The Effects of Barium, Strontium and TEA Ions on the Production of Action Potentials in the Cheliped Muscle of the Crayfish[1),2)]

By

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(With 8 Text-figures)

Mechanisms of the peripheral inhibition of the crustacean muscle have been studied by many investigators (van Harreveld and Wiersma 1937, Marmont and Wiersma 1938, Hoyle and Wiersma 1958 a,b,c, Fatt and Katz 1953 a,b, Grundfest, Reuben and Rickles 1959, Dudel and Kuffler 1961). But it has never been clarified whether the inhibition is post-synaptic or presynaptic and what effects inhibitory nerve impulses exert on the muscle membrane or contractile elements. On the other hand, it is very interesting, in comparison with the skeletal muscle of vertebrates, that the crustacean muscle evoke neither the propagated action potential nor the propagated contraction in the normal environment. Contrary to the inhibitory phenomena, some alkaline-earth ions and tetraethylammonium chloride convert the graded response to all-or-none activity and produce the action potential propagated along the muscle fibre (Fatt and Katz 1953, Fatt and Ginsborg 1958, Werman, McCann and Grundfest 1961).

Therefore, the study of the properties of the crustacean muscle fibres under various conditions, apart from their nerve junctions, seems to be necessary in order to gain some further information on the inhibitory mechanism. In the present experiments, it is attempted, with the aid of electrophysiological technique, to gain some information on the effects of Ba^{++}-, Sr^{++}- and TEA-ions on the crayfish muscle fibres and on the neuromuscular system.

Material and Methods

Preparation: The adductor and the abductor muscle of the cheliped of the crayfish, *Cambarus clarkii* were used. The cheliped was removed from the animal at the point of natural autotomy. The shell of the inside half of the meropodite was entirely cut away with

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the underlying muscle and then the main nerve trunk which consisted of three bundles was exposed. As each motor nerve supplying the adductor and the abductor muscle was contained in different bundles respectively (van Harreveld and Wiersma 1939, Nagahama 1950), the nerve fibres could be used without isolation into a single axon. Except for the nerve bundle containing the motor axon which supplied the muscle in question, all bundles were cut off with the remaining half of the meropodite. To expose the abductor muscle, which was used most frequently, the half of the shell of the propodite with the underlying adductor muscle was removed. As this muscle consisted of a fairly thin layer of fibres running obliquely like pine-needles from the shell to the chitinous tendon, this shell was used as the chamber of the muscle. This preparation was mounted on a slide glass fixed with rubber bands, and immersed in the saline bath in a plastic chamber; the muscle fibres were viewed in transmitted light through a slit opened at the bottom of the shell (Fig. 1). To facilitate the impalement of the microelectrode, the connective tissue which covered the muscle fibres was removed carefully and the mucle was gently stretched by flexing the joint.

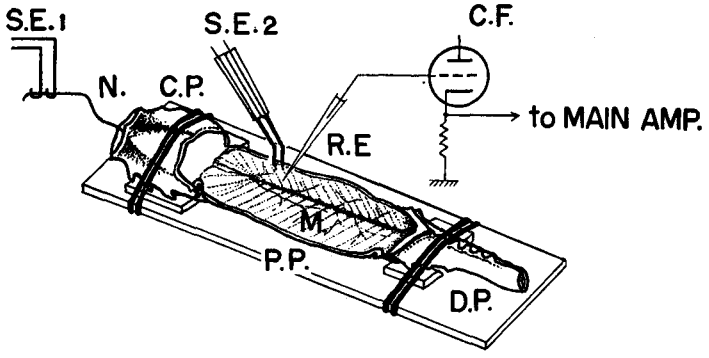

Fig. 1. Diagram of the abductor muscle preparation with recording and stimulating electrodes system. R.E.: glass capillary microelectrode; $S.E._1$ and $S.E._2$: Ag-AgCl type non-polarizable electrodes for indirect stimulation of the muscle through the nerve and for direct stimulation of the muscle itself, respectively; N.: nerve fibres supplying the abductor muscle (M.); C.P.: carpopodite; P.P.: propodite; D.P.: dactylopodite; C.F.: cathode follower.

The adductor muscle was exposed by opening a small hole in the shell. Other procedure was almost the same as that employed for the abductor muscle preparation.

Stimulation and recording: A square pulse generator was used as a stimulator. A single pulse or repetitive ones usually 0.2 msec. in duration were fed to a pair of stimulating electrodes of Ag-AgCl type through an isolating circuit from the ground to reduce the stimulus artifact. Indirect stimulation through the nerve, which was laid across a pair of stimulating electrodes, was made in the air. Direct stimulation to the muscle fibre was applied in the saline bath by means of a pair of Ag-AgCl electrodes covered with colophonium wax except for their tips. One end of the muscle fibre was put between the stimulating electrodes.

