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Membrane Constants and the Shape of Action Potential of the Frog and Crayfish Muscle Fibres¹⁾

By

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(With 12 Figures and 4 Tables)

The electric membrane constants of muscle or nerve fibre of the frog and crustacea were measured by Hodgkin and Rushton (1946), Fatt and Katz (1951, 1953), Tamasige (1950), and Washio and Kakiuchi (1962). But there is no report on the investigation of the relation between the electric membrane constants and the shape of action potential recorded with the intracellular microelectrode except the short report by Washio and Kakiuchi (1962).

The object of the present experiment is to measure the various electric membrane constants of muscle fibre with the intracellular microelectrodes, and to study the relation between these constants (in particular the resistance and capacity) and the change in shape of the action potential. It is also the main purpose to compare these values with the results obtained by Tamasige (1966) with the external microelectrodes.

The electric properties of the membrane change variously according to the external ionic component and concentration. The electric constants have a large difference in the value between the resting and active states. In the present work, the effect of different external cations such as TEA-, Ca-, Sr- and Ba-ions on the electric membrane constants was studied. It was also shown that these ions themselves enter the fibre with Na-ions when the action potentials were produced. The difference between the effects on the frog and crayfish muscle, and the characteristics of action of each ion on muscle fibre membrane are discussed.

Materials and Method

Preparation and experimental arrangement: Muscle fibres of semitendinosus muscle of the frog (*Rana japonica*, *Rana chensinensis*) and of dorsal extensor muscle of the crayfish

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(*Procambarus clarkii*) were used. The semitendinosus whole muscle was dissected from the hind leg of the frog. The complete preparation of the muscle had small pieces of tendon at both ends. It was spanned between the small glass hooks which were attached to the both side walls of a plastic chamber through the hole in the tendon. The normal frog Ringer's solution or other experimental solutions (see the below explanation) were filled in the chamber. The muscle was fixed to the chamber in just stout length, and then the membrane of the whole muscle was stripped off to facilitate the penetration of the microelectrode into the muscle fibre.

The abdomen of the crayfish was removed from the animal. To expose the abdominal extensor muscle, the external skeleton of the abdomen with the underlying abdominal flexor muscle was removed. It was mounted and fixed on a slide glass with rubber bands, and immersed in the saline bath in a plastic chamber; the muscle fibres were observed in transmitted light through a slit opened in the shell.

Application of electrical current and intracellular recordings of the electrical changes of the muscle fibre membrane were made with the two glass capillary microelectrodes filled with 3M KCl solution, the tip diameter about $1.0\ \mu$ (direct current resistance: 5–10 M Ω), connected to Ag-AgCl type non-polarizable electrode.

Experimental procedure: The two microelectrodes were inserted into the same fibre, the one to send current through the membrane, another to record the potential change across membrane. Direct stimulation of 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. A single pulse or repetitive ones usually 4–9 msec. in duration delivered from an

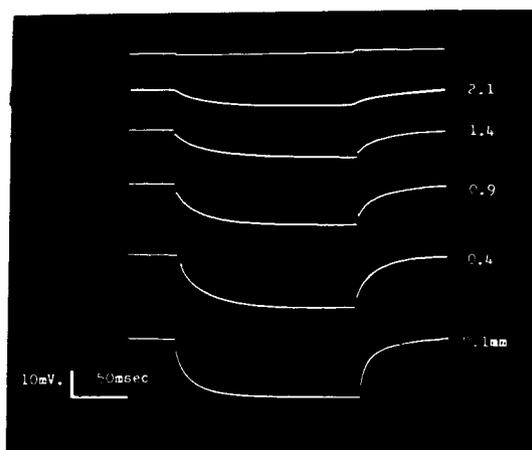


Fig. 1. Electrotonic potentials. *Rana japonica* muscle fibre. Top record; cathodic current pulse. The other records show membrane potential changes at the indicated distances from the point of current application.

electronic stimulator were fed to the pair of stimulating electrodes through an isolating circuit from the ground to reduce the stimulus artifact.

The microelectrodes were operated by separate manipulators and inserted into the muscle fibre under a binocular microscope (magnification $\times 37.5$). The distance between

electrodes tips were measured with an eyepiece micrometer. The entry of the electrodes into same fibre was indicated by the sudden appearance of the resting potential and by the presence of electrotonic potential with the recording electrode when rectangular current pulses, 0.1–0.3 μA intensity, 40–100 msec, duration were sent with the current sending electrode.

Current and potential across the fibre membrane were amplified through the separate channels and displayed on a dual-beam cathode ray oscilloscope and photographically recorded. The current intensity was measured from the potential difference across a 10 K Ω resistor. The technique for measurement in the present experiment was fundamentally the same as that employed by Fatt & Katz (1951, 1953).

Calculation: For the measurement of electric constants of muscle fibre membrane, the same technique of rectangular current pulse as that described by Hodgkin & Rushton (1946) and Fatt & Katz (1951, 1953) was employed. Electrotonic potential changes were produced by altering the distance between current applying electrode and voltage recording electrode.

