Voltage Clamp Analysis of Two Inward Current Mechanisms in the Egg Cell Membrane of a Starfish

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ABSTRACT   Ionic mechanisms of excitation were studied in the immature egg cell membrane of a starfish, *Mediaster aequalis*, by analyzing membrane currents during voltage clamp. The cell membrane shows two different inward current mechanisms. One is activated at a membrane potential of $-55 \sim -50$ mV and the other at $-7 \sim -6$ mV. They are referred to as channels I and II, respectively. A similar difference is also found in the membrane potential of half inactivation. Currents of the two channels can, therefore, be separated by selective inactivation. The currents of both channels depend on Ca^{++} (Sr^{++} or Ba^{++}) but only the current of channel I depends on Na^+. The time-course of current differs significantly between the two channels when compared at the same membrane potential. The relationship between the membrane current and the concentration of the permeant ions is also different between the two channels. The result suggests that channel II is a more saturable system. The sensitivity of the current to blocking cations such as Co^{++} or Mg^{++} is substantially greater in channel II than in channel I. Currents of both channels depend on the external pH with an apparent pK of 5.6. They are insensitive to 3 μM tetrodotoxin (TTX) but are eliminated totally by 7.3 mM procaine. The properties of channel II are similar to those of the Ca channel found in various adult tissues. The properties of channel I differ, however, from those of either the typical Ca or Na channels. Although the current of the channel depends on the external Na the amplitude of the Na current decreases not only with the Na concentration but also with the Ca concentration. No selectivity is found among Li^+, Na^+, Rb^+, and Cs^+. The experimental result suggests that Na^+ does not carry current but modifies the current carried by Ca in channel I.

INTRODUCTION

In some tissues, such as the squid giant axon (Hodgkin and Katz, 1949; see also Hille, 1970), the action potential is produced by an increase in the membrane permeability to Na ions whereas in some other tissues, such as crustacean

muscle fibers (Fatt and Ginsborg, 1958; Hagiwara and Naka, 1964), the increase in permeability is to Ca ions. Both mechanisms are found in a variety of tissues in different animals. The former is characterized by the term, "Na channel," and the latter by "Ca channel." Miyazaki et al. (1972, 1974 c) found that the membrane of the egg cell of a certain tunicate, Halocynthia, is electrically excitable and shows two separate inward current mechanisms. When the cell is surrounded by seawater one mechanism is activated at a membrane potential of about −45 mV, with the current carried mainly by Na ions. The other mechanism is activated at approximately zero membrane potential and the current is carried by Ca ions. Full-grown striated muscle cells of the same species show a pure Ca-dependent action potential. Kidokoro (1973) found that tissue-cultured myotube of rat includes both Na and Ca channels. On the other hand, the membrane of a rat skeletal muscle fiber shows a pure Na-dependent action potential. These findings suggest that the differentiation of the two channels may occur through the elimination of one of the channels that is present at early stages of development.

The membrane of the echinoderm egg is also electrically excitable (Miyazaki et al., 1974 a), and the action potential depends on both external Na and Ca ions. Most echinoderm egg cells are small, and the application of multielectrode techniques is usually difficult. However, the starfish, Mediaster aequalis, has giant eggs with diameters ranging between 0.8 and 1.2 mm. This size gives an excellent opportunity to analyze the ionic mechanisms of echinoderm egg cell membrane with voltage clamp technique. The first question asked was whether two distinctly different inward current mechanisms are also present in the membrane of the immature starfish egg cell. The experimental results of the present paper show convincingly that the answer is affirmative. The second question was whether these two mechanisms have properties of the typical Na and Ca channels found in nerve or muscle cells. The results show that one mechanism has most of the characteristics found for the typical Ca channel. However, the other mechanism differs from both the typical Na and Ca channels.

MATERIALS AND METHODS

Specimens of starfish, Mediaster aequalis, were collected in Southern California during the months of December to March. The animals were kept in an aquarium at 15°C. Before each experiment ovaries were isolated in seawater, and immature egg cells which spontaneously spawned from the ovaries were collected and used for the experiments.

Eggs were immobilized by being placed in a small cage made of thin glass rods mounted in a Lucite chamber. The chamber was perfused continuously and complete solution exchange required about 2 min. Therefore, a 5-min period was usually allowed for each new test solution before the measurements to ensure a complete exchange. All the experiments were performed at room temperature (21–22°C). The

compositions of the major solutions used are listed in Table I. Na-free solutions of various Ca concentrations were prepared by mixing Ca- Na-free solution (solution D) and 200 mM Ca solution (solution E) in appropriate proportions. Sr or Ba solution was obtained by replacing $CaCl_2$ with $SrCl_2$ or $BaCl_2$. In some experiments 537 mM Tris and 358 mM HCl in the solution B was replaced with 814 mM sucrose. In order to obtain 100, 200, or 400 mM Na with 0, 10, 25, or 50 mM Ca, solutions D, F, G, and H were mixed in appropriate proportions. Cs, Rb, and Li solutions were made by replacing NaCl in the Na solution with CsCl, RbCl, or LiCl, respectively. For the experiment with $CoCl_2$ an appropriate amount of $CoCl_2$ was added to the solution. This did not significantly increase the tonicity of the solution since the concentration

TABLE I

COMPOSITION OF SOLUTIONS

Solution	NaCl	CaCl₂	KCl	MgCl₂	Tris	HCl
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
Artificial seawater	470	10	10	50	—	—
Solution A (400 Na and 25 Ca)	400	25	10	50	57	38
Solution B (0 Na and 25 Ca)	—	25	10	50	537	358
Solution C (0 Na and 10 Ca)	—	10	10	50	564	376
Solution D (Na- Ca-free)	—	—	10	50	582	388
Solution E	—	200	10	50	222	148
Solution F	400	50	10	50	12	8
Solution G	400	—	10	50	102	68
Solution H	—	50	10	50	492	328

The pH of the solution was adjusted to 7.7 with 10 mM Tris and HCl. At this pH approximately one-third of Tris is undissociated.

of $CoCl_2$ never exceeded 25 mM. The solution used for studying the pH effect had the following composition: NaCl, 378 mM; $CaCl_2$, 25 mM; $MgCl_2$, 50 mM; K-hydrogenphthalate, 10 mM; acetic acid, 10 mM; HEPES, 10 mM; Tris-maleate, 10 mM; glycine, 10 mM. The pH was adjusted to 4, 5, 6, 7, 8, or 9 by adding an appropriate amount of NaOH. The final Na concentration ranged between 423 mM (pH 9) and 380 mM (pH 4.0). The alteration of the NaCl concentration from 423 to 380 mM at pH 7.7 did not cause any appreciable change in the inward current when the Ca concentration was 25 mM. Therefore, the above difference in the Na concentration among different pH solutions was ignored.

Two glass micropipettes filled with 3 M KCl were used for recording the membrane potential and for applying currents through the membrane, respectively. The technique and precautions for micropipette insertion were the same as that described by Miyazaki et al. (1974 c). A chlorided silver plate in the saline served as the indifferent electrode. A third KCl-filled micropipette was introduced into the external saline and the membrane potential was recorded as a potential difference between this electrode

and the intracellular potential electrode. The resistance of the glass pipettes ranged between 3 and 8 MΩ. The membrane current was recorded by a current-voltage converter inserted between the indifferent electrode and ground. The voltage clamp arrangement was essentially similar to that used for the barnacle muscle fiber (Hagiwara et al. 1974). The output of the feedback amplifier of the voltage clamp had a linear range of ±150 V. This was sufficient to pass enough current through the glass pipette. The rise time constant of the voltage step was about 2 ms, which was much faster than the initial change of the membrane current. The interior of the cell was considered equipotential. This was occasionally examined by observing the membrane potential simultaneously through another glass pipette inserted into the cell, but remote from the other two intracellular pipettes. Potential changes recorded through the two recording pipettes were always indistinguishable for sweep speeds used in the present experiment.

RESULTS

Action Potential of Egg Cell Membrane

When the cell was immersed in the artificial seawater, which contained 10 mM K, the resting membrane potential was -73 ± 3 mV (SD $n = 10$) and the resting membrane behaves as a K electrode (Hagiwara and Takahashi, 1974; Miyazaki et al., 1974 b). Changes in concentration of Na, Tris, Ca, Mg, or Cl did not result in any significant change in the resting potential.

Records A 1 and B 1 of Fig. 1 obtained in 400 mM Na and 25 mM Ca show that a positive shift of the membrane potential to about -50 mV gives rise to an action potential. The rising phase of the action potential shows two steps, one at about -50 mV and the other at $-6 \sim -7$ mV. The two steps can be interpreted by considering two inward current mechanisms having different membrane potential dependences. A depolarization to -50 mV activates the first mechanism and this results in a regenerative current. When the potential change due to this current reaches $-6 \sim -7$ mV the second mechanism is activated and this produces an additional depolarizing potential change.

