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TWO TYPES OF CALCIUM CHANNELS IN THE SOMATIC MEMBRANE OF NEW-BORN RAT DORSAL ROOT GANGLION NEURONES

BY S. A. FEDULOVA, P. G. KOSTYUK AND N. S. VESELOVSKY

From the A.A. Bogomoletz Institute of Physiology, Academy of Sciences of the Ukrainian S.S.R., Kiev, U.S.S.R.

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SUMMARY

1. Ca^{2+} inward currents evoked by membrane depolarization have been studied by the intracellular dialysis technique in the somatic membrane of isolated dorsal root ganglion neurones of new-born rats. In about 20% of the investigated cells a hump has been detected on the descending branch of the current–voltage curve, indicating the presence of two populations of Ca^{2+} channels differing in their potential-dependent characteristics.

2. An initial less regular component of the Ca^{2+} current was activated at membrane potentials from −75 to −70 mV. Its amplitude reached 0·2–0·9 nA at 14·6 mM-extracellular Ca^{2+}. The activation kinetics of this component could be approximated by the Hodgkin–Huxley equation using the square of the m variable. τ_m varied in the range from 8 to 1 ms at potentials between −60 and −25 mV ('fast' Ca^{2+} current).

3. The second component of the Ca^{2+} current was activated at membrane depolarizations to between −55 and −50 mV. It could be recorded in all cells investigated and reached a maximum value of 1–7 nA at the same extracellular Ca^{2+} concentration. This component decreased rapidly during cell dialysis with saline solutions. The decrease could be slowed down by cooling and accelerated by warming the extracellular solution. Intracellular introduction of 3′,5′-cAMP together with ATP and Mg^{2+} not only prevented the decrease but often restored the maximal current amplitude to its initial level. The activation kinetics of this component could also be approximated by a square function, τ_m being in the range 16–2·5 ms at membrane potentials between −20 and +3 mV ('slow' Ca^{2+} current).

4. The fast Ca^{2+} current inactivated exponentially at sustained depolarizations in a potential-dependent manner, τ_h varying from 76 to 35 ms at potentials between −50 and −30 mV. The inactivation of the slow Ca^{2+} current studied in double-pulse experiments was current-dependent and developed very slowly (time constant of several hundreds of milliseconds). It slowed down even more at low temperature or after substitution of Ba^{2+} for Ca^{2+} in the extracellular solution.

5. Both currents could also be carried by Ba^{2+} and Sr^{2+}, although the ion-selecting properties of the two types of channels showed quantitative differences. Specific blockers of Ca^{2+} channels (Co^{2+}, Mn^{2+}, Cd^{2+}, Ni^{2+} or verapamil) exerted similar effects on them.

6. The existence of metabolically dependent and metabolically independent Ca^{2+} channels in the neuronal membrane and their possible functional role are discussed.

INTRODUCTION

Extensive data about the functioning of a potential-dependent Ca^{2+} channel have been obtained during recent years on the somatic membrane of mollusc neurones. The use of intracellular dialysis (perfusion) was very important in this respect as it opened up the way for reliable separation of Ca^{2+} currents (Kostyuk, Krishtal & Pidoplichko, 1975; Kostyuk & Krishtal, 1977*a*, *b*; Akaike, Lee & Brown, 1978 and others). Further studies of this question on neuronal membranes of other animal species, especially mammalian, are of obvious importance. Intracellular dialysis can be successfully used for neurones of higher animals (Veselovsky, Kostyuk, Krishtal & Pidoplichko, 1977). The application of this technique to isolated neurones from rat dorsal root ganglia has shown that membrane depolarization also elicits specific Ca^{2+} currents in their somatic membrane; however, these currents were unstable and disappeared, as a rule, soon after the start of cell dialysis. The reason for this instability seems to be a high dependence of the functioning of Ca^{2+} channels on the intracellular level of cyclic nucleotides, which are washed out (or destroyed) during cell perfusion with saline solutions. In fact, intracellular introduction of exogenous 3′,5′-cAMP together with ATP and Mg^{2+} largely stabilized the Ca^{2+} conductance in the dialysed cells (Fedulova, Kostyuk & Veselovsky, 1981; Kostyuk, Veselovsky & Fedulova, 1981*c*). The discovery of such a stabilizing effect made possible a detailed study of Ca^{2+} channels in the somatic membrane of mammalian neurones. The results obtained are presented in this paper.

METHODS

Isolation of rat dorsal root ganglion neurones

Experiments were carried out on neurones isolated from dorsal root ganglia of 5–10 day old rats. The isolation technique did not differ from that already described (Kostyuk, Veselovsky & Tsyndrenko, 1981*d*). After decapitation and exposure of the spinal cord, five caudal pairs of the ganglia were extirpated and placed in Eagle medium (pH 7·4) containing 0·1% pronase (Serva). In this solution the ganglia were transferred into a shaker for 15 min at 32 °C. After treatment they were placed in fresh Eagle medium for 30 min at the same temperature and then stored in a refrigerator at 4 °C. During such storage the cells retained their excitability up to 48 h. Immediately before the experiment one of the ganglia was placed under a stereoscopic microscope and single cells were isolated mechanically with needles. The cell diameter varied between 30 and 40 μm; no functional identification of isolated neurones was made. Standard measurements of ionic currents were carried out at 20–22 °C.

