O_2 can diffuse into an isolated muscle is governed by the balance between rates of O_2 consumption and O_2 diffusion. Maximum diffusion distances for resting muscles, calculated using Eq. (3), are shown as a function of temperature in Figure 1. The distances were calculated for a surface

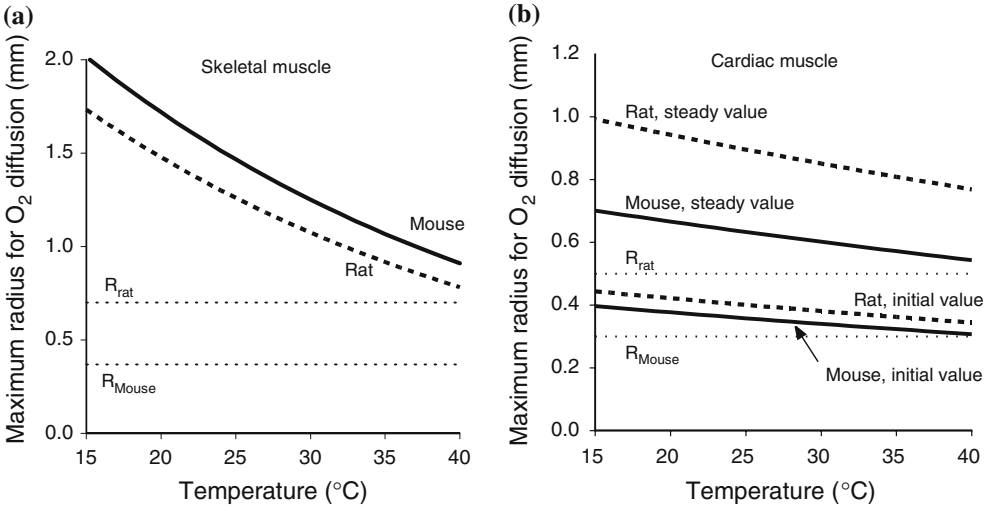

Fig. 1. Calculated radii to which O_2 will diffuse in resting skeletal and cardiac muscle. The maximum radius to which O_2 could diffuse is shown as a function of temperature for soleus/EDL muscles of rat (dashed line) and mouse (solid line). Resting metabolic rates do not differ between soleus and EDL muscles from the same species (Table 1). It was assumed that P_{O_2} at the muscle surface was 0.95 atm. The horizontal dashed lines indicate typical radii of whole rat (R_{Rat}) and mouse (R_{Mouse}) muscles. At physiological temperatures, the maximum radius for O_2 diffusion into resting rat skeletal muscle, but not that of mouse, is close to the typical muscle radius. (b) Same analysis for papillary muscles from rat (dashed lines) and mouse (solid lines). Resting metabolic rate of these muscles decreases with time after dissection. The lower pair of curved lines were calculated using the likely metabolic rates immediately after isolation of the muscles; the upper pair of lines are for the steady metabolic rates attained after $\sim$1 h for mouse papillary muscles and $\sim$2 h for rat papillary muscles. Diffusive O_2 supply is unlikely to be adequate to meet the resting metabolic demands of whole rat papillary muscles (radius, indicated by line labelled R_{Rat}, greater than radius to which O_2 will diffuse) immediately after dissection for any temperature between 15 and 37°C. The radius of mouse papillary muscle is less than the maximum O_2 diffusion radius at all temperatures <40°C regardless of time after isolation.

P_{O_2} of 0.95 atm (i.e. bathing solution equilibrated with a gas mixture containing 95% O_2) and for a range of temperatures encompassing typical experimental temperatures and physiological temperatures. The maximum distance to which O_2 can diffuse decreases with increasing temperature. For mouse skeletal muscles, the calculated maximum O_2 diffusion distance is always much greater than the radius of a whole mouse soleus or EDL muscle (Figure 1a). In contrast, the maximum diffusion distance into rat skeletal muscle is similar to the typical whole muscle radius at physiological temperatures (Figure 1a).

