FIBRE HETEROGENEITY IN THE CLOSER AND OPENER MUSCLES OF CRAYFISH WALKING LEGS

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Summary

1. The closer and opener muscles in the third walking legs of the three crayfish *Pacifastacus leniusculus*, *Procambarus clarkii* and *Astacus leptodactylus* are composed of fibres which differ in histochemistry, electrophysiology and morphology. Three major groups of fibres (A, B and C) were distinguished.

2. Group A fibres react weakly to histochemical stains for myofibrillar ATPase (mATPase) activity characteristic of fibres with slow shortening speeds. In the opener muscle, they are innervated by the opener excitor (OE) and the specific opener inhibitor (OI). In the closer muscle, group A fibres are innervated by the common inhibitory neurone (CI) in addition to single (slow closer excitor, SCE) or double excitatory (SCE and fast closer excitor, FCE) innervation. Group A fibres have the largest excitatory junction potentials (EJPs), the longest membrane time constants (τ) and the longest sarcomeres. They are located at the very distal and proximal ends of both muscles.

3. Group B fibres show higher mATPase activity than group A fibres. In the opener muscle, they are innervated by OE and OI; in the closer muscle, they receive double excitatory (SCE and FCE) and CI innervation. Single SCE and OE EJPs are small; those caused by FCE are larger. τ is shorter than in the other two fibre groups. Sarcomere lengths lie between those of group A and C fibres. Group B fibres are found along the entire lengths of both muscles.

4. Group C fibres exhibit the highest mATPase activity (characteristic of fibres with fast shortening velocity) which, in contrast to the ATPase of group B fibres, is not resistant to alkaline preincubation at pH10.05. In the closer, these fibres lack innervation by CI, otherwise the innervation pattern is identical to that of group B fibres. EJP size is similar to that of group B fibres; τ ranges between values for group A and B fibres. Sarcomere lengths are the shortest of all the fibre types. Group C fibres constitute the majority of the fibres in the two muscles and mainly occupy the central regions.

Introduction

The basis for division of labour within crustacean leg muscles lies in the heterogeneity

of their fibres. This diversity is expressed in morphological, neuromuscular, histochemical and biochemical properties (for reviews see Govind and Atwood, 1982; Rathmayer and Maier, 1987). Correlations between different variables have been established in a number of studies (Atwood and Bittner, 1971; Sherman and Atwood, 1972; Costello and Govind, 1983; Rathmayer and Hammelsbeck, 1985; Maier *et al.* 1986; Rathmayer and Maier, 1987; Wiens *et al.* 1988). Although the heterogeneity of muscle composition has been confirmed in various leg muscles of crabs and in the dimorphic claws of lobster and snapping shrimp (for reviews, see Govind and Atwood, 1982; Govind *et al.* 1987; Rathmayer and Maier, 1987), surprisingly little information exists on the composition of limb muscles in crayfish. The occurrence of different fibre properties has been noted in electrophysiological studies (claw opener: Iravani, 1965; Bittner, 1968*a,b*; Bittner and Sewell, 1976; opener of the first walking leg: Linder, 1974; Parnas *et al.* 1982), but a histochemical investigation of the closer muscle in two crayfish species reports a uniform composition of this muscle with regard to its myofibrillar ATPase (mATPase) activity (Mearow and Govind, 1986).

The aim of the present study was to characterize the fibre heterogeneity of the opener and closer muscles in walking legs of several crayfish species. This was achieved by measuring several variables in the same identified fibres. For the histochemical characterization, we determined the mATPase activity (Ogonowski and Lang, 1979; Govind *et al.* 1981; O'Connor *et al.* 1982; Maier *et al.* 1984) and the presence of isoforms of this enzyme on the basis of differences in pH stability. The existence of mATPase isoforms in other crustacean muscles has been demonstrated by several authors (Silverman and Charlton, 1980; Tse *et al.* 1983; Maier *et al.* 1984; Govind *et al.* 1986; Li and Mykles 1990). In addition, in the present investigation we recorded neuromuscular properties, such as innervation patterns, excitatory junction potential (EJP) amplitudes, facilitation and decay time of EJPs, and sarcomere lengths for the histochemically characterized fibres.

The results show the existence of three distinct fibre groups. Because their occurrence and distribution are similar in the three crayfish species studied, a general principle of organization for the two muscles is inferred.

Some of the results have been published previously in abstract form (Günzel and Rathmayer, 1988).

Materials and methods

Preparation

The experiments were performed on pristine third walking legs of the crayfish *Pacifastacus leniusculus* (Dana), *Procambarus clarkii* (Girard) and *Astacus leptodactylus* (Esch.). Legs were obtained by inducing autotomy.

For electrophysiological recording, opener muscles (8 for *Pacifastacus*, 4 for *Procambarus*) and closer muscles (4 for *Astacus*, 12 for *Pacifastacus*, 8 for *Procambarus*) were used. A muscle was exposed by removing small pieces of cuticle above its tendon. The small nerve bundles containing the axons that innervate the two muscles were isolated in the meropodite by splitting the main nerve trunks with the aid of

fine glass needles. The motor axons innervating the closer muscle (slow closer excitor, SCE; fast closer excitor, FCE; common inhibitor, CI) and the opener muscle (opener excitor, OE; opener inhibitor, OI; common inhibitor, CI) could be stimulated separately. For stimulation, either hook or suction electrodes were employed. Stimulation and intracellular recording techniques were conventional.

