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Initial mechanical efficiency of isolated cardiac muscle

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Summary

The aim of this study was to determine whether the initial mechanical efficiency (ratio of work output to initial metabolic cost) of isolated cardiac muscle is over 60%, as has been reported previously, or whether it is approximately 30%, as suggested by an estimate based on the well-established net mechanical efficiency (ratio of work output to total, suprabasal energy cost) of 15%. Determination of initial efficiency required separation of the enthalpy output (i.e. heat + work) into initial and recovery components. The former corresponds to energy produced by reactions that use high-energy phosphates and the latter to energy produced in the regeneration of high-energy phosphates. The two components were separated mathematically. Experiments were performed *in vitro* (30°C) using preparations dissected from rat left ventricular papillary muscles (*N*=13). Muscle work output and heat production were measured during a series of 40 contractions using a contraction protocol designed to mimic *in vivo* papillary muscle activity. Net mechanical efficiency was 13.3±0.7%. The total enthalpy output was 2.16 times greater than the initial enthalpy output, so that initial mechanical efficiency was 28.1±1.2%.

Key words: muscle energetics, heat production, efficiency, cardiac muscle.

Introduction

Two groups of biochemical reactions underlie muscle contraction: those that consume high-energy phosphates, called initial reactions, and those that regenerate high-energy phosphates, called recovery reactions. Muscular efficiency is the ratio of mechanical work produced to the metabolic energy consumed in the production of that work. The energy consumption term can either incorporate just the initial energy costs, giving the initial mechanical efficiency (e_I), or can encompass the net energy cost (the energetic equivalent of the oxygen consumed), giving the net mechanical efficiency (e_N). e_I is of interest because it provides insights into the fundamental mechanism of energy conversion by myosin cross-bridges.

The efficiency of mechanical work generation by cross-bridges in cardiac muscle is poorly established because it is difficult to experimentally separate the initial and recovery energy costs. The kinetics of recovery metabolism in cardiac muscle are so rapid that even the energy used within the time course of a single twitch includes a significant recovery metabolism component (Gibbs et al., 1967; Mast and Elzinga, 1990). Peterson and Alpert (1991) subtracted the presumed recovery heat component from the energy output recorded during isotonic shortening of rabbit papillary muscles and concluded that the maximum e_I in rabbit papillary muscles was 65%. This value is high compared with an estimate based on reported values of e_N. e_N of isolated cardiac muscle is typically ~15% (Gibbs et al., 1967; Syme, 1994; Mellors et al., 2001; Mellors and Barclay, 2001). The magnitude of the net metabolic cost of a series of contractions is typically twice that of initial metabolism (e.g. Mast et al., 1990) so e_I should be ~2-fold greater than e_N; that is, about 30%.

Both the approaches described above for determining e_I contain elements of uncertainty. For example, Peterson and Alpert (1991) implicitly assumed that the energy output associated with shortening was synchronous with shortening, but, at least in isometric contractions, a substantial fraction of the initial energy output associated with a single twitch appears late in the contraction, during force relaxation (Mast and Elzinga, 1990). To estimate e_I from e_N, it must be assumed that the ratio of energy output from recovery processes (*R*) to energy output from initial processes (*I*) is the same in isometric contractions and contractions with shortening because the *R*:*I* ratio in cardiac muscle has only been measured using isometric contractions (Mast et al., 1990). Although it seems reasonable to assume that the *R*:*I* ratio is independent of contraction type, the only published comparison of the *R*:*I* ratio in isometric and working contractions, which was made using mouse skeletal muscle (Woledge and Yin, 1989), revealed that the ratio was greater in shortening contractions (1.25) than in isometric contractions (1.0). It seems unlikely that such an effect could underlie the combination of e_I=65% and e_N=15% in cardiac muscle, because this would require the *R*:*I* ratio in working

contractions to be an improbable 2.5. This emphasises the uncertainty attached to the high e_I value reported by Peterson and Alpert (1991).

It is important to establish whether initial efficiency is over 60% or just 30% because, although the latter can be easily accommodated within a cross-bridge model using known mechanical properties of cardiac cross-bridges, the former cannot. For example, an e_I of 30% is consistent with each cross-bridge converting ~20% of the free energy from hydrolysis of one ATP molecule into work (for details of this calculation, see Discussion). This is quantitatively consistent with a cardiac cross-bridge model in which each cross-bridge cycle is associated with splitting of one ATP and in which the mean cross-bridge force and power stroke are approximately 2 pN and 10 nm, respectively. These values for cross-bridge force output and displacement correspond to those measured in experiments using isolated contractile proteins from cardiac muscle (Van Buren et al., 1995; Sugiura et al., 1998). If these cross-bridge forces and displacements are correct but e_I is >60% then there must be approximately two cross-bridge cycles performed using the energy from each ATP molecule. Alternatively, if there is a one-to-one coupling between ATP-splitting cycles and cross-bridge cycles, then an e_I of 60% could only come about if the product of cross-bridge force and power stroke were twice that calculated from *in vitro* measurements from cardiac cross-bridges (Sugiura et al., 1998).

