CONTRACTILE PROPERTIES OF OBLIQUELY STRIATED MUSCLE FROM THE MANTLE OF SQUID (*ALLOTEUTHIS SUBULATA*) AND CUTTLEFISH (*SEPIA OFFICINALIS*)

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Summary

The mechanical properties of obliquely striated muscle fibres were investigated using thin slices of mantle from squid *Alloteuthis subulata* and cuttlefish *Sepia officinalis*. Brief tetani or twitch stimuli were used as this pattern is likely to occur during jetting of the intact animal. The length–active force relationship for twitches and tetani (0.1 s, 50 Hz) was similar to that of vertebrate cross-striated fibres. Passive force at the length giving maximum tetanic force was $0.13\pm0.05P_0$ (mean ± S.E.M., N=6, where P_0 is maximum isometric tetanus force) and increased steeply at longer lengths. Peak force in a brief isometric tetanus (0.2 s, 100–150 Hz) was $262\pm16\,\text{mN}\,\text{mm}^{-2}$ cross-sectional area of wet tissue (N=6) for squid, and $226\pm19\,\text{mN}\,\text{mm}^{-2}$ (N=7) for cuttlefish. The force–velocity relationship for isotonic shortening during twitches of squid mantle slices was a 'double hyperbolic' relationship as described for cross-striated fibres by Edman. Fitting Edman's equation to the results gave: P^*=1.18±0.07, $V_{\max}$=2.43±0.11$L_{\text{tw}}\,\text{s}^{-1}$ and $1/G$=0.69±0.13 (N=8), where P^* is the intercept on the force axis expressed relative to P_{tw}, peak isometric twitch force, $V_{\max}$ is the intercept on the velocity axis, L_{tw} is the length at which P_{tw} is produced and G is the constant expressing curvature. The large values of $1/G$ indicate that the force–velocity relationship is not very curved. Maximum power was produced during shortening at $0.45\pm0.03P_{\text{tw}}$ (N=8). Maximum power during twitch contraction was $18.3\pm1.7\,\text{mW}\,\text{g}^{-1}$ wet mass or, expressed in relative units, $(V/V_{\max})(P/P_{\text{tw}})$, where V is the velocity during shortening and P is the force during shortening, was 0.16 ± 0.01 (N=8), which is higher than that of many cross-striated locomotor muscles.

Key words: muscle contraction, power, obliquely striated muscle, mantle muscle, cephalopod muscle, squid, cuttlefish, *Alloteuthis subulata*, *Sepia officinalis*, force–velocity relationship.

Introduction

The impressive jet propulsion of cephalopod molluscs is powered by contraction of mantle muscle fibres. Within the mantle, muscle fibres are arranged in two planes, as illustrated in Fig. 1A. Contraction of radial fibres thins the mantle wall, so increasing mantle diameter and drawing water into the mantle cavity, whilst contraction of circular fibres expels the locomotor jet *via* the exhalant siphon.

The mantle of squid and cuttlefish contains two main types of circular fibre, distinguished by mitochondrial content, blood supply and diameter (Bone *et al.* 1981). Mitochondria-rich fibres form thin inner and outer layers, whilst a larger central zone consists of smaller mitochondria-poor fibres (Fig. 1A). This distinct zonation of fibre types is reminiscent of the organisation of fast and slow fibres within the fish myotome (see, for example, Bone *et al.* 1986) and offers the possibility of examining the physiology of the two fibre types separately.

In cuttlefish, at least, the two types of circular fibre have different functional roles (Bone *et al.* 1994*a*).

All mantle fibres are obliquely striated and consist of a central mitochondrial core surrounded by a spiral array of myofilaments (see review by Bone *et al.* 1994*b*). Contraction involves a change in obliquity from approximately 6–12 ° at resting length to 14–18 ° in contracted fibres (Hanson and Lowy, 1957). These fibres are therefore rather different in design from cross-striated muscle fibres, and our aim in this study was to determine whether these differences are reflected by differences in contractile behaviour.

Several previous studies have described the force responses of cephalopod intact half-mantle preparations to stimulation of the stellar nerves (e.g. Prosser and Young, 1937; Wilson, 1960). The results from such studies are difficult to interpret since it is unclear which muscle fibre types are innervated by

the various components of the stellar nerves (see Gilly *et al.* 1996).

The most similar experiments to those reported here were made long ago by Marceau (1904–5, 1906*a*,*b*) using electrical stimulation to evoke mechanical responses in small strips of circular muscle from squid and cuttlefish mantle. He found little difference between the mechanical responses of cephalopod obliquely striated muscle and the cross-striated fibres of the frog gastrocnemius. His results, remarkable for their time, were not, however, sufficient to obtain force–velocity relationships for cephalopod muscle. Only a few further mechanical studies have been made. Lowy and Millman (1962) have reported some mechanical properties of the funnel retractor muscles of cuttlefish and octopus. More recently, Usher (reported in Bone *et al.* 1994*b*) made preliminary observations on slices of mantle. We report here a more detailed study of the mechanical properties of the mitochondria-poor, central circular fibres from squid and cuttlefish.

Materials and methods

Squid *Alloteuthis subulata* (Lamarck) and cuttlefish *Sepia officinalis* (L.) were trawled off Plymouth, UK, and maintained at the Marine Biological Association laboratory under closed circulation conditions at 11 °C. Animals were killed by decapitation, and a piece of the ventral mantle wall was glued to a plastic stage using cyanoacrylate cement (gel form). The tissue was incubated in modified artificial sea water (modified ASW) containing (in mmol l^{-1}): NaCl, 450; MgCl$_2$,10; EGTA, 10; Hepes, 10; pH 7.8 for 15–20 min at 4 °C. Thin slices of tissue (0.1–0.2 mm) were then cut from the central zone of muscle fibres using a vibratome (Campden Instruments Ltd, UK). A Peltier cooling system maintained a constant temperature of 4±0.5 °C during slicing. Slices were transferred to standard artificial sea water (ASW) containing (in mmol l^{-1}) NaCl, 470; KCl, 10; CaCl$_2$, 10; MgCl$_2$, 50; glucose, 20; Hepes, 10; pH 7.8 and stored at 4 °C. Keeping the mantle and the slices cold prior to the experiments improved viability, as did rinsing the preparations occasionally with modified ASW during the experiments.

