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THE CONTRACTILE PROPERTIES OF A CRAB RESPIRATORY MUSCLE

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SUMMARY

1. Contraction of scaphognathite muscle L2B of the green crab *Carcinus maenas* is strongly dependent on stimulus number and frequency. Single, supramaximal stimuli evoke little or no tension. When stimulated with shocks in either short bursts (10 stimuli in 0·5 s or less) or long bursts (5 s of stimulation), the isometric tension from the muscle increases with increasing stimulus frequency to a maximum at about 150 Hz at 15 °C, beyond which tension declines with further increase in stimulus frequency.

2. There can be facilitation of both contraction and relaxation between short bursts of stimuli. Facilitation of contraction is seen as increasing tension on successive bursts of a series, even when the interburst interval is long enough for relaxation to be completed during the interval. Interburst facilitation lasts at least 10 s. Facilitation of relaxation is seen as progressively faster relaxation from burst to burst of a series, and relaxation to lower tension levels when the interburst interval is so short that relaxation is incomplete in the interburst interval.

3. Maximum isometric tension occurs at muscle lengths slightly longer than the longest muscle length reached *in vivo*. Tension declines rapidly with changes in muscle length away from the optimum length. The maximum isometric tension was about 12 N cm^{-2}.

4. The maximum shortening velocity of a tetanically activated muscle was determined as 1·9 lengths s^{-1} (L s^{-1}) by extrapolation of force–velocity curves to zero force and 3·3 L s^{-1} by slack test measurements.

5. The scaphognathite muscle would be classified as a slow or tonic muscle on the basis of its requirements for multiple stimulation to reach full activation, and as a moderately fast muscle on the basis of its force–velocity properties.

INTRODUCTION

A scaphognathite (SG = gill bailer) of a decapod crustacean is one of a pair of bilateral blade-like appendages which lie within channels anterior to the branchial chamber. Up and down sculling movements of the two SGs pump water across the gills. The repetitive, stereotyped beating of the SGs has attracted considerable attention from workers interested in rhythmic behaviour patterns and their neural

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control, and there is a rather large literature covering various aspects of SG structure and function. The anatomy of the SG and the innervation pattern of its muscles have been described in some detail for several crustaceans (Pasztor, 1968; Pilkington & Simmers, 1973; Young, 1975; Pilkington & MacFarlane, 1978). Activity patterns in the motor neurones to the levator and depressor muscles have been characterized during slow and rapid beating and during forward and reversed beating, particularly in the crab *Carcinus maenas* (Pilkington & Simmers, 1973; Young, 1975; Mercier & Wilkens, 1984*a*,*b*). The effects of sensory input on the SG beating pattern, on coordination between right and left SGs, and on coordination between SGs and the heart have been described for the lobster *Homarus americanus* (Wilkens & Young, 1975; Young & Coyer, 1979). The central organization of the motor neurones and their interactions with interneurones which collectively form the pattern generator have been studied in several crustacean species (Mendelson, 1971; Pilkington & MacFarlane, 1978; Simmers & Bush, 1980, 1983*a*,*b*; DiCaprio & Fourtner, 1984). Pharmacological control of the central pattern generator and of the peripheral muscles have been investigated (Berlind, 1977; Mercier & Wilkens, 1985; Wilkens, Mercier & Evans, 1985). The overall performance of the system – the rate of SG beating, the water pressure developed, the volume of water pumped, the total work expended and the efficiency of the pump – has been characterized, again especially for *C. maenas* (Cumberlidge & Uglow, 1977*a*,*b*; Mercier & Wilkens, 1984*a*,*b*; Wilkens, Wilkes & Evans, 1984). Earlier work is reviewed by Wilkens (1976).

One link between the central pattern generator and the final mechanical performance which has been inadequately characterized for the SG system is the mechanical performance of the muscles involved – the relationships between neural input and muscle contraction, the contractile properties of the muscles as a function of muscle length and muscle load, and the mechanical power output of the individual SG muscles. It has been shown that the excitatory junctional potentials (EJPs) evoked in muscle fibres by single motor neurone impulses are small, and repetitive motor neurone input is required to activate the muscle sufficiently to produce significant contraction (Pasztor, 1968; Pilkington & MacFarlane, 1978; Moody-Corbett & Pasztor, 1980; Mercier & Wilkens, 1984*a*,*b*). Normally the muscles are activated with short bursts of impulses in the innervating motor neurones. Mercier & Wilkens (1984*a*,*b*) demonstrated that muscle contraction (force generated or distance shortened) during activation by bursts of impulses is a function of impulse number and frequency within the bursts and of the interval between bursts. These studies provide a background for interpreting the functional significance of different motor neurone output patterns from the central nervous system, but more quantitative information is needed to evaluate fully the relationships between activation patterns and mechanical performance. The first part of the study described below extends the observations of Mercier & Wilkens (1984*a*,*b*) on the relationships between activation patterns and muscle contraction.

The second part of the study deals with the length–tension and force–velocity relationships of an SG muscle. With few exceptions, previous studies on muscle dynamics have been with muscles which give large twitch responses and have

relatively high ratios of twitch tension to tetanic tension. The SG muscles, as indicated above, give vanishingly small twitches and require multiple stimulation at a rather high frequency to become fully activated. On the basis of the characteristics of neuromuscular transmission, the SG muscles would be termed 'slow' or 'tonic' (see, for examples, Atwood, 1973; Govind & Atwood, 1982; Hoyle, 1983). It was of interest to determine if these slow neuromuscular properties extended to contraction dynamics as well. In fact, as will be shown, the shortening velocity of a fully activated SG muscle is respectably fast. Slow neuromuscular transmission properties need not be associated with slow contraction dynamics.

Two features of the SG muscles make them particularly favourable preparations for studying contractile properties of crustacean muscles. First, unlike most crustacean muscles, the SG muscles apparently lack inhibitory innervation (Pasztor, 1968; Pilkington & MacFarlane, 1978; Moody-Corbett & Pasztor, 1980; Simmers & Bush, 1983*b*). Thus it is possible to activate the muscle with nerve stimulation without the complexities which might be introduced by the co-activation of excitatory and inhibitory inputs to the muscle. Second, the SG muscles are parallel-fibred muscles. Limb and trunk muscles in crustaceans are often pinnate. With parallel-fibred muscles it is not necessary, when determining muscle stress, to correct measured forces and cross-sectional areas for the off-axis orientation of the fibres. The experimental convenience of a simple innervation pattern and of parallel fibres are partially offset, however, by the small size of the SG muscles. In moderately large specimens of *C. maenas*, the larger SG muscles weigh only about 2 mg, which poses some problems for manipulation and measurement.

MATERIALS AND METHODS

Green crabs, *Carcinus maenas* (L.), were obtained at the Marine Biological Laboratory, Woods Hole, MA where the research was carried out. Animals were kept in running sea water at 17–22 °C until they were used, which was usually within 2 weeks of collection. The experimental animals were all male and ranged in size from 12 to 26 g.

The muscle studied was muscle L2B (Young, 1975), which is a levator of the SG. Muscle L2B is a broad, thin muscle made up of parallel fibres which originate proximally on a ridge of the endophragma and which insert distally on a hatchet-shaped sclerite (see fig. 2 in Young, 1975). This muscle was chosen because it is one of the larger SG muscles and because the sclerite upon which it inserts forms a convenient attachment point for a transducer.

The SG and its muscles were exposed by removing the limbs, the dorsal and ventrolateral exoskeleton, and the internal tissues of the digestive, reproductive and circulatory systems. The dissection was done with the animal pinned out in chilled (10–15 °C), running sea water. The running water carried away the fluids which oozed from the hepatopancreas as its various lobes were cut and removed. Early in the dissection the distal levator nerve to the SG was uncovered from the dorsal side and freed from surrounding circulatory vessels and hepatopancreas. The nerve was

cut proximally, leaving a distal length of nerve of 1–1·5 cm. Muscle L2B was exposed from the dorsal aspect by removing the thin cuticle which overlies it, and by removing two thin muscles (muscle PC of Young, 1975, and an undescribed muscle) which lie more dorsally. At this point in the dissection the SG blade was pushed into a fully depressed position and the *in situ* length of the extended levator muscle was measured with an ocular micrometer. The side of the muscle which faces posterior and lateral is slightly longer than that which is anterior and medial. The lengths of the posterior–lateral and the anterior–medial margins of the muscle were separately measured and averaged to obtain a single value for the *in situ* muscle length. This length was termed the reference length and was used in setting the length of the isolated muscle during later measurements.

