J. exp. Biol. ***185****, 195–206 (1993)*
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EFFICIENCY OF ENERGY CONVERSION DURING SINUSOIDAL MOVEMENT OF RED MUSCLE FIBRES FROM THE DOGFISH *SCYLIORHINUS CANICULA*

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Accepted 13 August 1993

Summary

Bundles of red myotomal muscle fibres isolated from dogfish were electrically stimulated at 12˚C. Peak twitch force was 54% of that produced by a brief isometric tetanus. Relaxation was slower than in white fibres, but much faster than would be expected for the tonic fibres found in amphibian muscle. These two results indicate that the red fibres in dogfish are slow, but not tonic, in their behaviour.

Net work output and heat production were measured during complete cycles of sinusoidal movement. The following variables were kept constant: peak-to-peak movement, about 7% of the muscle fibre length; tetanus duration, 33% of the mechanical cycle time; stimulus frequency, 40Hz. The frequency of movement and the timing of the stimulation were varied for each preparation to find the conditions optimal for power output and those optimal for efficiency (the ratio of net work output to total energy output as heat+work). To achieve either maximum power or maximum efficiency, the tetanus must start while the muscle fibres are being stretched, before the beginning of the shortening part of the mechanical cycle. The highest power output was produced during movement at 1.02Hz. The highest efficiency, 0.507±0.045 (±S.E.M., *N*=9), was at 0.61–0.95Hz. The efficiency is higher than that previously measured during sinusoidal movement of white fibres; the difference, 0.095±0.045 (±S.E.M. of the difference, d.f. 20), is statistically significant at the 5% level.

Introduction

The recognition that animals contain a variety of muscle fibre types has a long history (reviewed by Needham, 1971). In fish, the fibres can be broadly divided into red and white, which in many species are well segregated in the body. Thus, fish are particularly suitable for investigations of how the different fibre types are used during

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Key words: muscle, fish, dogfish, efficiency, mechanical power, heat, *Scyliorhinus canicula.*

locomotion. Electromyographic (EMG) studies in swimming fish show that the white fibres are used in fast swimming and the red ones in slow swimming (Bone, 1966; Grillner, 1974).

Experiments on isolated fibres of each type show that the white fibres have a higher velocity of shortening and power output than the red fibres (Bone *et al.* 1986; Rome *et al.* 1988). Fibre speed is clearly related to *in vivo* function. For example, red fibres of the carp are too slow to contribute power during the startle response (Rome *et al.* 1988).

However, the suitability of a given fibre type for a particular movement depends on more than its speed. The fibre must not only provide work for the movement, but must do so efficiently, that is with the conversion into work of a large fraction of the chemical energy turnover. Like maximum power, maximum efficiency would be expected to occur at a different speed of shortening in each fibre type. Does maximum efficiency occur at the speeds used during *in vivo* locomotion? There are too few measurements of efficiency to answer this question.

We have previously measured the efficiency of energy conversion by the white muscle fibres from dogfish during movement at different speeds (Curtin and Woledge, 1991, 1993). Here we report a study of the efficiency of contraction of the red fibres of the dogfish. Mos *et al.* (1990) have investigated motor unit activity in swimming dogfish, and this information is used here to establish the efficiency for these *in vivo* conditions.

Materials and methods

Dogfish, *Scyliorhinus canicula* (L.), from the holding tanks at the Citadel Hill site of the Plymouth Marine Laboratory, were killed by decapitation followed by pithing. Large fish ranging in length from 640 to 720mm, were selected. Bundles of fibres were dissected under saline from thin slices of the red myotomal muscle next to the skin along the midline in the section of the fish just behind the anus. A piece of myoseptum at each end of the bundle was held in a platinum foil clip. The saline solution contained ($mmol\,l^{-1}$): NaCl, 292; KCl, 3.2; $CaCl_2$, 5.0; $MgSO_4$, 1.0; Na_2SO_4, 1.6; $NaHCO_3$, 5.9; urea, 483. The composition is based on the standard Plymouth elasmobranch saline.