Rectangular preparations (see Fig. 1A,B) were dissected from the slices using an ophthalmic scalpel. The final dimensions of the tissue preparations used for the experiments were as follows: length 7.1–12.4 mm, approximate width 5 mm, and thickness 0.1–0.2 mm. T-shaped aluminium foil clips were used to attach one end of the preparation to a force transducer [either an AE801 element (SensoNor a.s., Horten, Norway) or an ST 01 force transducer (Devices)] and the other end to a servomotor arm (Series 300B dual-mode servo system, Cambridge Technology Inc., MA, USA). The servomotor controlled muscle movement during the experiments. The tissue was continuously superfused with a thin film of aerated standard ASW. Temperature was maintained at 11±0.5 °C. This temperature was the same as that of the aquarium tanks in which the animals were kept and was within the normal temperature
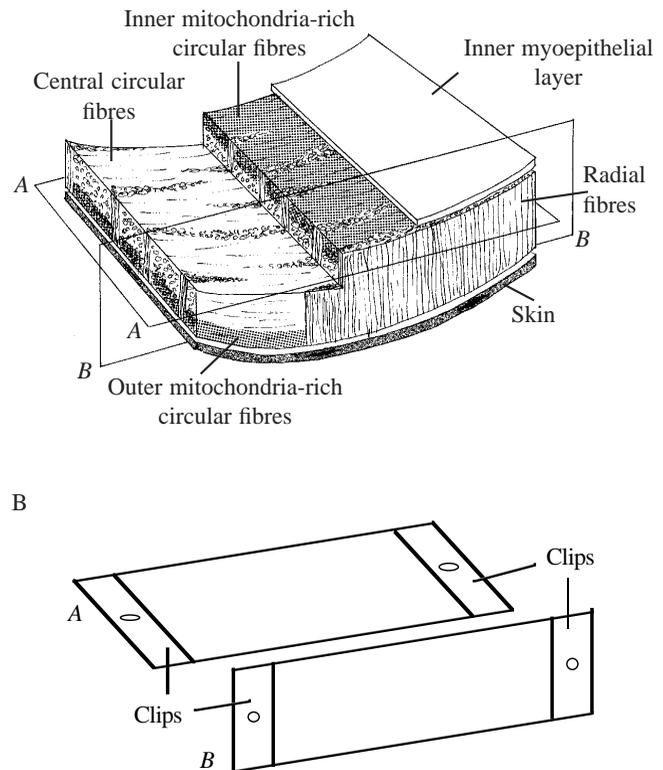

Fig. 1. (A). Diagram of a portion of the ventral mantle from the cuttlefish showing radial and circular muscle fibres; that of the squid is essentially identical. Central circular fibres lie between the outer and inner circular fibre layers. The majority of experiments were made on slices cut in plane *A*. Experiments on radial fibres were made on slices cut in plane *B*. (B) Diagrams showing the locations of the clips attached to preparations made from slices cut in plane *A* and plane *B*. In both cases, the long axis of the circular fibres is oriented from clip to clip.

range that the animals experience in the sea (8–18 °C). The preparation was stimulated using rectangular current pulses (model DS7 stimulator, Digitimer Ltd, UK) delivered *via* large platinum plate electrodes of sufficient size to cover the surfaces of the preparation. The stimulus was applied across the thickness of the slice, minimising the stimulus current required. Brief tetani or twitch stimuli were used as this pattern is likely to occur during jetting of the intact animal. Stimulation, motor position and force were controlled and recorded by a 'ViewDac' (Keithley, UK) sequence with a data-acquisition board (Lab Master D.M.A. Scientific Solutions Inc., USA).

Isometric contractions

Muscle length was adjusted such that a transient passive force was just apparent. The stimulus strength–twitch response relationship was then investigated (2 ms stimuli, 120 s between successive stimulations). Occasional control stimulations of constant current amplitude were given to monitor the degree of decline of twitch force as the trial progressed. At the end of a trial, the stimulus current was adjusted to a level 50 % higher than that required to elicit a maximal twitch response.

The length–force relationship of the preparation was

established for tetanic stimulation (50 Hz, 100 ms). At the end of a series, the length of the muscle slice was adjusted to that giving peak tetanic force (L_0).

The stimulus frequency–force relationship was determined using 200 ms tetani, at 5–200 Hz with 300 s intervals between successive tetani.

Force–velocity relationship

The force–velocity relationship was investigated using isotonic shortening during twitches of squid preparations. Supramaximal stimuli were first used to determine the optimum length (L_{tw}) for twitch force. Peak isometric twitch force is P_{tw}. Isotonic shortening was produced with the motor operating in its force-clamp mode. The velocity of shortening V was measured from the records of motor position (see Fig. 9). Occasional isometric control stimulations were applied to monitor the decline of force as the experiment progressed.

Curve fitting

Hill's equation (Hill, 1938) and Edman's equation (Edman, 1988; see below) were fitted to the force–velocity data for each muscle slice using the 'Solver' function of MS-Excel 5 to minimise the sum of the squares of the deviations of predicted velocity from the observed value.

The form of Hill's equation we used was:

$$V = V_{max}P*(P* - P)/(GP + 1), \quad (1)$$

where P is the force during shortening/P_{tw}, V is the velocity of shortening (units: $L_{tw}\,s^{-1}$), and the adjustable constants are V_{max}, $P*$ and G. V_{max} is the intercept on the velocity axis, $P*$ is the intercept on the force axis and G is the constant expressing curvature (=P_0/a in Hill's nomenclature, 1938, where P_0 is the peak tetanic force and a is a constant). All data points were included and given equal weighting, and the fit was not constrained to pass through P=1.0.

The form of Edman's equation we used was:

$$V = [V_{max}P*(P* - P)/(GP + 1)]\{1 - [1/(1 + e^{-k_1(P+k_2)})]\}, \quad (2)$$

where k_1 and k_2 are constants and the other elements are defined in Hill's equation. The values of $P*$, V_{max} and G were found by fitting Hill's equation to data for P in the range 0–0.78. The values of the remaining constants, k_1 and k_2, were found by fitting Edman's equation to the full set of data points (including P>0.78).

Preparation size

The length of the preparation at L_{tw} or L_0, as appropriate, was measured with an eyepiece micrometer under a dissecting microscope (approximate error 0.05 mm). The preparation was fixed (5 % glutaraldehyde in 75 % standard ASW, buffered with 0.5 mol l^{-1} sodium cacodylate), dried to constant mass at 70 °C, and weighed using a microbalance (approximate error 5 %). Specific force was expressed as mN mm^{-2} cross-sectional area of tissue, where area was calculated as volume/length. Volume was calculated from the dry mass of the tissue by assuming a density of 1.06 g cm^{-3} (Mendez and Keys, 1960)

and the wet mass:dry mass ratio determined for cuttlefish of 4.14±0.09 (mean ± S.E.M., N=10).

Most experiments were concerned with the contractile properties of the circular fibres and were performed using slices cut in plane *A* (see Fig. 1). The cross-sectional area calculated as described above includes the cross-sectional area of circular fibres, which is the quantity of interest, and also a contribution from cut fragments of radial fibres. The area occupied by radial fibre fragments and the total cross-sectional area were measured in sections of squid mantle fixed in glutaraldehyde (see above) and stained with Toluidine Blue. The thickness of the radial fibre layer measured at a number of locations in two different radial layers was 38.6±1.2 µm (mean ± S.E.M., N=14) and the distance from centre to centre of neighbouring radial layers measured in two sections was 136.8±2.3 µm (N=13). Thus, the radial fibres made up 28 % of the total cross-sectional area.