Intracellular recordings of the electrical changes of the muscle fibres were made with a glass capillary microelectrode filled with 3M KCl solution, tip diameter less than 1.0μ (direct current resistance: 5–20 megohms) (Ling and Gerard 1949). The microelectrode

was inserted into the muscle fibre with the aid of a micromanipulator under a binocular microscope. The microelectrode was connected with an input stage amplifier (cathode follower) and after amplification with a balanced direct coupled amplifier, potential changes were fed to the dual beam cathode ray oscilloscope. Particularly for recording of neuromuscular junction potentials, a high gain amplifier was used and a sensibility of 10 mm/mV on the cathode ray tube was obtained. The indifferent electrode was of Ag-AgCl type, having a considerable area, dipped in the saline bath.

Solution: The physiological solution for the crayfish (van Harreveld 1936) having the following ionic composition was employed; Na: 79.3 mM, K: 2.9 mM, Ca: 4.7 mM, Mg: 0.6 mM, Cl: 131.7 mM, buffered to pH=7.2 by Na-bicarbonate. The experimental solutions at various barium and strontium concentrations were made by adding $BaCl_2$ and $SrCl_2$ to van Harreveld's solution without changing the ionic composition of it. TEA test solution was made by the substitution of tetraethylammonium chloride for equivalent amounts of NaCl of van Harreveld's solution in various proportions. For examining the effect of hypertonic solution, a solution at twice salts concentration of van Harreveld's solution or sucrose which was mixed with van Harreveld's solution at various concentration was used.

All experiments were carried out at room temperature (19°–24°C).

Results

Indirect stimulation of the muscle through its nerve

Resting membrane potentials of the adductor and the abductor muscle ranged from 62 mV to 80 mV (inside negative). The value of the resting potentials varied in the different muscle fibres even in the same animal, and it was very difficult to measure the resting potentials accurately because of the difficulty in microelectrode impalement into the muscle fibre owing to obstructions of nerve branches and connective tissue over the fibres.

Adductor muscle: The adductor muscle receives two kinds of excitatory nerve, a fast nerve fibre and a slow one (van Harreveld and Wiersma 1936). A single shock stimulus to the fast nerve evoked twitch contraction in the muscle. In the present experiment, the slow and the fast axon were stimulated simultaneously. As is shown in figure 2, the adductor muscle responded electrically and mechanically to a single shock stimulus. Spike-like electrical changes of 6 mV in amplitude and 50 msec. in duration were elicited. Those depolarizations consisted of neuromuscular junction potentials (j.p.s.) and action potentials of the muscle. When both the frequency and the intensity of stimulation were increased, the size of individual junction potentials hardly changed. However, according as the frequency of stimulation increased, maintained depolarizations began to be set up in the muscle fibres at about 10 stimuli/sec. and the height reached 15 mV at 60 stimuli/sec. accompanied with a powerful sustained contraction as the result of the facilitation of the muscular excitement.

Abductor muscle: As the abductor muscle was supplied by only a single slow nerve fibre, it was a convenient material to use for investigating a pure slow contraction. The abductor muscle did not show contraction in response to a single

shock stimulus of the slow axon, but by repetitive stimuli of more than two pulses a weak contraction of the muscle could be elicited.

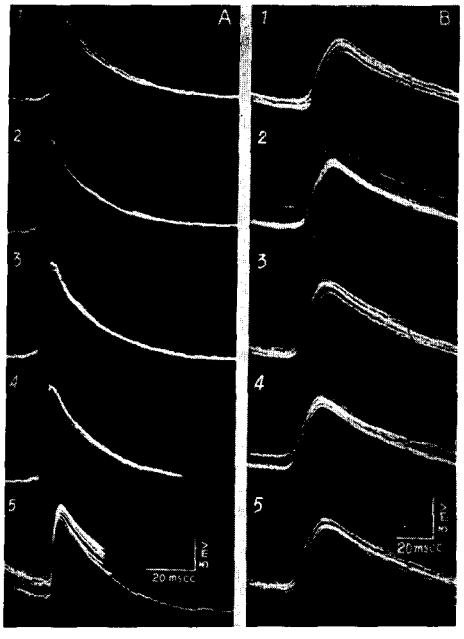

Fig. 2.