The experiments were carried out at 12°C with the fibre bundle mounted horizontally between a force transducer (Cambridge Technology, Inc. model 401) and a combined motor (Vibration Generator, model 101 Ling Dynamic Systems Ltd, Royston, UK) and length transducer (Variable transformer DFg2.5, RS646-460). Motor position was controlled by the sine-wave output of a function generator gated by a Digitimer. The bundle was in contact with a thermopile that measured fibre temperature.

The preparation was electrically stimulated end-to-end. In each experiment, the stimulus strength–twitch tension relationship was investigated by varying both the stimulus voltage and the pulse duration to establish supramaximal stimulus strength.

The fibre length–tension relationship was also investigated so that appropriate initial and final lengths, near the plateau of the length–tension relationship, could be chosen for the tetani with sinusoidal movement. The aim was to use lengths giving high force and, consequently, high power. The mean peak-to-peak movement was 7.2±0.4% (±S.E.M.,

N=9), expressed as a percentage of the middle length during movement, which was 7.05±0.36mm (±S.E.M., *N*=9).

For six of the nine fibre preparations, the force responses to a single stimulus, and 0.4 s of tetanic stimulation at frequencies of 5, 10, 20, 40, 50 and 60Hz, were also recorded. A stimulus frequency of 40Hz, which produced 97.1±0.7% (±S.E.M., *N*=6) of the force produced with 60Hz stimulation, was chosen for use in the experiment with sinusoidal movement.

The tetanus duration was kept fixed at 33% of mechanical cycle time. This duty cycle was chosen on the basis of observations of the durations of bursts of activity recorded from motor neurones in swimming spinal dogfish (Mos *et al*. 1990).

The timing of the tetanus within the mechanical cycle was designated as the 'stimulus phase', which we defined as the time from the beginning of shortening to the beginning of stimulation expressed as a percentage of the duration of the mechanical cycle (see Fig. 2). Thus, if stimulation started before shortening, the stimulus phase was negative.

Cycles of movement were performed while stimulation, motor position, force and temperature of the preparation were recorded. There was a recovery period of 3min between such trials. Values of net work, average mechanical power, heat and efficiency reported here are from the first complete cycle of movement and stimulation (see example in Fig. 2). The stimulus phase and the frequency of sinusoidal movement were varied; the amplitude of movement was kept constant.

Records were also made of the force and temperature changes during isometric tetani at the long, middle and short lengths.

Measurements of energy output

A record of force and temperature change during sinusoidal movement of the unstimulated muscle was subtracted from the corresponding records produced by the stimulated muscle to give a record of active force and temperature change. The work was calculated by integrating the product of the active force record and the differentiated record of length change. The mechanical power during a cycle of movement was found by dividing work by cycle duration. Note that this gives the average mechanical power during one complete cycle of movement, and that it is the net power calculated from the work done by the muscle fibres and any work that may have been done on them during the part of the mechanical cycle when they were stretched.

Heat output was determined from temperature changes detected by a thermopile made by vacuum deposition of antimony and bismuth on a mica substratum as described by Mulieri *et al.* (1977). Each thermocouple produced 83.2 $\mu V\,^{\circ}C^{-1}$ temperature difference. There were four thermocouples per millimetre along the length of the thermopile, and usually the output from a 3mm length of thermopile was recorded.

Temperature records were converted to heat and corrected for heat loss using characteristics determined for each fibre preparation by passing a known current through the whole thermopile. This produces a known quantity of heat due to the Peltier effect. An exponential function was fitted to the time course of heat loss following a period of Peltier heating to give values for the heat loss characteristics and the heat capacity of the fibres.

In eight experiments, force was also recorded during a contracture in high-K^+ solution, which contained (mmol l^{-1}): NaCl, 146; KCl, 310; $CaCl_2$, 2.5; $MgSO_4$, 0.5; Na_2SO_4, 0.8; $NaHCO_3$, 2.95; urea, 483.

