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# Na AND Ca COMPONENTS OF ACTION POTENTIAL IN AMPHIOXUS MUSCLE CELLS

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## SUMMARY

1. The ionic mechanism of the action potential produced in lamella-like muscle cells of amphioxus, *Branchiostoma californiense*, was investigated with intracellular recording and polarization techniques.

2. The resting potential and action potential overshoot in normal saline are $-53 \pm 5$ mV (S.D.) and $+29 \pm 10$ mV (S.D.) respectively.

3. The action potential is eliminated by tetrodotoxin (3 $\mu$M) and by replacing NaCl in the saline with Tris-chloride but maintained by replacing Na with Li.

4. After elimination of the normal action potential by tetrodotoxin or replacing Na with Tris, the addition of procaine (7·3 mM) to the external saline makes the membrane capable of producing a regenerative potential change.

5. The peak potential of the regenerative response depends on external Ca concentration in a manner predicted by the Nernst equation with Ca concentrations close to normal.

6. The Ca dependent response is reversibly suppressed by Co or La ions.

7. Similar regenerative responses are obtained when Ca is substituted with Sr or Ba.

8. It is concluded that two independent mechanisms of ionic permeability increase occur in the membrane of amphioxus muscle cell, one to Na and the other to Ca.

## INTRODUCTION

The action potential of a frog skeletal muscle fibre is produced by an increase in membrane permeability first to Na ions and then to K ions (Nastuk & Hodgkin, 1950). The surface depolarization spreads into the transverse tubular system (Huxley & Taylor, 1958) and this, in some way, results in a release of Ca ions from the sarcoplasmic reticulum (Ebashi & Lipmann, 1962; Weber, Herz & Reiss, 1963; Jöbsis & O'Connor, 1966).

In contrast, the action potential of a crustacean muscle fibre results from an initial increase in membrane permeability to Ca ions (Fatt & Katz, 1953; Fatt & Ginsborg, 1958; Hagiwara & Naka, 1964). In this case the Ca ions entering during an action potential probably contribute to the contraction (Ashley & Ridgway, 1970) even though the release of Ca ions from the sarcoplasmic reticulum may still be important.

It is known that in the protochordate, amphioxus, the myotomes consist of lamella-like sheet of striated muscle cells about $1 \sim 2\,\mu$m thick, 10–300 $\mu$m wide and about 600 $\mu$m long (Peachey, 1961; Flood, 1968). Previous electronmicroscopic studies have shown that neither transverse tubules nor clear sarcoplasmic reticula are found in amphioxus muscle cells (Peachey, 1961). This suggests that the calcium necessary for twitches may enter through the surface membrane during the action potential. Thus, an action potential in amphioxus muscle may include a significant increase of the membrane permeability to Ca ions. Previous electrophysiological studies show that these cells have inside negative resting potentials (Geduldig, 1965) and an overshooting action potential associated with a twitch (Guthrie & Banks, 1970) as in the frog twitch muscle.

The present study was intended to analyse the ionic mechanism of the action potential in the myotomal muscle cells of amphioxus (*Branchiostoma californiense*). A preliminary report has been published (Hagiwara, Henkart & Kidokoro, 1971).

## METHODS

*Materials.* Specimens of amphioxus, *Branchiostoma californiense*, were obtained from the coast of Southern California. They were 1–3 cm long.

*Electrical recording.* After decapitation and removal of the skin, the specimen was fixed on its side with fine needles on the transparent silicon rubber floor of a lucite chamber filled with normal saline. The temperature of the bath was kept at 10–14° C by a thermo-electric cooling unit and was continuously monitored. 3 M-KCl-filled glass micropipettes were introduced into muscle cells perpendicular to the body surface under transmitted illumination. It was necessary to remove the connective tissue sheath covering the myotome. Membrane potential changes were recorded while an electric current pulse was applied to the impaled cell membrane through the recording pipette. To compensate the *IR* drop across the pipette, a Model M 4A Electrometer (W. P. Instruments) was used in conjunction with a differential amplifier. The earth electrode was a silver–silver chloride pellet placed in the chamber. The applied current was monitored with a conventional current-to-voltage converter circuit using a high input impedance operational amplifier.