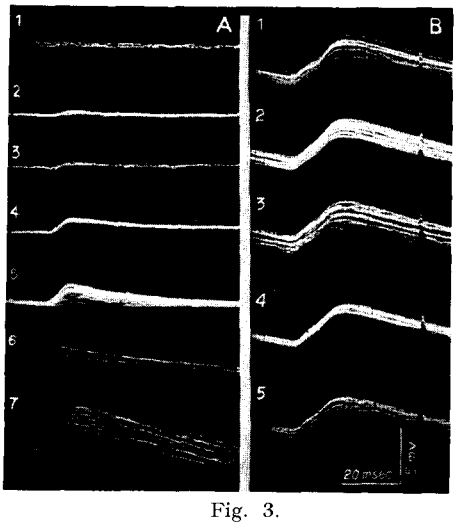

Fig. 3.

Fig. 2. Junction potentials intracellularly recorded from the adductor muscle fibre in response to repetitive stimulation of the nerve. Each record resulted from many successive sweeps synchronized with the frequency of stimulation. A: Frequency of stimulation was increased, 1 stimulus/sec in 1, 3 stimuli/sec in 2, 7 stimuli/sec in 3, 10 stimuli/sec in 4, and 20 stimuli/sec in 5, the intensity of stimulus being kept constant. B: Intensity of stimulation was increased from 1 to 5, when the frequency of stimulation (20 stimuli/sec) was kept constant.

Fig. 3. Neuro-muscular junction potentials recorded intracellularly from the abductor muscle fibre in response to repetitive stimuli of slow nerve fibre. A: Stimulus intensity being kept constant, the stimulus frequency was increased, 1 stimulus/sec in 1, 3 stimuli/sec in 2 and 3, 6 stimuli/sec in 4, 8 stimuli/sec in 5, 10 stimuli/sec in 6 and 20 stimuli/sec in 7. B: The frequency of stimulation being kept constant at 20 stimuli/sec, the intensity of it was gradually increased from 1 to 5. Time and potential scales of all records are common.

In the same way, no electrical responses of the muscle fibres to a single shock stimulus of the slow axon were detectable. But the amplitude of the electrical changes (viz. slow junction potentials) gradually increased according to increase in the frequency of repetitive stimuli (Fig. 3). The slow junction potentials (s.j.p.s.) recorded here were about 0.5 mV in amplitude and 50 msec. in duration when the nerve was stimulated at frequency of 3 per sec., 1 mV at 8 stimuli/sec.

and at last exceeded 2 mV at 10 stimuli/sec. At higher frequencies of repetitive stimuli (more than 10 stimuli/sec.), non-propagated action potentials (about 5 mV in amplitude) were set up in addition to the s.j.p.s. accompanied with a weak local contraction of the muscle. The slow nerve-abductor muscle system was highly facilitatable and according to the increase in the stimulus frequency, the individual j.p.s. were accompanied with the maintained depolarization, which were as much as 3.5 mV in amplitude at 50 stimuli/sec., 8 mV at 8 stimuli/sec. and 11 mV at 90 stimuli/sec. On the mechanical response of the muscle, the same tendency as in the electrical response was seen that the local contractions summated to a notable contraction as the stimulus frequency was increased.

As is seen in figure 3, when the stimulus frequency was increased, the stimulus intensity being kept constant, facilitation occurred in the production of s.j.p.s. while the maintained depolarization and contraction height of the muscle increased (Fig. 3, A). On the contrary, when the stimulus intensity was increased, the stimulus frequency being kept constant (at 20 stimuli/sec. which was sufficient to evoke a complete contraction of the muscle), no change in the electrical (individual j.p.s. and the maintained depolarization) and the mechanical responses were noticed (Fig. 3, B). These results revealed that in the neuromuscular system of the abductor muscle the stimulus frequency was a more important factor than the stimulus intensity. In other words, it may be considered that, in this neuromuscular system, the slow nerve fibre shows all-or-none response and in accordance with increase in frequency of stimulus, more nerve endings which are distributed over the muscle fibres are excited, consequently the magnitudes of electrical and mechanical responses of the muscle fibre increase.

As the abductor muscle was supplied by only a slow nerve fibre and its preparation could be made easily, in the following experiments, only the abductor muscle preparation was used.

