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CONTRACTILE PROPERTIES OF A HIGH-FREQUENCY MUSCLE FROM A CRUSTACEAN

III. MECHANICAL POWER OUTPUT

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Summary

The mechanical power output during oscillatory contraction was determined for the flagellum abductor muscle of the crab *Carcinus maenas* using the work loop technique. Measurements were made at 10 Hz, which is the normal operating frequency of the muscle. The temperature was 15 °C. Increasing the number of stimuli per cycle (given at an interstimulus interval of 3.3 ms) decreased the number of cycles required to reach a work plateau and increased the work per cycle at the plateau to a maximum at 4–5 stimuli per cycle. The maximum mechanical power output was 9.7 W kg^{-1} muscle (about 26 W kg^{-1} myofibril). The optimum strain for work output (5.7 %) was close to the estimated muscle strain *in vivo* (5.2 %).

Introduction

The sustained mechanical power output of muscle during repetitive contraction has been measured from a number of vertebrate and invertebrate muscles using the work loop technique. In the work loop approach, a muscle is subjected to cyclic length change (strain) and is stimulated phasically in the strain cycle. The area of the loop formed when muscle force is plotted against length over a full cycle is the net mechanical work done by the muscle or on the muscle over that cycle (e.g. Josephson, 1985, 1993).

A large number of variables affect mechanical power output from a muscle, and dealing appropriately with the multidimensional parameter space is a major difficulty in work loop studies. Work output during a cyclic contraction varies with the cycle frequency, the strain amplitude and trajectory, the muscle length upon which the strain is superimposed, the pattern of muscle stimulation, the phase of the stimulation in the strain cycle and, because of muscle facilitation and fatigue, the cycle chosen for analysis. Deterioration of the preparation and limits on experimental time generally prevent full analysis of all the possible permutations of parameter values. The usual goal in work loop

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studies is to estimate the mechanical power output during a given *in vivo* activity. Typically, one or two variables are chosen as being of primary interest, for example muscle temperature and/or operating frequency, some of the other parameters are maintained constant at values estimated to be characteristic of normal performance, and the values of the remaining parameters are varied systematically in order to find that set of values which maximizes work output.

The worth of an estimate of *in vivo* power output from an *in vitro* work loop analysis must depend on the appropriateness of the chosen values for relevant parameters, some of which may be difficult to measure directly and precisely. The pattern of muscle activation must often be selected somewhat arbitrarily. Muscle electromygraphic (EMG) recordings may be available as a guide to the phase and total duration of muscle electrical activity in a cycle, but in muscles composed of large numbers of motor units, as are vertebrate muscles, EMG patterns generally do not give information either on the number of times that a single motor unit is activated during a cycle or on the duration of activation of individual units. Direct measures of both strain amplitude and trajectory are often unavailable. Fortunately, many of the parameters in work loop studies – including muscle length, strain and stimulus phase – have rather broad optima for work output, so small errors in the estimates of these parameters are unlikely to be of great importance (e.g. Josephson, 1985; Stokes and Josephson, 1988; Syme and Stevens, 1989).

The two preceding papers (Josephson and Stokes, 1994; Stokes and Josephson, 1994) on the flagellum abductor (FA) of the crab *Carcinus maenas* provide an unusually complete evaluation of the relevant parameters affecting work output for a muscle. The normal operating frequency (about 10 Hz), the number of times each motor unit to the muscle fires per cycle (1–5) and the interval between the motoneurone impulses of a burst (3–4 ms) have all been directly measured, as has the extent of muscle shortening (5.2 %) during a normal contraction (Josephson and Stokes, 1994; Stokes and Josephson, 1994). The trajectory of the cyclic strain and the phase of muscle activation in the strain cycle have not been analyzed. The following study takes advantage of the background information available about the FA in evaluating the mechanical power output of this muscle during normal operation and in comparing optimal values for parameters measured *in vitro* with those actually occurring *in vivo*. The principal questions asked are: (1) what is the maximum power output of the muscle and how does this compare with that from other invertebrate and vertebrate muscles; (2) how does variation in the number of motoneurone action potentials per cycle, as occurs during normal activity, affect work per cycle; and (3) does the optimum strain for mechanical power output match the strain during normal muscle operation.

