45

STRAIN, MUSCLE LENGTH AND WORK OUTPUT IN A CRAB MUSCLE

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Summary

The relationships between muscle length, fractional change in length (strain) and work output during cyclic contraction were examined in scaphognathite levator muscle L2B of the green crab *Carcinus maenas* (L.). The muscle was subjected to sinusoidal strain at 2 Hz and to phasic stimulation in the strain cycle.

At an average length and stimulus phase which are optimum for net work output, the work from muscle L2B during shortening rises to a peak or a plateau with increasing strain. The failure of shortening work to increase continuously with strain is due, in part, to the greater shortening velocity associated with greater strain, and to the consequent reduction in muscle force during shortening at higher velocity. The work required to re-lengthen a muscle following contraction is a complex function of strain, with an initial peak followed by a work minimum and then a monotonic rise in work with further increase in strain. The early work minimum is a result of shortening inactivation which reduces muscle force and thus the work which must be done to re-lengthen the muscle. Because shortening work rises to a peak or plateau with increasing strain while lengthening work, for the most part, increases with strain, there is a sharp optimum strain (about 8 %) for net work output.

Muscle relaxation becomes slower with increasing muscle length. As muscle length is increased, fusion of tension from cycle to cycle becomes more pronounced and shortening inactivation becomes a more important determinant of optimum strain.

Introduction

The isometric, tetanic tension of a skeletal muscle varies with muscle length, as is portrayed in familiar length–tension diagrams. If a skeletal muscle were to be stimulated tetanically and allowed to shorten very slowly, its force throughout shortening would be expected to lie on or very close to the isometric length–tension curve. The work done during a very slow shortening would be equal to the area between that portion of the length–tension curve traversed by the muscle and the line of zero force. Increasing the distance of shortening would increase the

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amount of work done. Thus, the work output during very slow shortening is expected to increase monotonically with the amount of strain (= fractional length change).

Mechanical work output has been measured from muscles subjected to sinusoidal strain at frequencies approximating those of normal *in vivo* operation (e.g. Stokes & Josephson, 1988). If the muscle is stimulated phasically in the length cycle such that it is active and its tension is high during shortening whereas it is nearly or completely relaxed and its tension low during lengthening, the muscle does work on each shortening–lengthening cycle. The amount of work done under these conditions does not increase monotonically with the amplitude of the imposed strain. Rather, there is a distinct optimum strain for work output (see Fig. 1C). The value of the optimum strain varies with cycle frequency, muscle temperature and stimulation pattern (e.g. Josephson, 1985b; Stokes & Josephson, 1988; R. D. Stevenson & R. K. Josephson, unpublished results).

The presence of an optimum strain for work output during cyclic contraction has been demonstrated for a number of muscles, including katydid, locust and moth wing muscles (Josephson, 1985a,b; Mizisin & Josephson, 1987; R. D. Stevenson & R. K. Josephson, unpublished results), a crab respiratory muscle (Stokes & Josephson, 1988), the frog sartorius muscle (Stevens, 1988) and the rat diaphragm (Syme & Stevens, 1989). Indeed, it appears likely that an optimum strain for power output at any given operating frequency is a general feature of all muscles. It would be sensible, in the design of animals, for muscles to be attached to skeletal elements such that the length changes experienced by the muscles during critical activities involving power output are close to the optimum strain for the muscles. Thus, the optimum strain for muscle work output is likely to be an important evolutionary determinant of animal morphology.

That there is an optimum strain for work output is clear; why there should be an optimum strain is less so. This study considers the effects of strain on work output from a crab muscle. It will be shown that a number of factors contribute to the relationship between strain and work output; these include: the force–velocity relationship for the muscle, the length–tension relationship, passive compliance, shortening inactivation and length-dependent changes in time course of contraction.

Materials and methods

The muscle used was scaphognathite levator L2B from the crab *Carcinus maenas*. The techniques used to prepare this muscle, to record its mechanical responses, and to measure its length, mass and area have been described previously (Josephson & Stokes, 1987; Stokes & Josephson, 1988). Muscle lengths were controlled and forces were measured with a Cambridge 300H ergometer (Cambridge Technology, Cambridge, MA 02140). The muscle was activated by stimulating its motor nerve with a suction electrode. The stimuli were 0·5 ms

current pulses whose intensity was 1·5–2 times that needed to evoke a maximum contraction to a burst of 10 stimuli. All measurements were made at 15°C.

Work measurements were obtained from muscles subjected to sinusoidal length change at 2 Hz, which is approximately the frequency at which mechanical work output per cycle is maximal (Stokes & Josephson, 1988). The muscle length upon which the sinusoidal length change was superimposed will be termed the average muscle length. The amplitude of the length change, the strain, is measured from minimum to maximum muscle length. A strain of 10 % indicates a length excursion from 5 % below to 5 % above the average muscle length. The muscles were stimulated phasically on each cycle with a burst of 10 shocks in 100 ms. Each experimental trial consisted of 10–12 cycles of length change and muscle stimulation. Individual trials were separated by 2 min intervals. The cycle chosen for analysis was, in each case, the tenth cycle of a trial. There was considerable burst-to-burst facilitation during the early cycles of a trial (Fig. 1A). By the tenth cycle the responses had reached an approximate steady state. Force and position values for the tenth cycle were digitized (12-bit resolution, one force-position pair of samples each 0·4 ms) and analyzed with a computer. The work done either by the muscle or on the muscle for the cycle was determined as the area of the loop formed by plotting muscle force against position over a full cycle (Fig. 1B). The area between the line of zero force and the lengthening limb of the work loop, from minimum to maximum muscle length, is the work required to stretch out the muscle on that cycle; and the area between the line of zero force and the shortening limb of the loop, from maximum to minimum length, is the work done by the muscle in shortening.