The purpose of the present study was to determine both the net and initial mechanical efficiencies of cardiac muscle during steady contractile activity. To do this, the enthalpy produced by rat papillary muscles during and after a series of contractions was partitioned into initial and recovery components using a mathematical analysis described previously (Mast et al., 1990). This overcomes the problem of assuming that work output and the associated energy output are synchronous because all the energy produced by the muscles during and after the series of contractions was measured. The analytical method also calculated the *R*:*I* ratio using the energetic data recorded during the protocol in which the muscles were performing work, thus avoiding the need to extrapolate from reported values obtained using isometric contraction protocols. Furthermore, a contraction protocol designed to simulate *in vivo* strain patterns was used (Mellors and Barclay, 2001). Most previous studies of cardiac efficiency using isolated preparations have used less realistic protocols (e.g. Gibbs et al., 1967; Peterson and Alpert, 1991).

Materials and methods

The rat left ventricular papillary muscle preparation and the techniques for recording energy output from them have been described in detail previously (Baxi et al., 2000; Mellors et al., 2001; Mellors and Barclay, 2001) and will be only briefly described here.

Papillary muscles were dissected from the left ventricle of hearts removed from adult, male rats (*Rattus norvegicus* L.). Rats were first rendered unconscious by inhalation of chloroform and then killed by cervical dislocation. All animal handling procedures complied with the requirements of the Monash University Animal Ethics Committee. Muscles were bathed in oxygenated (95% O_2/5% CO_2) Krebs–Henseleit solution of the following composition: 118 mmol l^{-1} NaCl; 4.75 mmol l^{-1} KCl; 1.18 mmol l^{-1} KH_2PO_4; 1.18 mmol l^{-1} $MgSO_4$; 24.8 mmol l^{-1} $NaHCO_3$; 1.6 mmol l^{-1} $CaCl_2$; 10 mmol l^{-1} glucose. During dissection, 30 mmol l^{-1} butanedione monoxime (BDM) was added to the solution to optimise the recovery of mechanical function after dissection. Following dissection of the muscle, further dissection was performed to give a thin preparation of uniform cross-section. The preparation characteristics (mean ± S.E.M., N=13) were: mass, 2.63±0.3 mg; length, 4.53±0.3 mm; cross-sectional area, 0.55±0.04 mm^2; radius (assuming circular cross-section), 0.41±0.02 mm. During experiments, solution temperature was maintained at 30°C.

Small, platinum loops were tied to either end of the preparation and placed over hooks on two tungsten connecting rods, one attached to a strain gauge force transducer (SensoNor 801, Horten, Norway) and the other to the lever arm of an ergometer (Cambridge 300H, Cambridge Instruments, MA, USA). The preparation lay along a thermopile that was 4 mm long, contained 16 antimony–bismuth thermocouples and had an output of 0.81 mV deg.$^{-1}$ (Barclay et al., 1995; Mellors et al., 2001). The muscles were stimulated using rectangular electrical pulses delivered to the muscle *via* fine platinum wires that contacted the platinum loops attached to either end of the muscle.

Measurements of energy output

Enthalpy output was used as an index of muscle energy use. Contracting muscles produce energy as both mechanical work and heat; enthalpy output is the sum of the work and heat produced. Work output was calculated from records of muscle force output and change in muscle length. Heat output, excluding basal heat production, was calculated from the changes in muscle temperature, measured using the thermopile, during and after a series of contractions. Temperature records were corrected for heat lost from the muscle during recording and then multiplied by muscle heat capacity to determine the heat output. Rate of heat loss and effective heat capacity (i.e. the combined heat capacity of the muscle, any adhering solution and the thermopile under the muscle) were determined from the time course of cooling of the preparation after the muscle had been heated using the Peltier effect (Kretzschmar and Wilkie, 1972).

Contraction protocol

At the start of each experiment, the stimulus strength required to elicit maximum twitch force and the length at which twitch force was maximal (L_{max}) were determined. During the remainder of the experiment, a contraction protocol that was designed to closely match the *in vivo* strain dynamics of papillary muscles was used (Mellors and Barclay, 2001). It

consisted of 40 twitches delivered at a frequency of 2.2 Hz. During each contraction cycle (total duration 455 ms), muscle length was held constant for the first 45 ms after the stimulus was applied, then was allowed to decrease at a constant velocity for ~160 ms through an amplitude of ~10% L_{max}, and finally was stretched back to L_{max} at constant velocity for the remainder of the cycle (Fig. 1A). The timing of these length changes allowed force relaxation to be complete just prior to the start of the stretching phase of each cycle. The net efficiency measured using this protocol (Mellors and Barclay, 2001) is the same as the maximum net efficiency measured using either isotonic contractions or contractions with sinusoidal length changes (Mellors et al., 2001).