The preparation was kept at room temperature in a modified van Harreveld (1936) solution of the following composition: $205\,\mathrm{mmol\,l^{-1}}$ NaCl, $5.4\,\mathrm{mmol\,l^{-1}}$ KCl, $10\,\mathrm{mmol\,l^{-1}}$ $CaCl_2$, $2.6\,\mathrm{mmol\,l^{-1}}$ $MgCl_2$, $5\,\mathrm{mmol\,l^{-1}}$ glucose, buffered with $10\,\mathrm{mmol\,l^{-1}}$ Trizma (Sigma) and NaOH to pH7.4.

For measurement of facilitation of excitatory junctional potentials (EJPs), the excitatory axons to the opener and the closer muscle were stimulated with trains of 10 pulses at a frequency of 35Hz. The membrane time constant τ was evaluated as the decay time of the summated EJPs to 37%. Facilitation (f_n) was calculated by comparing the amplitude of the nth EJP (a_n) in a train with that of the first EJP (a_1) according to the formula:

$$f_n = (a_n/a_1) - 1.$$

After successful electrophysiological recording, fibres were marked intracellularly by ionophoretic injection of NBT (4-nitroblue-tetrazolium chloride, Boehringer) from a microelectrode (Maier *et al.* 1986). This enabled the identification of recorded fibres in subsequent frozen sections of the muscle made for the histochemical characterization of the fibres.

Histochemistry

The preparations (4 for *Astacus*, 23 for *Pacifastacus*, 5 for *Procambarus*) were quickly frozen in liquid nitrogen. The muscles were cross-sectioned serially ($20\,\mu\mathrm{m}$) at $-30°C$ in a cryomicrotome (2800 Frigocut, Reichert-Jung). Staining procedures for mATPase activity and preincubation for testing the pH stability of the mATPase were performed at room temperature as described by Maier *et al.* (1984). The mATPase activity was determined at pH8.4 and 9.4. At pH8.4, the incubation medium was buffered with $10\,\mathrm{mmol\,l^{-1}}$ Taps [*N*-tris(hydroxymethyl)methyl-3-aminopropanesulphonic acid, Sigma]. The pH stability of the mATPase was tested by preincubating the sections at pH10.05 for 10min before transferring them into the reaction medium at pH9.4.

Measurement of sarcomere lengths

After sections had been cut from a frozen muscle preparation for the histochemical identification of the fibres, the remaining part of several muscles (10 for *Pacifastacus*, 5 for *Procambarus*) was freeze-dried on silica gel at $-30°C$ for at least 8 days. Histochemically identified fibres were teased out of the dried muscle. Single dried fibres were glued to two glass needles with nitrocellulose dissolved in acetone, maintaining their original length. The fibres were subsequently submerged in a relaxing solution (composition according to Moisescu and Thieleczek, 1978). The sarcomere lengths were determined from the diffraction pattern generated by a laser beam (632.8nm, 4mW) illuminating the fibre. Because the different refractive indices of the A- and I-bands act as

a diffractional grating, the sarcomere length can be calculated from the distance between the interference maxima (see Zite-Ferenczy and Rüdel, 1978).

Results

Histochemical characterization

In sections of the closer and opener muscles stained for mATPase activity after incubation at pH8.4, three fibre populations could be distinguished (Fig. 1A,B). One group of fibres (marked with an asterisk in Fig. 1A) stained only slightly, indicating low mATPase activity. They were designated as group A. Another group (termed C fibres) exhibited heavy staining indicative of high mATPase activity (dark fibres, such as 1, 2 and 5 in Fig. 1B). The third group, called B fibres (see Fig. 1A and fibres 3 and 4 in Fig. 1B) stained with medium intensity. When the same staining procedure was performed at pH9.4 (Fig. 1C,D), fibres of group A (asterisks) again stained lightly, but a distinction between groups B and C was no longer possible because both showed similarly heavy staining. When the sections were preincubated at pH10.05 (Fig. 1E,F) and then stained for mATPase activity, group B fibres still showed a positive staining reaction (see Fig. 1E and fibres 3 and 4 in Fig. 1F). Group C fibres showed no (fibres 1, 5) or only weak (fibre 2) staining (Fig. 1F). Obviously, the mATPase of this fibre type is no longer stable at a pH of 10.05. Because of the low mATPase activity of fibre group A, it was not possible to determine the pH stability of its mATPase. Unlike results obtained with crab muscle fibres (Maier *et al.* 1984), preincubation of the sections at acidic pH (pH4.5–5.5) was not effective in separating fibre groups. Between pH5.5 and pH5 the staining intensities were identical to those without preincubation. Between pH5 and pH4.5 all fibres lost their mATPase activity and did not stain.

On the basis of differences in the total mATPase activity and especially because of differences in the stability at pH10.05, the existence of at least three different isoforms of the mATPase within the fibres of the two muscles can be inferred. The three fibre groups were found with identical characteristics and distributional patterns in the homologous muscles of all three species investigated. Fig. 2 shows a map of the fibre distribution for the closer muscle of *Pacifastacus leniusculus*. Group A fibres (white) form the smallest population. They are found exclusively at the distal and proximal ends of the muscle. Group B fibres (hatched) occur along the whole length of the muscle, whereas group C fibres (black) occupy most of the central region and comprise the majority of the fibres in both muscles. A similar distribution of the three groups is found in the opener muscle.