For cardiac muscle, the difference in temperature sensitivities between resting metabolism (Q_{10} = 1.3) and O_2 diffusivity (Q_{10} = 1.06) is less than the corresponding difference for skeletal muscle (Q_{10} values, 2 and 1.06, respectively) and consequently O_2 diffusion distances for cardiac muscle are less temperature sensitive than those for skeletal muscle. Resting metabolic rate of both rat and mouse papillary muscles varies substantially with time *in vitro* (for a review, see Gibbs and Loiselle, 2001). Resting metabolic rate of isolated papillary muscles decreases exponentially, with time constants (at 27°C) of 20 min for mouse (Widén and Barclay, 2005) and 60 min for rat (Gibbs and Loiselle, 2001). In Figure 1b, diffusion distances are given for both the high initial rate and the eventual steady rate. The analysis indicates that at 37°C it is unlikely that O_2 diffusion can support the high initial resting metabolism of whole rat papillary muscle (muscle radius > maximum diffusion distance) and that the margin between diffusion distance and muscle radius is small for mouse papillary muscle. The eventual steady resting metabolic rates are low enough that O_2 diffusion would be adequate to meet the metabolic requirements of both rat and mouse papillary muscles.

O_2 supply during steady contractile activity

Contractile activity raises rate of O_2 consumption above the resting value. When a muscle begins a regime of steady contractile activity, its rate of O_2 consumption increases with time towards an eventual steady value. For analyses of maximum diffusion distances during steady activity it was assumed that muscles were performing maximal tetanic contractions and that energy cost is proportional to the duration and frequency of contraction (Crow and Kushmerick, 1982; Kushmerick and Meyer, 1985; Barclay *et al.*, 1993, 1995; Reggiani *et al.*, 1997).

Calculated maximal diffusion distances in skeletal muscle decrease with increasing contraction duty cycle. This reflects the effects of a constant O_2 diffusivity while rate of O_2 consumption increases with duty cycle. For mouse soleus and EDL muscles, the calculated maximum diffusion distances are greater than whole muscle radii only at duty cycles below 0.1 at 35°C and 0.3 at 25°C. If a fibre bundle preparation with a radius 0.1 mm (i.e. a preparation 10 mm long with a mass of 0.3 mg) were used instead of a whole muscle, then O_2 diffusion would be capable of supporting the metabolic rate across the full duty cycle range.

Rat soleus and EDL muscles have lower steady-state O_2 consumption rates than the equivalent mouse muscles (Table 1) so that maximum O_2 diffusion distances, at a given duty cycle and temperature, are greater than those for mouse muscle; however, maximum diffusion distances are also less than whole muscle radius at all but the lowest duty cycles (Figure 2b). Therefore, for diffusive O_2 supply to be adequate to meet steady-state metabolic demands of contracting isolated rat soleus and EDL muscles, it is essential to have a preparation with a radius less than that of the whole muscle.