Stimulus heat

Each preparation was immersed in normal saline containing, in addition, 18mmol l^{-1} procaine to make the fibres inexcitable. Records were then made of heat during tetanic stimulation for each of the durations previously used. The records were corrected for heat loss in the usual way to determine the stimulus heat. This was subtracted from the heat recorded during stimulation of the active fibre preparation. On average, the stimulus heat was 43±7% (± S.E.M., *N*=9) of the total heat.

Fibre bundle size

At the end of each experiment, the length of the fibre bundle was measured under the stereomicroscope and the bundle was removed from the thermopile (mean fibre length 7.05±0.36mm, ±S.E.M., *N*=9). Myosepta and any other non-fibre material were carefully removed, and the fibres were dried at room temperature. Dried fibre bundles were weighed on a Cahn electrobalance (mean dry mass 0.436±0.053mg, ±S.E.M., *N*=9). The total cross-sectional area of the fibres was estimated as dry mass × ratio of wet to dry mass/density. The wet to dry mass ratio has not been measured in red fibres, so the value for white fibres (4.9±0.05, ±S.E.M., *N*=9, Curtin and Woledge, 1993) was assumed to apply. Density was assumed to be 1mg mm^{-3}. Mean cross-sectional area of fibres estimated in this way was 0.310±0.04 mm^2 (±S.E.M., *N*=9).

The maximum isometric force was normalized by dry mass and length of the fibres and the mean value for the nine fibre bundles was 0.338±0.038 N m g^{-1} drymass (S.E.M., *N*=9). This is probably an underestimate of the true capacity of the fibres to produce force because some fibres were likely to have been damaged during dissection and may not have been producing force. The value of red fibre force is about 40% of the value we found previously for white muscle fibres from dogfish (0.873±0.078 N m g^{-1} drymass, S.E.M., *N*=7) which was normalized using the mass of only the active muscle fibres. This ratio, 40%, is similar to that found by Bone *et al.* (1986) in a comparison of the force produced by skinned preparations of these two fibres types.

Because the red fibres are so small and are held together tightly in the bundle, we could not estimate the fraction of active fibres in preparations using methods that are reliable for white fibres. Therefore, rate of total energy output (power+heat rate) was used as an estimate of the size of the active part of each preparation, since for a standard set of conditions this quantity can reasonably be expected to be directly proportional to the amount of active tissue. We used the highest rate of energy output, chosen from among the values during the optimum power output at each frequency of movement, as the estimate of the size of the active part of each preparation. The heat rate, power and rate of total energy output of each preparation were normalized in this way to remove variation between results for different preparations that are due to differences in the amount of active contractile material in the fibre bundle.

Results

Stimulus frequency and isometric force

Fig. 1A shows the force developed by a red muscle fibre preparation in response to either a single stimulus or brief burst of stimuli, at various frequencies up to 60Hz. The responses to 50 and 60Hz stimulation were the same. Mos *et al.* (1990) recorded action potentials at frequencies up to and above 60Hz in motoneurones of spinal dogfish during swimming at tailbeat frequencies in the range used here. Thus, the action potential frequencies observed in the spinal fish were sufficient to produce maximum force. In our experiments, the peak force for a single stimulus was 54±6% (S.E.M., N=6) of that produced by 60Hz stimulation. Fig. 1B shows, on the same time scale, similar observations for a white muscle preparation. The response of the red fibres to a single stimulus is a somewhat smaller proportion of the maximum force observed and the rates of rise of tension and of relaxation are clearly lower. Correspondingly, stimuli at 10 and 20 Hz produce a fused tetanus in the red muscle, but not in the white muscle. The stimulus frequency we used for the observations during sinusoidal movements was 40 Hz; this produced, in isometric contractions, 97±1% (S.E.M., N=5) of the maximum force.