Sarcomere length

The sarcomere lengths varied considerably among the fibres (opener 5–13 μm, closer 6–16 μm). On average, sarcomeres of group A fibres were longest, those of group C fibres shortest (see Table 1). Although there was considerable overlap of values for the three groups, the differences in mean sarcomere lengths for the three fibre groups in a given muscle were significant ($P<0.05$, unpaired *t*-test). In the opener muscle, all fibre groups generally had shorter sarcomere lengths than those of corresponding groups in the closer
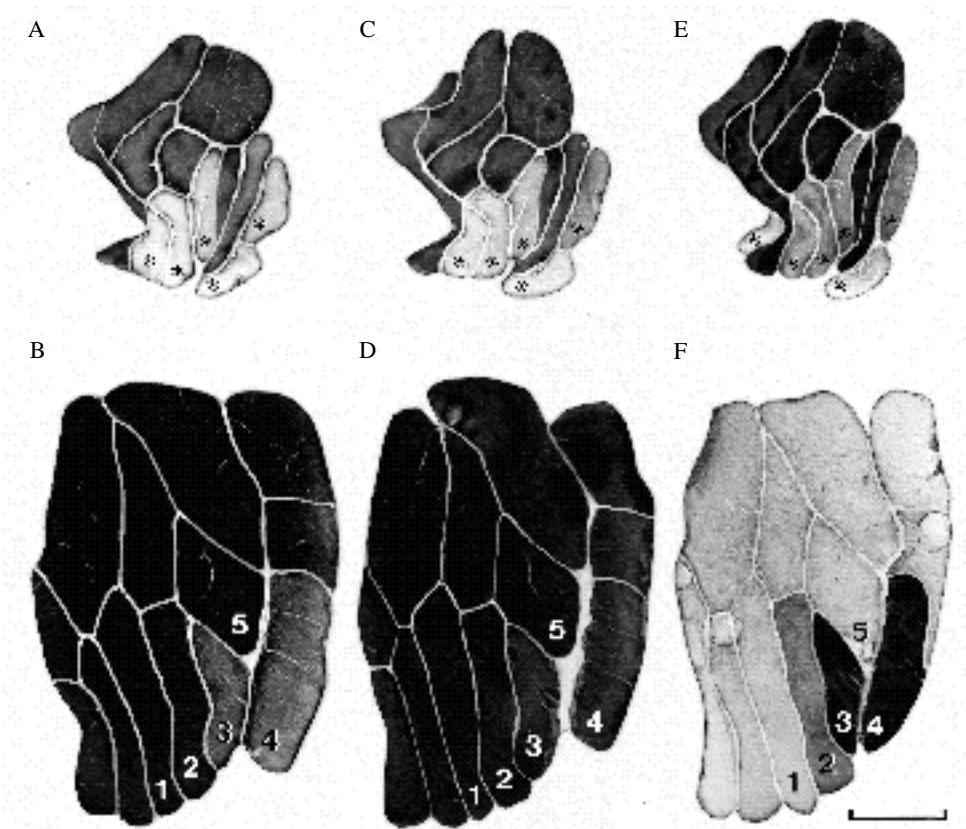
Fig. 1. Sections through the distal (A, C, E) and central regions (B, D, F) of a closer muscle in *Pacifastacusleniusculus*, stained for mATPase activity at pH8.4 (A and B), pH9.4 (C and D) and for pH stability of the mATPase after preincubation at pH10.05 (E and F). Asterisks indicate fibres with low mATPase activity (group A). Fibres 1–5 are identical to those in subsequent sections, belonging to group B (3 and 4) and C (1, 2, 5). Dorsal side up. Scale bar, 200 μm.

muscle. To determine that this was not due to different degrees of stretching of the two muscles during fixation, some legs were frozen with the dactylopodite in either a maximally opened or a maximally closed position. Thus, the two muscles could be compared when both maximally stretched or shortened. In both states, the sarcomeres in the opener muscle were always shorter than those of comparable fibre groups in the closer.