Records A 2 and B 2 of Fig. 1 were obtained from the same cell after the external NaCl had been replaced with isosmolar Tris-Cl. The removal of the external Na reduced the rate of rise of the action potential and at the same time the time-course of the falling phase became substantially faster. As will be described later these changes in the action potential correspond to the changes of the inward current during voltage clamp observed upon the removal of the external Na. These changes suggest that Na ions have a significant contribution to the action potential. The fact that a significant action potential is produced in the absence of the external Na^+ shows that ions other than Na^+ also play an important role as charge carriers during the action potential. As will be described later, this ion species is Ca^{++}. Records A 3 and
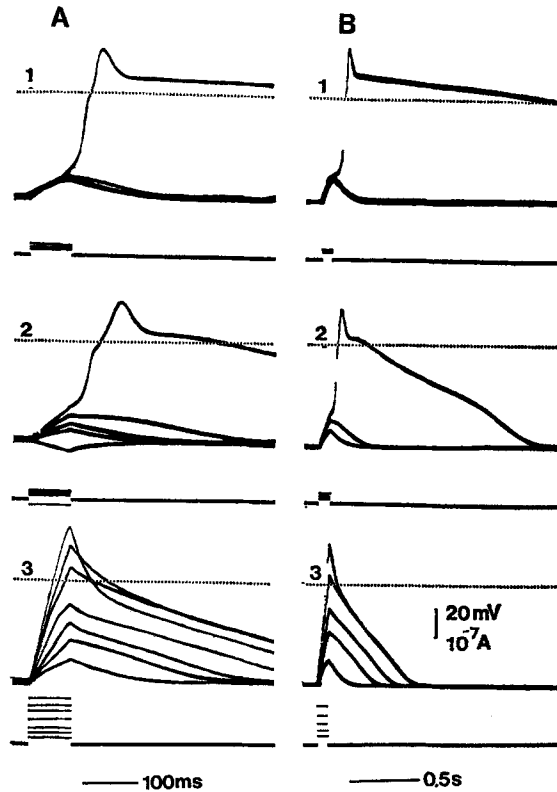
FIGURE 1. Potential changes of the egg cell membrane to a brief outward current pulse (75 ms). Records in B were obtained with a slower sweep speed. 1, in 400 mM Na and 25 mM Ca (solution A). 2 in zero Na and 25 mM Ca (solution B). 3 in Na- Ca-free solution (solution D). A dotted line in each record indicates the reference potential level. Cell diameter, 1 mm.

B 3 show potential changes after replacement of both the external $CaCl_2$ and NaCl with isosmolar Tris-Cl. Regenerative potential changes were no longer observed.

Membrane Current during Voltage Clamp

The membrane currents obtained during voltage clamp and the current-voltage relations obtained from the membrane currents are shown in Figs. 2 and 3, respectively. Currents obtained at membrane potentials more negative than the resting potential are common for three different solutions and characterized by anomalous or inward-going rectification (see traces -86 and -97 mV in Fig. 2). This result is similar to that observed in other starfish eggs (Hagiwara and Takahashi, 1974; Miyazaki et al. 1974 b). The membrane current during rectification includes instantaneous and time-dependent com-
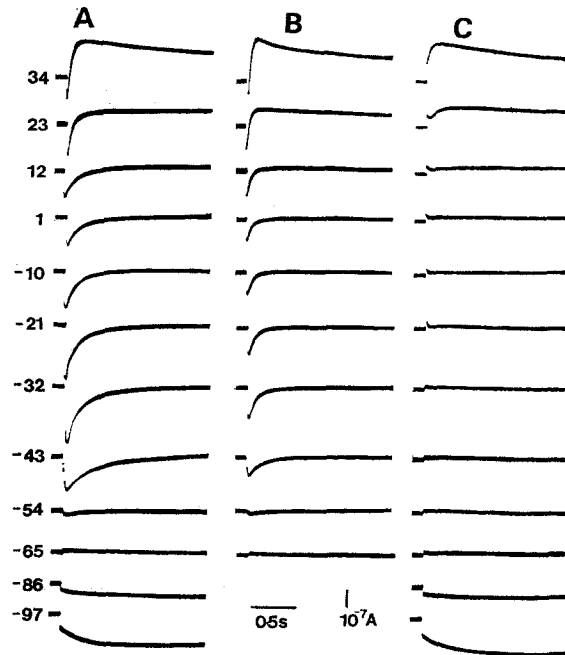
FIGURE 2. Membrane currents of the egg cell during voltage clamp. The membrane potential during the clamp is listed to each current recording. Holding potential was the resting potential (−73 mV). A, in 400 mM Na and 25 mM Ca (solution A). B, in zero Na and 25 mM Ca (solution B). C, in Na- Ca-free solution (solution D). Cell diameter, 1 mm.

ponents. The former appeared within 2 ms after the onset of the voltage pulse, while the latter developed slowly and reached final amplitude after several hundred milliseconds.

For membrane potentials more positive than −55 mV the initial capacitative current was followed by a transient inward current in 400 mM Na and 25 mM Ca and also in zero Na and 25 mM Ca. After reaching its peak the inward current decayed in about 1 s. The relation at the peak of the inward current (Fig. 3, continuous lines) has a negative slope resistance in two different ranges of membrane potentials, one between −55 and −30 mV and the other between 0 and +20 mV. Hence, two maxima of the peak inward current were found, one at about −30 mV and the other at about +20 mV. This corresponds to the two steps in the rising phase of the action potential and indicates the presence of two inward current mechanisms having different membrane potential dependences. The removal of the external Na reduced the peak amplitude of the inward current. The time-course of the inward current became substantially faster. As described already, corresponding changes are found in the action potential. The transient inward current was no longer found in Na- Ca-free solution. For membrane potentials between
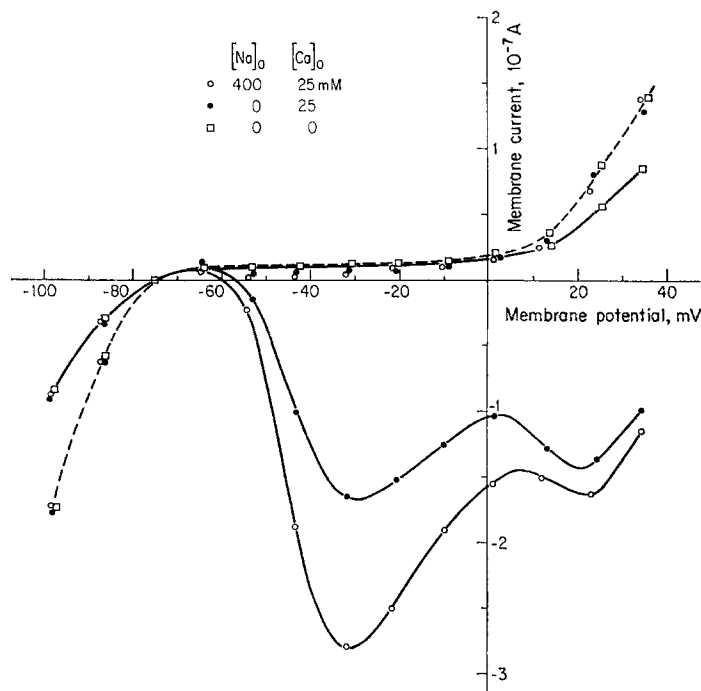
FIGURE 3. Current-voltage relation of the egg cell membrane during voltage clamp. Open circles represent data in 400 mM Na and 25 mM Ca; filled circles, those in zero Na and 25 mM Ca and open squares, those in Na- Ca-free solution. Those on a broken line are steady-state currents obtained just before the termination of a 1.7-s voltage pulse and those on continuous lines represent relations at the peak of the inward current. For the experiment in Na- Ca-free solution those on a continuous line are instantaneous currents obtained just after the initial capacitative current since no initial inward current occurs in this solution. This is also the case for the data obtained in the other two solutions in the range of membrane potentials more negative than −60 mV. Holding potential, −76 mV. Cell diameter, 1 mm.

resting and zero, the membrane current in Na- Ca-free solution was outward and of a small amplitude. The current was practically time invariant and its amplitude coincided with that of the steady-state current obtained in 400 mM Na and 25 mM Ca as well as in zero Na and 25 mM Ca. This time-invariant current was almost voltoge independent. The current-voltage relation often tended to show a slightly negative slope resistance in this range of membrane potentials. A similar negative slope resistance in the current-voltage relation has been observed in tunicate egg cell membrane (Miyazaki et al., 1974 *d*) as well as in membrane of other starfish eggs (Miyazaki et al., 1974 *b*).

Outward-going rectification appeared in the steady-state current-voltage relation when the membrane potential exceeded the zero potential level in all

three solutions. The amplitude of the steady-state outward current at a given membrane potential was common to the three cases. Records obtained in Na- Ca-free solution show that the membrane current during outward-going rectification includes instantaneous (continuous line) and time-dependent (broken line) components. The latter increased gradually with time during the maintained voltage pulse. At large positive membrane potentials (see traces +34 mV in Fig. 2) the outward current reached its maximum amplitude and then declined to the steady-state amplitude during the maintained voltage pulse. This suggests that there is a fast inactivating component of the outward current.

If the outward currents carried by Na^+ and Ca^{++} are neglected, and if the removal of the external Na^+ and Ca^{++} does not alter the current carried by ions other than Na and Ca, the peak amplitude of the inward current carried by Na^+ and Ca^{++} can be calculated by subtracting the current obtained in the Na- Ca-free solution from the current with Na and Ca. Since the current in the Na- Ca-free solution is practically time invariant between -70 mV and $+10$ mV, the amplitude of the peak inward current carried by Na^+ and Ca^{++} can be obtained as the difference between the observed peak inward current and the current at the final steady state at which membrane currents with and without Na and Ca coincide. For more positive membrane potentials the current in the Na- Ca-free medium is time dependent. Therefore, the peak inward current carried by Na^+ and Ca^{++} was estimated by actual subtraction. As will be described later the amplitude of the fast inactivating component of the outward current seems to increase as the external Ca concentration increases. In other words, the current carried by ions other than Na^+ and Ca^{++} may be altered by the removal of the external Na and Ca. This may result in an error of the estimation of the peak amplitude of the inward current for the range of relatively large positive membrane potentials. The error, however, seems to be small if the external Ca concentration is below 25 mM. This is because the amplitude of the fast inactivating outward current is likely to be small in this range of Ca concentration and because the outward current is likely to develop more slowly than the inward current.