Materials and methods

The basic procedures used in isolating the muscle and in preparing it for mechanical recordings are described in the preceding paper (Stokes and Josephson, 1994). The muscle was mounted vertically in a stirred, temperature-controlled (15±0.5 °C) bath. The nerve to the muscle was stimulated through a suction electrode using 0.5 ms shocks at an intensity about twice that needed to activate both of the motor units of the muscle. The

distal end of the muscle was attached to an ergometer (model 300H, Cambridge Instruments Co., Cambridge, MA), which was used to change muscle length and to measure muscle force. The muscle was subjected to sinusoidal strain and phasic stimulation in the strain cycle. The cycle frequency (10 Hz) and the interstimulus interval (3.3 ms) when there were multiple stimuli per cycle were chosen to be consistent with those during normal activity (Josephson and Stokes, 1994). The values of average muscle length, muscle strain and stimulus phase were selected empirically to be those that maximized work output per cycle. Values of muscle force and muscle position were collected with an analogue-to-digital converter at a sampling rate of one force–position pair each 0.3 ms and stored in a computer.

Bursts of 20 cycles of strain and associated muscle stimulation were given regularly at 2 min intervals. The eighteenth cycle was chosen for analysis. Each set of determinations with a new stimulus pattern began with a burst of isometric contractions using that pattern. The isometric responses were used to determine the delay from stimulation onset to the tension peak; this delay was used in calculating the stimulus phase (Stokes and Josephson, 1988). The search procedures used to find the optimal muscle length, strain and stimulus phase for work output with a given stimulus pattern were those outlined in Stokes and Josephson (1988). In brief, values were estimated for optimal stimulus phase and strain. The muscle length was varied systematically in successive trials in steps of 0.125 mm until the optimum length was found. At this length, the muscle strain was varied systematically on successive trials in steps of approximately 0.05 mm to find the optimum strain and, finally, the stimulus phase was varied in steps of about 6 % to find the optimum phase. If the value determined for either optimum strain or optimum phase differed by more than two steps from the initially estimated optimum, the newly determined value for the discrepant optimum was used and the whole procedure was repeated, beginning with a re-determination of optimum length.

After the optimum conditions had been identified, a trial using these conditions was collected and stored for later cycle-by-cycle analysis (see Fig. 2). In four preparations, the work and power output were first determined using one stimulus per cycle, and in subsequent determinations the number of stimuli per cycle was increased progressively to five. In another four preparations, the order was reversed, and the work and power output were determined first for five stimuli per cycle and subsequent sets of trials were with decreasing stimulus numbers. It required 10–20 trials (20–40 min) with each new stimulus pattern to determine the optimum phase, strain and average muscle length. Using equal numbers of preparations in which determinations were performed with an increasing number of stimuli per cycle and with a decreasing number of stimuli per cycle balanced, in part, the effects of progressive fatigue over the time course of these relatively long experiments.

After the optimum parameters had been determined for five stimuli per cycle, work output per cycle was determined using seven stimuli per cycle at the same muscle length, phase and strain found to be optimum for five stimuli per cycle. With seven stimuli per cycle, the total stimulation period per cycle (20 ms) is 20 % of the cycle duration. In this respect, the values with seven stimuli per cycle are comparable to work and power measurements made earlier with another muscle from the crab in which the duty cycle for stimulation was also 20 % (Stokes and Josephson, 1988). In many preparations, the final

trial was one which began with two stimuli per cycle at the conditions found to be optimal for work with two stimuli, but in which the number of stimuli per cycle was reduced to one after about 10 cycles. The purpose of such trials was to evaluate how rapidly work output declined following a reduction in the frequency of neuronal activation.

At the end of an experiment, the muscle was fixed in 70 % alcohol while still attached to the ergometer. The muscle was then removed, its length measured, and it was stored in alcohol. The measured muscle length was used to determine the length during the experiments and, from this, the absolute length optimum for work output at each stimulus condition. Muscle lengths are expressed as a fraction of the reference length, which is the longest length reached by the muscle *in vivo*, that with the flagellum fully adducted. Later, the muscle was rehydrated in saline and weighed. Rehydrated masses were multiplied by 1.32 to correct for loss of mass during alcohol-fixation and storage (Stokes and Josephson, 1994).