*Saline.* The compositions of the normal and the major modified salines are listed in Table 1. The compositions of other salines will be described in the Results. Various pre-cooled test solutions were applied from one side of the chamber while the excess solution was withdrawn from the other side. Twenty ml. of the test solution was added to the 3 ml. bath to completely exchange the solution. Tetrodotoxin (TTX) (Sankyo Pharmaceutical Co.) was used at a concentration of 3 $\mu$M. Procaine hydrochloride, $CoCl_2$ or $LaCl_3$ was added to solutions at concentrations below 10 mM;

this did not increase the tonicity of the solution significantly. When $CoCl_2$ or $LaCl_3$ were used, HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane-sulphonic acid)-NaOH buffer was used. For La experiments the pH of the external saline was adjusted to 7·0 instead of 7·7 to avoid the precipitation of $La(OH)_3$. Tris-salt of EGTA (ethyleneglycol-bis [$\beta$-aminoethylether-*N'*,*N'*-tetra-acetic acid) was often added to Ca-free saline at a concentration of 1 mM.

TABLE 1. Compositions of the saline used

| | NaCl | $CaCl_2$ | $MgCl_2$ | KCl | Tris-OH | HCl | $BaCl_2$ |
|---|---|---|---|---|---|---|---|
| Normal saline | 393 mM | 9·3 mM | 48·4 mM | 9·0 mM | * mM | mM | — |
| Ca saline | 393 | 57·7 | — | 9·0 | * | — | — |
| Ca-free saline | 393 | — | 57·7 | 9·0 | * | — | — |
| Tris saline | — | 9·3 | 48·4 | 9·0 | 501 | 306 | — |
| Ca-Mg-free Tris saline | — | — | — | 9·0 | 608 | 372 | — |
| Ba saline | — | — | — | 9·0 | * | * | 319·7 |

Tris-OH = Tris(hydroxymethyl)-amino-methane.

* The pH of salines except Tris and Ca-Mg-free Tris salines was adjusted to 7·7 by adding 10 mM-Tris-HCl or HEPES-NaOH buffer. The pH of the Tris or Ca-Mg-free Tris saline was adjusted to 7·7 by adding a small amount of HCl or Tris-OH.

## RESULTS

### *Resting and action potentials in normal saline*

The resting potential of amphioxus muscle cells often diminished shortly after impalement by a micropipette. This was probably due to the small size of these cells (1–2 $\mu$m thick). In about 10% of the penetrations the initial amplitude of the resting potential was maintained for more than 1–2 min. In these cells the action potential was produced by applying depolarizing current pulse. When the pulse altered the membrane potential to a critical level, a spike was initiated as shown in Fig. 1*A*. In one experiment resting and spike potentials were observed from four successive specimens in normal saline. All-or-none spike potentials were obtained in thirty-five impalements while the initial amplitude of the resting potential was maintained. Fig. 1*C* shows histograms of the resting potential and the overshoots of spike obtained with this sample and the correlation between them. The overshoots were measured from recordings obtained when the spike was produced just after the termination of a short current pulse (as shown by Fig. 3*A* 1). This procedure was adopted to avoid errors originating from a slight imbalance in the bridge circuit. The average resting potential was $-53 \pm 5$ mV (S.D.). The average values of the overshoot and the critical membrane potential for the initiation of a spike were $+29 \pm 10$ mV (S.D.) and $-37 \pm 7$ mV (S.D.) respectively. Flood (1968) described three different types of muscle cells, i.e. superficial, intermediate and deep lamellae. Guthrie & Banks (1970) suggested that different types

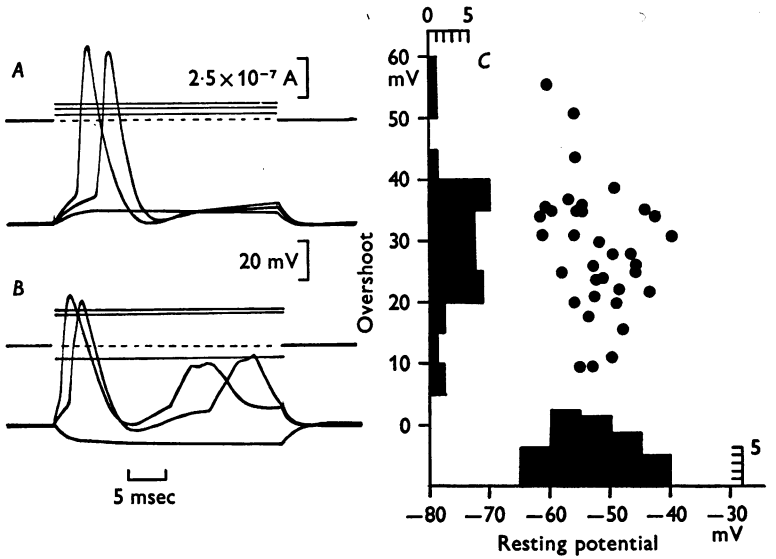


Fig. 1. *A* and *B*, action potentials of muscle cell membrane in normal saline. The upper trace indicates the potential level outside the cell and the amplitude of the applied current. *C*, histograms of resting potential amplitudes and the action potential overshoot. The plot in the centre shows the correlation between the overshoot and the resting potential.

