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Effects of Reduction of Calcium on Electrical Activities of Muscle Membrane[1]

By

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(*With 6 Text-figures*)

The effects of change in calcium concentration on the electrical properties of excitable tissues have been studied by many investigators. Some of the main facts established are that an increase in the calcium concentration in the external medium raises the threshold of stimulation (Jenerick and Gerard, 1953, in the frog muscle), and the membrane resistance (Cole, 1949, in the squid giant axon ; Tamasige, 1951, in the isolated frog muscle fibre).

Reduction in the calcium concentration has the converse effects and, thus, leads to spontaneous or repetitive firing of action potential. The progressive increase in frequency and eventual failure of the action potential following the further reduction of calcium concentration have also been found in the squid axon by Frankenhaeuser and Hodgkin (1957). This repetitive response was also seen in the crustacean muscle in normal saline under the prolonged current stimulation (Fatt and Katz, 1953). The responses of a model analogous to the squid giant axon were recently mathematically expressed and the parameters involved were computed by Huxley (1959), and it was found that the model axon responds in a very complicated fashion to stimulation in a low-calcium medium. These computed responses need to be proved experimentally. However, the role of calcium ions on the electrical activity, and hence, on the permeability change during activity has been revealed somewhat in the squid giant axon.

In the fresh preparation of the isolated frog muscle fibre, repetitive contractions in response to a prolonged direct current stimulation have been described by Tamasige (1953), and this repetitive contraction is known to occur more markedly in low-calcium Ringer's solution. There is, however, no detailed information on the electrical activity of the frog muscle fibre during the repetitive contraction induced by a prolonged direct current stimulus.

The experiments described here were carried out on the frog muscle fibre to detect the change in electrical excitability of the muscle in low calcium solution and to compare the responses of the muscle fibre to prolonged stimulus in normal and in low-calcium solutions.

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Material and method

Single muscle fibres were isolated from the iliofibralis muscle of the frog *Rana japonica*. Mounted in a transparent plastic chamber filled with the Ringer's solution, the fibre was impaled at the middle of its length with a microelectrode. The two-fold usage of a single intracellular electrode for stimulation and potential recording was achieved by placing the muscle fibre together with the inserted electrode in one arm of a Wheatstone bridge. This method was in principle identical with that at first introduced by Bishop (1927) and modified by Araki and Otani (1955). Prolonged cathodic currents of various intensities were applied through the microelectrode to the muscle membrane and from the same electrode the electrical response of the fibre was transmitted to a couple of balanced cathode-followers. The electrical response was displayed, through a three stage amplifier, on one beam of the oscilloscope.

The maximum intensity of stimulation applied was 9.5×10^{-8} amperes with a duration of 30 milliseconds. Current calibration was performed by the voltage drop across a resistance placed in one arm of the Wheatstone bridge. A twin-electrode system was also used when it was necessary. The maximum intensity applied in the system was 1.45×10^{-7} amperes and duration was the same as that in the single electrode.

The Ringer's solution had the following ionic composition, expressed in m.mol/l, Na, 126.4 ; K, 2.0 ; Ca, 1.3 ; Cl, 127.6 ; H_2PO_4, 2.0 ; HCO_3, 1.4. The reduced calcium-Ringer's solution was made by mixing the isotonic calcium-free Ringer's with the normal Ringer's at various volume ratios. All solutions were buffered with isotonic $NaHCO_3$ solution to pH=7.2. Most of the experiments were carried out at room temperature.

Results

Rheobase and chronaxie : When a cathodic rectangular pulse was delivered through the microelectrode, an action potential of the muscle fibre was produced, provided that the current was sufficiently above the threshold intensity. The moment of its initiation varies according to the intensity of stimulus. The time required for the initiation of action potential after making the stimulus current is measured as the reaction time on the photographic records and plotted against the current intensity.

