Pflugers Arch – Eur J Physiol (2005) 450: 209–216
DOI 10.1007/s00424-005-1408-4

CARDIOVASCULAR SYSTEM

C. Widén · C. J. Barclay

Resting metabolism of mouse papillary muscle

Received: 3 February 2005 / Accepted: 4 March 2005 / Published online: 29 April 2005

Abstract The aims of this study were to measure the resting metabolic rate of isolated mouse papillary muscles and to determine whether diffusive O_2 supply is adequate to support the resting metabolism. Resting metabolism of left ventricular papillary muscles was measured in vitro (27°C) using the myothermic technique. The rate of resting metabolism declined exponentially with time towards a steady value, with a time constant of 18 ± 2 min ($n = 13$). There was no alteration in isometric force output during this time. The magnitude of the resting metabolism, which depended inversely on muscle mass, more than doubled following a change in substrate from glucose to pyruvate and was increased 2.5-fold when the osmolarity of the bathing solution was increased by addition of 300 mM sucrose. Addition of 30 mM 2, 3-butanedione monoxime affected neither the time course of the decline in metabolic rate nor the eventual steady value. Analysis of the diffusive oxygen supply to the isolated preparation indicated that small papillary muscles (mass < 1 mg), which have a very high resting metabolic rate early in an experiment, are unlikely to be adequately oxygenated.

Introduction

The isolated mouse papillary muscle is useful for determining the physiological consequences of heart-focused genetic manipulations. Papillary muscles are composed of ventricular myocytes aligned along the long axis of the muscle, making them ideal for measurement of ventricular muscle force generation, work output and energy expenditure [3, 4, 9, 13, 18]. The only source of O_2 for an isolated muscle preparation is diffusive supply from the muscle surface. If the rate of O_2 consumption is sufficiently high, diffusive O_2 supply may be inadequate to oxygenate the entire muscle cross-section, leaving an anoxic region in the centre of the muscle. An anoxic region of muscle will eventually be unable to generate force or use energy, thus reducing mass-specific values for these variables, and, as observed in ischaemic cardiac muscle, contracture is likely to develop.

The adequacy of diffusional O_2 supply is governed by the balance between the rate at which O_2 diffuses into the muscle, quantified by the diffusivity, and the rate at which the tissue consumes O_2. The probability of an anoxic core forming is enhanced by conditions that increase metabolic rate relative to O_2 diffusivity. Muscles from small animals, such as mice, have inherently high metabolic rates compared with muscles from larger animals. Furthermore, it is common for investigations using mouse papillary muscles to be performed at 37°C [6, 15, 31]. Metabolic rate is more temperature sensitive than O_2 diffusivity [30] so higher experimental temperatures increase the probability of anoxic core formation.

In most studies that have used mouse papillary muscles, there appears to have been a poor appreciation of the potential limitations of diffusive O_2 supply [6, 15, 31, 34, 36]. In one study in which the size of muscle required to avoid anoxia was assessed [33], the assessment was based on published data for rat papillary muscles but the metabolic rate of mouse cardiac muscle is likely to be greater than that of rat cardiac muscle [12]. Another study used metabolic data from isolated beating mouse hearts to analyse O_2 diffusion into isolated mouse trabeculae [35]. However, the rate of O_2 consumption used in those authors' calculations is in error through not appreciating that the original value [14] was expressed per gram dry weight. Consequently, those authors [35] overestimated the rate of O_2 consumption by a factor of ~5 and concluded, incorrectly, that even thin mouse trabeculae (half-thickness ~0.11 mm) would

C. Widén · C. J. Barclay (✉)
School of Physiotherapy and Exercise Science,
Griffith University, PMB50 Gold Coast Mail Centre,
Gold Coast, QLD, 9726, Australia
E-mail: c.barclay@griffith.edu.au
Tel.: +61-7-55528240
Fax: +61-7-55528674

become anoxic at metabolic rates occurring during contractile activity. These two examples highlight the fundamental problem in the analysis of diffusional O_2 supply to isolated mouse cardiac muscles: the metabolic rate of these muscles has not been measured.

Metabolism can be divided into a resting component, corresponding to the metabolism required to support cellular processes other than those related to contractile activity, and a contractile or active component, representing the metabolism associated with excitation, activation and contraction. In cardiac muscle, resting metabolism accounts for 20–35% of the metabolism of a beating heart [12], a much higher fraction than for other striated muscles. The first factor to be considered in assessing the viability of in vitro preparations is the ability of diffusive O_2 supply to meet the metabolic demands of the resting muscle. The aims of the present study were to measure the resting metabolic rate of mouse papillary muscles and to assess the ability of O_2 diffusion to meet the resting energy demands.

