Electrical and Mechanical Responses in Deep Abdominal Extensor Muscles of Crayfish and Lobster

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ABSTRACT Electrical and mechanical studies have been made of the deep abdominal extensor muscles, medial (DEAM) and lateral (DEAL), of crayfish and lobster. The medial muscle responds to direct (intracellular) and indirect stimulation with a transient membrane depolarization which exhibits the properties of a propagated non-decremental action potential but does not overshoot the zero level. The amplitude is about 30 mv in crayfish and 50 mv in lobster. It is followed by a fast all-or-none twitch whose duration at 20°C is 30 to 50 msec. and whose developed tension is 500 gm/cm^2 or about half the tetanic value. Membrane potential is K^+-dependent and immersion in high K^+ induces a brief transient tension rise as in other twitch-type muscles. The action potential and twitch are normal even if all external Na^+ is replaced with sucrose but vary with external Ca^{++}, the action potential increasing 8 to 10 mv for a twofold increase in Ca^{++}. The lateral muscle (DEAL) is much slower and responds to intracellular stimulation only with an electrotonic or a local response. Mechanical responses and relaxation speeds are slow with minimal duration of contraction of 0.5 to 2 seconds. Immersion in high K solutions induces large maintained tensions. Sarcomere length in the fast DEAM is uniform and about 2 μ at rest, but in the DEAL speed is less and sarcomere length is greater averaging about 4.5 μ but with a mixed population of fibers.

The studies described here relate to the electrical and mechanical properties of a set of crustacean muscles which consistently display a propagated action potential and an all-or-none twitch.

In general, electrical stimulation of crustacean muscles will not initiate a propagated action potential (Wiersma, 1961) but rather a local depolarization followed by a contraction of part of the cell. The tension developed depends on strength, duration, and frequency of the stimuli (Hoyle and Wiersma, 1958 *a*; Wiersma, 1961). Even in fast muscle, junction potentials are usually recorded (Wiersma, 1961) and only after long or repetitive stimuli are propagated action potentials recorded (Fatt and Katz, 1953). They occur

only in the presence of calcium ions (Fatt and Katz, 1953; Hagiwara, Chichibu, and Naka, 1964) or other bivalent cations such as strontium or barium (Fatt and Ginsborg, 1958). When sodium ions are replaced by tetra-ammonium salts, large and long action potentials are obtained (Fatt and Katz, 1953; Fatt and Ginsborg, 1958). The muscles most thoroughly investigated have been the extensor and flexor in the meropodite in the walking limb (Girardier, Reuben, and Grundfest, 1962; Atwood, 1963), the extensor of the carpopodite (Fatt and Katz, 1953; Fatt and Ginsborg, 1958), the opener and the closer of the claw (Hoyle and Wiersma, 1958 *a*, *b*, *c*; Atwood 1963), the contractor epimeralis (Orkand, 1962 *a*, *b*), and the accessory flexor muscle of the walking leg (Dorai Raj, 1964; Atwood and Dorai Raj, 1964). These seem to be slow muscles, the contraction lasting from a few hundred milliseconds up to several seconds. Furthermore, in studies using direct intracellular stimulation the stimuli were quite long (generally 50 msec. Fatt and Katz, 1953; 1 to 2 sec., Orkand, 1962 *a*) and rarely gave rise to a spike.

The fast flip movement of the crayfish and lobster tails led us to investigate the abdominal muscles, of which two extensors were selected because the anatomy and mechanics are so much simpler than for the flexor group. Propagated action potentials and twitches were indeed regularly recorded from one of these mucles in response to indirect and direct stimulation. The experiments confirm that such crustacean muscle potentials are not sodium-dependent but are determined by the calcium ion concentration.