Examples of the series of records taken with the muscle fibres in the normal Ringer's fluid are shown in Fig. 1 and 2. The intensity-reaction time relation in the normal Ringer's is shown in Fig. 3. The curve in Fig. 3 is hyperbolic, and can be regarded as analogous to the intensity-time relationship of excitation. This indicates that the intensity-duration relationship can be expressed by the Weiss' formula. Thus, both the chronaxie and rheobase were obtained from the graph.

The responses and the intensity-duration relationship of the muscle fibre in low calcium-Ringer's fluid are also exemplified in Fig. 4 and 5 where the concentration of the calcium in the fluid is made 0.67 m.mol/l. The rheobase in the normal Ringer's fluid is $(7.5 \pm 1.6) \times 10^{-9}$ amperes and in 0.67 m.mol/l calcium-Ringer's fluid it is $(7.8 \pm 2.3) \times 10^{-9}$ amperes. The chronaxie is 3.25 ± 0.2 in the normal fluid and 3.47 ± 1.3 in 0.67 m.mol/l calcium fluid respectively.

The effect of calcium lack and of calcium concentration lower than 0.67 m.mol/l upon the excitability could not be studied because the muscle fibre in such a low calcium concentration elicited vigorous repetitive firings lasting a few minutes at the time of impalement and then rapidly ceased to fire.

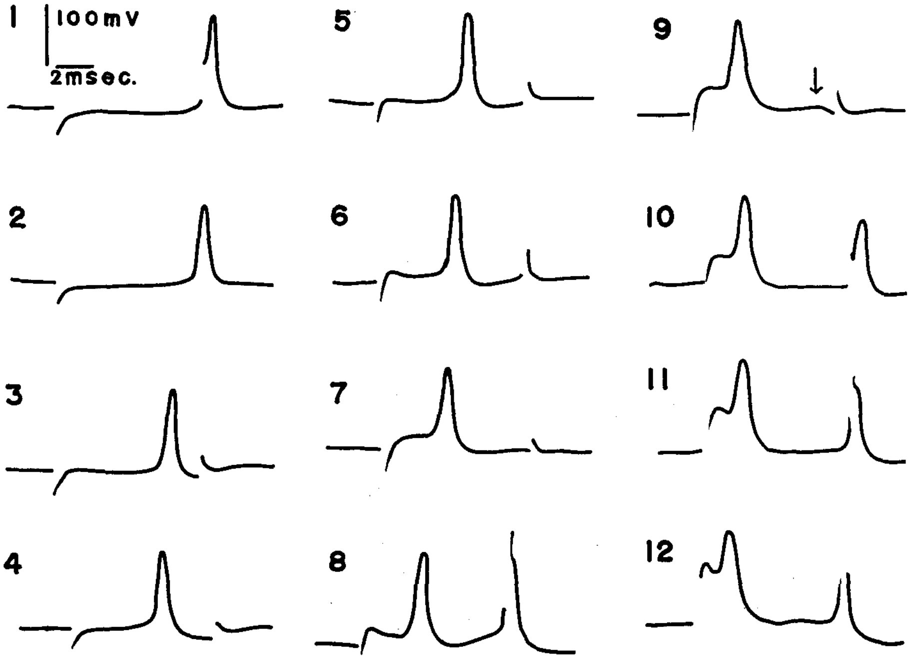

Fig. 1. A series of the responses of a single muscle fibre placed in the normal Ringer's fluid. The lapse of time from the making of the stimulating current (duration : 7.8 msec.) to the initiation of the action potential becomes shorter as the intensity of the stimulus increases. Note a small depolarization before the second action potential (No. 8). Arrow in No. 9 indicates the small depolarization which eventually fails to fire the action potential.

Size of action potential: In the normal Ringer's fluid, the action potential amplitude measured 116 mV as an average of thirteen fibres, which is in good agreement with those reported by many authors (e.g. 119 mV by Nastuk and Hodgkin, 1950). The mean value obtained in 0.67 m.mol/l calcium Ringer's fluid was 103 mV. As to the resting potential, no appreciable change could be observed due to the reduction of calcium concentration.