Neuromuscular properties

Closer muscle

The closer muscle is innervated by two excitatory neurones (slow closer excitor, SCE; fast closer excitor, FCE) and a branch of the common inhibitor (CI). SCE was found to innervate all fibres of the muscle. Group B and C fibres and one population of the group A fibres (A_1) receive double excitatory innervation through SCE and FCE. Excitatory
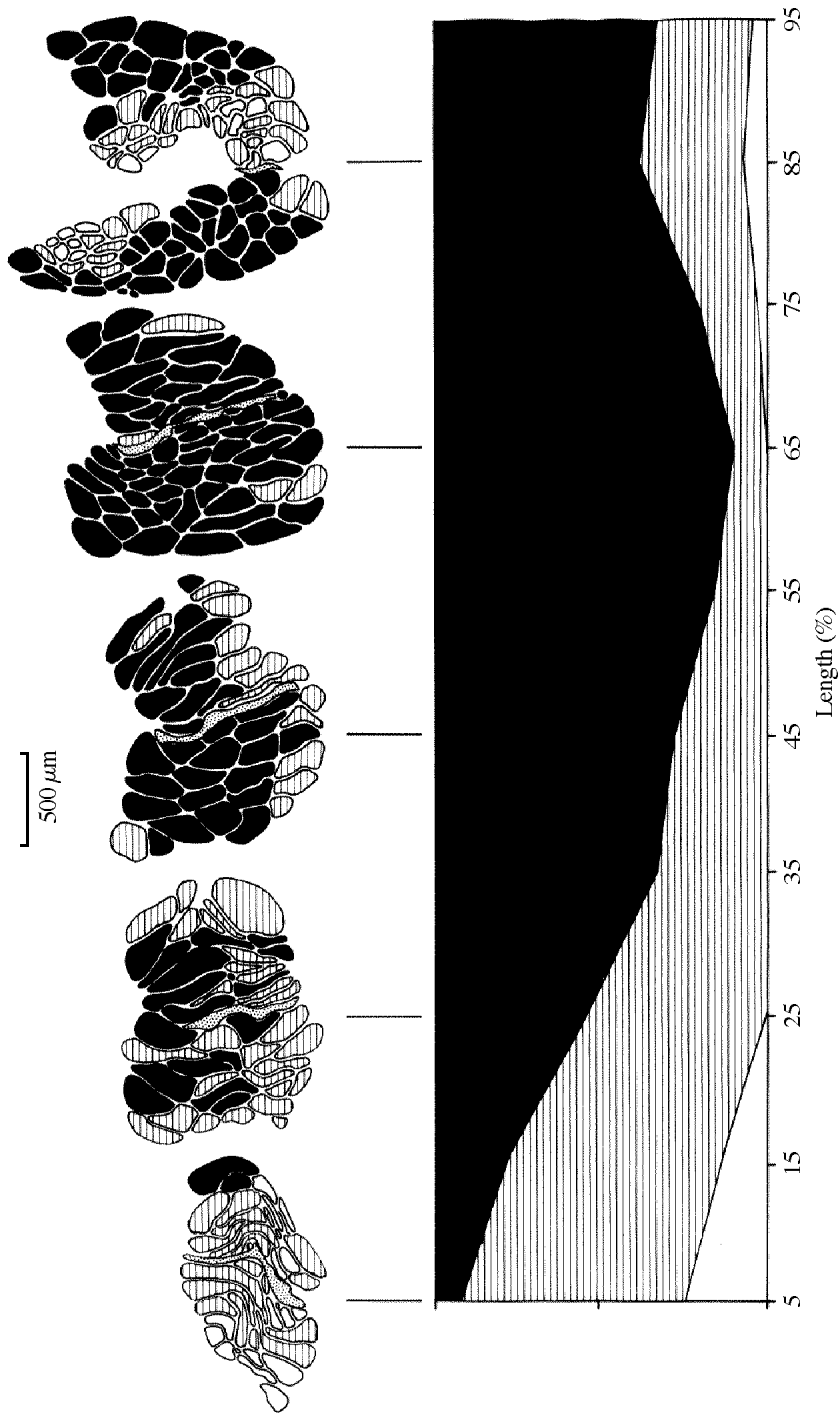
Fig. 2. Distribution of fibres belonging to three different groups in the closer muscle of *Pacifastacus leniusculus* according to histochemical mATPase staining. White, group A; hatched, group B; black, group C. In the upper part of the figure, five representative cross sections from different positions along the muscle length are shown. The percentage distribution of the three fibre groups and the position of the sections with regard to muscle length (lines) are shown in the lower part.

innervation of the rest of the A population (A_2) is solely through SCE. CI supplies all group A fibres, regardless of whether they receive single or double excitatory innervation, and the group B fibres (see Fig. 3).

Whereas inhibition exerted by CI activity was always prominent in group A fibres, group B fibres showed only very weak inhibition. Often it was detectable only during high-frequency stimulation of both SCE and CI, when a small decrease in input resistance of the muscle fibre produced a small decline in the summated EJPs (Figs 4 and 6).

The mean resting membrane potential of the muscle fibres did not differ significantly among the different fibre groups (Table 1). The membrane time constant τ showed statistically significant differences between the three fibre groups (Table 1, $P<0.05$, unpaired t-test). The amplitude of single EJPs showed variations from preparation to preparation, but in all preparations A_2 fibres exhibited the largest EJP amplitudes (see Table 1 for comparison). Fig. 4 shows examples of junctional responses upon repetitive stimulation of SCE (upper traces in recordings), SCE together with CI (middle traces) or CI alone (lower traces). The recordings shown were obtained from seven fibres belonging to group A, B or C. These fibres were subsequently identified histochemically. Their positions in the distal part of the closer muscle are indicated by lines in the cross section of the muscle shown in the upper part of Fig. 4.

The EJPs evoked by repetitive stimulation of SCE or FCE at 35Hz showed different amounts of facilitation, depending on fibre type (Fig. 5). In general, there was a similar inverse relationship between the amplitude of single EJPs and facilitation as reported for other preparations (Bittner, 1968*a*; Atwood and Bittner, 1971). Facilitation of EJPs (calculated from the tenth EJP in each train, f_{10}) during SCE stimulation was largest in group B and smallest in group A_2 fibres. In group A_1 and C fibres, facilitation values ranged between these values (Table 1). The extent of facilitation during FCE stimulation was small in group A_1 and medium in group B and C fibres (Table 1). The differences were highly significant ($P<0.01$, unpaired t-test).