METHODS

Recording Responses of the muscles were studied in relation to excitation applied directly to a selected muscle fiber through an intracellular electrode or indirectly by nerve stimulation. Two microelectrodes filled with 3 M KCl were inserted into the same fiber as described by Fatt and Katz (1953). Electrodes with 10 to 20 MΩ resistance and low tip potentials were used for recording and those with 5 to 10 MΩ resistance for stimulation. In order to be certain that both electrodes were in the same cell hyperpolarizing pulses were applied, and only if they were in one cell was an electrotonic response observed. Membrane characteristics were investigated by recording the amplitude and shape of the pulse at a series of distances from the stimulating electrode, and resistance was calculated from the equation given by Fatt and Ginsborg (1958).

Tension was recorded by means of an isometric tension transducer (RCA 5734 mechano-electrical transducer).

Preparation The experiments were conducted on the deep extensor abdominal medialis (DEAM) and on the deep extensor abdominal lateralis (DEAL) (Pilgrim and Wiersma, 1963) of the crayfish *Orconectes virilis* and of the lobster *Homarus americanus*. In most experiments only the muscles from the second and third segments were used.

To expose the muscles for direct stimulation, the tergal portion of the shell was

excised and the epidermis and connective tissue were carefully removed so that the muscles remained attached to their insertions. A small piece of the exoskeleton containing the muscle insertions was isolated at the anterior end and connected to the transducer by means of a small alligator clip. Several fibers remained attached to this piece of exoskeleton but with intracellular stimulation only one fiber responded as shown by sampling the electrical events in surrounding fibers. When it was desired to stimulate the muscles through their nerves, a ventral approach was preferred. In this case the dorsal part of the abdomen was removed by two longitudinal sections through the dorso-lateral lines so that the DEAM and DEAL, with their nerves, remained attached to the dorsal excised portion. The DEAM is innervated by two main trunks one of which is divided into two main branches supplying the central region of the muscle. The other trunk lies at the posterior end of the segment and innervates some of the muscle fibers in its own segment, some in the next segment. Only the central trunk was used in these experiments. The nerves were lifted on platinum electrodes for stimulation and the muscle fibers were then impaled from the ventral side. Pulse durations used for excitation were 0.05 and 0.5 msec. for nerve and muscle respectively. In the case of nerve stimulation an undetermined number of muscle fibers were excited and therefore no analyses were made of tension production in the bundle of fibers attached to the transducer.

Solutions Van Harreveld (1936) solution was used for the crayfish and the solution given by Fatt and Katz (1953) (for *Carcinus*) was used for the lobster. Changes in ionic concentration of K^+, Ca^{++}, and Mg^{++} were balanced by appropriate changes in the sodium concentration. Sodium itself was replaced by sucrose or tetraethylammonium chloride (TEA) as indicated by Fatt and Katz (1953). Experiments were carried out at room temperature (20–22°C).

RESULTS

Deep Extensor Abdominal Lateralis Muscle (DEAL) This muscle gave graded electrical and mechanical responses to direct or indirect stimulation. The fibers were parallel and ran from one segment to the next (Pilgrim and Wiersma, 1963). In the crayfish the fiber diameter averaged 300 μ and length ranged from 6 to 8 mm. Sarcomere length at rest is usually between 4 and 5μ but occasional fibers show 2 μ sarcomeres. The resting potential was 60 $\pm$ 2 mv, and calculated membrane resistance ranged from 2000 to 5000 ohm-cm with a space constant of 2 to 4 mm.

Very few fibers responded to a short intracellular stimulus lasting 0.5 msec. using the maximum current available for a stimulus isolation unit SIU4 (Grass Instrument Co.). Tension appeared when pulse duration was increased and the responses to 50 msec. depolarizing pulses are shown in Fig. 1 *a*, *b*, *c*. The duration of contraction varied over a range from 500 msec. to several seconds but in all cases the tension developed in response to one pulse was only from 20 to 50 gm/cm^2. With repetitive stimulation at 50 pulses/sec. a much stronger contraction resulted (Fig. 1 *d*). There were a few fibers of the DEAL

which responded to short internal stimuli with propagated action potentials (Fig. 2) and these were probably associated with the occurrence of the occasional fibers with short sarcomeres.