Results

Isometric twitches

The relationship between stimulus strength and twitch force is summarised in Fig. 2. Squid preparations were generally activated at lower stimulus strength than were cuttlefish preparations of similar thickness. The current required to elicit a supramaximal response was high (range 20–80 mA for all experiments). It is most likely that the muscle fibres in the slice preparations were stimulated directly, rather than *via* their nerve endings. Bone and Howarth (1980) presented evidence that the transmitter in the circular muscle fibres of squid and cuttlefish is L-glutamate, and that its action can be reversibly blocked by 2-amino-4-phosphonobutyric acid (2-APB). We confirmed that 2-APB at a concentration of 20 µmol l^{-1} (in ASW, buffered to pH 7.8) reliably inhibited glutamate contractures in our squid and cuttlefish preparations (N=5). In
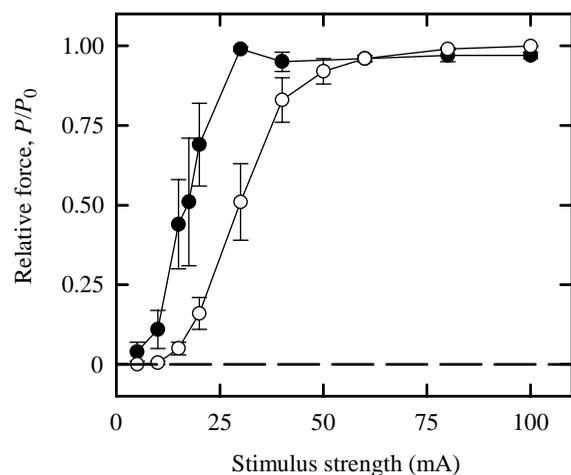

Fig. 2. Relationship between stimulus strength and twitch force. Force is expressed relative to the maximum twitch force produced by the preparation. Mean values ± S.E.M. (N=10) for squid (●) and for cuttlefish (○).
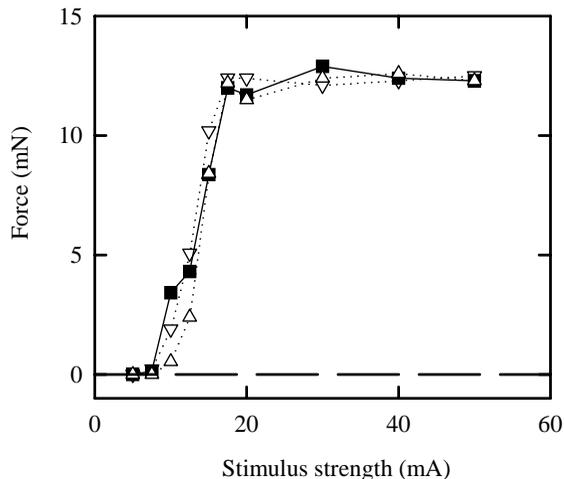
Fig. 3. The effect of the glutamate antagonist 2-APB on twitch force in a single preparation from squid. The stimulus strength–twitch force relationships in standard ASW ($\triangledown$), ASW + 20 μmol l^{-1} 2-APB ($\blacksquare$) and after return to standard ASW ($\triangle$) are shown.

general, stronger stimuli are required to activate muscle cells directly rather than indirectly *via* the release of transmitter from nerve endings. Thus, in our experiments, if the electrical stimuli were acting indirectly *via* the release of transmitter from nerve endings, higher stimulus strengths would be required in the presence of 2-APB than in the absence of the antagonist. However, in four out of five trials on preparations from squid, 2-APB did not affect the relationship between stimulus strength and twitch force (Fig. 3), indicating that the stimulus was directly depolarizing the muscle cells. In trials on six preparations from cuttlefish, 2-APB did not reduce the twitch force produced in response to stimuli at a current strength that was supramaximal in the absence of 2-APB. The full range of stimulus strengths was not tested in the presence of 2-APB in these experiments on cuttlefish.

Coupling between cells

Octanol (2 mmol l^{-1} in ASW, buffered to pH 7.8, diluted from 2 mol l^{-1} stock octanol in 0.5 v/v dimethyl sulphoxide), which is known to uncouple gap junctions (Johnston *et al.* 1980; Spray *et al.* 1985), was used to investigate cell coupling in both squid and cuttlefish. The stimulus strength–twitch force relationship was tested in the presence and absence of octanol. In the presence of octanol, stimuli at each strength were applied at 30 s intervals until twitch force stabilized. Fig. 4 shows the stimulus strength–force relationship with and without octanol for a preparation from squid. Even at 100 mA stimulus strength, a supramaximal response was not observed in any octanol-treated preparation (cuttlefish $N=6$, squid $N=4$). Extensive washing by superfusion with standard ASW resulted in at least a partial recovery of force in each preparation used.

Isometric contractile properties
Length–force relationship

The length–force relationship was investigated using thin

slice preparations. Passive force was examined in detail in experiments on cuttlefish slices. Some stress-relaxation of passive force was evident at longer muscle lengths, and it was necessary to allow approximately 2–3 min for passive force to stabilise between stimulations. A small but stable passive force was apparent at L_0, which was 13.0±5.0 % (mean ± S.E.M., $N=6$) of peak force P_0 in a 200 ms tetanus. The slope of the passive force *versus* length relationship increased steeply with length, becoming constant at lengths beyond L_0 (Fig. 5A). Interestingly, the stiffness of the linear part of the relationship varied considerably between preparations, ranging from 63 to 163 kPa or from 0.42 to 2.02(P/P_0)/(L/L_0). Mean values were 133.6±3.2 kPa and 1.36±0.25(P/P_0)/(L/L_0) (mean ± S.E.M., $N=6$). Fig. 5B shows length–active tetanic force results for one cuttlefish preparation.

Fig. 6 shows a summary of the results for tetani (50 Hz for 100 ms) of cuttlefish (Fig. 6A) ($N=6$) and squid (Fig. 6B) ($N=9$) and twitch stimulation of squid (Fig. 6C) ($N=9$). The squid results showed that the length giving maximum twitch force was slightly shorter (by 6.8±0.5 %L_0, mean ± S.E.M., $N=9$) than that giving the highest tetanic force. The solid lines in Fig. 5B and Fig. 6 are curves fitted using an approach similar to that of Edman and Reggiani (1987). A fourth-order polynomial was used for lengths near that giving maximum force and a linear fit at longer and shorter lengths. Maximum force is produced over a range of lengths (the 'plateau' region of the length–force relationship). The ends of the plateau are smooth curves, indicating heterogeneity within individual preparations of the types described by Edman and Reggiani (1987).

Evidence for muscle fibres acting in series

The lengths of circular fibres in squid (maximum approximately 1.4 mm) and cuttlefish (maximum approximately 1.2 mm) are considerably shorter than the length
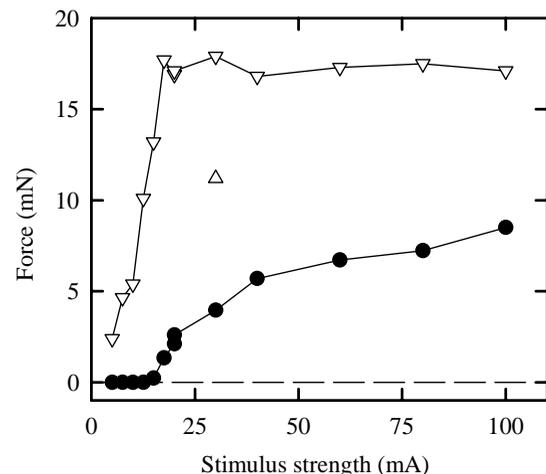

Fig. 4. The effect of octanol on twitch force in a single preparation from squid. The stimulus strength–twitch force relationships for this preparation in standard ASW ($\triangledown$), ASW + 2 mmol l^{-1} octanol ($\bullet$) and after return to standard ASW ($\triangle$) are shown.