Selective Inactivation

The foregoing results indicate that the cell membrane has two inward current mechanisms. The current-voltage relations (Fig. 3) indicate that the inward currents of the two mechanisms start to become significant at different membrane potentials, one at about -55 mV and the other at $-6 \sim -7$ mV. The results illustrated in Fig. 4 show that inactivation of the two mechanisms also occurs at different membrane potentials. The inward current associated with a -10-mV pulse was inactivated almost completely at a conditioning membrane potential of -35 mV. However, at the same conditioning potential, a substantial fraction of the inward current remained for a test pulse of $+22$ mV
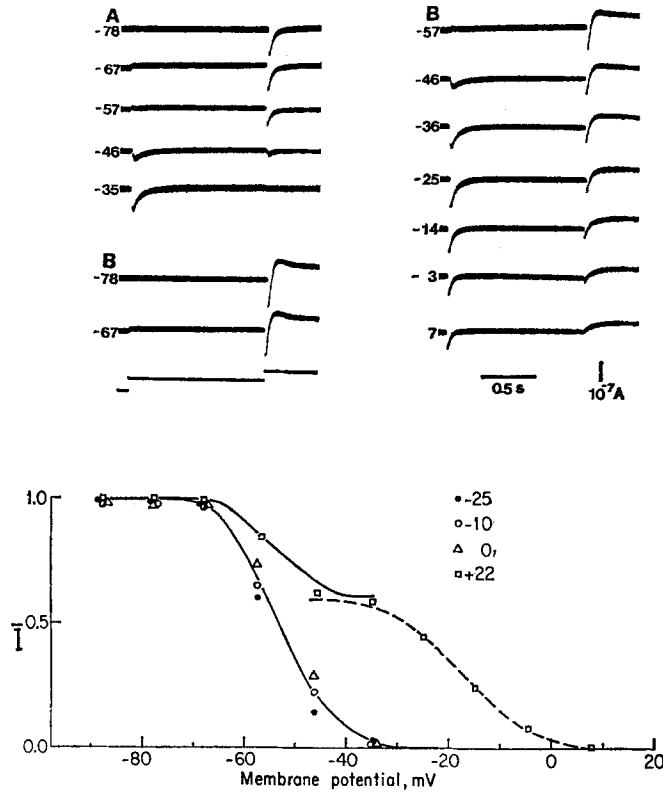
FIGURE 4. Inactivation of the inward current. Top, a conditioning voltage pulse is followed by a test pulse. The membrane potential during the test pulse is -10 mV in A and $+22$ mV in B. The membrane potential during the conditioning pulse is listed by each trace. The holding potential, -73 mV. Diameter, 950 μm. Bottom, the relative peak amplitude, $\bar{I}$ of the inward current is plotted against the conditioning membrane potential. The amplitude obtained when the conditioning membrane potential is -80 mV is taken as unity. Membrane potentials during the test pulse were -25, -10, 0, and $+22$ mV. Two continuous lines and a broken line were drawn according to Eq. 1 with $V_h = -53$ mV and $k_h = 5$ mV, and $V_h = -18$ mV and $k_h = 7$ mV, respectively.

and a complete inactivation occurred at about $+7$ mV. In order to examine the inactivation quantitatively the peak amplitude of the inward current carried by Ca^{++} and Na^+ was estimated by subtracting the current recorded in the Na- Ca-free saline under the same conditions of membrane potential. The corrected peak amplitude was normalized at a membrane potential of -80 mV. The relation between the relative amplitude $\bar{I}$ and the conditioning membrane potential (Fig. 4, bottom) can be expressed by (Hodgkin and Huxley, 1952 b):

$$\bar{I} = \left\{ 1 + \exp\left(\frac{V - V_h}{k_h}\right) \right\}^{-1}, \tag{1}$$

where V_h is the membrane potential at which I becomes one half and k_h is a constant characterizing the shape of the relation. For test pulses more negative than 0 mV the I-V relation shows a single step. The continuous line in Fig. 4 was drawn from Eq. 1 choosing $V_h = -53$ mV and $k_h = 5$ mV. When the membrane potential of the test pulse was $+22$ mV the inactivation occurred in two steps. The relation during the first step is similar to that found for test pulses at more negative membrane potentials. The second step was characterized by Eq. 1 with $V_h = -18$ mV and $k_h = 7$ mV (illustrated with a broken line in Fig. 4). Thus, the membrane potential at half inactivation for one mechanism is 35 mV more positive than that of the other. The corresponding difference observed in several other preparations ranged between 33 and 37 mV. A similar difference has been found in the membrane potential for the activation of the two mechanisms. In order to describe the two inward current mechanisms we shall simply use the terms "channel I" and "channel II" in this paper, the former being activated or inactivated at a membrane potential 30 $\sim$ 40 mV more negative than that of the latter. The usage of a term "channel" does not necessarily imply that the ion permeation is actually mediated through channel-like structures of the membrane. The significant difference of V_h found for the two channels indicates that a conditioning membrane potential can be found at which the current of channel I is nearly completely inactivated while that of channel II is kept intact.

Effects of Ca Ions

CHARGE CARRIERS   As mentioned already a substantial inward current of channel I as well as II remains after replacing the total Na with Tris. It is concluded that this current is carried by Ca ions but not by either Tris or Mg ions for the following reasons. (a) The replacement of NaCl with isosmolar sucrose gave the same inward current as that obtained by replacement with Tris-Cl. This indicates that Tris ions are not charge carriers. In most of the present experiments Tris was used to replace Na, but in a few cases such as that shown by Fig. 11 I sucrose was used. (b) The removal of 50 mM Mg in the solution resulted in an increase rather than a decrease in the inward current. This indicates that Mg ions are not charge carriers. As will be described later, Mg^{++} acts as a blocking cation for the inward current. (c) The inward current increased with increasing external Ca concentration. Fig. 5 A and B show the currents obtained at membrane potentials of -30 and $+14$ mV when the Ca concentration was 0, 10, 25, and 100 mM. At -30 mV the inward current increased as the Ca concentration increased. When the membrane potential became positive the fast inactivating component of the outward current appeared. Record B indicates that the amplitude of this component increased with increasing external Ca concentration. The amplitude of the inward current increased from 10 to 25 mM but no inward current was
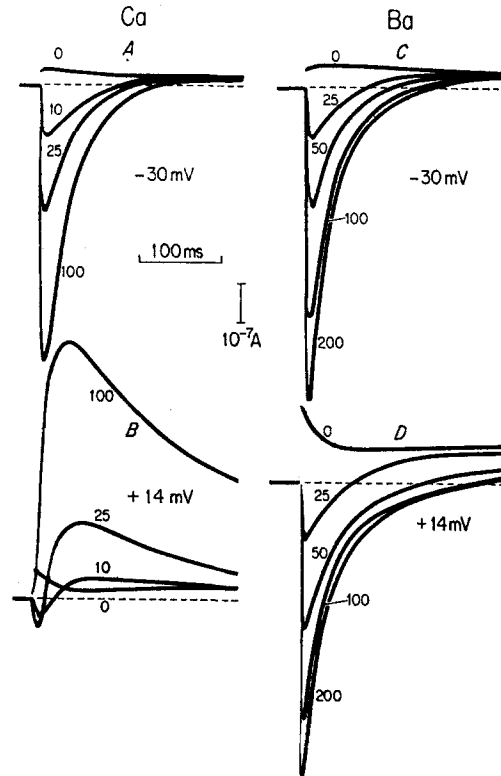
FIGURE 5. Membrane currents during voltage clamp. A and B, currents obtained at 0, 10, 25, and 100 mM Ca in Tris media. The membrane potential during voltage clamp was −30 mV for A and +14 mV for B. Holding potential, −74 mV. Diameter, 900 μm. C and D, currents obtained at 0, 25, 50, 100, and 200 mM Ba in Tris media. The membrane potential during voltage clamp was −30 mV for C and +14 mV for D. Holding potential, −73 mV. Diameter, 900 μm.

detected at 100 mM Ca in this preparation. This is interpreted in the following way. The inward current always increases as the Ca concentration increases. The fast inactivating component of the outward current also increases with the Ca concentration but its rate of increase is greater. Therefore, the net inward current may become smaller at high Ca concentration. The amplitude of the fast inactivating outward current as judged from maximum amplitude of the net outward current varies considerably among different preparations even at the same Ca concentration. The fast inactivating outward current created difficulties in obtaining the peak amplitude of the inward current carried by Ca^{++} at large positive membrane potentials. To obtain the peak amplitude, preparations having a smaller fast inactivating outward current were selected.