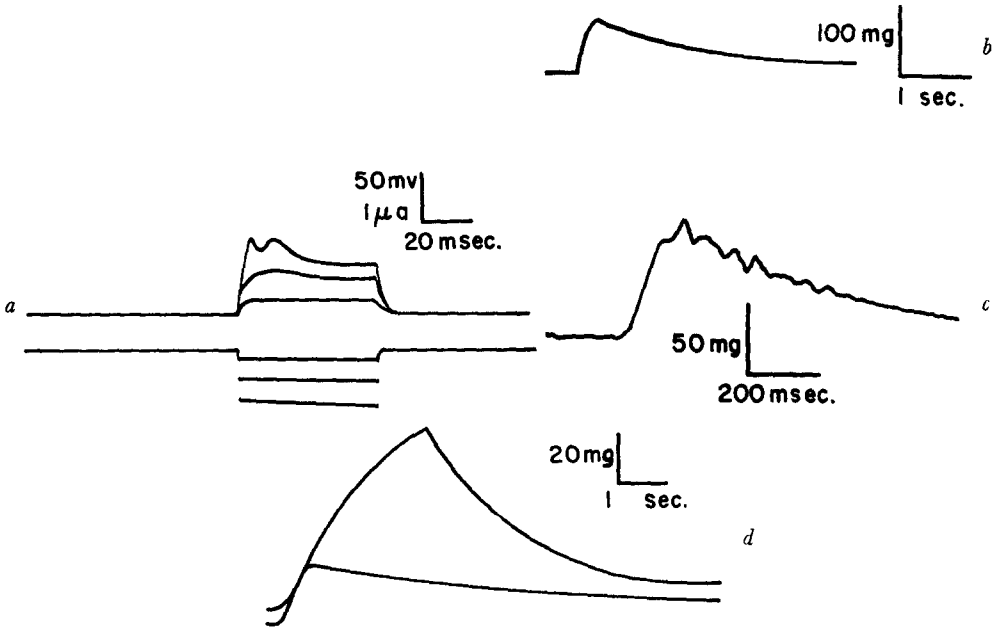

FIGURE 1. Electrical and mechanical responses of single fibers of crayfish DEAL muscle to stimulation with an intracellular electrode. (*a*) Electrotonic potential and local response (upper traces) induced by depolarizing current (lower traces). (*b* and *c*) Maximal tensions in response to 50 msec. pulses of current. Relaxation times varied usually between 0.5 and 2.5 sec. A few fibers responded with a rapid twitch. (*d*) Tension produced in response to single pulse and to repetitive stimulation (60 pulses/sec).

After immersion in TEA Ringer's with zero sodium, the fiber responded to an 0.5 msec. pulse with a large, 80 mv action potential with overshoot and a plateau lasting 120 msec. as in the extensor muscle of the carpopodite (Fatt and Katz, 1953). The tension developed was much larger, about 700 gm/cm^2.

Deep Extensor Abdominal Medialis Muscle (*DEAM*) The medial muscle fibers are also long but are twisted like a thread (Pilgrim and Wiersma, 1963) and show little interfibrillar or connective tissue. Their dimaeter is 150 to 200 μ in crayfish and 600 to 700 μ in lobster, while sarcomere length was uniformly 2 μ. The resting membrane potential averaged 65 $\pm$ 3 mv, and the membrane resistance in the crayfish was between 500 and 1000 Ω-cm with a space constant of 0.5 to 1.5 mm.

In contrast to the DEAL, these fibers responded uniformly to very brief depolarizing intracellular pulses (0.1 to 0.5 msec.) with an action potential

and a fast maximal twitch-like contraction lasting 30 to 60 msec. (Fig. 3). The amplitude of this action potential was only 25 to 30 mv, and its duration 8 to 10 msec. in the crayfish, and it did not overshoot the zero base line. Similar results were obtained in the lobster but with deflections usually between 40 to 50 mv, again with no overshoot.