of lamellae might show different amplitudes of the resting potential. In the present work, however, no analysis was made of the relation between recording depth and membrane potential. Fig. 1*C* shows that a smaller overshoot tends to be associated with a smaller amplitude of resting potential. The correlation coefficient between the overshoot and the amplitude of the resting potential is 0·4 and this is statistically significant at the 5% level. Some low resting potentials and overshoots observed in the present study could be due to injury of the membrane caused by electrode penetration. This indicates that the actual amplitudes of the resting potential and overshoot may be somewhat larger than our average values. Prolongation of a suprathreshold current pulse after the initiation of a spike potential produced a second spike. The amplitude of the second spike was often smaller than that of the first spike as shown in Fig. 1*B*.

*Resting membrane constants*

The effective d.c. resistance between the inside and the outside of the muscle cell ranged between 0·8 and 1·2 MΩ. The time constant of the membrane was estimated by assuming an exponential time course of the membrane potential change during polarization with an inward constant current pulse of a small amplitude. It ranged between 1·2 and 2·0 msec.

Such values are substantially smaller than those of frog twitch skeletal muscle fibres (Fatt & Katz, 1951) or crustacean muscle fibres (Fatt & Katz, 1953) determined by the same procedure. The difference could be attributed to the lack of tubular system in amphioxus muscle.

*Electrotonic coupling.* To test for electric coupling between muscle cells membrane potentials of two fibres were recorded simultaneously when current pulses were applied to one of them. In each segmental muscle the lamella-like muscle cells (1–2 $\mu$m) are stacked together above one another in the dorso-ventral direction. Two pipettes impaled cells separated by 30–100 $\mu$m along the dorso-ventral axis. There were, therefore, a number

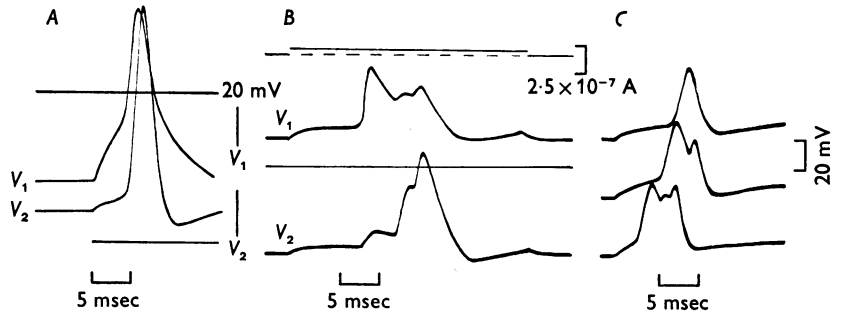


Fig. 2. *A* and *B*, simultaneous recordings from two muscle cells in the same myotome in normal saline. An outward polarizing current was applied to the cell 1 ($V_1$). The top trace in record *A* shows the zero potential level for $V_1$ as well as $V_2$. A bar below trace $V_2$ indicates the duration of applied pulse. *C*, three traces show potential changes obtained from the same cell. The intensity of the applied outward current increases from top to bottom.

of muscle cells between the two impaled cells. In twenty-one cases the resting potentials of the two simultaneously impaled cells remained more negative than $-45$ mV so that measurements could be made. In eleven cases no sign of electrical spread was seen either for spike or electrotonic potentials. Propagation of spike potentials from the polarized cell to the other was found in the remaining ten cases. Spread of slow electrotonic potentials was detected in approximately one-third of the latter. In Fig. 2*A*, $V_1$ shows the spike potential of the directly polarized cell and $V_2$, the spike potential propagated to the second cell. A delay of about 1·2 msec is found between these two spikes.

After a prolonged impalement by a micropipette the cell membrane often became incapable of responding to an outward current pulse with a normal overshooting spike potential. This was usually associated with a decrease in resting potential. Outward current pulses, however, sometimes produced small spike-like potential changes in such cells. One example is shown in Fig. 2*C*. The number of small spike-like potentials produced by a