Analysis of initial and recovery energy output

Cumulative work output (Fig. 2A) was calculated by summing the net work produced in each contraction. Net work was calculated by determining the area enclosed by a plot of force output as a function of change in muscle length and was thus the difference between the work done during shortening and the work done on the (relaxed) muscle to stretch it back to L_{max}. This method excludes contributions to the work performed during shortening by both parallel and series elastic elements (Mellors et al., 2001).

Enthalpy output was partitioned into initial and recovery components using the procedure described by Mast et al. (1990). This method uses the *rate* of enthalpy output and, in the present study, this was calculated by numerical differentiation of the cumulative enthalpy output (Fig. 2B). Although this creates a noisy signal, the analysis is sufficiently robust that the noise had little quantitative effect on the energy partitioning. Full details of the energy partitioning procedure and its underlying assumptions have been presented by Mast et al. (1990). Note that these authors demonstrated that this procedure gives the same result for partitioning energy output whether applied to data obtained during an energetic steady state or to data obtained during the transition from rest to a steady state, the case used in the present study.

The partitioning method (Mast et al., 1990) determines the amount of recovery heat produced per unit of initial heat produced (*R*:*I* ratio). It requires calculation of the time constant (τ) of the decline in rate of heat output after the contractions have ended. This was done by fitting, using the Levenberg–Marquardt method, a single exponential function through 50 s of rate of heat output data recorded after the final contraction (Fig. 2B). Using τ, the *R*:*I* ratio and the time course of changes in rate of enthalpy output, the time course of changes in rate of recovery heat output can be calculated. An iterative procedure was used to find the value of the *R*:*I* ratio that provided the closest match between the calculated time course of recovery heat output and the measured, post-contraction decline in heat rate (i.e. when it can be assumed that all the heat produced is recovery heat; Fig. 2B). This *R*:*I* ratio was then used to divide the heat rate data during the contraction series into initial and recovery components. The

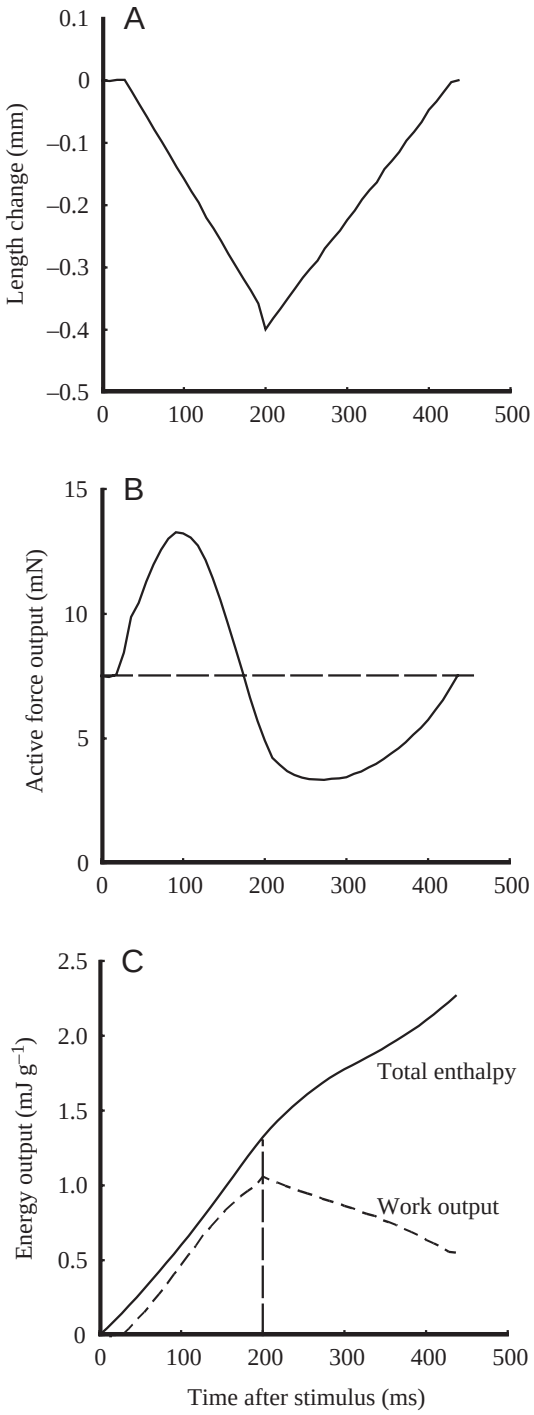

Fig. 1. An example of records, averaged over the last 10 cycles (4.54 s) of the contraction protocol, from one papillary muscle (mass, 3.1 mg; L_{max}, 5.9 mm). Stimulus was applied at 0 ms. (A) Change in muscle length. The muscle was initially held isometric, was then allowed to shorten at constant velocity through an amplitude of 10% L_{max} and was then lengthened at constant velocity. (B) Absolute force output. The horizontal broken line indicates the passive force at L_{max}. The force was lower after shortening because the passive force was lower at the short length. The muscle was relaxed during most of the lengthening phase. (C) Time course of work output and total enthalpy output (work + heat). The vertical broken line indicates the time at which shortening ended.

calculated rates of initial energy output and recovery energy output were then integrated (Fig. 2C), and the cumulative total of each variable was used to calculate the initial and net mechanical efficiencies.