Fig. 6 shows typical recordings from the different fibres of the closer muscle during stimulation of either SCE or FCE, or SCE in combination with CI. The differences in amplitude and facilitation properties of junctional potentials, the lack of FCE innervation of group A_2 fibres and the absence of CI effects in group C fibres are evident. Qualitatively similar results were obtained during stimulation at 55 and 75Hz.

Opener muscle

The opener muscle is supplied by one excitatory (opener excitor, OE) and two inhibitory neurones, the specific inhibitor of the opener (OI) and a branch of the common inhibitor (CI) (Wiens, 1985). All fibres are innervated by OE and OI. The distribution of CI was not explored in detail in this study, but Wiens (1985) has shown that the proximal slow fibres receive CI innervation. It can be assumed that they are identical with the proximal group A fibres described here (see Fig. 3).

As in the closer muscle, the resting membrane potentials of the fibres of the opener muscle did not differ significantly in the three fibre groups (Table 1). As in the closer, the membrane time constants were longer in group A than in group B and C fibres (Table 1, $P<0.05$, unpaired t-test).

Table 1. *Comparison of myofibrillar ATPase activity (mATPase), sarcomere length (SL), membrane potential (V_M), membrane time constant (τ), innervation pattern, range of amplitude of single EJPs and facilitation of the tenth EJP in a train of 35Hz stimulation (f_{10}) in the three fibre groups of the closer and opener muscle of* Pacifastacus leniusculus

	Closer				Opener		
	A		B	C	A	B	C
	A_1	A_2					
mATPase at pH8.4	Low		Medium	High	Low	Medium	High
SL (μm)	10.8±2.2		8.5±1.8	7.7±1.3	8.7±1.8	7.7±1.9	6.5±1.0
	N=39/10		N=81/10	N=110/10	N=20/5	N=25/5	N=28/5
V_M (mV)	−75.2±10.4		−77.2±11.9	−79.1±6.4	−73.9±8.2	−76.5±6.1	−72.9±6.4
	N=37/8		N=22/6	N=20/8	N=50/5	N=27/5	N=22/5
τ (ms)	113±55		46±14	62±22	118±39	42±14	63±28
	N=23/7		N=15/6	N=7/3	N=36/5	N=19/5	N=17/5
Innervation	SCE, FCE, CI	SCE, CI	SCE, FCE, CI	SCE, FCE	OE, OI, CI	OE, OI	OE, OI
EJP (mV)							
SCE/OE	0.8±0.7	3.4±2.0	0.3±0.3	0.6±0.6	1.6±0.8	0.4±0.4	0.7±0.6
	0.3 to 3.2	1 to 7.6	0.1 to 0.8	0.1 to 1.7	0.5 to 3.7	0.1 to 0.8	0.1 to 1.6
	N=20/6	N=39/10	N=47/12	N=23/8	N=54/5	N=28/5	N=25/5
FCE	3.3±3.6	–	0.9±0.4	0.6±0.5	–	–	–
	0.7 to 7.6	–	0.4 to 1.4	O.2t 1.3	–	–	–
	N=17/6		N=21/9	N=19/8			
f_{10}							
SCE/OE	4.5 to 10	−0.2 to +4	7 to 20	3 to 10	−0.2 to +2.5	4 to 15	1 to 6.5
	N=16/6	N=33/10	N=44/12	N=22/8	N=54/5	N=28/5	N=25/5
FCE	−0.5 to +0.75	–	1 to 4	2 to 8	–	–	–
	N=15/6		N=18/9	N=19/8			

Except for facilitation, means ± S.D. are given.

The number of fibres (*x*) and of muscles (*y*) used is indicated as *N=x/y*.

SCE, slow closer excitor; FCE, fast closer excitor; CI, common inhibitor; OE, open excitor; OI, opener inhibitor (specific).

The amplitudes of single EJPs varied considerably in different preparations. They differed significantly in the different fibre types ($P<0.05$, unpaired *t*-test), those of group A fibres usually being 3–4 times larger than those of group B and C fibres (Table 1). Fig. 7 shows sample recordings of EJPs from fibres belonging to the three different groups during stimulation of OE (upper traces) and OE together with OI (lower traces). Facilitation of the EJPs (Fig. 5) was always greatest in group B, intermediate in group C and smallest in group A fibres (Table 1). The differences among the fibre groups were highly significant ($P<0.01$, unpaired *t*-test).