The resting metabolism of cat and rabbit papillary muscles has been measured previously. Resting metabolic rate declines exponentially with time during an experiment [25, 27, 28]. For instance, the resting metabolic rate of rat papillary muscles was ~12 mW g^{-1} 1 h after the heart was removed from the animal but 2 h hours later it had decreased to ~7 mW g^{-1} and subsequently remained at that value. Potentially, this characteristic could result in a transient, central anoxia in the muscles early in an experiment, when metabolic rate is high, with the possibility that ischaemia-reperfusion damage then occurs as the resting metabolic rate declines and O_2 supply becomes adequate. Therefore, in the current study, the time course of changes in resting metabolism was characterised.

Materials and methods

Papillary muscle preparation

Papillary muscles were dissected from the left ventricle of hearts from 6- to 12-week-old male Swiss mice. The mice were rendered unconscious by inhalation of 80% CO_2-20% O_2 gas mixture [21] and killed by cervical dislocation. All animal-handling procedures were approved by the Griffith University Animal Ethics Committee. The chest was opened, the heart rapidly excised and transferred to oxygenated (95% O_2-5% CO_2) Krebs-Henseleit solution at ~22°C and of the following composition (mM): 118 NaCl, 4.75 KCl, 1.18 KH_2PO_4, 1.18 $MgSO_4$, 24.8 $NaHCO_3$, 2.5 $CaCl_2$, 10 glucose. The heart was massaged with the apex removed to facilitate removal of blood and then placed in Krebs solution containing 30 mM 2,3-butanedione monoxime (BDM) (Sigma, St. Louis, Mo., USA) to inhibit contractile activity. BDM protects muscles from dissection damage and does not alter energetic or mechanical properties once washed out [20]. The left ventricle was opened, a papillary muscle dissected free and T-shaped aluminium clips [10, 11] attached to the tendon at one end of the muscle and to a piece of ventricular wall at the other.

Experimental apparatus for myothermic investigations

In the experimental chamber, the clip at one end of the muscle was connected to a semi-conductor force transducer (AE801, SensorOne, Sausalito, Calif., USA) and the other end held in a clamp. The muscle lay along the active thermocouples of a thin-film, antimony-bismuth thermopile [2, 32] that was used to measure changes in muscle temperature from which heat production was calculated. Two thermopiles were used in this study. The recording regions of the two thermopiles were 4 mm and 5 mm long, contained 16 and 20 thermocouples and produced 1.30 and 1.21×10^{-3} V $°C^{-1}$ respectively. Preparations were placed on the thermopile so that the section of ventricular wall and the clip holding that end of the preparation were not on the recording region. The ends of the thermopile had thermocouples that were not connected to the recording region to ensure that heat loss from the preparation was uniform along its length and prevented thermal gradients from developing along the preparation. Muscles were stimulated via two platinum wire electrodes placed on either side of the preparation. After mounting in the chamber, muscles were stretched until the passive force was ~5 mN mm^{-2}, which corresponds to a sarcomere length of ~2.1 μm (see Fig. 4 in [35]). In a preliminary experiment, muscle length was set to that giving a passive force of 5 mN mm^{-2} and then the muscles were fixed in ethanol [19]. After 24 h, the muscles were transferred to glycerol, small bundles of myocytes were teased free and their sarcomere length measured using the diffraction pattern formed when He-Ne laser light was shone through the myocytes. The mean sarcomere length was 2.12 ± 0.03 μm (mean ± SEM; $n = 5$ preparations).

Heat measurements

The thermopile was enclosed in a glass chamber containing Krebs-Henseleit solution. The chamber was submerged in a 40-l, temperature-controlled water bath (F-10-HC, Julabo Labortechnik, Seelbach, Germany) maintained at 27°C. The thermopile output was proportional to the difference in temperature between the muscle and the frame of the thermopile. The frame was kept at a constant temperature by contact with the temperature-controlled reservoir. The rate of muscle heat output was calculated from the difference in muscle temperature between that measured when the chamber was full of solution (heat produced by the muscle dissipates into the solution so muscle temperature equals the chamber temperature) and that measured when the solution was drained from the chamber (air is a poor conductor of heat so muscle temperature increases until

rate of heat loss through the thermocouples to the frame equals the rate of heat production). The chamber was aerated continuously with 95% O_2-5% CO_2 that had been thermally equilibrated and saturated with water vapour.

The thermopile output was low-pass filtered (cut-off frequency 100 Hz) and amplified using a series arrangement of two low-noise amplifiers (15C-3A, Ancom Instruments, Cheltenham, Glos., UK; SR560, Stanford Research Systems, Sunnyvale, Calif., USA). The outputs of the force transducer and thermopile were sampled at 220 Hz, digitised using a 12-bit A/D converter (DAS-1802AO, Keithley Instruments, Cleveland, Ohio, USA), displayed on a monitor and stored on disk. Software developed using Test Point (Capital Equipment Corporation, Burlington, Mass., USA) was used to control data recording and to analyse the data.

