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Effect of Calcium and Calcium Channel Blockers on Transient Outward Current of F76 and D1 Neuronal Soma Membranes in the Subesophageal Ganglia of *Helix aspersa*

R. Bal, M. Janahmadi, G.G.R. Green, D.J. Sanders
Department of Physiology, University Medical School, Newcastle Upon Tyne, NE2 4HH, UK

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Abstract. Twin-electrode voltage-clamp techniques were used to study the effect of calcium and calcium channel blockers on the transient outward current in isolated F76 and D1 neurones of *Helix aspersa* subesophageal ganglia in vitro (soma only preparation with no cell processes). On lowering extracellular Ca^{2+} concentration from 10 to 2 mM or removing extracellular calcium from the bathing medium, the threshold for this current shifted in a negative direction by 11.5 and 20 mV, respectively. On the other hand, increasing the extracellular Ca^{2+} concentration from 10 to 20 and to 40 mM shifted the steady-state inactivation curves in positive directions on the voltage axis by 7 and 15 mV, respectively. Upon application of calcium channel blockers, Co^{2+}, La^{3+}, Ni^{2+} and Cd^{2+}, transient potassium current amplitude was reduced in a voltage-dependent manner, being more effective at voltages close to the threshold. The current was elicited even at a holding potential of −34 mV. The specific calcium channel blockers, amiloride and nifedipine did not shift the activation and steady-state inactivation curves and did not reduce the transient outward current amplitude. It was concluded that the transient outward current is not dependent on intracellular Ca^{2+} but that it is modulated by Ca^{2+} and di- and trivalent ions extracellularly. The effects of these ions are very unlikely to be due to a surface charge effect because the addition of La^{3+} (200 μM) completely reverses the shift in a hyperpolarizing direction when the extracellular Ca^{2+} concentration was reduced from 10 to 1 mM and additionally shifts the kinetics further still in a depolarizing direction. The responses seen here are consistent with a specific effect of di- and trivalent ions on the transient outward current channels leading to a modification of gating.

Correspondence to: R. Bal

Key words: Transient outward current — Calcium — Di- and trivalent ions — Channel gating

Introduction

The transient outward current generated by depolarization after a conditioning hyperpolarization was first characterized (Hagiwara, Kusano & Saito, 1961) and was later described kinetically by Connor and Stevens (1971*a*) in molluscan neurons. A current with similar properties has subsequently been demonstrated in many other invertebrate and vertebrate neuronal preparations. The transient potassium current is known to regulate firing frequency (Connor & Stevens, 1971*b*) and also modulates synaptic transmission (Daut, 1973).

In the majority of outward potassium current studies, di- and trivalent cations, such as Co^{2+}, Cd^{2+}, Ni^{2+} and La^{3+} have been used as calcium channel blockers both to assess calcium dependency of K-currents and to isolate these currents from calcium inward currents. However di- and trivalent ions have long been known to shift the steady-state activation and inactivation curves in depolarizing directions on the voltage axis for sodium and calcium channels. These effects have also been demonstrated for K-currents in a number of preparations and the effects of these ions have, in most cases, been attributed to a screening of the negative charge at the membrane surface (Frankenhaeuser & Hodgkin, 1957). On the other hand, not all the experimental results can be explained by this theory, so an alternative mechanism, the binding of a modulator to specific sites on channel proteins needs to be investigated.

In this study, we have investigated the effects of varying $[Ca]_o$ and of two types of calcium channel blockers, the ionic types, Co^{2+}, Cd^{2+}, Ni^{2+} and La^{3+}, and organic types, amiloride and nifedipine on the transient

outward current in isolated somata of F76 and D1 snail neurons. The former calcium channel blockers have similar charge distribution to calcium ions and the latter ones can not have a surface charge effect due to their structure. We also have studied the effect of low ionic strength on the transient outward current.

Material and Methods

Animal and Dissection

Specimens of *Helix aspersa* (British garden snail) were supplied by Blades Biological (UK) and ranged in weight from 3 to 5 g. For dissection an animal was pinned onto a cork board in an extended position. The ganglionic mass with its main peripheral nerves and aorta were dissected out from the animal and then pinned by the nerves and the edges of the connective tissue into a Sylgard-grounded recording chamber (Dow Corning, Midland, MI). The overlying two layers of connective tissue covering the ganglia were gently torn using two pairs of fine forceps, exposing the right and left ganglia where the F76 and D1 neurones are situated. The F76 and D1 neurons were then isolated; no enzymes were used for isolation. Before impalements, neurons were equilibrated for two hours in normal snail Ringer.