Intracellular dialysis. This was performed by a standard technique (Kostyuk & Krishtal, 1977*a*; Kostyuk, Krishtal & Pidoplichko, 1981*b*). Single cells (30–40 μm in diameter) were placed in a conical pore on the tip of a V-shaped 20–30 μm thick polyethylene tube which carried the 'intracellular' solution. The angle of the pore was 40–50° and the diameter of its internal opening 7–12 μm. The destruction of the membrane patch facing the pore opening was performed by a hydrostatic pressure increase in the perfusing system; it could be facilitated by using Ca^{2+}-free solution. The time necessary for complete replacement of the intracellular ionic composition varied between 10 and 20 s as estimated from the changes in the amplitude of transmembrane currents.

Solutions. The composition of the basic and modified extracellular solutions is given in Table 1. Their pH was kept at 7·3 by Tris chloride. For elimination of Na^+ currents the preferable substitute for Na^+ appeared to be choline (in equimolar concentration). To block Ca^{2+} currents, Co^{2+} (5 mM),

Mn^{2+} (5 mM), Cd^{2+} (0·5 mM) or Ni^{2+} (5 mM) were used. The osmolarity of the solution was maintained by replacement of choline chloride. Verapamil was used in concentrations of $1 \cdot 10^{-4}$–$3 \cdot 10^{-4}$ g/ml. Co^{2+}-containing solutions were prepared immediately before the experiment.

Cell dialysis was performed with Tris phosphate (150 mM) solution of pH 7·4 which, if necessary, was changed for one of the investigated solutions (see text).

TABLE 1. Composition of the extracellular solutions (mM)

	Basic solution	Modified solutions				
Salts	1	2	3	4	5	6
NaCl	140	—	—	—	—	—
$CaCl_2$	2	2	5·4	14·8	—	—
$MgCl_2$	2	2	2	2	2	2
Choline chloride	—	140	132	118	118	118
Tris Cl	5	5	5	5	5	5
$BaCl_2$	—	—	—	—	14·8	—
$SrCl_2$	—	—	—	—	—	14·8

Electric measurements. These were performed as described by Kostyuk *et al.* (1981*d*). The time taken for charging the membrane was in the range of 100–300 μs. It could be lowered to 50–70 μs by compensation of the series resistance. The leakage currents reached 0·1–0·2 nA and were subtracted automatically.

RESULTS

General description

The inward currents in the somatic membrane of dorsal root ganglion neurones from new-born rats measured in the basic solution 1 had complicated potential dependence and kinetics, as was described earlier (Kostyuk *et al.* 1981*c*, *d*; Fedulova *et al.* 1981). The removal of Na^+ from the external solution led to elimination of the fast component of the inward current, thus simplifying the observed picture. After such a removal a Ca^{2+}-dependent inward current has been recorded in all neurones investigated (480). In 20 % of these neurones this current consisted of two components which differed in potential dependence and kinetics (Fig. 1*A*). An initial less regular low-amplitude component was activated in solution with a normal concentration of external Ca^{2+} (2 mM) at membrane potentials of −75 to −70 mV. It had a clearly time-dependent inactivation. The second high-amplitude component was recorded in almost all cells. In solution with normal Ca^{2+} concentration (2 mM) this component was activated at membrane potentials of −55 to −50 mV and its inactivation developed extremely slowly (see details below). Changes in external Ca^{2+} concentration affected the amplitude of both components. Examples of current–voltage curves obtained for one of the investigated cells at 2, 5·4 and 14·8 mM-external Ca^{2+} are given in Fig. 1*B*. The hump on the descending branch of the curves clearly separates two potential-dependent components of the Ca^{2+} conductance differing in their activation potentials. The activation of the second one began at depolarizations at which the first current component had already reached its maximum value. In the range shown in this Figure the amplitude of both components depended linearly upon external Ca^{2+} concentrations; at higher concentrations current saturation was observed. The