Experiments with each new preparation began with measurement of tension rise time, a value that was used in calculations of stimulus phase, and measurement of the relative muscle length, stimulus phase and strain that were optimum for work output (see Stokes & Josephson, 1988). Because of the position of the muscle and the ergometer, the absolute muscle length could not be measured easily until the end of an experiment. Therefore, it was not possible when doing experiments to adjust the length to be a desired fraction of the muscle resting length or of the optimal length for work output. Rather, measurements were made at the length determined to be optimum for work output or at a fixed distance, typically 0·75 mm, longer or shorter than the determined optimum. The muscles used were about 10 mm long (mean optimum length = 10·4 mm, s.d. = 0·9 mm, $N = 36$), so an absolute length change of 0·75 mm is about 7·5 %. Each of the general observations reported below was replicated at least five times, each time with a different preparation. To facilitate comparisons, many of the figures (Figs 1, 2, 5, 8, 9) are based on data from the same preparation.

Results

Strain and work at optimum muscle length

At that average muscle length for which work output was maximal, the

relationship between muscle strain and the work output per cycle was a rather steeply rising and then falling curve (Fig. 1C). The optimum strain at the test frequency of 2 Hz was about 8 % (mean = 8·2 % for this series, S.E. = 0·4 %, $N = 8$; see also fig. 4 in Stokes & Josephson, 1988).

The total work per cycle (Fig. 1C) is the sum of the work done by the muscle in shortening (positive work) and the work required to lengthen the muscle again after it has shortened (negative work). For convenience, both shortening and lengthening work will be plotted and discussed as absolute values. For example, the term 'minimum' as applied to a lengthening work curve will indicate a point on a curve at which the absolute value of the lengthening work is less than in surrounding regions.
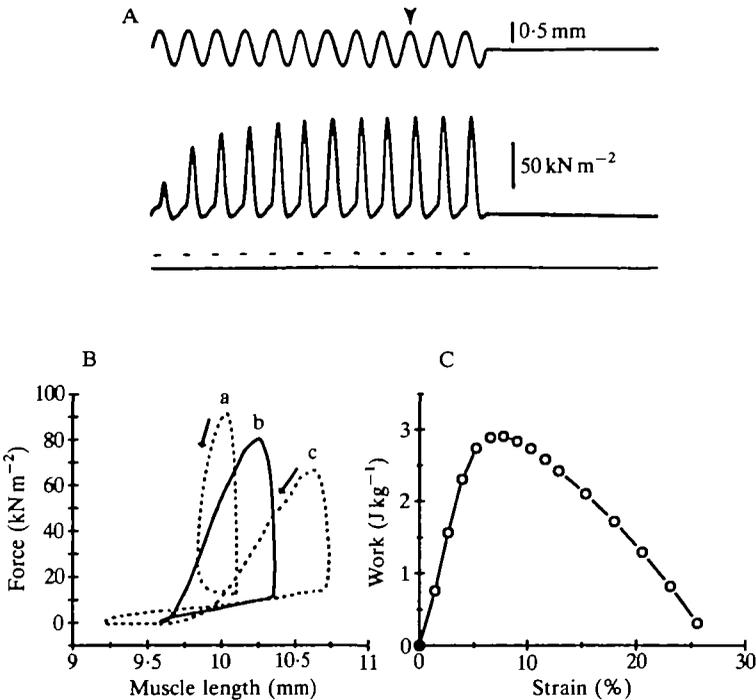

Fig. 1. (A) Muscle force (middle trace) during imposed sinusoidal strain (upper trace, frequency = 2 Hz) and phasic stimulation (lower trace, markers indicate bursts of 10 shocks in 100 ms). (B) Work loops at different strains. The average muscle length and stimulus phase were optimum for work output. Loop b is from the tenth cycle (marked by arrow) of Fig. 1A above. Loops a and c are plotted with dotted lines to aid discrimination. The strains in a–c were 2·7, 7·8 and 15·3 %. The net work output for the three loops was 1·56, 2·86 and 2·03 J kg^{-1}, respectively. Note that the loop at the largest strain (c) is complex with a counterclockwise component at longer lengths and a clockwise component at shorter lengths. (C) Muscle strain and work output per cycle. The average muscle length and the stimulus phase were optimum for work output at optimum strain. This is from the same preparation as Fig. 1A,B. Each point in this and in Figs 2 and 3 is the average of a determination from a set of trials at ascending strain and a determination from a set at descending strain with the same muscle.