pulse usually increased with increasing current intensity. The three traces in Fig. 2*C* were obtained from the same cell at three different intensities of applied current. The number of peaks in the response increased from one to three with the increases in the current intensity. Each peak occurred in an all-or-none fashion. This suggests that each small spike-like potential change results from the electrotonic spread of a spike potential either in part of the impaled cell some distance away from the insertion such as the 'ventral root' fibre (Flood, 1968), or in one of the cells electrotonically coupled to the cell recorded from. Two recordings in Fig. 2*B* were obtained simultaneously from a pair of electrotonically coupled cells after the membranes of both cells had slightly deteriorated. An outward current pulse was applied to one of them. In both cells the electrotonic potential produced by the current gave rise to a multi-peak response. The amplitude of the earliest peak of the directly polarized cell ($V_1$) is much larger than that of the second cell ($V_2$) whereas the relation is reversed for the amplitudes of the following two peaks. These records suggest that the earliest peak originated in a cell closer to the directly polarized cell while the following two arose in some cell(s) closer to the second cell. These results indicate the presence of electrotonic coupling between muscle cells. Although the spike potential may conduct through electrotonic junctions, the spread seems to be limited to a small region around a stimulated cell. This is also suggested by microscopic observation that the contraction produced by the stimulation of a single cell is localized to a relatively small region of each myotome. No clear structures corresponding to electrotonic coupling, such as gap junctions, have been demonstrated by electronmicroscopy (Flood, 1968).

*Effects of Na ions on the action potential*

The effect of removal of the external Na ions upon the action potential was observed by replacing the NaCl in the normal saline with iso-osmolar Tris-chloride (Tris-saline). Total replacement invariably rendered the cell membrane incapable of producing all-or-none action potentials (Fig. 3*B*1.) The change was reversed when the preparation was brought back into normal saline. A similar suppression of the action potential was also found under tetrodotoxin (TTX). Fig. 3*A*1 and 2 were obtained from two different muscle cells of the same preparation before and after application of TTX in the normal saline at a concentration of 3 $\mu$M. The recovery of the action potential from TTX was usually incomplete.

When the external Na ions were replaced with Li ions, the action potential was maintained. The overshoot and the time course of the action potential in Li media were almost identical to those found in Na media. These results indicate that the action potentials of amphioxus muscle cells are produced by an increase of the membrane permeability to Na ions

and, therefore, so far, the ionic mechanism of the action potential is similar to that found for frog twitch muscle fibre (Overton, 1902; Nastuk & Hodgkin, 1950; Nakajima *et al.* 1962).

*Regenerative potential changes due to Ca permeability*

Although the all-or-none action potential was eliminated, small graded responses to outward current pulses were often observed after prolonged perfusion with Tris-saline (see Fig. 3*B*1). This suggests that an outward

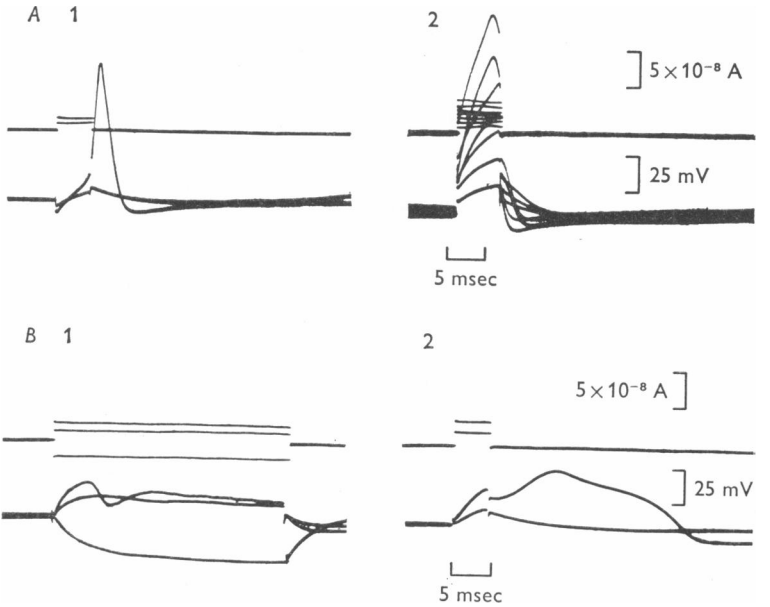


Fig. 3. *A*, action potential of a cell obtained in the normal saline (1) and potential changes of a different cell of the same preparation produced by outward current pulses after adding TTX (3 $\mu$M) in the external solution (2). *B*, potential changes of cell membrane produced by current pulses in Na-free Tris saline (1) and a regenerative potential change obtained from another cell of the same preparation after adding procaine (7·3 mM) in the external solution (2). The upper trace of each record shows the potential level outside the cell as well as the intensity of the applied membrane current.

current pulse may also produce a permeability increase to Ca ions. The permeability increase to those ions, however, does not result in an all-or-none potential change, probably because it is too small to overcome the increase in K permeability. Therefore, we attempted to suppress the increase in K permeability. It has been shown that procaine at a relatively low concentration in the external solution suppresses the increase in K conductance in several tissues (Shanes *et al.* 1959; Taylor, 1959; Hagiwara

& Nakajima, 1966; Hagiwara, Hayashi & Takahashi, 1969). The records in Fig. 3*B*1 and 2 were obtained from different cells of the same preparation before and after 7·3 mM procaine was added to Na-free Tris-saline. A regenerative potential change with a slow time course appeared under procaine.