CURRENT CARRIED BY ca^{++}, sr^{++}, OR ba^{++} Besides Ca^{++}, the inward current is carried bv Sr^{++} or Ba^{++} but not by Mg^{++} or Co^{++}. Three sets of

recordings in Fig. 6 A were obtained from the same cell when the external Na-free solution contained 10 mM of Ca (solution C), Sr or Ba. The time-courses of the membrane currents are similar among the records obtained in the three solutions when the membrane potential was more negative than +9 mV. Records obtained at +20 and +31 mV show that the outward current in the Ca solution develops more rapidly and reaches an amplitude significantly greater than that found in either Sr or Ba solution. The amplitude of the outward current in Ca declined gradually during the maintained voltage
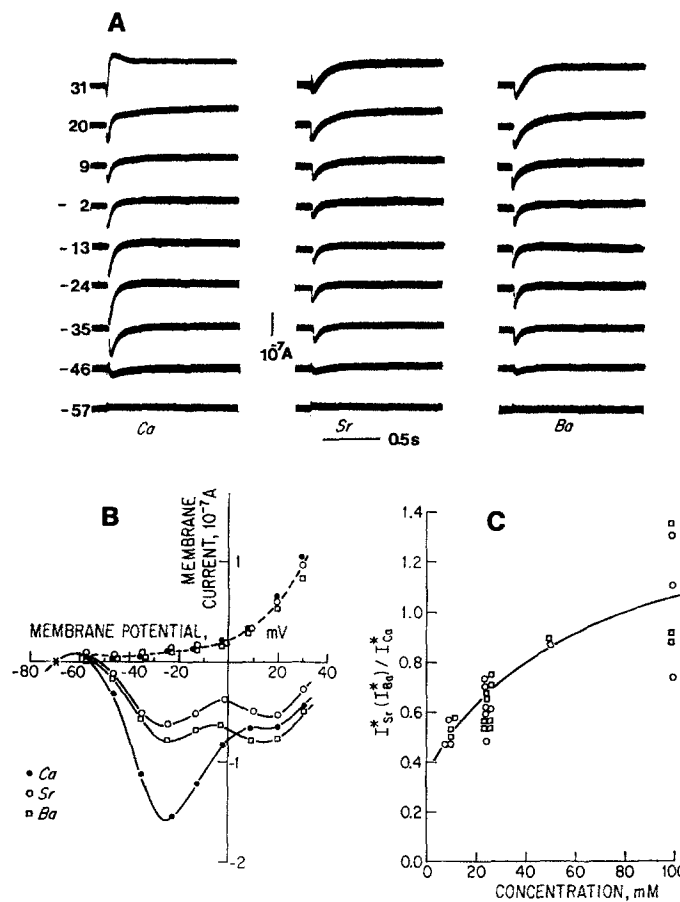

FIGURE 6.   (A) Membrane currents in Ca, Sr, and Ba media. The external solution contained no Na and 10 mM Ca, Sr, or Ba. Holding potential, −72 mV. Diameter, 900 μm. Membrane potential during voltage clamp is listed by each trace. (B) Current-voltage relations obtained from records in A. Continuous lines, at the peak of the inward current and a broken line, just before the termination of a 1.2-s voltage pulse. (C) Maximum amplitudes (I^*_{Ca}, I^*_{Sr}, and I^*_{Ba}) of the corrected peak inward current for channel I were obtained in Ca, Sr, and Ba solutions and I^*_{Sr}/I^*_{Ca} (open circles) and I^*_{Ba}/I^*_{Ca} (open squares) are plotted against the concentration $C = [Ca]_o = [Sr]_o = [Ba]_o$. The continuous line shows the tendency of change.

pulse and became equal to that in Sr or Ba solution at the steady state. This suggests that the fast inactivating component of the outward current, which is marked in Ca media, is practically absent or is at least much less marked in Sr and Ba media. The magnitude of the fast inactivating outward current was shown to increase with increasing Ca concentration. This is, however, not the case in Ba media as shown by Fig. 5 C and D. Neither the record obtained at -30 mV (C) nor that obtained at $+14$ mV (D) shows a significant fast inactivating outward current even when the Ba concentration is 200 mM.

The current-voltage relations for peak and steady-state conditions for the traces in Fig. 6 A are given in Fig. 6 B. The amplitude of the peak inward current showed maxima at -25 mV and at $+15$ mV in the Ca, Sr, and Ba solutions. However, the maximum amplitude itself is different in the three cases. The maximum amplitude of the current carried by Ca^{++}, Sr^{++}, or Ba^{++} through channel I was estimated by measuring the amplitude of the peak inward current from the level of the steady-state current. They are denoted as I^*_{Ca}, I^*_{Sr}, and I^*_{Ba}, respectively. I^*_{Sr} and I^*_{Ba} are substantially smaller than I^*_{Ca} in the case shown in Fig. 6 B and ratios I^*_{Sr}/I^*_{Ca} and I^*_{Ba}/I^*_{Ca} are 0.47 and 0.50, respectively. When similar experiments were performed at varying concentrations of $C = [Ca^{++}]_o = [Sr^{++}]_o = [Ba^{++}]_o$ it was found that those ratios depend on C. The result is summarized in Fig. 6 C where I^*_{Sr}/I^*_{Ca} and I^*_{Ba}/I^*_{Ca} obtained in different preparations were plotted against concentration. Although considerable variation is found among ratios obtained at a given C the result clearly indicates that both I^*_{Sr}/I^*_{Ca} and I^*_{Ba}/I^*_{Ca} increase with C. The corresponding ratios for channel II were measured in several preparations after selectively inactivating the current of the low threshold channel. The current carried by Ca^{++}, Sr^{++}, or Ba^{++} was then estimated by subtracting the current obtained in the Na- Ca-free solution. I^*_{Sr}/I^*_{Ca} and I^*_{Ba}/I^*_{Ca} for channel II were 0.90 ± 0.26 and 0.85 ± 0.25 ($n = 4$) at $C = 10$ mM and 0.92 ± 0.26 and 1.00 ± 0.26 ($n = 4$) at $C = 25$ mM. These values obtained at low concentrations of C are comparable to those obtained at higher concentrations for the current of channel I.

If the permeation of Ca^{++} through the Ca channel includes the binding of Ca^{++} to a site near or on the channel (Hagiwara and Takahashi, 1967 a; Hagiwara et al., 1974; see also Hille, 1974) the current $I_{Ca}(V)$ carried by Ca^{++} at membrane potential V can be given by:

$$I_{Ca}(V) = I_{Ca\,max}(V) \cdot \frac{[Ca^{++}]_o}{[Ca^{++}]_o + K_{Ca}(V)}, \qquad (2)$$

$I_{Ca\,max}$ being I_{Ca} expected when $[Ca^{++}]_o$ approaches infinity and K_{Ca} the dissociation constant of the site to Ca^{++}. The ratio I^*_{Ba}/I^*_{Ca} is then given by:

$$\frac{I^*_{Ba}}{I^*_{Ca}} \doteq \frac{I^*_{Ba\,max}}{I^*_{Ca\,max}} \cdot \frac{C + K^*_{Ca}}{C + K^*_{Ba}}, \qquad (3)$$

where $C = [Ba^{++}]_o = [Ca^{++}]_o$ and stars indicate the values at the membrane potential at which I_{Ba} and I_{Ca} become maximal. If one assumes that the ion species having a higher affinity is less mobile through the channel the dependence of I^*_{Sr}/I^*_{Ca} or I^*_{Ba}/I^*_{Ca} on C found in the present work can be explained by referring to Eq. 3. If the affinity of the site to Ba^{++} is lower than that to Ca^{++} and if the mobility of Ba^{++} through the channel is greater than that of Ca^{++}, $I^*_{Ba\ max}/I^*_{Ca\ max} > 1$ and $K^*_{Ca}/K^*_{Ba} < 1$. I^*_{Ba}/I^*_{Ca} should then increase with an increasing concentration C as in the present case. The fact that I^*_{Sr}/I^*_{Ca} and I^*_{Ba}/I^*_{Ca} obtained for channel II at low concentrations are comparable to those obtained at high concentrations for channel I suggests that K^*s for the former are substantially smaller than those for the latter. In other words channel II seems to be a more saturable system than channel I is. Table II presents actual values of I^*_{Ca} at various Ca concentrations.

TABLE II

MAXIMUM AMPLITUDES FOR MEMBRANE CURRENTS CARRIED BY
Ca^{++} OR Na^{++}

$[Ca^{++}]_o$	10 mM	25 mM	50 mM
	$\mu A/cm^2$	$\mu A/cm^2$	$\mu A/cm^2$
I^*_{Ca} (channel I)	3.3 ± 1.0 ($n = 7$)	5.6 ± 1.5 ($n = 8$)	12.3 ± 4.0 ($n = 5$)
I^*_{Ca} (channel II)	3.5 ± 1.3 ($n = 5$)	4.3 ± 1.2 ($n = 5$)	—
I^*_{Na} at $[Na^+] = 400$ mM	1.6 ± 0.9 ($n = 8$)	5.3 ± 2.3 ($n = 7$)	6.5 ± 2.7 ($n = 5$)

The surface area of the egg cell was calculated from its diameter by assuming its spherical shape. Our preliminary electronmicroscopic study shows numerous villus-like structures of the surface membrane. Each villus is about 0.5 μm. If these are taken into consideration the actual surface area becomes significantly greater than that calculated under the above assumption.

