J. Membrane Biol. 35, 239–256 (1977)
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Long-Lasting Inward Current in Snail Neurons in Barium Solutions in Voltage-Clamp Conditions

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Received 7 January 1976; revised 21 September 1976, 3 January 1977

Summary. The inward membrane current was recorded under voltage clamp from nonbursting neurons of the snail *Helix pomatia* in Na-free solutions containing Ba ions but no other divalent cations. The inward current was separated into two components: (*i*) an early fast inactivating component and (*ii*) a smaller long-lasting component. Both components were dependent on the external Ba concentration. It is concluded that both components of the inward current are carried by Ba ions. The activation of the early fast inactivating component of the inward current occurred at more positive membrane potential than that of the long-lasting component. The shape of the inactivation curve for the peak value of the inward current was similar to that for the long-lasting component. The potentials of half-inactivation for the peak value of the inward current and for its long-lasting component were −28 and −22 mV, respectively. The blocking effect of Co^{++} on the early fast inactivating component was substantially greater. In some neurons after treatment with 15 mM Co^{++} only the long-lasting component was recorded. The activation kinetics of the long-lasting component of the inward current were analyzed using the Hodgkin–Huxley equations. The results could be explained by assuming that two components of the inward current in Na–Ca-free solution with Ba ions flowed through the two different channels. The significance of the long-lasting inward current for the normal spike generation is discussed.

In molluscan neurons, Ba^{++} rapidly increases membrane resistance, causes slow depolarization, and prolongs the action potentials (Gerasimov, Kostyuk & Maiskii, 1965; Meves, 1968). Similar effects of Ba^{++} have been observed in many nerve and muscle cells (Werman & Grundfest, 1961; Sperelakis, Schneider & Harris, 1967; Koketsu & Nishi, 1969). All these effects can be attributed to a decreased K^+ conductance. In barnacle muscle fibers, the suppressing effect of Ba upon K conductance has been demonstrated under voltage-clamp conditions (Hagiwara, Fukuda & Eaton, 1974). The explanation for this effect may be that Ba, having nearly the same crystal or singly hydrated radius as K, may plug K^+-selective channels in the membrane (Mullins, 1961;

Werman & Grundfest, 1961; Sperelakis & Lehmkuhl, 1966; Krnjevic, Pumain & Renaud, 1971).

The effect of Ba on some axons appears to be very similar to that of Ca (Narahashi, 1966; Blaustein & Goldman, 1968; D'Arrigo, 1973). Fatt and Ginsborg (1958), Hagiwara and Naka (1964), and Hagiwara, Fukuda and Eaton (1974) regard Ba as an efficient carrier of the inward current through the Ca channel in crustacean muscle fibers. The role of Ba ions as charge carriers was also studied on molluscan neurons (Gerasimov *et al.*, 1965; Geduldig & Junge, 1968; Meves, 1968; Eckert & Lux, 1976), on neurons of bull-frog sympathetic ganglia (Koketsu & Nishi, 1969), on egg cells of a starfish (Hagiwara, Ozawa & Sand, 1975) and on egg cells of a certain tunicate (Okamoto, Takahashi & Yoshii, 1976).

The experiments reported here suggest that Ba ions can carry the inward current in snail nonbursting neurons through the early fast inactivating channel and through the slow inactivating channel. The main interest was to identify and characterize the slowly inactivating channel. Recently, somewhat similar channels have been found in molluscan bursting pacemaker neurons (Gola, 1974; Eckert & Lux, 1976), in isolated snail neurons (Kostyuk, Krishtal & Pidoplichko, 1975), and in frog skeletal muscle fibers bathed in a solution containing Ba ions (Bernard, Cardinaux & Potreau, 1976).

Materials and Methods

The circumesophageal ring of ganglia was dissected from the snail *Helix pomatia* and fixed in a chamber under normal snail saline. The connective tissue covering the dorsal surface of the ganglia was carefully removed. The experiments were done at room temperature (20–22 °C) on large identified nonbursting nerve cells in parietal ganglia (Sakharov, 1974). Fig. 1 is a diagram of the ganglia showing the cells used for this study. The compositions of the principal external solutions used are shown in Table 1.

A solution with the desired concentration of Ba was made by mixing solutions B and C or D and E in appropriate proportions. On addition of $CoCl_2$ to external solutions with 25 mM Ba and 20 mM tetraethylammonium (TEA), the equivalent amount of Tris-HCl was removed. The saline containing Co was made on the day of the experiment in order to minimize the amount of the trivalent form.

The voltage-clamp was a modification of the "point" clamping system first reported by Hagiwara and Saito (1959*a*). The device for voltage clamp was similar to that described by Chamberlain and Kerkut (1969). Two microelectrodes were introduced simultaneously into the same nerve cell, one to apply current and the other to record changes in the membrane potential. The microelectrodes were filled with 3 M KCl and had tip potentials less than 5 mV. The resistances of current electrodes ranged between 5 and 10 MΩ. In our experiments the observed rise-time of the membrane voltage step was usually less than 500 μsec and the decay time of the capacitive charging current between 1