A similar result was also obtained with procaine after the all-or-none action potential in normal Na had been eliminated by TTX. The series of

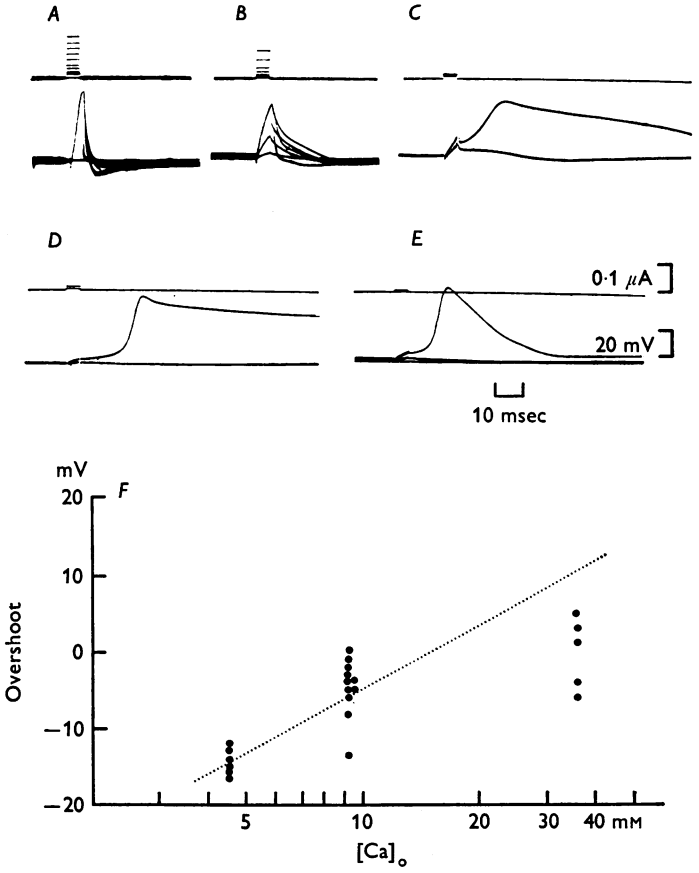


Fig. 4. *A–E*, potential changes produced by a short outward current pulse in five different cells of the same preparation when the external saline contained TTX (3 μM). *A*, in Ca free saline. *B*, Ca-free saline with 7·3 mM procaine. *C–E*, the Ca concentration was 4·6, 9·2 and 36·6 mM respectively and the external solution contained 7·3 mM procaine for all these three cases. The upper trace of each record (*A–E*) shows the potential level outside the cell as well as the intensity of the applied current. *F*, relationship between the overshoot of the procaine-induced Ca action potential and the external Ca concentrations. The data were obtained from the same preparation.

records in Fig. 4 shows that such a regenerative potential change is produced by an increase in Ca permeability. TTX of 3 $\mu$M in the normal saline eliminated the normal action potential as described before. Then the preparation was exposed to a prolonged perfusion (30 min or more) with Ca-free saline containing 57·7 mM-$MgCl_2$ (Table 1). In most experiments of this type, 1 mM-EGTA was added to Ca-free saline. Record *A* of Fig. 4 shows potential changes of the membrane in response to a short current pulse in Ca-free saline containing TTX. No regenerative responses are seen. Record *B* of the same Figure was obtained after adding 7·3 mM procaine to the Ca-free external saline with TTX. No regenerative responses were produced even in the presence of procaine. On the other hand, a slower decay of membrane potential change following the termination of the current pulse indicates that the K conductance increase had been suppressed. Records *C*, *D* and *E* were obtained from three different muscle cells of the same preparation as the one used for records *A* and *B*, when the external Ca concentration was raised from 0 to 4·6, 9·2 and 36·6 mM respectively by replacing $MgCl_2$ in the Ca-free saline (containing no EGTA) with $CaCl_2$. The restoration of Ca to the external solution made the membrane capable of producing prolonged action potentials. The peak potential level and the maximum rate of rise of the action potential both increased with increasing external Ca concentration. The lack of any regenerative potential change in the absence of external Ca ions does not seem to be due to any deterioration of the membrane. Similar responses were obtained by adding Ca even after several hours of immersion in the Ca-free saline. No significant differences were found between the effective membrane resistances of the cell in normal Ca saline and Ca-free salines containing 57·7 mM-Mg. The alteration of the Mg concentration in Ca free saline containing TTX and procaine from 57·7 mM either to 24 mM or 100 mM did not render the membrane capable of producing all-or-none potential changes. Therefore, the above results suggest that the increase of the membrane permeability occurs only to Ca ions and not to Mg ions.

