Inactivation of Calcium Current in the Somatic Membrane of Snail Neurons

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Abstract. The decline of calcium inward currents evoked by a long-lasting membrane depolarization was studied on isolated snail neurons internally perfused with a K^+ - free solution. Two exponential components superimposed on a steady inward current could be distinguished, a slow decline with a time constant of several hundreds of milliseconds, observed at all the testing potentials used, and a fast one with a time constant of several dozens of milliseconds, which appeared at depolarizations to about -10 mV and above. When the calcium current was blocked by extracellular Cd^{2+} or verapamil, an outward current could be recorded at the same depolarizations. Subtraction of the latter current from the total current, recorded prior to the blockage, largely reduced the fast component of the decline of the total current. An increase in pH_i from 7.3 to 8.1 led to the elimination of both the outward current and the fast component of the calcium current decline. The slow component remained practically unchanged, with its rate depending upon the current amplitude. It was slowed following intracellular administration of EDTA, and after equimolar substitution of Ba^{2+} for Ca^{2+}. It is concluded that the fast component of the calcium inward current decline is mainly due to the superposition of the outward current produced by low selective channels. Only the slow component represents an actual decline of the inward current through calcium channels; it is due to ion accumulation at the inner surface of the cell membrane.

Key words: Calcium channel — Inactivation — Non-specific outward current — Intracellular pH

Introduction

The somatic membrane of both invertebrate and vertebrate neurons possesses a potential-dependent calcium conductance based on the presence of ionic channels which are selective for divalent cations (Kostyuk et al. 1975; Standen 1975; Eckert and Lux 1976; Kostyuk and Krishtal 1977a; Akaike et al. 1977; Fedulova et al. 1981 and others). Like other transmembrane currents, the calcium

inward currents decline (inactivate) during sustained depolarization. It has been shown that the decline can be described by the sum of two exponentials (Kostyuk and Krishtal 1977; Ashcroft and Stanfield 1982); this may indicate involvement of at least two independent processes. Two basic mechanisms of inactivation of transmembrane ionic currents have been suggested: one potential-dependent, similar to that suggested for the sodium inward current in the axonal membrane (Hodgkin and Huxley 1952); and another Ca-dependent, caused by the entry of Ca^{2+} ions into the cell (Brehm and Eckert 1978; Tillotson 1979). It seems that both of them govern calcium channel behaviour in different preparations. However, there is a considerable discrepancy among different investigators in the evaluation of the possible role of each of them (cf. Fox 1981; Brown et al. 1981; Plant and Standen 1981; Ashcroft and Stanfield 1982). Considerable difficulties in investigating Ca channel inactivation arise from the presence of Ca-dependent outward current. In has been suggested that at least a portion of the decline of Ca currents in molluscan nerve cell membrane is due to the parallel activation of this outward current (Connor 1979; Kostyuk 1980; Byerly and Hagiwara 1982).

In the present work, carried out on intracellularly perfused snail neurons, the kinetics of the inward current decline was analysed in detail during long-lasting depolarizing membrane potential shifts and in double-pulse experiments. The use of the cell perfusion technique enabled a much better separation of the individual components of transmembrane currents. Some preliminary results have already been published (Doroshenko and Martynyuk 1982; Doroshenko et al. 1982b).

Materials and Methods

Experiments were performed on non-identified isolated neurons of the snail *Helix pomatia* during winter and spring. The techniques of cell isolation, intracellular perfusion, voltage clamping and recording of transmembrane ionic current did not differ from those previously described (Kostyuk et al. 1981). The standard extracellular solution contained (in mmol.l^{-1}): 30 $CaCl_2$; 4 $MgCl_2$; 80 Tris-Cl (pH 7.5—7.7). Equimolar substitutions of Ca^{2+} ions for other divalent cations were used when necessary. In order to block calcium currents, Cd^{2+} ions or verapamil were introduced into the extracellular solution. The standard intracellular solution contained: 120 mmol.l^{-1} Tris-Asp; and in some experiments 20 mmol.l^{-1} TEA-Br were added (pH 7.3). Intracellular pH changes were produced by adding Tris-OH to the dialysing solution. Changes in temperature were produced by passing the extracellular solution through a thin polyethylene tubing, placed in a special cooling chamber, prior to entering the perfusion chamber at different rates. The temperature of the solution was controlled by a thermistor sited in the vicinity of the cell.