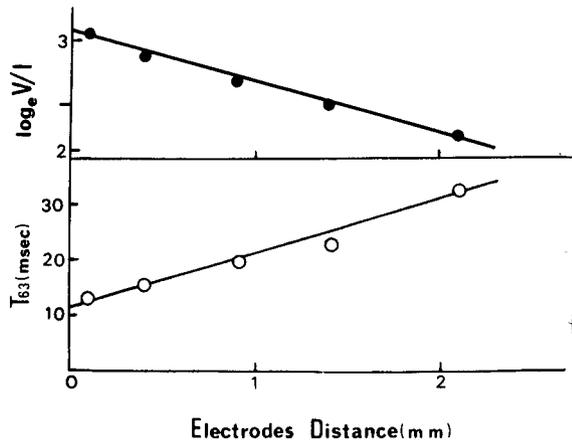


Fig. 2. Electrotonic potentials. *Rana japonica* muscle fibre. Abscissa; distance between intracellular 'current' and 'voltage' electrodes. Upper part; spatial decrement of steady anelectrotonic potential. Ordinate; relative size of electrotonic potential in logarithmic scale. Lower part; variation of '63%-time' of electrotonic potential with distance (used for determining membrane time constant). Ordinate; '63%-time' in msec.

For a distance x between the two internal electrodes, the steady potential change V_x recorded at the voltage electrode was related to the steady current I flowing through the current electrode. The current, the steady-state potential, and the time required to reach 63% of this potential value (T_{63}) were recorded at each separation. In each fibre, transfer resistance R_x (the ratio of the potential at electrode separation x to the current applied) was plotted against electrode separation. One of these graphs is given in Fig. 2. The "y intercept" of the drawn line gives the input resistance (R_0) of the fibre. The following equation are given to that line by Fatt & Katz.

$$\frac{V_x}{I_0} = R_x = 1/2 \sqrt{r_m/r_i} e^{-x/\sqrt{r_m \cdot r_i}} = R_0 \cdot e^{-x/\lambda} \quad (1)$$

where r_m is the resistance of 1 cm length of fibre membrane, r_i is the resistance of 1 cm length of the intracellular fluid. $\sqrt{r_m/r_i}$ = the length constant λ , $1/2\sqrt{r_m \cdot r_i}$ = the effective input resistance R_0 ; i.e. V/I_0 at zero electrode separation, I_0 is the current applied. And the equation (1) is also transformed into the following form, $\log V/I = -1/\lambda \cdot x + \log R_0$. Consequently, the resistance per unit length is given by $r_i = 2 R_0/\lambda$ and the resistance of a unit length of membrane is $r_m = 2\lambda R_0$. The resistance of the intracellular fluid can be determined from r_i if the fibre is assumed to be a circular cylinder of radius a ; $R_0 = r_i(\pi a^2)$. Similarly, the specific resistance of the membrane is $R_m = r_m(2\pi a)$. Thus, by plotting the decrement of potential with distance, the input resistance and space constant can be directly determined, and then, from these parameters, the specific membrane resistance and internal resistance.

The method used here to determine the specific capacitance of the membrane consists of measuring the time the electrotonic potential took to reach 63% of its steady-state value. Hodgkin and Rushton (1946) have shown empirically that in nerve axons a plot of $T_{1/2}$ against x approximates a straight line. Such plot of T_{63} were made here against electrode separation. One of these graphs is shown in Fig. 2. The "y intercept" of the line, T_{63} , is a measure of the membrane time constant. The specific capacitance of the membrane were calculated from the relation, $R_m \cdot C_m = \tau_m$, where τ_m is the time constant of the membrane and C_m the capacity of 1 cm² of membrane.

Solutions: The Ringer's solution for the frog employed contained 120.0 mM NaCl, 2.0 mM KCl, 1.2 mM CaCl₂ and 2.0 mM Na-phosphate. The physiological solution for the crayfish (van Harreveld's solution) consisted of 202.0 mM NaCl, 5.25 mM KCl, 14.0 mM CaCl₂, 2.67 mM MgCl₂ and 2.8 mM NaHCO₃. Isotonic test solutions of the various ions were used. They were made by substitution of isotonic solution of TEA, CaCl₂, SrCl₂ and BaCl₂ for equivalent amounts of solution of NaCl in various proportions. The bathing solution was changed by draining off with pipette and then by careful introduction of new solution. The old solution was washed out by replacing the new solution several times. All the test solutions used for the crayfish muscle always contained 10⁻³g/ml procain hydrochloride to reduce the effect of nervous stimulation.

All the experiments were made at room temperature of 20-23°C

Results

Electric constants of the resting fibre The electric constants in the several experimental solutions were determined. The values obtained are shown with those in the normal solution in Table 1.