Comparison of different species

The three different fibre groups were found to have identical histochemical and electrophysiological properties in the closer and opener muscles of third walking legs of

the three crayfish species investigated (*Pacifastacus leniusculus, Procambarus clarkii* and *Astacus leptodactylus*). This suggests a common principle in the composition and in the fibre arrangement of these muscles in reptantian crustaceans.
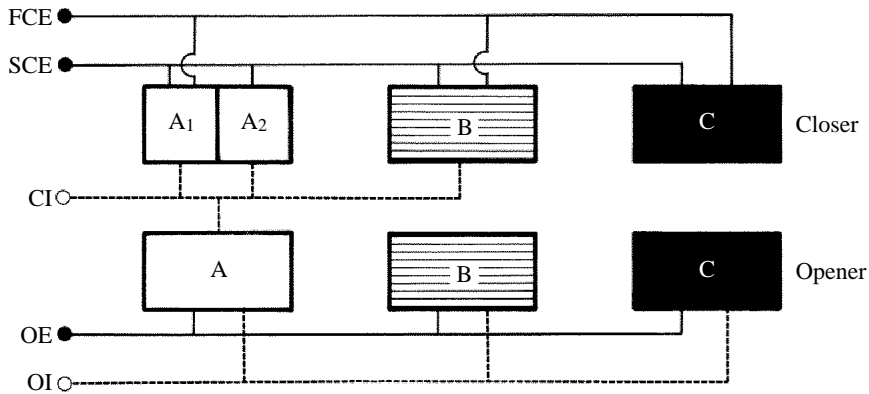

Fig. 3. Innervation pattern of the different fibre groups in the closer and opener muscles. CI, common inhibitor; FCE, fast closer excitor; SCE, slow closer excitor; OE, opener excitor; OI, opener inhibitor (specific).
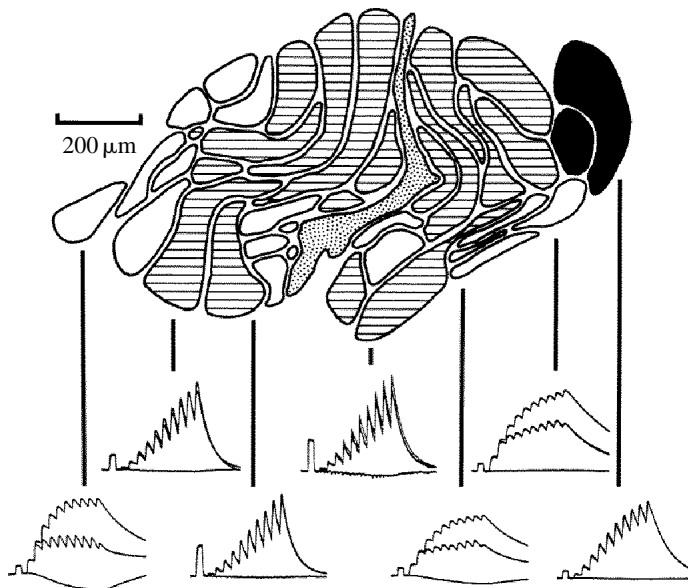

Fig. 4. Junctional responses from identified fibres in the distal part of the closer muscle of *Pacifastacus leniusculus* belonging to the three different groups. Shading as in Fig. 2. Upper traces, EJPs elicited during stimulation of SCE at 35Hz. Middle traces, simultaneous stimulation of SCE at 35Hz and CI at 70Hz. Lower traces, stimulation of CI alone at 70Hz. Inhibition in group B fibres is hardly visible at these frequencies. Calibration pulse is 2mV, 20ms.
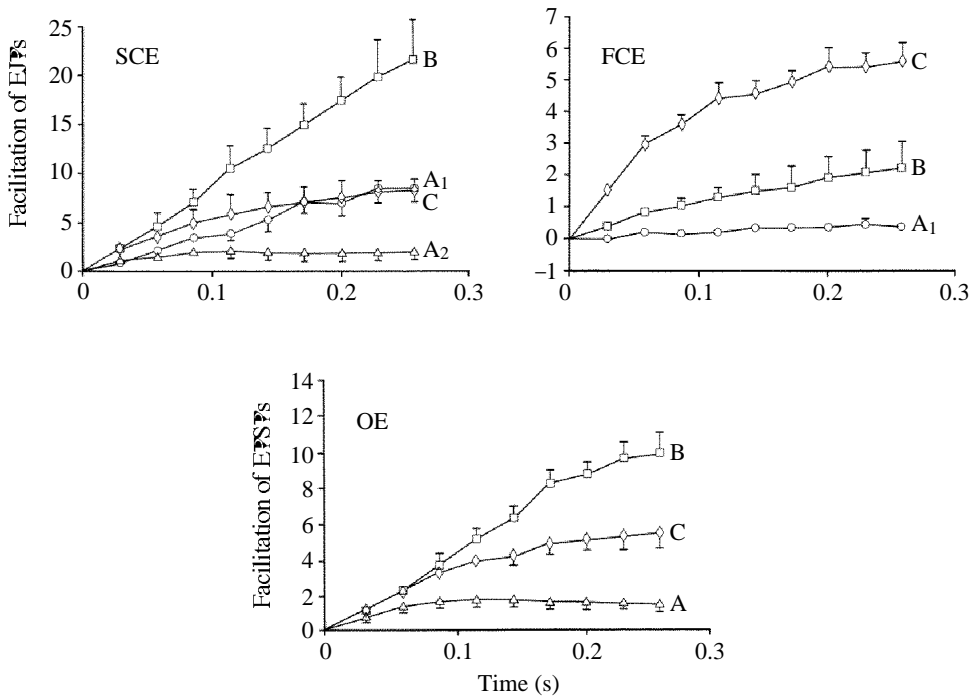
Fig. 5. Facilitation of EJPs in fibres of different groups in *Pacifastacus leniusculus* during stimulation of SCE, FCE and OE with a train of 10 pulses at 35Hz.