The small values of these potentials raised the question whether they were true propagated non-decremental action potentials. Hoyle and Smyth (1963) showed that, in the giant barnacle muscle, transient potential changes as high as 60 mv will decrement with distance; and Furshpan and Wiersma (1954) argued that spikes evoked by nerve stimulation in the contractile part of the stretch receptor in the crayfish abdomen were local junction events since the latent period was the same when the potential was recorded at different points in the same fiber. However, the possibility of propagated action potential

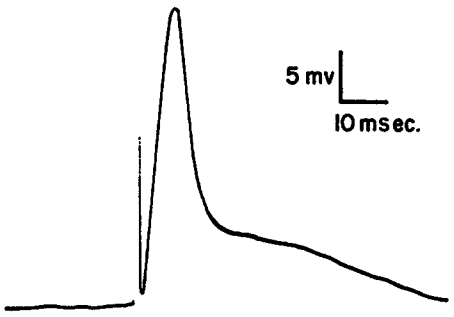

FIGURE 2. Action potential recorded in one of the few fast DEAL muscle fibers in crayfish which respond to a single stimulus of 0.5 msec. duration with a spike. Resting potential 60 mv, zero base line not shown.

cannot be dismissed (Furshpan, 1955; Wiersma, 1961). In the present experiments the potentials were shown to be non-decremental by recording at a series of distances from the internal stimulating electrode (Fig. 4 *a* and *c*). The variation of latency with distance showed the potentials to be propagated with velocities of 0.8 to 1 m/sec. in crayfish and 1 to 2 m/sec. in lobster.

It was possible that antidromic nerve stimulation distributed the response to the multiple nerve endings so a number of experiments were made to discount this possibility. The effect of nerve stimulation was itself first studied by lifting a nerve trunk on a pair of platinum electrodes. The results illustrated in Fig. 3 *b* and *d* show that the action potential and mechanical responses produced in the crayfish medial bundle (DEAM) were similar to those with direct stimulation. The latent periods with direct and indirect stimulation in lobster muscle were compared and Fig. 4 *b* shows that with nerve stimulation there was almost no change in the latent period of the muscle when the intracellular electrode was moved by 1 cm along the fiber whereas with direct stimulation (Fig. 4 *c*) there was a marked change in latency corresponding to a conduction velocity of 1.2 m/sec. If antidromic excitation were induced by intracellular

stimulation, no significant change in latency would be expected with distance (since propagation in the nerve is quite fast). Furthermore, as a result of the pattern of crustacean innervation with one nerve fiber supplying many muscle fibers, antidromic stimulation would be expected to excite many fibers.