Effects of TEA-ions (Table 1 and Fig. 3): After addition of TEA-ions at various concentrations to the bathing fluid, the membrane resistance values were generally larger than that of the normal solution. However, in the Na-free solution, the membrane resistance slightly decreased than that of Na-containing solution at a high concentration of TEA. Such a tendency was also seen in the cases of the other three kinds of ions. While, the membrane capacity values had smaller value than those in the normal solution.

The addition of 50 mM TEA to the crayfish solution caused the resistance value of the fibre to be approximately twice times higher. In 120 mM TEA (isotonic

Table 1. Effect of TEA-ions on electric constants of the frog and crayfish muscle fibres. τ_m ; time constant, λ ; length constant, Rm and Cm; specific membrane resistance and capacity, NR; Normal Ringer solution for frog. NS; Solution for crayfish. F.; frog, C.; crayfish. Each value is an average of data from more than 6 fibres.

TEA ⁺⁺ conc. (mM)		τ_m (msec)		λ (mm)		Rm ($\Omega \cdot \text{cm}^2$)		Cm ($\mu\text{F}/\text{cm}^2$)	
F.	C.	F.	C.	F.	C.	F.	C.	F.	C.
0(NR)	0(NS)	12.0	2.3	2.4	1.7	2,800	1,000	4.3	2.3
15.6	20	12.7	2.3	2.6	2.3	3,360	1,700	3.8	1.4
31.3	50	14.4	2.2	2.9	2.6	4,000	2,274	3.6	1.0
62.5	100	15.7	2.0	3.0	2.8	4,480	2,670	3.5	0.8
120 (Na-free)	200 (Na-free)	15.7	1.8	3.0	2.7	4,400	2,450	3.6	0.7

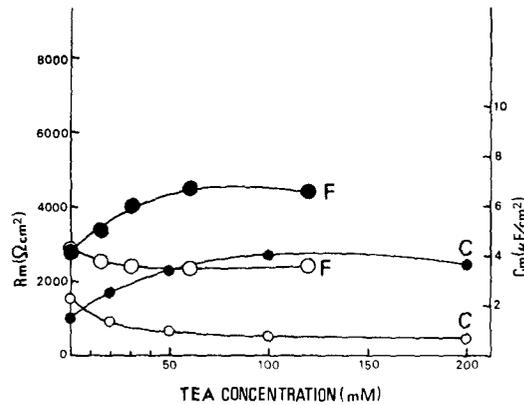


Fig. 3. Graphical expression of Rm and Cm values in TEA-ions derived from the values of Table 1. F; frog, C; crayfish.

—●—:Rm —○—:Cm

TEA-chloride) solution the resistance of the frog muscle fibres rose up to 4,400 $\Omega \cdot \text{cm}^2$ and in 200 mM TEA (Na-free) solution the membrane resistance of crayfish muscle was about 2,500 $\Omega \cdot \text{cm}^2$.

Effect of Ca-ions (Table 2 and Fig. 4): In the Ca-ions rich solution, the resting potential was liable to be reduced by injury of the muscle fibre membrane caused by penetration of the microelectrode. But an attempt was made to measure the resistance of only the fibres which had a value of resting potential about 90 mV for the frog muscle and 76 mV for the crayfish muscle at different concentrations of Ca-ions.

A considerably greater increase in the resting resistance appeared especially in the frog muscle when the Ca concentration was increased. When the Ca concentra-

tion was decreased to a half of the normal, the resting resistance were decreased and the membrane capacitance increased. The membrane capacitance measured can be seen to be lower at the higher Ca concentration. Sometimes the smaller values of the resting potentials were observed. It may due to the greater effect of leakage around the electrode tip with higher Ca concentration when the membrane resistance of the resting fibre was higher.

Effect of Sr-ions (Table 3 and Fig. 5): Effect of Sr-ions on the membrane constants was investigated in the same way as that of above ions at various concentrations.

The shape of electrotonic potentials obtained in the solution containing Sr-ions was different from those obtained in the cases of the other ions, and it had a rugged plateau. It may be thought that the Sr-ions may change the properties of the muscle fibre membrane and made them unstable. In this experiment, the membrane resistance R_m and the membrane capacitance C_m also increased in

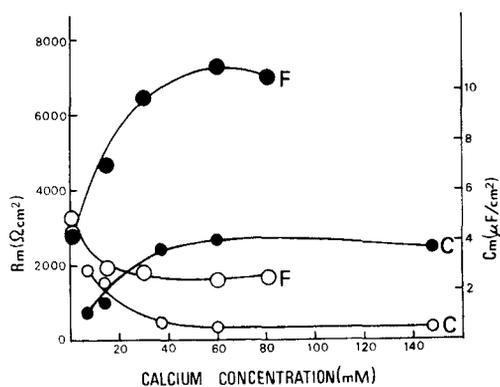


Fig. 4. The same as Fig. 3. in Ca-ions derived from Table 2.

Table 2. Effect of Ca-ions on electric constants. half; solution containing Ca of half volume of the normal solution. Each value is an average of data from more than 6 fibres.