Calculation of rate of resting heat production

Resting metabolic rate was defined as the rate measured when the muscle was mechanically quiescent and had been so long enough for metabolic activity associated with any preceding contractile activity to have ceased. In preliminary experiments, it was found that after a series of contractions active metabolic rate decreased with a time constant of 15 s at 27°C. Therefore, contractile activity must have ended at least 75 s prior to measurement of resting metabolism. In the current study, muscles performed a brief series of twitches for the measurement of contractile force after each measurement of resting metabolism. The muscle was then quiescent for 10 min before the next measurement.

The rate of heat production of the resting muscles (A_R) was calculated from the change in thermopile output (ΔV, corrected for amplifier gain) that occurred upon draining the solution from the muscle chamber using the following formula [16].

$$A_R = \frac{\Delta V \times k \times C}{n \times \alpha} \quad (1)$$

where n is the number of thermocouples, α the Seebeck coefficient (μV °C^{-1} couple^{-1}), k the rate of heat loss from the muscle (s^{-1}) and C the heat capacity of the muscle and any adhering saline (mJ °C^{-1}). C and k were determined from the time course of cooling following heating of the muscle using the Peltier effect [22].

To check the resolution of the method, a series of measurements were made using a dead papillary muscle (stored in ethanol for 24 h prior to measurements and then rehydrated in saline). The resting heat rate was -0.1 ± 0.4 mW g^{-1} (mean ± SD; $n = 21$ measurements). For comparison, the minimum measured resting heat rate for live preparations was 8.4 ± 1.6 mW g^{-1}.

Experimental protocols

The thermopile system required 15 min for thermal stabilisation before the first measurement of resting metabolism could be made. Measurements were then made at 10-min intervals for 40 min. In experiments in which the effect of metabolic substrate (glucose or pyruvate) on resting metabolism was to be determined, the protocol was performed as described above using one substrate and, 40 min after the first measurement, the solution changed to one containing the other substrate and the measurement protocol repeated. The order of presentation of the substrates was alternated in successive experiments.

Sucrose was used to study the effect of hyperosmolarity on resting metabolism. For these experiments, three measurements of resting heat rate were made in the standard Krebs solution and then that solution was replaced with Krebs containing 300 mM sucrose for the following three measurements. The solution was then changed back to the standard Krebs for three more measurements (see Fig. 3a).

Data normalisation and statistical analysis

At the end of an experiment the preparation was removed from the experimental apparatus, the clips cut off, the muscle lightly blotted and its mass determined using an electronic balance (Cahn 25, Cahn Instruments, Cerritos, Calif., USA). The average cross-sectional area was calculated assuming a density of 1.06 g cm^{-3}. Active force was normalised by cross-sectional area.

All data are presented as means ± SEM. The significance of variations in metabolic rate with muscle mass were analysed using one-way ANOVA. Comparisons of substrates and the effect of hyperosmolarity were made using Student's t-test for paired samples. Decisions concerning significance were made at the 95% level of confidence.

Analysis of diffusive O_2 supply

The radius to which O_2 can diffuse (the critical radius R_C) was calculated using the equation derived by Hill [16, 17].

$$R_C = \sqrt{\frac{4K\ Y_0}{A_R}} \quad (2)$$

where K is the diffusivity of O_2 in muscle (cm^2 atm^{-1} min^{-1}), Y_o the partial pressure of O_2 (PO_2) at the muscle surface (atm) and A_R the rate of O_2 consumption of the resting muscle (cm^3 min^{-1} g^{-1}). In this model it is assumed that the papillary muscle is a cylinder of uniform radius, that negligible O_2 diffusion occurs through the ends of the cylinder, and that rate of O_2 consumption is constant. K is

2.37×10^{-5} cm^2 atm^{-1} min^{-1} at 22.8°C [30] and was adjusted to 27°C using a Q_{10} of 1.06 [30], giving a value of 2.43×10^{-5} cm^2 atm^{-1} min^{-1}. The PO_2 in the chamber solution was measured using an O_2-sensitive microelectrode (OX500, Unisense, Aarhus, Denmark) and was 0.95 atm. Rates of resting metabolism were converted to rates of O_2 consumption using an energetic equivalent of 19 mJ μl^{-1}. This was calculated on the basis that molar gas volume at 27°C is 24.6 l and the enthalpy change for glucose oxidation is 2,803 kJ mol^{-1}. The contribution of myoglobin-facilitated O_2 diffusion to O_2 supply was not included in the model because myoglobin contributes little to total O_2 flux within isolated papillary muscles when the external PO_2 is >0.2 atm [26].

Results

Resting metabolic rate of papillary muscles depended on time and muscle mass

The rate of heat output from resting papillary muscles decreased with time during an experiment (Fig. 1). The records in Fig. 1a show thermopile records from one muscle at 10-min intervals. The amplitude of the record is proportional to resting heat rate. The rate of heat production declined with time and the difference between successive 10-min intervals decreased as the experiment progressed. The time course of the decline could be well described by a single exponential function with an asymptote approximating the eventual steady value [$A_R(\infty)$, Eq. 3].

$$A_R(t) = A_R(0)e^{-t/\tau} + A_R(\infty) \quad (3)$$

where $A_R(0)$ is the first value measured in an experiment (i.e. 15 min after placing the muscle in the chamber) and τ the time constant. Figure 1b illustrates that A_R eventually stopped declining and became fairly steady. The mean time constant was 18±2 min (n=13) so that ~90 min was required before A_R attained a steady value (Fig. 1b). Despite the changes in A_R, there was no significant effect of time on isometric force production during the time course of the experiments.