Results

Increasing the number of stimuli per cycle increased the work output per cycle (Table 1) and the rate at which work output per cycle rose through successive cycles early in a bout of activity (Figs 1, 2). The change in work output was great when the number of stimuli per cycle was increased from one to two, or from two to three, but further increasing the number of stimuli per cycle to four, five or seven resulted in little or no additional increase in work. The maximum work per cycle with four stimuli per cycle averaged 0.94 J kg^{-1} and that with seven shocks per cycle 0.97 J kg^{-1}. With one stimulus

Table 1. *Maximum power and optimum operating conditions for muscles activated with a varying number of stimuli per cycle*

	Stimuli per cycle					
	1	2	3	4	5	7
Power (W kg^{-1})	2.4	7.6	9.0	9.4	9.3	9.7
	(0.4)	(1.0)	(1.1)	(1.1)	(1.1)	(1.2)
Optimum length (% maximum *in vivo* length)	100.7	101.3	101.2	101.2	100.4	
	(0.7)	(0.8)	(0.8)	(1.1)	(1.4)	
Optimum strain (%)	5.28	5.66	5.76	5.73	5.96	
	(0.35)	(0.13)	(0.17)	(0.19)	(0.18)	
Optimum phase (%)	20.0	20.4	22.4	23.4	25.1	
	(1.3)	(1.9)	(1.2)	(0.6)	(0.7)	

Values are mean (S.E.M.).

The cycle frequency was 10 Hz, the stimulus interval within bursts was 3.3 ms and the cycle analyzed was the eighteenth of a burst.

Sample size was eight, except for the column with one stimulus per cycle, where it was five. With three preparations, the work per cycle was too small with one stimulus per cycle to evaluate the optimum muscle length, phase and strain. Including the power output from the three excluded preparations would reduce the average power with one stimulus per cycle to about 1.6 W kg^{-1}.

The increases in optimum strain and optimum phase with increasing number of stimuli per cycle are both statistically significant ($P<0.05$ that linear regression coefficient of strain or phase against number of stimuli $\leqslant 0$).

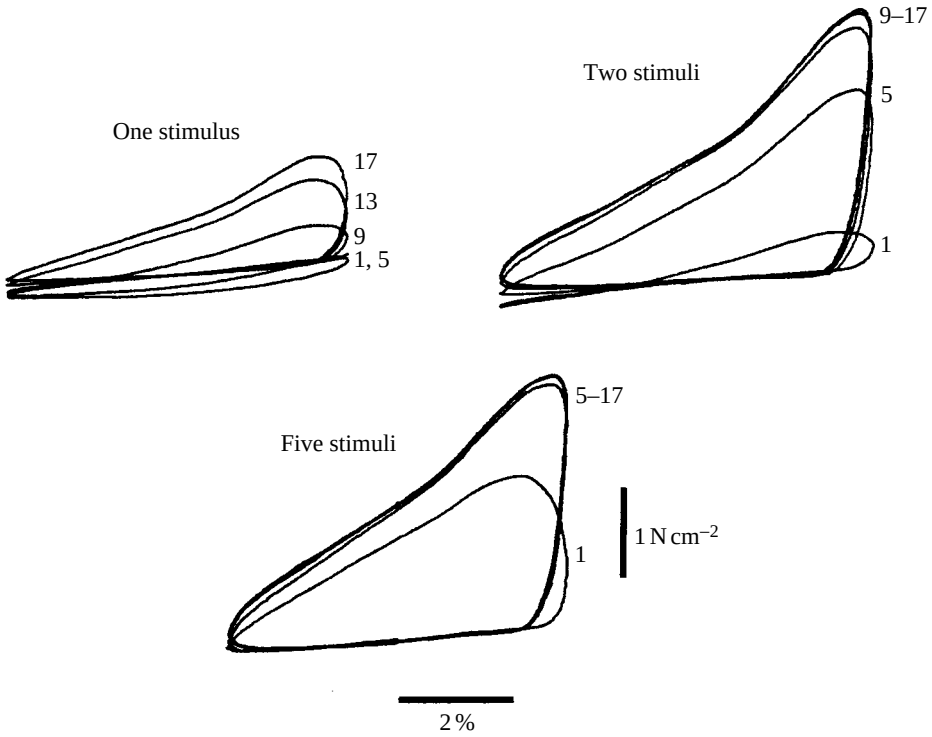

Fig. 1. Work loops with one, two and five stimuli per cycle. Cycles 1, 5, 9, 13 and 17 are shown for each case. All the loops were anticlockwise except the loops for cycles 1 and 5 with single stimuli per cycle, which were clockwise (i.e. on the early cycles with one stimulus per cycle the muscle absorbed rather than produced net work).

per cycle, the work per cycle was initially negative (the muscle absorbed rather than produced net work). Work did not become positive until about the tenth cycle and on average was still increasing by the eighteenth cycle (Fig. 2). With two or more stimuli per cycle, the work output reached a plateau or came close to a plateau by 9–12 cycles. Decreasing the number of stimuli per cycle from two to one at the tenth cycle caused an immediate reduction in work, but not to the level that would have pertained had the trial begun with single stimuli. Subsequently the work per cycle declined and after about five cycles the work output was no different from that in trials in which there had been a single stimulus per cycle throughout (Fig. 2).

