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TRANSIENT AND DELAYED POTASSIUM CURRENTS IN THE EGG CELL MEMBRANE OF THE COELENTERATE, *RENILLA KOELLIKERI*

BY S. HAGIWARA, S. YOSHIDA AND M. YOSHII

From the Department of Physiology, Ahmanson Laboratory of Neurobiology, B.R.I., and Jerry Lewis Neuromuscular Research Center, University of California, Los Angeles, California 90024, U.S.A.

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SUMMARY

1. The properties of the fast-inactivating or transient K current and the slowly inactivating or delayed K current of the membrane of immature eggs of the colonial marine coelenterate, *Renilla Koellikeri*, were studied by using voltage clamp and intracellular dialysis techniques.

2. The transient current is activated when the membrane potential becomes more positive than $-25 \sim -20$ mV (resting potential, -72 ± 5 mV) whereas the activation potential of the delayed current is $-10 \sim 0$ mV. These potentials are independent of either $[K^+]_o$ or $[K^+]_i$.

3. The inactivation of the transient current is rapid and is almost complete for membrane potentials more negative than the activation potential while it is slow for the delayed current and incomplete within a few seconds.

4. Both currents show similar reversal potentials which are predominantly determined by the K concentration gradient across the membrane.

5. The sensitivities of the conductance upon the internal K concentration differ between the two currents, suggesting that the interaction between the site and ions in the membrane channels differs between them.

6. Neither current is a Ca-activated K current.

7. 4-AP suppresses the transient current at concentrations substantially smaller than those that suppress the delayed current while TEA shows no effect on either current.

8. Intracellular application of pronase or tannic acid at relatively high concentrations does not alter the inactivation of either current.

9. The membrane includes a voltage-dependent Ca permeability which results in action potentials under current-clamp conditions.

INTRODUCTION

The increase in the K conductance of the squid axon initiated by a step depolarization shows a delayed onset and little inactivation if the voltage change is maintained. In the neurone soma membrane of the mollusc, *Onchidium*, Hagiwara, Kusano & Saito (1961) found a K conductance which inactivates rapidly during

voltage pulses. This fast inactivating or transient K conductance was also found later in snail neurone somata by Connor & Stevens (1971) and Neher (1971) and analysed extensively by them. The transient K conductance has since been found in various preparations other than molluscan neurones, i.e. cat motoneurones (Barrett & Crill, 1972), crustacean walking leg axons (Connor, 1975), starfish eggs (Miyazaki, Ohmori & Sasaki, 1975), hippocampal pyramidal neurones (Johnston & Hablitz, 1979) and cardiac muscle fibres (Kenyon & Gibbons, 1979*a*, *b*; Siegelbaum & Tsien, 1980). These findings suggest that the transient K conductance is a physiological property widely distributed among different tissues of different animals.

The membrane of the eggs of the sea pansy (*Renilla Koellikeri*) shows both the transient and the delayed K conductance increase. This gave us an excellent opportunity to compare and contrast the properties of the transient and the delayed K currents. The major experimental techniques employed were voltage clamp and intracellular dialysis.

METHODS

Materials. Immature eggs of the colonial marine coelenterate, sea pansy, *Renilla Koellikeri* Pfeffer, were used. Animals were collected off the coast of southern California during the period from May to August.

Preparation of eggs. The ovaries were dissected out of the animal and the eggs were freed from their follicular envelopes by treating with 0·1 % pronase (Calbiochem-Behring Corp., La Jolla, CA) in normal saline for 15–20 min. The eggs were covered with a jelly coat 50–80 μm thick. The eggs were spherical and had an average diameter of about 350 μm. When the internal dialysis technique was used, this jelly coat was removed to obtain a good contact between the cell surface and the glass wall. This was performed either by treating eggs with 0·5 % trypsin (Sigma Chemical Co., Saint Louis, MO) in the normal saline for 15–25 min, or mechanically by using a thin polythene tubing with a platinum wire coil inside. The egg was sucked in and out of the tubing so that it passed through the coil repeatedly; the shear exerted by the coil removed the jelly coat.

Recording. Voltage-clamp as well as current-clamp experiments were performed either by the conventional two intracellular micropipette technique, or by a method using internal dialysis. The two micropipette technique was similar to that described previously (Hagiwara, Ozawa & Sand, 1975). Intracellular glass pipettes were filled with 3 M-KCl and had a resistance of 5–10 MΩ. The membrane current was measured as an *IR* drop across 1 MΩ resistor inserted between the current electrode and the output of the feed-back amplifier (Hagiwara & Yoshii, 1980).

The techniques of voltage clamp by internal dialysis with a glass funnel were similar to those described before (Takahashi & Yoshii, 1978; Hagiwara & Yoshii, 1979). The diameter of opening at the tip of the funnel was 100–150 μm. The egg was introduced inside the glass funnel after coating the inside of the funnel with liquid silicone (curing agent of Sylgard 184, Dow Corning Corp.). The shunt resistance through the space between the cell membrane and the inner surface of the funnel was relatively low because of the remaining jelly coat and ranged between 0·3 and 0·5 MΩ. The series resistance in the voltage clamp was estimated from the initial jump of the potential when a brief constant current pulse (10 msec) was applied and was compensated electronically. The series resistance determined in this way ranged between 1 and 5 kΩ.

Solutions. The composition of the major external and internal solutions used are listed in Table 1. External solutions of various K concentrations were prepared by mixing two appropriate solutions. Chloride-free solutions were obtained by replacing Cl with methanesulphonate. The pH of the external solutions was buffered at 7·6 ~ 7·7 with either Tris or HEPES. The external solution was perfused and a complete exchange of the solutions required only a few minutes.