Sinusoidal movement

Fig. 2 shows a set of example records of length, stimulation, force and work for one fibre bundle, for movement at 0.952Hz. The stimulus phase was −10%; that is, the tetanus started 0.105s before the start of shortening. These are the conditions that gave the maximum mechanical power for this fibre bundle at a constant movement of 5.8% fibre length and stimulus duty cycle of 33%. The values of work, power and efficiency were measured for the period (one mechanical cycle) between the vertical broken lines in Fig. 2.

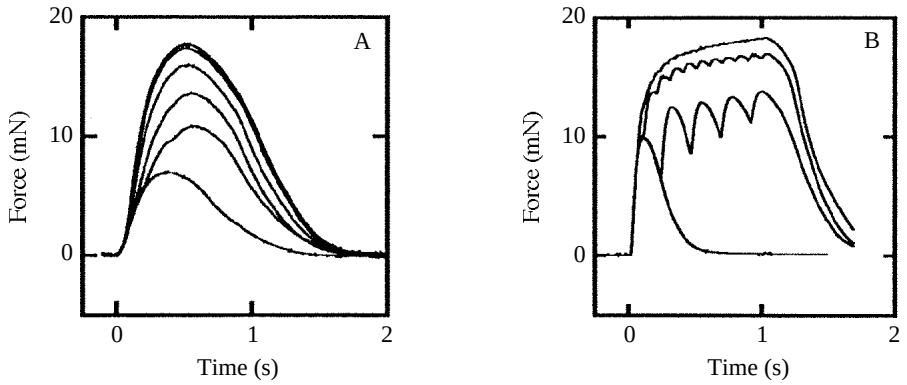

Fig. 1. Example records of force produced by red fibres (A) and by white fibres (B) under isometric conditions. Superimposed records of a twitch and brief tetani with different stimulation frequencies. Tetanic stimulation of red fibres in A was for 0.4s at 5, 10, 20, 40, 50 and 60Hz. Force records at 50 and 60Hz were identical. Tetanic stimulation of white fibres in B was for 1.0s at 5, 10 and 20Hz.