Ca ⁺⁺ conc. (mM)		τ_m (msec)		λ (mm)		R_m (Ω . cm ²)		C_m (μ F/cm ²)	
F.	C.	F.	C.	F.	C.	F.	C.	F.	C.
0.6(half)	7(half)	9.4	2.0	2.0	1.5	1,920	720	4.9	2.8
1.2(NR)	14(NS)	12.0	2.3	2.4	1.7	2,800	1,000	4.3	2.3
15		13.5		3.1		4,660		2.9	
30	37	17.4	1.7	3.6	2.7	6,440	2,433	2.7	0.7
60	60	17.5	1.4	3.9	2.8	7,280	2,667	2.4	0.5
81.2 (Na-free)	148 (Na-free)	17.4	1.2	3.8	2.7	7,010	2,480	2.5	0.5

Table 3. Effects of Sr-ions on electric constants. Each value is an average of data from more than 6 fibres.

Sr ⁺⁺ conc. (mM)		τ_m (msec)		λ (mm)		R _m ($\Omega \cdot \text{cm}^2$)		C _m ($\mu\text{F}/\text{cm}^2$)	
F.	C.	F.	C.	F.	C.	F.	C.	F.	C.
0(NQ)	0(NS)	12.0	2.3	2.4	1.7	2,800	1,000	4.3	2.3
15	14	23.8	4.4	2.9	2.2	4,100	1,610	5.8	2.7
30	37	26.8	11.8	3.0	2.6	4,400	2,320	6.1	5.1
60	60	30.0	16.9	3.1	2.8	4,610	2,590	6.5	6.5
80	143	28.7	16.2	3.0	2.7	4,410	2,490	6.5	6.5
(Na-free)	(Na-free)								

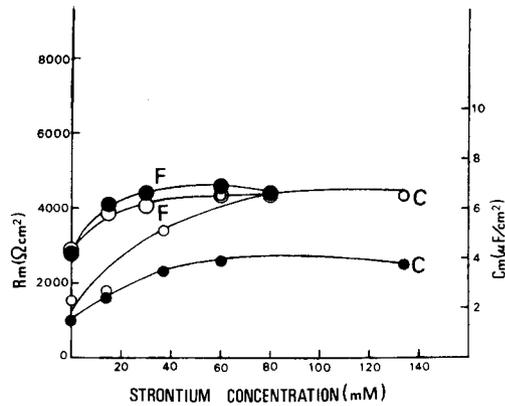


Fig. 5. The same as Fig. 3. in Sr-ions derived from Table 3.

proportion to the increase of concentration of Sr-ions similarly to the result obtained from the crayfish muscle in Ba-ions.

Effect of Ba-ions (Table 4 and Fig. 6): The effects of Ba-ions on the membrane constants were examined in the same way as those of TEA-, Ca- and Sr-ions. The Ba-ions prolonged the duration of electrotonic potential showing a plateau according to their concentration especially in the crayfish muscle with an accompanying increase in the potential size. According to the increase of Ba-ions concentration, the membrane resistance values markedly increased. In the solution of 60 mM Ba-ions, it was particularly high. The other constants, the time constant τ_m and the length constant λ was greatly increased in proportion to the increase of Ba-ions concentration except the isotonic pure Ba-solution for the frog muscle and the Na-free solution for the crayfish muscle, while the membrane capacitance C_m decreased in the frog muscle, but increased considerably in the crayfish muscle.

There were some cases where after the addition of Ba-ions at any concentration the shape of the electrotonic membrane potentials recorded at several points

Table 4. Effect of Ba-ions on electric constants. Each value is an average of data from more than 6 fibres.

Ba ⁺⁺ conc. (mM)		τ_m (msec)		λ (mm)		Rm ($\Omega \cdot \text{cm}^2$)		Cm ($\mu\text{F}/\text{cm}^2$)	
F.	C.	F.	C.	F.	C.	F.	C.	F.	C.
0(NR)	0(NR)	12.0	2.3	2.4	1.7	2,800	1,000	4.3	2.3
15	14	15.2	23.4	3.7	2.5	6,620	2,018	2.3	11.6
30	37	16.9	30.1	4.1	2.8	8,060	2,510	2.1	12.0
60	60	17.0	31.8	4.2	2.8	8,500	2,629	2.0	12.1
80 (Na-ree)	134 (Na-free)	17.1	27.2	0.4	2.7	7,830	2,347	2.2	11.6

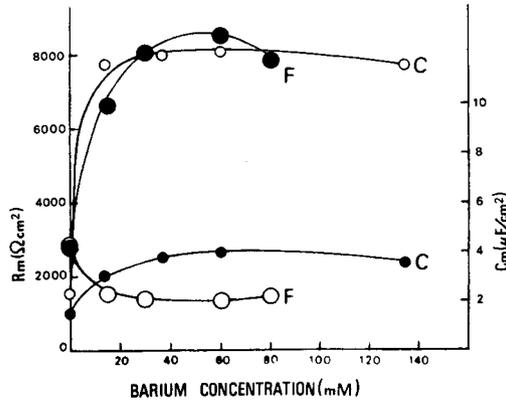


Fig. 6. The same as Fig. 3, in Ba-ions derived from Table 4.