Repetitive firing of action potential: In Fig. 1, No. 8 the second action potential appears at the end of the stimulation. In the normal Ringer's fluid, such repetitive firings of the action potential in response to the prolonged stimulation could be observed when the preparation was very fresh.

It seems quite important to note that the second one initiates before the cessation of stimulation. This fact eliminates the possibility that the second action potential is the 'off-effect' of the stimulation. The intervals between the two action potentials did not seem to depend much upon the intensity of the stimulation.

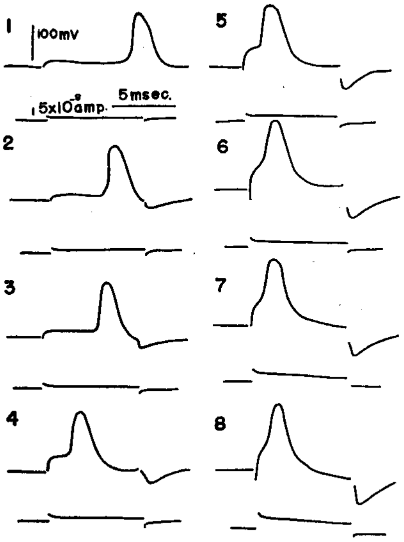

Fig. 2.

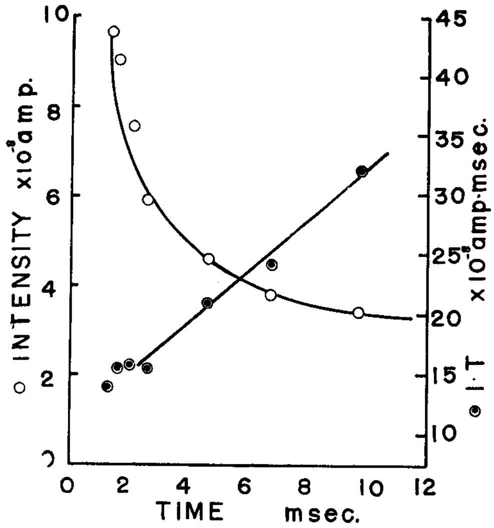

Fig. 3.

Fig. 2. Another example of the series of the responses in the normal Ringer's fluid used for the measurement of the intensity-duration relationship. This also shows the dependence of the latency of the action potential upon the stimulus intensity.

Fig. 3. The intensity-duration relationship for the action potential in the normal Ringer's fluid. The hyperbolic curve of this relationship is replotted in I·T vs. T relation and the rheobase and the chronaxie were measured.

The other fact of interest in the series is that the amplitude of the second action potential diminishes in proportion to the increment of the stimulation intensity. The process of the gradual depolarization developing into the second action potential is clearly seen in Fig. 1, No. 6, 7 and 9. This depolarization may be infered to be the abortive local response and seems to be of the same nature as the response of the medullated nerve to the subthreshold current (Katz, 1947).

Repetitive response which seldom appeared in the normal Ringer's fluid could be seen more frequently in a solution of low calcium concentration. The muscle fibre in 0.67 m.mol/l calcium solution responded always with repetitive firing to the prolonged stimulation.

Moreover, the second and third action potentials appeared regularly and the intervals between each successive two spikes were kept constant. In 0.67 m.mol/l calcium-Ringer's solution the interval between the first and the second was 9.0 msec. and that between the second and the third was 10.6 msec. and these intervals were not altered by the change in the intensity of stimulation.

There was, moreover, a progressive decrease in the size of the action potential, the later ones becoming smaller in size. And the rate of the decrease was augmented as the intensity of the stimulation increased.

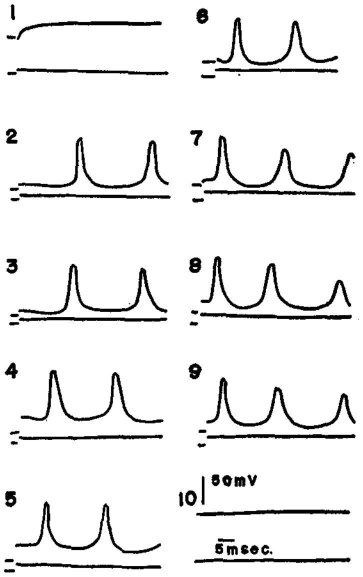

Fig. 4.