Transmembrane currents were digitized using an A/D convertor (frequency of 1 kHz), and stored on a magnetic tape. As the currents showed a comparatively slow time course, such a frequency was sufficient for a reliable analysis of their kinetics. Analog compensation of the leakage current through the membrane and a shunting resistance (between the cell membrane and the wall of the dialysing pore) was made previous by passing a rectangular voltage pulse through the corresponding electronic circuit to the summing input of the operational amplifier (see Kostyuk and Krishtal 1977a). To subtract capacitative currents, digital summation of the current responses to equal de- and hyperpolarizing

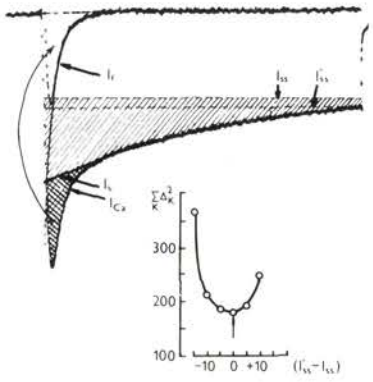

Fig. 1. Diagram illustrating the procedure of approximation of the inward current decline by exponential functions. The figure presents an example of the calcium inward current (I_{Ca}) recorded at an approximately 1 sec long depolarizing membrane potential shift. I'_{ss} is the real value of the current at the end of depolarization, I_{ss} is the calculated level of the stationary current corresponding to the minimal value of $\sum_K \Delta_K^2$ (see text). I_s is the slow exponent approximating the terminal part of (I_{Ca}—I_{ss}), I_{s_0} is its initial amplitude (at the beginning of the test pulse). The cross-hatched area shows the fast current component remaining after subtraction of ($I_{ss} + I_s$) from the total I_{Ca}. It is approximated by I_f. The inset shows the dependence of $\sum_K \Delta_K^2$ (ordinate) on the deviation of I'_{ss} from I_{ss} (abscissa).

membrane potential displacements was used. Only results obtained on cells showing steady values of leakage current over the measured range of testing potential displacements were taken for the analysis. The data were fed from the magnetic tape into an Electronics D3-28 microcomputer for processing. The computer was used, in particular, for a semiautomatic approximation of the current decline by two or more exponential functions. As the calcium current did not reach steady-state level even following 1 sec depolarizing shifts, determination of the "true" value of the latter was included in the approximation procedure. The sequence of approximation steps is illustrated in Fig. 1: 1) The current value at the end of the depolarizing shift was taken as the apparent value of the stationary current (I'_{ss}). This value was subtracted from the total current, and the rest (hatched area) was plotted in a semilogarithmic form. 2) The terminal part of the plot obtained was approximated by a straight line using the least squares technique. 3) From this line, the corresponding exponential function was reconstructed, and the sum of square deviations of the actual current curve from the reconstructed exponential was calculated over the chosen time interval $\left(\sum_K \Delta_K^2\right)$. 4) The value of I'_{ss} was changed in fixed steps and all the procedures described under 1—3 were repeated to find the "true" value of the stationary current (I_{ss}) using the minimal value of $\sum_K \Delta_K^2$ (see inset in Fig. 1). 5) Having determined I_{ss}, the time constant, τ_s, of the most slowly declining component of the current was calculated. 6) This component was subtracted from the total current recorded (cross-hatched area), and the whole approximation process started again for the rest of the current until the next faster component was determined (except that the stationary level of the inward current was not determined). The value of τ_f for this component was calculated, etc. Time constants of the exponential components of the current decline largely differed, and their separation did not cause any problems.

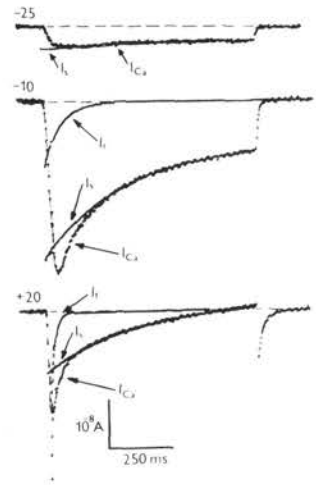

Fig. 2. Inward currents recorded at long depolarizing membrane potential shifts from a neuron placed in sodium-free extracellular and perfused with potassium-free intracellular solutions: approximation of current decline by exponential functions. Testing potentials (V_t) are indicated near the respective records. *A*: $V_t = -25$ mV: $\tau_s = 300$ msec; *B*: $V_t = -10$ mV: $\tau_s = 260$ msec; $\tau_f = 52$ msec; *C*: $V_t = +20$ mV: $\tau_s = 380$ msec, $\tau_f = 15$ msec. Holding potential -50 mV, 20 °C.

The choice of the test pulse duration influences to some extent the results of the τ_s determination: if it exceeds the τ_s value by a factor of 2—3, then the values of τ_s determined at different test pulse durations would differ from each other by 5—10 %.