along a muscle fibre was different from the trapezoidal electrotonic potential shape. Werman, McCann and Grundfest (1961), using two recording electrodes, observed the same fact (differences in shape of potentials) from the insect muscle in effects of Ba-ions 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, the electrotonic potentials recorded at a point from the stimulating electrodes were irregular; the shape of the potential which did not seem to propagate equally, was not constantly trapezoidal. It is conceivable that the stimulating current which flowed between electrodes changed the property of the muscle membrane near them and electrotonic potential could not propagate normally through such places. The muscle membrane bathed in the solution of Ba-ions at a high concentration may be especially liable to be affected by exposure to the electrical current.

In every above case, in the isotonic medium containing pure TEA-Cl, CaCl₂, SrCl₂ or BaCl₂ for the frog muscle and in the Na-free solution for the crayfish

muscle, the membrane resistance R_m were little smaller than that in Na-containing solution of these cations at higher concentration. But the effect of Na-lack can not be explained yet.

Action potentials elicited by direct stimulation of the muscle fibres. In recording the action potential of the frog muscle fibres, to eliminate the possibility of tearing the membrane with the microelectrode tip during the twitch movement of fibre, the hypertonic solution, prepared by adding 120 mM of NaCl to the normal solution, was used. The hypertonic solution did not completely eliminate the mechanical response. Test experiments were carried out to investigate whether the hypertonic solution caused appreciable changes in the action potentials. The results obtained from such test experiments confirmed that hypertonicity does not appreciably affect the shape of the action potentials. However, it was observed that the hypertonic solutions increased the resting membrane potential by approximately 8 mV.

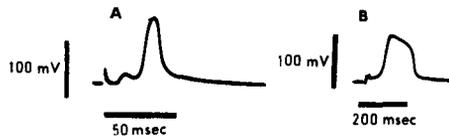


Fig. 7. Action potential A; the frog muscle fibre in a hypertonic Ringer's solution, B; the crayfish muscle fibr in procain containing van Harreveld's solution

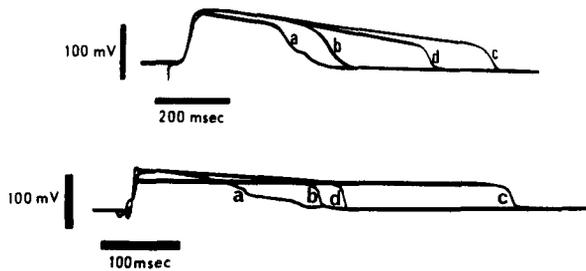


Fig. 8. Action potentials recorded in TEA-ion solutions. Upper trace; superimposed traces of the records at TEA concentration (in mM) of 15.6 (a), 31.3(b), 62.5 (c), and 120 (Na-free, d) in the frog muscle fibre. Lower trace; the same traces at concentration (in mM) of 20(a), 50(b), 100(c), and 200 (Na-free, d) in the crayfish muscle fibre.

Fig. 7 A shows the action potential of the frog muscle fibre in the hypertonic Ringer solution. The record of this action potential had a shape similar to that observed by other reseachers (Caputo & Gimenez, 1967).

The action potential of the crayfish muscle in van Harreveld's solution was also recorded. Normally graded responses to direct stimulation were converted into all-or-none action potential when the muscle fibres were bathed in the

procain-containing van Harreveld's solution for about one-half hour. The shape of the action potential during the early period was irregular and complicated with secondary peaks. With time the response became stable and a smooth propagated action potential was produced.

The propagated action potential of the procain-treated fibres consisted of a fast rising phase (19–20 V/sec), a plateau phase of slow repolarization (lasting up to several hundred milliseconds), and then steep falling phase (0.6–6V/sec, except for very stale preparation). An overshoot of 10 mV was seen unless the preparation became stale.

Effect of TEA-ions: The relation between the action potential and TEA concentration was examined. The result of the experiment is shown in Fig. 8. The action potentials that were recorded in the frog and crayfish muscle fibres immersed in TEA solution had a rectangular shape, similar to that described by

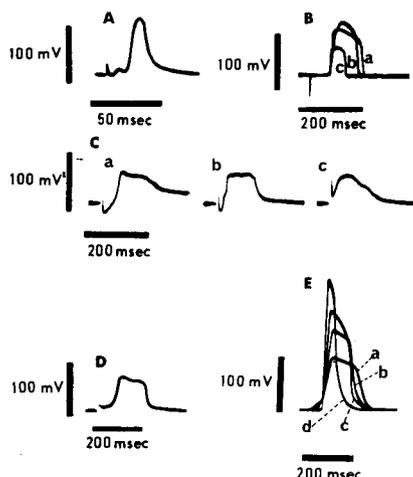


Fig. 9. Effects of external Ca-ions on action potential. A-C; records of the frog muscle fibre at Ca concentrations (in mM) of 0.6 (half, A), 15(B, a), 30(B, b), (60 B,c), and 81.2 (Na-free, C, a-c). D-E; records of the crayfish muscle fibre at Ca concentrations (in mM) of 7 (half, D), 14(normal, E, a), 37(E, b), 60(E, c), and 148(Na-free, E, d).