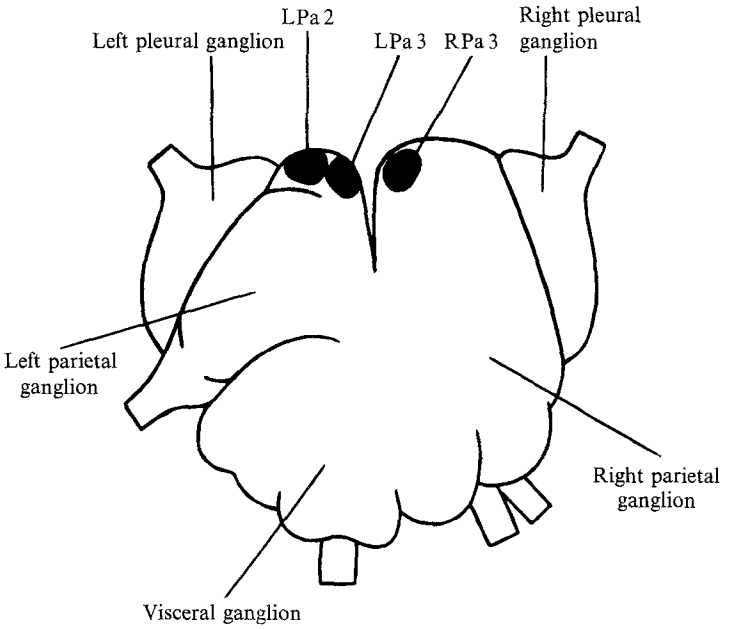

Fig. 1. Diagram of the visceral complex of ganglia of *Helix pomatia*. The position of the cells used in this study are indicated (*see* Sakharov, 1974)

Table 1. Composition of bathing solutions (concentrations in mM)

	NaCl	KCl	$CaCl_2$	$BaCl_2$	Tris base	TEA-Cl
A. Normal saline	80	4	10	—	10	—
B. Na—Ca-free Tris saline	—	4	—	—	95	—
C. Ba saline	—	4	—	60	15	—
D. Na—Ca-free Tris saline with TEA	—	4	—	—	75	20
E. Ba saline with TEA	—	4	—	50	10	20
F. Ba saline TEA rich	—	4	—	25	10	52

Tris base was added neutralized with HCl. Final pH of all solutions 7·7.

and 2 msec (the membrane capacitance was 10–20 nF). All the Figures have been plotted directly from the data with no correction for the leakage conductance. The membrane was depolarized using voltage steps or voltage ramps of constant dv/dt. In the second type of voltage clamp the recorded current includes a constant capacitive current. The stepwise voltage change has the advantage that the effects of potential and time on the membrane current can easily be separated. Using ramp voltage clamp the current-voltage relations were directly measurable. But in this case the effects of potential and time on membrane current are not so clearly discernible. The ramp clamp was used in the study

of the slow inward current in molluscan bursting neurons (Gola, 1974; Eckert & Lux, 1976).

In voltage-clamp studies of neuronal somata the axonal inward current from unclamped regions of the axon obscured the membrane current measurements (Hagiwara & Saito, 1959*a*; Chamberlain & Kerkut, 1969; Magura, Kiss & Krishtal, 1971; Kostyuk, Krishtal & Doroshenko, 1974; Standen, 1975*b*). The axon was inexcitable in Na-free solution (Magura *et al.*, 1971; Wald, 1972; Standen, 1975*b*) and under these conditions the axonal inward current disappeared.

In mollusc ganglia, several layers of glial cells and their processes are found to be interposed between the inner face of the nerve sheath and the surface of the giant neurons (Sattelle & Lane, 1972). It is important to know how fast and completely the ionic environment of the neuronal surface is changed in experimentation.

The effect of saline changes on the peak value of the inward current was observed within 2–4 min (Standen, 1975*b*). This observation suggests the absence of any barrier to the movement of ions localized in a glial layer.

Results

The Inward Current in Na—Ca-Free Solutions with Ba Ions

Fig. 2 shows current records obtained under voltage clamp from the cell bathed in: a normal solution (*A*), a Na—Ca-free solution with 10 mM Ba (B_1), a Na—Ca-free solution with 30 mM Ba (B_2), and Na—Ca-free solution with 60 mM Ba (B_3).

Decrease of the peak value of the transient inward current was observed in Na—Ca-free solution with 10 mM Ba. As can be seen from Fig. 2(*D*) the inward current became progressively larger with increasing Ba concentration. The rise of the peak value of the inward current occurs within 5 min after increasing Ba concentration.

The current-voltage relations of the peak of the inward current were shifted along the voltage axis in the positive direction with increasing Ba concentration (Fig. 2*C*). This shift may be due to the stabilizing effect of Ba ions.

As shown in Fig. 2(*D*), the relation between the maximum of the inward current intensity and Ba concentration was not linear. The peak of the current intensity became less sensitive to Ba in the range of high concentration. A similar saturation effect was found for Ca current in a barnacle muscle fiber (Hagiwara & Takahashi, 1967; Hagiwara, 1973), in a starfish egg cell (Hagiwara *et al.*, 1975), and in egg cell of a certain tunicate (Okamoto *et al.*, 1976). A saturation of the calcium channel responsible for Ca-mediated potassium activation has been recently observed in *Helix* neurons (Meech & Standen, 1975).