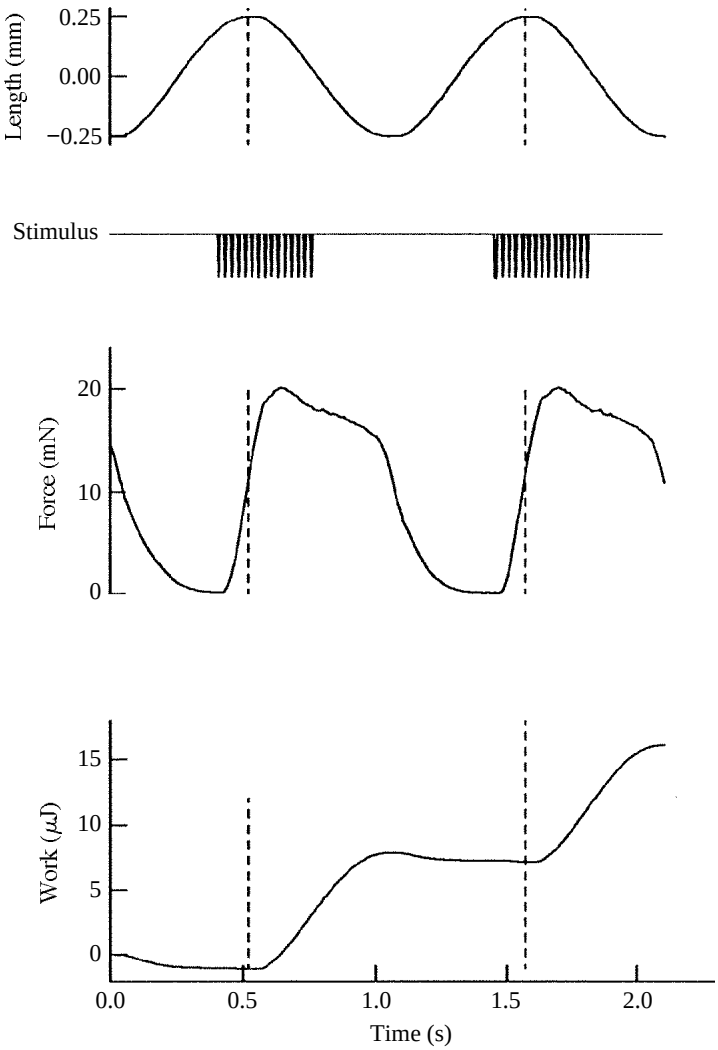

Fig. 2. Sample records of length, stimulus and force during sinusoidal movement at 0.952Hz with a brief tetanus during each cycle of movement. The vertical broken lines mark the beginning and end of the mechanical cycle for which values are reported. On the length record, a positive slope indicates muscle lengthening, a negative slope muscle shortening. The peak-to-peak amplitude of movement was 8.6% of the middle fibre length. In this example, the stimulus phase was −10%; that is, the time the stimulation started was 10% of the mechanical cycle time before shortening started. Active force is shown. Force produced during sinusoidal movement of the resting fibres was small and has been subtracted. The bottom panel shows the time course of mechanical work, which was calculated as described in the Materials and methods section.

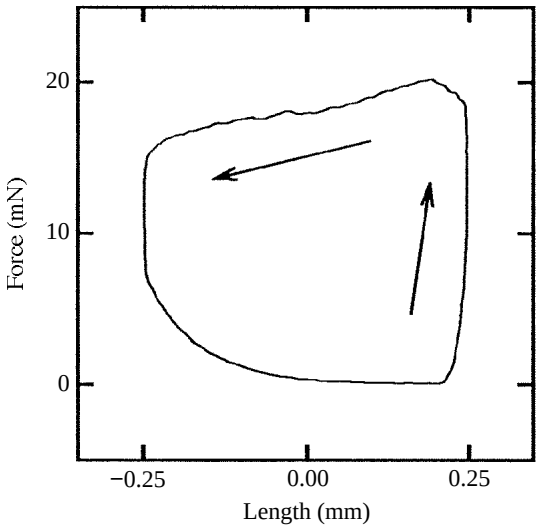

Fig. 3. A work loop formed by plotting force against length for the records shown in Fig. 2 between the two broken vertical lines. The arrows show the direction of progress around the loop. The net work for the cycle is the area enclosed by the loop.

The record shows that force fell after stimulation ended as the muscle shortened; by the end of shortening, it had fallen to about 70% of its peak value. Thereafter, force fell even more rapidly as the stretch part of the mechanical cycle started, and force had reached zero before stimulation started again.

The work output as a function of time, which was calculated from the length and force record as described in the Materials and methods section, is shown in the bottom panel of Fig. 2. Note that the work decreases when the muscle is stretched while it is producing force. Force is plotted against length during this cycle in Fig. 3. The net work is the area enclosed by this loop. The mechanical power in the cycle was found by dividing the net work in by the cycle duration; thus, the value of mechanical power we report is the average power during one complete cycle.

In each experiment, five different frequencies of sinusoidal movement between 0.61 and 1.67Hz were used, and the stimulation phase was varied in steps of 5% to find the conditions that gave maximum power and those for maximum efficiency, at constant amplitude of movement and stimulus duty cycle. Fig. 4 summarizes the values of work, mechanical power and efficiency measured on the same fibre bundle used for the records in Figs 2 and 3. For each frequency of movement, the range of stimulus phases was usually sufficient to encompass the values of stimulus phase optimal for work, for power and for efficiency. When the stimulation starts earlier or later in the cycle, there is less work, power or efficiency. The ‘optimal stimulus phases’ varied with the mechanical cycle frequency; to be optimal, the stimulation had to start earlier in the mechanical cycle as the mechanical frequency increased. It is also noteworthy that the optimal stimulus phases are all negative. As has been found in muscle from other animals, stimulation always had to start during the stretch part of the cycle to give maximum work and

