

CONTRACTILE PROPERTIES OF FISH FAST MUSCLE FIBRES

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SUMMARY

A study has been made of the contractile properties of fast muscles isolated from the myotome of the cod (*Gadus morhua* L.) and the pectoral fin of the cuckoo ray (*Raja naevus*). Cod has polyneuronally and cuckoo ray focally innervated fast fibres. Both types respond to a single stimulus (1 msec, 6 V) with a twitch. However, the effect of stimulation frequency on tension development is very different in the two muscles. Typical tetanic fusion frequencies on multiple stimulation are 5–10 Hz for ray and 40–50 Hz for cod. Maximal isometric tensions require stimulation frequencies in excess of 200 Hz for cod as compared to only 20 Hz for skate. Twitch tetanus ratios are 0.46 for ray and 0.19 for cod muscle.

It is concluded that the membrane of polyneuronally innervated fibres is relatively inexcitable and that full activation may require simultaneous and perhaps asynchronous recruitment of a number of different motor neurones. Polyneuronal innervation may well give some additional flexibility to power development by the locomotory musculature.

Key words: fish, fast skeletal muscle, contractile properties.

INTRODUCTION

In general, the power requirements of swimming rise as a function of body-size and velocity (Webb, 1975). Thus burst speeds require proportionally more effort than steady swimming. In the 'older' fish groups (elasmobranchs, holosteans, chondrosteans and some teleosts with primitive taxonomic features, e.g., *Clupeiformes gonorynchiformes*) the different energetic requirements of steady and burst swimming has led to a complete anatomical and functional division between the fast and slow motor systems (Bone 1964, 1970). Thus in dogfish (*Scyliorhinus canicula*) sustained swim-

ming is entirely supported by a thin myotomal strip of tonically active red muscle fibres (Bone, 1966). In contrast, the bulk of the musculature, composed of phasically active white fibres, is reserved for burst swimming (Bone, 1966). In dogfish, red and white muscle have a similar pattern of innervation and electrophysiological properties to respectively the tonic (multiple innervation) and fast twitch fibres (focal innervation) of other vertebrates (Bone, 1964; Stanfield, 1972).

However, in most 'modern' teleosts there is not a simple division of labour between red and white muscles and both are recruited at sustainable swimming speeds (Johnston, 1977; Bone, 1978; Johnston and Moon, 1980). For example, the threshold speed for recruitment of white fibres in saithe (*Pol-lachius virens*) is only 1.8 lengths/s (Johnston and Moon, 1980). Interestingly, electronmicrographs recorded from red and white muscles at 2–3 lengths/s are similar in appearance and amplitude (150 μ V peak to peak). Much larger spike-like potentials (1 mV to peak) are recorded from white muscle at higher speeds and during bursts of acceleration (Johnston and Moon, 1980). The fast muscles of most teleosts are unique among vertebrates in having multi-terminal innervation (Bone, 1964). For example, each fibre of the scorpeaniforme fish *Myoxocephalus scorpius* receives 8–22 motor terminations from 2–5 separate axons from each of four spinal nerves (Hudson, 1969). *Myoxocephalus* fast fibres are capable of producing two distinct kinds of electrical response on stimulation of spinal nerves, (a) local junction potentials and (b) propagated action potentials (Hudson, 1969).

There have been very few studies of the mechanical properties of fish locomotory muscles. In the present study the contractile properties of fast muscles from species with focally (*Raja naevus*) and multi-terminally (*Gadus morhua*) innervated fibres are compared.

METHODS

All experiments were carried out at 15°C. The following Ringer solutions were employed: A. Cuckoo ray (*R. naevus*) 312 mM NaCl, 4 mM KCl, 5 mM CaCl₂, 450 mM urea, 10 mM Tris-maleate, pH 7.4.

B. Cod (*G. morhua*) 142 mM NaCl, 2.6 mM KCl, 2.7 mM CaCl₂, 1 mM MgCl₂, 18.5 mM NaHCO₃, 3.2 mM NaH₂ PO₄, pH 7.4. Fibre bundles, about 0.02 cm² in cross section, were rapidly dissected from the anterior dorsal myotomal muscle of the cod and the ventral pectoral fin ray of the cuckoo ray. Tension was recorded using a strain gauge (0–100 g) (Devices, Ltd.). Fibre bundles were attached to the transducer by small tungsten hooks inserted into the myosepta (cod) or tendon (ray). Stimulation was ended by silver electrodes (1 msec pulse width; 6 V) and the muscle strip was super-perfused with oxygenated Ringer. Responses were recorded on a storage oscilloscope or pen recorder (Gould Advance Instruments, U.S.A.). In some experiments fibre bundles were soaked for 15–20 min in Ringer solution containing 5×10^{-5} M tubocurarine prior to use.