Effects of Ba⁺⁺-, Sr⁺⁺- and TEA-ions on the neuromuscular system: When the preparation was treated with a solution at a low concentration of Ba⁺⁺- and Sr⁺⁺-ions (15–20 mM in the bath fluid), propagated action potentials of high amplitude and of long duration were evoked in the muscle fibre by a single shock stimulus to the nerve, accompanied with the twitch contraction of the muscle. Especially, in van Harreveld's solution whose Ca⁺⁺-ions were completely replaced by equivalent amounts of Ba⁺⁺-ions, a large propagated action potential (95 mV in height, more than 80 msec. in duration and junction potential was 34.5 mV) which has an overshoot of 7 mV was recorded with the muscle fibre. On the contrary, in the presense of Ba⁺⁺- and Sr⁺⁺-ions at a high concentration (50 mM–100 mM in the bath fluid), though the stimulation of the nerve once or twice elicited mechanical and electrical responses in the muscle fibre only immediately after the treatment, the muscle fibre soon began to show neither electrical nor mechanical responses to the indirect stimulus through the nerve, but both the nerve and the muscle themselves were still excitable. These facts indicate that Ba⁺⁺- and Sr⁺⁺-ions at a high

concentration block the neuromuscular transmission.

However, TEA which was substituted for Na^+ in the bath fluid could convert the graded response of the muscle to all-or-none activity as did the Ba^{++}- and Sr^{++}-ions. It did not block the neuromuscular transmission, even at a high concentration. Indirect stimulation through the nerve evoked large propagated action potentials of the muscle followed by all-or-none mechanical responses of all-or-none fashion.

Direct stimulation of the muscle fibres

Effect of Ba^{++}-ions: After addition of Ba^{++}-ions at various concentrations (15 mM, 30 mM and 50 mM) to the bath fluid, their action on the muscle fibre was investigated by means of direct stimulation of the muscle fibre. According to the increase of Ba^{++}-ion concentration, the test solution became a little more hypertonic. But under microscopical observation, there was no great shrinkage of the muscle fibre, even in 50 mM Ba^{++}-solution.

As is shown in Table 1, the variation of values of resting potentials in different individual fibres in the same muscle was not striking and the change in the resting potentials was not proportional to the concentration of applied Ba^{++}-ions. However, in proportion to the increase of Ba^{++} concentration, the graded responses being converted to all-or-none activity both electrically and mechanically, the amplitude of the action potential markedly increased. This effect of Ba^{++}-ions was much more striking than that of Sr^{++}-ions.

In 15 mM Ba^{++}, a spike potential could not be obtained by a single shock stimulus to the muscle and in the mechanical response only a graded local contraction was observed. 30 mM Ba^{++} completely converted the graded responses to all-or-none activity and a trapzoidal large action potential which has overshoot

Table 1. Effects of Ba^{++}-, Sr^{++}- and TEA-ions on the resting and action potential

Test solution		Resting Potential mV	Action potential		
			Amplitude mV	Duration msec	Max. rate of rise V/sec
$BaCl_2$*	15	58.9— 89.7	—	—	—
in	30	54.5— 67.5	61.3— 75.0	37.2— 50.0	5.5— 20.1
mM	50	61.6— 95.0	61.0— 96.7	35.0—104.5	80.8—315.0
$SrCl_2$*	20	63.0— 73.0	35.0— 49.0	23.0— 32.0	81.8—120.5
in	30	62.1— 81.6	42.0— 73.8	27.7— 93.8	127.0—332.0
mM	100	64.0— 86.0	67.7— 97.0	34.8— 57.0	280.0—537.0
TEA-Cl**	33	54.0— 86.3	74.2—101.0	52.9— 57.6	5.6— 15.2
in	50	62.5—116.6	36.7— 76.0	72.0—152.0	11.3— 26.3
%	100	66.7— 73.8	85.6— 90.6	96.7— 99.2	4.6

* $BaCl_2$ and $SrCl_2$ are added to van Harreveld's solution.

** TEA-Cl is substituted for NaCl in van Harreveld's solution at proportions as shown in the table.

beyond the zero level and long plateau following the spike (75 mV in height and 50 msec. in duration) was recorded accompanied with a strong contraction of the muscle. Ba^{++}-ions at a high concentration (over 50 mM in the bath fluid) had more striking action and caused a marked increase in the size of the action potential, which exceeded 96.7 mV in amplitude and 104.5 msec. in duration, especially the plateau tended to become prolonged (Fig. 4).