As can be seen from Fig. 2(B_2) the long-lasting inward current

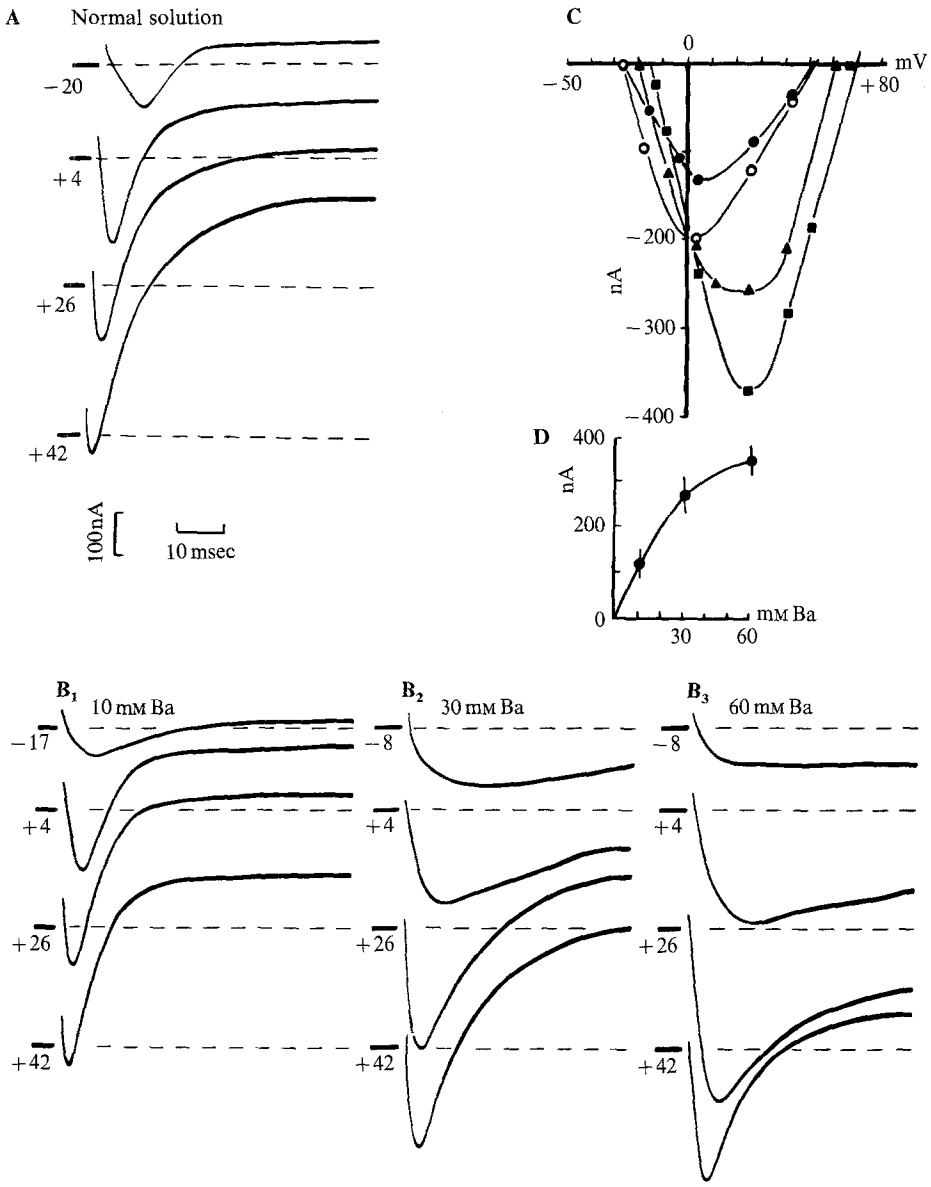

Fig. 2. Membrane currents associated with voltage clamp of snail neuron in Na−Ca-free solutions with Ba. (*A*) The neuron was first in normal solution. The external solution was then exchanged into Na−Ca-free solution with 10 mM Ba (B_1), 30 mM Ba (B_2), and 60 mM Ba (B_3). The number listed for each current record is the membrane potential in mV during the voltage pulse. The holding membrane potential was −45 mV. The records in *A*, B_1, B_2 and B_3 were taken from the same cell. (*C*) Current-voltage relations of the peak of the inward current. Open circles: normal solution; filled circles: 10 mM Ba; triangles: 30 mM Ba; squares: 60 mM Ba. (*D*) Results of five cells showing the effect of different external Ba concentration on the maximum of inward current. Bars: 2 × SEM

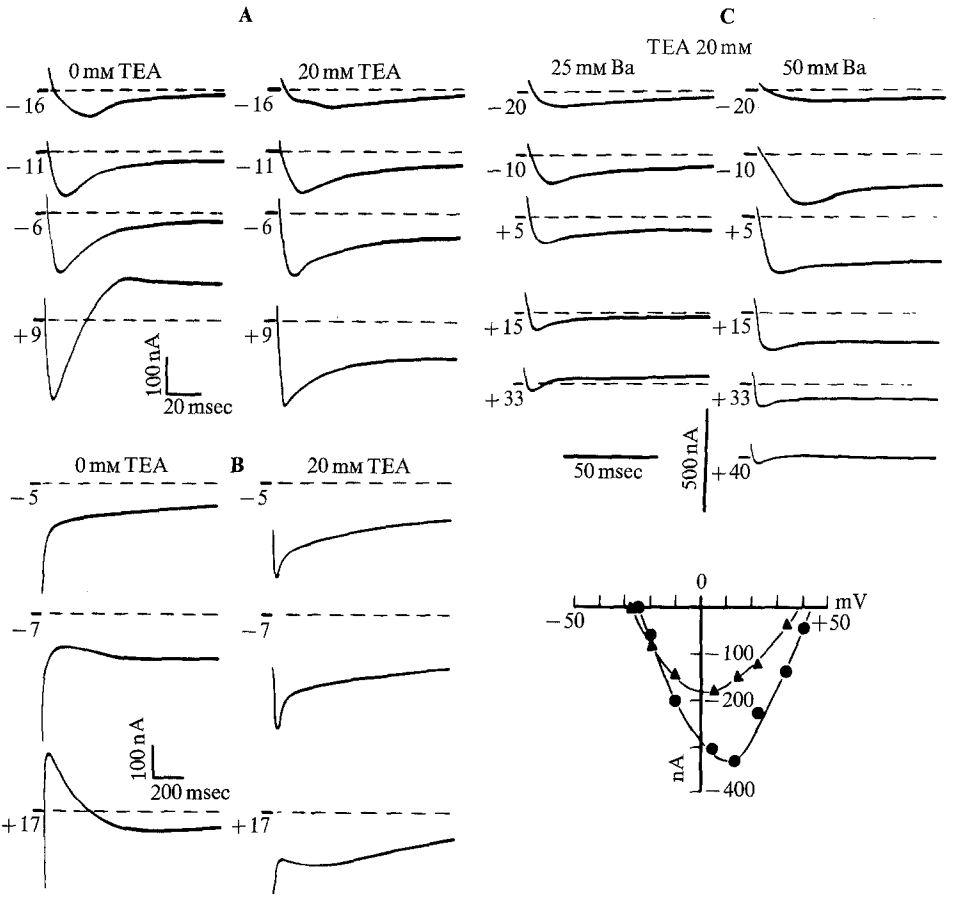

Fig. 3. (*A*, *B*) The effect of 20 mM TEA on the membrane current in the solution with 50 mM Ba. Records in (*B*) were obtained with a slower sweep speed from another neuron. (*C*) The effect of Ba concentration in solution with 20 mM TEA on the inward current. Below, current-voltage relation of the peak of the inward current in solution with 25 mM Ba (triangles) and in solution with 50 mM Ba (circles). The number listed for each current record is the membrane potential in mV during the voltage pulse. The holding potential was −45 mV in *A*, *B* and *C*