The Ca^{2+} concentration of the internal solution was buffered at 0·2 μM with 5 mM-EGTA (ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetra-acetic acid) and 2·5 mM-Ca gluconate. Glutamate was used as the major anion. Solutions of various K concentrations were prepared by mixing 400 mM-K and K-free arginine solutions in appropriate proportions. Glucose was added to maintain

TABLE 1. Composition of solutions

(A) External solutions (mM)

	NaCl	KCl	$CaCl_2$	$MgCl_2$	$MnCl_2$	Tris.OH	HCl	HEPES
Normal	460	10	10	50	—	10	7·5*	—
10 mM-Mn, Ca-free	460	10	—	—	10	—	—	5*
400 mM-K	70	400	10	50	—	10	7·5*	—
K-free	470	—	10	50	—	10	7·5*	—

(B) Internal solutions (mM)

	NaOH	KOH	EGTA	Ca gluconate	PIPES	L-glutamic acid	Arginine	Glucose
Standard	20	200	5	2·5	10	390†	200	170
400 mM-K	20	400	5	2·5	10	390†	—	170
K-free	20	—	5	2·5	10	390†	400	170

* Used for titration to pH 7·6–7·7.
† Used for titration to pH 7·0.

the tonicity. The pH of the internal solutions was adjusted to 7·0 with PIPES. The K concentration of 200 mM in the standard solution was based on measurements of the actual internal K concentration, with glass micropipettes filled with K^+-selective ion exchange resin (Corning 477317). The K activity was observed as a potential difference between the K^+-selective electrode and a 3 M-KCl-filled glass micropipette, both intracellular. Two measurements gave the internal K concentrations of 203 and 191 mM. The following chemicals were also used in the experiments: tetraethylammonium chloride (TEA-Cl; Eastman Kodak Co., Rochester, N.Y.) and 4-aminopyridine (4-AP) and tannic acid (Sigma Chemical Co., St Louis, MO).

All of the experiments were performed at room temperature (20 ~ 22 °C).

RESULTS

Potential changes under current clamp

The average resting membrane potential of the immature egg of *Renilla Koellikeri* immersed in the normal saline containing 10 mM-K was -72 ± 5 mV (S.D., $n = 12$). The resting membrane behaved as a nearly perfect K electrode for $[K^+]_o$ above 10 mM. The replacement of either the external Na^+ with $Tris^+$ or the external Cl^- with methanesulphonate$^-$ produced no appreciable change in the resting potential.

When an outward current pulse was applied through the second intracellular pipette, the membrane depolarized with an almost linear time course (Fig. 1*A*). The rate of the depolarization increased with increasing current intensity. Once the potential reached about -20 mV, it tended to remain at this level. As will be described below, this is due to the activation of the 'transient K current'. A further increase in the current intensity caused an additional slow rise which gave rise to a spike-like potential followed by an undershoot. As the current intensity was increased, the threshold was reached earlier and, consequently, the spike latency became shorter; however, the potential level at the peak of the spike was relatively unaltered. A prolonged suprathreshold current produced repetitive spike potentials (Fig. 1*B*).

To study the ionic basis of the spike potentials, the effects of alterations of the external solution were examined. The amplitude of the spike potential was not altered when the external Na^+ was replaced by $Tris^+$. In contrast, the replacement of 10 mM-$CaCl_2$ in the bath with $MnCl_2$ significantly reduced the peak potential level of the spike (Fig. 1*C*). In this solution the response to a prolonged current was no longer repetitive spikes, but rather oscillatory potentials of decreasing amplitudes. Increasing the external Ca concentration to 100 mM in Na-free (Tris) medium greatly increased the potential at the peak of the spike (Fig. 1*D*). The results suggest that the inward current mechanism responsible for the spike is predominantly a voltage-dependent increase of the Ca permeability.

Membrane currents during voltage clamp

The membrane currents during voltage clamp were investigated to determine the properties of the membrane conductance underlying the potential changes observed during current clamp. Membrane currents obtained during voltage clamp of the cell in normal saline with the holding potential equal to the resting potential (-76 mV) are illustrated by the continuous traces in Fig. 2. For clamp potentials near the resting potential, the initial capacitative current was followed by a small, time-independent outward current. At a potential of about -20 mV a *transient outward current*

appeared. As the membrane potential was made more positive, the peak amplitude of the transient current increased, the rise time became shorter and the rate of the fall of the current was increased. When the potential approached the zero level, the *delayed outward current* became apparent. When the holding potential was shifted from −76 to −30 mV, the transient outward current was totally inactivated while the delayed outward current remained unaffected (traces illustrated by open circles in Fig. 2). The decay of the outward current was seen when the voltage pulse was

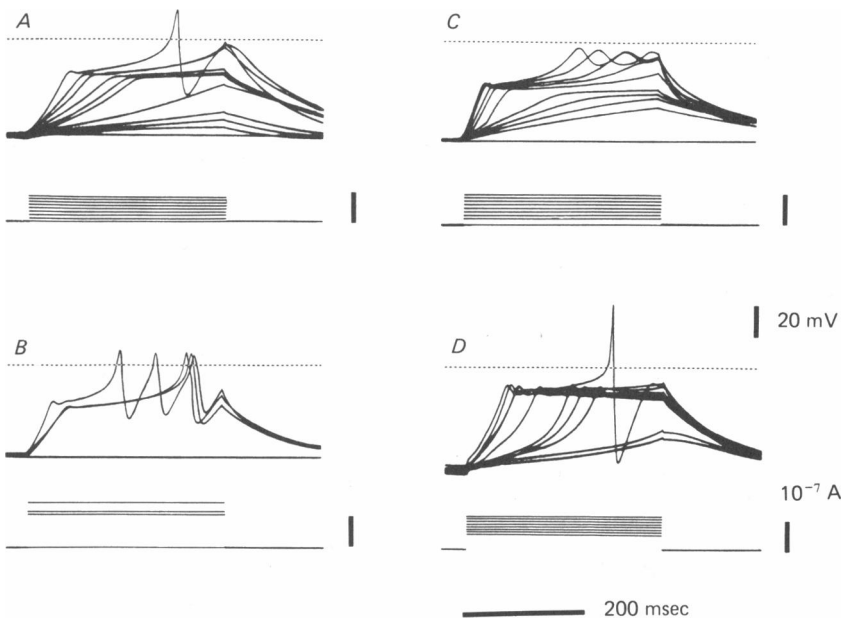

Fig. 1. Membrane potential changes of *Renilla* egg obtained under current clamp. *A* and *B*, in the normal saline. *C*, in Mn saline. *D*, at the 100 mM-Ca, in Tris solution. All the records were obtained from the same egg. The dotted trace in each record indicates the reference potential level. The upward direction in the voltage trace represents inside positivity of the cell and in the current trace outwardly directed membrane current. This is common for all Figures in this paper.

prolonged (Fig. 3*A*) but the rate of decline was substantially smaller than that of the transient current. In other cells (Fig. 3) the delayed outward current was smaller compared with the transient current than was the case in the cell used to obtain the records of Fig. 2. Such variations were found throughout the present experiments; they may be related to the developmental stages of the oocyte. However, no systematic study was made of this relationship.