Calculation of mechanical efficiency

Efficiency was defined as the ratio of work output to metabolic energy cost, expressed as a percentage. Net mechanical efficiency (e_N) was defined as the ratio of the net mechanical work output during the series of contractions (W_N) to the total, suprabasal enthalpy produced during and after the series of contractions. Enthalpy output was the sum of the total heat output (Q_T) and the net work performed (W_N):

$$e_N = \frac{W_N}{Q_T + W_N} \times 100 . \quad (1)$$

Initial mechanical efficiency (e_I) was defined as the ratio of W_N to the total initial enthalpy output. The total initial enthalpy output was the sum of W_N and the cumulative total of the calculated initial heat output (Q_I):

$$e_I = \frac{W_N}{Q_I + W_N} \times 100 . \quad (2)$$

In the Discussion, consideration is given to thermodynamic efficiency, which is the ratio of work output to free energy change and is given the symbol ε.

Preparation oxygenation

The adequacy of oxygenation was assessed by calculating the change in partial pressure of O_2 (P_{O_2}) through the cross-section of a cylindrical muscle. The analysis, which has been described in detail previously (Loiselle, 1985a; Baxi et al., 2000), was based on Hill's analysis of diffusion into a cylinder (Hill, 1928) but incorporated a realistic relationship between the rate of mitochondrial oxygen consumption and P_{O_2} (Loiselle, 1985a). The analysis assumes that muscles are uniform cylinders, that metabolic rate is constant and that diffusion of O_2 into the ends of the cylinder is negligible. Steady-state rate of O_2 consumption was calculated from the rate of enthalpy output measured during the last three steady-state contraction cycles, when the muscles were close to an energetic steady state (i.e. muscle temperature was the same at the start of successive cycles; Paul, 1983). An energetic equivalent of 20 mJ μl^{-1} O_2 was used to convert enthalpy measurements into equivalent O_2 consumption.