Fatt & Katz (1953) for action potentials in crab muscles exposed to various substituted ammonium compounds, and were similar in shape to those obtained from Ba treated muscle fibres, which were trapezoidal ones and had long plateau. The amplitude of the action potential in different fibres ranged between 90 mV and 120 mV. The resting potential had a mean value of 90 mV (ranged 83–97 mV) in frog muscle and of 78 mV (range 73–91 mV) in crayfish muscle fibres. The duration of the action potential, measured between points on the rising and falling

phases at one half the peak amplitude, varied 50 to 200 msec, and increased with the TEA concentration.

It is interesting that the duration of action potentials obtained from the muscle treated with the substitution of high concentration of TEA for NaCl was much longer than Na-free TEA solution. These results coincides with the previous experiment reported by Murayama and Yamashita (1962).

Effect of Ca-ions: The relation between the action potential and the Ca concentration was examined in the frog and crayfish muscle fibres. The results of the experiment are shown in Fig. 9. When the frog muscle was bathed in isotonic Ca solution, the completely propagated action potential could not be recorded but an incompletely propagated response often remained (see Fig. 9. C). In crayfish muscles, an increase in Ca concentration above the normal level (14 mM) increased the amplitude and maximum rate of rise of the action potential.

In both the frog and crayfish muscles, action potentials showed considerable reduction in duration depending on increase of Ca-concentration, and some reduction in amplitude.

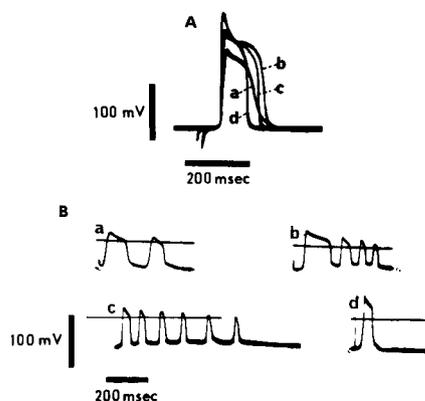


Fig. 10. Effects of Sr on action potentials. A; superimposed traces of the records at Sr concentration (in mM) of 15(A, a), 30(A, b), 60(A, c), and 80(Na-free, A, d) in the frog muscle fibre. B; action potentials of the crayfish muscle fibre recorded in salines containing Sr of 14 mM(B, a), 37 mM(B, b), 60 mM(B, c), and 134 mM(Na-free, B, d).

Effect of Sr-ions: The records of action potentials on the muscle fibres bathed in the solution of Sr-ions at various concentrations are shown in Fig. 10. The same tendency as that in the case of Ca-ions were observed. However, the increase in concentration of Sr-ions were less effective on the shape of action potential than that of Ca-ions. It is characteristic in this case that overshooting spikes were elicited many times by a single stimulus. Especially in the case of crayfish muscle at a high concentration, the spikes were elicited repeatedly. The

duration of the action potential shortened a little but the amplitude increased in proportion of increased concentration of Sr-ions. The falling phase showed a wide variation in different fibres. It was frequently composed of a number of steps. This was probably caused by differences in the duration of the active state of the membrane in different areas along the fibre.

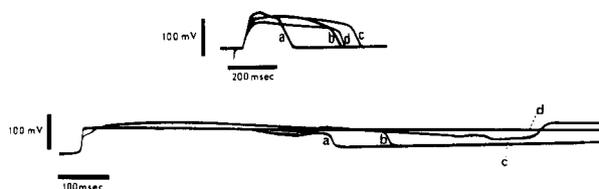


Fig. 11. Action potentials recorded in Ba solutions. Upper traces; superimposed traces of the records at Ba concentration (in mM) of 15(a), 30(b), 60(c), and 80(Na-free, d) in the frog muscle fibre. Lower trace; the same traces of the crayfish muscle fibre at concentration (in mM) of 14(a), 37(b), 60(c), and 134(Na-free, d).

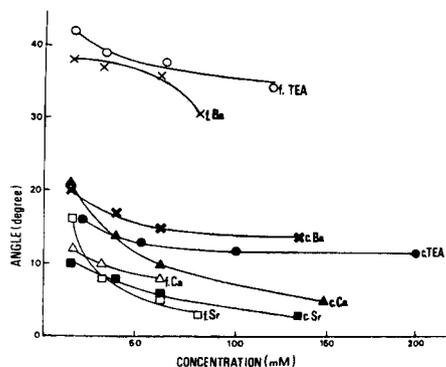


Fig. 12. Angle between tangents at the points of maximum rate or rise and fall of action potential recorded at the same sweep speed.