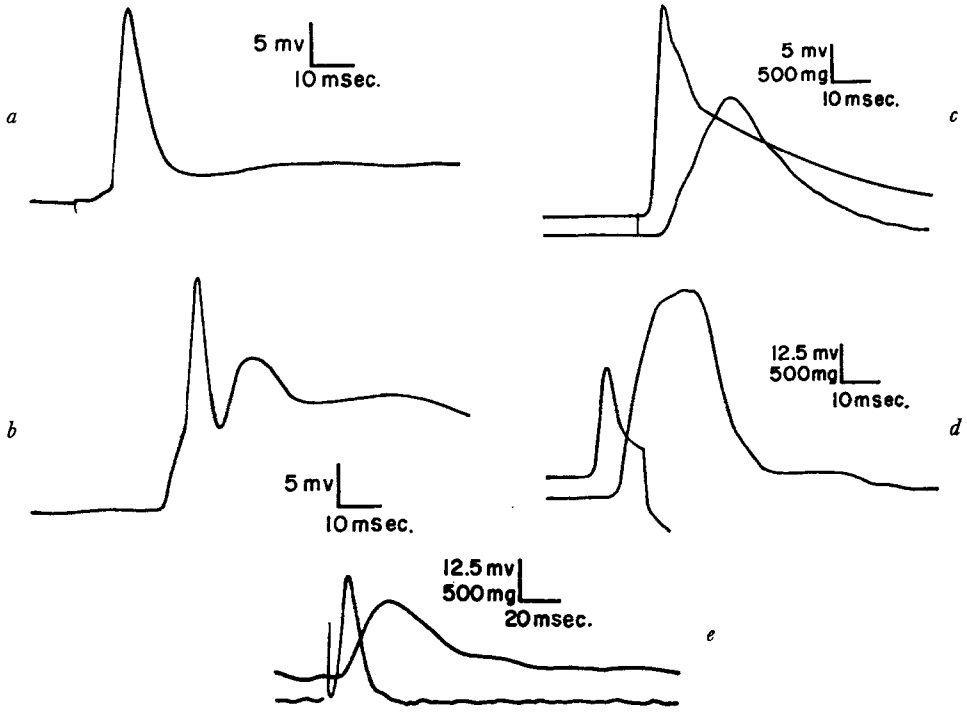

FIGURE 3. Electrical and mechanical responses in crayfish DEAM muscle fibers. (*a*) Action potential in response to a direct intracellular stimulus of 0.5 msec. duration. (*b*) Action potential in response to a single impulse (0.05 msec. duration) applied to the nerve showing a prepotential which gives rise to a spike at 10 mv depolarization. The later waves are movement artefacts. (*c*) Action potential and mechanical activity with intracellular stimulation of 0.5 msec. duration. (*d*) Action potential and mechanical activity following nerve stimulation with 0.05 sec. pulse. No prepotential is observed, probably because the recording electrode was away from a myoneural junction. (*e*) Lobster DEAM muscle fiber: action potential and electrical activity in response to intracellular stimulus of 0.5 msec. duration.

Measurements were made in neighboring fibers and activity could be recorded only in the fiber excited. A direct attempt to record any antidromic excitation was made with the nerve lifted on the platinum wire electrodes but no activity could be detected in the nerve when the muscle was stimulated intracellularly.

Evidence was also provided by causing a block of the neuromuscular junction in the fast medial muscles. It was found that the toxins produced by the chrysomonad *Prymnesium parvum* (see review by Parnas, 1963) blocked the

crayfish neuromuscular junction without affecting nerve transmission or contraction of the DEAM to direct stimulation (Parnas and Abbott, 1965). The DEAM was soaked in bathing solution containing just enough toxin to block