The membrane currents obtained with negative voltage pulses (Fig. 3*B*) demonstrated the presence of *anomalous* or *inward rectification* in the membrane of the egg of *Renilla*. The amplitude of the inward current just after the capacitative current (not shown in Fig. 3*B*) was significantly greater than that obtained with a positive voltage pulse of the same amplitude. The inward current then increased with time to reach a final steady-state value. In other words, both the instantaneous and

steady-state currents show inward rectification (Fig. 3*C*). These results show that the general properties of the anomalous rectification are similar to those found in eggs of starfish (Hagiwara, Miyazaki & Rosenthal, 1976).

The fact that the peak potential level of the spike is relatively insensitive to the

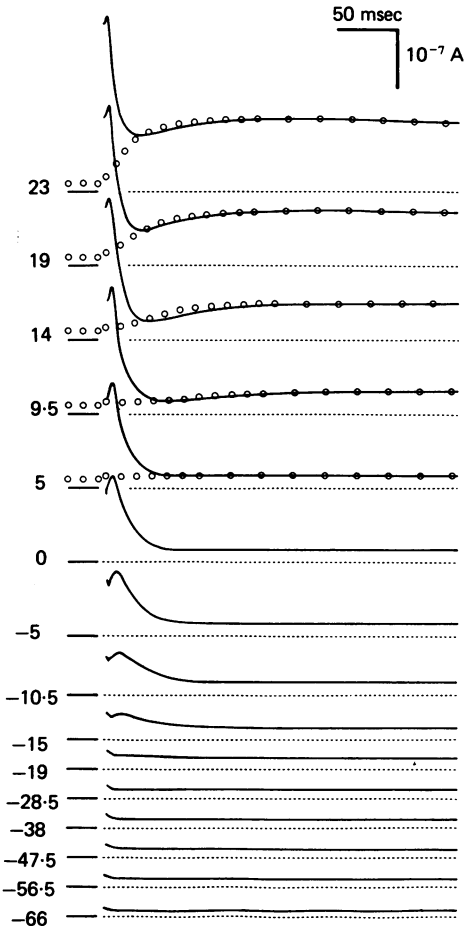

Fig. 2. Membrane currents obtained during voltage clamp with two intracellular micropipettes from *Renilla* egg immersed in the normal solution. Figures indicate membrane potentials in mV. Holding potential, the zero-current potential (−76 mV) for continuous traces and −30 mV for traces illustrated with open circles. Note that the transient outward currents were totally inactivated at a holding potential of −30 mV, whereas there were almost no changes in the delayed outward currents.

intensity of the applied current suggests that positive voltage pulses activate the inward current mechanism. The relation between the voltage and the current at 80 msec from the onset of the voltage pulse has a region of negative slope (Fig. 3*C*). This is presumably responsible for the initiation of spike potentials. No net inward current was, however, recorded in the normal solution containing 10 mM-Ca. As

mentioned already, the replacement of the external 10 mM-Ca with Mn diminished the spike potentials. This replacement, therefore, should diminish the component of the inward current carried by Ca^{2+}. The membrane currents in a cell before and after this replacement are shown in Fig. 4 *A* and *B*. The results show that a small component

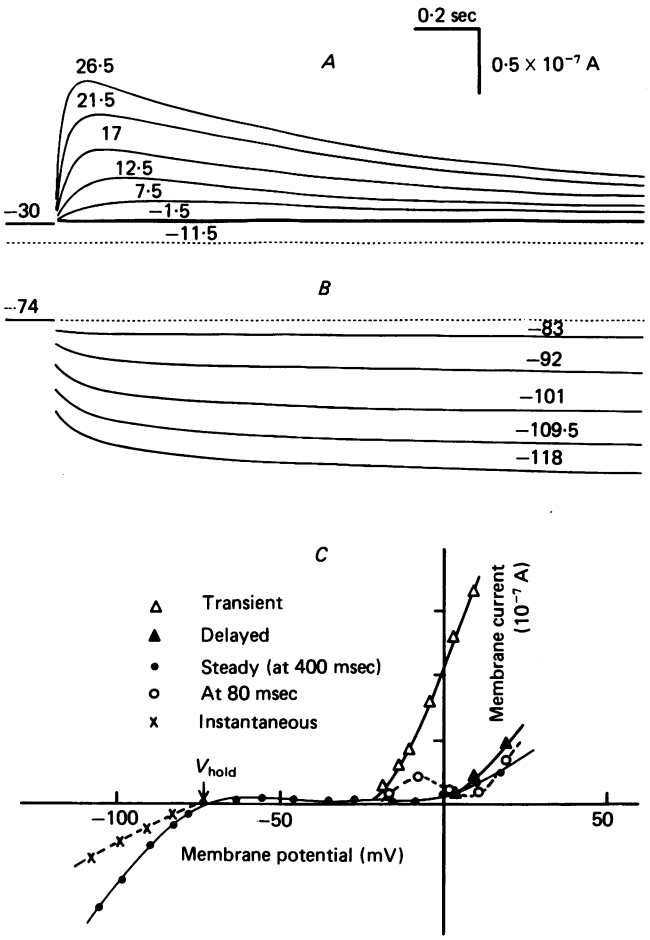

Fig. 3. Membrane currents of *Renilla* egg immersed in the normal solution obtained during voltage clamp with two intracellular micropipettes. *A*, the holding potential, was −30 mV. *B*, the holding potential was the zero-current potential (−74 mV). *A* and *B* were obtained from the same cell. Figures listed indicate membrane potentials in mV. *C*, current–voltage relations obtained from another cell in the normal saline. The holding potential was the zero-current potential (−72 mV).

of inward Ca current normally overlapped the falling phase of the transient current and the rising phase of the delayed current. Plots of the peak amplitudes of the transient and delayed outward currents obtained before and after this replacement as a function of the membrane potential are shown in Fig. 4*C*. The removal of the Ca current did not alter significantly the peak amplitude of either current. This

excludes the possibility that the transient outward current and the major fraction of the delayed current were produced by Ca^{2+} influx into the cell.