TIME-COURSE OF Ca CURRENT   As mentioned above the fast inactivating outward current is found when the membrane potential is positive and its amplitude increases with an increasing Ca concentration. This makes it difficult to study the time-course of the Ca current in this range of membrane potentials. As shown by Fig. 5 C and D the fast inactivating outward current is small in Ba media and does not increase when the Ba concentration is increased. Therefore, the above difficulty can be overcome if the current carried by Ba^{++} is observed instead of that carried by Ca^{++}. Figs. 5 A and C and 6 A show that the time-course is common for currents carried by Ca^{++} and Ba^{++} at least for the range of negative membrane potentials. Therefore, it seems reasonable to assume that their time-courses are also similar for the range of

positive membrane potentials where the fast inactivating outward current masks the inward current in Ca media.

The currents carried by Ba^{++} at 200 mM solution obtained by subtracting those in Ba-free solution are illustrated in Fig. 7 on logarithmic scale (broken lines). The currents of channels I (thin continuous lines) and II (thick continuous lines) were then separated by using selective inactivation technique. The time-course of the current can be characterized by the activation and inactivation processes such as $m^n \cdot h$ in the case of a squid axon. In the present case, however, no attempt was made to obtain m, h, and their time constants. The result, nevertheless, suggests that the time constant of activation as well
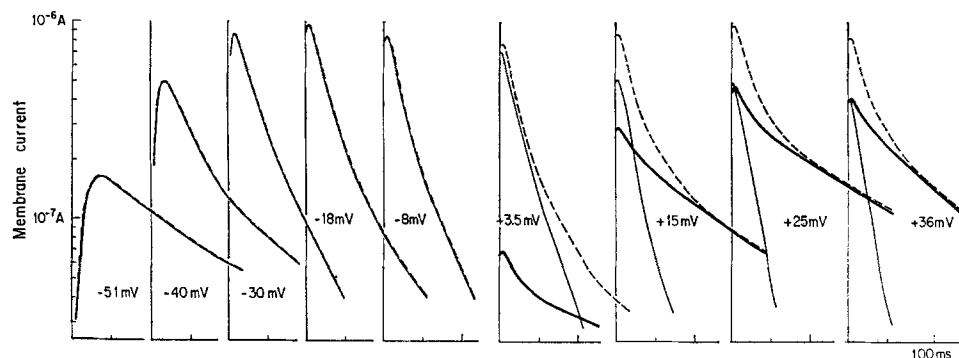

FIGURE 7.   Time-course of the inward current. The external Na-free solution contained 200 mM Ba. Currents carried by Ba^{++} were obtained by subtracting currents recorded in the Na- Ca-free solution (solution D). The inward current is plotted in the positive direction on logarithmic scale. Broken lines, corrected total currents obtained at a holding potential of -76 mV. Thick continuous lines, currents of channel II obtained at a holding potential of -35 mV. Thin continuous lines, currents of channel I.

as inactivation decreases as the membrane potential becomes more positive in a manner similar to that found in most adult excitable tissues. The inactivation process of current of channel II is substantially slower than that of the current of channel I obtained at the same membrane potential. In other words there is a distinct difference in the temporal behavior between currents of the two channels. Channel II can, therefore, be referred to as the slow channel.

EFFECT OF CONCENTRATION   Currents of the two channels were obtained as before when the Ba concentration was 25, 50, 100, and 200 mM in the absence of Na. The results show that the time-course of the current at a given membrane potential is practically independent of the Ba concentration. The plot of the peak amplitudes against the membrane potential (Fig. 8 A) shows that the peak inward current of channel I became maximal at about -20 mV and that this was practically independent of the Ba concentration. The corresponding membrane potential for the maximum current of channel II was
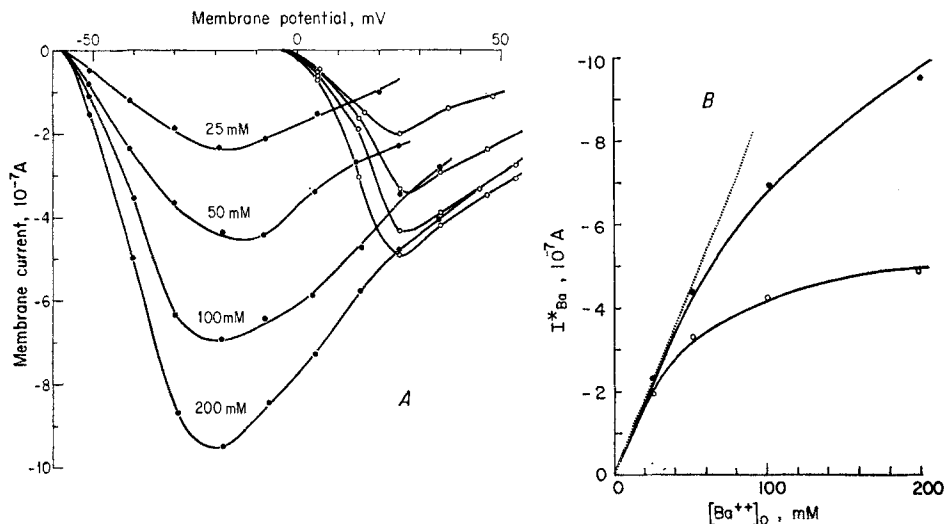
FIGURE 8.   (A) Peak amplitude of the current carried by Ba^{++} is plotted against the membrane potential for channel I (filled circles) and for channel II (open circles). They were obtained by subtracting the currents recorded in the Na- Ca-free solution. Currents of channel II were obtained at a holding potential of −35 mV. Currents of channel I were obtained by subtracting the current of channel II from the corrected total current obtained at a holding potential of −73 mV. Diameter, 900 μm. (B) Relations between the maximum amplitude of the peak inward current and the external Ba concentration. Data obtained from the experiment shown in A. Filled and open circles, current at −20 mV and at +25 mV, respectively. Continuous lines were drawn according to Eq. 2 with K_{Ba} = 213 mM (filled circles) and 73 mM (open circles).

about +25 mV. The amplitude I^*_{Ba} of the current at −20 mV for channel I and I^*_{Ba} at +25 mV for channel II were plotted against the Ba concentration in Fig. 8 B. The continuous lines were calculated from Eq. 2 with K_{Ba} = 213 mM for channel I and K_{Ba} = 73 mM for channel II. The experimental results agree reasonably well with this equation. The dissociation constant for channel I is greater than that of channel II by a factor of about 3. This result is consistent with the prediction made from the result obtained for I^*_{Ba}/I^*_{Ca}.

Effects of Na Ions

TIME-COURSE AND CURRENT-VOLTAGE RELATION   When the Na$^+$ in the Na-Ca solution is removed by replacing with Tris or sucrose a significant fraction of the inward current disappears. This fraction of the membrane current was the same for Tris and sucrose substitution and is tentatively defined as the "Na current." Traces illustrated with thicker lines in Fig. 9 A show Na currents obtained from this definition, whereas those illustrated with thinner lines represent Ca currents. The Na and Ca currents differ in their time-courses, i.e., the Na current decays with a much slower time-course. The
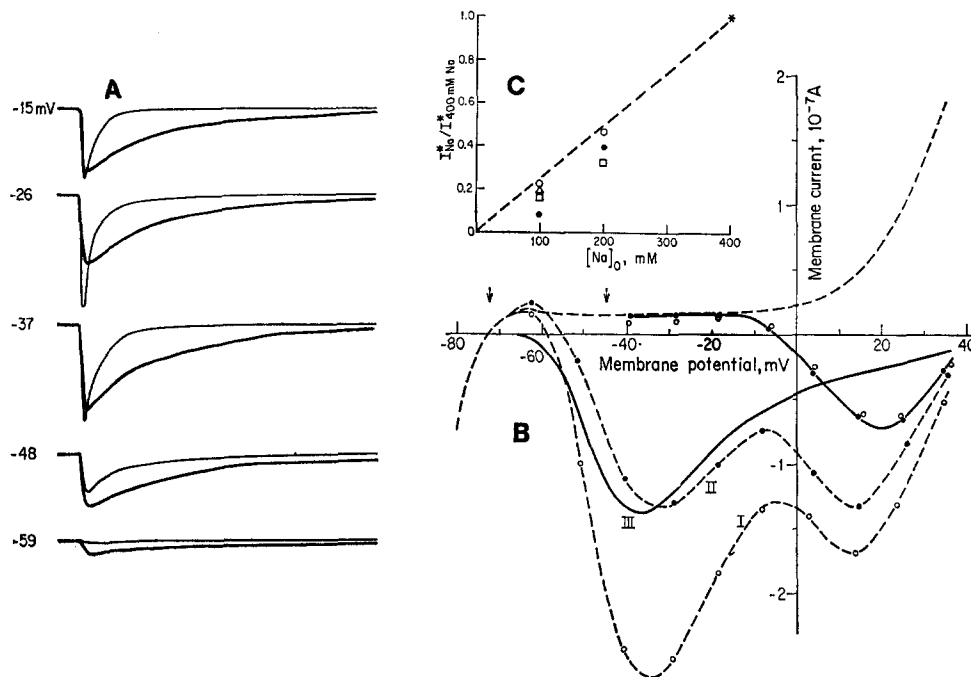
FIGURE 9. (A) Na current (thick lines) and Ca current (thin lines) obtained in solution A (400 mM Na and 25 mM Ca). The former was obtained as a difference between currents recorded with and without 400 mM Na at 25 mM Ca and the latter, as a difference between those obtained with and without 25 mM Ca in the absence of Na. Holding potential, − 76 mV. Diameter, 1 mm. (B) Current-voltage relations of the cell. I, in 400 mM Na and 25 mM Ca. II, in no Na and 25 mM Ca. III is the difference between I and II and represents the relation at the peak of the Na current. Open and filled circles with a continuous line show relations obtained at the peak of the inward current in 400 mM Na and 25 mM Ca, and zero Na and 25 mM Ca, respectively, after inactivating the current of channel I by holding the membrane potential at −45 mV. They show that the current of channel II is unaffected by the removal of Na. The steady-state current voltage relation is shown by a broken line on the top of the figure. Holding potential, −75 mV. Diameter, 900 μm. (C) The maximum amplitude of the peak Na current is plotted against [Na]$_o$. The amplitude at 400 mM Na is taken as unity for each preparation. The Ca concentration was 25 mM throughout.