After the muscle length had been measured, the hatchet-shaped sclerite was dissected free from the remaining exoskeleton of the schaphognathite. Muscles D1C and L2B′, which also attach to the sclerite, were transected. All the remaining levator and depressor muscles except for L2B were cut and the entire blade of the SG was removed. The preparation was then transferred to a dish filled with crab Ringer's solution (compositon given in Mercier & Wilkens, 1984*b*) and pinned to a Sylgard resin layer in the bottom of the dish, with the muscle in either a vertical or a horizontal orientation as was required. The muscle was attached to a transducer by slipping a hook at the end of a fine insect pin around the hatchet-shaped sclerite upon which the muscle inserts. The other end of the pin was attached to a transducer. In experiments in which only isometric tension was measured, the transducer used was a strain gauge made from a pair of Pixie semiconductor elements (see Miller, 1979, fig. 4-4 for details on construction). The resonant frequency of the transducer and the attached insect pin was approximately 1·5 kHz. In experiments involving force–velocity and quick-release measurements the transducer was a Cambridge Model 300H ergometer (Cambridge Technology, Cambridge, MA 02140). The transducer was mounted in a manipulator so that the position of it and the attached muscle could be moved and the muscle length adjusted. The motor nerve to the muscle was stimulated with an *en passant* suction electrode. The stimuli were 0·5 ms voltage pulses usually at twice the strength required to activate the motor axon in the nerve with the highest threshold. A cooling coil built into the experimental dish was used to hold constant the temperature of the solution surrounding the muscle. The muscle temperature ranged from 14·6 to 15·4 °C between experiments but varied by no more than 0·2–0·3 °C during a single experiment.

Muscle mass was determined from muscles which had been fixed in 70 % ethanol. We found it difficult to separate cleanly fresh muscles from their origins and insertions. Since the muscles are small, tissue loss during dissection could lead to significant errors in determining the muscle size. After alcohol fixation, the muscles separate cleanly from the exoskeleton and dissection errors are minimized. Alcohol fixation was also used to determine the length of a muscle during an experiment when this would otherwise have been difficult. The muscle was mounted vertically in those experiments in which the Cambridge ergometer was used, and it was difficult to

measure accurately the muscle length with an ocular micrometer. In these experiments the muscle was fixed in place at the end of the experiment, while it was still attached to the transducer. After 15–30 min of fixation, the muscle was freed from the transducer, the animal was unpinned and placed in a convenient orientation, and the length of the fixed muscle was measured. Fixed muscles from all experiments were stored in 70% ethanol for several days to several weeks. They were then rehydrated overnight in crab Ringer's solution and weighed to the nearest 0·01 mg on a torsion balance. There is some mass loss associated with ethanol fixation and rehydration. To estimate the extent of this loss, a set of 14 mandibular muscles was removed from crabs during preparation of muscle L2B. The mandibular muscles were weighed while fresh, stored in 70% ethanol for 3–4 weeks, rehydrated in crab saline overnight, and reweighed. The mass of the rehydrated muscles averaged 73·5% of their original mass (S.E. = 1·1%). Although the mandibular muscles were about 10 times larger than the SG muscles of interest, we have assumed that the fractional mass loss associated with fixation and rehydration is similar in the two muscle types and have used the relative mass loss in the mandibular muscles as a correction factor in estimating the original wet mass of the SG muscles. The muscle cross-sectional area was determined as the ratio of muscle mass to muscle length.

Measurements were obtained from 37 muscles. The average length of these muscles was 6·89 mm (S.D. = 0·63) and the average mass, corrected for loss during fixation, was 1·94 mg (S.D. = 0·51 mg).

Additional experimental details are given in appropriate places in the text.

RESULTS

Neural control of contraction

The relationships between the strength, frequency and pattern of neuronal stimulation and the evoked muscle tension were examined in several ways. Stimuli of varied strength were used to determine the number of motor units in the muscle. The effect of stimulus frequency on evoked tension was examined using bursts of stimuli which were long enough, except at low frequencies as it turned out, for the tension to reach a steady state. Since the SG muscles are normally activated with short, repetitive bursts of nerve impulses, particular attention was given to the muscle tension evoked by short bursts of stimuli. The effects of frequency were investigated using stimulus bursts with a constant number of stimuli but with the shocks at differing intervals. Facilitation between bursts was characterized using bursts of stimuli at a fixed stimulus frequency and number but at varying interburst intervals. Finally, muscle contraction was measured in response to repetitive bursts of stimuli over a range of interburst intervals. The stimulus number within these bursts was constant but the frequency was varied so that the stimulation period occupied a constant fraction of the burst cycle. The last activation pattern closely approximates to that measured from the muscle *in vivo* (Young, 1975; Mercier & Wilkens, 1984*b*).

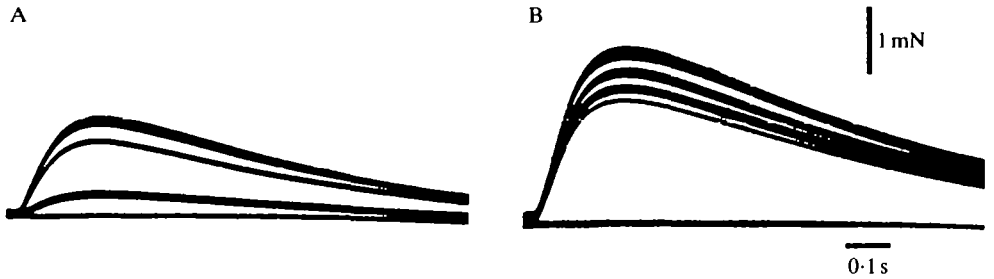

Fig. 1. Motor units in the scaphognathite muscle. The motor nerve to the muscle was stimulated with a pair of shocks every 2·5 s. The interval between the shocks of a pair was 6 ms. The stimulus intensity was initially well above threshold. After a number of stimuli had been given and a steady-state response reached, the stimulus intensity was slowly reduced, causing individual motor units to drop out as the stimulus dropped below their threshold. (A) A muscle with at least three motor units. (B) A muscle with at least four motor units.

The number of motor units

Attempts to determine the number of motor units in muscle L2B on the basis of the number of tension steps in response to stimuli of gradually changed intensity proved difficult. Almost no preparations gave detectable tension in response to single stimuli, and therefore it was necessary to use multiple stimuli. Multiple stimuli can pose problems. If the stimulus intensity is near the threshold of a motor unit, the unit may not be activated by each stimulus of a burst. Therefore the tension evoked by a burst of three or more stimuli to a motor unit can be expected to be somewhat graded with stimulus intensity near the threshold for activation of that motor unit. This, we assume, is not the case with pairs of stimuli, since failure to respond to either of the shocks of a pair should lead to an abrupt cessation of response in muscles such as muscle L2B which normally do not give contractions in response to single stimuli. We therefore used pairs of stimuli in examining the number of motor units in the muscle.

The paired stimuli were separated by 6 ms. The pairs were repeated regularly at intervals which ranged from 2 to 5 s in different preparations. Initially the stimulus intensity was set well above threshold for all the motor units of the muscle. After the tension responses to the individual stimulus pairs had reached a steady state, the stimulus intensity was gradually reduced, causing motor units to drop out of the response.

In several preparations the tension decreased in three discrete steps as the stimulus intensity was gradually reduced; indicating the presence of at least three motor units (Fig. 1A). The estimate is a minimum since motor units with very close thresholds may not have been distinguished. In two preparations there appeared to be four tension steps and therefore at least four motor units (Fig. 1B). This result confirms earlier findings from axon staining and intracellular recording that muscle L2B is innervated by three and sometimes four motor axons in the crabs *Nectocarcinus antarcticus* and *C. maenas* (Pilkington & MacFarlane, 1978; Mercier & Wilkens,

1984*b*). The thresholds of the different motor units were quite similar, suggesting that the motor axons are of similar size.

Stimulus frequency and maximum isometric tension

Muscles were stimulated with 5-s trains of stimuli. The trials were spaced regularly at 2-min intervals to maintain the preparation in a steady-state condition. The interstimulus intervals (ISI) tested ranged from 50 to 2 ms and were presented in order of decreasing ISI in successive trials. The muscles were held at reference length or slightly longer. There was considerable variation in the tension produced by different muscles of this series. When we made these measurements we did not realize the strong dependence of muscle tension on muscle length (see below), and we did not take care to set all muscles at an optimum length or at the same fraction of their optimum length; this accounts in part for the variation from preparation to preparation. To prevent results of strong muscles from swamping those of weaker muscles, the tension from a muscle of this series is expressed as relative tension, defined as the maximum tension at a test frequency divided by the tension produced with stimulation at a 10-ms ISI in the same preparation.

The maximum tension reached during a 5-s burst of stimuli increased with decreasing interstimulus intervals to a maximum, beyond which tension declined with further shortening of the interstimulus interval (Fig. 2). With the set of intervals tested, tension was maximum at an interstimulus interval of 6·7 ms, equivalent to a frequency of 150 Hz. With interstimulus intervals of 10 ms or less the tension reached a peak within the first 1–2 s of stimulation and there was often a subsequent decline in tension. With intervals of 15 ms and longer the tension rose continually during the 5-s stimulation period (Fig. 2). Undoubtedly the relative tension achieved with low-frequency stimulation would have been greater than that measured had a longer stimulation period been used.