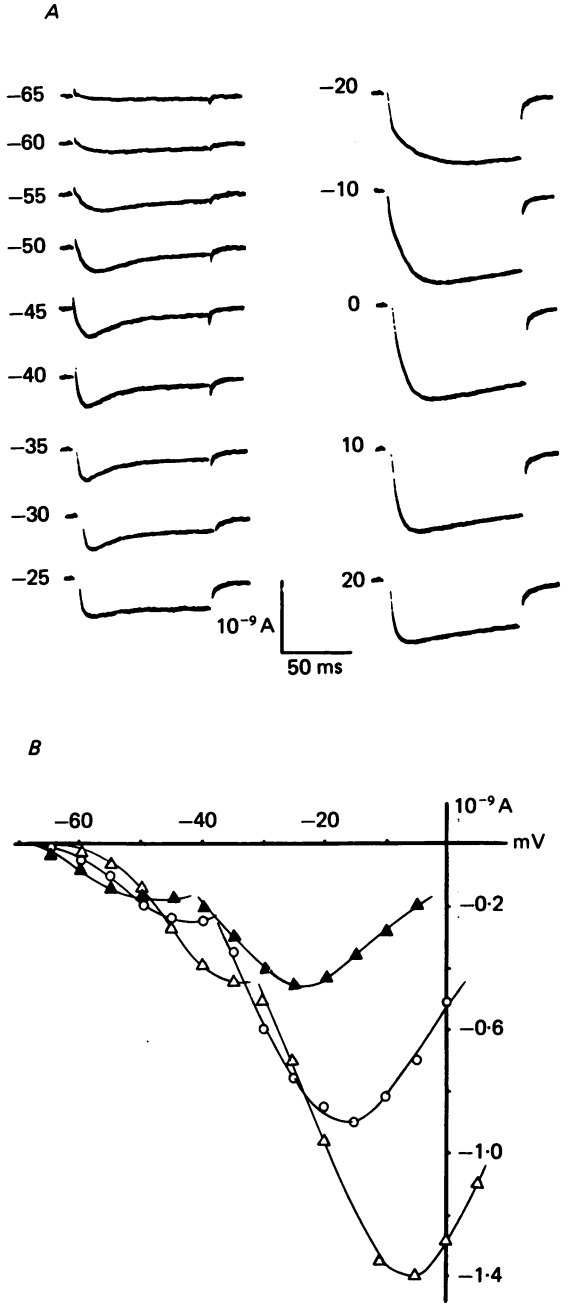

Fig. 1. Inward Ca^{2+} currents in the somatic membrane of rat dorsal root ganglion neurones. Intracellular dialysis by Tris phosphate (150 mM). Holding potential −90 mV. *A*, experimental traces of inward Ca^{2+} currents in Na^{+}-free outside solution with 14·8 mM-Ca^{2+}. Numbers near current curves indicate membrane potential in mV. *B*, current–voltage characteristics for the somatic membrane of one of the investigated neurones obtained in solutions with different external Ca^{2+} concentration (▲, 2 mM; ○, 5·4 mM; △, 14·8 mM).

amplitude of the first inactivating component varied between 0·1 and 0·3 nA in solutions with normal Ca^{2+} concentration, but only in some neurones did it reach 0·5 nA. The maximal amplitude of the second non-inactivating component was 0·5–2 nA in solution of the same Ca^{2+} concentration. To record both components more reliably, all experiments were carried out in solutions with 14·8 mM-external Ca^{2+}. The maximal amplitudes of both components increased 3–3·5 times under these conditions (see Fig. 1*B*).

As has already been reported (Fedulova *et al.* 1981), a characteristic feature of Ca^{2+} currents in the somatic membrane is their extreme instability during cell dialysis with saline solutions (contrary to high stability of Na^+ currents). This progressive decline affected mainly the second non-inactivating component of the Ca^{2+} current and could be slowed by lowering the temperature. At temperatures in the range 5–32 °C the rate of ion replacement in the cell (controlled, for instance, by changes in outward K^+ currents) was not considerably altered. Thus, the deceleration of Ca^{2+} conductance decline in the course of cell dialysis cannot be explained simply by washing out of the cell some substances necessary for the maintenance of channel activity. It obviously reflects the development of changes in some biochemical reactions which keep the channels in a functioning state under normal conditions. Taking into account the data available about the important role of cAMP-dependent phosphorylation of cellular proteins in the maintenance of many cellular functions, one may assume that a certain balance between phosphorylation and dephosphorylation of Ca^{2+} channel proteins is also necessary for channel activity. When the cell is dialysed with saline solution, a rapid depletion of cAMP (as well as of other substances) may take place, and a shift towards dephosphorylation may render the Ca^{2+} channels inactive.

The experimental results are in favour of this suggestion. A very effective way of stabilizing the second component of the Ca^{2+} conductance was to add 3′,5′-cAMP together with ATP and Mg^{2+} to the perfusing solution. As a result, not only did the decline in current amplitude stop, but a quite stable restoration of the initial level of Ca^{2+} conductance could be achieved. The stabilizing effect was not related to any changes in the membrane leakage, as has been shown by continuous control of the latter with hyperpolarizing membrane potential shifts. After restoration the amplitude of Ca^{2+} currents started to decline again but at a much slower rate than without introduction of these substances into the cell.

The effectiveness of the described procedure depended on the time which elapsed after the beginning of cell dialysis. If the introduction of cAMP started at a moment when the Ca^{2+} conductance was already greatly reduced, its restoration was more difficult and no complete recovery could be achieved. The restoring effect depended on the concentration of substances used. Variation of cAMP between 10^{-6} and 10^{-4} M has shown that the optimum concentration for cAMP is 5×10^{-5} M; its further increase did not improve the effect.

Fig. 2 shows the described effect of cAMP in combination with ATP and Mg^{2+}. As can be seen from this Figure, the dependence on the intracellular level of these substances was obvious only for the second long-lasting component. The initial inactivating component of the Ca^{2+} current changed very slowly in the course of cell perfusion and the addition of cAMP did not have any appreciable effect on it. It should be indicated that some stabilization of the inactivating component of the Ca^{2+}

conductance could also be observed after addition of only ATP and Mg^{2+} to the perfusing solution; however, subsequent addition of cAMP greatly potentiated their action (see Fig. 3).

It should be noted that it was not possible to use the different rates of decrease of the maximal amplitudes of both components of the Ca^{2+} inward current (see Fig. 2)