peaks of the two currents, however, occur at approximately the same time. Therefore, the peak amplitude of the Na current can be obtained as a difference between the peak amplitudes of the inward currents found with and without Na. Relations I and II in Fig. 9 B show those for the peak inward current obtained with and without 400 mM Na at 25 mM Ca. The difference between I and II (relation III) represents the relation at the peak of the Na current and shows a single maximum of the current in the range of the membrane potential where the Ca current of channel I becomes maximal. The

result, therefore, suggests that the Na contribution is restricted only to channel I. This can be examined by observing the current of channel II after inactivating the current of channel I. The result illustrated in Fig. 9 B shows that the current of channel II was unaltered by the removal of Na. The current-voltage relation at the peak of the Na current, however, differs from that of the Ca current of channel I in a few points. The activation potential at which the Na current starts to appear is slightly more negative than that of the Ca current. In the case shown by Fig. 9 A the Na current is significant but practically no Ca current is seen at -59 mV. The membrane potential of the maximum Na current was also more negative than that of the maximum Ca current. In other words, the entire current-voltage relation for the Na current is shifted from that for the Ca current along the membrane potential axis by about -10 mV. The error in the measurement of membrane potential due to the junction potential between the solution and a 3 M KCl-filled external electrode is at most 2–3 mV in the present experiment and, therefore, the above shift of the current-voltage relation cannot be attributed to differences in the junction potential.

EFFECT OF Ca   The result described above indicates that the inward current of channel I depends on both Na$^+$ and Ca^{++} in the external saline. When the Ca^{++} was removed from the solution by replacing it with Tris$^+$ or Mg^{++}, the entire inward current disappeared even when the Na concentration was significant. This was not due to any irreversible damage of the cell membrane produced by the removal of Ca^{++} since a complete recovery of the inward current was observed upon returning to the original solution. The above experimental result indicates that the Na current, defined as the current which disappears upon the removal of the external Na, depends upon the external Ca^{++} concentration. Fig. 10 I shows that the Na current at 10 mM Ca was substantially smaller than that at 25 mM Ca. But the increase of the Ca concentration from 25 to 50 mM did not result in any further increase in the Na current. Therefore, the Na current seems to increase with the external Ca concentration up to levels of about 25 mM. The above effect of Ca upon the Na current is not due to any change in inactivation. The membrane potential of half inactivation (V_h) of channel I observed at 10 and 25 mM Ca in a single preparation was -54 mV at 400 mM Na and 25 mM Ca, -53 mV at zero Na and 25 mM Ca, -56 mV at 400 mM Na and 10 mM Ca, and -56 mM at zero Na and 10 mM Ca and so appears to be almost independent of the alteration of the Ca concentration from 10 to 25 mM. Table II shows the maximum values of the peak Na current (I^*_{Na}) obtained at 400 mM Na with different Ca concentrations. The ratio between the maximum amplitudes of the peak Na and Ca currents showed a considerable variation among different cells. I^*_{Na}/I^*_{Ca} was 0.5 $\pm$ 0.5 $(n = 9)$ at 400 mM Na and 10 mM Ca and 1.0 $\pm$ 0.5 $(n = 8)$ at 400 mM Na and 25 mM Ca.
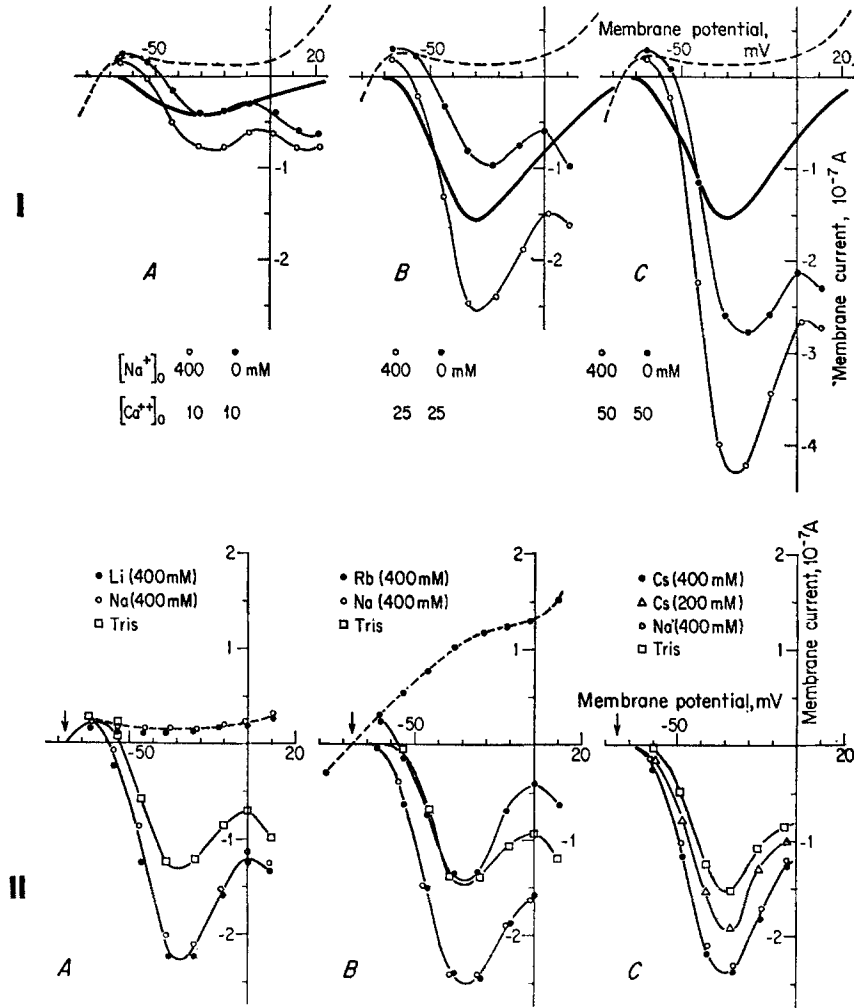
FIGURE 10. (I) Effect of [Ca]$_o$ upon the Na current. Continuous and broken lines represent current-voltage relations at the peak of the inward current and at the steady state (1.7 s after the onset of the voltage pulse), respectively (this applies also to II). Three sets of relations were obtained from the same cell. A thick continuous line in each set represents the relation at the peak of the Na current obtained as a difference between the currents with and without Na. Holding potential, -76 mV. Diameter, 900 μm. (II) Effects of Li$^+$, Rb$^+$, and Cs$^+$ upon the Na current. The Ca concentration was 25 mM throughout. (A) Effect of Li$^+$. Holding potential, -77 mV. Diameter, 900 μm. (B) Effect of Rb$^+$. The upper continuous line for Rb represents the relation at the peak of the inward current while the lower continuous line for Rb, that of the corrected peak inward current. The relations for Na and Tris are also those of the corrected peak inward current. Holding potential, -77 mV. Diameter, 900 μm. (C) Effect of Cs$^+$. Relations between the corrected peak amplitude of the inward current and the membrane potential. Holding potential, -80 mV. Diameter, 900 μm.

NA CONCENTRATION   The amplitude of the Na current decreased with decreasing external Na concentration. The peak amplitude of the Na current was obtained at 100, 200, and 400 mM Na when the Ca concentration was 25 mM. The reduction of the Na concentration was performed either by replacing NaCl with Tris-Cl or sucrose and both methods gave the same results. The maximum amplitude of the peak Na current, I^*_{Na}, occurs approximately at the same membrane potential independently of the Na concentration. Fig. 9 C summarizes the result obtained with four preparations. The result shows that I^*_{Na} increases with the Na^+ concentration. The relation is slightly concave upward.

SELECTIVITY AMONG Na^+, Li^+, Cs^+, AND Rb^+ Replacement of the Na^+ in the external solution with Li^+ resulted in no observable changes in the membrane current. The result in Fig. 10 IIA shows that the amplitude of the peak inward current was unaltered by the replacement of Na with Li. Fig. 10 IIB shows the current-voltage relations obtained at 400 mM Rb. The amplitude of the steady-state current was much greater than that obtained at 400 mM Na since the Rb permeability of the resting cell membrane is significant (Hagiwara and Takahashi, 1974). When Ca^{++} was removed from the Rb solution the inward current disappeared. Membrane currents between −70 and 0 mV were practically time invariant and their amplitudes were equal to those of the steady-state current found when Ca was present. This result was the same as that found when Ca was removed from the Na or Tris solution. Therefore, the corrected peak inward current in Rb solution was obtained as the difference between the observed peak inward current and the steady-state current. The result shows that the amplitude of the corrected peak inward current in 400 mM Rb is almost identical to that of the corrected peak current in 400 mM Na. Fig. 10 IIC shows corrected peak inward currents in 400 mM Na, 400 mM Cs, 200 mM Cs, and Tris. The Cs and Na currents are almost equal when Na is replaced with Cs on a mole-to-mole basis. The result also shows that the Cs current decreases with decreasing Cs concentration.