RESULTS

White muscle fibres from cuckoo ray and cod show very different stimulation characteristics (Table I, Fig. 1). Both fibres respond to a single supra-maximal stimulus with a fast twitch. However, both the rate of development and relaxation of twitch tension is greater in cod than ray muscle. On multiple stimulation ray muscle produces fused tetani at 5–10 Hz and maximum tetanic tension at around 20 Hz stimulation (Fig. 1). In contrast, cod muscle produces individual twitches at 10 Hz with no evidence of summation. The multi-terminally innervated fibres of cod require stimulation frequencies of 200–300 Hz to produce maximum tensions and show fused tetani at 35–45 Hz (Table I). Essentially similar results were obtained both in the presence and absence of tubocurarine, indicating that the muscle fibre bundles were stimulated directly and not via the nerves. Interestingly the myotomal preparations rapidly fatigue and become inexcitable on repeated multiple stimulation. In contrast, fin and jaw muscle preparations from fish maintain 90% of initial isometric tensions even after more than one hundred short tetani given over the course of a day's experiments (Flitney and Johnston, 1979). Absolute tensions are far lower than those obtained for amphibian and mammalian muscles, as has previously been noted for other fish muscle preparations (Flitney and Johnston, 1979).

DISCUSSION

Bone and Johnston (unpubl. observ.) found that isolated white fibre bundles from the dogfish myotome produced maximum isometric tensions at stimulation frequencies of around 20 Hz and fused tetani at 8 Hz. Similar results have been obtained for cuckoo ray white muscle in the present study (Table I). In contrast, very different stimulation characteristics have been reported for multi-terminally innervated teleost muscle. Flitney and John-

TABLE I

Contractile properties of white muscles from cuckoo ray (*R. naevus*) and cod (*G. morhua* L.).

Parameter	Cuckoo ray	Cod
$t_{1/2}$ (msec) development twitch tension	25.6 ± 3.3	15.4 ± 1.0
$t_{1/2}$ (msec) relaxation twitch tension	60.3 ± 13.5	21.2 ± 1.2
Twitch tension (g/cm ²)	63.7 ± 10.3	10.4 ± 1.6
Tetanic fusion frequency (Hz)	5–10	35–45
Stimulation frequency to give peak tension (Hz)	20	200–300
Peak tetanic tension (g/cm ²)	138.6 ± 33.1	54.4 ± 7.7
Twitch : tetanus ratio	0.46 ± 0.04	0.19 ± 0.03

Values represent mean ± S.E. for eight muscles.

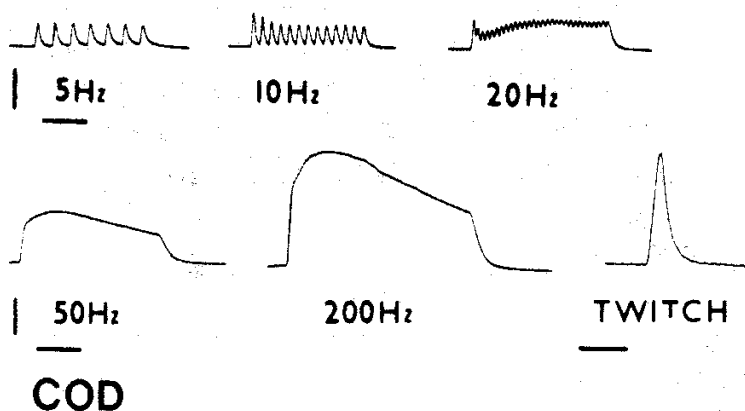
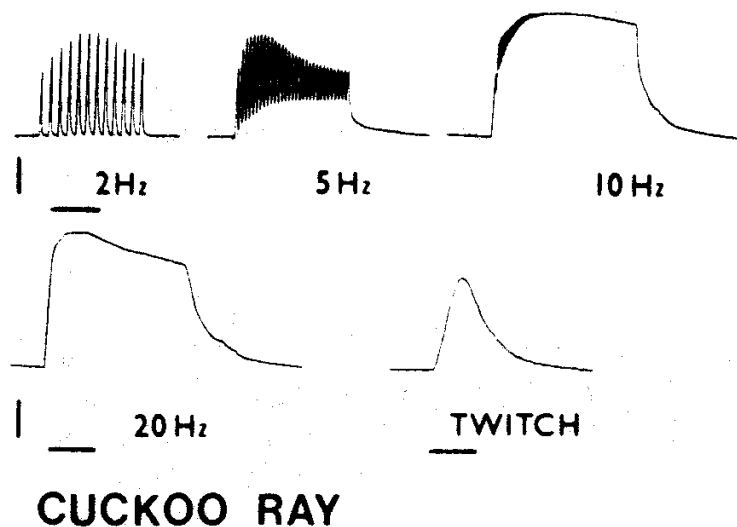