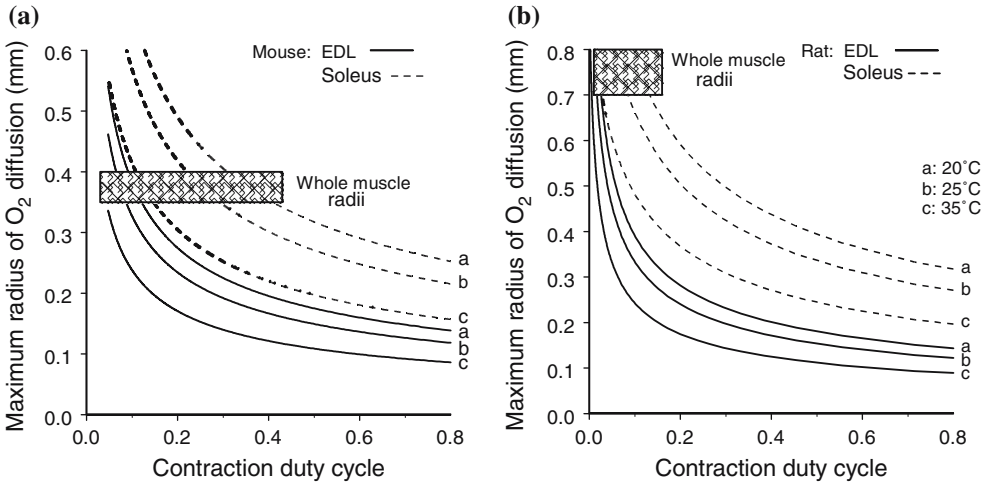

Fig. 2. Maximum O_2 diffusion distances during steady-state contractile activity in mouse and rat skeletal muscle. The maximum distance to which O_2 can diffuse was calculated for steady-state activity of mouse (a) and rat (b) skeletal muscles at various duty cycles and temperatures. Duty cycle was defined as contraction duration/time between start of successive contractions. Rates of O_2 consumption included both resting and active components (Table 1). Typical whole muscle radii are indicated by the cross-hatched regions. For each muscle type, the relationship between maximum O_2 diffusion radius and duty cycle is shown for temperatures of 20°C (lines labelled "a"), 25°C ("b") and 35°C ("c"), a range which spans temperatures typically used for *in vitro* studies of mammalian skeletal muscle. Increasing either duty cycle or temperature, or both, reduces the maximum distance into a muscle to which O_2 can diffuse.

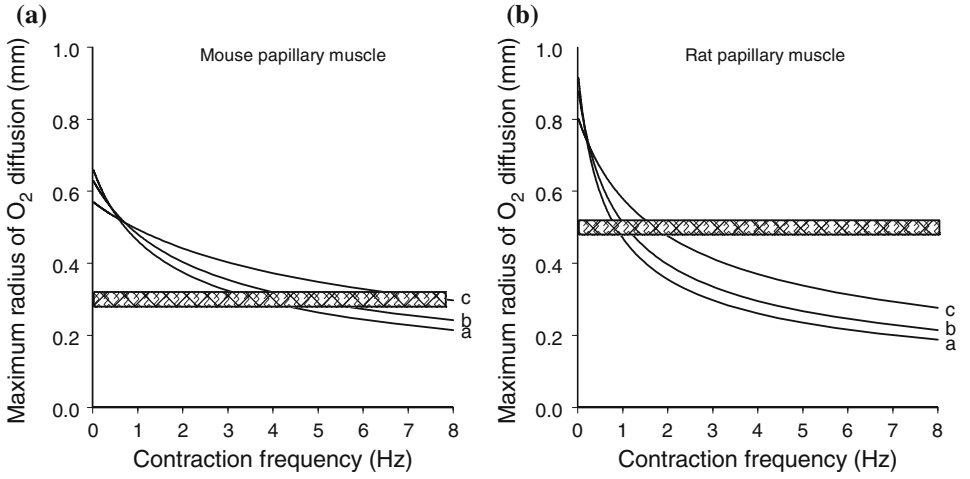

Fig. 3. Maximum O_2 diffusion distances during steady-state contractile activity in mouse and rat cardiac muscle. Diffusion distances calculated for papillary muscles of mouse (a) and rat (b) as a function of contraction frequency. It was assumed that resting metabolic rate had declined to a steady value. Calculated for 22°C (line labelled "a"), 27°C ("b") and 37°C ("c"). Cross-hatched bars show typical whole muscle radii. O_2 supply is adequate when maximum radius of O_2 diffusion is greater than the muscle radius (i.e. when lines are above hatched bars for whole muscles).

Maximum diffusion distances for papillary muscles are shown as a function of contraction frequency (i.e. twitches s^{-1}) in Figure 3. As for skeletal muscle, maximum diffusion distance decreases with increasing contraction frequency. However, the effects of temperature on diffusion distances are less marked than those for skeletal muscle. This reflects the differing temperature sensitivities of resting and active metabolism for cardiac muscle. Resting metabolic rate increases with temperature (Q_{10} = 1.3, Gibbs and Loiselle, 2001) whereas active metabolic rate (energy cost per twitch) decreases with increasing temperature (Q_{10} = 0.6, Buckberg *et al.*, 1977). At low contraction frequencies, resting metabolism makes a substantial contribution to the total metabolic rate and thus the temperature sensitivity of total metabolism is dominated by the Q_{10} for resting metabolism. Consequently, maximum diffusion distances at low contraction frequencies decrease with increasing temperature. At high contraction frequencies, active metabolism is the most important contributor to total metabolism and, because active metabolic rate declines with increasing temperature, maximum diffusion distances at high contraction frequencies increase as temperature increases. The overall effect is that maximum O_2 diffusion distances are much less temperature sensitive than those for skeletal muscle.