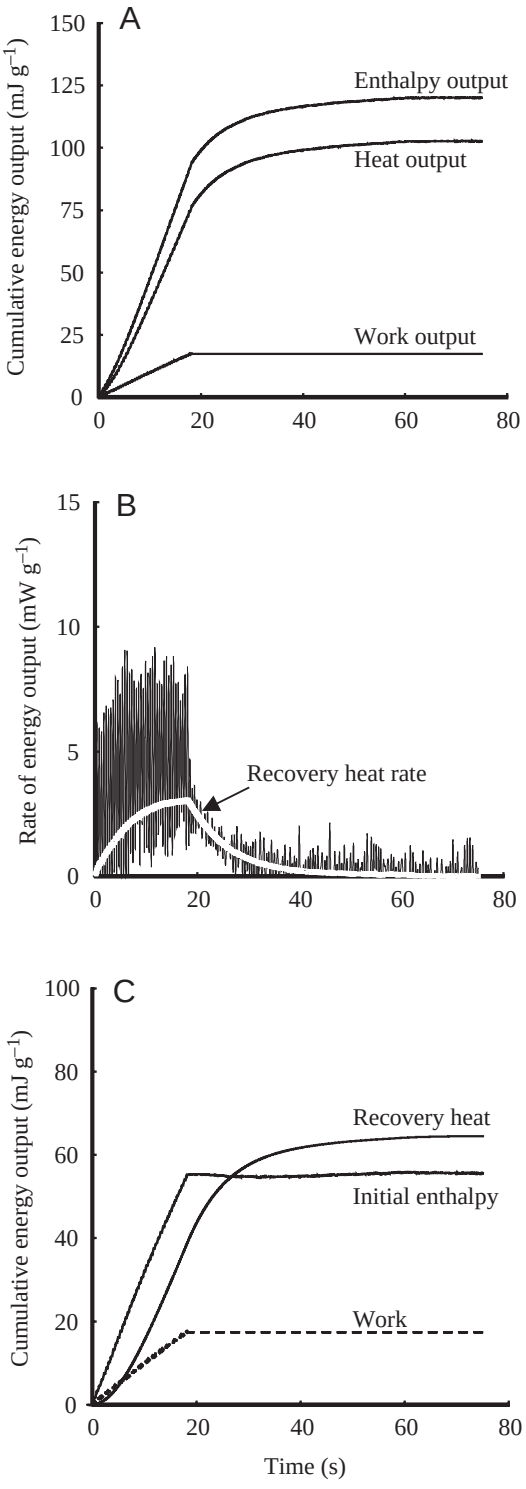

Fig. 2. Records of muscle energy output and illustration of steps in analysis. (A) Cumulative outputs of work, heat and enthalpy (i.e. heat + work) from a rat papillary muscle preparation (mass, 2.97 mg; length, 5.0 mm) during and after a series of 40 twitches delivered at 2.2 Hz. (B) The time course of rate of enthalpy output, calculated by differentiation of the cumulative enthalpy record in A. A single exponential was fitted through the signal recorded after the contractions had ended, indicated by the declining white line from 18.8 s onwards, to determine the time constant for the decline in rate of recovery heat output (τ=8.4 s). The calculated rate of recovery heat output during the contractions is also shown (white line between 0 s and 18.8 s). The R:I ratio for this preparation was 1.16. (C) The calculated cumulative initial enthalpy output (i.e. initial heat output + work output) for all 40 contractions and recovery heat output. Work output is also shown (broken line).

Results

An example of the time courses of change in muscle length, force output and energy output within each contraction cycle was obtained by averaging the data for each variable in each contraction cycle across the last 10 cycles (Fig. 1). During this time, the mechanical and energetic variations were similar in successive cycles; that is, the muscle was close to being in an energetic steady state (Paul, 1983). The muscle produced work while shortening during the first half of the cycle and then had work done on it (by the ergometer) to lengthen it during the second half of the cycle. The net work output is the difference between the work performed by the muscle and that performed on the muscle and is the cumulative value of the work output at the end of the cycle. The maximum work output during the cycle was reached at the end of shortening. Maximum work can include contributions from elastic elements in parallel and in series with the contractile apparatus but, because the force output was the same at the start and end of each cycle, elastic elements made no contribution to net work. Enthalpy was produced throughout the cycle but was produced more rapidly while the muscle was shortening than during the lengthening phase of the cycle.

The denominator of the definition of e_N incorporated all the suprabasal enthalpy output associated with generating work. Mechanical work was produced only during the contraction series, but the associated enthalpy output was produced both during, and for 40–50 s after, the contraction series (Fig. 2A).

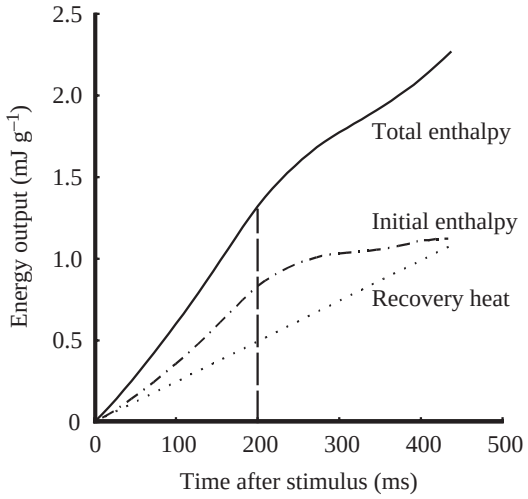

Fig. 3. The calculated time courses of production of initial and recovery enthalpies. The total enthalpy record is the same as that in Fig. 2C. Records are the averages of those for the last 10 cycles of the contraction protocol. The vertical broken line indicates the time at which shortening ended. Initial enthalpy is the sum of the work output and initial heat output. All the energy from recovery processes appeared as heat. In this example, ~75% of the initial enthalpy was produced by the end of shortening. Note that, although there was slightly more initial enthalpy than recovery enthalpy produced in the cycle, recovery enthalpy output continued after the contractions were finished so that the total recovery enthalpy output was greater than the total initial enthalpy output.

The mean net mechanical efficiency was 13.3±0.7% (mean ± S.E.M.; N=13 muscles).

Each enthalpy output record was partitioned into initial and recovery components. The calculated initial enthalpy was produced, like the work, only during the contraction series (Fig. 2C). At the start of the contraction series, the rate of recovery enthalpy output increased slowly compared with the immediate onset of initial enthalpy output. The rate of recovery heat output became constant only near the end of the contraction series (Fig. 2B). Once the contraction series was complete, the rate of recovery enthalpy output decreased exponentially with a mean time constant of 10.9±1.1 s (Fig. 2B).

Within each steady-state cycle, the calculated initial enthalpy was produced largely while the muscle was shortening, and little was produced during the lengthening phase of the cycle (Fig. 3). Across all the muscles tested, 95.0±2.4% of the initial enthalpy had been produced by the end of the shortening phase during the last 10 cycles. The rate of recovery heat output was constant during the steady-state cycles (Fig. 3).