Fig. 4*F* shows the relation between the peak potential level of the procaine-induced action potential under TTX and the external Ca concentration. The data were obtained from cells of the same preparation when the amplitude of the resting potential at the time of recording was more than 45 mV. The broken line in the Figure represents a slope of 28 mV for a tenfold change in Ca concentration which is expected from the Nernst equation. Although the points are somewhat scattered the result shows that the increment of the average peak potential level agrees with this slope at least for the range of the Ca concentrations close to the normal value (between 9·3 and 4·6 mM). This indicates that the procaine-induced action potential is produced as a result of a permeability increase to Ca

ions. The procaine-induced action potential in Tris-saline showed similar behaviour with changes in external Ca.

Although the peak potential level of the procaine-induced action potential behaves like a Ca electrode in the range of concentration close to the normal value, there were deviations with higher concentrations. As seen in Fig. 4*F*, the increment of the peak potential became smaller than that expected from a Ca electrode as the concentration was increased. The response tended to saturate. When the Ca concentration was increased to a very high level (more than 100 mM), the regenerative response itself often disappeared. Similar phenomena have also been observed for 'Ca spikes' of various tissues such as crustacean muscle fibres (Fatt & Ginsborg, 1958) or the presynaptic terminals of squid giant synapses (Katz & Miledi, 1969). The saturation phenomenon of the overshoot of the 'Ca spike' has been analysed in a barnacle muscle fibre and it is suggested that the important Ca ions are those at sites somewhere near or at the fibre membrane rather than those in the bulk solution (Hagiwara & Takahashi, 1967). The saturation found for the overshoot in the present experiment may indicate that the Ca permeability increase also depends on the Ca at membrane sites in amphioxus muscle.

In the experiment illustrated in Fig. 4, the procaine-induced Ca action potential disappeared in a relatively short time (30 min) in Ca-free saline containing no EGTA. However, small but significant regenerative responses were often seen in some preparations even after a prolonged perfusion (30 min) with Ca-free saline. If a linear extrapolation is made of the relation between the peak potential level and the logarithm of the Ca concentration the amplitude of the response found in nominally Ca-free saline suggests that in fact the Ca concentration in the vicinity of the membrane was up to 1 mM. This concentration appears too large to be accounted for by contamination. The tentative explanation of this phenomenon is that Ca ions extruded from inside the cell accumulate at the sites even in nominally Ca-free saline.

*Effects of other polyvalent cations*

*Sr and Ba.* The preceding results show that an outward current pulse initiates a permeability increase of the membrane to Ca but not to Mg. Among other divalent cations examined, a similar permeability increase was found for Sr and Ba. Sr and Ca behaved very similarly. Slightly different behaviour of the membrane was found with Ba. As mentioned, the normal action potential disappeared in Tris-saline. When $CaCl_2$ and $MgCl_2$ in this solution were replaced with an equivalent amount of $BaCl_2$, the membrane became capable of producing an all-or-none action potential. Records *A*, *B* and *C* in Fig. 5 were obtained from three different cells

of the same preparation at three different Ba concentrations (33·5, 106·6 and 319·7 mM). Solutions were obtained by mixing Ca-Mg-free Tris-saline and Ba saline in appropriate proportions. The potential level at the peak of the action potential increased with increasing Ba concentration. The relation between the overshoot amplitudes and the Ba concentration is shown in Fig. 5. The data were obtained from cells of the same preparation. The average value of the peak potential increases with a slope of about 28 mV for a tenfold increase in Ba concentration, showing that the membrane becomes permeable to Ba ions, and the regenerative potential observed can be called a 'Ba action potential'. The Ba action potential differs from the Ca action potential in the following two respects: (1) regenerative responses in the Ba media were obtained in the absence of procaine. This is probably due to the fact that Ba ions suppress the increase of K conductance resulting from depolarization (Werman & Grundfest, 1961; Fatt & Ginsborg, 1958; Hagiwara & Naka, 1964); (2) the peak potential level increased almost linearly with the logarithm of the Ba concentration up to high concentrations. This may also be due to the suppressing effect of Ba on the K conductance. If this effect increases with an increasing Ba concentration the relation between the overshoot and the concentration for the Ba action potential should show a slope greater than that found for the Ca action potential. Another possibility is that