The above results indicate that the membrane of the egg of *Renilla* includes the transient and delayed outward currents, the inward current of anomalous rectification

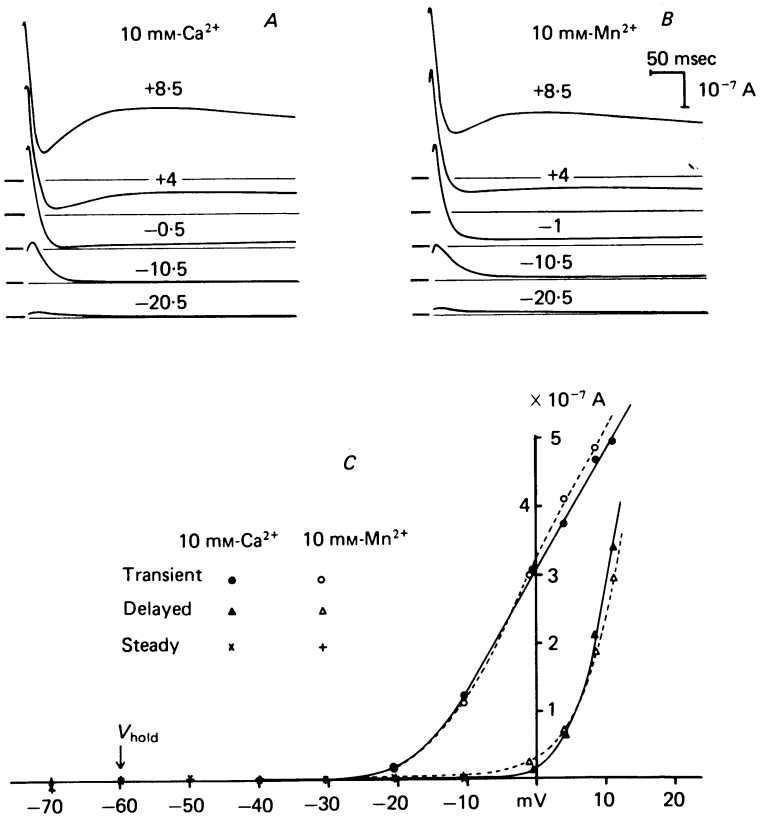

Fig. 4. Membrane currents of *Renilla* egg obtained during voltage clamp with two intracellular micropipettes. The holding potential was −60 mV. *A*, in normal saline and, *B*, after replacing 10 mM-$CaCl_2$ with 10 mM-$MnCl_2$. *C*, the current–voltage relations at the peaks of the transient and delayed outward currents before (continuous lines) and after the replacement (dashed lines). Steady-state currents for negative membrane potentials were obtained at the end of a 400 msec voltage pulse.

and the inward Ca current. This paper concerns the properties of the transient and delayed outward currents. The remaining two currents are not analysed further in this paper.

Inactivation of the outward current

Transient current. One of the remarkable properties of this current is its fast inactivation. To analyse the time course of inactivation, the two-step voltage-clamp technique was used. The membrane potential was held at a relatively large negative potential at which the inactivation was totally removed. The potential was then

changed to a new level for a variable duration (conditioning pulse) and this was followed by a test pulse to −5 mV. The peak amplitude of the current produced by the test pulse was normalized by dividing it by that obtained in the absence of the conditioning pulse. The normalized amplitude h declined with time and time course could be described by a single exponential (Fig. 5*A*), i.e.

$$h = h_\infty + (1 - h_\infty) \exp(-t/\tau_h), \tag{1}$$

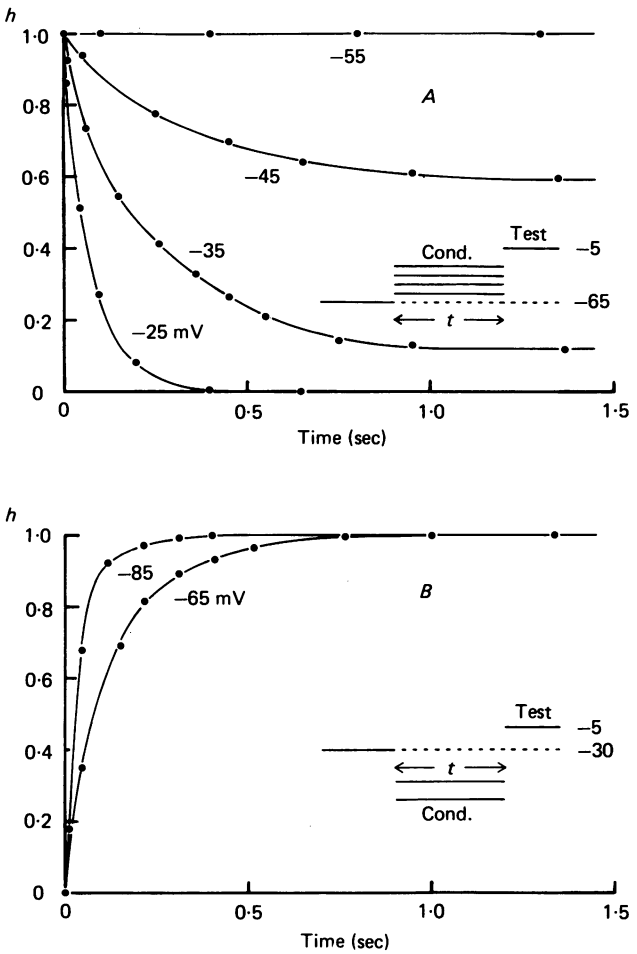

Fig. 5. Time courses of inactivation of the transient current obtained in the normal saline by using a two-step voltage-clamp technique. Explanation, see text.

where τ_h is the time constant and h_∞ is the value of h expected at $t = \infty$. A plot of the values of h_∞ obtained from several preparations as a function of the membrane potential is given in Fig. 6*A*. The relationship between h_∞ and V can be expressed by

$$h_\infty = \{1 + \exp[(V - V_h)/v]\}^{-1}, \tag{2}$$

where V_h is V at $h_\infty = 0{\cdot}5$ and v represents a parameter for the steepness of the curve. The curve in Fig. 6*A* was drawn by assuming that $V_h = -41$ mV and $v = 4$ mV. The time constant, τ_h, decreased as the membrane potential was made more positive. The relationship obtained in several cells between τ_h and the membrane potential is shown in Fig. 6*B*.