The absolute value of A_R depended on muscle mass, being greater for small muscles than large muscles. To illustrate this, muscles were sorted according to mass and mean values calculated for each group (Table 1, Fig. 2). The mean resting metabolic rate of muscles with mass <1 mg (mean mass 0.74±0.03 mg; n=3) was 47±11 mW g^{-1} compared with 14±2 mW g^{-1} for muscles >2 mg (mean mass 2.6±0.2 mg; n=4). There was no significant difference in the time constant for decline in rate among the groups and the slope of a line through a plot of the individual data as a function of muscle radius did not differ significantly from zero (r^2=0.04, n=13).

On eight occasions, two papillary muscles from the same heart were tested. In those cases, the second muscle was left attached to the ventricular wall of the isolated heart for ~2 h, exposed to oxygenated Krebs, before it was transferred to the experimental chamber. The initial values of A_R for the second muscles were appropriate for their mass, as judged by data from muscles that were tested shortly after excision of the heart, and A_R declined with the same time course as for the other muscles.

The effect of BDM on resting metabolic rate

To determine whether changes in either cross-bridge activity or Ca^{2+} cycling may have contributed to the decline in rate with time, the resting metabolism of four muscles was measured with 30 mM BDM in the Krebs solution. BDM at this concentration inhibits both cross-bridge cycling and Ca^{2+} cycling [1]. A_R still declined exponentially with a time constant of 17±4 min (n=4).

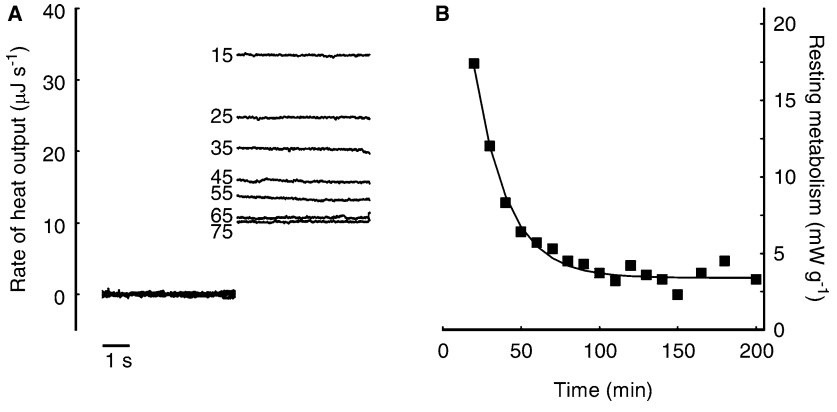

Fig. 1a,b Example of decline in resting heat rate with time during an experiment. **a** Thermopile records from one papillary muscle (mass 2.06 mg; length 4.6 mm) at different times during an experiment. The time (in min) at which each recording was made is given *beside* the record. The sections of records on the *left* are the baseline measurements made while the muscle was immersed in solution. The sections of the right were made after the solution had been drained. **b** Resting metabolic rate, normalised by muscle mass (*filled square*) (mass 1.78 mg; length 3.9 mm) measured at 10-min intervals. An exponential curve (*solid line*) was fitted through the points fitted by least-squares regression. The time constant was 20 min and the steady value was 3.6 mW g^{-1}

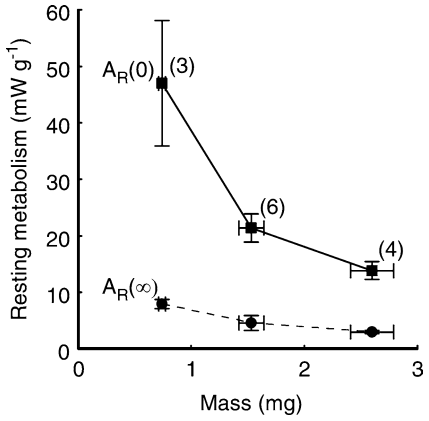

Fig. 2 Effect of muscle mass on resting metabolic rate. Muscles were divided according to wet mass into three groups (< 1 mg, 1–2 mg, > 2 mg) and for each group, the mean resting metabolic rate is plotted as a function of mean mass. The number of muscles in each group is shown in *parentheses*. Data are shown for the first measurement made in an experiment (*filled square*; *solid line*) and for the estimated steady values (*filled circle*; *dashed line*). The steady values were estimated from the exponential curves fitted to the data for each muscle (Fig. 1b). Means ± SE. The effect of muscle mass on resting metabolic rate was significant

Although the experiment was not specifically designed to determine whether the magnitude of A_R at the start of an experiment is affected by BDM, the mean value in the first measurement (9 ± 2 mW g^{-1}; mean mass 2.2 ± 0.6 mg) was comparable to that for muscles of similar mass measured without BDM. After measuring A_R in the presence of BDM for ~90 min, the solution was changed to Krebs without BDM and, after a 15-min interval (sufficient for mechanical activity to recover), A_R was measured again. At that time, A_R measured without BDM present did not differ significantly from that measured previously with BDM.