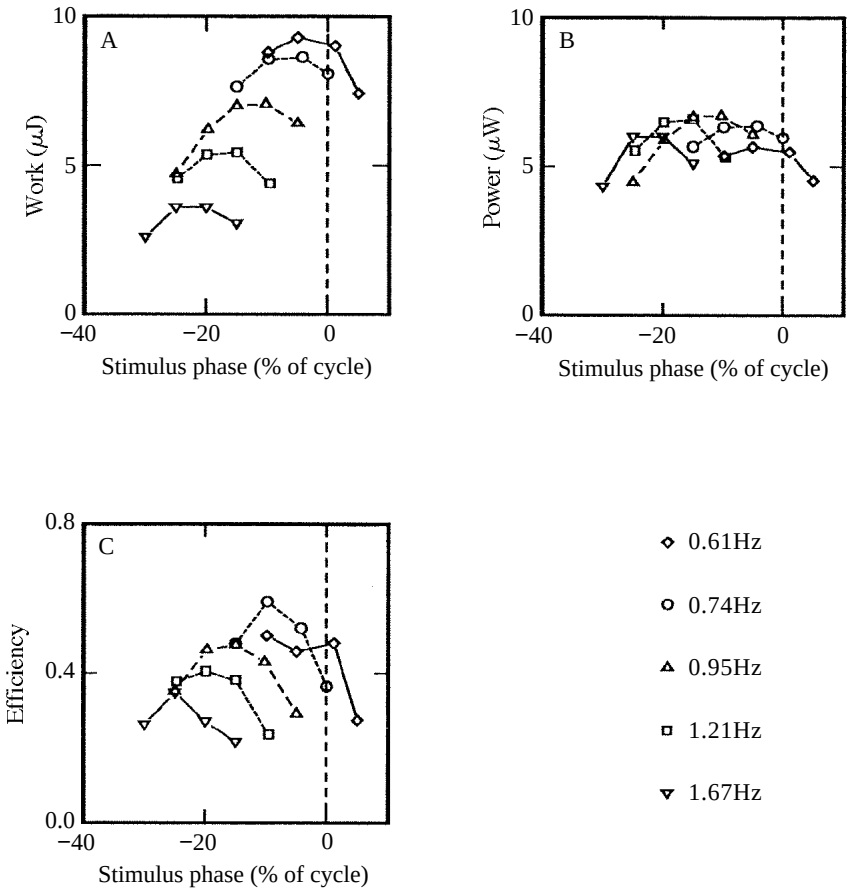

Fig. 4. Values of work (A), power (B) and efficiency (C) for one fibre bundle during sinusoidal movement with various phases of stimulation. Results for five frequencies of movement are shown. The vertical broken line marks zero stimulus phase, for which stimulation and shortening start at the same time.

maximum power (Josephson, 1985; Altringham and Johnston, 1990). We find that the same is true for maximum efficiency.

Highest power output

Power is expressed relative to the rate of energy output by the preparation to remove variation between preparations due to differences in the amount of active tissue in the preparations (see Materials and methods). The mean of the highest peak relative power output by each fibre bundle, irrespective of mechanical frequency, was 0.292±0.026 (S.E.M., N=9) produced during sinusoidal movement at 1.02±0.066Hz (S.E.M., N=9). At this sinusoidal frequency, the average velocity of movement during shortening (or lengthening) was 0.147 fibrelength s^{-1} and the maximum was 0.231 fibrelength s^{-1}.