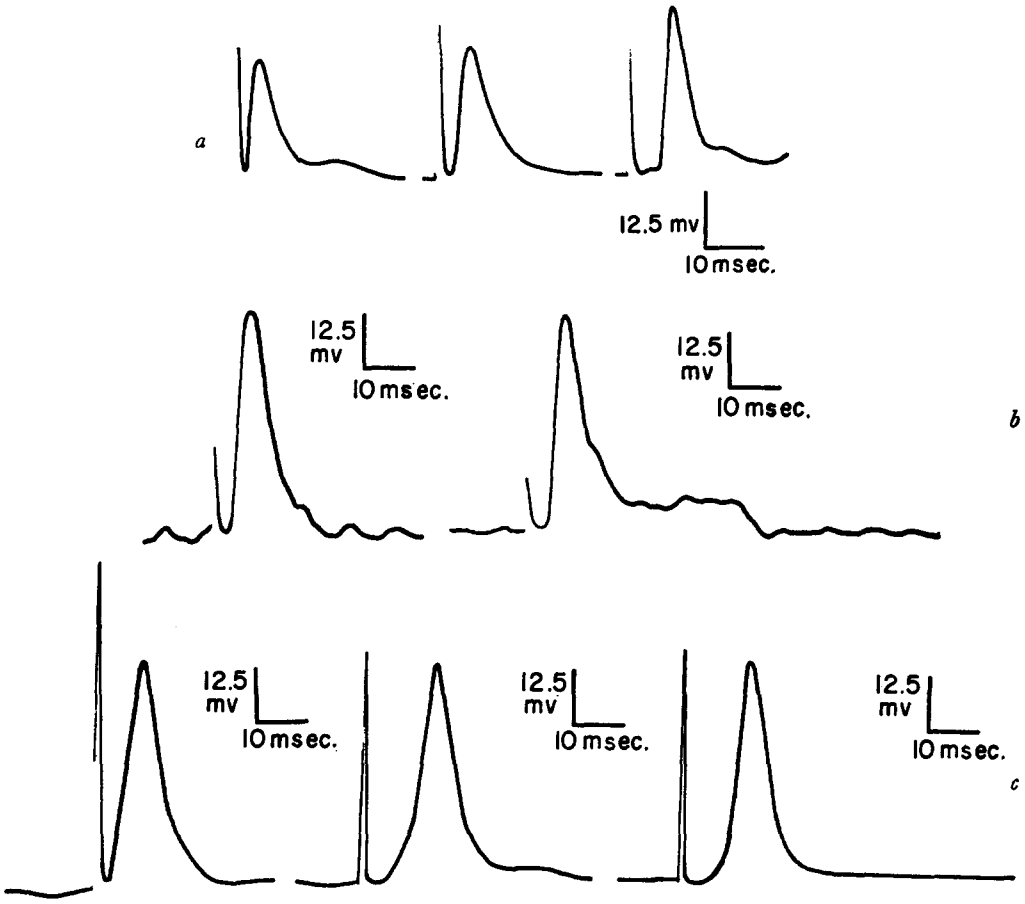

FIGURE 4. Studies on propagation of electrical activity in DEAM muscle fibers. (*a*) Crayfish muscle with internal stimulating electrode fixed and recording electrode positioned 200μ, 1 mm, and 3.2 mm distant. (*b*) Lobster muscle stimulated indirectly with electrode in the middle of the fiber in the record to the left, and moved 1 cm along the fiber in the record to the right. Note that the latent periods are almost identical. (*c*) Lobster muscle with intracellular stimulation and electrodes separated by 0.8 mm, 5 mm, and 1 cm respectively.

the junction (about 1 γ/ml of crude extract). Stimulation with an intracellular electrode in the muscle still produced an action potential with no change in threshold and gave a normal twitch although nerve stimulation gave no response. Higher concentrations of toxin caused both nerve and muscle to become inexcitable.

Ionic Requirement for the DEAM Action Potential Changes were made in the composition of the bathing fluid and it was found that removal of potassium or magnesium left unaltered the muscle responses. In the case of sodium depletion, isotonicity was maintained by addition of sucrose, and even with zero external sodium the electrical and mechanical responses remained unchanged after soaking for an hour (although excitability decreased over several hours). On the other hand, removal of calcium ions resulted in immediate decrease of the action potential (Fig. 5) and addition of EDTA up to a concentration of 3 mM in the calcium-free solution eliminated completely the electrical response. When the treated muscle was washed and placed in a nor-

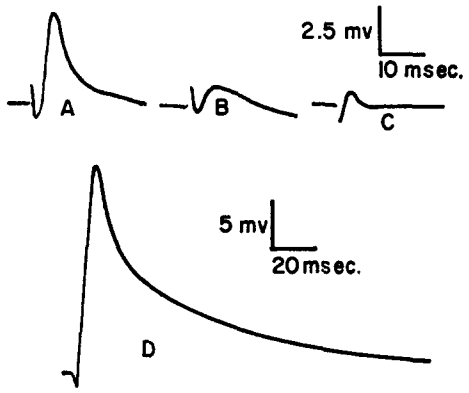

FIGURE 5. Effect of calcium on the action potential recorded in crayfish DEAM with intracellular stimulation. (A) Reduced response after 2 minute wash with calcium-free Ringer's; (B) after 10 minutes in calcium-free solution; (C) after addition of 3 mM EDTA; (D) after washing in bathing fluid containing four times normal Ca^{++} concentration. The normal potential is seen in Fig. 4 *a*.

mal bathing solution or one containing high calcium (four times normal), the action potential was restored. An attempt was made to determine the dependence of the magnitude of the action potential on external calcium concentration. Over a range of calcium from 0.5 to 4 times normal, the action potential increased 8 to 10 mv for each doubling of calcium concentration. However, in high concentrations of calcium (16 times normal) the activity of this muscle was blocked as reported for the contractor epimeralis of the crayfish (Orkand, 1962 *b*).