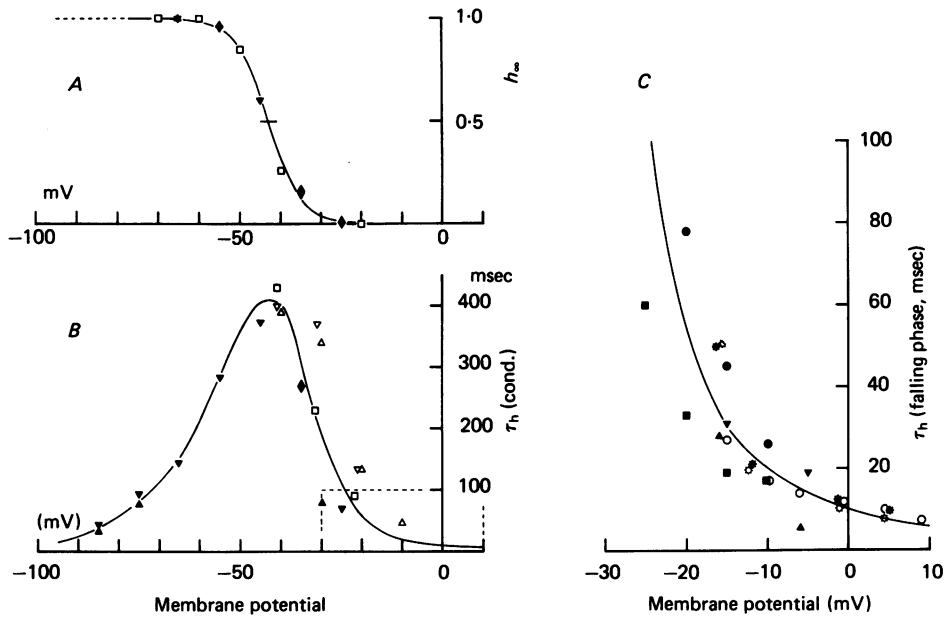

Fig. 6. *A*, steady-state inactivation curve for the transient current. *B*, inactivation time constant obtained with two-step voltage clamp (shown in Fig. 5) is plotted against the membrane potential during the conditioning pulse. *C*, inactivation time constant obtained from the decay of the transient current. Each symbol denotes data from a single cell. Open symbols show data obtained from cells immersed in normal saline, whereas filled symbols are for cells in 10 mM-Mn solution. The continuous line in *C* is identical to the curve in *B* for the corresponding range of membrane potentials. All filled triangles in *B* for membrane potentials more negative than −40 mV represent data obtained from recovery.

The results illustrated by Fig. 6*A* show that the inactivation was almost totally removed at $-55 \sim -60$ mV and that almost complete inactivation occurred at $-30 \sim -25$ mV. The kinetics of activation of the transient current were not analysed in detail because the time resolution of the present method was inadequate. The activation started at $-20 \sim -25$ mV at which potential h_∞ was already very close to zero. In other words, there was little overlap between the inactivation and the activation curves along the voltage axis.

The decay of the transient outward current was well approximated by a single exponential when the membrane potential was negative. The relationship between the time constant of the falling phase of the transient current and the membrane potential is shown in Fig. 6*C*. At positive membrane potentials, the late phase of the decay was likely to be contaminated by both the inward Ca and the delayed outward

currents. In these ranges of membrane potentials, only the early period of decay was used for the curve fitting, and the decay was assumed to be described by a single exponential. To test for a contribution of the inward Ca current, time constants found in experiments done in Mn^{2+} media (Fig. 6*C*, filled symbols) were compared to those measured in normal solution (10 mM-Ca; Fig. 6*C*, open symbols). No significant differences were detected. The τ_h values obtained with the conditioning pulse technique (Fig. 6*B*) and the decay time constants of the current (Fig. 6*C*) show relatively good agreement (see right corner of Fig. 6*B*).

To study the kinetics of the recovery of the transient current after inactivation, the membrane potential was held at −30 mV so that the transient current was almost totally inactivated. Following this, the membrane potential was clamped to a more negative potential for varying time periods (conditioning pulses) and then the peak amplitude of the current produced by the test pulse (−5 mV) was measured. The time course of the recovery of the response was approximated by a single exponential (Fig. 5*B*). The time constant decreased as the membrane potential of the conditioning pulse became more negative. All filled triangles plotted in Fig. 6*B* for membrane potentials more negative than −40 mV represent values of τ_h obtained from recovery. The result shows that the time constant of inactivation is a bell-shaped function of the membrane potential. Values of τ_h at membrane potentials near −45 mV obtained with the conditioning positive voltage-pulse technique and by observing the recovery from inactivation coincide reasonably well.

Delayed current. The delayed current also declined when the voltage pulse was long. The decline was probably dependent upon the membrane potential rather than a secondary effect resulting from the change in the driving force. A large prolonged outward current carried by K^+ may increase the K concentration just outside the membrane when the cell is in the normal saline containing 10 mM-K. This did not seem to be the major cause of decline since similar declines for the outward current were observed in 400 mM-K media in which the change in the K concentration just outside the membrane should be negligible. The details of the inactivation of the delayed outward current were not analysed. The amplitude of the current was not altered when the holding poential was shifted from −75 mV to −20 mV, which was very close to the membrane potential at which the activation of the delayed current started. Therefore, the activation and inactivation may overlap substantially along the voltage axis. In other words, there could be a significant potential range in which the inactivation is incomplete. In contrast, the inactivation is always complete for the transient outward current.