Results

Inward current decay components

Fig. 2 presents recordings of transmembrane currents elicited by prolonged (of the order of 1 sec) depolarizing shifts of the membrane potential after the removal of Na^+ ions from the outside and K^+ ions from the inside of a cell placed in a Ca-containing solution. The principal charge carriers during the current flow are calcium ions.

The kinetics of these currents showed a series of important features: a) Inward currents which appeared at small depolarizations (Fig. 2*A*) were of remarkable constancy and showed only a slight decay at a sustained depolarization of the membrane; b) At stronger depolarizations (Fig. 2*B*), the decay of the intensified currents during sustained depolarization became more evident; however, later on this decay slowed down and the current very slowly approached a stationary level. c) At very strong depolarizations (Fig. 2 *C*) the rate of the initial decay became

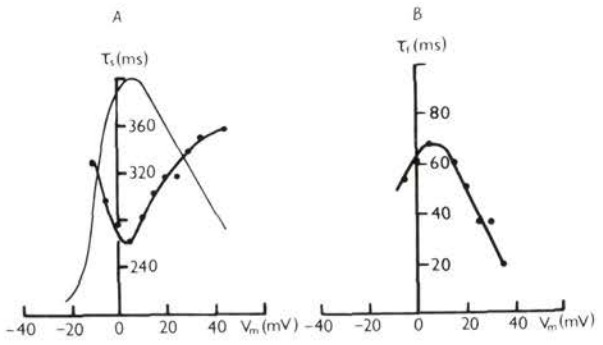

Fig. 3. Potential-dependence of the time constants of the slow (*A*) and fast (*B*) exponential decays of the inward current. Abscissa — testing potential (mV), ordinate — decay time constants. The thin line in *A* represents the current-voltage characteristic for the peak value of the inward calcium current in the same cell. Holding potential −50 mV, 19 °C.

extremely high, and at the end of the depolarizing shift the direction of the current reversed.

In accordance with the reported data (Kostyuk and Krishtal 1977; Ashcroft and Stanfield 1982) the current decay could be approximated, in general, by sum of two exponential functions with different time constants. The slow exponential decline of the inward current (I_s) with a time constant of several hundreds of milliseconds (τ_s) was observed at all the testing potentials used. The fast component of the decline (I_f), having a time constant of several dozens of milliseconds (τ_f), became superimposed on the slow one at stronger depolarizations (to about −10 mV and over).

Potential-dependence of the decay time constants

The time constant of the slow decline of the inward current (τ_s) varied with changes in testing membrane potential in a typical U-shaped manner (Fig. 3*A*). A comparison of this curve with the current-voltage curve for the calcium inward current in the same cell, (Fig. 3*A*, thin line), definitely indicates the existence of a correlation between both events. The fastest decline rate corresponds to the maximal value of the calcium current, indicating that the slow component of the current decay may somehow be related to the accumulation of Ca^{2+} ions at the inner surface of the cell membrane, as suggested by Brehm and Eckert (1978) and Tillotson (1979).

Figure 3*B* shows the potential dependence of the time constant of the fast decline (τ_f). The difference between this bell-shaped and the previously discussed U-shaped curve is quite obvious, suggesting that the two components differ in the nature of their underlying processes.

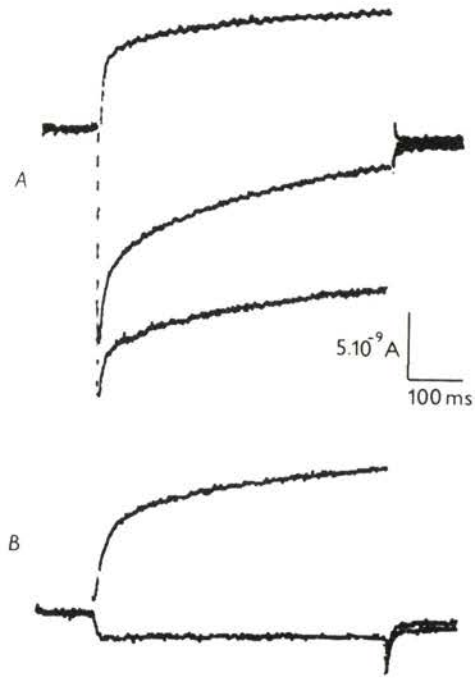

Fig. 4. Demonstration of the presence of a non-specific outward current disturbing the time course of the inward current. *A* — the effect of subtraction of the non-specific outward current obtained after the blocking of the calcium current with 0.2 mmol.l^{-1} verapamil (1) from the total membrane current (2). Trace 3 is the result of this subtraction. *B* — the effect of pH_i elevation from 7.3 to 8.2 on the nonspecific outward current. *Upper trace:* membrane current obtained with 1.5 mmol.l^{-1} Cd^{2+} ions in the extracellular solution with pH_i 7.3, *lower trace*: the same after pH_i raised to 8.2. Holding potential −50 mV, 20 °C.