The mean value of the ratio of recovery enthalpy output to initial enthalpy output that provided the best match to the recorded data was 1.16±0.03. That is, the total amount of recovery heat produced was slightly greater, on average, than the total initial enthalpy produced. The net enthalpy output is the sum of the initial and recovery components and was thus 1+1.16=2.16-fold greater than the cumulative total of the initial enthalpy produced. This difference was reflected in the initial mechanical efficiency; the mean value of e_I was 28.1±1.2% (N=13).

Discussion

The purpose of this study was to estimate the initial mechanical efficiency using a method that avoided assumptions about the relative time courses of work output and initial enthalpy output, that did not depend on *R*:*I* values calculated for isometric contractions and that required that muscles contract in a manner similar to that which occurs *in vivo*. e_I was calculated to be ~28%, less than half the value reported by Peterson and Alpert (1991) but consistent with estimates based on published e_N values and the *R*:*I* ratio determined from isometric contractions.

Comparison of results with those from previous studies

The *R*:*I* ratio determined in the present study (mean value, 1.16) was similar to both that reported previously for rabbit papillary muscles (1.10; Mast et al., 1990) and the theoretical value (1.13; Woledge et al., 1985, p. 219). Mast et al. (1990) used isometric contractions, whereas in the present study working contractions were used. A comparison of *R*:*I* ratio for isometric and working contractions of mouse soleus muscle (a slow-twitch muscle) found the ratio to be significantly greater during a working contraction (1.25) than during isometric contractions (1.0; Woledge and Yin, 1989). Although the

present study did not explicitly examine this phenomenon, comparison with the work of Mast et al. (1990) provides no evidence to support the idea that, in cardiac muscle, the type of contraction performed has a substantial effect on the *R*:*I* ratio.

The rate of decline in recovery metabolism of papillary muscles in the present study (time constant, ~11 s) is consistent with values reported previously for cardiac muscle. For example, Van Beek et al. (1999) measured time constants between 6 s and 12 s for changes in rate of O_2 consumption of isolated rabbit hearts (28°C) subjected to abrupt changes in work load.

To account for the difference between e_I calculated in the present study and that reported by Peterson and Alpert (1991), the work output in the earlier study was substantially greater than that in the present study and/or the initial energy output was substantially less. Peterson and Alpert (1991) calculated the work done by the muscle during the shortening phase of the isotonic contraction (active isotonic force output × distance shortened). We have previously shown that, as long as an appropriate correction is made for work performed by parallel elastic elements, the work output during an isotonic contraction is equal to the net work as calculated in the present study (Mellors et al., 2001). Although Peterson and Alpert (1991) did not correct for parallel elastic work, their estimate of work output was conservative because they used the passive force at L_{max} as the active force baseline and thus they most likely underestimated contractile element work output.

The most likely possibility is that Peterson and Alpert (1991) underestimated the initial heat production associated with shortening. They measured only the heat produced between the start of contraction and the end of shortening. The basis of this method was the assumption that all the energy associated with the shortening is produced within the time course of shortening. It is possible that this assumption does not hold for cardiac muscle. For instance, during an isometric twitch, over half the initial enthalpy output arising from cross-bridge cycling appears during force relaxation (Mast and Elzinga, 1990). Thus, measuring the enthalpy output only during shortening probably led Peterson and Alpert (1991) to underestimate initial energy consumption. In the present study, shortening continued until force relaxation was complete, and the majority of the initial enthalpy was produced within this time (Fig. 3).

Oxygenation of papillary muscles

Supply of O_2 to isolated preparations is by diffusion from the surrounding solution. Hill (1928) introduced the idea that the centre of a muscle may become anoxic if diffusive O_2 supply cannot match metabolic demands. An analysis of the adequacy of O_2 supply was performed to check: (1) whether anoxia was likely to have occurred in the present study and (2) whether the radius of the preparations had any effect on efficiency.

The analysis of O_2 diffusion into papillary muscles (Fig. 4A) indicated that during steady-state activity the P_{O_2} in the centre of even the largest muscles used would be in excess of the levels required to impair mitochondrial oxidative phosphorylation (1 kPa; for a review, see Loiselle, 1982). For 11 of the 13 preparations used, the estimated P_{O_2} in the centre of the muscles was 10 kPa. Note that the analysis was made assuming the muscles were in a steady state whereas during