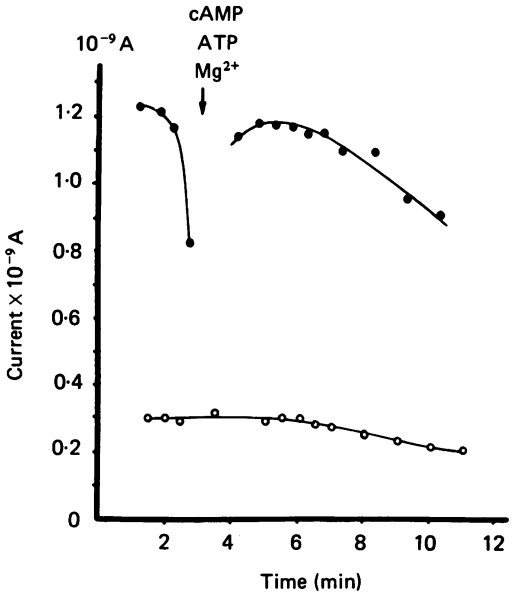

Fig. 2. The effect of intracellular introduction of cAMP (1×10^{-6} M), ATP (2 mM) and Mg^{2+} 5 mM) into a dialysed neurone on the maximal amplitude of the two components of Ca^{2+} inward current. Arrow indicates the time of introduction of cAMP, ATP and Mg^{2+}: ●, for slow component, ○, for fast component. Abscissa, time after beginning of cell dialysis; ordinate, values of the currents.

for their complete separation. As indicated above, the initial ratio between the maximal amplitudes of inactivating and non-inactivating Ca^{2+} currents was 2–10. Although the rate of decline of the maximal amplitude of the inactivating component was much less than that of the non-inactivating component, the decrease of the latter occurred at a time when reliable recording of the inactivating component was already difficult.

The discovery of a way to stabilize Ca^{2+} current in dialysed cell membranes created the necessary conditions for more thorough studies of the properties of the corresponding ionic channels. Most measurements were made at a temperature of 20–22 °C at which the stability of currents was quite sufficient.

Activation kinetics

The activation of both components of the Ca^{2+} current was approximated by the Hodgkin–Huxley equation using a simplified method for determination of the power of the m variable (Mironov, 1982). The presence of inactivation was not taken into

account in the calculations because of its quite slow development. For both components the square power could be used successfully.

Fig. 4 presents the potential dependence of τ_m for the initial and second component of the Ca^{2+} current measured in four neurones. The value of τ_m for the initial (inactivating component) could be determined only at those test potentials at which

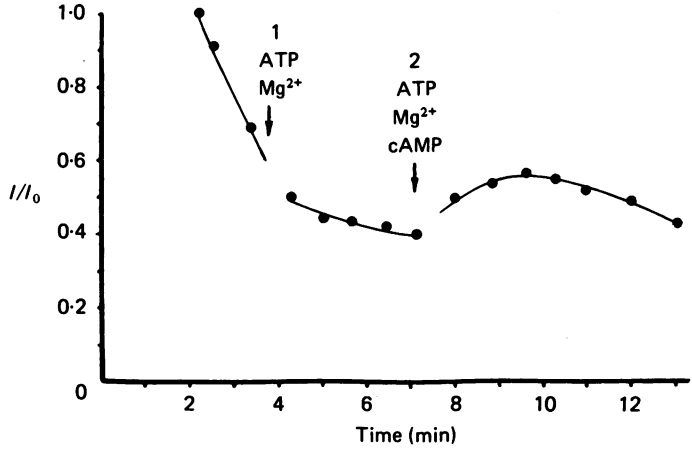

Fig. 3. Partial stabilization of the second (slow) component of the Ca^{2+} current during introduction of ATP (2 mM) and Mg^{2+} (5 mM) into a dialysed neurone and restoring effect of additional introduction of cAMP (50 μM). Arrows indicate the time of introduction of ATP, Mg^{2+} (1) and cAMP (2) into the dialysing solution.

the much stronger second non-inactivating component was not yet present (between -60 and -30 mV). The value of τ_m for the second component exceeded that for the initial one about twice. Therefore the two components could be designated 'fast' and 'slow' Ca^{2+} currents $I_{Ca,f}$ and $I_{Ca,s}$.

The activation time constants changed between 1·2 and 2·0 times for 10 °C change in temperature (measurement for maximum current amplitudes).

Inactivation kinetics

The inactivation of $I_{Ca,f}$ during sustained depolarization could be approximated by a single exponential. Its time constant τ_h was about 75 ms at a test potential of -55 mV and decreased with increasing depolarization, as can be seen from Fig. 5. It was impossible to follow the changes in τ_h at depolarizations above -30 mV because of the superposition of the slow current.

Inactivation of $I_{Ca,s}$ during sustained depolarization developed extremely slowly. Two exponentials could be distinguished qualitatively in its time course. It was difficult to measure their time constants precisely because of the harmful effect of prolonged depolarization on the membrane; the first was about 850–950 ms and the second one about 2·7–3·3 s (test potential -10 mV).

The change in time constants of current inactivation for a 10 °C temperature alteration was 1·5–2·0 and 2–3 times for the first and second exponentials, respectively.

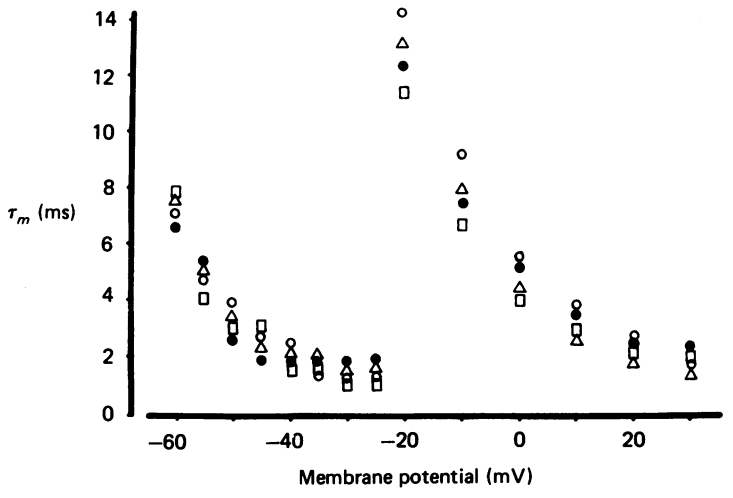

Fig. 4. Potential dependence of activation time constants $\tau_{m,t}$ and $\tau_{m,s}$ of the two components of Ca^{2+} inward current. Measurements were performed in four different neurones dialysed with Tris phosphate solution (150 mM) ●, ○ or Tris phosphate (140 mM) + ATP (2 mM) + Mg^{2+} (5 mM) △, □.