The effect of hyperosmolarity on resting metabolic rate

A characteristic of resting metabolism in striated muscles from other species is that it increases with increasing osmolarity of the bathing solution (e.g. [29]). To see whether this also applied to mouse papillary muscles, resting metabolism measured in normal Krebs solution was compared with that measured in the presence of the impermeant solute sucrose (300 mM ≡ 300 mOsmol l^{-1}, a threefold increase in osmolarity). In the hyperosmotic solution, A_R increased greatly (Fig. 3a) and force output (Fig. 3b) was almost abolished. The mean resting heat rate with sucrose was $247 \pm 20\%$ ($n = 4$) of that in normal Krebs solution. Upon returning the muscle to normal solution, both A_R and force output returned to previous values within 15 min.

Table 1 Characteristics of mouse papillary muscle preparations

	< 1 mg	1–2 mg	> 2 mg
Number of muscles	3	6	4
Wet mass (mg)	0.74 ± 0.03	1.5 ± 0.1	2.6 ± 0.2
Length (mm)	2.8 ± 0.3	3.8 ± 0.2	4.2 ± 0.4
Cross-sectional area (mm^2)	0.26 ± 0.02	0.39 ± 0.04	0.61 ± 0.09
Radius (mm)	0.28 ± 0.01	0.35 ± 0.02	0.44 ± 0.03

The effect of metabolic substrate on resting metabolism and force output

Two exogenous substrates are commonly used in experiments with isolated papillary muscles: glucose and pyruvate. In all preparations studied ($n = 10$), resting metabolic rate was higher with pyruvate than with

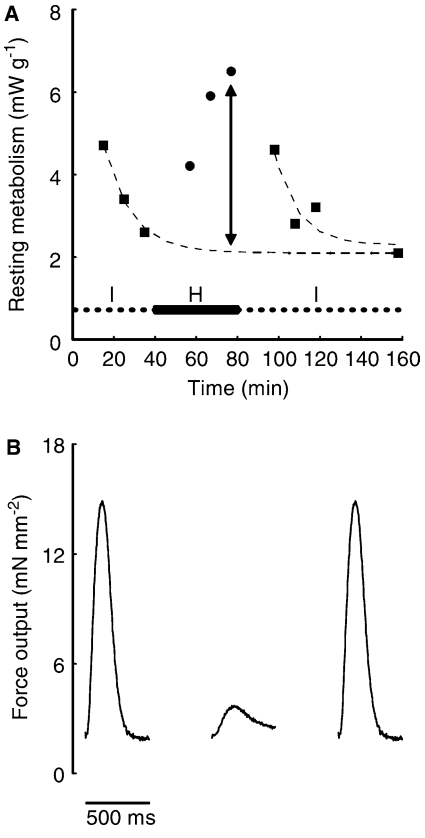

Fig. 3a,b Example of the effect of hyperosmolarity on resting heat output and force production. **a** The protocol used to determine the effect of hyperosmolarity on resting metabolism. The first three measurements of resting metabolism (*filled squares*) were made in the presence of isosmotic saline (*I*). The exponential decline in rate was fitted with a curve (*dashed line*). Three measurements were then made in the hyperosmotic solution (time of exposure is indicated by *H* and *solid horizontal line*; resting heat rate, *filled circles*), followed by another four in isosmotic solution. The magnitude of the hyperosmotic stimulation of resting metabolism (*arrow*) was determined by comparing the resting metabolism with that predicted by extrapolation of the exponential fitted through the first three points (*dashed line*). Hyperosmolarity caused a three-fold increase in resting heat rate in this example. **b** Records of the time course of isometric force production made before (*left*), during (*middle*) and after (*right*) exposure to the hyperosmolar solution. Hyperosmolarity reversibly decreased isometric force production

glucose. The mean difference (A_R with pyruvate minus A_R with glucose) was significant and was 2.8 ± 0.8 mW g^{-1}, which corresponded to the value in pyruvate being $210 \pm 50\%$ of that in glucose. There was no significant difference in the isometric force output between the two substrates [mean values: pyruvate 20 ± 3 mN mm^{-2}; glucose 19 ± 2 mN mm^{-2} ($n = 9$)].

Discussion

The above data are the first measurements of the metabolism of mouse papillary muscles. The primary purpose of this study was to obtain data that could be used to assess the adequacy of diffusive O_2 supply. In addition, the characterisation of resting metabolism of mouse papillary muscles provides some further insight into the metabolism of in vitro preparations of cardiac muscle and these will be discussed first.