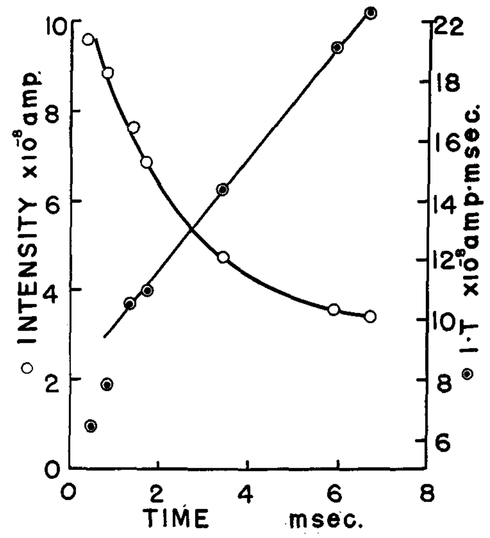

Fig. 5.

Fig. 4. A series of responses of muscle fibres in 0.67 m.mol/l calcium-Ringer's fluid. Repetitive firing of the action potential appears quite regularly. Note the constant intervals between the successive spikes and the decrease in amplitude of the action potential which becomes progressively apparent as the stimulus intensity increases.

Fig. 5. The intensity-duration relationship and the replotted I·T vs. T relation of the responses in 0.67 m.mol/l calcium-Ringer's solution.

Oscillatory subthreshold response : When the repetitive firing occured in the normal or low-calcium Ringer's fluid, small gradual depolarizations preceding the second and the later action potentials were often observed (Fig. 1, 4). This was more clearly seen when the action potential eventually failed to fire (Fig. 1, No. 9). This small depolarization also appeared in response to the stimulus precisely controlled to just the threshold level.

The records of the small depolarizations are illustrated in Fig. 6, where the responses are recorded from the muscle fibre placed in normal Ringer's fluid.

Though the latent period, the time interval between the set-up of the stimulating current and the first peak of the subthreshold oscillation, depended on the stimulus intensity, and became shorter as the intensity increased, the period of the subthreshold oscillation was kept rather constant and was not very different from the interval between the repetitive action potentials. The time course of this subthreshold oscillatory response was identical with that of the small gradual depolarization period preceding the second and the later action potentials. This coincidence leads us to conclude that the subthreshold oscillation is the cause of the repetitive firing of the action potential in the muscle fibre under prolonged stimulation.

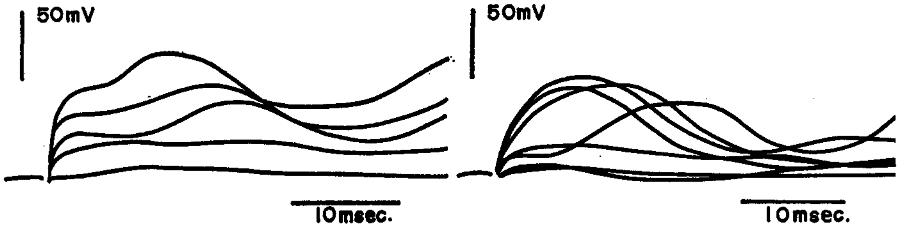

Fig. 6. Damped oscillatory response to the stimulus at the intensity of the threshold level. Two examples in normal Ringer' solution. Note the sudden appearance of the oscillatory response caused by a slight increase in the stimulus intensity, and also the constant intervals between the peaks of the oscillation. In these fibres a slow depolarization of non-repetitive nature is also recorded in response to the threshold stimulation.