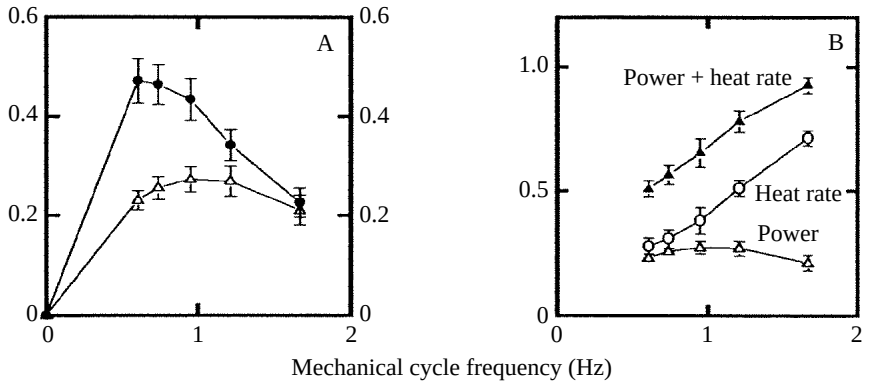

Fig. 5. (A) Variation of efficiency (●) and relative power (△) with frequency of sinusoidal movement. Each point is the mean for all fibre bundles of the maximum efficiency found by varying stimulus phase. Power is that for the stimulus phase giving maximum efficiency. (B) Mean rate of total energy output (power+heat rate), mean heat rate and mean power output for conditions giving the efficiencies shown in A. Values of power, heat rate and total energy output for each fibre bundle are expressed relative to rate of total energy output by that fibre bundle to remove variation between preparations due to differences in the amount of active contractile material in the bundles (see Materials and methods). Bars are ± 1 S.E.M. The number of values included in each mean is nine.

Efficiency

As explained in Materials and methods, we used the ratio of power to rate of total energy output as a measure of efficiency. For each frequency of sinusoidal movement, the value of the highest efficiency for each preparation was identified and Fig. 5A shows the means along with the relative power for the same conditions. Efficiency was high and almost equal at the two lowest frequencies of movement at which 46% and 47% of the total energy output appeared as work. In seven of the nine preparations, the efficiency was greater during 0.74Hz movement than during 0.61Hz movement. Thus, in most cases, the range of sinusoidal frequencies included that optimal for efficiency.

The values of heat rate, power and rate of total energy output for the stimulus phase giving maximum efficiency at each sinusoidal frequency are summarized in Fig. 5B. Note that all of the values have been expressed relative to the rate of total energy output.

The maximum efficiency did not occur at the same frequency of movement in all the preparations; it occurred at 0.61Hz for two, at 0.74Hz for four and at 0.95Hz for three preparations. We have selected, for each preparation, the highest efficiency value, irrespective of frequency, and the average of these maximum observed efficiency values is 0.507±0.045 (S.E.M., N=9).

K^+ contractures

In eight experiments, K^+ contractures were obtained after observations with sinusoidal movement had been completed. The force was 87±5% (S.E.M.) of the force in an isometric tetanus during stimulation at 60Hz for 0.4s.

Discussion

Red fibres: slow but not tonic

We have found that all of the red muscle preparations showed a clear mechanical response to a single stimulus. This was surprising because Stanfield's (1972) voltage-clamp study of dogfish muscle fibres showed that the properties of the red fibres covered a wide range. In many red fibres, he observed the membrane currents required for an action potential, but other, apparently equally viable, red fibres did not show these currents. This second group of red fibres seemed to be reminiscent of the slow tonic fibres in frog muscle, which do not produce action potentials. From Stanfield's results, we expected to find some 'tonic fibre behaviour' in our red fibre preparations. Characteristic features of the contractile behaviour of tonic fibres include the following. (a) They produce very little, if any, force in response to a single stimulus. (b) Force is produced when the junctional potentials in response to repeated stimulation summate to produce a sufficient membrane depolarization; when the frequency of repeated stimuli is increased, the depolarization, and therefore the force, increases. (c) At the end of stimulation, the force relaxes 50–100 times more slowly than relaxation of twitch fibres (Kuffler and Vaughan Williams, 1953; Lännergren, 1975).

In fact, all the red fibre preparations responded to a single stimulus with a force of about half that in a maximal tetanus (50 and 60Hz stimulation). Force increased with stimulus frequency in a way similar to that seen in white fibres, which Stanfield (1972) has shown all conduct action potentials. Relaxation of the red fibres was not dramatically slower than for the white fibres.