Comparison with other studies

A prominent characteristic of the resting metabolism of papillary muscles is that it declines with time (Fig. 1). This has been observed in papillary muscles from cats and rats [27] but not guinea-pigs [8, 27]. For papillary muscles from rats, the time constant for the decline in resting heat rate is greater [~60 min at 27°C; 24, 27] than that for mouse papillary muscles (~20 min at 27°C). The cause of the decline in resting metabolism with time remains obscure. It is not a reflection of a dying muscle, because the mechanical performance does not alter with time, nor does it represent an irreversible or only slowly reversible depression of metabolic capacity, because the resting metabolism can be stimulated several-fold by changing substrate from glucose to pyruvate and increasing the osmolarity of the bathing solution (Fig. 3). The decline is related to isolation of the muscles, since it probably does not occur in isolated hearts (for a detailed discussion, see [12]). Gibbs and Loiselle [12] have suggested that the decline in resting metabolism may arise from accumulation of an inhibitory endothelial factor that accumulates in the absence of microvascular circulation. Our data suggest that the rate does not decline from the time the heart is excised, as previously suggested [12, 24, 27] because in our experiments the resting metabolism was the same whether muscles were tested within 20 min of excision of the heart or had remained in the open, superfused ventricle for 2 h before dissection. This suggests that the decline in metabolic rate is initiated by dissection of the papillary muscle from the ventricle. This raises the possibility that the resting metabolic heat rate is high initially due to dissection damage, as has been described for skeletal muscle [5]. The only part of the papillary muscles that necessarily incurred damage during dissection was the end that attached to the ventricular wall (one of the clips holding the preparation was crimped onto a piece of ventricular wall muscle) but heat output from that section of the preparation was not included in our recordings. Thus, it is unlikely that the high metabolic rate recorded at the start of an experiment was related to dissection damage. The cause of the change in resting metabolic rate with time in isolated papillary muscles thus remains unclear.

The inverse relationship between muscle size and resting metabolism has been described previously for rabbit papillary muscles [28], although in the current study the magnitude (both absolute and relative) of this effect was greater than that for rabbit muscles. An obvious explanation for such an effect is that larger muscles are unable to get enough O_2 by diffusion to support their metabolism, so an anoxic core develops. However, this explanation has been shown to be incorrect, by both modelling and experiment [23, 26, 28]. In fact, as will be demonstrated in the following section, it is the small preparations used in the current study that are most likely to be O_2 diffusion limited.

Adequacy of diffusive oxygen supply

The main purpose of these experiments was to obtain data to enable an assessment of the adequacy of diffusive O_2 supply to meet the metabolic requirements of resting mouse papillary muscles. If the metabolic rate of an isolated muscle is high compared with the rate at which O_2 diffuses into the muscle, an anoxic region may develop in the core of the muscle. Furthermore, if an anoxic region subsequently becomes oxygenated, for example as metabolic rate declines with time, then the cellular characteristics associated with ischaemia-reperfusion damage (for a review, see [7]) may become apparent.

The results of the analysis of diffusive O_2 supply are illustrated in Fig. 4 in which the resting metabolic rates of the muscles used in this study are plotted as a function of muscle radius. Data are shown both for the first values measured (Fig. 4a) and for the estimated steady values (Fig. 4b). The curved lines indicate the calculated critical radius (R_C; Eq. 2) for each combination of radius and metabolic rate. For muscles lying to the right of these lines, O_2 diffusion would have been inadequate to meet the O_2 demand. For the metabolic rates measured early in an experiment, the calculations indicate that six muscles may have had inadequate O_2 supply (Fig. 4a). Three of these were muscles with masses below 0.8 mg. Once resting metabolic rate had declined to its steady value, all the muscles used were below the critical radius for their metabolic rate.

This analysis illustrates that despite the small size of the mouse papillary muscles, at early times in an experiment their metabolic rate can be sufficiently high for the formation of an anoxic core and, in an apparent paradox, this is particularly likely to occur for the smallest muscles because they have high metabolic rates. Furthermore, because the resting metabolic rate is