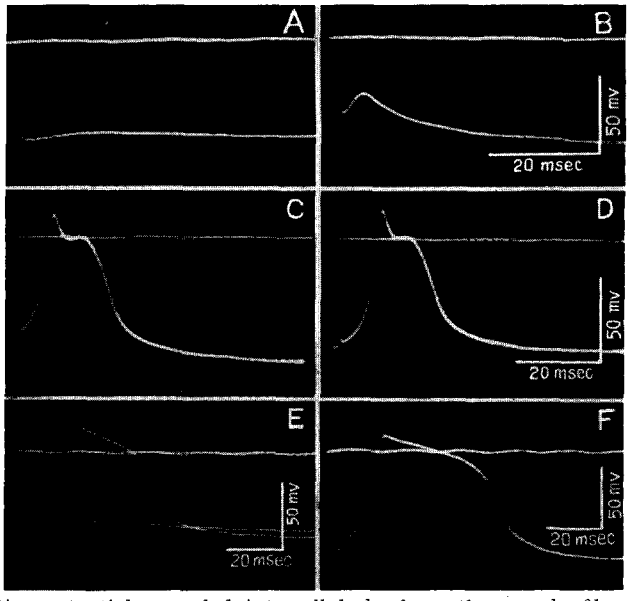

Fig. 4. Action potentials recorded intracellularly from the muscle fibres treated with Ba^{++}-ions at various concentrations: (A and B) 15 mM $BaCl_2$, (C and D) 30 mM $BaCl_2$, (E and F) 50mM $BaCl_2$ added to the bath fluid.

The threshold for eliciting an observable response of the muscle fibres decreased with increase in concentration of Ba^{++}-ions. Those trapzoidal action potentials obtained in the present experiments were similar to those obtained from the other crustacean muscles and insect muscles (Fatt and Ginsborg 1958, Werman, McCann and Grundfest 1961).

The refractory period of the muscle fibre treated with Ba^{++}-ions was generally much longer in the production of propagated action potentials, especially in the case of those at a high concentration. Figure 5 shows that when the muscle fibre treated with 50 mM Ba^{++} was stimulated at a time interval of 30 sec., the following action potentials after the first gradually decreased both in amplitude and in duration, because the successive stimuli were applied to the muscle in its relative refractory period.

Moreover, there were some cases where after the addition of Ba^{++}-ions at a

high concentration (50 mM) the shape of the action potentials recorded at several points from the shell to the tendon along a single muscle fibre was different from the trapzoidal action potential (Fig. 6). This fact was not seen in effects of Ba^{++}-ions at lower concentrations. Werman, McCann and Grundfest (1961), using two recording electrodes, observed the same fact as that described above (differences in shape of potentials) from the insect muscle in effects of Ba^{++}-ions

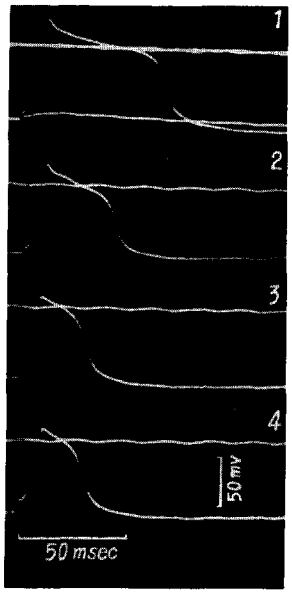

Fig. 5.

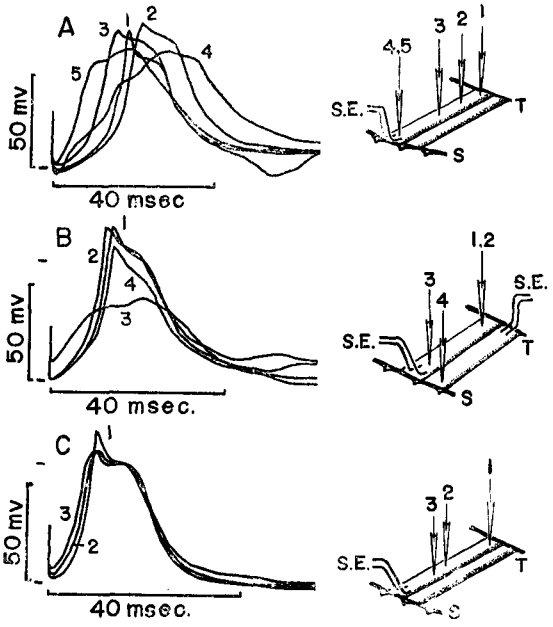

Fig. 6.

Fig. 5. Records show refractoriness of 50 mM Ba^{++}-treated muscle fibre. When successive stimuli at time intervals of 30 sec. were given, the duration and amplitude of action potentials decreased gradually from top to bottom.