There was a small but consistent increase in optimum stimulus phase with increasing number of stimuli per cycle, and a small but inconsistent increase in optimum strain (Table 1). The average muscle length which was optimal for work and power output was about 1 % greater than the maximum *in vivo* length of the muscle (Table 1).

Discussion

Maximum power

The maximum power output of the FA muscle, 9.7 W kg^{-1}, is similar to that measured

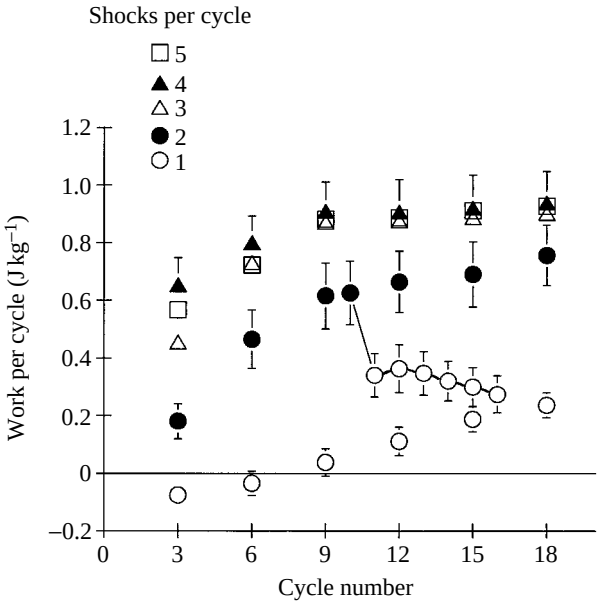

Fig. 2. Work per cycle throughout a train of imposed length change and stimulation. Values are shown as mean (N=8 except with one stimulus per cycle, where N=5). Vertical lines are standard errors where these are larger than the symbol (standard errors are not shown for three and five stimuli per cycle). The muscle length, strain and stimulus phase were those found to be optimal for work output on the eighteenth cycle. The seven joined points are from six preparations in which the number of stimuli per cycle was reduced from two to one during the middle of a trial. The positioning of these points along the x-axis is approximate. The switch from two stimuli to one stimulus per cycle was controlled manually. The average transition came at the tenth cycle, but the range was from the seventh to the twelfth cycle.

earlier from a respiratory muscle of the same species (8.8 W kg^{-1}, Stokes and Josephson, 1988). The power outputs of both the FA and the respiratory muscle were rather low compared with values obtained using comparable techniques from insect and bird flight muscles (50–104 W kg^{-1}; Josephson, 1985; Mizisin and Josephson, 1987; Malamud *et al.* 1988; Stevenson and Josephson, 1990; Biewener *et al.* 1992), from fish myotomal muscles (13–35 W kg^{-1} for white muscle; Altringham and Johnston, 1990; Johnson and Johnston, 1991; Johnson *et al.* 1991; Moon *et al.* 1991; 5–38 W kg^{-1} for red muscle; Altringham and Johnston, 1990; Rome and Swank, 1992; Rome *et al.* 1993), from mammalian diaphragm muscles (27–50 W kg^{-1}; Syme and Stevens, 1989; Altringham and Young, 1991), from amphibian limb muscles (30–35 W kg^{-1}; Stevens, 1988; Stevens and Syme, 1989) or from fast fibres of lizard limb muscle (20–154 W kg^{-1} depending on temperature; Swoap *et al.* 1993). Two factors contribute to the seemingly low power output of the crab muscles: the small fraction of the volume of these muscles that is occupied by myofibrils and the temperature at which the measurements from the crab muscles was made, which was lower than that in most of the other studies except those with some of the fish muscles.

Myofibrils make up about 44 % of the volume of fibres from the FA muscle and the

fibres constitute about 85 % of the whole muscle volume (Stokes and Josephson, 1992). Thus, the fraction of a whole muscle that is made up of myofibrils, those components which actually produce the mechanical power, is only 37 %. The power output of 9.7 W kg^{-1} muscle corresponds to 26 W kg^{-1} myofibril.