When sodium ions were replaced by TEA the action potential was much larger than normal (75 mv) with overshoot beyond the base line. The potential was very prolonged and the associated tension rise was larger and lasted longer than in the control (Fig. 6). This experiment seemed important in order to show that the muscle membrane, which in normal saline displays only a small

action potential, is nevertheless quite capable of supporting a large transient change with an overshoot.

Mechanical Activity of the DEAM The responses were similar to those which characterize the fast phasic striated muscles of vertebrates: an all-or-none twitch with almost symmetrical rising and relaxation phases; a total twitch duration of from 30 to 60 msec. at 20°C; fusion of tension at stimulus

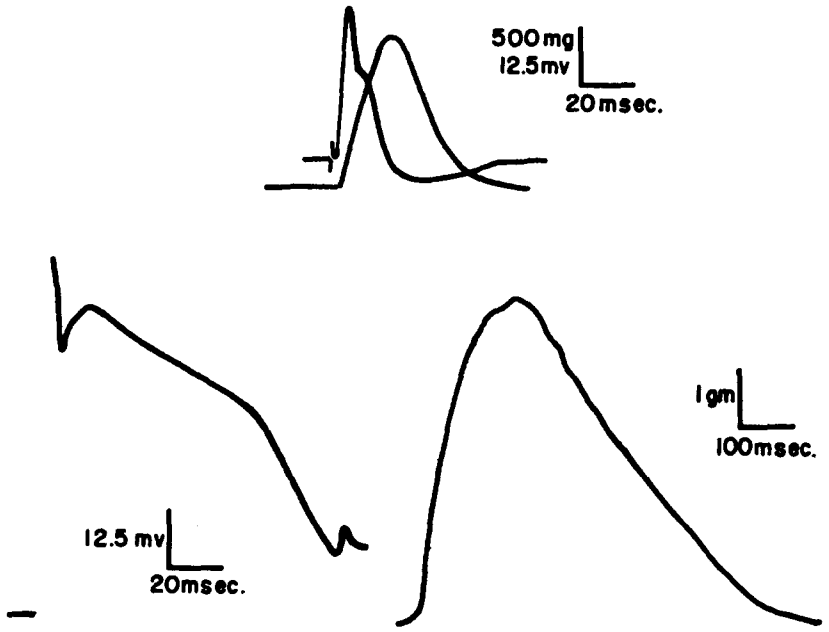

FIGURE 6. Effect of TEA on the action potential and mechanical activity in crayfish DEAM: control responses in normal bathing solution in upper curves; action potential recorded in TEA solution at lower left, and mechanical activity at lower right. Resting potential 50 mv, but zero base line is not shown. Note the overshoot in TEA solution which is absent in the control.

frequency of 60 pulses/sec. with a tetanus-twitch ratio of 2 to 1; and tetanic tension of greater than 1 kg/cm^2. This contrasted with the slow graded responses of the lateral muscle, which displays the characteristics of a tonic fiber. The differences in speed are also reflected in sarcomere lengths which are uniformly about 2 μ in the DEAM, and about 5 μ for the DEAL compared with about 10 μ for the limb muscles.

The phasic and tonic fibers of vertebrates differ in their response to solutions containing a high concentration of KCl. Single fibers of frog sartorius muscle produce a transient tension lasting only a few seconds when exposed to such a solution (Hodgkin and Horowicz, 1960) but the slow tonic fibers of rectus

abdominis or ileofibularis give prolonged contractions (Kuffler and Vaughan Williams, 1953). Comparable measurements, were, therefore, made on the DEAM (fast) and DEAL (slow) muscles of crayfish following immersion in bathing fluid containing 50 mM K^+. The DEAM produced a small tension with noticeable fibrillation but the response was transient and tension dropped to zero within a few seconds. Tension in the DEAL muscle, on the other hand, rose more slowly but was maintained for many minutes in the high K^+ solution as in the tonic type muscles.