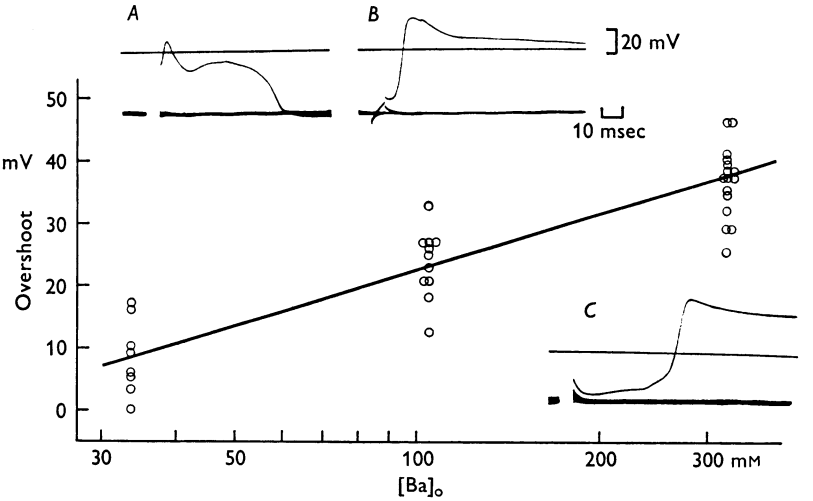


Fig. 5. *A–C*, action potentials of the cell membrane obtained in Ba media. The Ba concentration was 33·5 in *A*, 106·6 in *B* and 319·7 mM in *C*. The external solutions did not contain Na, Ca, Mg or procaine and the tonicity is adjusted with Tris-Cl. The upper trace of each record shows the potential level outside the cell. The graph shows the relation between $[Ba]_o$ and the overshoot of the Ba action potential. All data were obtained from the same preparation.

the affinity of Ba to the membrane site is smaller than that of Ca so that a saturation is not reached even at relatively high concentrations. These results show that the permeability increase of the membrane normally occurring to Ca ions can occur also to Sr and Ba ions. This is also the case in other excitable membranes which undergo a Ca permeability increase.

*Co and La.* It has been shown in various tissues that the conductance increase of the membrane to Ca ions is suppressed by small amounts of transition metal ions such as Co and Mn (Hagiwara & Nakajima, 1966; Hagiwara & Takahashi, 1967) or La (M. P. Henkart, in preparation). A similar suppression by Co or La was found for the procaine-induced Ca action potential of the present preparation. The suppression was reversed when Co or La was removed. It was noted above that small regenerative potential changes frequently persisted for a relatively long time in Ca-free saline. Addition of 5 mM-$CoCl_2$ to the Ca-free solution invariably abolished the regenerative response in a relatively short time (10 min). A similar but more intense effect was found for $LaCl_3$. Addition of 1 mM-$LaCl_3$ abolished the regenerative response usually in a period even shorter than that found for 5 mM-$CoCl_2$. These results support the idea that the Ca ions important for the Ca action potential are probably those occupying sites somewhere near or at the membrane. Co or La ions suppress the Ca action potential by occupying those membrane sites.

*Effects of the Ca concentration, Co and La on the normal action potential*

The evidence for a procaine-induced Ca action potential strongly indicates that a similar increase of the membrane conductance to Ca ions occurs during the normal action potential which primarily results from an increase in Na conductance. The Ca conductance, however, does not seem to contribute significantly to the overshoot of the normal action potential. No significant decrease in the normal overshoot was found after a prolonged immersion of the preparation (30 min–1 hr) in Ca-free saline containing 57·7 mM-$MgCl_2$. This also was the case when either 1 mM-EGTA or 5 mM-$CoCl_2$ was added to the Ca-free saline. In one experiment resting potentials and spike overshoots of twenty muscle cells in the Ca-free saline containing 1 mM-EGTA (Tris salt) were compared with those of another twenty muscle cells of the same preparation in the normal saline before the application of the Ca-free saline. The average resting potential and the overshoot were $-51 \pm 5$ mV (S.D.) and $25 \pm 9$ mV (S.D.) in the normal saline and $-52 \pm 4$ mV (S.D.) and $28 \pm 8$ mV (S.D.) in Ca-free saline. The inward membrane current carried by Na ions during the normal action potential is probably much greater than that carried by Ca ions so that the Ca permeability increase shows only a negligible effect on the overshoot of the normal action potential.

## DISCUSSION

The action potential of amphioxus muscle cells in normal saline is primarily produced by a conductance increase of the membrane to Na ions in a manner similar to that found in squid giant axon or a frog twitch muscle fibre. The evidence is the following. (1) The action potential is eliminated by removing Na from the external solution (Hodgkin & Katz, 1949; Nastuk & Hodgkin, 1950). (2) The action potential can be maintained in Li media (Hodgkin & Katz, 1949: Overton, 1902). (3) Tetrodotoxin suppresses the action potential at low concentrations (Narahashi, Moore & Scott, 1964; Nakajima, Iwasaki & Obata, 1962).