Duty cycle dependence of duration of contractile activity before anoxia develops

The solution to Eq. (1) with a time varying rate of O_2 consumption (Eq. 4) provides a profile of variations in intramuscular P_{O_2} with time and radial distance from the muscle centre (Figure 4). As the rate of O_2 consumption increases progressively after the start of activity, P_{O_2} at all distances below the muscle surface decreases and continues to do so until either a steady state is reached, in which the minimum intramuscular P_{O_2} is greater than 0, or until P_{O_2} reaches 0. In the latter case, it is of interest to the experimenter to know how long it will take, after the start of activity, before an anoxic region begins to develop at the muscle

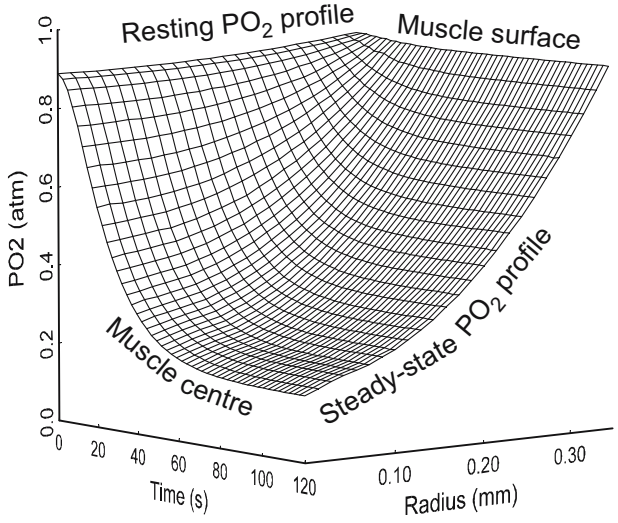

Fig. 4. Temporal and radial dependencies of P_{O_2} during rest to steady-state transition. The intramuscular P_{O_2} profile was calculated for mouse soleus muscle (radius, 0.35 mm; temperature, 27°C; surface P_{O_2}, 0.95 atm) at 0.2 s intervals during the transition from rest to steady contractile activity. The muscle was at rest initially; the P_{O_2} profile for that state is shown by the upper left border of the surface. The simulated activity was 1 s tetani repeated at 5 s intervals. As the activity commenced, P_{O_2} at the centre of the muscle declined from an initial value of 0.87 atm to a steady value of ~0.2 atm after 120 s. The surface P_{O_2} was kept at 0.95 atm throughout. This is equivalent to assuming that O_2 supply to the muscle surface was not a limiting factor.

centre. This time was calculated for mouse EDL and soleus muscles as a function of contraction duty cycle for various combinations of experimental temperature, muscle radius and surface P_{O_2} (Figure 5).

Raising temperature decreases the time, at a given duty cycle, before central anoxia begins to develop (Figure 5a and b). For example, a whole mouse soleus muscle could contract at a duty cycle of 0.5 for ~60 s at 20°C before anoxia develops but for only ~12 s at 35°C (Figure 5a). The higher active metabolic rate of the fast-twitch EDL muscle means that anoxia will develop more quickly for a given temperature and duty cycle. Reducing muscle radius, for example by using fibre bundles instead of whole muscles, is the most