Fig. 6. Three examples of tracings of action potentials obtained from Ba^{++}-treated muscle fibre at different positions as shown in the right figures. S.E.: stimulating electrodes. S.: shell of the propodite. T.: tendon. (A) 50 mM $BaCl_2$, 10 minutes' treatment, (B) 50 mM $BaCl_2$, 50 minutes' treatment, (C) 30 mM $BaCl_2$, 50 minutes' treatment. Note that normal propagation is not seen in action potentials recorded from near the stimulating electrode in 50 mM Ba^{++}, but is seen in 30 mM Ba^{++}.

and have expressed the opinion that marked irregularities in propagation are due to spatial non-uniformities in the Ba^{++}-treated membrane. In the present experiment, only the action potentials recorded near the stimulating electrodes were irregular, whilst in case of recording at a distance from the stimulating electrodes, the shape of the action potentials which seemed to propagate equally,

was constantly trapzoidal. It is conceivable that stimulating current which flowed between electrodes changed the property of the muscle membrane near them and action potential could not propagate normally through such places. The muscle membrane treated with Ba^{++}-ions at a high concentration may be especially liable to be affected by the electrical current.

Effect of Sr^{++}-ions: Effect of Sr^{++}-ions on the membrane was investigated in the same way as that of Ba^{++}-ions. Measurements made on the muscle fibres treated with Sr^{++}-ions at various concentration are summarized in Table 1. Sr^{++}-ions were less effective than Ba^{++}-ions, nevertheless at a high concentration Sr^{++}-ions gave results similar to those of Ba^{++}-ions which converted the graded response to all-or-none type. Namely, 20 mM Sr^{++}-ions produced only insignificant action potentials, which were 35–49 mV in amplitude (Fig. 7, A and B) and not accompanied with a strong contraction of the muscle; while in 30 mM Sr^{++}, the graded response of the muscle was completely converted to all-or-none activity, and the propagated action potential was produced which attained to 73.8 mV in amplitude with the overshoot, accompanied with powerful contraction (Fig. 7, C and D). At a high concentration (100 mM Sr^{++}), a tendency toward increase of the effect was seen, the action potential produced exceeded 97 mV and overshooting spikes were elicited very frequently (Fig. 7, E and F).

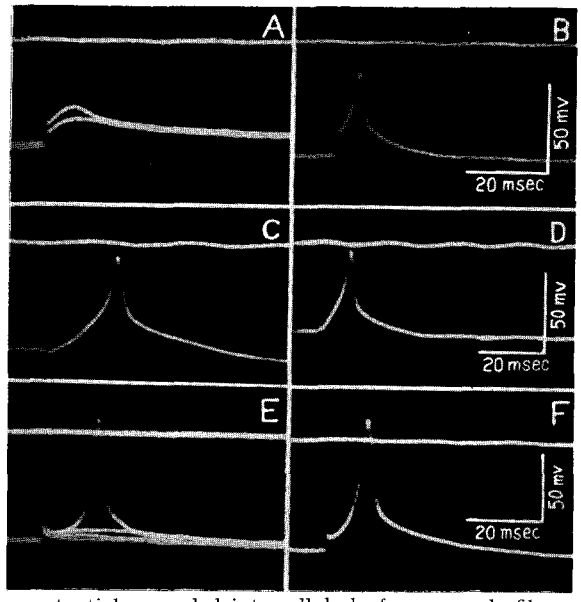

Fig. 7. Action potentials recorded intracellularly from muscle fibres treated with Sr^{++}-ions at various concentrations: (A and B) 20 mM $SrCl_2$, (C and D) 30 mM $SrCl_2$, (E and F) 100 mM $SrCl_2$ added to the bath fluid. Time and potential scales of records A-B, C-D and E-F are common.

However, the shape of action potentials obtained in Sr^{++}-ions was greatly different from those obtained from Ba^{++}-treated muscle and also from the results obtained in the crayfish muscle (Fatt and Ginsborg 1958) and the insect muscle (Werman, McCann and Grundfest 1961) in the presence of Sr^{++}-ions. The action potentials obtained in the present experiment were in the shape of a spike which rose at a greater rate and had no long plateau following it. The duration of the spike was not very long and negative after-potential followed it. It is not clear why those differences took place in the shape of action potentials between the results obtained from the present experiment and others. There may be some unknown differences in the detailed points in the experimental conditions.

Effect of TEA: When TEA-ions were substituted for Na^+ in the external medium, it converted the graded response of the muscle fibre to all-or-none type response as Sr^{++}- and Ba^{++}-ions did; they also enabled the muscle fibre to give very large and long propagated action potentials accompanied with a very strong and long-lasting contraction and a slow relaxation. In contrast to Ba^{++}- and Sr^{++}-ions, it took more than 1 hour for TEA to produce the effect on the muscle fibre; the time required for appearance of its effect did not depend on the concentration of TEA.