appeared in solutions with 30 mM Ba (records at −8 and +4 mV). This current significantly increased in solution with 60 mM Ba [Fig. 2(B_3)].

The inward current in Ba solutions was obscured by an outward K current which was not completely suppressed by Ba ions. The separation of the long-lasting inward current from K current has been attempted by using TEA. TEA blocks the delayed outward current in molluscan neurons (Hagiwara & Saito, 1959*b*; Neher & Lux, 1972). Fig. 3(*A*, *B*) shows that in solution with 50 mM Ba and 20 mM TEA the long-lasting

inward current increased. Such effect of TEA was more distinct at more positive test potentials. (A solution with 50 mM Ba and 30 mM Tris-HCl was used as reference.)

Fig. 3(*B*) demonstrates the effect of TEA on the inward currents at a slower sweep speed. The time course of the inward current in solution with TEA was quite distinct from that in a reference solution. After the inward current reached its peak it decreased rather rapidly within the first 50 msec. One can see a second gradual decay of the inward current. The analysis of the decaying phase of the inward current using the instantaneous current-voltage relationship is difficult in molluscan neurons. The capacitative surge associated with a sudden change in the voltage significantly obscures the tail of the inward current. One may assume that the decaying phase of the inward current is due to inactivation.

From the results of the experiments it seems reasonable to suggest that the inward current in Na—Ca-free solution with Ba ions has two components: *i*) an early fast inactivating component, and *ii*) a long-lasting (slow inactivating) component. As can be seen from Fig. 2(B_3) the long-lasting component appeared at more negative membrane potentials than the fast inactivating component. The overlap of the two inward current components occurs at the beginning of the test voltage pulse. The long-lasting component of the inward current was observed in 80% of the neurons studied.

Variations of the relative magnitude of the fast inactivating component and the long-lasting component of the inward current were observed. Fig. 3(*C*) shows the inward current traces in TEA solutions with 25 and 50 mM Ba. The fast inactivating component was relatively small in the case of Fig. 3(*C*). The increasing Ba concentration from 25 to 50 mM increased the maximum peak value of the inward current by a factor of about two. This observation seems difficult to reconcile with the results presented in Fig. 2. One may suggest that the long-lasting inward current shows no indication of saturation in Ba concentrations below 50 mM. In solution with 10 mM Ba the residual outward current significantly masked the long-lasting inward current even after the treatment with TEA. The long-lasting inward current quickly decreased in some neurons during the experiment.

Inactivation of the Inward Current by Conditioning Depolarization

In this set of experiments the solution with 25 mM Ba and 52 mM TEA was used in order of more complete suppression of outward currents.