Tension as a function of stimulus frequency in short bursts

Muscles were held at or slightly longer than reference length. On each trial the muscle was stimulated with five bursts of stimuli at an interburst interval of 5 s. There were always 10 stimuli per burst. The ISI ranged from 2 to 50 ms in different trials. The trials were spaced at 2-min intervals. The trials began with a test at an ISI of 10 ms, and every third trial thereafter was again with 10 ms ISI. Thus each trial with a new test ISI was immediately preceded by or followed by a trial in which the ISI was 10 ms. To compensate for differences in absolute strength between preparations and for changes in responsiveness with time throughout a series, all force values are expressed relative to those in the closest trial with 10 ms ISI.

The maximum tension reached in response to a burst of 10 stimuli rose with decreasing ISI to a maximum at 6·7 ms (150 Hz), beyond which peak tension dropped progressively with further decrease in the ISI (Fig. 3). The results plotted in Fig. 3 are for the first burst of the set of five given at each stimulus interval. The plot of relative force against ISI for the fifth burst of the series was essentially identical to that for the first burst. In several of the preparations of this series the

relaxation rate following a burst of stimuli became progressively faster following the successive bursts of a trial, especially between the first and subsequent responses (Fig. 3). Such facilitation of relaxation is considered in greater detail below.

Facilitation between bursts

The muscle was held at or slightly greater than rest length and stimulated with bursts of stimuli at varied interburst intervals (IBI). Each stimulus burst contained 10 stimuli at an ISI of 10 ms. Each trial consisted of five stimulus bursts (four at the longest interval tested). Individual trials were spaced at 2-min intervals. Facilitation was measured as the ratio of the tension evoked on burst n of a trial divided by the peak tension on the first burst of the trial.

The muscles of this experiment fell into two groups: those in which the tension evoked on the first stimulus burst of each trial was moderately large (range = 1·5–7·4 N cm^{-2}, average = 3·1 N cm^{-2}, $N = 5$ muscles) and in which facilitation from burst to burst was modest; and those in which the tension evoked by the first burst was much lower (range = 0·09–0·45 N cm^{-2}, average = 0·22 N cm^{-2}, $N = 3$), and in which facilitation from burst to burst was much more pronounced. Results

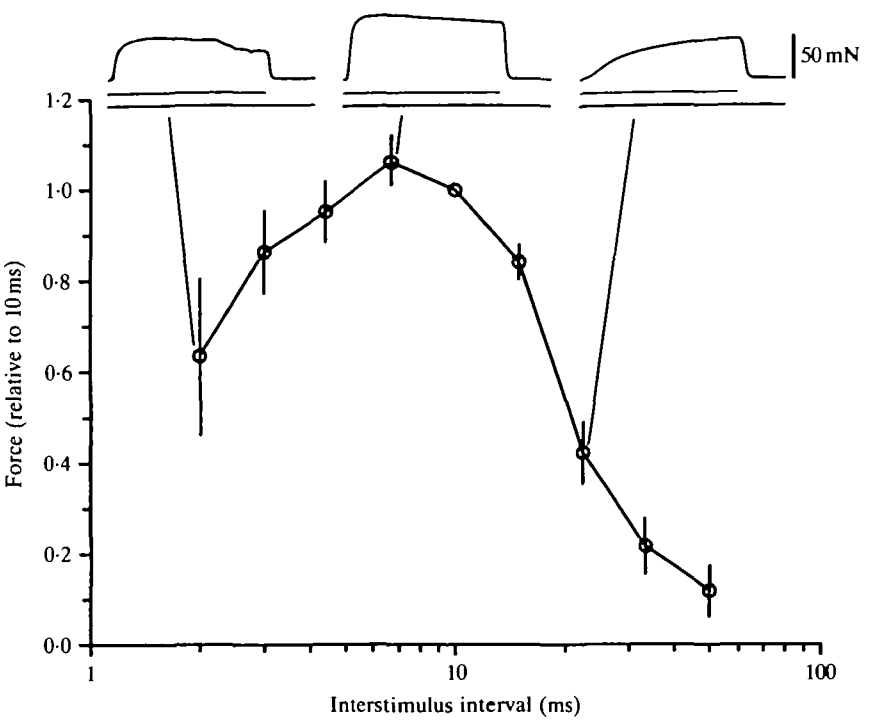

Fig. 2. Isometric tetanic tension as a function of stimulus frequency. Muscles were stimulated for 5 s at interstimulus intervals (ISI) ranging from 2 to 50 ms. The upper set of traces show responses from an individual animal (stimulus markers are shown beneath the tension traces). The plotted symbols below are the forces relative to that measured with an ISI of 10 ms. The plotted values are means of six preparations. Vertical lines are ±S.E.

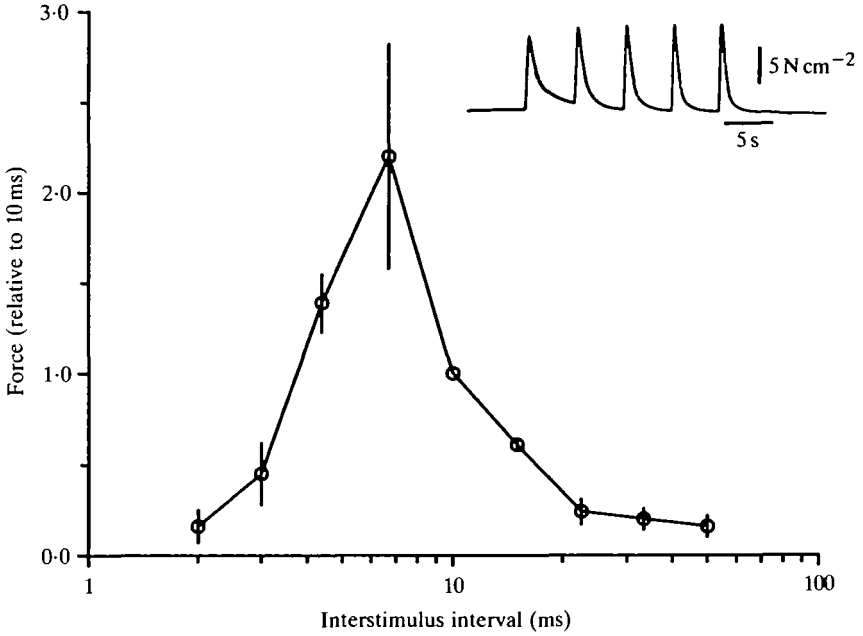

Fig. 3. Isometric tension during short bursts of stimuli as a function of the interstimulus interval (ISI) within the bursts. There were 10 shocks per burst, and the bursts were repeated at 5-s intervals to give a set of five bursts. The inset is an example of the tension responses at an ISI of 6·7 ms. The open circles are the peak forces during the first burst of each set, expressed as the force at a given ISI relative to that at an ISI of 10 ms. Vertical bars are ±S.E. ($N = 8$ preparations).

from these two groups are plotted separately in Fig. 4. Although the absolute values of the facilitation measure are quite different in the two groups of muscles, the general shapes of the facilitation curves are similar. There was progressive facilitation during the set of bursts, and the response on the fifth burst was greater than that on the fourth which, in turn, was greater than that on the third and so on. Facilitation was more pronounced with short interburst intervals than with long ones but there was still measurable facilitation with interburst intervals as long as 11·5 s.

Stimulation for a constant fraction of the burst cycle

Mercier & Wilkens (1984*b*) noted that the number of motor neurone impulses per cycle to muscle L2B of *C. maenas* is largely independent of SG beating frequency. Further, the impulse frequency within a burst is proportional to the interburst frequency. A corollary of these two observations, a constant number of impulses per burst and impulse frequency proportional to burst frequency, is that the burst duration is inversely proportional to the burst frequency, and therefore that the stimulus bursts occupy a relatively constant fraction of the burst cycle. This confirms earlier observations of Young (1975) who found that the impulse bursts to various SG muscles each occupied a relatively constant fraction of the SG beating cycle. According to Young, muscle L2B is active for about 20% of the cycle.

In the following experiment, muscle L2B was activated in patterns mimicking normal activation patterns. The muscle was stimulated with repetitive bursts of stimuli at burst frequencies (0·83–5 Hz) which covered the range of normal beating frequencies (Hughes, Knights & Scammell, 1969; Mercier & Wilkens, 1984*a*). The bursts all contained 10 stimuli, which is within the normal range of pulses per burst (Mercier & Wilkens, 1984*b*), and the interval between individual stimuli was adjusted so that the total stimulation period was always 20% of the burst cycle duration.