Effect of Ba-ions: As is shown in Fig. 11, with Ba substituted for Na, the action potentials were of greater duration than those obtained in the muscle in the normal solution. Especially in the crayfish muscle, the duration markedly increased with the Ba-concentration. However in this case, it may be thought that procain contributed to the prolongation of the duration. In isotonic BaCl_2 -solution for the frog muscle and Na-free Ba-ions solution for the crayfish muscle, the duration of action potentials were shorter than that obtained in the solution of the substitution of high concentration of Ba for Na, similarly to the result obtained in the TEA-ions.

Action potential angle: Fig. 12 shows the angle formed by the tangent at the point of maximum rate of rise of action potential with that of fall of it. The angle decreased in proportion to the increase in concentration of the above divalent ions and TEA-ions both in the frog and crayfish muscle.

Discussion

It had previously been shown that the values of membrane resistance of the frog muscle fibre are $7 \times 10^3 \Omega \cdot \text{cm}^2$ (Tamasige, 1950) and $4 \times 10^3 \Omega \cdot \text{cm}^2$ (Fatt and Katz, 1951) from the frog, $116 \Omega \cdot \text{cm}^2$ (Fatt and Katz, 1953) from the crab, and $1,000 \Omega \cdot \text{cm}^2$ (Fatt and Ginsborg, 1958) from the crayfish. In the present experiment, the membrane resistance values of $2,800 \Omega \cdot \text{cm}^2$ of the frog and $1,000 \Omega \cdot \text{cm}^2$ of the crayfish were obtained. The difference between the value of $2,800 \Omega \cdot \text{cm}^2$ and those obtained by the former researchers may be caused by the different temperature and other experimental conditions. However, the difference between the values of $7-8 \times 10^3 \Omega \cdot \text{cm}^2$ (20–30°C) measured by Tamasige (1950) using extracellular electrodes and of $2,800 \Omega \cdot \text{cm}^2$ obtained from the present experiment may be caused by the difference between the extracellular and intracellular electrodes. While the value of crayfish muscle membrane resistance measured in the procaine containing solution in this experiment is in agreement with that measured by Fatt and Ginsborg in van Harreveld's solution (1958). However, these values are considerably larger than that of the crab muscle membrane, which is $116 \Omega \cdot \text{cm}^2$, measured by Fatt and Katz (1953). This difference may be based on the different media between the marine and fresh water animal (Tamasige, 1948).

For the membrane capacity, the following values have been reported, $8 \mu\text{F}/\text{cm}^2$ for the frog muscle fibre (Eccles, 1952, Fatt and Katz, 1951), $42 \mu\text{F}/\text{cm}^2$ for the crab muscle fibre (Fatt and Katz, 1952) and $20 \mu\text{F}/\text{cm}^2$ for the crayfish muscle fibre (Fatt and Ginsborg, 1958). In this experiment, a little smaller values such as $4.3 \mu\text{F}/\text{cm}^2$ for the frog and $2.3 \mu\text{F}/\text{cm}^2$ for the crayfish were obtained. Especially in the case of the crayfish muscle, a considerably smaller value was obtained which is one tenth of the value measured by Fatt and Ginsborg (1958). This may be caused by the effect of procaine which might make the solution hypertonic and might change the membrane properties.

The increase of the resting membrane resistance owing to the increase of TEA concentration may be one of the causes of prolongation of the action potential duration. Such relation was also seen in the case of Ba-ions; the effect of Ba on prolongation of the duration was much more striking. And this fact that the prolongation of action potential caused by external TEA-ions have been shown by many investigators. The action of TEA is to increase the ionic permeability of the muscle membrane during activity temporarily, and consequently facilitate the influx of cations into the cell interior at the beginning of action potential, and the ions once flowed into the cell cannot flow out again easily outside the cell because of high membrane resistance. Moreover, K-ions which flowed out the cell during

activity cannot return back into the cell easily. From such reasons, the duration of action potential may be prolonged. This is obvious from the fact which has been suggested by Beaulieu and Frank (1967) that, at the vertebrate nerve-muscle junction, TEA has a specific action facilitating movement of calcium ions through the membrane, and the well-established action of TEA blocks the potassium current (Katz and Miledi, 1969). And it may support the former notion that the sharpness of the angle between the tangents at the points of maximum rate of rise and fall of action potential in proportion to TEA concentration increase appears to be caused by the increase in the resting membrane resistance.

The shortening of duration of action potential when all the external Na-ions were substituted for TEA as compared with the TEA solution at high concentration containing sodium, may be based on the fact that, in a sodium free medium, there is a gradual loss of sodium from the cell (Katz and Miledi, 1969). The same condition were seen in the case of Ba-ions.

Generally, calcium ions have an increasing effect on the membrane resistance. In the above experiment, a very large increase in the membrane resistance with increase of Ca concentration were observed. The fact was observed in the resting state, however, it is considered that one of the causes of increase in the amplitude of action potential in Ca solution is the increase in membrane resistance. It is also supported from the results that, the more Ca-ions are concentrated, the sharper is the maximum rate of rise and fall of action potential. The same relation is also seen in the case of Sr-ions.