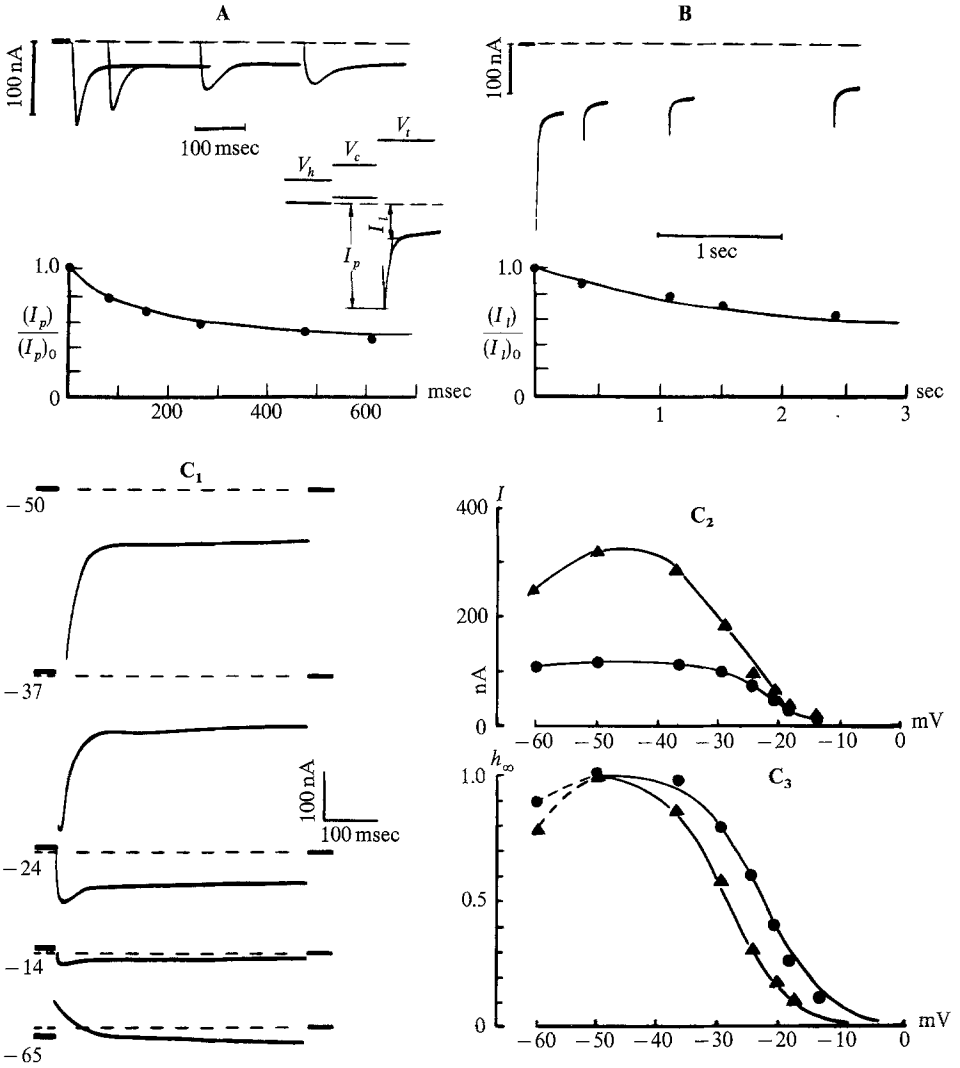

Fig. 4. The effect of a conditioning change in membrane potential on the peak value of the inward current (I_p) and its long-lasting component (I_l) in solution with 25 mM Ba and 52 mM TEA. (*A*) Time course of early inward current inactivation. Current records (above) are shown when the command voltage pulse follows a prepulse of variable duration. The left-hand record was taken without a prepulse; the remaining records are shown superposed each with a delay from the onset of the left-hand record equivalent to the duration of its own prepulse. Holding potential (V_h): −45 mV; conditioning prepulse level (V_c): −30 mV; test potential level (V_t): +2 mV. The broken line represent the holding current at holding potential. For the lower curve, $(I_p)/(I_p)_0$ is plotted against the conditioning pulse duration (t), where $(I_p)_0$ is the peak of the inward current at t=0. The inset diagram illustrates a method of measuring I_p and I_l. (*B*) Time course of the long lasting component inactivation. Holding potential: −40 mV; conditioning prepulse level: −24 mV; test potential level: −5 mV. (C_1) Dependence of the inward current upon the conditioning prepulse level. Test potential was −3 mV. The number listed for each current record is the conditioning membrane potential. Duration of the conditioning shift of the membrane potential was 5 sec. (C_2) The peak of the inward current (I_p) (triangles) and its long-lasting component (I_l) (circles) are plotted against the conditioning potential. C_1 and C_2 from the same cell. (C_3) Steady-state relation for inactivation of I_p and I_l (from results presented in C_2)

Fig. 4(*A*) shows the effect of duration of conditioning prepulse on the inactivation of the early component of the inward current. Conditioning depolarization to −30 mV causes exponential decrease in the peak value of the inward current with the time constant of 120 msec. The effect of such conditioning depolarization upon the long-lasting component of the inward current was negligible [*see* upper part of Fig. 4(*A*)].

Fig. 4(*B*) demonstrates the exponential decrease in the long-lasting component of the inward current with time constant of 1.4 sec at conditioning depolarization to −24 mV. The continuous lines in Fig. 4(*A*) and (*B*) were calculated from the Hodgkin and Huxley (1952*a*) expression:

$$\dot{I}/\dot{I}_0 = I_\infty/\dot{I}_0 + (1 - \dot{I}_\infty/\dot{I}_0)\exp(-t/\tau_h),$$

where $\dot{I}/\dot{I}_0$ is the inward current ($\dot{I}$) relative to inward current with no conditioning prepulse ($\dot{I}_0$), $\dot{I}_\infty$ is the steady-state value of $\dot{I}$, t is the duration of the prepulse, and τ_h is the time constant of inactivation.

Fig. 4(C_1) shows the dependence of the inward current upon the conditioning prepulse level.

The duration of prepulse was 5 sec. This value is long enough for inactivation of early and long-lasting components of the inward current to reach a steady-state level. The peak value of the inward current reached maximum at a conditioning potential of −50 mV [Fig. 4(C_2)]. Conditioning depolarization of −35 mV decreased only the peak value of the inward current. More positive conditioning shifts of the membrane potential caused suppression of the peak value and the long-lasting component of the inward current. The inward current decreased when conditioning potential was more negative than −50 mV [Fig. 4(C_2)]. The fast outward current was recorded when the conditioning prepulse was −65 mV. This current in molluscan neurons has been described by Hagiwara, Kusano and Saito (1961), Connor and Stevens (1971), Neher (1971) and Gola (1972). The blocking effect of TEA on the fast outward current is small (Neher & Lux, 1972).

In Fig. 4(*B*) the peak value of the inward current and its long-lasting component was plotted as a function of the conditioning potential. These results were normalized and the inactivation curves were obtained (Fig. 4*C*). In the range of conditioning potential values from −50 to −10 mV inactivation curves may be calculated by the Hodgkin and Huxley (1952*a*) equation