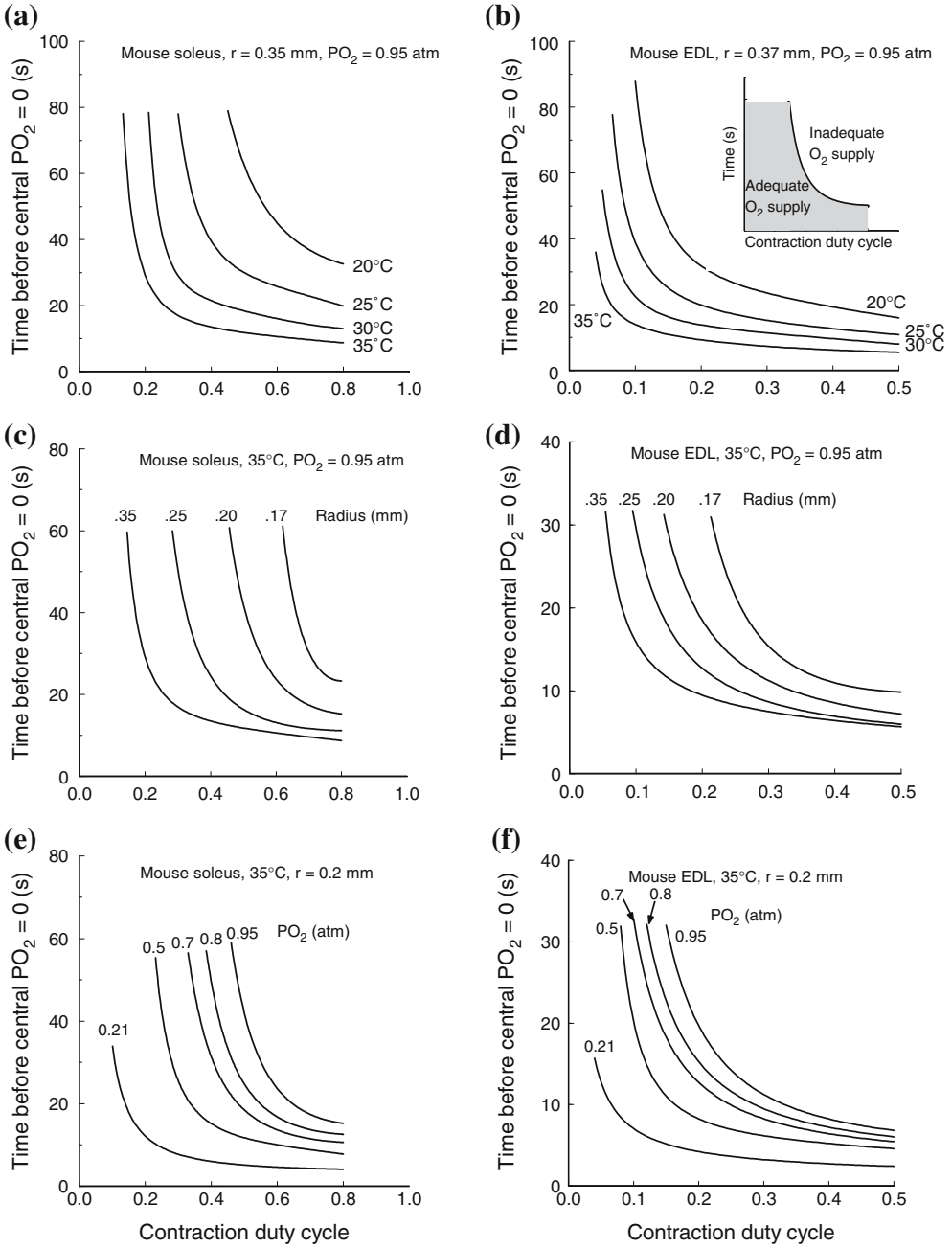

Fig. 5. Time between start of contractile activity and development of central anoxia. Calculated times for central P_{O_2} of mouse skeletal muscle preparations to decrease to 0 during the transition from rest to steady activity is shown as a function of duty cycle. The metabolic demands of combinations of duty cycle and duration of contractile activity that lie to the left of the curved lines can be met by diffusive O_2 supply (see insert in panel b); combinations that lie to the right of the curves will result in development of anoxia. Note the different duty cycle ranges for soleus and EDL muscles. Results are shown for soleus (graphs on left) and EDL (right). (a, b) Effects of temperature on time before central anoxia develops at different duty cycles. Muscle radii were taken as those typical of whole muscles. Increasing temperature decreases the time before anoxia develops. (c, d) Effect of varying muscle radius at a temperature of 35°C and surface P_{O_2} of 0.95 atm. Radii (mm) are indicated at the upper end of each curve. Decreasing radius increases the time before central anoxia develops. (e, f) Effect of varying P_{O_2} at muscle surface at a temperature of 35°C and radius of 0.2 mm (i.e. approximately half the radius of a whole muscle). Decreasing surface P_{O_2} decreases the time before central anoxia develops.

effective means of increasing the metabolic demands that can be met by diffusive O_2 supply (Figure 5c and d). The effects of variation in P_{O_2} at the muscle surface on O_2 supply are also of interest because it is not always possible to attain full equilibrium between the gas mixture and the solution (e.g. Barclay and Loiselle, 1992). Over the likely experimental range of solution P_{O_2} values (assuming the solution is aerated with 95% O_2) of 0.7–0.95, the effects of varying surface P_{O_2} are considerable (Figure 5e and f). For instance, an EDL preparation with a radius of 0.2 mm could contract at a duty cycle of 0.2 for ~20 s before central anoxia developed with a surface P_{O_2} of 0.95 atm but for only ~13 s if P_{O_2} was 0.7 atm.