Demonstration of the presence of a net outward current

It was suggested earlier that the fast component of the current decline may be due to the development of an outward current superimposed on the calcium inward current which, itself, inactivates very slowly if at all (Connor 1979; Kostyuk 1980; Byerly and Hagiwara 1982). The appearance of the fast phase of the current decay with the bell-shaped potential dependence of its time constant at increased depolarizations, combined with the reversal of the current direction at high depolarizing shifts, clearly indicates the presence of an outward current regardless of the absence of K^+ ions inside the cell. This current may be carried by $Tris^+$ or some of other ions present in the solutions, and it is therefore called "non-specific" (see Kostyuk and Krishtal 1977a). The outward current could be recorded directly when the calcium inward current was eliminated by the addition of verapamil or

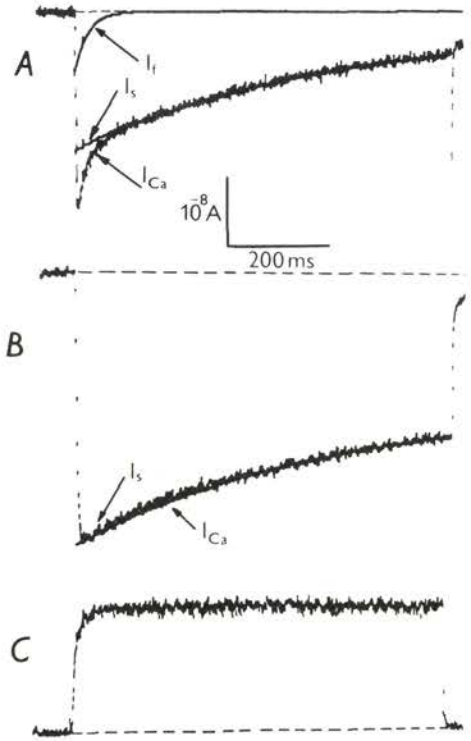

Fig. 5. The effect of elevated pH_i on the calcium inward current. *A*: inward current (I_{Ca}) obtained at pH_i 7.3 during membrane depolarization to +5 mV. I_s, I_f — the slow ($\tau_s = 460$ msec) and fast ($\tau_f = 26$ msec) exponents approximating the I_{Ca} decay, respectively. *B*: current obtained during the same depolarization after raising pH_i to 8.1. Note the disappearance of the fast component of the I_{Ca} decay (the new $\tau_s = 500$ msec). *C*: result of substraction of *B* from *A*, representing the blocked non-specific outward current. Holding potential −50 mV, 18 °C.

cadmium (Fig. 4) to the extracellular solution. Trace 2 in Fig. 4*A* represents an initial calcium inward current recorded during depolarization of the membrane to +10 mV. After the addition of verapamil, the same depolarization generated an outward current (trace 1).

Trace 3 (Fig. 4*A*) represents the result of the subtraction of trace 1 from trace 2, i. e. the corrected inward current. Such subtraction resulted in the disappearance of the reversal of the inward currents, in the diminution of their fast decline, and in an increase in their maximal amplitude.

These results do not leave any doubts about the important role of a non-specific outward current in the rapid decline of the inward current during the initial period of membrane depolarization. The observation that the subtraction of the non-specific current recorded after the blocking of the calcium channels from

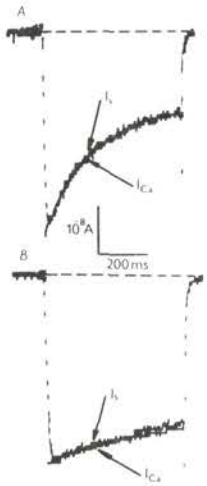

Fig. 6. Changes in kinetics of the inward current decay after introduction of 10 mmol.l^{-1} EDTA in the intracellular solution. *A* — before introduction; *B* — after the introduction. In both cases the decay of the current could be perfectly approximated by a single exponent. *A*: $\tau_s = 210$ msec. *B*: $\tau_s = 520$ msec. Holding potential −50 mV, testing potential +5 mV, pH_i 8.2, 12 °C.

the total current does not produce a complete disappearance of this effect (see curve 3, Fig. 4*A*), may have several reasons. 1) Drugs used to block calcium channels may also to some extent affect the channels passing the non-specific outward current. 2) The channels passing the non-specific current may be sensitive to the presence of Ca^{2+} ions at the inner surface of the cell membrane, and the blocking of the calcium inward current may affect their functioning via a decrease in the internal calcium concentration. Both possibilities would affect the results of current subtraction, and hinder an exact estimation of the time course of the pure calcium current.