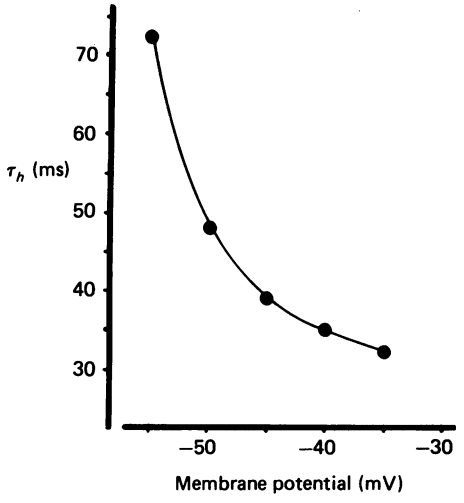

Fig. 5. Potential dependence of the inactivation time constant of the fast component of the inward Ca^{2+} current in one neurone. Abscissa – membrane potential, ordinate – time constant.

After equimolar replacement of Ca^{2+} by Ba^{2+} in the external solution the second inactivation component slowed down considerably (by a factor of 2), although the first one was not much affected.

Fig. 6*A* and *B* presents records of the slow currents carried by Ca^{2+} and Ba^{2+} in the same cell and the separation of their decay into exponential components.

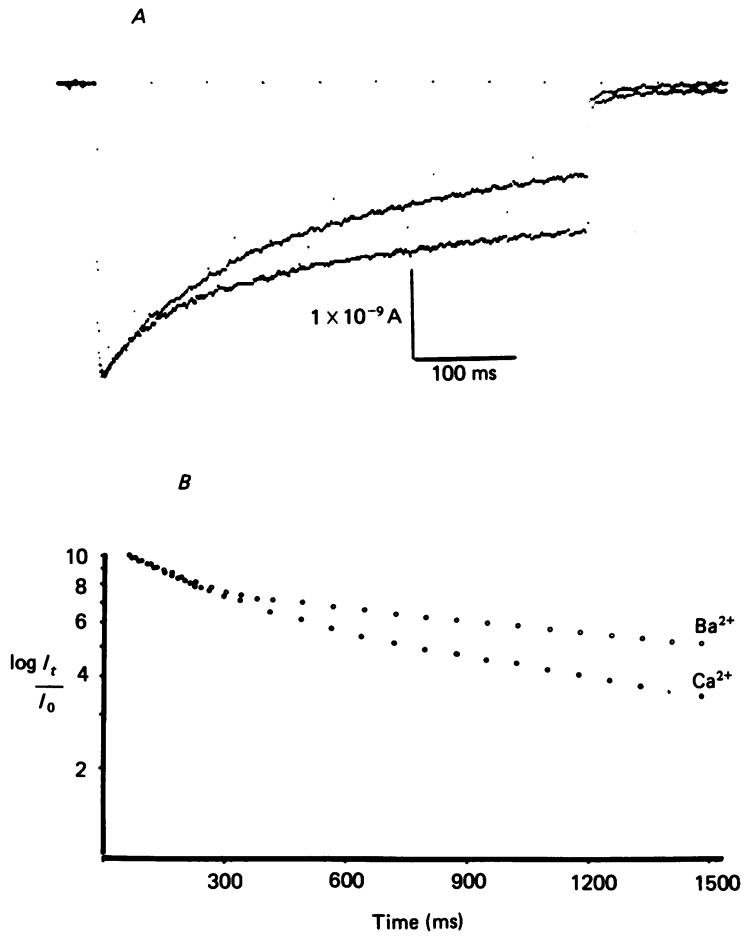

Fig. 6. Change in the time course of the slow Ca^{2+} current inactivation after equimolar replacement of Ca^{2+} (14·8 mM) by Ba^{2+} in the external Na^{+}-free solution: *A*, comparison of normalized current curves at the maximum of the current–voltage characteristic in each solution. Dotted line is a zero line. *B*, the decay of these currents plotted on a semilogarithmic scale.

At large depolarizations (to more than +30 mV) the time course of Ca^{2+} current decay was complicated by the appearance of a very fast component (time constant of several tens of milliseconds) which could be attributed to the activation of a non-specific outward current (see Kostyuk & Krishtal, 1977*a*; Doroshenko, Kostyuk & Martynyuk, 1982*a*). For this reason it was more convenient to study the inactivation of $I_{Ca,s}$ in two-pulse experiments, in which following a variable depolarizing conditioning pulse a second pulse (corresponding in amplitude to the peak of the current–voltage curve) was applied after a definite interval. Fig. 7*A* presents the relation between the test-pulse current and the Ca^{2+} current evoked by the conditioning pulse. The test-pulse current was normalized to its value in the absence of the conditioning pulse. The curve has an obvious minimum which corresponds to