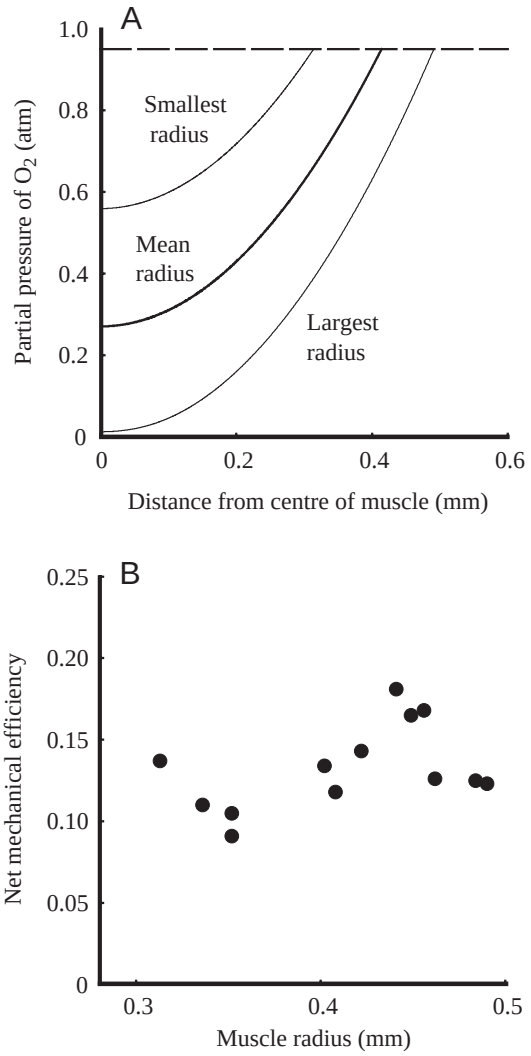

Fig. 4. Analysis of O_2 supply to isolated papillary muscles. (A) Partial pressure of O_2 (P_{O_2}) profile through the cross-section of a cylindrical muscle. 1 atm=1.013×10^5 Pa. The thickest line shows the P_{O_2} profile for a preparation with a radius equal to the mean value (0.41 mm) of those used in the study. The results for the smallest and largest preparations used (upper and lower lines, respectively) are also shown. Analysis was performed using the following parameters: P_{O_2} at muscle surface, 0.95 atm (horizontal broken line); diffusivity of O_2, 2.39×10^{-5} cm^2 atm^{-1} min^{-1} at 27°C, adjusted to 30°C using a Q_{10} of 1.04 (Mahler et al., 1985); steady-state rate of active metabolism, 9.7 mW g^{-1} (present study; equivalent rate of O_2 consumption, 29 µl min^{-1} g^{-1}); basal metabolic rate, 4.4 mW g^{-1} at 27°C (Baxi et al., 2000); Q_{10}=1.31 (Loiselle, 1985b). (B) The relationship between net efficiency and the muscle radius for all preparations. The slope of a straight line fitted through the data did not differ significantly from zero.

experiments muscles progressed from rest to close to a steady state, so the calculated central P_{O_2} values would tend to underestimate the actual P_{O_2}. The results of the analysis are consistent with the idea that anoxia was unlikely to have occurred in the preparations used in this study.

As a further check, and one that does not depend on assumptions concerning O_2 diffusion through muscle, rates of metabolism or muscle geometry, an analysis was performed to see whether there was any relationship between the radius of the preparations and net efficiency (Fig. 4B). There was no significant correlation between these variables (r^2=0.2, P>0.05), indicating that muscle size did not affect net efficiency. Therefore, the results of both the analysis of a model for oxygen diffusion into isolated muscles and the analysis relating muscle size to measured efficiency support the idea that anoxia had no effect on efficiency.

Cross-bridge thermodynamic efficiency

It is possible, knowing e_I, to estimate the thermodynamic efficiency of cross-bridge energy conversion (ε_{CB}); that is, the fraction of the energy produced by hydrolysis of one ATP molecule that is converted into work during one cross-bridge ATP-splitting event. If e_I is 28%, the enthalpy of PCr splitting is 35 kJ mol^{-1} (Woledge and Reilly, 1988), the free energy change of ATP hydrolysis in cardiac cells is 60 kJ mol^{-1} (Kammermeir et al., 1982) and it is assumed that 80% of the initial energy cost can be attributed to cross-bridge cycling (reported values range from 70% to 85%; Gibbs et al., 1988; Alpert et al., 1989; Schramm et al., 1994), then the cross-bridge thermodynamic efficiency is (28/0.8)×(35/60)≈20%. The fraction of energy output assumed to be related to cross-bridge activity has only a small influence on this value: if cross-bridge cycling accounted for 70% of the energy use then ε_{CB} would be 23%, and if cross-bridge cycling accounted for 85% of initial energy use then ε_{CB} would be 19%. If one ATP were used in each cross-bridge cycle, providing 100×10^{-21} J of free energy, then an ε_{CB} of 20% would correspond to 20×10^{-21} J work per cross-bridge cycle.