Fig. 1. Isometric contractions of bundles of white muscle fibres at different stimulation frequencies. Top: cuckoo ray (*R. naevus*) pectoral muscle. Bottom: cod (*G. morhua*) dorsal myotomal muscle. Stimulation voltage (6 V, 1 msec pulse width). Vertical scale bar represents tension (30 g/cm² (cuckoo ray), 15 g/cm² (cod)). Horizontal scale bars represents 1 s (cuckoo ray), 500 msec (cod) (multiple stimulation) and for twitches 100 msec. Temperature 15°C. Note the lower tetanic fusion frequency and lower stimulation frequency required to elicit maximum tension in ray compared to cod muscle.

ston (1979) studied both red and white fibre bundles isolated from adductor operculi muscles of *Tilapia mossambica*. Red fibres from *Tilapia* do not respond to a single stimulus but give graded contractions at frequencies in excess of 5–10 Hz. Both fibre types produce graded fused tetani, reaching a maximum at 300–400 Hz. Unloaded speeds of shortening are 2.6 lo s⁻¹ for white and 1.5 lo s⁻¹ for red fibres at 18°C. The present study represents the first report of the stimulation characteristics of multi-terminally innervated white myotomal fibres. In general, the results obtained in Table I are comparable to those reported for *Tilapia* jaw muscle (Flitney and Johnston, 1979).

From these results it would appear that the rate of tension development in multi-terminally innervated fibres is limited as much by the muscle membrane properties as by its intrinsic maximum speed of shortening (except at very high stimulation frequencies). In *Myoxocephalus* fast fibres Hudson considered that each end plate is derived from a separate axon (Hudson, 1969). Histological and electrophysiological evidence for polyneuronal innervation has also been obtained for a number of other teleosts with multi-terminally innervated fast fibres (Bone, 1978). It seems likely that full activation of polyneuronal muscles requires simultaneous and perhaps asynchronous activity of a number of different motor neurons. This may well give an additional flexibility to power development by the musculature over and above that produced by the hierarchical recruitment of motor units of different sizes. In fish with multi-terminally, as opposed to focally innervated fast muscles, there is not such a sharp transition between sustainable swimming speeds and those leading rapidly to fatigue (Bone, 1975).

However, it seems paradoxical that a single fibre type should be able to operate efficiently over such a wide range of shortening speeds. Activation of muscle contraction in fish and other vertebrates involves the direct binding of Ca^{2+} to troponin C on the thin filament (Lehman and Szent-Gyorgyi, 1975). However, there is some evidence that Ca^{2+} levels can modulate both force production and the speed of shortening in skeletal muscle. Julian (1971) reported that the force-velocity relationship of frog muscle fibres, made permeable to Ca^{2+} by brief treatment with glycerol and detergent, was altered at pCa values above 6.09. For example, at pCa values above 6.09, the steady force produced was 37% and the V_{max} 47% of that at pCa 5.49 (Julian, 1971). In polyneuronal muscles the release of calcium from the SR and hence tension development is presumably dependent on the degree of depolarization of the muscle membrane which is in turn determined by the number of motor neurones activated and their firing frequency. If a similar dependence of velocity of shortening on pCa applies to fish muscle, then a relatively inexcitable membrane would provide a mechanism for changing muscle properties at low firing frequencies, such as might be obtained during slow speed swimming.

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