Fig. 5. Length–force relationship of a single preparation from a cuttlefish. Force is expressed relative to the peak force P_0 for the stimulation pattern used here, and preparation length is expressed relative to the optimal length for active force production, L_0. (A) The points show the passive force produced without stimulation. The line is the linear best fit for lengths greater than L_0. (B) The active force produced in response to tetanic stimulation (100 ms at 50 Hz). The line is a fourth-order polynomial fit.
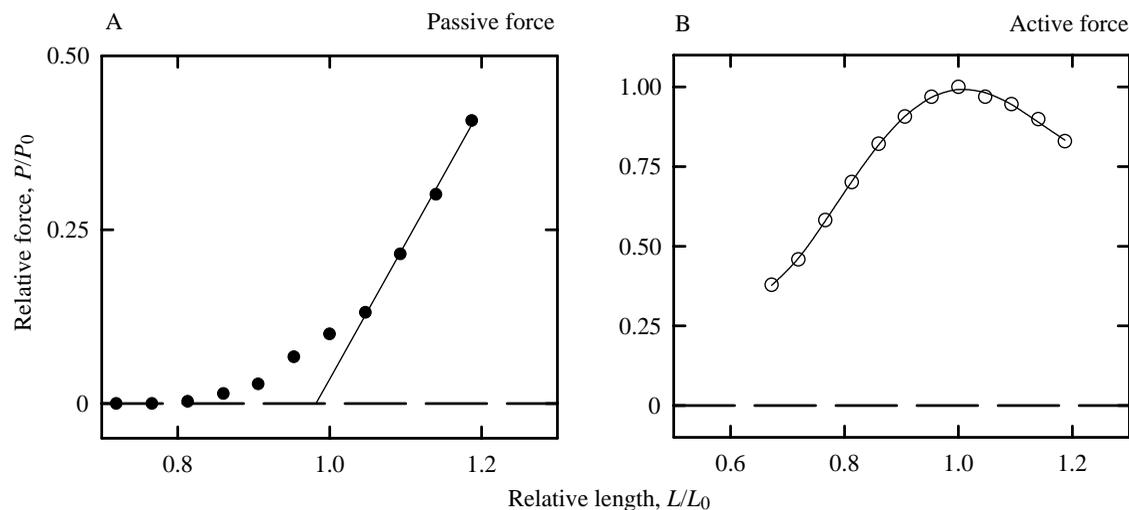
of the mantle slices we used (7.1–12.4 mm). The extent to which the fibres operate in series (where fibre forces do not add) or in parallel (where fibres forces add) was examined in the following way. The twitch force was measured at the length giving maximum force, then the length of the preparation was reduced by approximately 50 % by cutting a portion from its end. A new clip was attached to the cut end and the length giving maximum twitch force was measured anew and force recorded. On the most extreme assumption that all the fibres are acting in parallel, the force for all the fibres will add. When a hypothetical preparation of fibres 1.2 mm long is cut to reduce its length from 10 to 5 mm, the force would be reduced to 43 % of its original value. Force is reduced because intact fibres have been completely removed and the cut damages some fibres. If the fibres are acting in series, the expected result is very different. Assuming that damaged fibres are inextensible, reducing the preparation length from 10 to 5 mm would not affect force at all. (Clearly the cut fibres may be extensible and allow intact fibres in series with them to shorten. On average, the damaged fibres would amount to approximately 12 % of the total length in a 10 mm long piece and approximately 24 % in a 5 mm long piece.) When the length of seven cuttlefish preparations was reduced to 51.8±2.3 % of the original L_{tw} (from 8.8±0.3 to 4.5±0.2 mm, mean ± S.E.M., $N=7$), peak twitch force was not significantly affected. Force was 92.8±9.03 % of that produced by the original preparations (mean ± S.E.M., $N=7$). Therefore, the operation of fibres in the mantle slices is much closer to that expected for fibres acting in series than to that for fibres acting in parallel.

Effect of activating radial muscle fibres

The radial fibres of the mantle, which act antagonistically to the circular fibres (Fig. 1A,B), contract in response to acetylcholine but are insensitive to L-glutamate (Bone *et al.* 1982). The effect of cholinergic activation of the radial musculature on the length of six cuttlefish preparations was investigated. In order to obtain intact radial fibres, the slices were cut perpendicular to the surface of the mantle (plane *B* in Fig. 1), i.e. at right angles to those (plane *A* in Fig. 1) used in all the other experiments reported in this paper. Following measurement of twitch force at L_{tw} (the length giving maximum twitch force), the preparation was removed from the muscle bath and placed in a small dish. The ends of the preparation were not held, so the preparation was at its 'slack' length. Slack length (in the direction parallel to the long axis of the circular fibres) was 0.87±0.02L_{tw}, as measured with the eye-piece graticule of a stereomicroscope. The bathing solution was changed to ASW containing 20 μmol l^{-1} acetylcholine, buffered to pH 7.8, which immediately caused a rapid extension from the 'slack' preparation length. Preparation length was remeasured. Experiments were performed on six preparations which extended by more than 20 % from their slack length (0.87±0.02L_{tw}) to 1.08±0.02L_{tw} (mean ± S.E.M., $N=6$). Acetylcholine was also applied to slices cut in a plane *A* (Fig. 1A,B), parallel to the surface of the mantle (i.e. as in all other experiments reported in this paper). In these slices, which contain no intact radial fibres, acetylcholine superfusion had no observable effect on the length of the preparation, nor upon twitches evoked by electrical stimuli.