The work during muscle shortening increased rapidly with increasing strain either to an approximate plateau or to a maximum with a subsequent decline in work following further increases in strain (Figs 2C, 3). The work required to
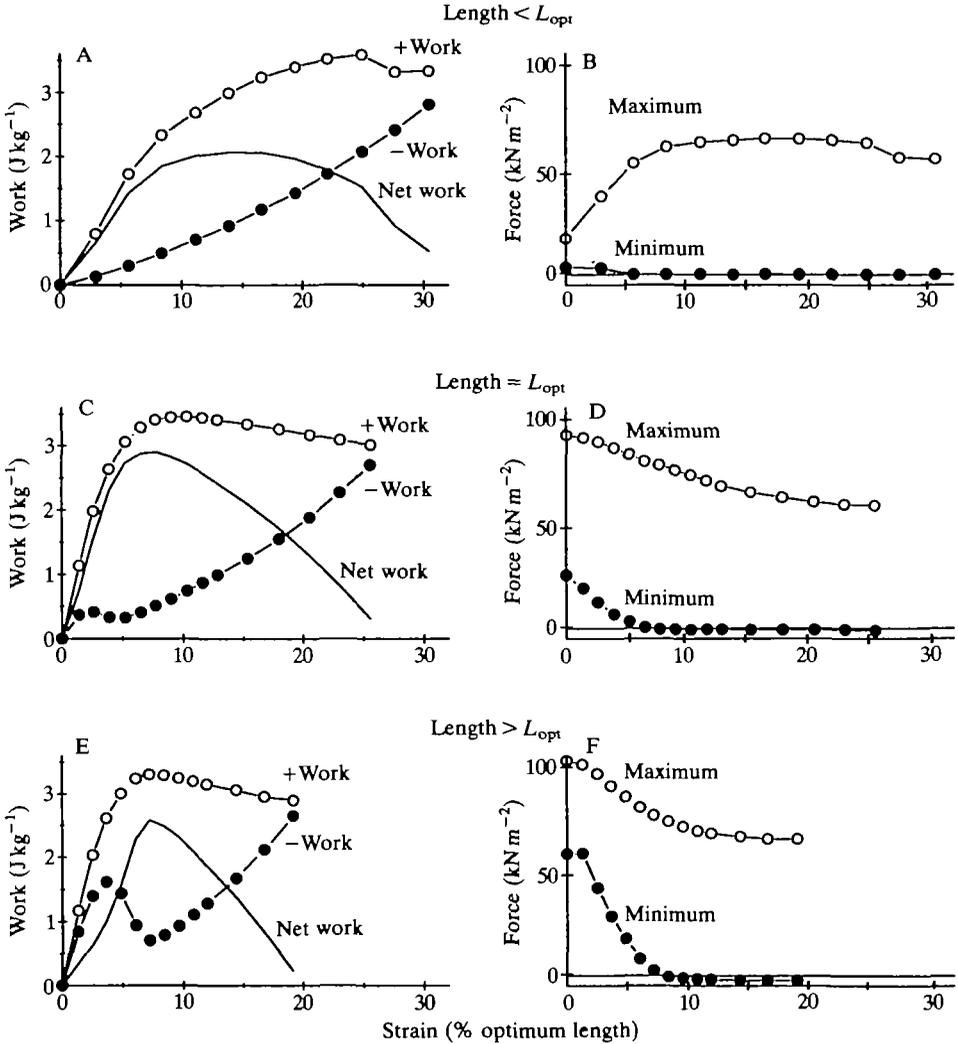

Fig. 2. (A,C,E) The relationship between muscle strain and the work done by the muscle in shortening (+ work), and the work required to restretch the muscle (− work). The curves without symbols are the net work. The average muscle lengths were (A) 0·75 mm less than the optimum, (C) the optimum for work output, (E) 0·75 mm greater than the optimum. The net work curve in C is the same curve as in Fig. 1. The strain in A and E, and in other figures at lengths other than the optimum length, is calculated using the optimum length; i.e. a strain of 10 % indicates a length change equal to 10 % of the optimum length even when the average muscle length was longer or shorter than the optimum. (B,D,F) Maximum and minimum force during the work loops which generated the data of Fig. 2A,C,E, respectively.

lengthen the muscle initially rose with strain, but with further increase in strain there was a distinct dip in the curve to a work minimum at a strain of 5–10 % (Figs 2C, 3). The work then increased monotonically with strain. The early decline in work with increasing strain indicates that there was a decrease in the average force required to lengthen the muscle, even though the total amount of muscle stretch and the total rate of stretch both increased. It is because the shortening work reached a maximum or a plateau with increasing strain while the lengthening work increased nearly continuously with strain that the net work per cycle had an optimum strain for work output.

The changes in shortening and lengthening work with strain were a consequence of changes in the shape and position of the work loops. With increasing strain, there was a continuous decline in the maximum force during the cycle (Figs 1B, 2D). In the low strain range, up to about 6–8 % strain, there was also a decrease in the minimum force per cycle with increasing strain. At strains greater than 6–8 % the minimum force developed by the muscle was nearly zero for all strain values. The work loops were entirely or almost entirely counterclockwise for strains less than the optimum strain for work output. A counterclockwise loop indicates that the force during shortening was greater than that during lengthening, and
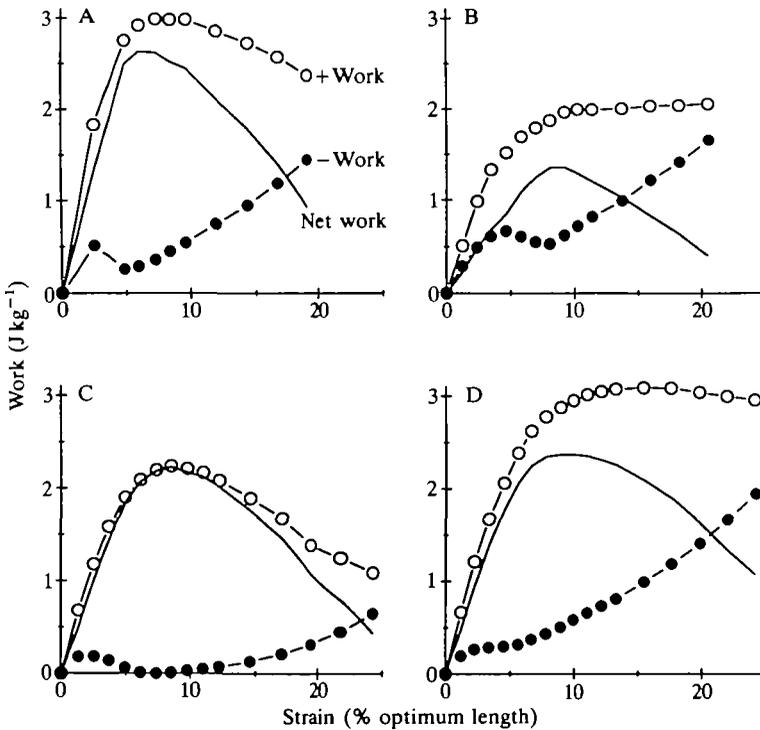