Effect of increased pH_i on inward current decay

For reasons stated above, other ways of eliminating non-specific outward current were searched for: changes in intracellular pH proved to be a very effective way. Fig. 4*B* illustrates the effect of an increased pH_i (from 7.3 to 8.2) on the non-specific outward current elicited by depolarization to +30 mV of a cell bathed in a solution containing 1.5 mmol.l^{-1} Cd^{2+} ions (upper trace). The lower trace represents a small inward current which was not blocked by Cd^{2+} ions; it was unmasked after the elimination of the outward current with a high pH_i.

Effects of an increased pH_i on the calcium inward current are shown in Fig. 5. Figure 5*A* shows a record of an inward current at an intracellular pH of 7.3, and

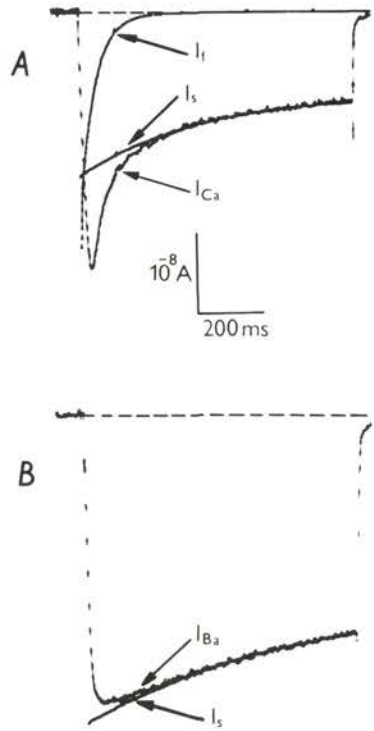

Fig. 7. Changes in kinetics of the inward current decay after equimolar substitution of Ba^{2+} for Ca^{2+} in the extracellular solution. *A*: Ca^{2+}; the current decline was separated into two exponents (τ_s = 180 msec, τ_f = 22 msec). *B*: Ba^{2+}; the fast component is practically absent (τ_s = 310 msec). Holding potential −50 mV, testing potential −15 mV, pH_i 7.3, 24 °C.

Fig. 5*B* shows a record from the same cell during the same depolarization following a pH_i rise to 8.1. This increase resulted in a complete elimination of the fast component of the current decline (practically at all the testing potentials). The maximal amplitude of the inward current increased, and its decline could now be approximated by a single exponent with a time constant of several hundreds of milliseconds, reaching a steady level. The new time constant practically coincided with the τ_s value for the normal pH_i current curve.

Figure 5*C* shows the result of a subtraction of corresponding records obtained at pH_i 8.1 and 7.3, respectively. The difference may be considered a true representation of the non-specific outward current.

Our data seem to support the suggestion that, during sustained depolarization, the fast component of the inward current decline is mainly due to the parallel development of an outward current. Its ionic nature is to be explained.

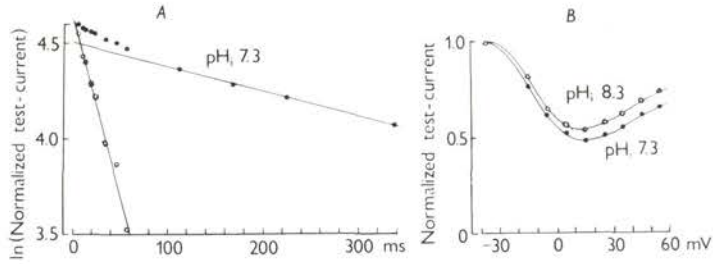

Fig. 8. Turn-off of the calcium currents in two-pulse experiments. *A* — time course of the turn-off of the calcium inward current at pH$_i$ 7.3. Interpulse interval 46.2 ms. Test-pulse duration 23.2 ms. Ordinate — logarithm of $(I_{test}/I_{test\,max}) \times 100$. For the logarithms of the difference currents the scale is the same but shifted 3 logarithm units up. Abscissa — duration of the conditioning pulse. Holding potential −50 mV, testing potential +15 mV, 23 °C. *B* — the effect of pH$_i$ elevation (from 7.3 to 8.3) on the potential-dependence of inactivation of the calcium inward current in two-pulse experiments. Ordinate — $I_{test}/I_{test\,max}$, abscissa — amplitude of the conditioning pulse. Conditioning pulse duration 280 msec, interpulse duration 50 msec. Holding potential −40 mV, testing potential +5 mV, 22 °C.