Relationship between stimulus frequency and force

The frequency–force relationships for cuttlefish and squid preparations were investigated using 200 ms tetani at frequencies up to 200 Hz. Fusion frequency was approximately 30 Hz for squid and 20 Hz for cuttlefish. Above the fusion frequency, multiple stimulation produced graded tetani, as illustrated by the cuttlefish records shown in Fig. 7A. In both preparations, force rose steeply between 5 and 50 Hz, reaching a plateau at a stimulation frequency of approximately 100 Hz (Fig. 7B). Peak specific tetanic force in this brief tetanus was 262±16 mN mm^{-2} cross-sectional area ($N=6$) for squid and 226±19 mN mm^{-2} cross-sectional area ($N=7$) for cuttlefish. The twitch:tetanus ratio was 0.18±0.01 ($N=6$) for squid and
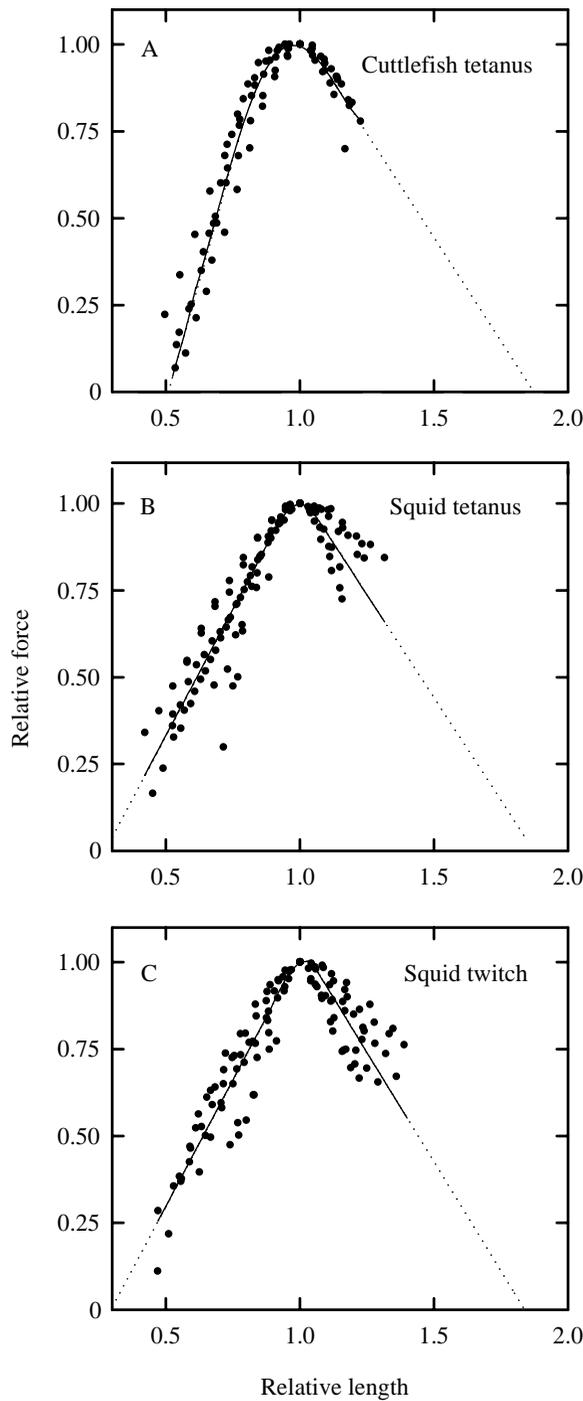
Fig. 6. Summaries of length–active force relationships for tetanic stimulation (100 ms at 50 Hz). (A) Six preparations from cuttlefish, (B) nine preparations from squid, and (C) twitch stimulation of squid (*N*=9). Force is expressed relative to the preparation's peak force for the same pattern of stimulation, and length is expressed relative to that giving this peak force. The lines are fitted curves (see text for further details).

0.10±0.02 (*N*=7) for cuttlefish (Fig. 8). Bath application of iso-osmotic KCl confirmed that the cuttlefish preparations did not contain a significant population of live fibres that were failing to respond to tetanic stimulation. Peak force produced during
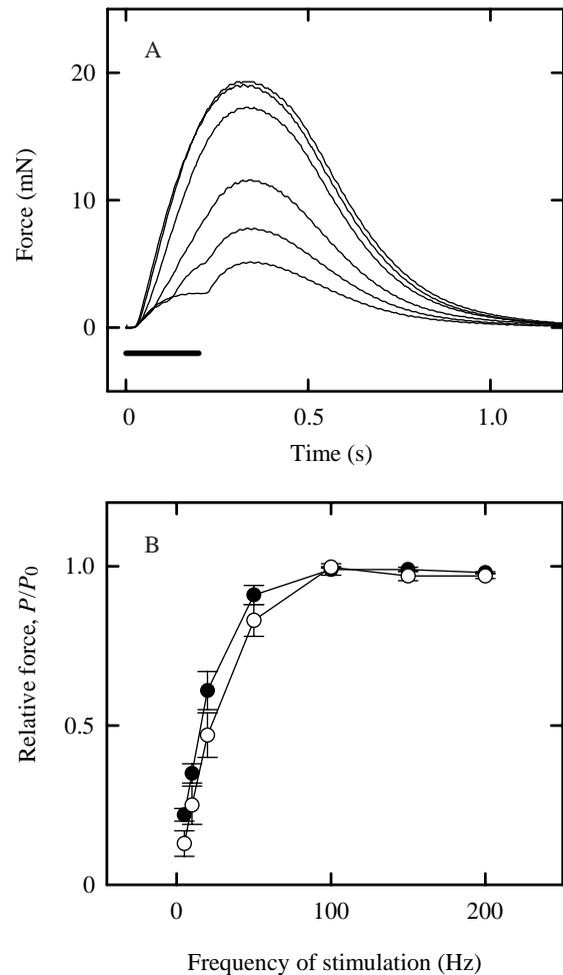

Fig. 7. Stimulus frequency–force relationship. (A) The traces show, for a single cuttlefish preparation, superimposed records of force produced in response to stimulation at frequencies of 5, 10, 20, 50, 100 and 150 Hz for 0.2 s (indicated by the horizontal bar). (B) Pooled stimulus frequency–force data. Force is expressed relative to the preparation's maximum observed force, P_0. Points show mean values ± S.E.M. for squid (●, *N*=6) and for cuttlefish (○, *N*=7).

KCl-induced contractures was $0.83±0.09P_0$ (mean ± S.E.M., *N*=7). These isometric mechanical properties of squid and cuttlefish preparations are summarised in Table 1.

Tetanus duration and force

It is clear from the records shown in Fig. 8 that force was still increasing and had not reached a 'plateau' value with 200 ms tetanic stimulation. To investigate how much more force could be produced with longer tetani, experiments were performed on seven preparations of squid mantle using tetani lasting 100, 200 and 500 ms. In only one preparation was force still increasing after 500 ms of stimulation. The peak force with 500 ms stimulation was 9.0±1.1 % (mean ± S.E.M., *N*=7) higher than with 200 ms of stimulation.

Relationship between force and velocity of shortening

The relationship between force and velocity was established

Table 1. *Isometric mechanical properties of thin slice mantle preparations of squid and cuttlefish*

	Squid (*N*=6)	Cuttlefish (*N*=7)
P_0 (mN mm^{-2} wet cross-sectional area)	262±16	226±19
P_{tw}/P_0	0.18±0.01	0.10±0.02
TPT (ms)	92±10	205±21
RT$_{50}$ (ms)	187±25	484±42

Values are means ± S.E.M.

P_0 is the peak force in a brief tetanus (0.2 s stimulation at 100–150 Hz); P_{tw}/P_0 is the twitch:tetanus ratio; TPT is time from onset of stimulation to development of maximum force during a twitch; RT$_{50}$ is half-relaxation time during a twitch.

Note that P_0 is certainly biased low (see Discussion).

for preparations from eight squid, using isotonic (force-clamp) shortening during twitch responses. Fig. 9 shows records (from a single preparation) of force and length during shortening at three levels of force-clamp and during an isometric contraction. Force and shortening velocity measurements are the mean value for a period close to the start of a release when force and velocity had stabilized. Peak isometric force varied by approximately 5 % during the experimental protocol (4.79±0.57 %, mean ± S.E.M., *N*=8). Peak isometric twitch force was 43.7±2.5 mN mm^{-2} cross-sectional area (mean ± S.E.M., *N*=8).