The muscles in this series were held at or slightly above reference length. Each trial consisted of a 10-s train of stimulus bursts. Trials were repeated at 2-min intervals. With each preparation the series began with stimulation at an interburst interval of 0·5 s, and every third trial thereafter was again with an IBI of 0·5 s. Thus each trial with a new IBI was either immediately before or immediately after one at an IBI of 0·5 s. To compensate for differences in absolute strength between preparations and for changes in responsiveness with time throughout a series, all force values are expressed relative to the peak force from the appropriate stimulus burst in the closest trial at 0·5 s IBI. Because of facilitation and summation, and in some preparations obvious fatigue, the maximum and minimum tension per cycle changed throughout the trains of stimulus bursts (Fig. 5). Two points in the train were chosen for analysis and to illustrate the responses: the fifth stimulus burst, which came at different absolute times after the onset of stimulation depending on

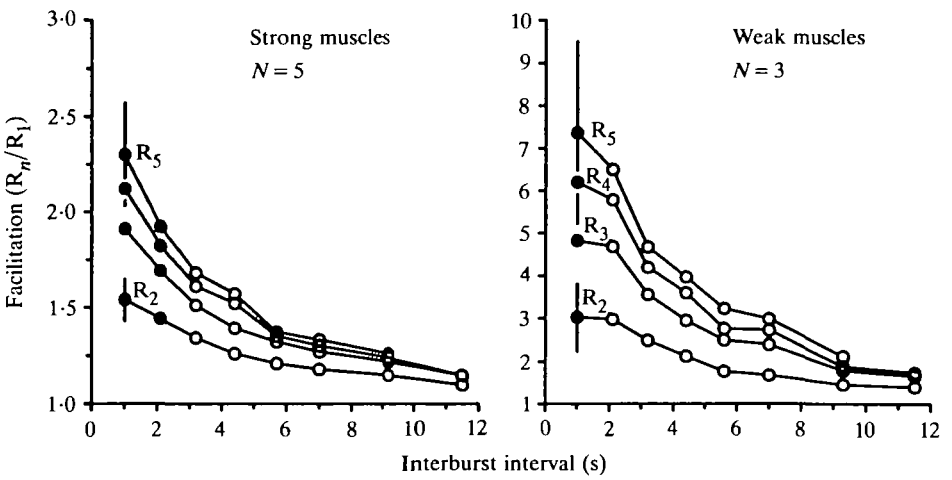

Fig. 4. Facilitation between bursts. Muscles were stimulated with a burst of 10 stimuli at an interstimulus interval (ISI) of 10 ms. The burst frequency ranged from 1 per s to 1 per 11·5 s. Facilitation was measured as the ratio of the peak force on bursts 2–5 of each set (R_n) to that of the first burst (R_1). Vertical bars, shown for a few points only, are ±S.E. Filled circles indicate values which are affected by mechanical summation because of incomplete relaxation between bursts. For the 'strong muscles' filled circles are used when there was some summation in two or more of the five preparations. With the 'weak muscles' filled circles indicate that there was summation in one of the three preparations.

the burst frequency; and the response closest to 10 s of stimulation, which came after a varying number of bursts, again depending on the burst frequency.

Within the set of interburst intervals tested, tension was greatest for bursts at an interval of 0·3 s, and declined at longer and shorter intervals (Fig. 6). This optimum

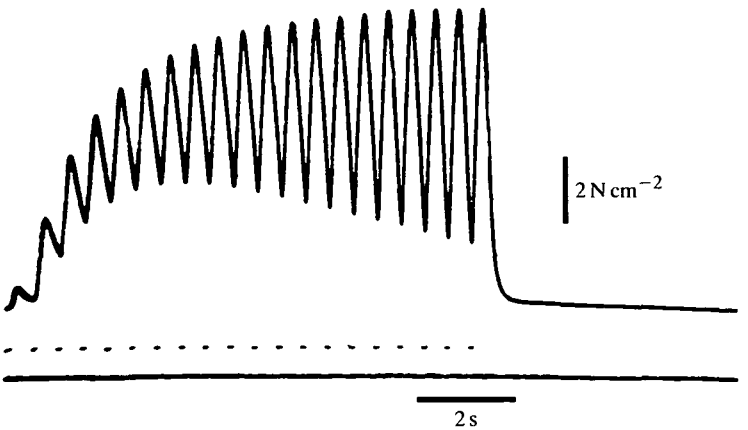

Fig. 5. Facilitation of contraction and relaxation during repeated bursts of stimuli. Here the bursts were at 2 s^{-1} and each burst was 10 stimuli at an interstimulus interval of 10 ms.

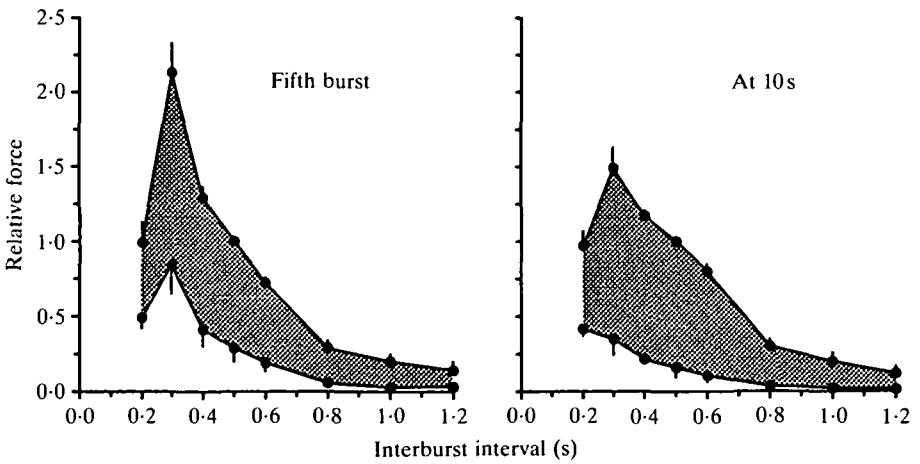

Fig. 6. Maximum and minimum tension during stimulation with bursts of stimuli at different interburst intervals. The basic responses were similar to that of Fig. 5. The muscle was stimulated with bursts, each containing 10 stimuli, with the interstimulus interval adjusted so that the total stimulation period was 20 % of the length of the burst cycle. The upper set of filled circles in each graph is the force at the tension peak, either for the fifth burst (left) or the burst closest to 10 s of stimulation (right). The lower set of circles is the force at maximum relaxation. The filled portion of the plots, therefore, is the force range during the burst cycle. All forces are expressed relative to the maximum force at a burst frequency of 2 s^{-1}. Vertical bars, shown in one direction only, are S.E. ($N = 8$ preparations).

frequency is slightly less than the maximum repetition frequency of the SG muscles *in vivo* (about 5 Hz, Hughes *et al.* 1969; Mercier & Wilkens, 1984*a*). At the optimum IBI of 0·3 s, and with 10 stimuli per burst and a burst duration equal to 20 % of the burst cycle length, the ISI within the burst was 6·7 ms. The optimum ISI for the stimulus protocol in this experiment is thus the same as that for long stimulation bursts and short bursts at a constant IBI (Figs 2, 3). Relaxation between bursts becomes progressively less complete as the interburst interval is reduced. However, the increase in maximum tension with decreasing IBI in the range 1·2–0·3 s is greater than the increase in the minimum tension between stimulation bursts, so the tension change per cycle which is available to drive the SG blade in its excursion increases with increasing maximum tension (Fig. 6).

A striking aspect of the responses to short, repetitive bursts of stimuli, especially at short interburst intervals, was facilitation, both of muscle contraction and of relaxation (Fig. 5). Early in a set of stimulus bursts the tension reached during the contraction peaks became progressively greater. At short interburst intervals there was summation so the tension minima between peaks initially increased, but later there was progressively greater relaxation. The tension minima became lower even while the tension peaks were still increasing. A consequence of the facilitation of contraction giving increased tension peaks and facilitation of relaxation giving deeper tension troughs was that the tension excursion on each cycle became progressively greater during the train. Although the mechanisms of facilitation of contraction and relaxation are unknown, their consequence, greater tension change per cycle, is certainly of functional significance, especially for a muscle like L2B which is normally repetitively active.