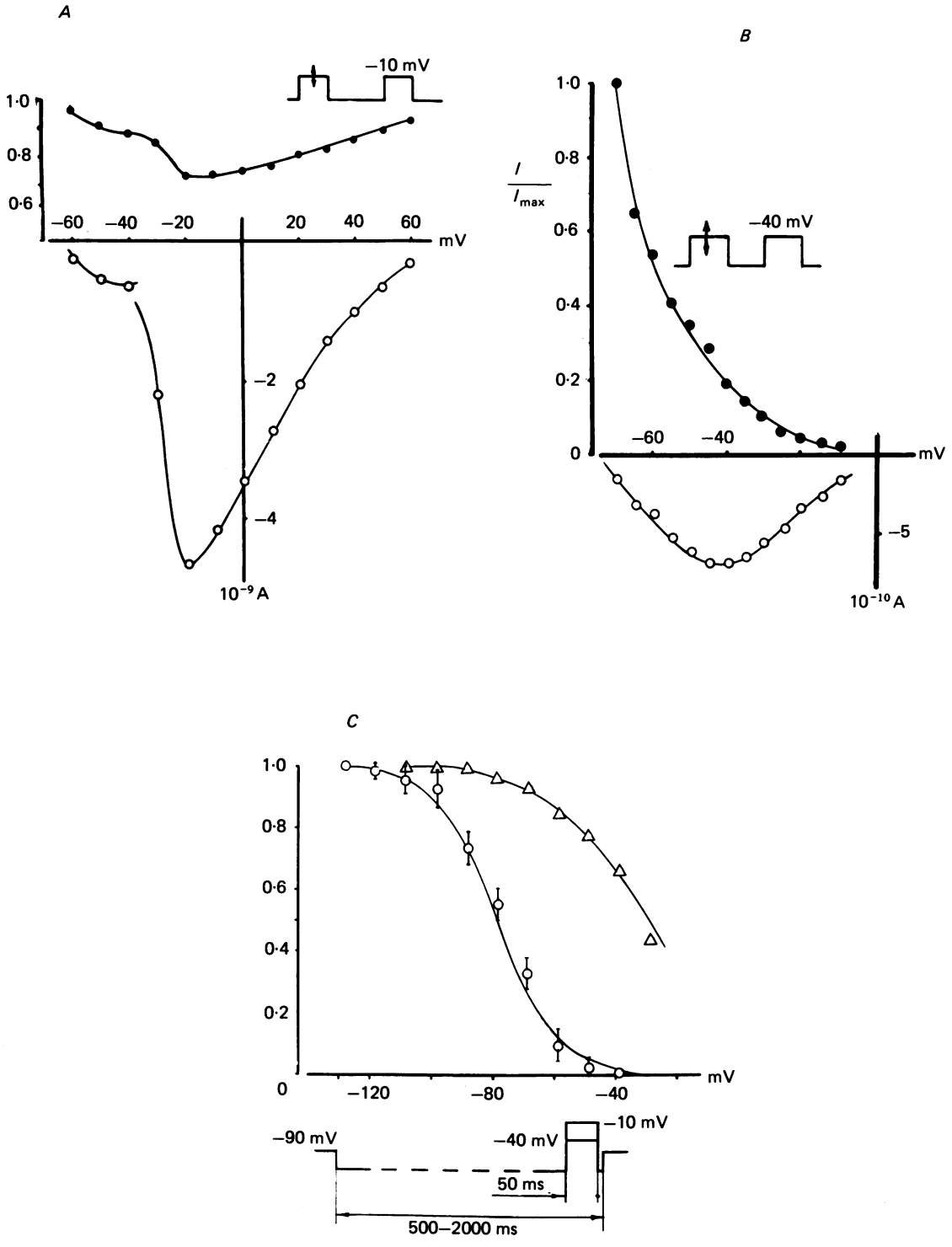

Fig. 7. For legend see opposite.

the peak of the current–voltage curve for the conditioning-pulse current, indicating that the decrease in the test-pulse current is related not to the level of membrane potential but to the value of the preceding inward Ca^{2+} current. This result is in agreement with similar data obtained for the inactivation of Ca^{2+} current in mollusc neurones (Eckert & Tillotson, 1981; Plant & Standen, 1981; Brown, Morimoto, Tsuda & Wilson, 1981; Doroshenko *et al.* 1982*a*) which were explained by self-blocking of Ca^{2+} channels due to entering Ca^{2+} ions.

Similar two-pulse experiments on $I_{Ca,f}$ have shown that this current decreases monotonically with the increase in conditioning depolarizing pulses up to 0 mV (Fig. 7*B*). Thus, no relation between the decrease of the test current and the amplitude of the conditioning current could be observed for $I_{Ca,f}$.

Steady-state inactivation

To determine the characteristics of steady-state inactivation of Ca^{2+} currents, the dependence of their maximal amplitude on the holding potential level was measured. The conditioning shift of the holding potential lasted 500 ms for $I_{Ca,f}$ and 1–2 s for $I_{Ca,s}$. The test-pulse potential was −40 mV for $I_{Ca,f}$ and −10 mV for $I_{Ca,s}$ (see inset in Fig. 7*C*).