Effect of intracellular EDTA on the inward current decay

If the slow component of the inward current decline represents an actual decrease in calcium current, which correlates with the current amplitude (Fig. 3*A*), one should expect that this decline would depend on the conditions of calcium accumulation inside the cell. The rate of the ion accumulation depends on the buffering capacity of the intracellular medium. To change the latter 10 mmol.l^{-1} EDTA were added to the intracellular solution. This was actually followed by an increase in the time constant of the slow decay of the inward current (Fig. 6*B*). Current records shown in Fig. 6*A*, *B* were taken at pH$_i$ 8.2: as shown previously at this pH$_i$ the fast component of the current decay becomes depressed. This finding supports the suggestion that the decline of calcium current is controlled by the rate of Ca^{2+} accumulation inside the cell.

Effect of replacing extracellular Ca^{2+} by Ba^{2+} on the inward current decay

Equimolar replacing of Ca^{2+} ions in the extracellular solution by Ba^{2+} ions caused an increase in the inward current maximal amplitude as well as definite changes in its time course. The fast component of the current decay considerably diminished or completely disappeared (Fig. 7*B*). The time constant of the slow decay increased (from 180 msec for the calcium to 310 msec for the barium inward current in the given cell) indicating that Ba^{2+} ions are much less effective in inducing a decrease in the inward current through calcium channels.

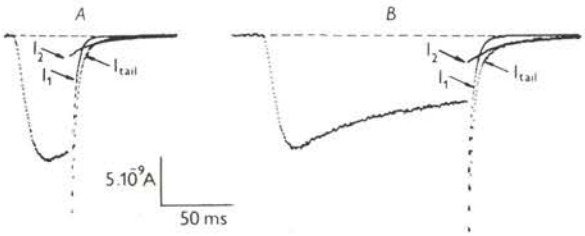

Fig. 9. Kinetics of the tail currents recorded in the same cell during repolarization to holding potential level after depolarizations of different duration. (The "tail" current, I_{tail}, is approximated with two exponents, I_1 and I_2, with time constants τ_1 and τ_2). *A* — testing depolarization for 38 msec, $\tau_1 = 5$ msec, $\tau_2 = 31$ msec, in *B* — testing depolarization for 145 msec, $\tau_1 = 4$ msec, $\tau_2 = 29$ msec. Holding potential −40 mV, testing potential +15 mV, pH_i 7.3, 6 °C. 4 mmol.l^{-1} K^+ were added to the extracellular solution.

Turn-off of inward current in two-pulse experiments

Since most of the results on calcium current inactivation have been obtained in two-pulse experiments (cf. Tillotson 1979; Eckert and Tillotson 1981) we performed similar experiments on intracellularly perfused nerve cells. Two types of membrane potential displacement protocols were used. 1) The conditioning and the testing depolarizing pulses of the same amplitude, corresponding to the maximum of the calcium current *I—V* curve, were separated by a constant delay (about 50 msec). The dependence of the test-pulse current amplitude on the duration of the conditioning pulse ("turn-off" process) was investigated. 2) The dependence of the test-pulse current amplitude on the amplitude of varying conditioning pulses of a constant duration was studied. The same delay as in the first protocol was introduced between the two pulses.

Time course of the turn-off process. The time course of the turn-off of the test inward current was studied using protocol. 1. As the duration of the conditioning pulse increased, the amplitude of the calcium inward current elicited by the test pulse decreased. Plotting of the logarithm of test current amplitude against the duration of the conditioning pulse revealed that at normal pH_i the turn-off process can be described by a sum of two exponents (Fig. 8*A*) — a situation resembling that with the current decay during sustained depolarization.

The time constant of the fast exponent (in the cell illustrated at $V_t = +15$ mV; $\tau_1 = 56$ msec) was close to that for fast decay of the Ca current during long-lasting depolarization, while the time constant of the slow exponent ($\tau_2 = 770$ msec) significantly exceeded τ_s values. The latter may be explained by the fact that in two-pulse experiments the logarithms of the total Ca currents during the test depolarizations were calculated, while for long-lasting Ca currents, only the difference between the total current and its steady-state component was taken.

When the cell was perfused with an intracellular solution of an increased pH, the fast exponent of the turn-off process largely diminished, while the slow one remained practically unchanged.