The foregoing result indicates that the mechanism responsible for the Na current shows little selectivity among different alkali cations. No experiment was performed to examine the K permeability. An increase in the external K concentration caused a large increase in the resting membrane conductance and this made it difficult to observe inward currents. Since no selectivities are found among Na^+, Rb^+, Cs^+, and Li, it is likely that the selectivity between K^+ and Na^+ is also small. The selectivity of Na^+ over $Tris^+$ is, however, highly significant.

Effect of Blocking Cations

Analysis of the Ca-dependent action potential in a barnacle muscle fiber has shown that various divalent cations such as Mg^{++}, Co^{++}, Mn^{++}, and Ni^{++}

have a blocking effect on the current of the Ca channel (Hagiwara and Nakajima, 1966; Hagiwara and Takahashi, 1967 *a*). The external saline of the starfish egg cell usually contains 50 mM Mg^{++}. Miyazaki et al. (1974 *a*) have shown that the amplitude of the action potential was increased by the removal of Mg^{++} from the external saline. They interpreted the increase as a recovery from the blocking by Mg^{++}. This was confirmed in the present work. The removal of 50 mM $MgCl_2$ from saline A or B by replacing with isosmolar Tris-Cl increased the peak amplitude of the current of channel II. The current of channel I was not much affected. The fact indicates that the sensitivity to blocking cations is different between currents of the two channels.

The difference in sensitivities between currents of the two channels was further examined by observing the effect of Co^{++} which has a blocking effect much greater than that of Mg^{++}. The experiment was performed in the solution in which Tris-Cl and $MgCl_2$ of solution B (25 mM Ca) had been replaced with 950 mM sucrose. This was to avoid the formation of a complex salt by Tris and Co^{++}. $CoCl_2$ was then added to the solution and the peak amplitude of the current carried by Ca^{++} was estimated by using the subtraction method and plotted for different membrane potentials in Fig. 11 IA. Fig. 11 IB shows currents of channel II obtained by selective inactivation technique. The result shows that the currents of both channels decreased with an increasing Co^{++} concentration. But the rate of decrease was much greater for channel II. The maximum peak currents, I^*_{Ca}'s, for channels I and II were normalized to their values in the absence of Co^{++} and plotted for different Co^{++} concentrations in Fig. 11 IC.

Analysis of the Ca-dependent action potential of a barnacle muscle fiber indicates that the blocking cation, Co^{++}, competitively occupies the site normally occupied by Ca^{++} even though Co^{++} is impermeant through the Ca channel (Hagiwara and Takahashi, 1967 *a*). If this is the case in the starfish egg cell membrane, the normalized maximum current, I^*_{Ca} is given by

$$ I^*_{Ca} = \left(1 + \frac{[Co^{++}]_o}{\overline{K}^*_{Co}} \right)^{-1}, \tag{4} $$

whereas $\overline{K}^*_{Co} = K^*_{Co} (1 + [Ca^{++}]_o/K^*_{Ca})$. K^*_{Ca} and K^*_{Co} are the dissociation constants of Ca^{++} and Co^{++} to the site and they are functions of membrane potential. Two broken lines in Fig. 11 C were drawn according to Eq. 4 with $\overline{K}^*_{Co} = 7$ and 1.7 mM, respectively. In two other preparations examined, K^*_{Co} for the two channels was 6.3 and 1.7 mM and 4.7 and 1.3 mM, respectively. The result, therefore, shows that the current of channel II is significantly more sensitive to Co^{++} than that of channel I. The ratio between K^*_{Co}'s of the two channels is 3.6 ∼ 4.1. A similar ratio has been obtained between K^*_{Ba}'s of the two channels. This indicates that the site of channel II has greater affinity to divalent cations compared with that of channel I.
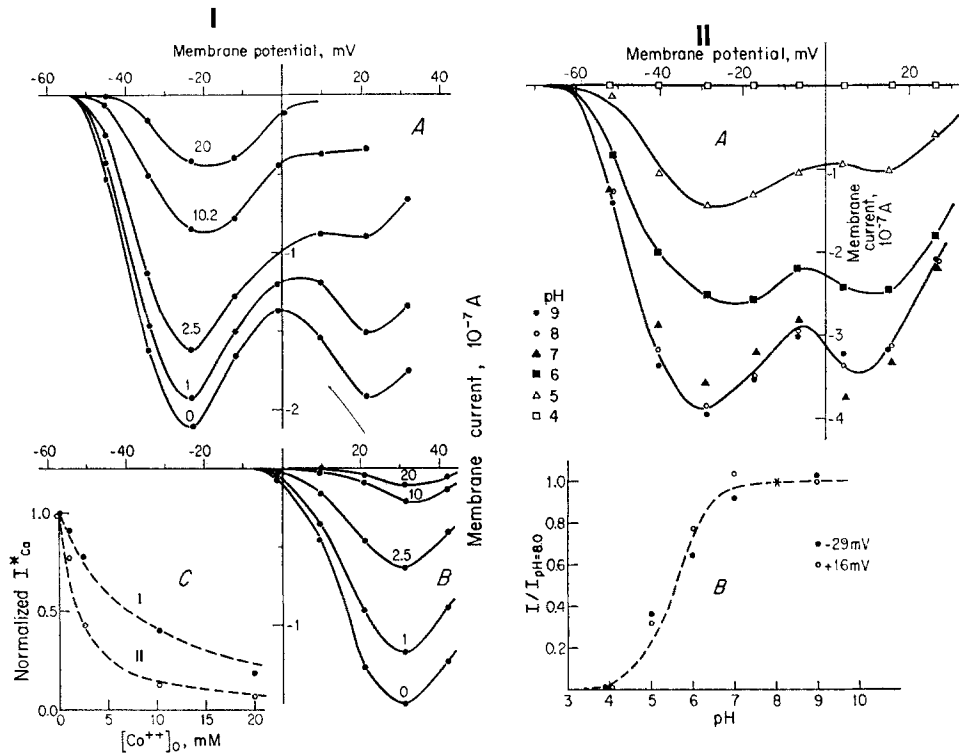
FIGURE 11. (I) Effect of Co^{++} upon the inward current. (A) Relations between the corrected peak inward current and the membrane potential at different Co^{++} concentrations (listed by each curve). The composition of the Co-free solution was 25 mM CaCl$_2$, 950 mM sucrose, 10 mM KCl, and no MgCl$_2$. Holding potential, −78 mV. Diameter, 800 μm. (B) Relations obtained from the same cell as that in A but at a holding potential of −40 mV. (C). The maximum amplitude of the corrected peak inward current is plotted against [Co^{++}]$_o$. The amplitude obtained in the absence of Co is taken as unity. Broken lines were drawn according to Eq. 4 with $\bar{K}^*_{Co}$ = 7 mM for channel I(I) 1.7 mM for channel II (II), respectively. (II) Effect of pH on the inward current. (A) Corrected peak inward current was plotted for different membrane potentials. Different symbols represent currents at different pH's. The external solution contained 25 mM Ca and 380 ∼ 423 mM Na. Holding potential, −80 mV. Diameter, 900 μm. (B) Amplitudes of corrected peak inward currents at −29 mV (filled circles) and +16 mV (open circles) are plotted against pH. The amplitude at pH 8 is taken as unity in both cases. The data were obtained from experiments shown in A. A broken line was drawn according to the Henderson-Hasselbalch equation with pK = 5.6.

The above experiment was performed in Na-free media. Similar experiments were also performed in Na media, in a solution containing 523 mM NaCl, 25 mM CaCl$_2$, and 10 mM KCl but no MgCl$_2$. $\bar{K}^*_{Co}$'s of channel I obtained in three preparations were 7.1, 7.7, and 8.3 mM, respectively, and are similar to those obtained in the absence of Na. When 523 mM NaCl in the

above solution was replaced with sucrose the maximum amplitude of the peak inward current of channel I became approximately one-half. This indicates that approximately 50% of the total current is represented by Na current. At 25 mM Ca in sucrose media the maximum peak inward current of channel I was reduced to less than 10% of the original value when the Co concentration was 25 mM. Subsequent replacement of sucrose with NaCl usually resulted in little increase in inward current. This suggests either that the flow of the Na current is coupled with that of the Ca current or that Na does not carry current but modifies the Ca current.