$$h_\infty = \left[1 + \exp\left(\frac{V - V_h}{k}\right)\right]^{-1},$$

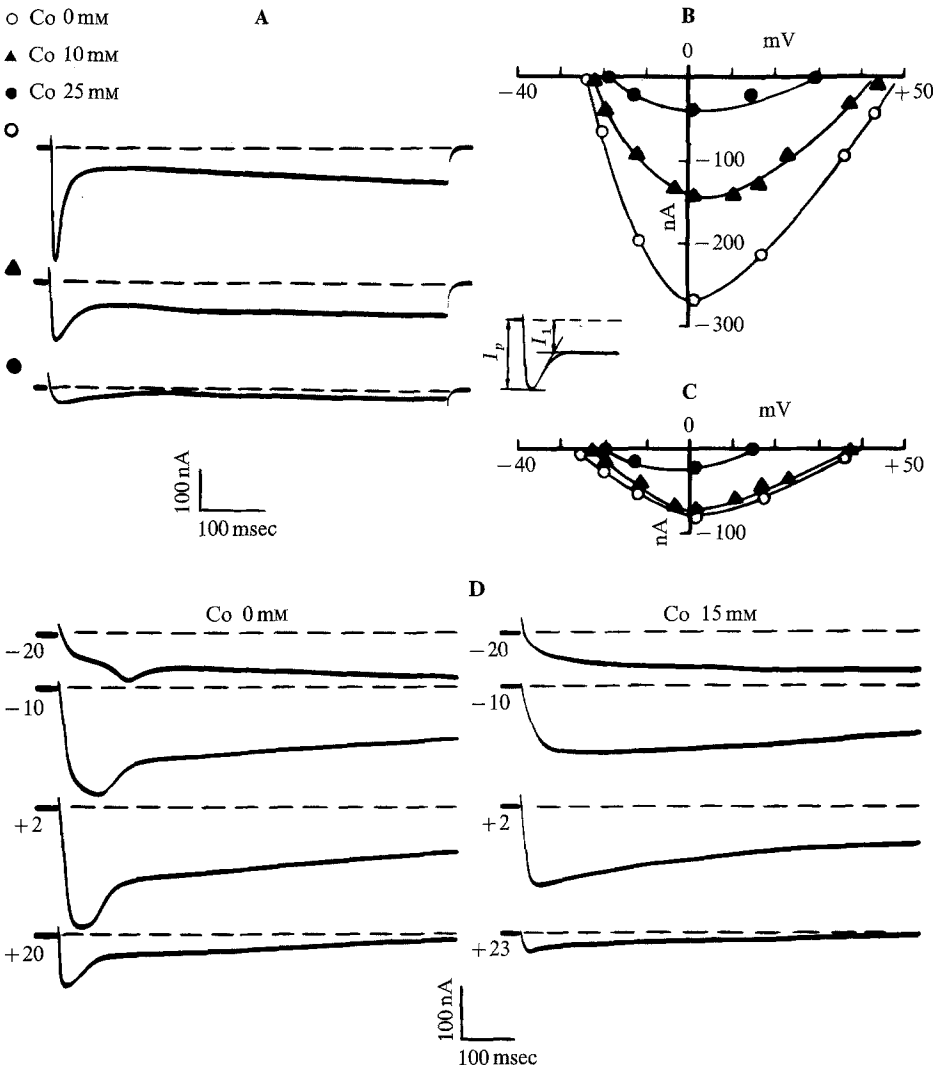

Fig. 5. (*A*) Effects of Co ions (10 and 25 mM) on the inward current in solution with 25 mM Ba and 20 mM TEA. Current records at −2 mV. Holding potential −45 mV. (*B*) Effect of Co ions on the current-voltage relations of the peak of the inward current (I_p). (*C*) Effect of Co ions on the current-voltage relation of the long-lasting component of the inward current (I_l). The inset diagram illustrates a method of measuring I_p and I_l. (*D*) Suppression of the early component of the inward current by 15 mM Co. The number listed for each current record is the membrane potential in mV during the voltage pulse. Holding potential: −40 mV

where V = membrane potential, V_h = potential at which $h_\infty = 0.5$, and k = shape parameter.

The potentials of half-inactivation (V_h) for the peak value of the inward current and for its long-lasting component were 28 and 22 mV, re-

spectively. The shape parameter (k) was taken as 5 mV in both diagrams. According to the results presented in Fig. 4 one may suggest that the voltage dependence of the long-lasting component inactivation is somewhat different from that of the fast inactivating component.

The deviation of experimental data from the Hodgkin-Huxley equation at conditioning potentials more negative than the resting potential was described by Geduldig and Gruener (1970), Kostyuk *et al.* (1974), and Standen (1975*b*). The most likely explanation is that it is caused by a component of the fast outward current. Standen (1975*b*) has shown that the deviation was abolished by TEA. In experiments described above, TEA (52 mM) did not abolish this deviation probably because of the low sensitivity of the fast outward current to the blocking action of TEA (*see* Neher & Lux, 1972).

The short conditioning depolarization was used for selective inactivation of the early transient inward current and separation of the slow inward current in bursting pacemaker neurons of *Helix* (Eckert & Lux, 1976). This method was unsuccessful in separation of the long-lasting inward current in Ba solution due to incomplete inactivation of the early fast inactivating component.

Effect of Co Ions upon the Inward Current in Ba Solution

Co^{++} blocks the Ca channel of the barnacle giant muscle fiber (Hagiwara & Takahashi, 1967), the calcium component of action potential in molluscan neurons (Geduldig & Junge, 1968; Standen, 1975*a*) and in other tissues (Reuter, 1973; Hagiwara *et al.*, 1975; Okamoto *et al.*, 1976). Co ions block the Ba current in the Ca channel of the barnacle muscle fiber (Hagiwara *et al.*, 1974). Fig. 5 shows that addition of 10 and 25 mM Co to the solution with 25 mM Ba and 20 mM TEA reduced the peak value of the inward current to 50% and 15%, respectively.

The effect of 10 mM Co upon the long-lasting inward current was rather small. In the solution with 25 mM Co the long-lasting inward current was reduced to 40% of the control value.

In some neurons the fast inactivating component of the inward current disappeared after the treatment with Co ions and only the long-lasting inward current was recorded (Fig. 5*D*).