Mechanical properties of tetanically stimulated muscle

Muscle length and tetanic tension

The effects of muscle length on its maximum isometric tension were examined in muscles tetanically stimulated with a 2-s burst of shocks at 150 Hz, a frequency which gives a maximum tetanic response (Fig. 2). Measurements of isometric tension with the muscle at 110 % of reference length, which is about the optimum length for tension generation, were made between each test at a different muscle length. The systematic interposition of measurements at 110 % of reference length allowed us to monitor the general condition of the preparation and to discontinue measurements when there was obvious decline in the performance of the muscle. A 2-min rest period was allowed between each length change and stimulation of the muscle. The muscle lengths tested began at 80–90 % of the reference length and continued, in 5 % steps, up to 150–180 % of reference length. Stretching the muscle to the longer test lengths generated considerable resting tension. The tension declined following the stretch, at first rapidly and then more slowly, and reached an approximate steady state by the end of the 2-min rest period. With each trial the passive force measured just before stimulation was subtracted from the peak force during stimulation to

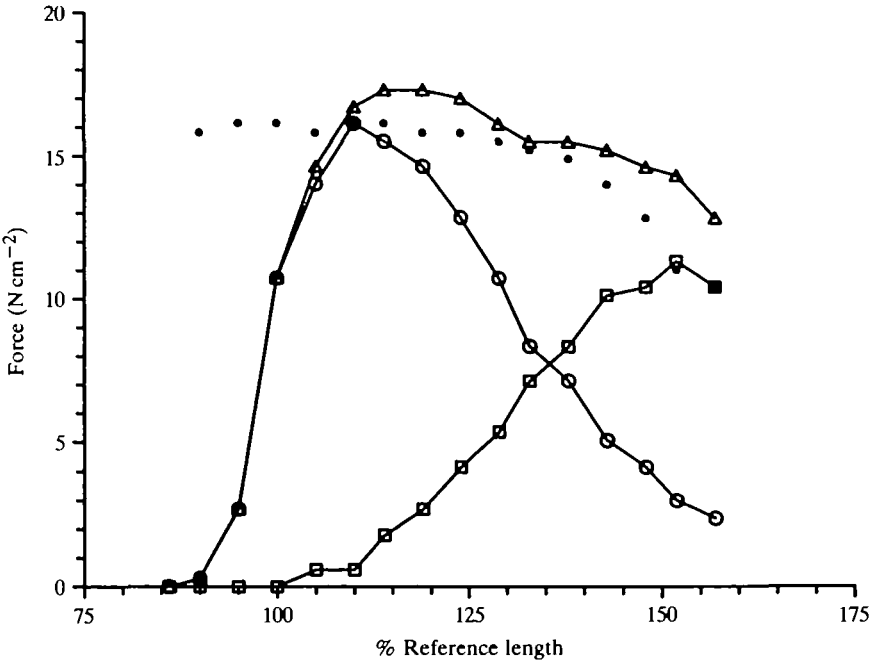

Fig. 7. Muscle length and maximum isometric tension. Passive force (□) is the force immediately before stimulation; peak force (△) is the maximum force during stimulation (150 Hz for 2 s); and the active increment (○) is the difference between the peak force and the passive force. Trials were given at a series of increasing muscle lengths. The muscle was returned to 110 % of reference length between each trial at a different length and tetanically stimulated. The small, filled circles give the active force increment for each of these control trials at 110 % of reference length, plotted at the muscle length of the following trial. Note that the control tension remained relatively constant until the muscle was stretched to more than about 140 % of reference length.

obtain the active tension increment. Length–tension curves were obtained from six muscles.

The length–tension curves for the SG muscle were sharply peaked (Fig. 7). Tension dropped rapidly on either side of an optimum length, especially on the shortening side. Active tension was zero or nearly zero at lengths less than 90 % of reference length. In five of the six muscles, the peak tension came at 110–120 % of reference length; in the sixth muscle at 130 %. We suspect that the 130 % value may be an error, perhaps an overestimate of optimum length because of undetected stretch of the skeletal attachments while mounting the muscle. In the six preparations the maximum isometric tension at optimum length averaged 13·2 N cm^{-2} (S.E. = 2·6 N cm^{-2}). During investigations of the force–velocity relationships of SG muscles (see below), the optimum muscle lengths for force production during tetanic stimulation were briefly determined for a number of muscles. These measurements were made without returning the muscle to a constant reference length between each test length. The maximum tetanic tension at optimum length from this second series of muscles was 11·3 N cm^{-2} (S.E. = 1·2 N cm^{-2}, $N = 7$).

Muscle force and shortening velocity

The force–velocity relationship of the SG muscle was determined with an ergometer set up so that the muscle contracted isometrically until the tension reached a pre-set level, usually 60–80 % of the maximum tetanic tension, at which time the muscle was released to an isotonic load (Fig. 8). If the isotonic load was larger than the pre-set release level, the muscle continued contracting isometrically beyond the release level until its tension reached the isotonic load. Muscle force and muscle length were displayed on an oscilloscope screen and photographed. Shortening velocity was determined from the slope of the length trace as a function of time. The slope was measured by drawing a straight-line tangent to the length trace and measuring the angle of this line with respect to the time axis. With light isotonic loads there were damped, oscillatory transients in the length trace immediately after the rapid shortening at the transition from isometric to isotonic contraction. The shortening velocity was measured after a delay of 10 ms from the onset of isotonic contraction, by which time the transient responses had subsided.

Each experiment began with a brief series of isometric, tetanic responses at different muscle lengths, given in order to find the muscle length at which tetanic tension was greatest. In these trials the stimuli were 2-s trains at 150 Hz. Individual trials were separated by 2 min. During the force–velocity measurements the resting muscle length was set at, or slightly longer than, that length giving maximum tension. During the force–velocity trials the muscle was again stimulated at 150 Hz.

Several sets of force–velocity measurements were made with each muscle. Each set began with an isometric measurement, followed by 2–4 isotonic measurements at decreasing isotonic load. Preparations were discarded in which the isometric tension in the successive determinations dropped by more than 20 % during the course of the experiment. The individual trials of a set were separated by 2 min. The maximum shortening velocity was determined by fitting a hyperbola (Hill, 1938) to the force–velocity points, excluding those points taken at forces greater than 80 % of the

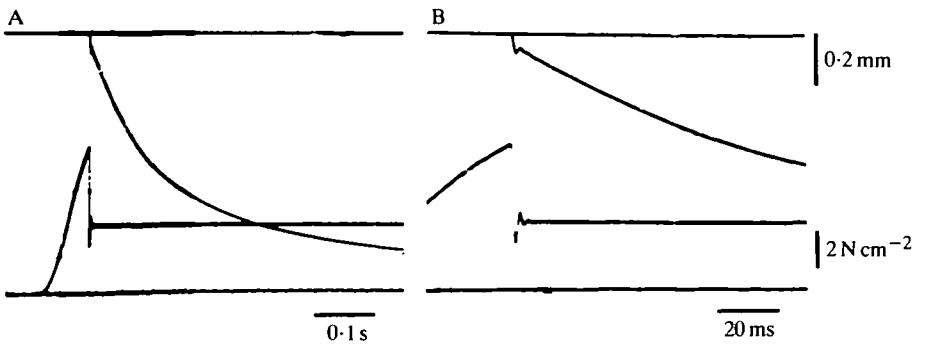

Fig. 8. Muscle shortening following quick release from an isometric to an isotonic contraction during tetanic stimulation. Increased force is an upward deflection (lower traces), muscle shortening is a downward deflection (upper traces). A sweep immediately before stimulation gives a baseline for the force and length records. B is identical to A but using a faster time base.

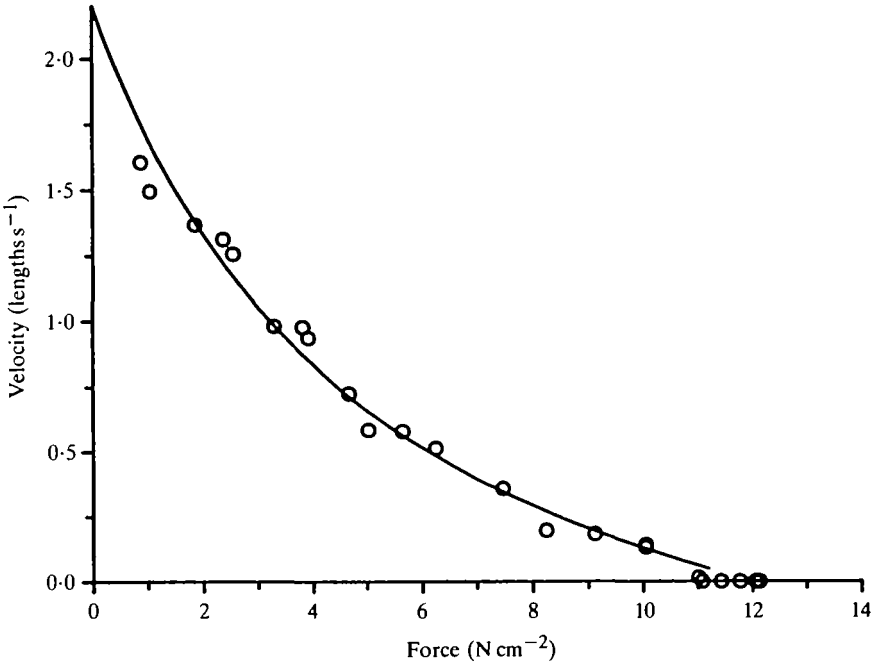

Fig. 9. Shortening velocity as a function of muscle load. The solid line is a Hill curve fitted to all data points at forces less than 80% of the average isometric tension.

maximum isometric tension (Edman, Mulieri & Scubon-Mulieri, 1976). Maximum shortening velocity (V_m) was determined from the intercept of the curve with the zero force axis. In several preparations there was some decline in the maximum tetanic tension during the several isometric trials scattered through the series. To compensate partially for changes in isometric tension, some of the force–velocity curves were plotted using normalized force as the abscissa. Specifically, force was expressed as the ratio of the isotonic force during a trial to the maximum force during the nearest isometric trial. The force–velocity data points were fitted quite well by a Hill curve (Fig. 9). The maximum shortening velocity (V_m) determined from the Hill curves averaged 1·88 L s^{-1} (S.E. = 0·06 L s^{-1}, $N = 7$). The values of a/P_0 for the Hill curves, which are a measure of curvature, averaged 0·41 (S.E. = 0·06).