Effect of pH

The effect of pH upon the inward current of the cell membrane was examined in solutions in which the Ca concentration was 25 mM and the Na concentration varied between 423 and 380 mM (see Methods). As mentioned before, the above variation in the Na concentration at pH 7.7 did not result in any significant change in the inward current. The amplitude of the corrected peak inward current relative to that obtained at pH 8.0 was plotted against the membrane potentials for different pH's in Fig. 11 IIA. It shows that the membrane potentials at maximum peak inward current do not change significantly as the pH of the external solution was altered. The peak inward currents at −29 mV and at +16 mV were plotted against pH in Fig. 11 IIB. The amplitude of the current became maximal at −29 mV and the current at this potential represents the current of channel I. The major fraction of the current at +16 mV should be the current of channel II. The result indicates (a) both currents show a similar dependence upon the external pH, and (b) the common relation resembles the titration curve of a weak acidic site whose pK is 5.6. A broken line in Fig. 11 IIB was drawn according to the Henderson-Hasselbalch equation with pK = 5.6. A similar result was obtained in three other cases examined and their pK's were 5.6, 5.5, and 5.6. This indicates that, as far as the pK is concerned, the molecular mechanisms for the two channels are common. This pK value is close to that obtained for the Na conductance in the frog Ranvier node (pK = 5.2, Hille, 1968) but different from that obtained for the Na conductance of a squid axon (pK = 6.5, Stillman et al., 1971).

Effects of Tetrodotoxin and Procaine

Tetrodotoxin (TTX) blocks the current of the Na channel of a variety of tissues at extremely low concentrations (50–100 nM, Narahashi et al., 1964) whereas the current of the Ca channel is not affected by 3 μM TTX (Ozeki and Grundfest, 1965; Hagiwara and Nakajima, 1966). The effect of TTX upon the membrane current of the starfish egg was examined in solution A containing 400 mM Na and 25 mM Ca. No changes were found in the mem-

brane current even when the TTX concentration was 3 μM. This result agrees with the finding by Miyazaki et al. (1974 *a*) that the action potential of a starfish egg cell is insensitive to TTX. The result indicates that both channels are insensitive to TTX.

Procaine is known to block the current of the Na channel at a concentration below 10 mM (Taylor, 1959). The current of the Ca channel is not affected at all at this concentration (Fatt and Katz, 1953; Hagiwara and Nakajima, 1966). Fig. 12 shows the effect of procaine upon the membrane current examined in solution A. The amplitude of the inward current of channel I as well as II was substantially decreased at 1.8 mM procaine. The inward current practically disappeared at 7.3 mM. The result indicates, therefore, that
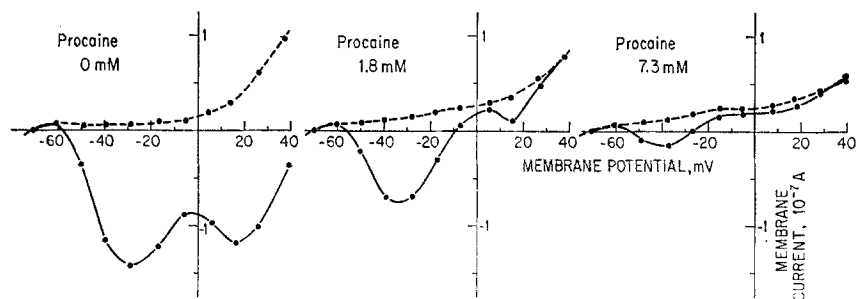

FIGURE 12.   Effect of procaine upon the inward current. Current-voltage relations of the cell at the peak of the inward current (continuous line) and at the steady state observed just before the termination of a 1.5-s voltage pulse (broken line). The external solution contained 400 mM Na and 10 mM Ca. Holding potential, −72 mV. Diameter, 750 μm.

the currents of both the channels are sensitive to procaine to approximately the extent seen for the Na current of various adult tissues.

DISCUSSION

The present experimental results show the existence of two inward current mechanisms in the membrane of a starfish egg cell. The criteria to distinguish the two mechanisms are the following. (*a*) *Membrane potential dependence*: They can be activated and inactivated separately by exploiting their different membrane potential dependencies. The two mechanisms are referred to as channel I and channel II in the present paper. (*b*) *Time-course of the membrane current*: The time-course of the current, especially that of its decay at a given membrane potential, is substantially slower in channel II than in channel I. (*c*) *Dependence of membrane current on the ion concentration*: The dependence is different between the two channels even when the charge carrier is only Ba^{++} in both channels. The dissociation constant K_{Ba} determined from the relation between the amplitude of the membrane current and the Ba concentration is substantially greater in channel I than in channel II. Consequently, the

current of channel II shows a marked saturation even when the concentration is not very high. (*d*) *Sensitivity to blocking cations*: The current of channel II is substantially more sensitive to blocking cations than that of channel I. (*e*) *Effect of Na^+*: The current of channel I depends on the external Na while that of channel II is insensitive to Na.

The behavior of the membrane current of channel II resembles that of the Ca channel found in various adult tissues producing Ca-dependent action potentials. The usual Ca channel is distinguished from the usual Na channel by various criteria. (*a*) The Ca channel is permeable to Ca^{++}, Sr^{++}, and Ba^{++} but not to monovalent cations; but the Na channel is permeable to monovalent cations and the permeability to divalent cations is very small (Baker et al., 1971). Channel II resembles the Ca channel in this respect. (*b*) The current of the Na channel increases linearly with an increase in the permeant ion concentration under usual conditions (Hodgkin and Huxley, 1952 *a*). In contrast, the Ca channel is a saturable system (Hagiwara and Takahashi, 1967 *a*; Hagiwara et al., 1974). The current of channel II shows a marked saturation even when the dilavent cation concentration is relatively low. (*c*) The current of the Ca channel is blocked by Co^{++} at a relatively low concentration and this is the case for the current of channel II. (*d*) The current of typical Na channels is blocked by 1 μM TTX as well as by 10 mM procaine whereas no effect is found for the current of the usual Ca channel. The current of channel II in starfish eggs is unaffected by 3 μM TTX but almost totally blocked by 7.3 mM procaine. The last pharmacological property of channel II is the only property so far different from that of the Ca channel in various adult tissues.

Although the current appears to depend on the external Na ions channel I of the starfish egg cell membrane differs from the usual Na channel in various respects. One of the differences is a high permeability to Ca^{++}, Sr^{++}, or Ba^{++}. Although the Na channel of the squid axon is permeable to similar divalent cations their permeabilities are very small compared with the Na permeability (Watanabe et al., 1967; Baker et al., 1971; Meves and Vogel, 1973). The Na channel shows marked selectivity among different alkali cations. For example, the permeability ratio P_{Cs}/P_{Na} is smaller than 0.05 in Na channels of a frog nerve fiber (Hille, 1972) or a squid giant axon (Chandler and Meves, 1965). Channel I of starfish eggs shows practically no selectivity among those monovalent cations. The current of channel I is insensitive to TTX and this property is also different from that of the usual Na channels. Since TTX insensitivity of the Na channel has been found in various other tissues (tissue-cultured myotube, Kidokoro, 1973; denervated muscle fiber, Redfern et al., 1970; Puffer fish muscle, Hagiwara and Takahashi, 1967 *b*; Kidokoro et al., 1974), this discrepancy does not seem to be serious. Procaine blocks the current of channel I at a concentration normally effective to adult Na channels. In

this respect the channel resembles the usual Na channel. The Na current identified as the difference of currents obtained with and without Na^+ depends on the external Ca concentration. The Na current is no longer detected when the external Ca is removed. The Ca current of channel I is blocked by Co^{++} at relatively high concentrations. After the Ca current is eliminated by Co^{++} addition of Na^+ to the external solution does not restore the inward current. If the current is carried by Na^+ the above fact indicates that the flow of Na^+ may be coupled with the flow of Ca^{++}. At present we have no evidence that the current is actually carried by Na^+. Therefore, it is more likely that Na^+ does not cross through the channel itself, but rather that it has an effect on the current by modifying the movement of Ca^{++}. In this respect channel I also resembles the Ca channel. Unlike the ordinary Ca channel the current of channel I is markedly reduced when the concentration of alkali cations is reduced by maintaining the tonicity with Tris-Cl or sucrose. As mentioned above, we suspect the modifying effect of alkali cations upon the Ca current of channel I. However, the same result can be interpreted by assuming that Tris or sucrose has the suppressing effect upon the Ca current of channel I.

Two inward current mechanisms have been found in the tunicate egg cell (Miyazaki et al., 1972). Recently voltage clamp type analysis (Okamoto et al.)[1] shows that channel II in the tunicate egg resembles the usual Ca channel and, therefore, the results are common between tunicate and starfish eggs. Unlike that of the starfish egg channel I of the tunicate egg resembles the Na channel in various respects such as the selectivity among alkali cations. In other words, the properties of channel I are different between the two preparations. It seems likely that Na channels similar to those of the tunicate egg are found in adult tunicate tissues. Action potentials of radial nerves of adult sea urchin (Sandeman, 1965; Millot and Okamura, 1968) and adult starfish (Binyon and Hasler, 1970; Podolskii, 1972) have been recorded. The ionic mechanisms of those action potentials are, however, not yet known. Therefore, it is difficult to discuss the differentiation of channels during development. If adult tissues of echinoderm have a Na channel similar to that of the squid giant axon or the node of Ranvier of a frog channel I of the egg cell may represent its precursor. If the action potential of adult starfish nerve is operated by a channel similar to channel I of the egg cell, the difference between channel I of the starfish egg and the typical Na channel is not a matter of development but rather of phylogenetic evolution.

Although an extensive analysis was not performed on the fast inactivating component of the outward current the results show that its amplitude increases as the Ca concentration increases. The transient outward current may be linked to the entry of Ca^{++} into the cell. It has been shown in a molluscan

neuron that the entry of Ca^{++} results in a transient increase of the K permeability of the membrane (Meech, 1972). The fast inactivating current in the present preparation may be caused by the same mechanism.

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