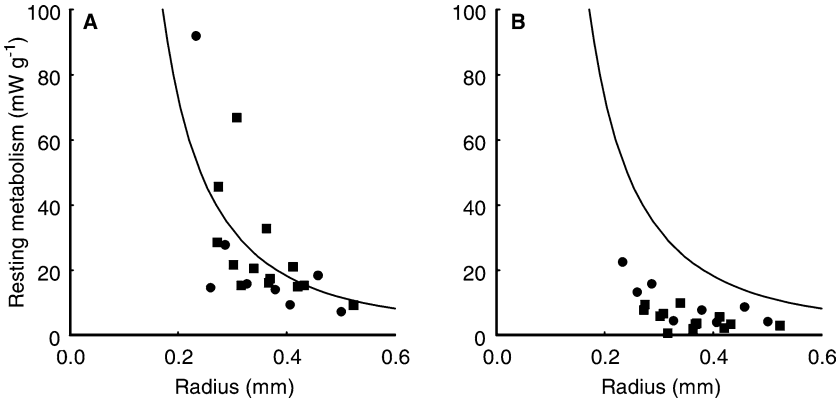

Fig. 4a,b Adequacy of diffusive oxygen supply to resting mouse papillary muscle. The resting metabolic rates measured 15 min after placing the muscles in the chamber (**a**) and the estimated steady value (**b**) plotted as a function of muscle radius. Muscle radii were calculated from the average cross-sectional area for each muscle. Data are shown for muscles with glucose (*filled squares*) and pyruvate (*filled circles*) as substrate. The *curved lines* are the critical radii for diffusive O_2 supply at 27°C; to the *left* of the curves, muscles are adequately oxygenated and to the *right*, O_2 supply would have been inadequate to maintain $PO_2 > 0$ throughout the muscle cross-section. Critical radii were calculated using Eq. 2

doubled if pyruvate is used as a substrate, achieving adequate oxygenation would be more difficult using that substrate; doubling metabolic rate reduces R_C by a factor of $\sqrt{(0.5)}$, i.e. ~0.7.

Experimental temperature also has an important effect on diffusive O_2 supply with lower temperatures favouring O_2 supply because resting metabolism is more temperature sensitive (Q_{10}~1.3; [25,28]) than O_2 diffusivity ($Q_{10} = 1.06$; [30]). Some mouse papillary muscle experiments have been performed at 37°C [6, 15, 31]. At that temperature, metabolic rate would be 1.3 times greater than the values in Fig. 4 and the critical radius would be decreased by a factor of $\sqrt{(1.06/1.3)}$, i.e. 0.9. The metabolic rate of contracting mouse papillary muscles has not yet been measured but contractile activity at physiological frequencies might be expected to double metabolic rate [12], decreasing the critical radius by an additional factor of 0.7. If we consider a mid-range papillary muscle (mass ~1.5 mg; radius 0.35 mm), at 27°C it has an eventual steady A_R of ~4.5 mW g^{-1} and R_C would be 0.8 mm ($R_C >$ actual radius implies adequate O_2 supply). Some 2 h after dissection, at 37°C, contracting and with pyruvate as substrate, total metabolic rate could be ~18 mW g^{-1} and R_C would be reduced to 0.4 mm but still greater than the actual radius. However, under those conditions R_C would be < 0.3 mm, favouring anoxic core formation, throughout the first 30 min in vitro. These calculations emphasise that the adequacy of diffusive O_2 supply cannot be taken for granted simply because the preparation is small.

Recommendations for performing experiments with isolated papillary muscles

In summary, even for quiescent mouse papillary muscles it is possible that diffusive O_2 supply is inadequate, especially shortly after removal of the muscle from the heart. On the basis of the data obtained in this study we would recommend the following procedures to minimise the chances of O_2 supply becoming inadequate: (1) avoid using very small papillary muscles (< 1 mg); (2) use glucose rather than pyruvate as substrate; (3) use a temperature lower than 37°C; (4) avoid or minimise contractile activity during the equilibration period after dissecting the muscle. Implementing recommendations 2–4 just during the time that A_R is high (the first 40–60 min after dissection) would also minimise the chances of developing anoxia early in an experiment.

Acknowledgements This work was supported by the Heart Foundation Research Centre, Griffith University.

References

1. Backx PH, Gao WD, Azan-Backx MD, Marban E (1994) Mechanism of force inhibition by 2,3-butanedione monoxime in rat cardiac muscle: roles of $[Ca^{2+}]_i$ and cross-bridge kinetics. J Physiol (Lond) 476:487–500
2. Barclay CJ, Arnold PD, Gibbs CL (1995) Fatigue and heat production in repeated contractions of mouse skeletal muscle. J Physiol (Lond) 488:741–752
3. Barclay CJ, Widén C, Mellors LJ (2003) Initial mechanical efficiency of isolated cardiac muscle. J Exp Biol 206:2725–2732
4. Baxi J, Barclay CJ, Gibbs CL (2000) Energetics of rat papillary muscle during contractions with sinusoidal length changes. Am J Physiol 278:H1545–H1554
5. Biron R, Burger A, Chinet A, Clausen T, Dubois-Ferriere R (1979) Thyroid hormones and the energetics of active sodium-potassium transport in mammalian skeletal muscles. J Physiol (Lond) 297:47–60
6. Bluhm WF, Kranias EG, Dillmann WH, Meyer M (2000) Phospholamban: a major determinant of the cardiac force-frequency relationship. Am J Physiol 278:H249–H255
7. Bolli R, Marban E (1999) Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 79:609–634
8. Daut J, Elzinga G (1988) Heat production of quiescent ventricular trabeculae isolated from guinea-pig heart. J Physiol (Lond) 398:259–275