Membrane capacitance values were decreased by TEA- and Ca- ions in the cases of frog and crayfish muscle fibre and by Ba- ions in the case of frog muscle fibre; the decrease may be based on the increase in the membrane thickness supported from the fact of the increase in the membrane resistance value, but the cause of the increase in membrane capacitance values by Sr-ions for the frog and crayfish muscle fibres and by Ba-ions for the crayfish muscle fibre is not yet obvious. However, it is considered that the difference in membrane properties between frog and crayfish muscle fibres may be related to the difference of change in the capacitance by such divalent cations.

The reason why the membrane resistance of crayfish muscle is lower than that of frog muscle can be explained by the higher ionic concentration and conductance of body fluid of the crayfish and the thinner membrane which can be estimated from the value of membrane capacitance.

The tendency of lowering the membrane resistance values when all the external Na-ions were substituted for these four kinds of ions may be caused by the efflux of intracellular Na-ions (Katz and Miledi, 1969). The action of these four ions in this experiment on action potential is the same for both the frog and crayfish muscle fundamentally, that is, the inflow of these cations into the muscle cell is the main factor to generate the action potential.

It has been shown that, in the frog muscle and the squid giant axon, the

main cations contribute to the formation of action potential is Na-ions, but in the crayfish muscle, the cations are Ca-ions (Takeda, 1967), and also in this experiment, the result to confirm this fact was obtained. The increase in the amplitude of the action potential with increasing concentration of Ca-ions is due to the rapid increase in Ca-ions influx into the cell at the beginning of action potential. This can be known from the steep grade of the rising phase of action potential. And, perhaps, the efflux of the intracellular Ca-ions may be performed at the same time. And the shortening of the duration of action potential in Ca solution may be caused by the fact that the Ca-ions cannot get into a cell deeply, so they extrude out immediately after the entrance, and the leaked K-ions may get back into the cell simultaneously. The same results were obtained from the smooth muscle of the guinea-pig taenia coli (Brading, Bülbring and Tomita, 1969) and *Nitella* (Barry, 1968). The similar process was observed also in the case of Sr-ions.

It has been shown that, when Sr-ions were contained in the external bathing solution, the membrane conductance during the action potential increased with increasing Sr concentration, in contrast to the conductance of the resting membrane, which decreased (Fatt and Ginsborg, 1958). Consequently, the main factor to elicit the action potential in Sr solution is Sr-ions likely as in the case of other cations, especially it may be thought that they contribute to the generation of action potential through the same process as Ca-ions do. However, in the bathing solution of Sr, an area of the membrane which is under exciting state undergo a wide change of ionic permeability, and since the Sr-ions are able to move in both directions across the membrane, moreover, the excitation of the membrane are propagated over and over again (Fatt and Ginsborg, 1958), there are a large possibility of alternate occurrence of depolarization and hyperpolarization. From these reasons, it has been observed that, in the Sr-ions solution, the action potentials were elicited several times successively for the only one stimulus.

Ba-ions are also considered to be the ions which increase the ionic permeability of the active membrane and flow into the cell abundantly as other cation's process. Especially like TEA-ions, the duration of the action potential was markedly prolonged.

Summary

1. The effects of TEA-, Ca-, Sr- and Ba-ions on the electrical membrane properties and on the shapes of action potentials of the frog and crayfish muscle fibres were investigated.

2. Electric resting membrane constants of the frog and crayfish muscle fibres were measured in the normal physiological solution and in the medium containing TEA-, Ca-, Sr- and Ba-ions in which these ions are substituted for Na-ions at various concentrations. These ions increased the specific resistance of the muscle fibre membrane, as their concentration was increased. Specific membrane capacitance values decreased in the solution of TEA- and Ca-ions for both the frog

and crayfish, and also in the solution of Ba-ions for the frog muscle fibre, and the value increased in the solution of Sr-ions for both frog and crayfish, and also in the solution of Ba-ions for the crayfish muscle fibre.

3. The action potential of muscle fibres in each medium of those above mentioned was also recorded. In the solution of TEA-and Ba-ions, the shape of it was trapezoidal and the duration was prolonged according to the concentration, and the prolongation may be caused by the increase of resting value of the membrane resistance. In the solution of Ca-ions, the shape for the crayfish muscle fibre showed a high amplitude and the duration was reduced according to the increased concentration. The results may relate to the increase of resting values of the membrane resistance. In the case of Sr-ions, the action potential was repeatedly elicited especially for the crayfish muscle. This may be based on the membrane labile nature in the Sr-ions medium.

4. The angle formed by the tangent at the point of maximum rate of rise of action potential with that of fall were measured. The angle is an indication of the sharpness of the spike potential. The angle reduced in proportion to the increase of ion concentration. It was concluded that the increase in the sharpness of action potential shape is proportional to the increase of the resting value of the membrane resistance.

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