A force–velocity curve for one preparation is shown in Fig. 10A, with the force expressed relative to the isometric twitch force. As described in the Materials and methods section, both Hill's (1938) and Edman's (1988) equations were fitted to the results for individual preparations. In six of the eight preparations, there was a distinct break in the force–velocity relationship at a relative force of 0.78 (Fig. 10A). Hence, in these six cases, Edman's double hyperbolic function gave a better fit to the data than Hill's single hyperbolic function. Table 2 shows the values of the

Table 2. *Force–velocity parameters for eight preparations from squid*

n	Preparation	*P**	V_{max}	$1/G$	k_1	k_2
26	1	1.20	2.07	0.67	16.1	0.92
17	2	0.98	2.71	0.45	700	0.98
24	3	1.16	2.28	0.39	16.9	0.95
27	4	0.98	1.98	1.54	500	0.99
29	5	1.34	2.56	0.48	500	0.98
39	6	1.49	2.78	0.51	22.9	0.96
35	7	1.29	2.35	0.72	39.9	0.99
19	8	1.02	2.69	0.78	39.6	0.86
Mean		1.18	2.43	0.69	299	0.95
S.E.M.		0.07	0.11	0.13	101	0.02
N		8	8	8	8	8

Values are from fits to Edman's double hyperbolic equation (equation 2; see text).

All forces up to and including isometric values were used in the fitting (see text).

n is the number of data points; *N* is the number of experiments.
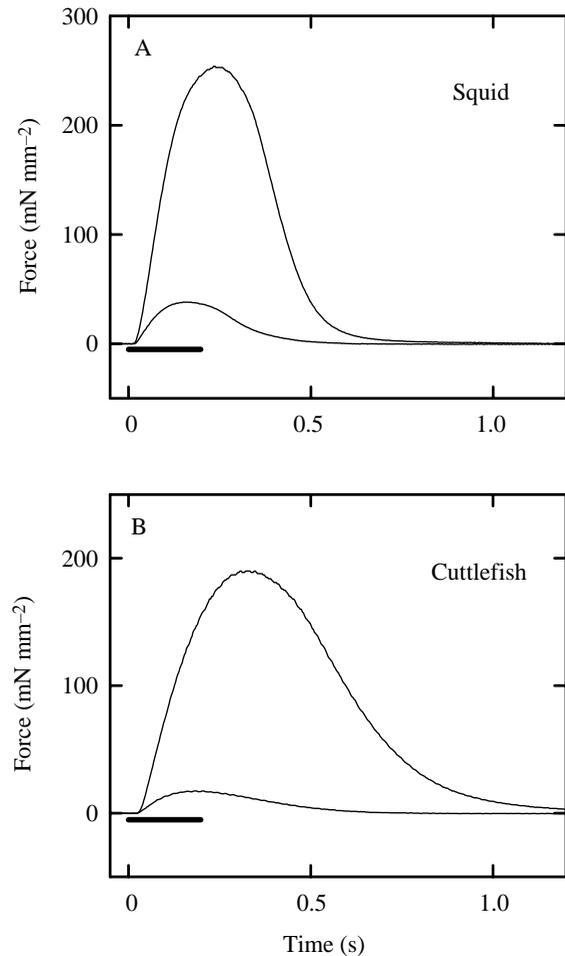

Fig. 8. Superimposed records of twitch and tetanic force (0.2 s tetanus at frequency giving maximum force, heavy horizontal line shows duration of tetanic stimulation) for (A) squid and (B) cuttlefish.

fitted parameters for Edman's equation for all eight experiments and their mean values.

The mechanical power output was calculated from the data points for force and velocity. Fig. 10B shows the power output of a single preparation. Maximum power was produced at 0.45±0.03P_{tw} (mean ± S.E.M., *N*=8). Maximum relative power output was 0.16±0.01(V/V_{max})(P/P_{tw}) (mean ± S.E.M., *N*=8). The results for all preparations show that maximum absolute power output was 18.3±1.7 mW g^{-1} wet mass (mean ± S.E.M., *N*=8).

Discussion

Our aim in this study was to determine whether obliquely striated muscle fibres show different mechanical properties from those well-documented for cross-striated fibres. We chose to examine cephalopods, since in the mantle of squid and cuttlefish it is easy to obtain obliquely striated fibre populations in suitable quantities for mechanical experiments. Obliquely striated fibres are not unique to cephalopods, however, and are found in a variety of animals, ranging from the pelagic tunicate
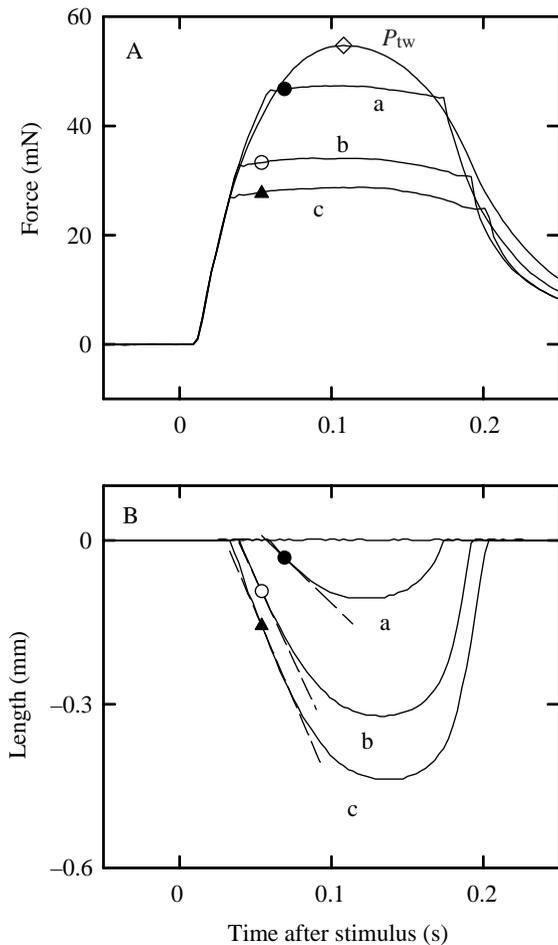
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Fig. 9. Twitch force (A) and velocity of shortening (B) by a preparation from a squid. Superimposed records of twitch force and length after a stimulus at time zero. The highest force record was obtained during an isometric (constant-length) twitch (P_{tw} is the peak force), and the others (a, b, c) were obtained during isotonic twitches with force clamped to three different values. The symbols mark the point at which force was measured on each record. The slopes of the broken lines on the length records give the velocity of shortening and the symbols mark the centre of the section, from which the slopes were measured.

Fig. 10. (A) Force–velocity relationship for a single squid preparation. Force is expressed relative to the isometric force from the preparation (mean from repeat twitches) and velocity is expressed in $L_{tw}\,s^{-1}$, where L_{tw} is the length at which peak isometric twitch force is produced. The broken line was fitted to data for relative forces below 0.78 using Hill's single hyperbolic function (see text). The solid line was fitted to the entire data set using Edmans's double hyperbolic equation (see text and Table 2, preparation 1). (B) Points show the relative power output of the preparation calculated from the data in A. The solid line was calculated from the solid line in A.

Doliolum gegenbauri Uljanin to annelids, nematodes and other molluscs. Their structure and distribution has been reviewed in some detail by Lanzavecchia (1977, 1981).

We discuss first the mechanical properties of the cephalopod fibres, and then consider some speculations.