Discussion

Histochemistry

The histochemical determination of mATPase activity is based on the precipitation of free inorganic phosphate by calcium and subsequent stepwise transformation of the precipitate into cobalt sulphide, which is dark in colour (Gomori, 1941). The staining intensity of individual muscle fibre sections depends on the amount of phosphate produced during the time of incubation in ATP-containing media. The experimental conditions (pH, ionic strength, temperature) influence both the activity of the mATPase and subsequent reactions of the staining procedure. Because precipitation of calcium phosphate occurs at alkaline pH, incubation of sections for mATPase activity determination is usually carried out at pH9.4 (Padykula and Herman, 1955; Ogonowski and Lang, 1979).

It is known from biochemical investigations (Hájek *et al.* 1973) that the pH optima of the mATPase activity from slow and fast abdominal muscles of crayfish are between pH 7 and 8. We therefore investigated mATPase activity at a pH of 8.4 (near to the pH optimum) and 9.4 (optimal phosphate precipitation) in alternating cross sections of a muscle. At pH8.4, three fibre populations (A, B and C) with different staining intensities could be distinguished, whereas at pH9.4, group B and C fibres stained equally. Experiments with preincubation at pH10.05 demonstrated a pronounced pH lability of
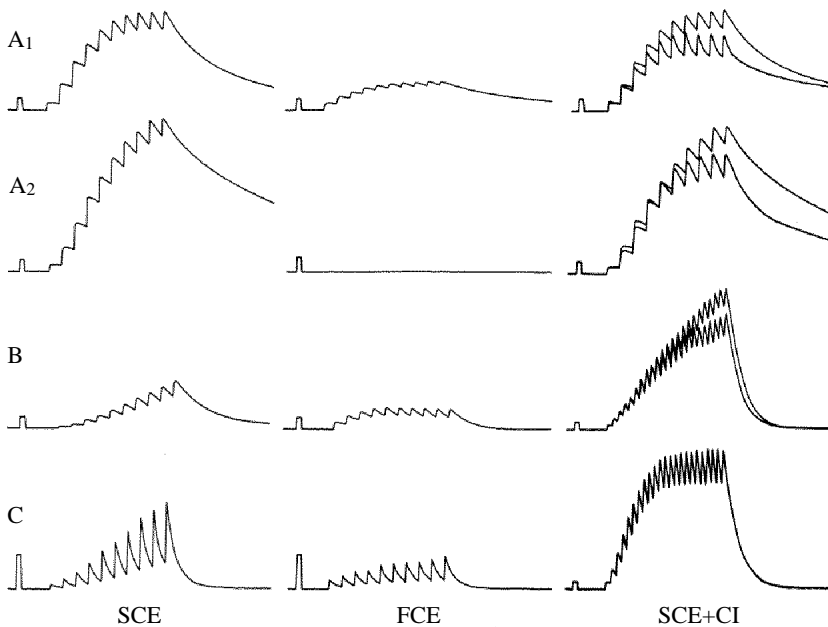
Fig. 6. Junctional responses from fibres of the closer muscle belonging to different types during selective stimulation of SCE or FCE at 35Hz. 10 sweeps averaged. The effects of stimulation of CI concomittantly with SCE are shown on the right. Two averaged traces are superimposed: one (upper) without, another (lower) with CI stimulation at different frequencies: A_1 and A_2: SCE, 35Hz; CI, 70Hz; B and C: SCE, 85Hz, CI, 90Hz. Calibration pulses 2mV, 10ms.

the mATPase activity in group C fibres in contrast to group B fibres. Thus, their equal staining intensity at pH9.4 can be explained by a partial inhibition of the mATPase activity in group C fibres at this pH. Different alkali labilities of the mATPase at pH9.4 might also explain why several authors (Lang *et al.* 1980; Govind *et al.* 1981; O'Connor *et al.* 1982; Maier *et al.* 1984) found no differences in mATPase activity among otherwise different fibre types, while others, who used a lower pH value (Tse *et al.* 1983; Rossi-Durand and Pagni, 1986), did find differences.

The mATPase activity is correlated with the maximum shortening velocity (Bárány, 1967; Barnard *et al.* 1971; Edman *et al.* 1988; Galler and Rathmayer, 1992; Müller *et al.* 1992): low activity is characteristic of slow fibres, whereas high activity is characteristic of fibres with fast shortening velocities.