Potential dependence of the turn-off process. When the amplitude of the conditioning shift of the membrane potential was changed (protocol 2), the magnitude of the test inward current also changed in a typical way: the larger the inward current during the conditioning pulse, the stronger the depression of the test current. The dependence of the test current amplitude on the conditioning voltage had the usual U-shaped form (Fig. 8*B*). Qualitatively, the same results were observed with either pH_i value. However, at normal pH_i the extent of the depression was slightly larger and the ascending phase of the dependence was less steep as compared to those observed at increased pH_i.

Inward current relaxation

During membrane repolarization to the holding potential level, an exponential tail current with a mean time constant of 1.9 ± 0.3 msec (measurements on 5 cells at 20 °C during repolarization to -50 mV) could always be distinguished. If a small amount of K^+ ions (4 mmol.l^{-1}) was added to the extracellular solution, another component of the tail current appeared. Its time constant was about 21 msec under the above conditions. Lowering the temperature slowed down the speed of the relaxation processes. At 6 °C, the time constant of the faster tail current was about 4—5 msec (τ_1 in Fig. 9), that of the potassium component was about 30 msec (τ_2 in Fig. 9). Under this condition it was impossible to detect a superfast tail current component described by Byerly and Hagiwara (1982).

In order to investigate whether the superposition of the non-specific outward current would change the described kinetics of the inward current relaxation, measurements of the latter were performed at two durations of the test pulse — corresponding to the peak of the current, and after the current had nearly reached the steady state (Fig. 9). The development of the outward current did not produce any detectable contribution to the tail current. The reason may be either its very small initial amplitude or its very fast relaxation.

Apart from the above tail current components, an additional very slowly decaying component could be observed in the presence of 4 mmol.l^{-1} K^+ ions in the extracellular solution. The time constant of this component was of the order of several hundreds of milliseconds, and its initial amplitude increased with the increase in the duration of the testing pulse.

Discussion

The data presented here confirm the complex nature of the decline of the calcium inward currents during sustained membrane depolarization (cf. Kostyuk and Krishtal 1977b; Doroshenko et al. 1978; Brown et al. 1981; Ashcroft and Stanfield 1982). The decline can be clearly divided into a fast (with a time constant of several dozens of milliseconds) and a slow (with a time constant of several hundreds of milliseconds) exponential component superimposed on a stationary current. The time constants of these components correspond to those reported earlier for mollusc neurons (Plant and Standen 1981; Byerly and Hagiwara 1982) and are somewhat slower than those recorded in insect skeletal muscle fibres (Ashcroft and Stanfield 1982). While the slow component of the decline could be observed at all testing potentials, the fast one only appeared at depolarizations to about -10 mV and over (Fig. 2). These exponential components could also easily be separated from each other by an increase in pH_i (Fig. 5), suggesting that they are of a different nature. For this reason, it will be more convenient to discuss them separately.

Most controversies concern the fast decay of the calcium current. It has been suggested to reflect calcium-dependent inactivation of the current (Brown et al. 1981; Plant and Standen 1981) as well as to be due to the development of an outward current superimposed on the slowly inactivating calcium inward current (Connor 1979; Kostyuk 1980; Byerly and Hagiwara 1982). Our results support the latter suggestion. First of all, in a cell intracellularly perfused with a K^+-free solution, a net outward current can be recorded when the calcium inward current is blocked (Fig. 4) (Byerly and Hagiwara 1982). It activates at the same depolarizations when the fast decay of the inward current appears. Its subtraction from the total membrane current significantly reduces the fast component of the current decay and leads to an increase in the inward current amplitude (Fig. 4*A*). The voltage dependence of its time constant has a bell-shaped form (Fig. 3*B*) which is usually found for activation of a potential-dependent current.

Furthermore, intracellular perfusion of a cell with a high-pH solution leads to the elimination of either the fast component of the calcium inward current decay or the outward current when the calcium current is beforehand blocked by extracellular cadmium or verapamil (Figs. 4 and 5). It has been shown earlier that changes in intracellular pH, both increase (Brodwick and Eaton 1978) and decrease (Nonner et al. 1980), may affect the potential-dependent inactivation of sodium inward current: its rate became reduced. However, the fast decay of the calcium inward current cannot be attributed to the potential-dependent inactivation for the reasons discussed in several previous papers (e. g. Plant and Standen 1981).