Isometric force characteristics

The muscle fibres in the mantle preparation are electrically coupled. Not only do the muscle fibres show extensive dye coupling (Bone *et al.* 1994*a*), but octanol also depresses twitch force. Even with direct muscle excitation, as in our experiments, it seems that some spread of excitation *via* gap junctions is necessary to activate all the muscle cells. All the pr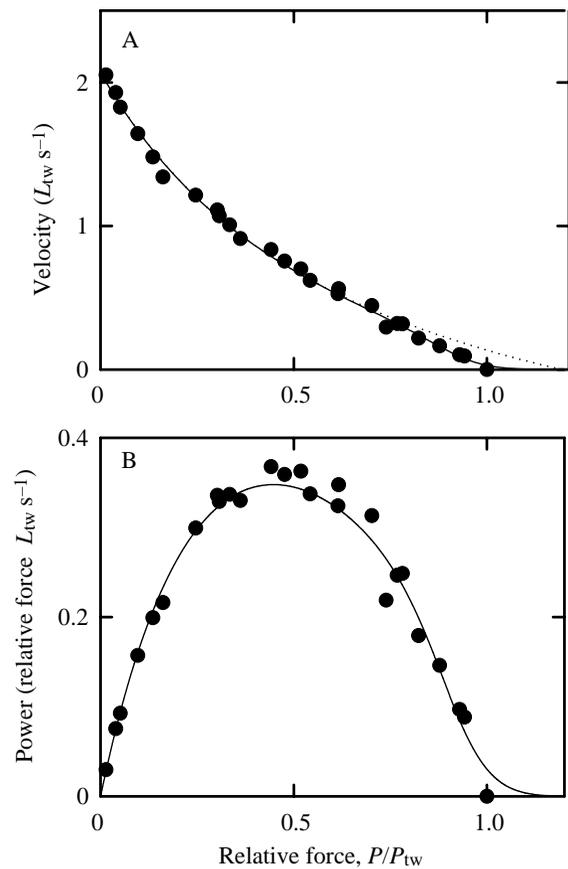eparations showed a clear mechanical response to a single stimulus. A single spike in a third-order giant axon innervating the mantle evokes a rapid strong contraction, and early work (Prosser and Young, 1937) suggested that the force was similar to that in tetani evoked by multiple stimuli to the giant axon. It was thus unexpected to find in mitochondria-poor muscle preparations that the twitch:tetanus ratio was small (0.1–0.18) and that maximum tension was not evoked until stimulus frequency was 200 Hz.

The peak isometric forces in brief (0.2 s) isometric tetani at optimum frequency were $262\pm16\,mN\,mm^{-2}$ cross-sectional area for squid and $226\pm19\,mN\,mm^{-2}$ cross-sectional area for cuttlefish preparations. For tetani of longer duration giving a plateau of force, the peak force was approximately 10 % higher than in a 0.2 s tetanus. These values are very similar to the maximum force produced by a variety of cross-striated muscles from vertebrates, e.g. white fibre bundles from dogfish *Scyliorhinus canicula*, $255\,mN\,mm^{-2}$ (Curtin and Woledge,

Table 3. *Variables used to calculate relative power outputs in Fig. 11*

Animal	Muscle	Temperature (°C)	Maximum power	1/G	Reference
Squid, *Alloteuthis subulata*	Mantle, circular fibres	11	0.152	0.69	This report
Carp, *Cyprinus carpio*	Red fibres	10	0.133	0.49	Rome and Sosnicki, 1990
Xenopus laevis	Iliofibularis, type 1 fibre	20	0.132	0.48	Lannergren *et al.* 1982
Rat	Extensor digitorum longus	30	0.125	0.43	Ranatunga, 1982
Mouse	Soleus	21	0.112	0.34	Barclay *et al.* 1993
Frog, *Hyla chryscelia*	Sartorius	25	0.105	0.30	McLister *et al.* 1995
Frog, *Hyla cinerea*	Sartorius	25	0.104	0.29	McLister *et al.* 1995
Dogfish, *Scyliorhinus canicula*	Myotomal, white fibres	12	0.092	0.24	Curtin and Woledge, 1988
Frog, *Hyla versicolor*	Sartorius	25	0.076	0.17	McLister *et al.* 1995

G is a constant expressing curvature (P_0/a in Hill's equation).

1988), or single fast twitch fibres from frog *Rana temporaria*, 287 mN mm^{-2} (Curtin and Edman, 1994).

Several considerations make it rather surprising that the squid mantle slices produce as much isometric force as dogfish muscle. If single muscle fibres were compared, similar forces might be expected, since force production depends upon the cross-sectional area of the myofilament lattice within the fibres and, in both squid and dogfish, few mitochondria interrupt the myofilament array. In squid fibres, the mitochondrial core occupies 6 % of the fibre cross-sectional area (Bone *et al.* 1981) compared with approximately 1 % in dogfish white fibres (Totland *et al.* 1981). However, our slice preparations cut in plane *A* (see Fig. 1), which contain mainly circular mantle fibres, certainly also contained fragments of rows of radial fibres and these make up approximately 28 % of the cross-sectional area. Slices cut in plane *A* also contain part of the extensive connective tissue lattice that surrounds the muscle fibres within the mantle, which is entirely lacking in dogfish white muscle fibres. These two components of the slices we studied mean that at least 28 % of the cross-sectional area of the slice did not contribute to the active force.

Further, apart from the fibres severely damaged on the cut surfaces of the slices, a proportion of fibres are damaged by cutting the ends of the slice, and the cut fibres would not be expected to contribute to the force. Muscle fibres in squid and cuttlefish are considerably shorter than the preparation length, so at the ends of the slice not many would be cut, and approximately 80–90 % would be expected to remain intact (calculated from reasonable assumptions about fibre length and preparation length) in the squid and cuttlefish preparations.

All of these considerations suggest that a proportion (probably at least 30 %) of the cross-sectional area of the squid and cuttlefish slice preparations did not produce active force. Taking this into account and adding the 10 % force that could be produced with longer stimulation, the maximum isometric forces could reasonably be expected to reach 400 mN mm^{-2} cross-sectional area of active tissue for squid and 350 mN mm^{-2} for cuttlefish.

The fact that force was not much affected by removing half of the length of the preparation indicated that the fibres along the length of the preparation are operating in series. That is, the forces of the individual fibres are not adding as they would if the fibres were operating in parallel. Thus, the parallel fibre arrangement is not the cause of the high force per cross-sectional area. A different possibility is that, in the mantle muscle of cephalopods, there are more crossbridges acting in
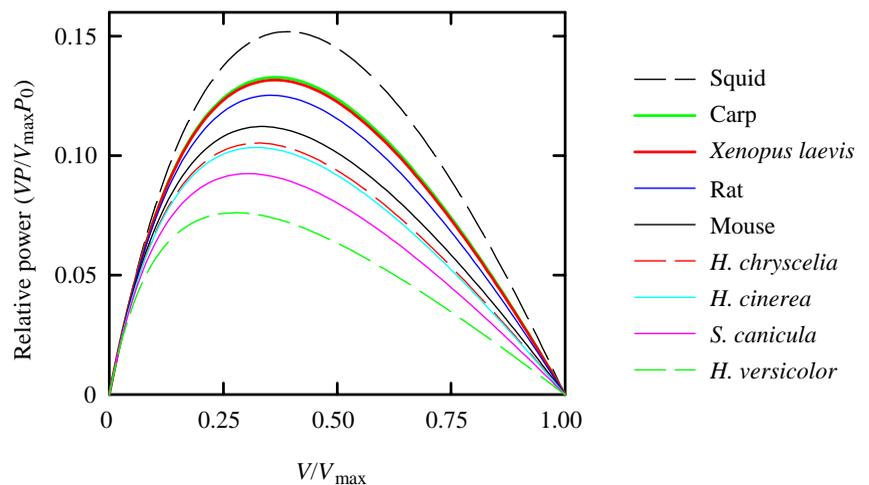