It seemed possible that our red fibre preparations might contain some fibres that were not responding at all to the electrical stimulation we gave; these might be a population of red fibres that does not conduct action potentials. This was tested by comparing the force in a K^+ contracture, which would cause all the fibres to contract, with that produced by electrical stimulation; the forces were very similar. This rules out the possibility that the red muscle preparation contained a significant fraction of viable fibres that are not activated by our electrical stimulation. Thus, our results indicate that each stimulus produced an action potential in all the live muscle fibres. As a neuromuscular blocking agent was not used, we have no evidence about whether the action potentials are from direct or indirect (*via* nerve endings) stimulation of the muscle fibres.

Our conclusion that red fibres in dogfish do produce action potentials is similar to that of Altringham and Johnston (1988) about the red fibres in the teleost *Myoxocephalus scorpius*.

Conditions giving maximum power

In the experiments reported here, the maximum power was produced at 1.02±0.66Hz (S.E.M. N=9). This is 29.3% of the frequency that gave maximum power in white fibres (Curtin and Woledge, 1993). The experiments are not completely comparable; the stimulus duty cycles were different in the two experiments and they were not optimized in either case. Nevertheless, it is worth noting that the ratio of the frequencies giving maxmum power is similar to the ratio, 0.316, of velocites of ramp shortening giving

maximum power in skinned red and white fibres from dogfish, 0.38 fibrelength s^{-1} and 1.24 fibrelength s^{-1}, respectively (Bone *et al.* 1986).

Efficiency

The maximum efficiency for the red fibres is significantly greater than that for the white fibres ($P<0.05$). The values are 0.507±0.045 (S.E.M., $N=9$) for red fibres and 0.412±0.021 (S.E.M., $N=13$) for the white fibres (Curtin and Woledge, 1993). The mechanical frequency at which the maximum efficiency is most often found in red fibres is 0.74Hz, and for white fibres it is 2.5Hz. So the use *in vivo* of the red muscle fibres for slow swimming (Bone, 1966; Grillner, 1974) is a strategy that can spare energy compared with using white fibres.

For both fibre types, the mechanical frequency giving the highest efficiency is lower than that required for maximum power output (Fig. 5A; Fig. 4A of Curtin and Woledge, 1993). So it clearly is an over-simplification to think in terms of a single mechanical frequency which gives the best muscle performance. When efficiency is crucial, a lower mechanical frequency must be used than when high power is required.

Why might the red fibres be more efficient than the white fibres? The red fibres may use less energy for processes other than transduction into work. For example, red fibres do not contain the soluble Ca^{2+}-binding protein parvalbumin, whereas the white fibres do. Thus, it is likely that red fibres require less Ca^{2+} release from the sarcoplasmic reticulum and therefore less ATP use for its re-uptake than do the white fibres. In the muscles where the ATP used for Ca^{2+} uptake has been measured, it amounts to about 28% of the isometric energy output (Woledge *et al.* 1985, p. 200). If this amount of energy was used for Ca^{2+} uptake in the white fibres, whereas in the red fibres the amount was negligible, most of the observed difference in efficiency would be explained.

Regardless of the amount of energy used for Ca^{2+} uptake, the efficiency of energy conversion by the myofibrils themselves in both white and red muscle is somewhat larger than the values we report here. As described in our earlier paper, the work done for each molecule of ATP split can be calculated from the efficiency observations reported here. The value for red fibres is 29×10^{-21} J. This can be compared with the value of 17.8×10^{-21} J of work done by an attached cross-bridge going through the working stroke in the cycle described by the Huxley and Simmons model (1971). It is clear that more than one such working stroke must be performed during each ATP-splitting cycle to account for the efficiency we have observed. This argument is stronger for the red fibres than for the white because the red fibres have higher efficiency.

We thank Dr J. V. Howarth, Dr Q. Bone and the staff of the Marine Biological Association, Plymouth, for their help and advice. We also thank the Browne Research Fund of the Royal Society, and the SERC for financial support for this work.

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