Discussion

An increase in concentration of calcium in Ringer's fluid raises the rheobase and lowers the chronaxie, and a fluid containing 13 m.mol/l calcium, which is ten times the normal in calcium concentration results in an increase (by 11 per cent) in the rheobasic current (Hisada, unpublished data). This result is in accordance with the observation made by Jenerick (1953) except that the high calcium Ringer's fluid ten times the normal almost doubled the threshold in his experiment. The discrepancy in the results given above may be accounted for by the difference in the stimulating methods. It seems highly probable that the increase in the rheobasic current is related to the increase in the membrane resistance.

An increase (35 per cent) of the membrane resistance with a tenfold increase of calcium concentration was reported by Tamasige (1951) within the range from 1 to 10 m.mol/l. In a lower concentration of calcium than that in the normal Ringer's fluid slight increases in both the chronaxie and the rheobase were detected in the present investigation.

The fact that in a lower calcium fluid the muscle fibre tends to fire repetitively in response to a small mechanical disturbance as well as to the electrical one seems to be contradictory to this finding. But it must be noted that such repetitive firing is the result of the subthreshold oscillation which gradually builds up and leads to the firing of the propagative action potentials. The rheobase measured relates only to the first action potential and does not directly relate to the damped oscillation thereafter induced.

The various types of mechanical responses of the frog msucle fibre to electrical stimulation have been thoroughly studied by Tamasige (1953). A train of several contractions of a fully propagative nature having a period of about 100 msec. was observed in a very fresh single muscle fibre in normal Ringer's fluid in response to the prolonged direct current stimulation.

The period of the repetition of the contraction is much longer than that of repetitive electrical response described here, about ten action potentials will correspond to one contraction. A possible explanation of this discrepancy, between the frequencies of mechanical and electrical repetitive responses, may be that after an all-or-none type contraction is produced by the first action potential, the successive action potential does not exert any effect on the previously existing contraction because of the refractory period. Then after recovering from the contraction, another contraction succeeds resulting from the action potential which occurs at that time. Thus, the frequency of the mechanical responses appears to have no relation to that of the action potential, but is determined by the length of refractory period.

In a train of action potentials elicited by prolonged stimulation, a progressive decrease in amplitude occurs and the rate of the decrease is augmented as the stimulus intensity is increased. The possible cause of this decrease may be explained as follows, the sustaining depolarization exerted by the prolonged stimulation during repetitive activity is not favourable for the recovery process of ionic permeability from the active state to the resting level, and, therefore, results in a residual increase of permeability. The height of the successive action potentials is affected and is diminished depending on the magnitude of the shift in the permeability for potassium and sodium ions (Hodgkin and Huxley, 1952).

Subthreshold osciallation is the results of fluctuation in permeability which is induced by the set-up of the stimulating current and developes spontaneously until it forms a damped oscillation while the current is applied. The computation of the electrical response of the model nerve by Huxley (1959) suggest that the oscillation in squid nerve is produced by the sodium and potassium permeability changes readily affected by the reduction of calcium concentration. The subthreshold oscillation observed in the frog muscle is likely to be of the same nature although it demands further investigation.

Summary

1. The effect of the reduction of calcium in the surrounding medium on the electrical activity of a single muscle fibre was studied with the aid of a single intra-

cellular microelectrode system, which was specially designed to perform both stimulating and potential recording simultaneously.

2. Both the rheobase and the chronaxie of the muscle fibre measured with prolonged stimulation slightly increase in low calcium Ringer's fluid.
3. The amplitude of the action potential becomes small in the low calcium Ringer's solution (13 mV decrease in 0.67 m.mol/l calcium-Ringer's fluid), but no appreciable change occurs in the resting potential.
4. With a prolonged stimulation, the muscle fibre in low calcium fluid shows a repetitive firing of the action potential with porgressively decreasing amplitude. There is no direct relation between the frequency of the repetition and the stimulus intensity.
5. A small depolarization preceding the action potential is observed and found to be responsible for the repetitive firing.
6. The relation between the repetitive contraction and action potential is discussed.

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