Fig. 3. Shortening work, lengthening work and net work for four other preparations (A,B,C,D) in which the work was analyzed the same way as in Fig. 2C. The average muscle length and stimulus phase were optimum for work output in each example.

therefore that the muscle did work on the apparatus during the cycle (i.e. work output was positive). Conversely, a clockwise loop indicates that the muscle force was greater during lengthening than during shortening, and therefore that work had to be done on the muscle by the apparatus to force the muscle through the cycle (i.e. net work output was negative). With strains longer than the optimum, the work loops acquired clockwise components (Fig. 1B). In the length regions for which the loops were clockwise, the muscle force was greater during lengthening than during shortening and work was absorbed rather than produced by the muscle. The area of the negative work region became increasingly large with increasing strain above the optimum, which in part accounted for the declining work with strain at large strain values.

Strain and work at short and long muscle lengths

Work output was strongly dependent on the average muscle length (Fig. 4). The work per cycle dropped sharply as the average muscle length was made shorter or longer than the optimum length. At a length 0·75 mm less than the optimum length (= 6·7–8·5 % below optimum length) the average work output was 53·9 % of that at the optimum length (s.e. = 3·4 %, $N = 18$). At a muscle length 0·75 mm greater than the optimum length, the work output averaged 51·4 % of that at the optimum length (s.e. = 4·1 %, $N = 18$). The work output was near zero or negative at lengths which differed from the optimum by more than about 15 %.

The relationships between strain and work were examined in detail at average muscle lengths 0·75 mm below and 0·75 mm above the optimum muscle length for work output. At the shorter muscle length the work during shortening rose with
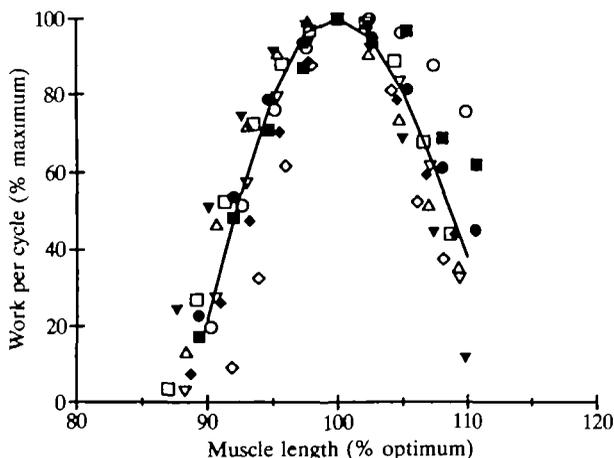

Fig. 4. Work output and average muscle length. The points were collected at estimated values for optimum strain and stimulus phase. The continuous line is for the preparation of Figs 1 and 2. The symbols are from nine other preparations. Each symbol type represents a different muscle. These nine preparations were selected because these were the muscles which had been examined over the widest ranges of muscle length.

increasing strain through most or all of the strain range examined (Fig. 2A). The work during shortening was examined at short muscle lengths in five preparations. In two of the five, there was some decline in shortening work at strains greater than about 20% (e.g. Fig. 2A); but in the other three preparations shortening work increased with strain to the largest strain tested which was about 30%. In all preparations the lengthening work, the work required to restretch a muscle after it had been allowed to shorten, increased monotonically with strain (Fig. 2A). At the shorter muscle length, the relationship between net work per cycle and strain had a very broad peak with a maximum at strain values appreciably higher than was the case with muscles at the optimum length (mean optimum strain at the short length = 14·4%, S.E. = 1·4%, $N = 8$). At the optimum strain and phase for work output, the work loops for the shorter muscle were wide and complex, with both a clockwise and a counterclockwise component (Fig. 5). The maximum force per cycle at the shorter muscle length rose with strain to a broad plateau, and minimum force per cycle was small at all strain levels (Fig. 2B).

If the average muscle length was increased to 0·75 mm above the optimum length for work output, the early peak and trough in the curve relating strain and lengthening work became much more pronounced than at the optimum length (Fig. 2E), and the minimum force per cycle at low strains was greater than at the optimum length (Fig. 2F). Otherwise the relationships between force, work and strain, and the shapes of the work loops, were much as they were at the optimum muscle length (Figs 2, 5).

Muscle length and contraction time course

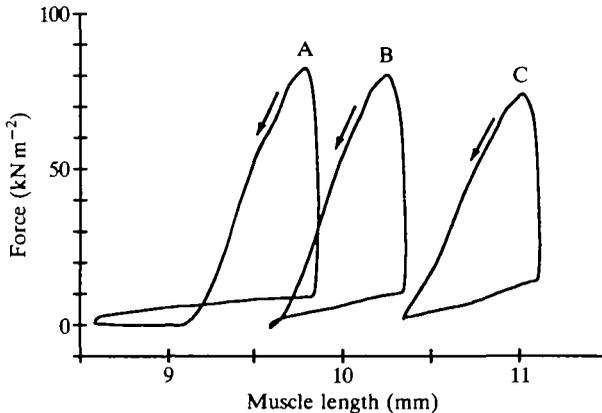Changing the muscle length did not alter the rise time of an isometric