Intracellular dialysis and effects of internal K

When the cell was immersed in the normal external saline, and the interior dialysed with the standard internal solution (200 mM-K), the zero-current potential was usually about $-20 \sim -10$ mV, which was substantially less negative than the resting potential observed with intracellular micropipettes. This was due to the shunt between the inner wall of the glass tubing and the cell surface. The membrane potential was held at −60 mV and then clamped to various potentials (Fig. 7*A*). The time courses of the membrane currents were similar to those obtained with voltage clamp with two micropipettes (Figs. 2 and 4). The currents at the peak of the transient

outward current and at the end of 400 msec voltage pulse are plotted against the membrane potential in Fig. 7*B*. Since the delayed outward current in this cell showed almost no decay in 400 msec for membrane potentials more positive than zero mV, the value at 400 msec was taken as that of the maximum amplitude of the delayed current. When the voltage clamp was performed with two micropipettes, the steady-state current was negligibly small for membrane potentials between −70 and

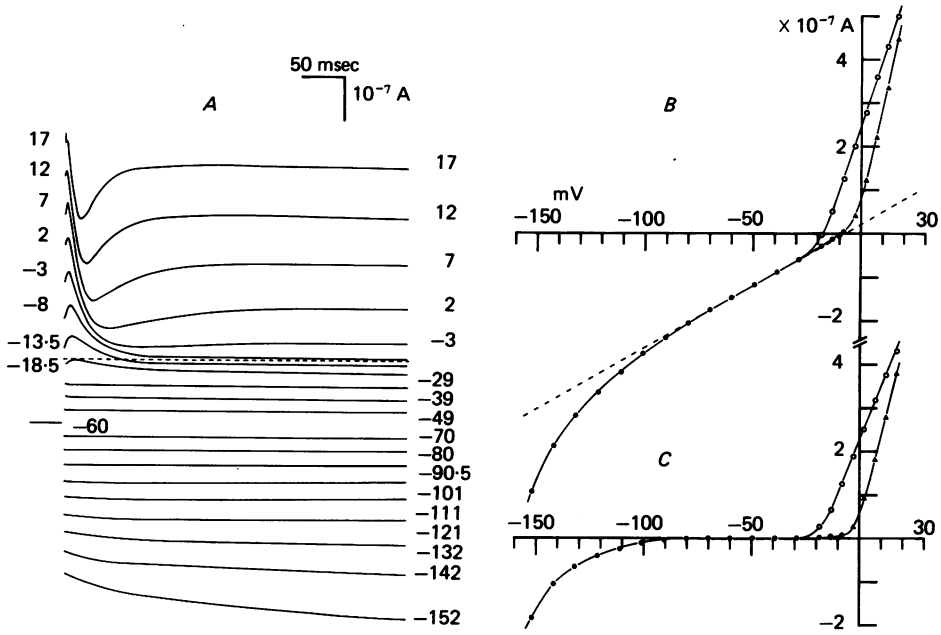

Fig. 7. *A*, membrane currents of *Renilla* egg obtained during voltage clamp with internal dialysis. The holding potential was −60 mV. *A*, dashed line, zero-current level. The figures show the membrane potentials in mV. The external solution was the normal solution and the internal solution was the standard solution. *B*, current–voltage relation at the peak of the transient current (open circles) and at the end of the 400 msec voltage pulse (filled circles). *C*, The same relation after subtraction of the shunt current (illustrated by dashed straight line in *B*).

−30 mV (see Fig. 3*C*). Therefore, the linear component of current illustrated by the dashed line in Fig. 7*B* was assumed to be the shunt current. The curves in Fig. 7*C* represent the current–voltage relations obtained after subtraction of this component. They are similar to those obtained with the two-micropipette voltage-clamp technique. The anomalous rectification was detected in the dialysed cell. There was, however, a tendency for it to become less prominent after prolonged internal dialysis.

Experiments similar to those described above were performed when the external K concentration was increased from the normal 10 to 400 mM by replacing NaCl with KCl (Fig. 8). The zero-current potential was then usually a small positive value. The membrane potential was held at −60 mV. Since the anomalous rectification was substantially reduced in the dialysed cell, the intensity of the inward current

necessary to hold the membrane potential at −60 mV was comparable to the case in which $[K^+]_o$ was 10 mM. The transient inward current first appeared at about −25 mV. Its amplitude was a maximum at about −15 mV (Fig. 8*A*, trace −15·5 mV). At −10 ~ 5 mV, the delayed inward current also appeared (trace −5·5 mV). At +4 mV, both currents became outwardly directed. At more positive membrane potentials (traces +9 and +14 mV) the transient current became unclear.

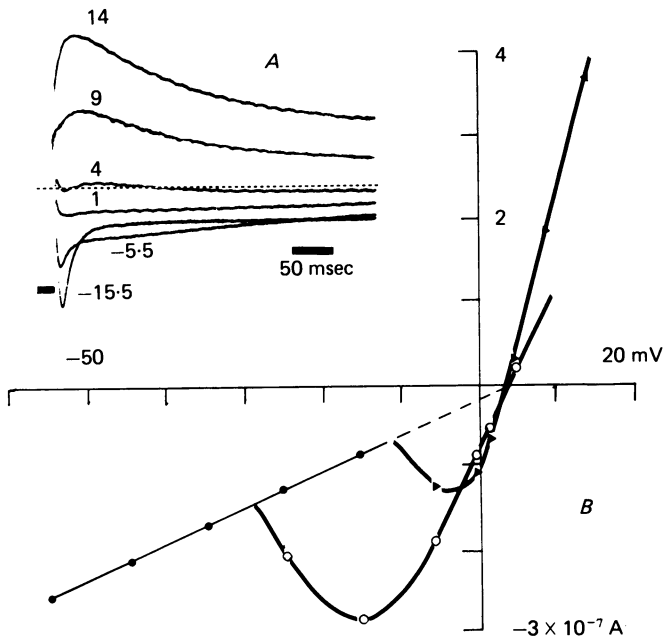

Fig. 8. *A*, membrane currents of *Renilla* egg obtained during voltage clamp with internal dialysis. The external solution was the 400 mM-K solution and the internal solution was the standard solution. Holding potential was − 60 mV. *A*, dashed line shows the zero-current level. The figures give the membrane potentials in mV. *B*, current–voltage relation at the peak of the transient current (open circles) at the peak of the delayed current (filled triangles) and at the end of the 400 msec voltage pulse (filled circles with dashed lines).