The blocking effect of Co upon the long-lasting inward current in Na—Ca-free Ba solutions may be obscured by the effect of Co ions on the residual K current. Meech and Standen (1975) showed that the outward current in *Helix* neuron was reduced by about 40% in the

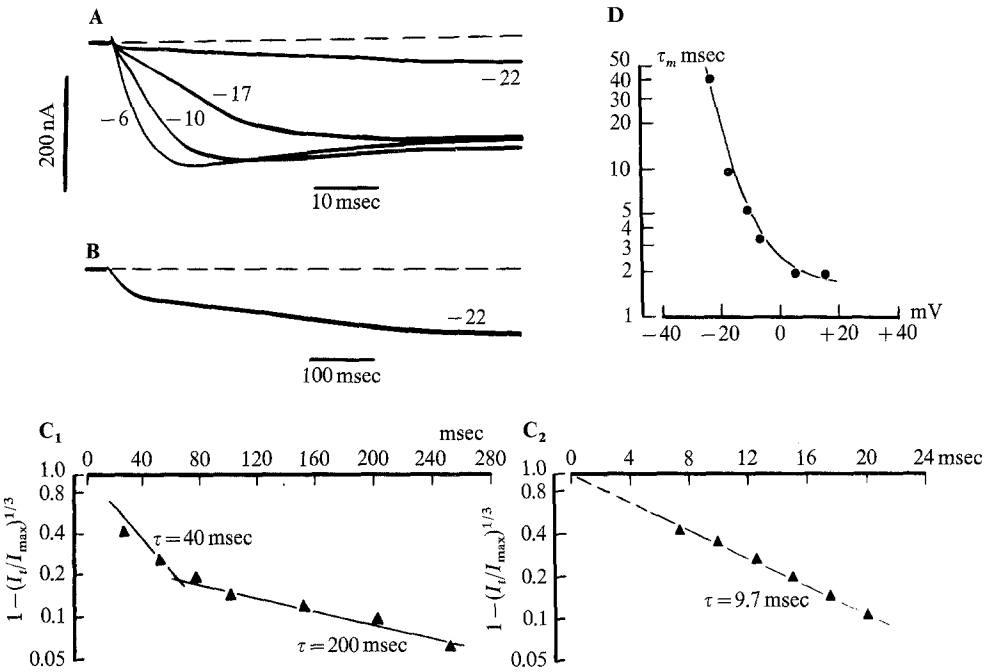

Fig. 6. Analysis of the activation kinetics of the long-lasting component of the inward current. (*A*) Examples of inward currents were analyzed in this Fig. The number listed for each current record is the membrane potential in mV during the voltage pulse. The holding potential was −45 mV. (*B*) Record at −22 mV was obtained with a slower sweep speed. (C_1 and C_2) The estimation of the time constants of the long-lasting inward current at −22 mV (C_1) and −17 mV (C_2). (*D*) The activation time constants as functions of the membrane potential. Ordinate: logarithmic scale in msec; abscissa: membrane potential in mV

presence of 10 mM Co in the bathing medium with 10 mM Ca. One may assume that a similar reduction of residual outward current occurs in Ca-free medium with Ba ions (*see* Meech, 1974).

Analysis of the Time Course of the Long-Lasting Inward Current Activation

At membrane potential more positive than −20 mV the overlap of the early and long-lasting inward current components obscured the time course of the long-lasting inward current activation. The selective inactivation technique is unsuccessful in complete separation of the long-lasting inward current. The potential dependency of the time course of the long-lasting inward current activation was studied in those neurons

where the fast inactivating component of the inward current was not observed after the treatment with 15 mM Co.

Fig. 6 shows that the activation kinetics of the long-lasting inward current are strongly voltage dependent. The rise-time of the long-lasting inward current decreased from about 400 msec during −22 mV pulses to about 30 msec during −17 mV pulses. The fast and slow phases of activation were observed at −22 mV (Fig. 6*B*). But in some experiments a secondary slow phase of activation was not observed.

The time course of the long-lasting inward current activation was analyzed in accordance with the Hodgkin-Huxley equations (Hodgkin & Huxley, 1952*b*; Okamoto *et al.*, 1976). This current can be formalized in terms of m and h parameters as follows:

$$i_{\mathrm{Ba}} = I_{\mathrm{Ba}}\, m^n h.$$

Here

$$m = m_0 + (m_\infty - m_0)\,[1 - \exp(-t/\tau_m)]$$

$$h = h_\infty + (h_0 - h_\infty)\exp(-t/\tau_h).$$

In the case of the long-lasting inward current n was exactly three. Since $\tau_h \gg \tau_m$

$$\ln\{1 - [i_{\mathrm{Ba}}(t)/i_{\mathrm{Ba}}(\infty)]^{1/3}\} = a - t/\tau_m$$

where $a = \ln(1 - m_0/m_\infty)$. As can be seen from Fig. 6(C_2)

$$\{1 - [i_{\mathrm{Ba}}(t)/i_{\mathrm{Ba}}(\infty)]^{1/3}\}$$

was found exactly on a straight line in semilogarithmic scale with slope of τ_m. [At −22 mV the time course of activation was determined by two time constants—*see* Fig. 6(C_1).] Fig. 6*D* shows the relation of the time constant for activation to the membrane potential.

Inward Current in TEA Solution With Ca Ions

Fig. 7(*A*) shows that the replacement of 25 mM Ba by 25 mM Ca in Na-free solution with 25 mM TEA caused the decrease in the long-lasting component of the inward current. In some neurons in solution with 25 mM Ca the long-lasting component was observed only in ramp voltage-clamp condition. As can be seen from Fig. 7(*B*) the long-lasting inward current caused the appearance of the negative slope in a current-voltage curve recorded in ramp voltage clamp at a rate of 13 mV/sec. In solution with 25 mM Ba the negative slope is more distinct than that in solution with 25 mM Ca.