Fig. 5. Work loops at different average muscle length. The strain for each loop was that giving maximum work output at that muscle length. The average muscle length in A was 0·75 mm less than the optimum length (= 7·5% less than optimum); in B the average length was at the optimum length, and in C the average length was 0·75 mm (= 7·5%) greater than the optimum length. The strain and work output for each loop were: (A) 12·8%, 2·50 J kg^{-1}; (B) 7·8%, 2·85 J kg^{-1}; (C) 7·8%, 2·51 J kg^{-1}.

contraction, but it did change the relaxation rate (Figs 6, 7). The time from peak tension to 50 % relaxation increased more than two times, and from peak tension to 90 % relaxation more than three times as the length was increased from 1 mm below to 1 mm above the optimum length for work output (Fig. 7A). There was also an increase in isometric tension with length (Fig. 7B). Muscle force and relaxation time did not increase *pari passu*. The increasing force nearly reached a plateau with increasing length, while the relaxation time increased approximately linearly with length (Fig. 7).

Shortening inactivation

When a muscle was held isometrically at the optimum length for work output and stimulated at 2 Hz, relaxation between bursts of stimuli was incomplete and there was considerable fusion of tension from burst to burst (Fig. 8A). It is this incomplete relaxation which produced the elevated minimum tension at zero strain in Fig. 2D. At muscle lengths longer than the optimum, there was even less relaxation between bursts, and a higher minimum tension at zero strain (Fig. 2F). Subjecting the muscle to sinusoidal strain, with a phase between stimulation and strain which was optimum for work output, reduced the minimum muscle tension. Muscle relaxation between bursts was more complete than for a muscle held at constant length (Figs 2D,F, 8B).

Part of the reduction in minimum force with applied strain might have been due to release of stretched series and parallel elastic elements during the imposed
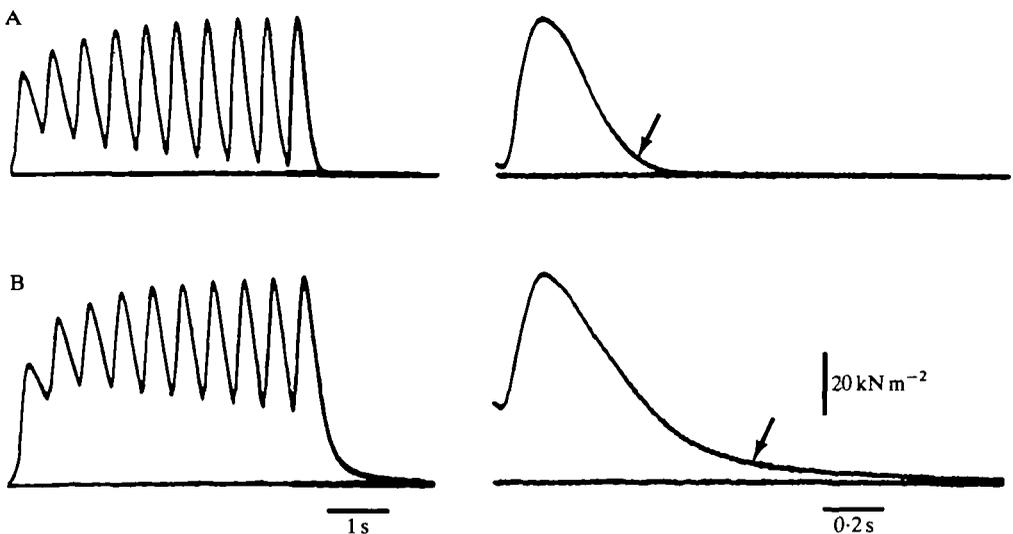

Fig. 6. The effect of muscle length on the amplitude and time course of isometric tension. (A) Muscle length 0·5 mm (= 5·2 %) less than the optimum length for work output. (B) Muscle length 1·0 mm (= 10·4 %) longer than the optimum length. The muscle was stimulated with bursts of stimuli (10 shocks in 100 ms) at a frequency of 2 Hz. The traces in the right column are the last contractions of each set at a higher sweep speed. The arrows indicate 90 % relaxation.

shortening. But this was not the entire reason for the decline in minimum tension with strain. Fig. 9A compares work loops at optimum average muscle length. Loop 1 was at low strain, loop 2 at the optimum strain for work output, and loop 3 was for the unstimulated muscle subjected to the same strain as that in loop 2.
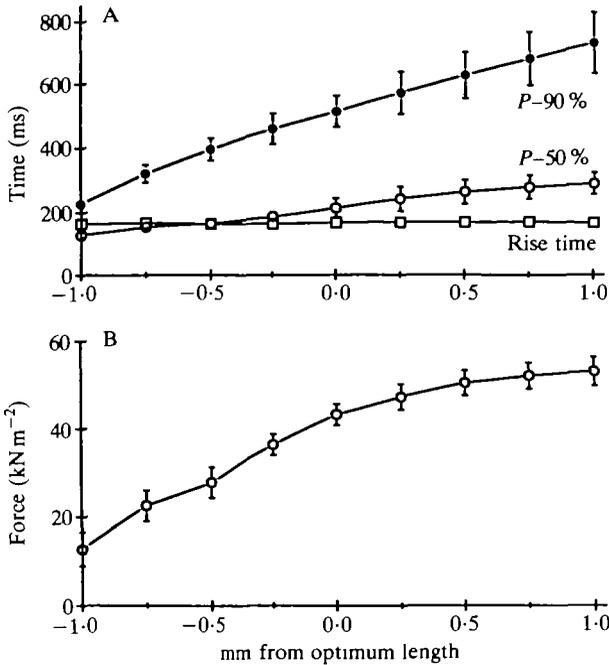