Action potentials evoked with substitution of TEA for Na^+ (Fig. 8) were similar in shape to those obtained from Ba^{++}-treated muscle fibres and from the heart, which were trapzoidal ones and had long plateau after the spike (Weidmann 1951). Measurements made on the muscle fibres treated with TEA at various concentrations are summarized in Table 1. Resting and action potentials of the muscle were measured 1 hour after the start of treatment. Such a tendency, as was

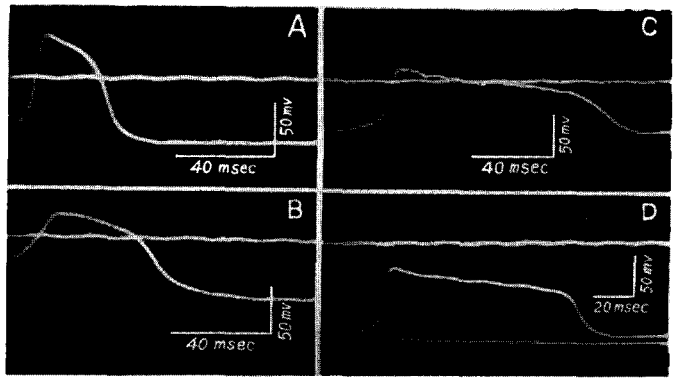

Fig. 8. Action potentials recorded from the muscle fibres treated with TEA solution at various concentrations: substitution of 33 per cent TEA for Na^+ in A, 100 per cent TEA for Na^+ in B and 50 per cent TEA in C and D for equivalent amounts of NaCl in the bath fluid. Each record was taken 1 hour after start of the treatment.

reported by Fatt and Ginsborg that the resting potential decreased according to increase of TEA, was not seen, because variation of the resting potentials in different fibres was much larger than the change of them depended upon the concentration of applied TEA.

With replacement of 33% NaCl by TEA, large propagated action potential (up to 96.4 mV in amplitude and 57.6 msec. in duration) with overshoot of the potential was evoked. Even when all NaCl was completely replaced with TEA, overshooting spike potentials (up to 90.6 mV in amplitude and 99.2 msec. in duration) of all-or-none fashion were recorded in response to a single shock stimulus. The size of action potentials recorded from TEA-treated muscle fibres did not much depend on the concentration of TEA, and action of TEA in production of overshooting spikes was already manifested almost completely even in case of TEA of a relatively low concentration (see Table 1).

However, it is interesting that the duration of action potentials obtained from the muscle treated with the substitution of 50% TEA for NaCl was much longer (115.5 msec.) than with the substitution of 33% (55.1 msec.) and 100% TEA (97.9 msec.) for NaCl.

The effect of TEA progressed irreversiblly; that does not mean that both mechanical and electrical responses increased gradually with time.

Effect of hypertonic solutions: It has been reported by Mueller (1958) that prolonged action potentials were obtained from the Ranvier's node of the frog nerve fibre in hypertonic solutions. In the present experiment, effect of hypertonic solutions of various degree, sucrose being added to van Harreveld's solution at concentrations from 50 mM to 1 M, was tested in the crustacean muscle fibres. Hypertonic solution at a high concentration (1M sucrose) made the muscle fibres shrink and become completely inexcitable. Even with hypertonic solutions of lower degrees (50 mM–100 mM sucrose in the bath fluid) and the solution at twice the salt concentration of van Harreveld's solution, no prolonged action potential was recorded, only graded responses being observed. This apparently indicates that the prolonged action potentials obtained from the muscle fibres treated with Ba^{++}- and Sr^{++}- ions were not due to the hypertonic effect.

Discussion

The observations that Ba^{++}-, Sr^{++}- and TEA-ions convert the graded responses of the muscle to all-or-none activity accompanied with propagated action potentials of a high amplitude and a long duration agree with those of Fatt and Ginsborg (1958) and others on crayfish or insect muscles.

Mechanism of Ba^{++}- and Sr^{++}-ion actions on the cell membrane are thought to be slightly different from that of TEA. From the facts that it takes more than one hour for TEA effect to appear after the application and that TEA effect progresses irreversiblly, it is assumed that TEA may change the properties of the

cell membrane and that consequently the ionic permeability increases. When TEA was completely substituted for the Na^+-ions of van Harreveld's solution, the muscle responded with propagated action potentials of a large amplitude and a long duration. This indicates that Na^+-ions are not an absolutely necessary factor in the production of the action potential which has overshoot of the potential from zero level, although the Na^+-ion has been thought to be an important factor in the mechanism (Hodgkin and Huxley 1952). Ca^{++}-ions may play an important role in production of the action potential under such circumstances that Ba^{++}-, Sr^{++}- or TEA-ions were substituted for Na^+-ions (Fatt and Ginsborg 1958).