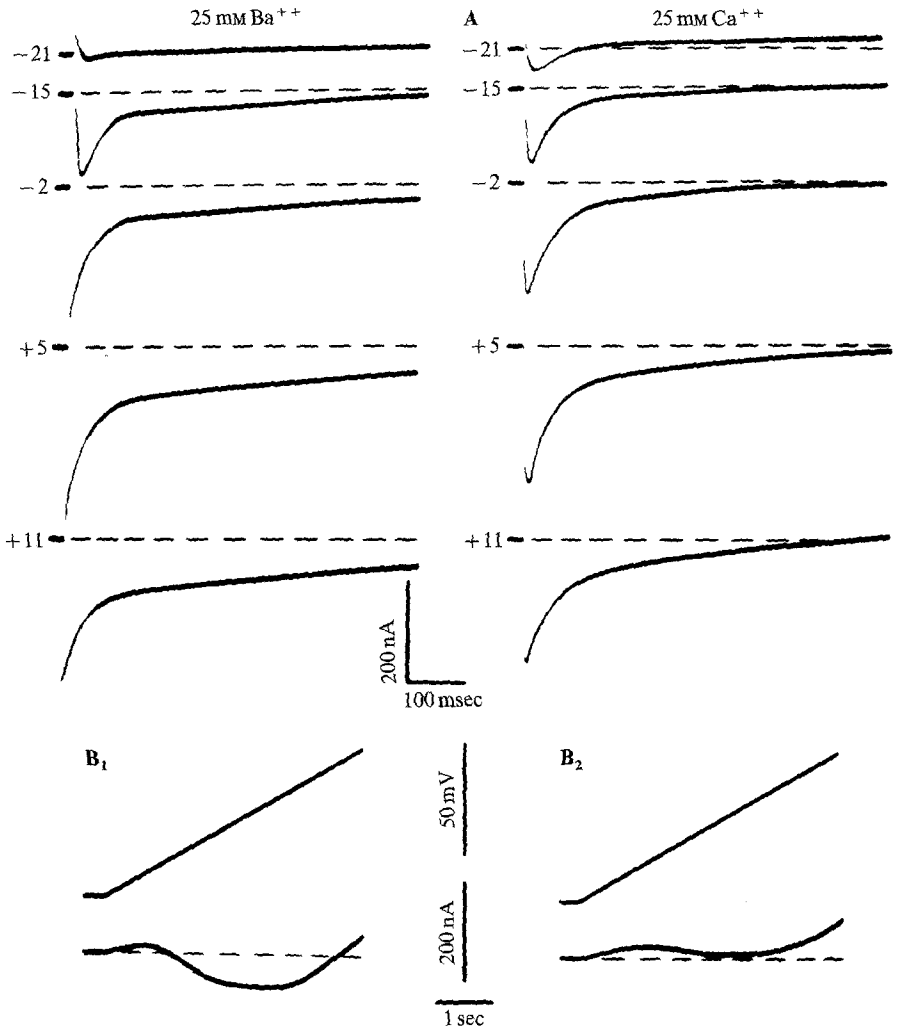

Fig. 7. (*A*) The effect of replacement of Ba with Ca ions in solution with 52 mM TEA on the inward current. The holding potential was −45 mV. The number listed for each current record is the membrane potential in mV during the voltage pulse. (B_1, B_2) Current-voltage relations obtained with ramp voltage clamp of 13 mV/sec in solution with 25 mM Ba (B_1) and in solution with 25 mM Ca (B_2). Above, membrane potential; below, current record. The records in *A*, B_1 and B_2 were taken from the same cell

The long-lasting component of the inward current was independent of the external Na concentration.

Discussion

Upon the results of the above experiments it seems reasonable to suppose that the inward current in molluscan neurons flows through two

different channels in Na-free solution containing Ba ions but no other divalent cations. They are referred to as the early fast inactivating channel and the long-lasting inward current channel. The criteria to distinguish the two channels are the following. (*a*) The early component of an inward current is activated at a more positive membrane potential than the long-lasting component. (*b*) The voltage dependence of the long-lasting component inactivation is somewhat different from that of the early fast inactivating component. (*c*) The blocking effect of Co ions is greater in the early inward current channel than in the long-lasting inward current channel. The long-lasting inward current seems to show no indication of saturation at Ba concentrations below 50 mM. The early inward current channel is a more saturable system. According to the theory developed by Hagiwara and Takahashi (1967), Hagiwara (1973), and Hagiwara *et al.* (1974, 1975), the binding of Ca (or Ba and Sr) to membrane sites is an important step in the permeation of these ions through the membrane. Hille (1975) recently suggested the existence of the saturable ion binding site within the Na channel.

One may speculate that early and long-lasting inward currents flow through "Ca channels". Ba ions are useful tools for the investigation of the long-lasting inward current channel because of its higher permeability to Ba than to Ca.

One may assume that the long-lasting inward current described above is similar to the slow inward current which had been described in bursting pacemaker neurons by Gola (1974) and Eckert and Lux (1976). Eckert and Lux (1976) concluded that the slow inward current is carried primarily by Ca. The slow current is greatly enhanced by Ba substitution for Ca. It was proposed that the slow inward current plays an important role in the generation of oscillatory waves in bursting pacemaker neurons (Gola, 1974; Eckert & Lux, 1976). (According to Barker and Gainer (1975) Na may play the major role in the generation of the bursting pacemaker potential activity.)

It seems reasonable to suppose that during the spike generation in nonbursting neurons in normal conditions the relatively small Ca inward current flows through the long-lasting inward current channel. One may suppose that this Ca current is important in activation of the calcium-mediated potassium channel (Meech, 1974; Meech & Standen, 1975). Earlier it was suggested that during the repetitive firing in molluscan neurons as the inactivation of the transport mechanism for sodium current progressed, the role of calcium ions as carriers of the inward current increased (Magura & Zamekhovsky, 1973).

Gerasimov *et al.* (1965) and Meves (1968) have found that action potentials of snail neurons developed a long plateau in solutions with Ba ions. It seems that the plateau is due to the suppression of K conductance by Ba ions and to the activation of the long-lasting inward current.

I would like to thank Professor P.G. Kostyuk for his kind support and valuable advice. I am greatly indebted to Professor S. Hagiwara for a number of useful suggestions concerning the material presented in this paper and much advice with the experiments and the manuscript. I am also greateful to Professor H.D. Lux for helpful discussion, and to Drs. A. Valeyev, E. Dolgaya and E. Kovaleva for their help.

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