Fig. 7. The effects of muscle length on (A) the time course of contraction and (B) its amplitude. The contraction measured in each case was the tenth response, which was the last response in a set of contractions like those in Fig. 7. P–50 % is the relaxation time measured from contraction peak to 50 % relaxation; P–90 % is the relaxation time to 90 % relaxation. Vertical lines are standard errors ($N = 6$ preparations).
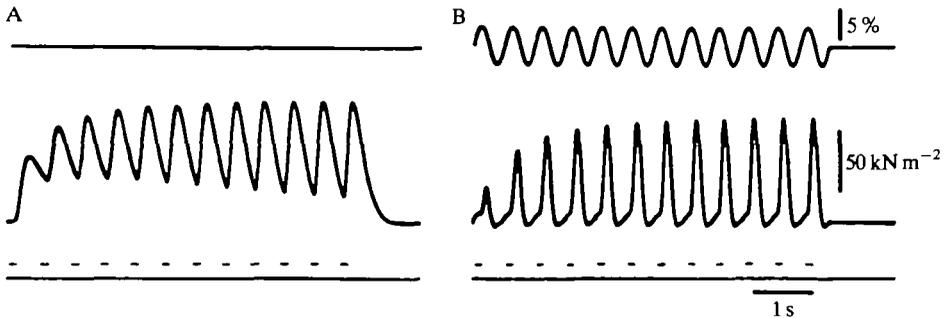

Fig. 8. Strain enhances muscle relaxation. (A) Isometric contractions to bursts of stimuli (10 shocks in 100 ms) at 2 Hz. (B) Muscle responses under the same stimulus conditions, but with imposed sinusoidal strain (6·5 %) at the phase giving optimum work output. The upper trace in each set is muscle length, the middle trace force, and the lower trace a stimulus marker.

Loops 1 and 2 are counterclockwise, loop 3 clockwise. During the lengthening portions of the cycles with stimulation (lower limbs of loops 1 and 2), the force at equivalent muscle lengths was less for the loop with the greater strain. If nothing else had happened within the muscle, the elastic elements which were released during shortening should have been equivalently restretched during lengthening, and at the same length during the lengthening phase the tension should have been similar in the two loops.

It should be noted that the force during the lengthening portion of loop 2 in Fig. 9A was not different from the force during lengthening of the unstimulated muscle (upper limb of loop 3). In the cycle of loop 2 the muscle was totally inactive at the end of the shortening, and no more resistant to stretch than an unstimulated muscle. Following the smaller shortening in the cycle with lower strain (loop 1), the muscle was still capable of bearing considerable tension. It appears that the greater the amount of allowed shortening, or possibly the greater the speed of shortening, the more closely the resistance of the muscle to subsequent stretch approached that of an inactive muscle. Shortening inactivated the contractile response of the muscle.

Muscle inactivation with shortening is seen even more clearly in muscles operating at lengths longer than the optimum for work output (Fig. 9B). At the longer length, the minimum tension between bursts of stimuli during isometric contraction was greater than at the optimum length, and the decline in minimum force with strain was more pronounced (compare Fig. 2D and 2F). Fig. 9B compares three loops at increasing strain from a stimulated muscle and one loop from the unstimulated muscle. In the loops with stimulation (loops 1–3), the greater the preceding shortening, the smaller the force at similar muscle lengths
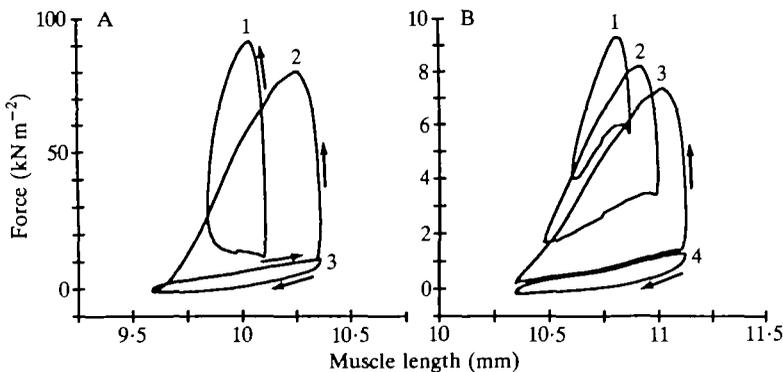

Fig. 9. Work loops at different strain. (A) Muscle length at the optimum for work output. The muscle was stimulated at the optimum phase for work output in loops 1 and 2; loop 3 is from the unstimulated muscle. The strain and work output for the three loops were: 1, 2·7%, 1·56 J kg^{-1}; 2, 7·8%, 2·86 J kg^{-1}; 3, 7·8%, −0·35 J kg^{-1}. (B) Muscle length 7·5% greater than the optimum length. Loops 1–3 are with phasic stimulation, loop 4 without stimulation. The strain and work output were: 1, 2·7%, 0·65 J kg^{-1}; 2, 5·2%, 1·61 J kg^{-1}; 3, 7·8%, 2·65 J kg^{-1}; 4, 7·8%, −0·43 J kg^{-1}.

during lengthening. At the longest strain, the muscle force during elongation was scarcely different from that of the unstimulated muscle (compare the lower limb of loop 3 and the upper limb of loop 4); following the longer strain, the muscle was essentially fully inactivated. Resistance to stretch may be defined as the slope of the line relating force and length (i.e. dP/dL where P is muscle force and L muscle length). Up to at least the midpoint of the lengthening half-cycle (muscle length = 10·75 mm in Fig. 9B), the slope of the force curve during lengthening is greater the smaller the total strain for the cycle. Thus, the resistance of the muscle to stretch was greater following small shortening than following large shortening. The decrease in stretch resistance with increase of shortening is another indication of shortening-induced inactivation of the muscle.