DISCUSSION

There has been a growing awareness of a spectrum of muscle fibers in respect to speed and sensitivity within many crustaceans (*e.g.* Atwood, 1963). Atwood and Dorai Raj (1964), for example, have recently shown that the accessory flexor muscles of large walking legs of male *Cancer magister* show a distribution of mechanical properties. Bundles of proximal fibers developed no tension in response to brief direct stimuli; and there was a gradual rise of tension during prolonged depolarization. The larger fibers on the distal region of the same muscles were much faster and produced tension in response to brief depolarizations, and although spikes occurred only occasionally, they were accompanied by a rapid twitch-type contraction. Fibers from the central portion of the muscle showed a distribution between those of the two end groups. However, in these leg muscles of crabs no tension appeared in response to a single nerve impulse and the fast distal fibers produced tension only when the nerve impulse frequency exceeded 25 per second. The present experiments show that fast phasic all-or-none twitch-type muscles can occur in addition to the better known ones which give graded contractions. No studies were made here of the properties of any inhibitory nerve fibers to the fast DEAM muscle, and the results presented show only that a single stimulus applied as a maximal nerve stimulation or applied intracellularly to the muscle produces an action potential and maximal twitch in the muscle.

Spikes have often been reported in crustacean muscles, but are not the usual occurrence. The motor nerves terminate at many junction points along the muscle (Takeuchi and Takeuchi, 1964) and nerve stimulation produces non-propagated junction potentials. When spikes are induced they are generally local and non-propagated (Furshpan and Wiersma, 1954). If excitation is applied through internal microelectrodes, spikes can be produced but only with prolonged depolarization (Fatt and Katz, 1953; Fatt and Ginsborg, 1958).

Propagated non-decremental responses occur consistently in the DEAM with direct and indirect stimulation without facilitation and in response to very short single pulses. The magnitude of the potentials is small but although they never reach the zero membrane level they still appear to conform to the char-

acteristics of action potentials and have been designated as such rather than as spikes. Kennedy and Takeda (1964) reported similar responses in abdominal flexor muscles of the crayfish and referred to them as secondary electrogenic responses. Propagation of these action potentials within the animal will not be important since excitation occurs simultaneously at junctions all along the fiber. The same conclusion was drawn by Furshpan (1955) for certain crustacean fibers and by Ginsborg (1960) for the anterior latissimus dorsi of the chick.

Hagiwara, Naka, and Chichibu (1964) and Hagiwara, Chichibu, and Naka (1964) have shown conclusively that, in the giant muscle fibers of barnacles, calcium ions carry current across the membrane but propagated calcium-dependent spikes appear only when the internal calcium ion concentration is artificially reduced: otherwise the membrane response is local and graded. In the DEAM muscle the action potential is the normal occurrence, which suggests that the free internal calcium level must be very low, while the demonstrated dependency of amplitude on external calcium confirms the calcium requirement. Sodium ions have been shown not to be essential for crustacean muscles (Fatt and Katz, 1953; Fatt and Ginsborg, 1958); and similar results have been reported by Bulbring and Kuriyama (1963) for the taenia coli of guinea pig. The possibility that an unknown ion is extruded from the cell has been discussed (Fatt and Katz, 1953) and the most probable ion would be chloride. However, this ion is freely permeable in crustacean muscle (Girardier *et al.*, 1963) and under normal conditions is probably in electrochemical equilibirum (Shaw, 1955) so that a sudden fast movement of this anion is unlikely.

The two muscles described differ significantly in their responses and also in their sarcomere sizes. In the case of the crustacean muscles, there seems to be a correlation between duration of contraction of the muscle and the sarcomere organization. This has been discussed in some detail by Atwood (1963) and by Atwood *et al.* (1964). The leg and claw muscles which are slow to relax have a sarcomere length of 10 μ (Girardier *et al.*, 1963); the lateral (DEAL) muscles relax more rapidly and have a sarcomere length of 5 μ; while the fast DEAM muscles have a sarcomere length of only 2 μ. We have no knowledge of what determines the length of a sarcomere, but it is unlikely that within a single species this relationship of sarcomere length to speed is accidental.

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