Fig. 11. Relative power output of some locomotor muscles calculated from force–velocity characterisitics {=[2+G–2√(1+G)]/G^2, see Woledge *et al.* 1985, p. 49}. Relative power = (*P*/isometric force)(*V*/V_{max}). Details and references are given in Table 3.

parallel because the thick filaments are longer than those in dogfish and frog muscle. The number of thick filaments per cross-sectional area of myofibril and the surface area of the thick filament per unit filament length are not very different in frog muscle (Squire, 1986, p. 214; Squire *et al.* 1990, p. 6) from those in squid funnel retractor mucle (Hanson and Lowy, 1957; values for mantle muscle are not known). The spacing of the crossbridges on the surface of the thick filament is remarkably uniform among species (Squire, 1986). Thus, the thick filament length may be the major structural factor responsible for a difference in the number of crossbridges contributing to force. In cross-striated vertebrate fibres, thick filaments are close to 1.5 μm long, e.g. 1.56 μm in frog (Craig and Offer, 1976; Page and Huxley, 1963), and they are even shorter in the cross-striated fibres in squid tentacles (Van Leeuwen and Kier, 1997). In contrast, in the obliquely striated fibres of squid tentacles, Kier (1985) has reported finding thick filaments as long as 2.8 μm. If they are the same length in the fibres of our squid mantle preparations, it would not be surprising that the isometric force we obtained was as high as it is. Unfortunately, we have no information about the thick filament length in the mantle fibres of any squid species.

The lengths of thick filaments in obliquely striated fibres have only been measured in a few species; it is interesting that in all cases they greatly exceed 1.5 μm. Values in Nematomorpha range from 6 μm (Rosenbluth, 1965) to 120 μm (Eakin and Brandenburger, 1974). In annelids, Mill and Knapp (1970) report values of 9.5 μm in the circular fibres of *Lumbricus terrestris*, whilst in molluscs, Hanson and Lowy (1961) observe oyster *Cassostrea angulata* thick filaments to be approximately 5 μm long.

Until the mechanical properties of other obliquely striated fibres have been examined, it remains unclear whether this fibre design is peculiarly specialised for high force production. So far as we are aware, the only measurements of isometric force production of such fibres are for the funnel retractors of *Sepia officialis* (220 mN mm^{-2} cross-sectional area) and *Octopus vulgaris* or *Eledone moschata* (510 mN mm^{-2} cross-sectional area) (Lowy and Millman, 1962).

Length–force relationships and the 'mantle spring'

The form of the active length–force relationship is similar to that of cross-striated fibres, increasing as preparation length increased up to $1.0L_0$, remaining fairly constant over a range of lengths and then decreasing at longer lengths. Thus, the length–force relationship is as expected for the behaviour of sliding filaments (Gordon *et al.* 1966).

A feature of the cephalopod system so far not considered, however, is that the mantle fibres contract and relax within the constraints of a remarkably abundant, three-dimensional connective tissue lattice. Gosline and Shadwick (1983) have argued on energetic grounds that the connective tissue lattice acts as an important elastic energy storage mechanism during jetting. Bone *et al.* (1994*a*) suggest that the connective tissue is also functionally relevant during the hyperinflation phase of the jet cycle, when radial muscle activity thins the mantle. However,

direct evidence for the role of the mantle spring in the operation of the mantle muscle fibres remains to be provided. Our simple experiments with mantle slices demonstrated that cholinergic activation of the radial musculature stretches the elasticity in parallel with the circular muscle fibres (Fig. 5A). This 'sets' circular muscle length slightly longer than L_{tw} (mean $1.08L_{tw}$) so that, when stimulated, the muscle fibres will shorten over the length range giving highest force (Fig. 5B). The steep increase in passive force at longer lengths may (in addition to storing elastic energy) prevent 'over-stretching' of circular fibres to lengths beyond the range for near-maximum active force generation. It should be noted that, in preparing the slices, the connective tissue lattice was damaged to some extent, and thus the elasticity we observe in our slices is not identical to that in the intact mantle.

The force–velocity relationship

It is striking that the shape of the force–velocity relationship of the obliquely striated fibres from the squid mantle is essentially similar to that typical of vertebrate cross-striated fibres. The maximum velocity of shortening found by extrapolating the force–velocity relationship, 2.4 preparation lengths s^{-1}, is rather low compared with that of some cross-striated locomotor muscles working at normal body temperature, e.g. type 1 fibres from *Xenopus laevis* iliofibularis, $9.2L_0$ s^{-1} (Lannergren *et al.* 1982), or extensor digitorum longus muscle from rat, $13.5L_0$ s^{-1} (Ranatunga, 1982). We measured V_{max} in twitches, but we would expect it to be the same during tetani, since V_{max} does not vary with the degree of activation (Ambrogi-Lorenzini *et al.* 1983).

The squid fibres we have examined are probably mainly active during rapid escape responses, when they drive the locomotor jet directly. In these escape responses, muscle power (the rate at which work is done) is more relevant than V_{max}. The small curvature of the force–velocity relationship indicates high relative power output $(VP)/(V_{max}P_{tw})$. Fig. 11 shows the relative power output of the squid mantle fibres along with similar data from a range of vertebrate cross-striated locomotor muscles. From this comparison, it is clear that squid muscle is able to produce high relative power compared with other animals. The squid results are for twitch stimulation, but this is unlikely to be responsible for the high relative power; Hill found similar values of a/P_0 (equivalent to $1/G$ in our terminology) for tetanic (Hill, 1938) and twitch (Hill, 1949) stimulation.

Fibre length changes

As Rosenbluth (1967) pointed out, changes in length in obliquely striated muscle fibres may take place by shear as well as by sliding of the thick and thin filaments past each other, so such fibres are capable of greater length changes than cross-striated fibres. Indeed, they are found in animals with a hydrostatic skeleton where there are large changes in shape. We have not found that force can be produced by cephalopod mantle fibres over a much larger range of lengths than could be achieved by cross-striated fibres. However, this would be worth further investigation, because the results shown in Fig. 6

cannot rule out the possibility that some force is produced at lengths even shorter than $0.5L/L_0$.

Conclusion

Our mechanical measurements of the obliquely striated muscle fibres in cephalopods have not revealed any very striking differences from the measurements made previously on vertebrate cross-striated muscle fibres. However, the mitochondria-poor fibres from squid and cuttlefish mantle produce relatively high force per unit cross-sectional area compared with vertebrate muscle fibres. It is possible that this may be a consequence of longer thick filaments than in the vertebrate muscle design.

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