Discussion

Some questions and partial answers

Why, when the average length is at or above the optimum for work output, does the work done during muscle shortening rise to a broad plateau or a peak rather than continue to increase with added strain (Figs 2C,E, 3)?

If the cycle frequency is constant, increasing the distance moved per cycle (i.e. the strain) increases the shortening or lengthening velocity in equivalent parts of the cycle. For the scaphognathite muscle, as with other muscles, there is an inverse relationship between shortening velocity and muscle force, a relationship typically expressed as a force–velocity curve (Josephson & Stokes, 1987). Thus, increasing strain increases shortening velocity and decreases muscle force during shortening. This decline in force limits work output, and is certainly part of the reason why shortening work does not increase continuously with increasing strain.

The net work per cycle is the area of the work loop created by plotting muscle force against length (e.g. Fig. 1B). With increasing strain the work loops are expanded along the length axis, while the decreasing force associated with increasing strain compresses the loops along the force axis. It is appropriate to ask if the vertical compression more than compensates for the horizontal expansion, as would be required if the explanation offered above for the failure of shortening work to increase continuously with increasing strain is correct. One way to approach this is to ask what would be the relationships between force, work and strain during sinusoidal shortening if one considered only the force–velocity properties of the muscle. The following model ignores changes in isometric force with muscle length and changing states of muscle activation with time or distance, and assumes that a single force–velocity relationship will obtain throughout muscle shortening.

The model: assume that during shortening:

$$(P + a)(V + b) = (P_0 + a)b ,\qquad (1)$$

(Hill, 1938) where P is force, V is shortening velocity and a, b, P_0 are constants. For muscle L2B at 15°C (Josephson & Stokes, 1987), $a = 4\cdot63\,\mathrm{N\,cm^{-2}}$, $b =$

$0\cdot77$ lengths s^{-1}, $P_0 = 11\cdot3\,N\,cm^{-2}$. Length change ($\Delta L$) is the difference between existing muscle length (L) and average muscle length ($\bar{L}$):

$$\Delta L = L - \bar{L}\,.$$

For sinusoidal length change:

$$\Delta L = \Delta L_0 \sin(\omega t)\,,$$

where ΔL_0 is the maximum value of ΔL, ω is the angular velocity (radians s^{-1}) and t is time.

$$V = d(\Delta L)/dt = \Delta L_0 \omega \cos(\omega t)\,.$$

Since

$$\cos(\omega t) = \pm\sqrt{1 - [\sin(\omega t)]^2}$$

and

$$\sin(\omega t) = \Delta L/\Delta L_0\,,$$

$$V = \Delta L_0 \omega \sqrt{1 - (\Delta L/\Delta L_0)^2}\,. \qquad (2)$$

Combining equations 1 and 2 and rearranging gives:

$$P = \frac{(P_0 + a)b}{\Delta L_0 \omega \sqrt{1 - (\Delta L/\Delta L_0)^2} + b} - a$$

and

$$\text{Work} = \int_{+\Delta L_0}^{-\Delta L_0} P dL\,.$$

The expected force throughout a shortening half-cycle and the shortening work over the half-cycle were determined with a computer program for evaluating mathematical functions (MathCAD, Cambridge, MA 02139). The parameters used in the force–velocity relationship were those determined earlier for the scaphognathite muscle (Josephson & Stokes, 1987). The expected force during muscle shortening is a U-shaped function of muscle length (Fig. 10). At the
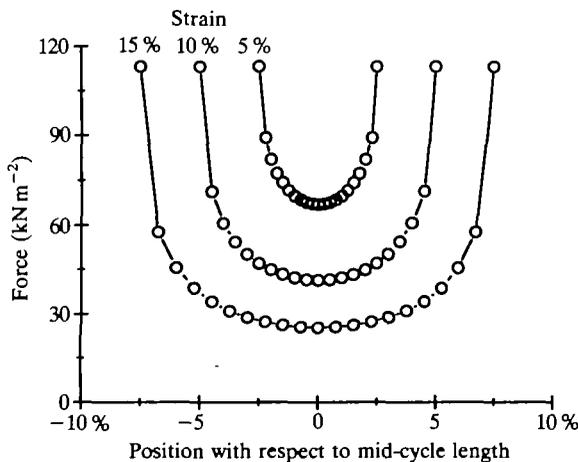

Fig. 10. Expected muscle force throughout shortening, based on measured force–velocity properties of the scaphognathite muscle and the assumption that the muscle is fully active throughout the shortening phase of the cycle.

maximum and minimum lengths, where the velocity is 0, the force is P_0. The minimum force through a cycle, as expected, declines with increasing strain. Interestingly, the U-shaped curves have rather flat bottoms. The combination of a hyperbolic force–velocity relationship and a sinusoidal length trajectory results in muscle force which is nearly constant over most of the shortening half-cycle. The work per cycle predicted by the model rises with strain to a maximum at about 15% strain, and falls thereafter (Fig. 11). The shape of the relationship between shortening work and strain is not greatly dissimilar in the model from that for real muscles. The work output predicted by the model is greater than that in real muscles, but not alarmingly so. It should be remembered that the model is based on a muscle which would be fully active through the whole of shortening, while in the experiments with real muscles the state of activation rises during stimulation and falls thereafter, and is presumably not maintained through the whole of the shortening half-cycle. The point which emerges from these considerations is that the force–velocity relationship for the crab muscle does, in itself, predict that shortening work should not rise continuously with strain.

Why does the work during shortening rise continuously with increasing strain, up to rather large strains, if the average muscle length is shorter than the optimum length (Fig. 2A)?