In Figure 6, the time before central anoxia begins to develop is shown for whole rat soleus and EDL muscles. Due to the larger radius of these muscles compared to those of the mouse, only protocols combining a low duty cycle and short period of activity can be used performed without the likelihood of central anoxia developing.

Does the presence of myoglobin enhance diffusive O_2 supply?

The influence of myoglobin on steady-state critical radii and on time before central hypoxia develops was determined by comparing solutions to Eqs. (1) and (6). The effects of including myoglobin-facilitated O_2 diffusion in the model were very small. For instance, steady state central P_{O_2} for a whole soleus muscle (radius 0.35 mm, 25°C) contracting at a duty cycle of 0.2 was only 0.4% higher with myoglobin than without (central P_{O_2} ~0.25 atm in both cases). The effects of myoglobin were most readily evident for contraction protocols that would be predicted to reduce steady state central P_{O_2} to very low levels (i.e. within the P_{O_2} range that myoglobin releases O_2). For example, for a soleus muscle (as above) contracting at a duty cycle of 0.285 in the absence of myoglobin, central P_{O_2} declined to 0 after 125 s of activity whereas if myoglobin were included in the model, central P_{O_2} remained non-zero (0.0036 atm or 2.7 mm Hg). For duty cycles that reduced central P_{O_2} to 0 more quickly, the steepness of the P_{O_2} gradients attenuated any beneficial effects of myoglobin.

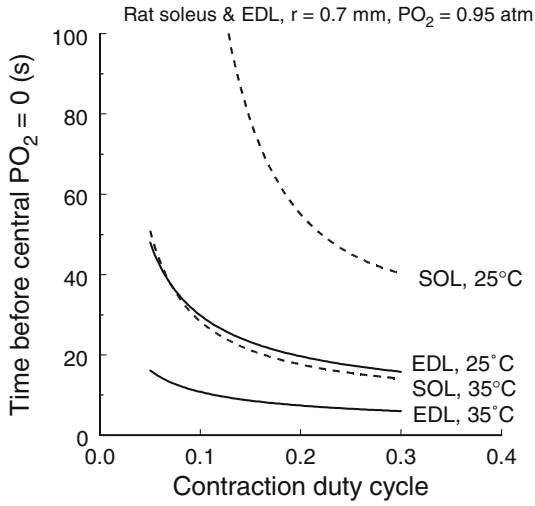

Fig. 6. Time between start of contractile activity and development of central anoxia for rat skeletal muscle. Calculated times after start of contractile activity before central P_{O_2} decreases to 0 as a function of contraction duty cycle for rat soleus (- - - -) and EDL (—) muscles. Muscle radius assumed to be 0.7 mm and surface P_{O_2} 0.95 atm. Data are shown for 25 and 35°C. The insert to Figure 5b provides a guide to interpreting these curves.

Discussion

The analysis presented here updates that provided by AV Hill (Hill, 1928, 1965: pp. 208–241). Whereas Hill's analysis dealt primarily with the classical isolated frog muscle preparation and provided analytical solutions to the diffusion equation for preparations of various geometries, the current analysis was restricted to cylindrical preparations and focused on mammalian preparations commonly used today. As is evident from Figures 1–6, ensuring preparation size and contraction protocol are compatible with adequate diffusive O_2 supply remains an important consideration for isolated muscle experiments. Perhaps one of the more surprising results of the analysis was the relative insensitivity of the steady-state critical radius for O_2 diffusion to temperature for papillary muscles. This arose from the different direction of temperature sensitivities of active and resting metabolisms in those muscles.

Overall, the analysis indicates that for all of the preparations considered, either only moderate duty cycles or contraction frequencies can be used or preparations with radii substantially below that of a whole muscle are required to ensure adequate O_2 supply across a reasonable range of duty cycles. The data in Figure 5 illustrate that reducing radius is the most effective means of enhancing diffusive O_2 supply. If investigations are to be made at physiological temperatures and contraction rates (e.g. Bluhm *et al.*, 2000; Barclay and Weber, 2004), then it is imperative that preparations substantially smaller than whole muscles are used. If radius cannot be altered, then reducing temperature also enhances O_2 supply in skeletal muscle. Although it can be difficult to ensure full equilibration of the external bathing solution with O_2, a small decrease in surface P_{O_2} has relatively modest effects on O_2 supply (Figure 5e and f). The analysis also revealed that the presence of myoglobin has such small effects on O_2 supply in isolated muscles that it would be of no practical benefit to diffusive O_2 supply. Loiselle (1987) reached a similar conclusion after calculating that although O_2 delivered from myoglobin may be an important source of O_2 for central regions of muscles (when central P_{O_2} was low), it could only account for a small fraction of the total oxygen consumed by an isolated papillary muscle.