The rate of muscle shortening following release to an isotonic load falls progressively with time after release (Fig. 8). A similar decline in the rate of shortening was also seen in afterloaded isotonic contractions, especially at low loads (in afterloaded contractions a muscle contracts isometrically until the force developed equals a pre-set load, after which contraction is isotonic shortening). The decline in shortening velocity is probably due in part to the muscle moving down the steep ascending limb of the length–tension curve as it shortens; the more the muscle shortens below the optimum length the lower is its capacity for generating force. Muscle deactivation upon shortening (see, for example, Edman, 1975) may also contribute to the declining rate of shortening during isotonic contractions.

Maximum shortening velocity, slack test

The maximum shortening velocity of the muscle was also determined using the slack test (Edman, 1979). In the slack test an isometrically contracting muscle is quickly released to a shorter length. The release distance is great enough for muscle tension to fall to zero (Fig. 10). The muscle then contracts at the new length. Until the slack is taken up and tension begins to reappear, the contraction at the new length is at zero load. A plot of release distance for several different distances against the delay between release and the onset of redeveloped tension has a slope which is the shortening velocity under zero load (Fig. 11). The shortening velocity determined with the slack test is usually designated V_0. In our slack test measurements the muscle length before release was at, or slightly longer than, that length giving maximum isometric tension. The average V_0 for the SG muscle was $3{\cdot}3\,L\,s^{-1}$ (S.E. $= 0{\cdot}4\,L\,s^{-1}$, $N = 9$). Six muscles from which satisfactory force–velocity curves were obtained were also used in slack test determinations. In these muscles the ratio V_m/V_0 averaged 2·03 (S.E. = 0·1). Values of maximum shortening velocity from the slack test are usually greater than those determined from the fit to a Hill curve (Edman, 1979; Josephson, 1984; Claflin & Faulkner, 1985) but the difference between V_m and V_0 for the SG muscle is unusually large.

DISCUSSION

Activation pattern and isometric contraction

Contraction of SG muscle L2B is strongly dependent on the pattern and number of activating impulses in motor neurones to the muscle. Single stimuli and multiple stimuli at low frequency, 30 Hz or less, evoke little contraction (Fig. 2). The

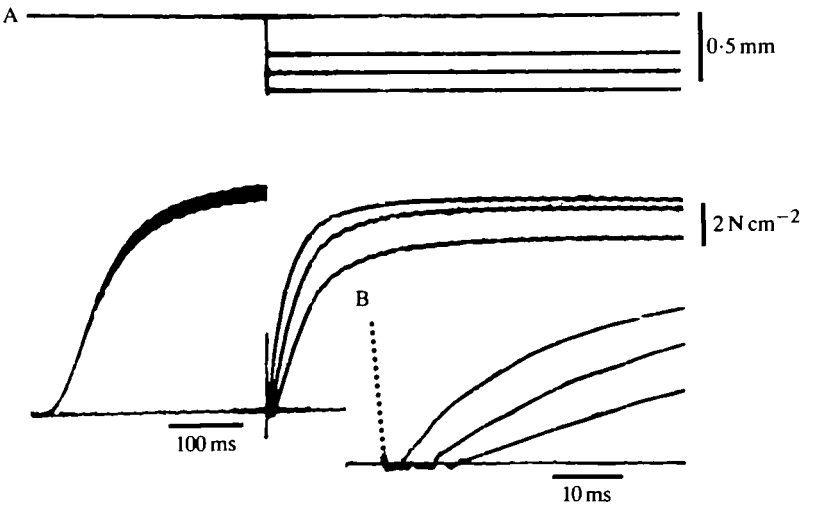

Fig. 10. Tension re-development following quick release. The upper set of traces in A is muscle position (shortening downwards), the lower set is force. (B) A segment of the force records, that immediately following release, using an expanded time base.

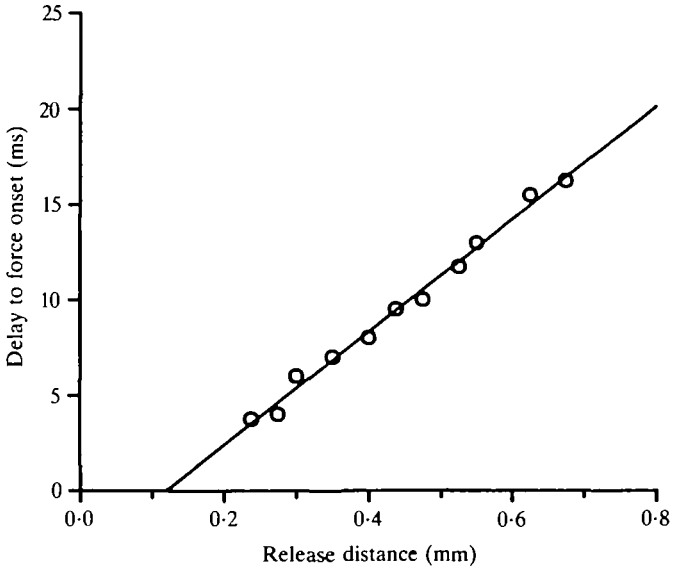

Fig. 11. The time taken until the onset of tension re-development as a function of release distance.

contractile response to either short or long bursts of stimuli increases with stimulus frequency to at least 150 Hz, beyond which the isometric tension declines with further increases in frequency (Figs 2, 3, 6).

Why the isometric tension should decline at high stimulus frequencies is not obvious. Were there both inhibitory and excitatory innervation to the muscle, as is often the case with arthropod muscles, the decline in response might be explained as due to a balance between inhibition and excitation, with inhibitory inputs being relatively more important than excitatory inputs at high stimulus frequencies and relatively less important at low frequencies. Available evidence, however, indicates that the SG muscles do not have inhibitory innervation (see, for example, Moody-Corbett & Pasztor, 1980). Reduced tension at high stimulation frequencies must have another explanation. It seems likely that the decline in response at high frequency is due to failure of the motor axons to follow faithfully the stimulating shocks. At very high frequencies, individual axons might be partially refractory and skip stimuli in a train, so the actual input frequency in a motor neurone would be less than the stimulation frequency. Or there may be conduction failure at axonal branch points during high-frequency activation, as has been observed in crayfish motor axons (Smith, 1980). Conduction failure at axonal branchings would reduce the volume of muscle reached and excited by an action potential. If declining response at high frequency is due either to motor neurone refractoriness to axonal stimulation or to conduction failure along the axons, the high-frequency decline need not be of functional significance to the animal. Conceivably the refractory period for normal inputs, determined by properties of the motor neurones at their synaptic regions within the central nervous system, could be longer than the refractory period in the axon, and the safety factor for axonal conduction at normal operating frequencies

could be sufficiently great for axonal properties not to be rate-limiting. The maximum firing rate recorded from the motor nerve serving muscle L2B is about 140 Hz (Mercier & Wilkens, 1984*b*). This rate may include spikes from several motor neurones in the nerve, and thus be greater than the maximum rate in any single motor neurone. It is likely that during normal operation the maximum firing rate of individual motor neurones to muscle L2B is well below 150 Hz and, therefore, that the motor units normally operate in a range in which tension increases monotonically with firing frequency.

During normal beating the SG muscles are activated by short bursts of impulses along their motor neurones. The responses of the muscle to short bursts can be expected to depend on the number of impulses forming the bursts, the interstimulus interval within the bursts (Fig. 3) and, because of facilitation between bursts, on the interval between adjacent bursts (Fig. 4). Facilitation between bursts was detectable for at least 10–12 s. Even longer facilitatory processes are seen in some crustacean muscles in which minutes of stimulation may be followed by heightened responsiveness lasting for many minutes (see, for examples, Sherman & Atwood, 1971; Jacobs & Atwood, 1981). We did not attempt to demonstrate long-term facilitation in muscle L2B.