The experimental results indicate that the membrane of amphioxus muscle cells also shows an increase in Ca permeability. Analysis of the procaine-induced Ca action potential shows: (1) the overshoot of the potential depends on the external Ca concentration in a manner predicted by the Nernst equation when the Ca concentration is close to its normal value (9·3 mM), but the overshoot–Ca concentration relationship tends to saturate as the concentration increases; (2) the permeability increase occurs also to Ba and Sr; (3) the permeability increase to Ca is suppressed by transition metal ions or La ions in the external solution; and (4) TTX at a high concentration of 3 $\mu$M has no effect on the permeability increase. These properties of the permeability increase to Ca in amphioxus muscle are similar to those found in other tissues such as crustacean muscle fibres (Fatt & Ginsborg, 1958; Hagiwara & Naka, 1964; Abbott & Parnas, 1965), presynaptic terminal of squid giant synapse (Katz & Miledi, 1969), molluscan neurones (Geduldig & Junge, 1968; Meves, 1968), some smooth muscles (Toida & Osa, 1965). But the increase of the Ca permeability found in amphioxus muscle cells differs from that found in a squid giant axon (Watanabe *et al.* 1967) in its sensitivity to TTX. The latter is abolished at low concentration of TTX.

The increase of the Ca permeability in the present study is evidenced by the presence of procaine-induced action potentials in the absence of a Na spike. This raises the following two questions. (1) Does procaine facilitate or even create an increase of the Ca permeability which is normally absent? This is unlikely for the following reasons. (*a*) It has been shown in the barnacle muscle fibre by using voltage–clamp technique that procaine does not affect the membrane current carried by Ca ions (Hagiwara *et al.* 1969). (*b*) Similar regenerative response occurs for Ba ions in the absence of procaine. Thus, the increase in Ca permeability is a property of the membrane before the application of procaine. (2) The second question is whether or not the same Ca permeability increase occurs in the presence of a normal Na spike. If the Ca permeability increase is a function of the

number of sites occupied by Ca and if Na competes with Ca at the same site, the increase of the Ca permeability in normal Na could be much smaller than that found in Na-free media. The fact that essentially the same procaine-induced Ca action potential is found in normal Na media under TTX indicates this is not the case. Studies of Ca spikes in barnacle muscle fibre have shown that the important Ca ions are those occupying sites near or at the membrane rather than those in the external solution (Hagiwara & Takahashi, 1967). The present results indicate that the same is true in amphioxus muscle cells.

Amphioxus muscle membrane has two independent mechanisms of permeability increase, i.e. increases in Na and Ca permeabilities. This property is similar to that found in Aplysia ganglion cells (Junge, 1967; Geduldig & Junge, 1968; Geduldig & Gruener, 1970) and frog heart ventricles (Niedergerke & Orkand, 1966; Hagiwara & Nakajima, 1966).

Under normal Ca concentration the peak of the procaine-induced Ca action potential almost reaches the zero membrane potential when the resting potential is $-70$ mV. If the membrane capacity is known the necessary minimum charge displacement during the action potential can be calculated. The present experiment, however, did not supply the data for the membrane capacity of an amphioxus muscle cell. Therefore, 1–2 $\mu$F/cm$^2$ was taken for the specific membrane capacity based on the assumption that the capacity is similar to that of the surface membrane of frog twitch muscle fibre (Falk & Fatt, 1964; Gage & Eisenberg, 1969; Nakajima & Hodgkin, 1970). The calculation shows that the charge displacement should be $0{\cdot}7$–$1{\cdot}4 \times 10^{-7}$ C/cm$^2$ per action potential. This indicates a minimum Ca influx of $3{\cdot}6$–$7{\cdot}3 \times 10^{-13}$ mole/cm$^2$ per action potential if the thickness of the muscle cell is taken as 1 $\mu$m. The influx should raise the internal Ca concentration by $0{\cdot}7$–$1{\cdot}5 \times 10^{-5}$ M. Therefore, the Ca influx may play an important role in excitation-contraction coupling (Hagiwara *et al.* 1971).

The lamella-like muscle cell in the amphioxus sends its process to the spinal cord to make a synapse. The muscle process is called 'ventral root fibre' (Flood, 1968). The biological significance of the Na spike in amphioxus muscle cell appears to be the conduction of an impulse from the synaptic region through the ventral root fibre, toward the muscle lamella which exerts a contraction whereas the Ca spike is important for excitation–contraction coupling.

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