Limitations of analysis

Equation (1) describes diffusion of O_2 through the walls of a cylinder, ignoring the contribution of O_2 diffusion through the ends of the cylinder. For a uniform cylinder, the ratio of the cross-sectional area of the two ends to the area of the cylinder walls equals the ratio of the radius to the length. For whole EDL or soleus muscles, this ratio is $\sim 0.4/10 = 0.04$ or 4%. For fibre bundles, the radius is smaller and the length little changed so the relative area of the ends would be smaller still. Therefore, ignoring O_2 diffusion through the ends of a cylinder is reasonable as this pathway could contribute little to overall O_2 supply.

Whole muscle preparations tend to be fusiform in shape so that the middle of the muscle is thicker than the average radius (used in the simulations). The assumption of uniform radius only has a small effect on predictions of the area of muscle adequately oxygenated. For instance, if the maximum radius of a fusiform muscle was 20% greater than the average radius and simulations showed that O_2 could diffuse to a distance equal to the average radius, then the area of the fusiform muscle that would be anoxic (radius = 0.2 × average radius) would be only 4% of the cross-sectional area at that position along the muscle's length.

Estimates of rates of oxygen consumption ignored the likelihood that muscles will fatigue, particularly at high duty cycles, which reduces energy cost (e.g. Barclay *et al*., 1993, 1995). For example, the energy cost of contractions by mouse EDL fibre bundles contracting with a duty cycle of 0.12 at 25°C decreased to 70% of its initial value after 30 contractions. This reduction in energy cost is equivalent to that produced by a 30% decrease in duty cycle and would increase the maximum radius to which O_2 can diffuse (i.e. moving leftwards along the lines in Figure 2). Therefore, the effect of ignoring the possible influence of fatigue on metabolic rate is that maximum diffusion distances and times before central anoxia develops may be underestimated, particularly at high duty cycles. A related consideration is that rate of O_2 consumption is unlikely to increase linearly with duty cycle across the full range of duty cycles because at some point the muscle's maximum oxidative capacity will be reached. In reality, the maximum rate of O_2 consumption by an isolated muscle is likely to be limited by the onset of fatigue; that is, over some range of duty cycles, rate of O_2 consumption will increase with duty cycle but beyond that range, further increases in duty cycle will cause sufficient fatigue that rate of O_2 consumption will decrease (Barclay and Weber, 2004).

Comparison to experimental data

Segal and Faulkner (1985) used maximum isometric twitch force as an index of adequacy of O_2 supply to isolated rat soleus and EDL muscles (radii, ~1 mm) incubated at different temperatures for 1 h. Over 60 min *in vitro* at either 35 or 40°C, but not at lower temperatures, twitch force declined substantially relative to the values measured when the muscles were first isolated. Using the data in Figure 1a, it would be predicted that diffusive O_2 supply would be adequate to meet the resting O_2 requirements of rat muscles of 1 mm radius only at temperatures below 32°C, which is consistent with the experimental data. Paradise *et al.* (1981) thoroughly mapped the relationships among twitch force, muscle radius, temperature, surface P_{O_2} and stimulation rate for papillary muscles from kittens. They defined conditions providing adequate oxygenation as those in which twitch force output did not decline relative to the values at high P_{O_2}. The current model was used to calculate critical radii under the conditions used in that study using published metabolic data for cat cardiac muscle (Loiselle and Gibbs, 1979). The calculated critical radii were, on average just 6.7% greater than the experimental values. The concordance between the results of these two studies and estimates using the diffusion model supports the notion that that the information obtained from the modelling is relevant to isolated muscle experiments.

It should be noted that the magnitude of declines in twitch and tetanic forces are unlikely to provide a quantitative indication of the degree of anoxia. For example, tetanic and twitch force output from rat skeletal muscle after 1 h at 40°C decreased by 25% and 50%, respectively (Segal and Faulkner, 1985). However, O_2 diffusion analysis predicts a central anoxic region ~0.25 mm in radius, which is 7% of the total muscle cross-sectional area and much smaller than the decline in force output.