9. Dietrich DL, Elzinga G (1993) Heat produced by rabbit papillary muscle during anoxia and reoxygenation. Circ Res 73:1177–1187
10. Donald TC, Reeves DN, Reeves RC, Walker AA, Hefner LL (1980) Effect of damaged ends in papillary muscle preparations. Am J Physiol 238:H14–H23
11. Ford LE, Huxley AF, Simmons RM (1977) Tension responses to sudden length change in stimulated frog muscle fibres near slack length. J Physiol (Lond) 269:441–515
12. Gibbs CL, Loiselle DS (2001) Cardiac basal metabolism. Jpn J Physiol 51:399–426
13. Gibbs CL, Mommaerts WF, Ricchiuti NV (1967) Energetics of cardiac contractions. J Physiol (Lond) 191:25–46
14. Gustafson LA, Van Beek JH (2000) Measurement of the activation time of oxidative phosphorylation in isolated mouse hearts. Am J Physiol 279:H3118–H3123
15. He H, Giordano FJ, Hilal-Dandan R, Choi DJ, Rockman HA, McDonough PM, Bluhm WF, Meyer M, Sayen MR, Swanson E, Dillmann WH (1997) Overexpression of the rat sarcoplasmic reticulum Ca^{2+} ATPase gene in the heart of transgenic mice accelerates calcium transients and cardiac relaxation. J Clin Invest 100:380–389
16. Hill AV (1928) The diffusion of oxygen and lactic acid through tissues. Proc R Soc B 104:39–96
17. Hill AV (1965) Trails and trials in physiology. Arnold, London
18. Hisano R, Cooper G (1987) Correlation of force-length area with oxygen consumption in ferret papillary muscle. Circ Res 61:318–328
19. Josephson R, Stokes D (1994) Contractile properties of a high-frequency muscle from a crustacean—mechanical power output. J Exp Biol 187:295–303
20. Kiriazis H, Gibbs CL (1995) Papillary muscles split in the presence of 2,3-butanedione monoxime have normal energetic and mechanical properties. Am J Physiol 269:H1685–H1694
21. Kohler I, Meier R, Busato A, Neiger-Aeschbacher G, Schatzmann U (1999) Is carbon dioxide (CO_2) a useful short acting anaesthetic for small laboratory animals? Lab Anim 33:155–161
22. Kretzschmar KM, Wilkie DR (1972) A new method for absolute heat measurement, utilizing the Peltier effect. J Physiol (Lond) 224:18P–21P
23. Loiselle D (1985) A theoretical analysis of the rate of resting metabolism of isolated papillary muscle. Adv Myocardiol 6:205–216
24. Loiselle DS (1985) The effect of temperature on the basal metabolism of cardiac muscle. Pflugers Arch 405:163–169
25. Loiselle DS (1985) The rate of resting heat production of rat papillary muscle. Pflugers Arch 405:155–162
26. Loiselle DS (1987) The effect of myoglobin-facilitated oxygen transport on the basal metabolism of papillary muscle. Biophys J 51:905–913
27. Loiselle DS, Gibbs CL (1979) Species differences in cardiac energetics. Am J Physiol 237:H90–H98
28. Loiselle DS, Gibbs CL (1983) Factors affecting the metabolism of resting rabbit papillary muscle. Pflugers Arch 396:285–291
29. Loiselle DS, Stienen GJ, van Hardeveld C, van der Meulen ET, Zahalak GI, Daut J, Elzinga G (1996) The effect of hyperosmolality on the rate of heat production of quiescent trabeculae isolated from the rat heart. J Gen Physiol 108:497–514
30. Mahler M, Louy C, Homsher E, Peskoff A (1985) Reappraisal of diffusion, solubility, and consumption of oxygen in frog skeletal muscle, with applications to muscle energy balance. J Gen Physiol 86:105–134
31. Meyer M, Bluhm WF, He H, Post SR, Giordano FJ, Lew WY, Dillmann WH (1999) Phospholamban-to-SERCA2 ratio controls the force-frequency relationship. Am J Physiol 276:H779–H785
32. Mulieri LA, Luhr G, Trefry J, Alpert NR (1977) Metal-film thermopiles for use with rabbit right ventricular papillary muscles. Am J Physiol 233:C146–C156
33. Redel A, Baumgartner W, Golenhofen K, Drenckhahn D, Golenhofen N (2002) Mechanical activity and force-frequency relationship of isolated mouse papillary muscle: effects of extracellular calcium concentration, temperature and contraction type. Pflugers Arch 445:297–304
34. Stull LB, Leppo MK, Marban E, Janssen PM (2002) Physiological determinants of contractile force generation and calcium handling in mouse myocardium. J Mol Cell Cardiol 34:1367–1376
35. Stuyvers BD, McCulloch AD, Guo J, Duff HJ, ter Keurs HE (2002) Effect of stimulation rate, sarcomere length and Ca^{2+} on force generation by mouse cardiac muscle. J Physiol (Lond) 544:817–830
36. Wang JF, Yan X, Min J, Sullivan MF, Hampton TG, Morgan JP (2002) Cocaine downregulates cardiac SERCA2a and depresses myocardial function in the murine model. Can J Physiol Pharmacol 80:1015–1021