Maximum isometric, tetanic tension for scaphognathite muscle L2B is achieved at muscle lengths 10–20% greater than the longest muscle length reached *in vivo* (Josephson & Stokes, 1987). The optimum muscle length for work output is slightly longer than the maximum *in vivo* length (Stokes & Josephson, 1988). The optimum length for work output is on the rising limb of the isometric length–tension curve, but not far from the maximum of this curve. The length–tension curve for the scaphognathite muscle is quite narrow, and the rising phase of the
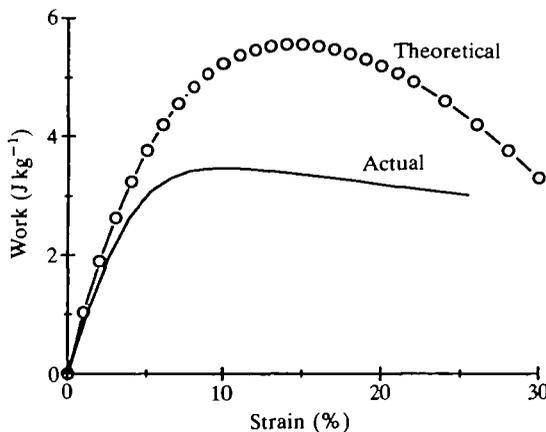

Fig. 11. Shortening work as a function of strain. Each point in the theoretical curve is a measurement of the area beneath a curve like one of the three shown in Fig. 10. The 'actual' shortening work is the upper curve in Fig. 2C.

curve is especially steep (Josephson & Stokes, 1987). The short muscle length examined was 7–8 % below the optimum length, which is significantly down on the ascending limb of the length–tension curve. For a muscle on the ascending limb of the length–tension curve, increasing strain can be expected to have two effects. It should increase the shortening velocity which, for reasons discussed above, should reduce the force throughout the shortening half-cycle. But increasing strain will also increase the maximum muscle length during the cycle and move the portion of the work loop that is at longer muscle lengths into a position on the length–tension curve that is more favorable for force production. Apparently the latter effect, increasing force because of operating at longer absolute muscle lengths for at least part of the loop, is more significant than decreasing force because of increasing velocity. At the short length, muscle force increases with strain or remains nearly constant through most or all of the strain range examined (Fig. 2B). It is because force does not decline with increasing strain at the short muscle length that work during shortening continues to increase with strain for muscles at the short length.

What causes the dip at low strain levels in the curve relating the work required to stretch a muscle and total muscle strain (Figs 2C,E, 3)?

At 2 Hz cycle frequency, and at low strain, muscle relaxation is incomplete in the intervals between stimulus bursts (Fig. 2D,F). During the early part of the lengthening half-cycle, muscle elongation is opposed by the residual force evoked by the preceding burst of stimuli. More work is required to stretch out the resisting muscle than would be required were the muscle fully relaxed. For strains up to 3–5 %, the lengthening work rises rapidly with strain. However, the increased strain also leads to greater muscle relaxation (Fig. 2D,F) and diminished resistance to stretch. For strain in the general range of 3–8 %, the reduction in muscle force and resistance to stretch with increasing strain dominates over the expected increase in work with increasing distance of stretch. In this range the lengthening work declines with strain, thus reversing the early increase in lengthening work with strain and creating a dip in the work curve. At still higher strains, the distance of elongation again becomes the major factor and the work again rises with strain.

The increased muscle relaxation with increasing strain is a manifestation of shortening inactivation. Inactivation of contractile activity induced by shortening has been reported for vertebrate skeletal muscles of several kinds (Jewell & Wilkie, 1960; Joyce & Rack, 1969; Edman & Kiessling, 1971; Briden & Alpert, 1972; Edman, 1975, 1980; Lannergren, 1978). Shortening inactivation and its lengthening counterpart, stretch activation, are particularly prominent features of insect asynchronous muscles (for reviews, see Pringle, 1978, 1981). It is stretch activation and shortening inactivation that allow oscillatory contraction of insect asynchronous muscle when these are activated while the muscle is attached to an inertial load. The functional significance of shortening inactivation in skeletal muscles other than insect asynchronous muscles has been uncertain. In the crab respiratory muscle, shortening inactivation can greatly decrease the work required to restretch a muscle following shortening and can significantly increase the net

work available from the muscle in the strain range 5–10 %, which is likely to be the strain range at which the muscle normally operates.

Why is the early dip in lengthening work absent at the short muscle length tested and why is it more pronounced at long muscle lengths than at the optimum length (Fig. 2)?

At the short muscle length, relaxation is fast enough to be complete or nearly so by the beginning of the lengthening portion of the cycle. Thus shortening inactivation, which is the phenomenon that produces the early dip in the lengthening work, is of little consequence, at least not at the 2 Hz cycle frequency. Increasing the muscle length decreases the rate of relaxation and increases the amount of tension fusion between adjacent bursts of stimuli. At longer muscle lengths relaxation is slower, tension fusion greater, and shortening inactivation and its consequence, a dip at low strain levels in the work required to restretch the muscle, become more prominent.

Prolongation of relaxation with increasing muscle length, similar to that described here for crab muscles, has been reported for frog muscles (Hartree & Hill, 1921; Jewell & Wilkie, 1960; Close, 1972), cat muscles (Rack & Westbury, 1969) and insect muscles (Josephson, 1973). An increase in myofibrillar calcium sensitivity with increasing muscle length has been found in a number of different muscles (for a review, see Stephenson & Wendt, 1984). An increase in calcium sensitivity would allow contraction at lower calcium levels as calcium is withdrawn from the cytoplasm during relaxation, and could be responsible, at least in part, for the prolongation of relaxation.

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