All these findings argue very strongly against the viewpoint that the fast decline reflects inactivation of calcium channels; they can be explained well by the

assumption that the fast decay of the calcium inward current is mainly due to superposition of a rapidly-developing non-specific outward current on the calcium inward current. Activation of ionic channels, giving rise to this current, may depend on the presence of calcium ions at the inner surface of the cell membrane, as the current is sensitive to the action of several factors which influence the calcium current (see also Byerly and Hagiwara 1982). The fact that the fast decay of the calcium current already occurs at negative test potentials can be explained on the assumption that $Tris^+$ or H^+ ions may carry the outward current (Doroshenko et al. 1978; Thomas and Meech 1982). As the intracellular concentration of $Tris^+$ ions (120—130 $mmol.l^{-1}$) exceeds the extracellular one (about 80 $mmol.l^{-1}$), the corresponding Nernst potential is negative (about -10 mV). For H^+, it equals to about -17 mV at the given pH_o (7.5—7.7) and pH_i (7.3) values.

The possible involvement of the outward current carried by H^+ ions into the fast decay of the calcium currents is supported also by the fact that both a lowered pH of the extracellular solution and an increased pH of the intracellular medium have similar effects (Doroshenko et al. 1978; Byerly and Hagiwara 1982).

It is worth mentioning that the presence of the fast decay of the inward current depends, to a great extent, on the nature of the current-carrying ions. When Ba ions are inward current carriers the fast decay of the currents is only slightly expressed, and very often, Ba currents practically do not contain it at all.

Obviously, only the slow component of the inward current decay during sustained depolarization can be considered as an expression of the true decrease of the current. It did not practically change following an increase in the pH_i. Several characteristics of this decay (U-shaped dependence of its time constant on the testing depolarization inversely related to the current-voltage characteristics, slowing down after the increase in the calcium-buffering capacity of the intracellular medium) are in conformity with the suggestion that it is mainly produced by an increase in the calcium ion concentration near the internal side of the cell membrane. However, it cannot simply be attributed to ion accumulation and subsequent changes in the corresponding electrochemical gradient, because, although barium currents through calcium channels are larger than calcium ones, they decline slower (see also Magura 1977). It is difficult to decide now whether this slow decrease of the current is due to a direct binding of penetrating ions to the inner surface of the membrane, or it is mediated by alterations of intracellular biochemical reactions caused by increased calcium ion concentrations. Recently it has been shown that calcium conductance of the nerve cell membrane strongly depends upon the intracellular cAMP metabolism which, in turn, is affected by changes in intracellular Ca^{2+} (Doroshenko et al. 1982a). On the other hand, it has been suggested that Ca-induced inactivation of alamethicin channels in lipid bilayers results from changes in the surface potential of the membrane produced by binding of Ca^{2+} ions (Hall and Cahalan 1982). This mechanism might explain the

difference in inactivation rates observed for calcium and barium inward currents, as it is known that barium ions are much less potent modulators of the membrane surface potential in snail neurons compared to calcium ions (Kostyuk et al. 1982). Some effects of changed pH_i on Ca binding to the cell membrane cannot be ruled out either.

The results of two-pulse experiments have confirmed the above conclusions. They have shown that, if preceded by conditioning current of different duration and at normal pH_i, the turn-off of the test pulse current develops in two phases differing by their rates. The faster phase of the turn-off process is affected by increased pH_i, while the slower one remains practically unchanged. These data and the fact that the rates of the inward current decrease, observed in two-pulse experiments, are similar to those observed during prolonged depolarization (taking into account the differing methods of determination of τ_s and τ_2 discussed above) suggest that the nature of the fast turn-off phase may be similar to that of the fast decay of the inward current during prolonged depolarization of the membrane, i. e. it may reflect the parallel activation of a non-specific outward current. The potential dependence of the test-pulse current shows some changes following an increase in the pH_i (Fig. 8*B*) also indicating the contribution of the non-specific outward current to the process of calcium current decrease during subsequent depolarizations of the membrane.

The inactivation of calcium currents in our experiments was never complete. At high pH_i, the remaining stationary component of the current amounted up to 50—60 % of its maximal value. It may reflect the attainment of an equilibrium between Ca^{2+} ion influx and their removal from the internal surface of the cell membrane; the presence of a special type of calcium channels which are free from inactivation cannot be ruled out either.

The data about tail-current kinetics obtained in the present experiments are in conformity with those reported previously (Kostyuk et al. 1981). We cannot agree with Byerly and Hagiwara (1982) who attributed tail currents with time constant almost identical to those found in our experiments to relaxation of ionic channels carrying non-specific current, because the tail currents of the latter (when recorded under conditions of blocked calcium inward current) were much smaller in their initial amplitude and (sometimes) of reversed direction. This is also substantiated by the finding that it was impossible to detect any difference in the relaxation kinetics of the inward current of various durations (Fig. 9).

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