There was often facilitation of relaxation during repetitive bursts, manifested by more rapid relaxation and relaxation to a lower tension level during successive interburst intervals (Figs 3, 5). Facilitation of relaxation has been described previously (Bullock, 1943), but it seems not to be a widely recognized phenomenon. The mechanisms leading to facilitation of relaxation are unknown. Several possibilities suggest themselves. It may be that successive junctional potentials become shorter, perhaps because of better synchronized transmitter release or because of increasing effectiveness of transmitter inactivation systems. Shorter periods of depolarization should lead directly to more rapid relaxation. Alternatively, and less interesting, the increased rate of relaxation might be a consequence of there being a heterogeneous population of muscle fibres which differ both in contraction time course and rate of fatigue. If slower fibres were the first to become fatigued during repetitive activation, successive responses could become dominated by more rapid fibres with quicker relaxation. It should be noted that there is no evidence for motor unit heterogeneity on the basis of the time course of responses. Indeed, the incremental tension responses which drop out during motor axon de-recruitment (Fig. 1) all seem to have similar time courses. But this does not rule out the possibility that the motor units, while similar among themselves, are individually heterogeneous with respect to the types of muscle fibres of which they are composed. Yet another possibility, and a considerably more interesting one, is that there is facilitation of the excitation–contraction coupling system such that calcium uptake by the sarcoplasmic reticulum becomes progressively enhanced, leading to increasingly rapid relaxation. Clarification of the mechanisms involved in the facilitation of relaxation might well uncover phenomena of basic importance for understanding the control of muscle contraction.

One of the stimulation paradigms used was to stimulate in bursts at varied interburst intervals, with the stimulus period occupying a constant fraction of the burst cycle. This pattern was chosen because it approximates the normal activation pattern of the muscle (Young, 1975; Mercier & Wilkens, 1984*a*,*b*). It should be noted, however, that it would be extremely difficult, if possible at all, to mimic accurately the normal activity inflow to muscle L2B using whole nerve stimulation. Nerve stimulation activates all elements in the nerve synchronously, and forces the same firing pattern on all axons for which the stimuli are suprathreshold. It is unlikely that axons in the nerve normally fire synchronously, even those which are active during the same portions of the SG cycle. In addition, nerve stimulation co-activates motor neurones which are not normally active in the same cycle. During normal activity, forward beating of the SG, which moves water forward across the gills and out near the antennules, is interrupted periodically by sequences of reversed beating. Different motor axons to muscle L2B participate in forward and in reversed beating (Young, 1975; Simmers & Bush, 1983*b*; Mercier & Wilkens, 1984*b*). With nerve stimulation, forward-beating motor neurones are activated synchronously with backward-beating motor neurones, a pattern which may never occur normally. Although the muscle activation patterns achieved with nerve stimulation may not precisely mirror those in an intact animal, there is no reason to believe that the muscle responses evoked are dissimilar in any major way from those that occur *in vivo*.

Length–tension relationships

SG muscle L2B is able to operate over only a rather narrow length range. In the length–tension curve of Fig. 7, the active force increment is 50% or more of its maximum value from 98% of reference length to 133% of reference length; i.e. over a length span of only 35% of the reference length. Among the six preparations from which length–tension curves were obtained, the equivalent length span over which the force was 50% or more of its maximum value was from 18% of the reference length in the narrowest curve to 42% of the reference length in the broadest curve (average = 35%, S.D. = 9%). The length–tension curve for muscle L2B is conspicuously narrower than length–tension curves published for other crustacean muscles (Zachar & Zacharova, 1966; April & Brandt, 1973; Eastwood, Wood & Reuben, 1978; Chapple, 1983). The rate of decline in maximum isometric tension as the muscle is shortened is particularly dramatic, especially when compared with frog limb muscles which, because of the many studies done with them, have become the benchmarks for muscle mechanics (Fig. 12). Several factors are thought to contribute to declining tension at shorter muscle lengths, including: collision of thin filaments in the middle of the sarcomere; interaction of thin filaments with inappropriately polarized portions of the thick filaments across the central bare zone of the thick filaments; and collision of thick filaments with the Z-lines (Gordon, Huxley & Julian, 1966). The rapid decline in isometric tension with decreasing length of the SG muscle suggests that there is steric hindrance involving thin filaments in the middle of the sarcomere, or collision of thick filaments with the Z-

line, after only a little shortening from what has been defined as the reference length. In normal operation, each sweep of the SG moves the appendage between upper and lower limits set by the walls of the chamber in which it moves. The SG system is a constant-amplitude pump. One assumes that the mechanics of the SG articulation are such that muscle L2B shortens little during each beat cycle, and that the muscle length normally remains in a region for which the capacity for tension development is high.

Shortening velocity

The SG muscle would be termed a slow or tonic muscle on the basis of the neural control of contraction. The twitch–tetanic tension ratio is vanishingly small and many stimuli are needed to achieve full tetanic tension. Presumably, because many stimuli are needed to activate the muscle fully, the tension rise during tetanic stimulation is moderately slow. The slow features of muscle activation, however, are not paralleled by slow contraction dynamics in the fully activated muscle.

The maximum shortening velocity of muscle L2B at 15°C was determined as $1{\cdot}9\,L\,s^{-1}$ from the velocity axis intercept of a Hill curve, and $3{\cdot}3\,L\,s^{-1}$ from slack test measurements. These values are not especially low when compared with other muscles. The maximum shortening velocity of muscle L2B was higher than that found for a hermit crab abdominal muscle ($0{\cdot}8\,L\,s^{-1}$) measured at a slightly lower temperature (12·5°C, Chapple, 1983). Woledge, Curtin & Homsher (1985) tabulated

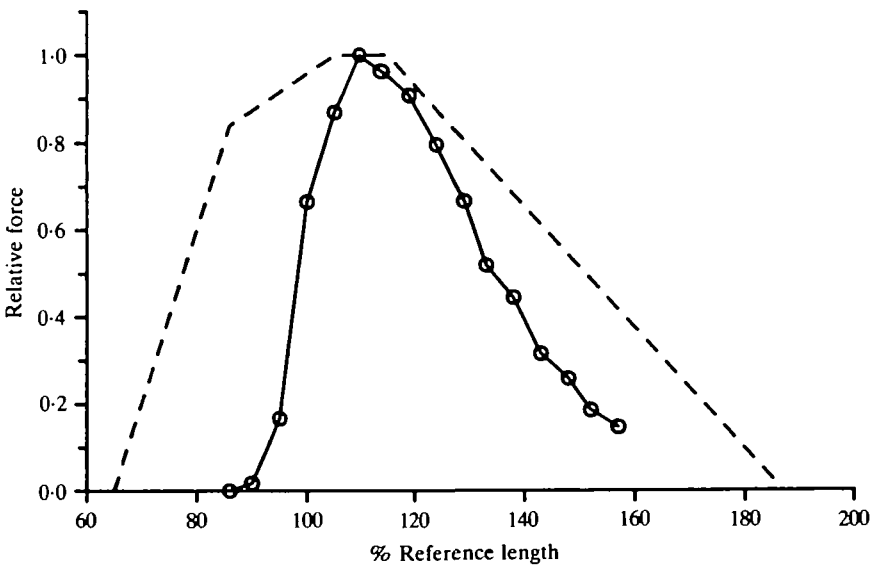

Fig. 12. Length–tension relationships for crab and frog muscle. The open circles and joining line are the active force increment from Fig. 7. The dotted line is based on the length–tension relationship for frog muscle fibres according to Gordon, Huxley & Julian (1966). The reference length for the frog values was taken as a sarcomere length of 1·95 μm. This value was chosen so that the middle of the force plateau for the frog fibre would coincide with the peak in the crab curve.

force–velocity parameters for a number of vertebrate muscles (their table 2.II). This set of data is not readily comparable with that from the SG muscle because the vertebrate values were generally collected at different temperatures from those of the crab experiments. Table 2.III of Woledge *et al.* (1985) gives temperature coefficients for a number of contractile properties of vertebrate muscles. The average Q_{10} for maximum shortening velocity in this table is 1·98 (mean of 14 table entries, range = 1·53–2·83). We used this value to estimate the maximum shortening velocity for the muscles of table 2.II of Woledge *et al.* (1985) at 15 °C. The average shortening velocity for all amphibian limb muscles or muscle fibres, excluding non-twitch slow fibres, corrected to 15 °C, was $5\,L\,s^{-1}$ (range = 3·0–7·1 $L\,s^{-1}$, 19 table entries). The average shortening velocity of mammalian muscle fibres in the table was $3{\cdot}3\,L\,s^{-1}$ (range = 1·1–6·1 $L\,s^{-1}$, 18 entries). Thus the maximum shortening velocity for the crab SG muscle is somewhat slower than that expected for amphibian muscle fibres, and within the range of mammalian muscle fibres. In addition, the maximum shortening velocity, based on Hill curves, for the SG muscle is faster than that of a rat soleus muscle, $0{\cdot}94\,L\,s^{-1}$ at 15 °C, based on a measured value of $1{\cdot}58\,L\,s^{-1}$ at 20 °C and a Q_{10} of 2·83 for the range 20–25 °C (Ranatunga, 1982), and slower than the rat extensor digitorum longus, $2{\cdot}5\,L\,s^{-1}$ at 15 °C, based on $3{\cdot}4\,L\,s^{-1}$ at 20 °C and a Q_{10} of 1·84 for the range 20–25 °C (Ranatunga, 1982). Although the SG muscle would be characterized as a slow or tonic muscle on the basis of the neural control of its contraction, it would not be described as a slow muscle on the basis of its shortening velocity.