Mearow and Govind (1986) reported in their paper on lobster leg muscles that the closer muscle of pristine (i.e. not regenerated) walking legs of two crayfish species (*Orconectes rusticus, Procambarus clarkii*) consists of uniform fibres with low mATPase activity. In that investigation, the mATPase activity was determined after incubating the sections for 30min at 4˚C. The time allowed for the reaction was probably too short to permit sufficient ATP splitting, so that differences in the mATPase activity (and therefore the existence of histochemically different fibre groups) did not become apparent. We incubated our sections for 15min at room temperature.
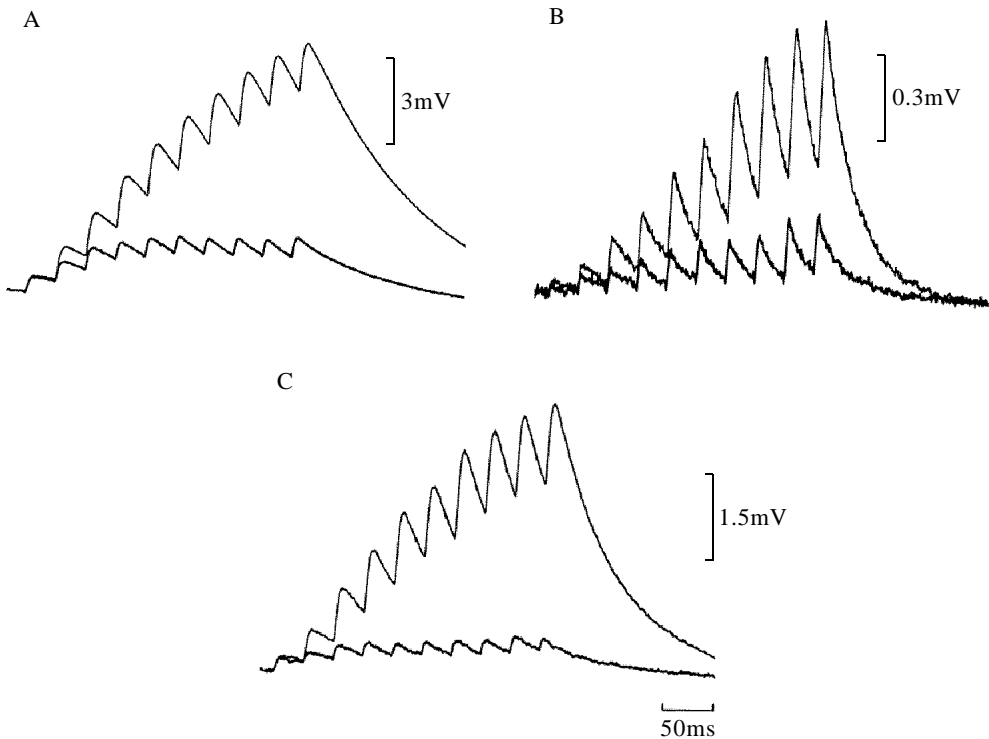
A

B

C

Fig. 7. Junctional responses from three different fibres in the opener muscle of *Pacifastacus leniusculus* during stimulation of OE at 35Hz (upper traces) and together with OI at 70Hz (lower traces). (A) group A fibre; (B) group B fibre; (C) group C fibre.

Sarcomere length

In order to determine sarcomere lengths in fibres which had previously been identified histochemically, muscle fibres had to be dissected from histochemically characterized muscles. To enable dissection of single fibres from a muscle that was frozen for cryosectioning, the muscle had to be freeze-dried. Application of the laser method then made it possible to measure sarcomere lengths of these fibres with a minimal risk of artificially changing the length of the fibres.

In the two leg muscles investigated, the mean lengths of sarcomeres differed. In general, fibres with low mATPase activity (and presumably a slow shortening velocity) had longer sarcomeres than fibres with high mATPase activity. Group A fibres in the opener muscle had the same mean sarcomere length as group B fibres in the closer, which have a much higher mATPase activity. This suggests that absolute values for sarcomere length alone are not indicative of whether a muscle fibre is of the slow or fast type. Costello and Govind (1983) also noticed that in the closer muscle of the lobster claw the rise time of contraction was not correlated with sarcomere lengths of the fibres.

Owing to the different angles of attachment between the muscle fibres and the tendon, the fibres of the distal and proximal regions (predominantly group A fibres) are only about half as long as the central fibres (group C and some group B fibres). Thus, in both

muscles, the fibre group with the shortest sarcomeres (group C) also had the longest fibres, providing the greatest number of sarcomeres in series. This, together with the high mATPase activity, should foster rapid contractions.

Neuromuscular properties

In the three crayfish species studied here, the fibres of the closer muscle exhibit only two patterns of excitatory innervation: either through SCE alone (A_2) or through SCE and FCE (A_1, B, C). No fibres were found with single FCE innervation. This is in contrast to the situation in the closer muscle of lobster walking legs where three types of innervation are present: fibres may be innervated only by SCE, only by FCE, or have double excitatory innervation (Mearow and Govind, 1986). Both patterns of excitatory innervation differ again from the situation in brachyuran crabs, where the fibres of the closer muscle receive either double excitatory or single FCE innervation (Rathmayer and Maier, 1987; Wiens *et al.* 1988). The distribution of the common inhibitor neurone (CI) also differs between Reptantia and Brachyura. In the crayfish closer, innervation by CI does not parallel that of SCE, as is the case in crabs (Rathmayer and Erxleben, 1983). Only the group A and B fibres in the crayfish are supplied by CI. Data correlating excitatory and inhibitory innervation through CI are lacking for the lobster closer muscle.

In the opener, only the proximal tonic fibres (presumably the group A fibres of the present study) receive CI innervation (Wiens, 1985), in contrast to crabs, where all fibres of the opener are innervated by CI (Wiens *et al.* 1988).

The histochemical determination of mATPase activity allowed us to discriminate between the three distinct fibre groups. Although the mean values of the other variables investigated (amplitudes and facilitation of EJPs, membrane time constants and sarcomere lengths) differed significantly among the different groups, they showed considerable variation among the fibres belonging to one group. Each variable taken alone would not suffice for a clear group separation. However, since these variables were always determined in fibres which had been individually identified histochemically, it was possible to match these properties with the histochemically assessed fibre groups.

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