The maximum work that could potentially be performed per cross-bridge cycle can be determined from the cross-bridge force-extension relationship (or T_2 curve; Huxley and Simmons, 1971) determined from quick-release experiments. Colomo et al. (1994) determined T_2 curves for frog atrial cells (10°C). Their data indicated that the maximum work per cross-bridge cycle would be ~8.5 $\mathbf{P}_0$ nm, where $\mathbf{P}_0$ is the maximum isometric cross-bridge force. If, as estimated above, maximum work per cross-bridge is 20×10^{-21} J, and this corresponds to the maximum cross-bridge work estimated from the T_2 curve for amphibian atrial cells, then maximum cross-bridge force would be 20×10^{-21} J/8.5×10^{-9} m=2.4×10^{-12} N. This force output is consistent with that determined for myosin from cardiac muscle (Sugiura et al., 1998).

The data presented by Colomo et al. (1994) are also consistent with cross-bridges having a working stroke (i.e. the filament movement generated by one cross-bridge in one attachment cycle) of ~10 nm. However, other experimental evidence has been interpreted as indicating that the cross-bridge stroke in cardiac muscle may be as great as 20–30 nm (see De Winkel et al., 1995 and references therein). For instance, De Winkel et al. (1995) calculated cross-bridge working stroke from the dynamic stiffness of skinned rat trabelculae (22°C) and concluded that it was at least 20 nm. It has been suggested (Gibbs and Barclay, 1995) that these values are consistent with high efficiency values, such as those reported by Peterson and Alpert (1991). If, however, ε_{CB} is only 20%, then such large working strokes would imply that maximum cross-bridge force output must be <1 pN, which is much smaller than values determined using isolated contractile proteins (Van Buren et al., 1995; Sugiura et al., 1998). Thus, large cross-bridge working strokes seem unlikely but it is important to clarify the amplitude of the working stroke of cardiac cross-bridges in intact muscle.

Efficiency of oxidative recovery

The magnitude of the difference in estimates of e_I between the present study and that of Peterson and Alpert (1991) also has significant implications for the thermodynamics of oxidative recovery processes. It is well established that e_N is <20% in cardiac muscle of both mammals (Gibbs et al., 1967; Mellors et al., 2001; Mellors and Barclay, 2001) and amphibians (Syme, 1994). e_N is approximately the product of the initial thermodynamic efficiency (ε_I, the ratio of work output to initial free energy change, including both that associated with cross-bridge cycling and ion pumping) and the efficiency with which mitochondria convert the free energy from metabolic substrate into free energy in ATP. This is because the free energy change and the enthalpy change for the recovery processes have almost the same value (for a detailed discussion, see Gibbs and Barclay, 1995). ε_I is the product of the initial mechanical efficiency and the ratio of ΔH_{PCr} and ΔG_{ATP} (=35/60≈0.6). If e_I were 60% (Peterson and Alpert, 1991) then ε_I would be 36%. e_N is 13% (present study), so the efficiency of mitochondrial energy conversion would be 13/36≈35%. However, the efficiency of the recovery processes appears likely to be much higher than this, probably between 70% and 80% (Gibbs and Barclay, 1995; Lou et al., 2000). This further supports the idea that it would be difficult to reconcile an e_I as high as 60% with the well-established values for e_N.

The data from the present study can be used to estimate the efficiency of the recovery processes. If e_I is 28% then ε_I≈16.8% and the efficiency of the recovery processes would be (13.3/16.8)×100=80%. This is similar to the value of 84% calculated from measurements of the O_2 consumed and heat produced by skeletal muscle fibres from the dogfish (*Scyliorhinus canicula*; Lou et al., 2000).

Conclusions

The initial efficiency of isolated cardiac muscle is ~30%, which is consistent with cross-bridges converting about 20% of the free energy change of ATP hydrolysis into work. These values are similar to those of other striated muscles (for a

review, see Gibbs and Barclay, 1998) and suggest that, despite its specialised function, the mechanism for converting chemical energy into mechanical energy in cardiac muscle does not differ fundamentally from that in other striated muscles. However, there are a number of reports of *net* efficiency values of human hearts of ~30% (for a review, see Gibbs and Barclay, 1995). Such values are consistent with the high value of initial efficiency proposed by Peterson and Alpert (1991) but are difficult to reconcile with the relatively low values determined in the present study. It is important to understanding human cardiac energetics that this apparent discrepancy between data from isolated cardiac preparations and human hearts *in vivo* be resolved.

List of symbols

ΔH_{PCr}	molar enthalpy change for hydrolysis of phosphocreatine
ΔG_{ATP}	molar free energy change for hydrolysis of ATP
ε_{CB}	thermodynamic efficiency of cross-bridge energy conversion
ε_I	ratio of work output to initial free energy change
e_I	initial mechanical efficiency
e_N	net mechanical efficiency
L_{max}	length at which twitch force is maximal
$\mathbf{P}_0$	maximum isometric cross-bridge force
Q_I	initial heat output
Q_T	total supra-basal heat ouput
R:I	ratio of enthalpy output from recovery processes to enthalpy output from initial processes
τ	time constant for post-contractile decline in rate of recovery heat output
W_N	net mechanical work output

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