Important differences have been detected in the steady-state inactivation of both currents. For $I_{Ca,f}$ it could be approximated by the Hodgkin–Huxley equation

$$h_\infty(V_m) = \frac{1}{1+\exp\left(\frac{V-V_{\frac{1}{2}}}{k_h}\right)}, \quad (1)$$

where $V_{\frac{1}{2}}$ is the membrane potential at which $h_\infty = 0{\cdot}5$ and k_h is the steepness coefficient; $V_{\frac{1}{2}}$ was −80 mV and k_h = 10 mV. In Fig. 7*C* the continuous line shows the dependence of steady-state inactivation of $I_{Ca,f}$ on membrane potential calculated according to eqn. (1); the dots are the experimental data from seventeen neurones.

For $I_{Ca,s}$ the detectable steady-state inactivation developed only at holding potentials above −80 mV when an influx of Ca^{2+} into the cell was already induced by the preceding depolarization. The decrease in $I_{Ca,s}$ became obvious at holding potentials above −40 mV, but under these conditions the measurement was difficult because of the appearance of side-effects (increase in leakage and development of the non-specific outward current).

Fig. 7. Investigation of both components of Ca^{2+} current by double-pulse technique. Pulse protocol shown in insets. Upper curve for *A* and *B*: abscissa, conditioning pulse value; ordinate, normalized amplitude of test pulse current (its amplitude without conditioning pulse taken as unity). Lower curve: current–voltage characteristic for inward current produced by conditioning pulse. *A*, slow component. Pulse duration 100 ms, interpulse interval 100 ms; potential of conditioning pulse was changed between −60 and +60 mV. *B*, fast component in one of the neurones which showed practically no slow component. Pulse duration 50 ms, interpulse interval 50 ms; potential of the conditioning pulse was changed between −70 and −10 mV. *C*, steady-state level of inactivation of the two components of the inward Ca^{2+} current in the somatic membrane: ○, fast component (mean for seventeen neurones, the curve drawn according to the Hodgkin–Huxley equation, see text); △, slow component (example for one of the neurones), curve drawn by hand.

Selectivity and pharmacological sensitivity

In order to determine the selectivity properties of Ca^{2+} channels generating the fast and slow calcium currents, Ca^{2+} in the external solution was replaced by equimolar amounts of Sr^{2+} or Ba^{2+} (solutions 5 and 6). This replacement increased the maximal amplitude of both currents, as shown in Fig. 8*A* and *B*. If we accept that the reversal

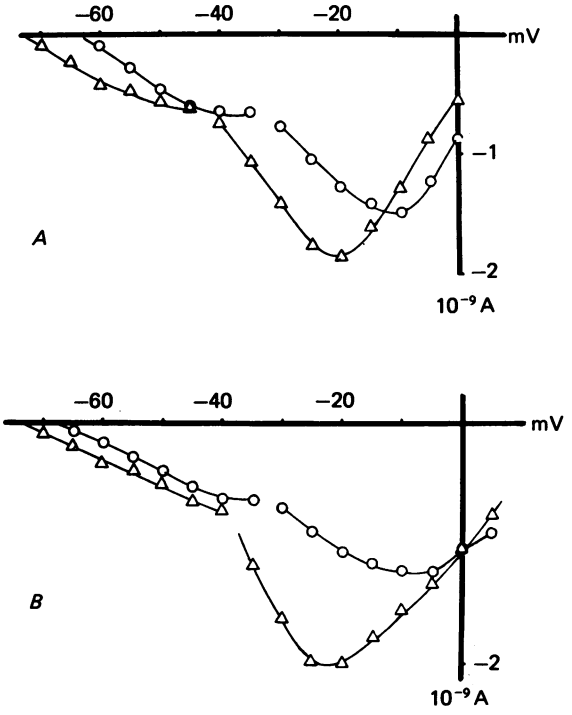

Fig. 8. Selectivity of the two components of the Ca^{2+} inward current: *A*, replacement of Ca^{2+} (14·8 mM) in the extracellular Na^{+}-free solution by an equimolar amount of Sr^{2+} (△); *B*, replacement of Ca^{2+} by an equimolar amount of Ba^{2+} (14·8 mM) (△). Holding potential −90 mV.

potential for the Ca^{2+} currents corresponds to very high positive membrane potentials, then their maximal amplitude can be considered proportional to conductance. With this approximation the relative permeability of the 'slow' Ca^{2+} channels to Ba^{2+}, Sr^{2+} and Ca^{2+} could be estimated as 1·8 : 1·3 : 1·0. It should be noted that the current–voltage curves for Ca^{2+} currents were shifted by about 20 mV compared with those for currents carried by Ba^{2+} or Sr^{2+}, indicating a stronger effect of Ca^{2+} on the surface charges of the neuronal membrane.

No obvious changes were noticed in the amplitude of $I_{Ca,f}$ after similar replacements of ions. Therefore, no quantitative data for the relative permeability of 'fast' Ca^{2+} channels can be presented; the current–voltage curve for this current was shifted by about 10 mV in the depolarizing direction in the presence of Ca^{2+}.

The action of known specific blockers of Ca^{2+} channels was also tested. No

substantial difference was found in the effect of Mn^{2+}, Ni^{2+}, Co^{2+} (5 mM) or Cd^{2+} (0·5–2 mM). Verapamil (10^{-4} M) definitely inhibited $I_{Ca,s}$ predominantly. However, no complete separation of the two components could be achieved in this way (see Fig. 9).