In this potential range the amplitude of the transient current became smaller than that of the delayed current and was overlapped by the rising phase of the delayed current. When the holding potential was shifted from −60 to −30 mV the transient current was inactivated. This resulted in an elimination of the initial few msec of the outward current at these membrane potentials. The current–voltage relations for the peak transient and delayed currents and also for the current at the end of a 400 msec voltage pulse (more negative than −15 mV) are shown in Fig. 8*B*. The linear current at 400 msec was assumed to be the shunt current. The extension of the linear relation crossed the transient and delayed current curves at almost the same point. In other words, the two currents actually reverse in direction at approximately the same membrane potential, which was +3 mV in this case. It was +11 mV in another

experiment. This was slightly less positive than the K equilibrium potential calculated from the Nernst equation (+18 mV) but not very far from it. Therefore, the results, nevertheless, suggest that the major charge carrier is K^+ for both transient and delayed currents. The results also show that activation potentials of both currents are not affected by change of the reversal potential.

The values of the reversal potential, E_r, could be determined by using a two-step voltage clamp. After either the transient or the delayed current was developed during the first voltage pulse the membrane potential was clamped to various different levels (the second pulse) to determine the reversal potential for the ensuring tail current. This method worked well for the delayed current, whereas some difficulties were found for the transient current. When the membrane potential of the second voltage step was more negative than −40 mV, the tail current of the transient current decayed so fast that its reversal could not be detected accurately. When the cell in the normal external saline (10 mM-K) was dialysed with standard internal solution containing 200 mM-K, E_r for the delayed outward current was about −70 mV. When the concentration of the internal solution was reduced by replacing the K glutamate with isosmolar arginine glutamate, E_r for the delayed current shifted in the positive direction and reached a new constant level in 10 ~ 20 min. Therefore, 15 min of dialysis was assumed to be sufficient for the exchange of the internal medium. The values of E_r for the delayed current obtained with the cell in the normal external saline are shown in Fig. 9*A*, when the K concentration of the internal solution was reduced from 200 mM to 100, 50 and 25 mM in succession. The values of E_r for the transient current were difficult to determine at $[K^+]_i$ = 200 or 100 mM. However, they coincided with those obtained for the delayed current within a few mV at $[K^+]_i$ = 50 and 25 mM. At $[K^+]_o$ = 400 mM and $[K^+]_i$ = 200 mM, both currents actually reversed in direction at almost the same membrane potential. Therefore, we conclude that E_r values of both currents were similar at any K concentration even though they might not be identical. The reversal potential changed with K concentration changes as expected for a K electrode (Fig. 9*A*). The dashed lines in Fig. 9*A* indicate the K equilibrium potentials calculated from the external (10 mM) and internal K concentrations. It is not clear whether the failure of the potential to follow the Nernst relation was due to a permeability to some ions other than K^+, or to the leakage of the dialysis system. This result, nevertheless, confirmed that the major charge carrier for the transient, as well as the delayed current, was K^+.

The current–voltage relations at the peak transient and delayed outward currents were obtained at $[K^+]_i$ = 200, 100 and 50 mM after subtracting appropriate shunt currents. The chord conductance, G, was calculated from

$$G = I/(V - E_r), \tag{3}$$

where I, V and E_r are the peak transient or delayed current, the membrane potential and the reversal potential determined from tail currents of the delayed current respectively. The relationships between the chord conductance and the membrane potential are shown in Fig. 9*B* and *C*. For these combinations of external and internal K concentrations, E_r was substantially more negative than the membrane potential at which either current is activated. Therefore, it was likely that the current was carried by movement of internal K^+. In other words,the value of G was expected to

depend on $[K^+]_i$. The value of G for the peak transient current at a given membrane potential did decrease with decreasing $[K^+]_i$ (Fig. 9*B*). However, these results were not sufficiently accurate to define a quantitative relationship between G and $[K^+]_i$. In contrast, for the peak delayed current G was relatively insensitive to $[K^+]_i$ over the range examined.

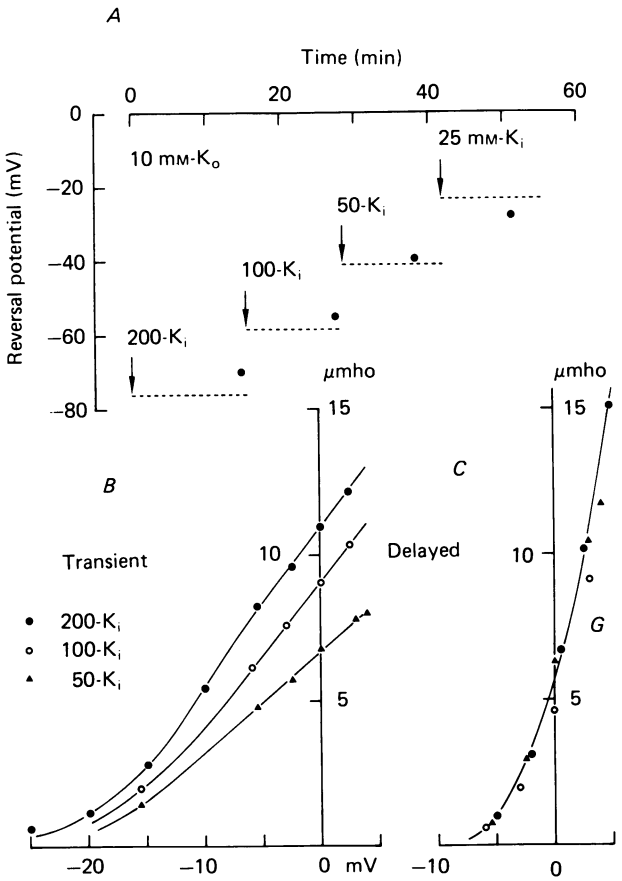

Fig. 9. *A*, reversal potentials of the delayed current obtained during two-step voltage clamp with internal dialysis. The external solution was the normal solution. The K concentration of the internal solution was diluted from 200 mM to 100, 50 and 25 mM in succession with K-free arginine glutamate solution. The cell was dialysed for about 15 min with each solution before the measurement. The dashed lines represent Nernst potentials calculated from the internal and the external K concentrations. *B* and *C*, conductances at the peaks of the transient (*B*) and the delayed current (*C*) calculated from eqn (3) for $[K^+]_i$ = 200, 100 and 50 mM.