Can whole muscles be used at physiological temperatures?

At physiological temperatures, diffusive O_2 supply to whole rat skeletal muscles is barely adequate to support resting metabolism (Figure 1a) and consequently almost any steady level of activity would produce central anoxia (Figure 2b). Whole muscles can be used without anoxia developing providing temperature and duty cycle are low (Figures 2 and 6). For example, the analysis indicated that a mouse soleus muscle at 20°C would be adequately oxygenated at duty cycles up to 0.3 (Figure 2a). However, adequate O_2 supply to a whole mouse EDL muscle could only be maintained for duty cycles below 0.1. If mouse muscles are to be studied using contraction protocols that simulate realistic locomotor muscle activity for rodents (duty cycles > 0.3; Gillis and Biewener, 2001), then preparations with smaller radii than whole muscles have to be used. Thus, the O_2 diffusion analysis indicates that for rat and mouse preparations, especially if they are to be used at physiological temperature and contraction rates, fibre bundle preparations rather than whole muscles should be used.

Consideration of O_2 supply is of particular importance in studies of muscle fatigue using isolated preparations. In a recent study, whole rat soleus muscles were fatigued using a duty cycle of 0.3 (i.e. 1 s stimulation per 3 s) for 5 min at 30°C (Kristensen *et al.*, 2005). An analysis such as that used to generate the data shown in Figure 6 was performed for those conditions and showed that central P_{O_2} would have declined to 0 in about 20 s and that once a steady state had been established ~60% of the muscle's cross-section would likely have been anoxic. In that experiment, the focus of the investigation was the effects of externally applied lactic acid on recovery from fatigue. Recovery of force generating ability in that case would be at least partly limited by diffusion of O_2 back into anoxic regions, complicating interpretation of the data. A more desirable experimental design would incorporate a smaller radius preparation to avoid central anoxia.

Can single muscle fibres become anoxic under normal experimental conditions?

Single frog muscle fibres have been used extensively to investigate contraction mechanisms. These preparations are typically oxygenated with room air (P_{O_2} at muscle surface 0.21 atm) and have radii of 50 to 100 μm (Ford *et al.*, 1977; Lombardi and Piazzesi, 1990). The first notable point is that at the low temperatures typically used for these preparations (0–5°C) O_2 consumption increases very slowly when activity commences (t = 540 s at 0°C; Hill, 1940). Even if a 100 μm radius fibre were contracting at 0°C with a duty cycle of 0.1 (e.g. Lou and Sun, 1994), then the central P_{O_2} would still be >0.17 atm (132 mm Hg) during steady state activity and if the temperature were raised to 10°C, central P_{O_2} would remain well above 0. Thus, with typical experimental contraction duty cycles, surface P_{O_2} maintained at 0.21 atm and low temperatures, it is unlikely that a single frog muscle fibre would become anoxic.

Allen and colleagues (e.g. Westerblad *et al.*, 1993; Chin *et al.*, 1997) have used single fibres from mouse fast-twitch muscles to investigate mechanisms of fatigue. These preparations have radii of ~25 μm and are bathed in solution equilibrated with 95% O_2 at 22°C. Even the most vigorous protocols used in those studies (e.g. 0.35 s tetani every 1 s), which cause considerable fatigue, central P_{O_2} would remain within 10% of the surface value. This further illustrates the notion that fatigue of isolated muscles, as indicated by reduced capacity to produce force, does not simply arise from anoxia and, conversely, changes in force output may not be a reliable means of assessing adequacy of O_2 supply.

Special consideration of resting metabolism in cardiac muscle

Resting metabolism of cardiac muscle is high compared to that of skeletal muscles and accounts for a relatively large fraction (25–30%) of total metabolism (for a review, see Gibbs and Loiselle, 2001). Furthermore, the resting metabolism is particularly high shortly after isolation of the muscles from the heart (e.g. Loiselle, 1985a, b; Widén and Barclay, 2005). The cause of this effect remains unclear (Gibbs and Loiselle, 2001). The current analysis indicates that for both rat and mouse papillary muscles diffusive O_2 supply may be inadequate to support the high initial resting metabolism (Figure 1b) and thus care must be taken during this time to optimise O_2 supply by, for example, minimizing contractile activity or lowering temperature.

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