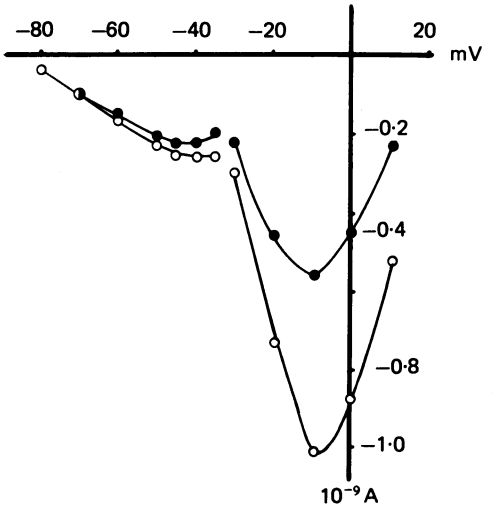

Fig. 9. Blocking effect of extracellularly applied verapamil (10^{-4} M) on the two components of I_{Ca}. External solution contained 14·8 mM-Ca^{2+}. Current–voltage characteristics were obtained before (○) and 2 min after application (●). Holding potential −90 mV.

DISCUSSION

The characteristics of the main ('slow') population of Ca^{2+} channels observed in the somatic membrane of rat sensory neurones are similar to those of previously studied Ca^{2+} channels in mollusc neurones (Kostyuk & Krishtal, 1977*a*; Akaike *et al.* 1978; Byerly & Hagiwara, 1982).

As in molluscs (Doroshenko, Kostyuk & Martynyuk, 1982*b*), in rat neurones the maintenance of stable Ca^{2+} conductance of the somatic membrane is related to the normal sequence of intracellular cAMP- and ATP-dependent reactions. Recent data obtained by intracellular administration of the catalytic subunit of cAMP-dependent protein kinase into perfused snail neurones indicate the importance of cAMP-dependent phosphorylation of some membrane proteins for the functioning of Ca^{2+} channels (Doroshenko, Kostyuk, Martynyuk, Kursky & Vorobets, 1984). In *Aplysia* neurones intracellular injection of the catalytic subunit from an intracellular micro-electrode potentiates 'Ca^{2+}' action potentials (Kaczmarek, Jennings, Strumwasser, Nairn, Walter, Wilson & Greengard, 1980). It should be noted that the described stabilizing effect of exogenous cAMP, ATP and Mg^{2+} on the Ca^{2+} conductance of dialysed neurones probably concerns only one of the mechanisms of intracellular regulation of Ca^{2+} channel activity. The continuing decline (although at a much slower rate) in the Ca^{2+} current amplitude indicates that, apart from the changes in the intracellular level of cAMP, ATP and Mg^{2+}, some other changes take place during dialysis. It may be a slow wash-out of membrane protein kinase, as the introduction of its exogenous

catalytic subunit restores Ca^{2+} conductance at a similar slow rate (Doroshenko *et al.* 1984). Another explanation for the continuous decline of Ca^{2+} current despite addition of cAMP and other substances might be the alteration of the intracellular cytoskeleton which plays some role in the maintenance of membrane excitability (Matsumoto & Sakai, 1979; Fukuda, Kameyama & Yamaguchi, 1981). Nevertheless, cAMP-dependent protein phosphorylation seems to play a special role in the control of Ca^{2+} conductance. This is obvious from the observation that the introduction of exogenous cAMP is a very effective way of stabilizing Ca^{2+} conductance in cells taken from new-born animals; in adult cells this effect is much less pronounced or even absent (Kostyuk, Fedulova, Mironov & Veselovsky, 1981*a*).

An important difference between the rat neuronal membrane and the membrane of snail neurones is the presence, in 20 % of the cells, of a different population of Ca^{2+} channels less dependent on cellular metabolism. The corresponding component of the Ca^{2+} current cannot be attributed to permeation of Ca^{2+} through some other type of channels (for instance, K^+ channels), as it can be blocked by specific Ca^{2+} channel blockers. Data about the presence of two different populations of Ca^{2+} channels have also been obtained (using a different experimental procedure) for the synaptosomes from rat brain (Nachshen & Blaustein, 1982). According to these data, the fast component of Ca^{2+} entry into synaptosomes inactivates (as in the somatic membrane), whereas the slow one does not. However, the relative permeabilities for different divalent cations found in synaptosomes differ from our data obtained for the somatic membrane. Two different types of Ca^{2+} channels are also reported for the membrane of mouse neuroblastoma cells. Initially, in this preparation Moolenaar & Spector (1978) and Spector (1981) detected Ca^{2+} channels similar to the fast Ca^{2+} channels described in the present paper. But recently, using block of outward currents (including the Ca^{2+}-dependent K^+ current) by quinidine, a second system of Ca^{2+} channels has been distinguished, probably similar to the slow channels in the somatic membrane (Fishman & Spector, 1981). It is of interest that in the blastoma cell only the slow channels (which according to our data are highly dependent on cyclic nucleotide metabolism and are predominant in normal cells) happen to be reduced in number. This allows one to suggest that the metabolically dependent Ca^{2+} channels depend on cellular processes which are no longer present after malignant transformation.

The substantial difference in the potential dependence of the two types of Ca^{2+} channels should be stressed. This difference has been detected recently during action potential measurements on neurones of immature rats (Murase & Randic, 1983). It should be pointed out that it is better to perform such experiments in Na^+-free solutions, since slow Na^+ channels resistant to tetrodotoxin may be present in many mammalian neurones (Kostyuk *et al.* 1981*d*). Obviously, the fast Ca^{2+} channels play an important role in near-threshold membrane phenomena, whereas the slow ones start to function in parallel with Na^+ channels only during the generation of a full-sized action potential.

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