On the other hand, as Ba^{++}- and Sr^{++}-ion concentrations in van Harreveld's solution were increased, the duration and the amplitude of produced action potential increased gradually. It may be assumed that Ba^{++}- and Sr^{++}-ions themselves have strong tendency to penetrate into the cell interior across the cell membrane during activity, in addition to the effects that Ba^{++}- and Sr^{++}-ions change the properties of the cell membrane and increase the ionic permeability during activity like TEA-ions.

It has been reported that propagated action potentials were obtained 1) from the frog muscle in an isotonic $CaCl_2$ solution (Tamasige 1956) and 2) from the insect and the lobster muscle fibres in the saline containing excess Ca^{++}-ions (Werman, McCann and Grundfest 1961). It seems that Ca^{++}-ions have the same effect as Ba^{++}- and Sr^{++}-ions. From those facts, it is suggested that it may be due to lack in amounts of Ca^{++}-ions in the bath fluid that in the crayfish muscle the all-or-none propagated action potentials is not evoked in the normal saline.

In addition, it may be thought that the mechanism of peripheral inhibition of Crustacea is contrary to the increase of excitability of the muscle membrane caused by the treatment of Ba^{++}-, Sr^{++}- or TEA-ions. It is likely that in the peripheral inhibition 1) the transmitter substance released from the inhibitory nerve endings may affect Ca^{++}-ions in the muscle membrane or van Harreveld's solution, and that 2) the property and consequently permeability during activity of the post-synaptic membrane of the muscle may change, whose sensibility to excitatory nerve impulses decreases.

It is of great interest that in the presence of Ba^{++}- and Sr^{++}-ions at higher concentrations the neuromuscular transmission was blocked, but not in the case of Ba^{++}- and Sr^{++}-ions at lower concentrations nor in TEA. Ba^{++}- and Sr^{++}-ions act to decrease permeability of the cell membrane. Too high concentrations of Ba^{++}- and Sr^{++}-ions may inhibit the increase in permeability of the post-synaptic membrane during activity.

Summary

1. Effects of Ba^{++}-, Sr^{++}- and TEA-ions on the electrical activities of the muscle fibres and neuromuscular system of the crayfish cheliped were studied.

2. Under the normal conditions, both the adductor and the abductor muscle showed only graded responses to not only indirect stimulation through the nerve but also to direct stimulation of the muscle, producing small junction potentials which could be facilitated by increase in the frequency of repetitive stimuli.

3. Graded responses of the muscle were converted to the propagated response of all-or-none fashion by Ba^{++}-, Sr^{++}- and TEA-ions in addition to van Harreveld's solution.

4. Ba^{++}- and Sr^{++}-ions at medium and high concentrations (over 30 mM) blocked neuromuscular transmission although they increased the excitability of the muscle fibre, but those facts were not observed in cases of the use of TEA-ions and Ba^{++}- and Sr^{++}-ions at lower concentrations.

5. When Ba^{++}-ions were applied at a concentration of over 30 mM in the bath fluid, large propagated action potentials which had frequently overshoots of the potential beyond zero level and had long-lasting plateau after a spike, were produced by a single shock stimulus to the muscle, accompanied with strong propagated contraction of the muscle. Effects of Ba^{++}-ions increased according to increase of Ba^{++}-ions concentration.

6. Similarly, in Sr^{++}-ions in the bath fluid (over 30 mM), propagated action potentials were obtained from the muscle, which also evoked strong propagated contraction. Overshooting spikes were elicited very frequently, but they were typical spike potentials and had no plateaus. Action of Sr^{++}-ions was a little less effective than that of Ba^{++}-ions.

7. When the muscle was treated with Ba^{++}- and Sr^{++}-ions at various concentrations, long-lasting refractoriness of the muscle was observed (over 30 seconds in 50 mM Ba^{++}).

8. When TEA-chloride was substituted for NaCl in the bath fluid at various proportions, prolonged and propagated action potentials which showed overshoots were frequently obtained from the muscle, followed by long-lasting contraction of the muscle. Even in the solution of complete replacement of the bath NaCl by TEA, prolonged action potentials were also obtained.

9. A possible explanation of the production of prolonged action potential and, besides, of the inhibitory phenomena is also presented.

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