Mitochondria are rather poorly represented in fish white muscle or fast amphibian limb muscle (e.g. Patterson and Goldspink, 1972; Mobley and Eisenberg, 1975; Johnston, 1982). In these muscles, and probably in fast fibres from lizard muscles as well, myofibrils make up more than 80 % of the fibre volume, and the power output per kilogram myofibril is probably not more than 20–30 % greater than the power output per kilogram muscle. We estimate that the power outputs of fast amphibian and fish muscles given above are equivalent to 17–46 W kg^{-1} myofibril and that from lizard limb muscle at 15 °C to about 26 W kg^{-1} myofibril. The power output per kilogram myofibril appears not to differ much in crab muscles and in amphibian or fish muscles.

Fibres from insect flight muscles, in contrast, have abundant mitochondria and, in synchronous flight muscles, abundant sarcoplasmic reticulum as well. In these muscles, myofibrils make up only about half the fibre volume (e.g. Mizisin and Ready, 1986; Novicki, 1989). The power output of insect flight muscles per kilogram myofibril can be expected to be about twice the power output per kilogram whole muscle, giving specific power outputs several times greater than the equivalent value for the crab FA. The power measurements given above for insect and bird flight muscles, and those for the diaphragm muscles as well, were made at 29 °C or above, those from the crab muscles were at 15 °C. Mechanical power output is strongly dependent on muscle temperature (Josephson, 1993). At least some of the difference in power output between the flight muscles and the diaphragm muscles on the one hand, and the crab muscles on the other, is attributable to the different temperatures at which the measurements were made.

Optimum parameter values for work output

The optimum number of stimuli per cycle for work output and the optimum cycle strain *in vitro* were in reasonable agreement with the values measured *in vivo*. This is reassuring as a validation of the work loop approach. The optimum strain for power output *in vitro* was about 5.7 %; the actual muscle strain during activity is estimated to be 5.2 % (Stokes and Josephson, 1994). In life, the motor units of the FA muscle are activated with a maximum of three, four and rarely five action potentials per cycle (Josephson and Stokes, 1994). Three or four stimuli per cycle produced nearly maximum work output in *in vitro* measurements, three shocks per cycle producing over 90 % and four shocks per cycle over 95 % of the maximum work output obtainable with longer stimulus bursts (Table 1).

The optimum muscle length was consistently slightly longer than the reference length (Table 1), which is perplexing. The reference length is the longest length reached by the muscle *in vivo*. If the optimum length is greater then the reference length, a muscle, oscillating about its optimum length, operates over a length range most of which is longer than muscle lengths ever reached in the animal. We think that there may be a systematic error in the measurement of optimum muscle length that tends to overestimate this parameter. Generally, the optimum muscle length for either tetanic force or power output tended to increase gradually through the course of a set of trials. We think that this

increase is probably due to slow stretch of the distal tendon or possibly of basal skeletal elements. Stretch of the tendon would increase the apparent muscle length as we measured it (see Stokes and Josephson, 1994). Thus, it is possible that the contractile portion of the muscle was at or below its *in vivo* length while the measured muscle length, which includes that of the tendon, was greater than that ever reached in the animal.

In a preceding paper (Josephson and Stokes, 1994) we suggested that muscles *in vitro*, under the conditions in which we investigated them, were more dependent on facilitation than are muscles *in vivo*. An enhanced dependence on facilitation would probably affect the cycle-to-cycle increase in work during the early portion of a work measurement trial and the number of cycles taken to reach a plateau. The fact that the work output reaches a plateau that is relatively independent of the number of stimuli per cycle (Fig. 2) suggests that muscle activation is becoming saturated and that work output at the plateau is not limited by the requirements for facilitation in neuromuscular transmission.

In some crustacean muscles, there is a curious hysteresis in the relationship between stimulus frequency and isometric force, such that the high force resulting from a high stimulus frequency is partially retained when the stimulus frequency is subsequently reduced. Thus, the sustained force at a low stimulation frequency is greater if the period at low frequency is preceded by one at high frequency (Blaschko *et al.* 1931; Wilson and Larimer, 1968; Wilson *et al.* 1970). The experiment illustrated by the connected points in Fig. 2 was carried out to determine whether there was a similar hysteresis in work output when the number of stimuli per cycle was reduced during a set of cyclic contractions. When going from two stimuli per cycle to one, there was an immediate drop in work output and then a slower drop until, after a few cycles, the work output with one shock per cycle was no different from what it would have been had the series contained only single shocks per cycle from the beginning. High work output, once established with multiple stimuli per cycle, cannot be maintained with single stimuli per cycle. There does not seem to be a work equivalent in the FA to the sustained force enhancement seen in some crustacean muscles following the transition from a higher to a lower stimulation rate.

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