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REFERENCES

APRIL, E. W. & BRANDT, P. W. (1973). The myofilament lattice: studies on isolated fibers. III. The effect of myofilament spacing upon tension. *J. gen. Physiol.* **61**, 490–508.

ATWOOD, H. L. (1973). An attempt to account for the diversity of crustacean muscles. *Am. Zool.* **13**, 357–378.

BERLIND, A. (1977). Neurohumoral and reflex control of scaphognathite beating in the crab *Carcinus maenas*. *J. comp. Physiol.* **116**, 77–90.

BULLOCK, T. H. (1943). Neuromuscular facilitation in scyphomedusae. *J. cell. comp. Physiol.* **22**, 251–272.

CHAPPLE, W. D. (1983). Mechanical responses of a crustacean slow muscle. *J. exp. Biol.* **107**, 367–383.

CLAFLIN, D. R. & FAULKNER, J. A. (1985). Shortening velocity extrapolated to zero load and unloaded shortening velocity of whole rat skeletal muscle. *J. Physiol., Lond.* **359**, 357–363.

CUMBERLIDGE, N. & UGLOW, R. F. (1977*a*). Heart and scaphognathite activity in the shore crab *Carcinus maenas* (L.). *J. exp. mar. Biol. Ecol.* **28**, 87–107.

CUMBERLIDGE, N. & UGLOW, R. F. (1977*b*). Size, temperature, and scaphognathite frequency-dependent variations of ventilation volumes in *Carcinus maenas* (L.). *J. exp. mar. Biol. Ecol.* **30**, 85–93.

DICAPRIO, R. A. & FOURTNER, C. R. (1984). Neural control of ventilation in the shore crab, *Carcinus maenas*. I. Scaphognathite motor neurons and their effect on the ventilatory rhythm. *J. comp. Physiol.* A **155**, 397–405.

EASTWOOD, A. B., WOOD, D. S. & REUBEN, J. P. (1978). Unusual thin and thick filament packing in a crustacean muscle. *J. Cell Biol.* **77**, 48–58.

EDMAN, K. A. P. (1975). Mechanical deactivation induced by active shortening in isolated muscle fibres of the frog. *J. Physiol., Lond.* **246**, 255–275.

EDMAN, K. A. P. (1979). The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres. *J. Physiol., Lond.* **291**, 143–159.

EDMAN, K. A. P., MULIERI, L. A. & SCUBON-MULIERI, B. (1976). Non-hyperbolic force–velocity relationship in single muscle fibres. *Acta physiol. scand.* **98**, 143–156.

GORDON, A. M., HUXLEY, A. F. & JULIAN, F. J. (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J. Physiol., Lond.* **184**, 170–192.

GOVIND, C. K. & ATWOOD, H. L. (1982). Organization of neuromuscular systems. In *The Biology of Crustacea*, vol. 3 (ed. H. L. Atwood & D. C. Sandeman), pp. 63–103. New York, London: Academic Press.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Ser.* B **126**, 136–195.

HOYLE, G. (1983). *Muscles and Their Neural Control.* New York: John Wiley & Sons.

HUGHES, G. M., KNIGHTS, B. & SCAMMELL, C. A. (1969). The distribution of P_{O_2}, and the hydrostatic pressure changes within the branchial chambers in relation to gill ventilation of the shore crab *Carcinus maenas* L. *J. exp. Biol.* **51**, 203–220.

JACOBS, J. R. & ATWOOD, H. L. (1981). Long term facilitation of tension in crustacean muscles and its modulation by temperature, activity, and circulating amines. *J. comp. Physiol.* **144**, 335–343.

JOSEPHSON, R. K. (1984). Contraction dynamics of flight and stridulatory muscles of tettigoniid insects. *J. exp. Biol.* **108**, 77–96.

MENDELSON, M. (1971). Oscillator neurons in crustacean ganglia. *Science* **171**, 1170–1173.

MERCIER, A. J. & WILKENS, J. L. (1984*a*). Analysis of the scaphognathite ventilatory pump in the shore crab *Carcinus maenas*. I. Work and power. *J. exp. Biol.* **113**, 55–68.

MERCIER, A. J. & WILKENS, J. L. (1984*b*). Analysis of the scaphognathite ventilatory pump in the shore crab *Carcinus maenas*. III. Neuromuscular mechanisms. *J. exp. Biol.* **113**, 83–99.

MERCIER, A. J. & WILKENS, J. L. (1985). Modulatory effects of proctolin on a crab ventilatory muscle. *J. Neurobiol.* **16**, 401–408.

MILLER, T. A. (1979). *Insect Neurophysiological Techniques.* New York: Springer-Verlag.

MOODY-CORBETT, F. & PASZTOR, V. M. (1980). Innervation, synaptic physiology, and ultrastructure of three muscles of the second maxilla in crayfish. *J. Neurobiol.* **11**, 21–30,

PASZTOR, V. M. (1968). The neurophysiology and respiration in decapod crustacea. I. The motor system. *Can. J. Zool.* **46**, 585–596.

PILKINGTON, J. B. & MACFARLANE, D. W. (1978). Numbers and central projections of crab second maxilla motor neurones. *J. mar. Biol. Ass. U.K.* **58**, 571–584.

PILKINGTON, J. B. & SIMMERS, A. J. (1973). An analysis of bailer movements responsible for gill ventilation in the crab, *Cancer novae-zelandiae*. *Mar. Behav. Physiol.* **2**, 73–95.

RANATUNGA, K. W. (1982). Temperature-dependence of shortening velocity and rate of isometric tension development in rat skeletal muscle. *J. Physiol., Lond.* **329**, 465–483.

SHERMAN, R. G. & ATWOOD, H. L. (1971). Synaptic facilitation: long-term neuromuscular facilitation in crustaceans. *Science* **171**, 1248–1250.

SIMMERS, A. J. & BUSH, B. M. H. (1980). Non-spiking neurones controlling ventilation in crabs. *Brain Res.* **197**, 247–252.

SIMMERS, A. J. & BUSH, B. M. H. (1983*a*). Central nervous mechanisms controlling rhythmic burst generation in the ventilatory motoneurones of *Carcinus maenas*. *J. comp. Physiol.* **150**, 1–21.

SIMMERS, A. J. & BUSH, B. M. H. (1983*b*). Motor programme switching in the ventilatory system of *Carcinus maenas*: the neuronal basis of bimodal scaphognathite beating. *J. exp. Biol.* **104**, 163–181.

SMITH, D. O. (1980). Mechanisms of action potential propagation failure at sites of axon branching in the crayfish. *J. Physiol., Lond.* **301**, 243–259.

WILKENS, J. L. (1976). Neuronal control of respiration in decapod crustacea. *Fedn Proc. Fedn Am. Socs exp. Biol.* **35**, 2000–2006.

WILKENS, J. L., MERCIER, A. J. & EVANS, J. (1985). Cardiac and ventilatory responses to stress and to neurohormonal modulators by the shore crab, *Carcinus maenas*. *Comp. Biochem. Physiol.* **82**C, 337–343.

WILKENS, J. L., WILKES, P. R. H. & EVANS, J. (1984). Analysis of the scaphognathite ventilatory pump in the shore crab *Carcinus maenas*. II. Pumping efficiency and metabolic cost. *J. exp. Biol.* **113**, 69–81.

WILKENS, J. L. & YOUNG, R. E. (1975). Patterns and bilateral coordination of scaphognathite rhythms in the lobster *Homarus americanus*. *J. exp. Biol.* **63**, 219–235.

WOLEDGE, R. C., CURTIN, N. A. & HOMSHER, E. (1985). *Energetic Aspects of Muscle Contraction*. London: Academic Press.

YOUNG, R. E. (1975). Neuromuscular control of ventilation in the crab *Carcinus maenas*. *J. comp. Physiol.* **101**, 1–37.

YOUNG, R. E. & COYER, P. E. (1979). Phase co-ordination in the cardiac and ventilatory rhythms of the lobster *Homarus americanus*. *J. exp. Biol.* **82**, 53–74.

ZACHAR, J. & ZACHAROVA, D. (1966). The length–tension diagram of single muscle fibers of the crayfish. *Experientia* **22**, 451–452.