Pharmacological effects

External application of 1 mM-4-amino pyridine (4-AP) in the normal external saline reduced the amplitude of the transient outward current to about 35% without affecting the delayed outward current. When the concentration was raised to 5 mM, the transient current was eliminated entirely. At this concentration, however, the

delayed current was slightly reduced. The effect of 4-AP was completely reversible. The effects of 4-AP on the transient and delayed currents are similar to those obtained in other preparations (Thompson, 1977; Byrne, 1980; Lisman, Swan & Fain, 1979). 30 mM-tetraethylammonium (TEA) ions applied externally or internally had no effects on either the transient or the delayed current in the present preparation. TEA is known to block the delayed K current in various other preparations (Fatt & Katz, 1953; Tasaki & Hagiwara, 1957; Hagiwara & Saito, 1959). Addition of 5 ~ 10 mM-CsCl to the standard internal solution did not cause any noticeable effect of either current. Internal Cs^+ is also known to block K channels of the squid giant axon (Bezanilla & Armstrong, 1972).

The kinetics of the inactivation for the transient current resemble those of the Na channel of axons. The inactivation of the Na channel can be removed by several treatments. Perfusion of the squid giant axon with 1–2 mg pronase/ml. removes the inactivation of the Na channel almost completely (Rojas & Armstrong, 1971; Armstrong, Bezanilla & Rojas, 1973). Tannic acid at concentrations less than 0·1 mM in the internal solution also removes the inactivation of the Na channel of the crayfish axon (Shrager, Macey & Strickholm, 1969). In the egg of *Renilla* pronase at 2 mg/ml. in the internal dialysing solution for 20 min was without effect on the rate of decline of the transient outward current. Pronase did reach the cell membrane in this time. Within 8 ~ 10 min after the onset of dialysis with pronase, the leakage current started to increase and the amplitude of the transient, as well as the delayed, outward current decreased. At about 20 min the cell membrane started to disintegrate, but the rate of fall of the transient outward current was still unaltered. Dialysis with an internal solution containing 0·5 mM-tannic acid for 20 min had no effect on either the amplitude or the rate of decline of the transient outward current. The results suggest that the molecular mechanisms of inactivation for the transient outward current and the Na channel may be different, even though their kinetics have a number of similarities.

DISCUSSION

The results indicate that several of the ion channels normally found in the membrane of adult tissues in various animals are also found in the membrane of the immature egg of the coelenterate, *Renilla Koellikeri*. They have also been found in the eggs of other animals (Hagiwara & Jaffe, 1979). At least three types of K permeabilities are present in the *Renilla* egg. They are associated with the anomalous or inward rectification, the transient or fast inactivating outward current and the delayed or slowly inactivating outward current. In addition, a voltage-dependent Ca permeability exists. At the present stage of analysis, it is not certain whether the membrane has one kind of Ca channel or the two kinds of Ca channels found in the starfish (Hagiwara *et al.* 1975). The presence of a voltage-dependent Na permeability has not yet been fully excluded.

In the *Renilla* egg, the amplitude of the Ca current is small in normal external saline, and the dominant currents are the K currents. Therefore, the analysis in this paper focused on the properties of the different K currents, namely, the transient and delayed K currents.

The inactivation of the transient current is rapid and is similar to that originally found for the Na channel of the squid giant axon (Hodgkin & Huxley, 1952). A similar

resemblance has also been demonstrated in the molluscan nerve cell soma (Neher, 1971; Byrne, 1980). The delayed K current inactivates substantially more slowly than does the transient current. The entry of Ca^{2+} in snail nerve cells produces a K current similar to the delayed current described in this paper (Meech & Standen, 1975). A K current similar to the transient current has been found in cardiac muscle (Kenyon & Gibbons, 1979*a*, *b*). In the calf Purkinje fibre, Siegelbaum & Tsien (1980) have shown that a large fraction of this current is a Ca-activated K current. However, the fact that the replacement of the external Ca with Mn produced no changes in either the maximum amplitude of the delayed current or the peak amplitude of the transient current indicates that neither current is a Ca-activated K current in the egg of *Renilla*.

The activation of the transient current starts at $-20 \sim -25$ mV, whereas that of the delayed current starts at $-10 \sim 0$ mV. These values are independent of either $[K^+]_i$ or $[K^+]_o$; in contrast the activation of the inward or anomalous rectification depends not only on the membrane potential but also on $[K^+]_o$ (Hagiwara & Yoshii, 1979). As already mentioned, similar transient and delayed currents are found in various neuronal preparations (Hagiwara *et al.* 1961; Connor & Stevens, 1971; Neher, 1971; Barrett & Crill, 1972; Connor, 1975; Lisman *et al.* 1979; Hablitz & Johnston, 1980; Byrne, 1980). In each case, the activation of the transient current occurs at a membrane potential more negative than that of the delayed current. The membrane potential at half inactivation of the transient current, V_h, was -41 mV in the egg cell of *Renilla*. Since the resting potential is about -72 mV, inactivation is absent in the resting membrane. In contrast, in various neuronal preparations of molluscs (Hagiwara *et al.* 1961; Neher, 1971; Connor & Stevens, 1971; Kostyuk, Krishtal & Doroshenko, 1975; Thompson, 1977) the transient current is almost inactivated at the resting potential; the *Aplysia* motoneurones mediating inking (Byrne, 1980) are an exception, however. The absence of inactivation for the transient current at the resting potential in the *Aplysia* motoneurones has a functional significance; however, in the egg cell the biological significance of the transient current itself is still unclear.

The dependence of the reverse potential upon the external and internal K concentrations suggests that both the transient and the delayed currents occur as a result of permeability increase of the cell membrane to K ions. The reversal potentials of the two currents are similar, but might not be identical; thus their ion selectivities may not be identical.

The experimental results show that the conductance for the delayed current at a given membrane potential is relatively insensitive to a decrease in $[K^+]_i$ from 200 to 50 mM, whereas the conductance for the transient current does decrease with a decrease in $[K^+]_i$. Various properties of K channels can be explained by a class of models assuming channels having multiple sites with multiple ion occupancy (Hille & Schwartz, 1978). In such models the conductance–concentration relation may show different forms depending upon the ion–site interaction. The present result suggests that membrane channels for the transient and the delayed currents differ in this respect.

The transient K current has been described in egg cells of both coelenterates and echinoderms. It is, however, difficult to point out its biological function in egg cells. It may be present to be functional after differentiation.

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