Solutions

Normal Ringer solution had the following composition (in mM): NaCl, 80; KCl, 4; $MgCl_2$, 5; $CaCl_2$, 10; glucose, 10; HEPES, 5, as described by Taylor (1987) (all these chemicals were obtained from BDH, UK and were AnalaR grade). The pH of the Ringer solutions was adjusted with TRISMA base (Sigma) to 7.4. Osmolarity was in the range of 212–216 mosM per liter and it was routinely checked using a Roebling osmometer (CAMLAB). The drugs were used by adding to the perfusing Ringer solution. In some experiments, Na^+ ions were replaced on an equimolar basis by choline chloride (BDH) or in some cases mannitol (Sigma) to suppress the sodium inward current. Replacement of Ca^{2+} ions by Co^{2+} was done on an equimolar basis. The Ringer solutions containing cobalt or nifedipine were kept in the dark by covering with foil after being freshly made up and experiments with these substances were carried out in dim light. Unless otherwise specified, 30 mM of tetraethylammonium ion (TEA) was added to the recording solution without disturbing the osmolarity (in substitution for NaCl) to suppress slow outward currents.

Recording Techniques and Equipment

Both voltage and current passing electrodes were freshly pulled from borosilicate glass capillaries with 1 mm external diameter and 0.58 mm internal diameter, which had a filament fused into the lumen (Clark Electromedical Instruments, UK), using a horizontal puller (Narishige). Microelectrodes were coated with Sylgard (184 silicone elastomer kit, Dow Corning). Both microelectrodes were filled with 3 M KCl and had tip resistances of 1–6 MOhm. Both current and voltage clamp experiments were performed using an Axoclamp-2A amplifier (Axon Instruments, Burlingame, CA). All recordings were performed at room temperature (20–24°C).

Generated data were filtered at 2000 Hz with a two-channel low pass variable filter (Kemo). Current and voltage records were sampled at 16 KHz and were digitized online using a 16 bit A/D converter (Tucker Davis- Technologies, TDT) and stored for further analyses. At the end of each experiment, the tip potential was measured. If it was greater than ±5 mV, the data were then discarded. Furthermore so as to minimize the errors due to tip potentials, microelectrodes with low tip resistance (1–6 MOhm) were utilized. For voltage clamp experiments a conventional two-electrode voltage-clamp technique was used. Curve fittings were performed using nonlinear least squares regression routines in MATLAB (The MathWorks, version 4.26c) and pClamp program (version 5.5). Leak currents were obtained by blocking all known ionic currents and assuming that the remaining current is the leak current. Leak currents were digitally subtracted from the data presented.

Activation and Inactivation Curves

The voltage dependency of activation and inactivation of the transient outward current was examined. The voltage dependency for inactivation was determined by using different holding potentials, mostly between −30 and −110 mV in 5 mV steps, and stepping to a fixed potential, which was dependent on the experiment, either +10 mV where all currents but the transient K outward current were suppressed or −25 mV where the transient potassium current can be studied in isolation in Na-free, Ca-containing Ringer. The relationship for activation was investigated with a holding membrane potential of −100 mV in order to remove inactivation of the transient K channels and then stepping to progressively more positive potentials. The data points for the steady-state inactivation curves were fitted using Boltzman functions of the form: $I/I_{max} = [1 + \exp(V_{Half} - V_{step})/K]^{-1}$ (i), in which I_{Max} is the maximum current; V_{Half} is the voltage at which the current is half inactivated and K is the slope of inactivation curve. Similarly, activation curves were fitted using Boltzman functions of the form: $I/I_{max} = [1 + \exp(V_{step} - V_{Half})/K]^{-1}$ (ii), where I_{Max} is the current amplitude that corresponds to maximum conductance, V_{Half}, the voltage at which the current is half activated, K is the slope factor.

Results

In this study, three groups of experiments are reported: (i) Changing the extracellular calcium concentration to assess the effect of extracellular calcium concentration on the transient outward current kinetic properties. (ii) Measuring the effect of low ionic strength on the transient outward current (iii) Applying ionic and organic calcium channel blockers extracellularly to investigate their effects on the steady-state activation and inactivation curves. The data reported here were collected from 67 neurons; 37 from F76 and 30 from D1. No differences between the cell types were detected and the data from both cells were pooled.

Effect of $[Ca]_o$ on the Transient Outward Current

The extracellular calcium concentration was varied in order to study how the transient outward current is affected by $[Ca]_O$. The activation threshold for the transient outward current was shifted by about 20 mV in a negative direction when extracellular calcium was removed. The amplitude of the holding current increased in all cases, accompanied by shifts of the current-voltage

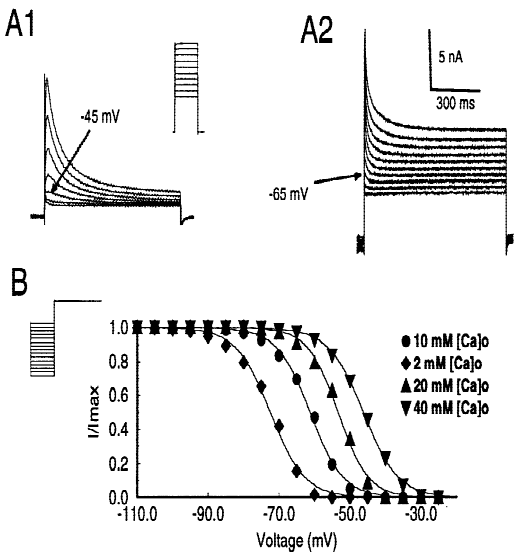

Fig. 1. Effect of changing $[Ca]_o$. (*A*) Effect of removal of calcium from bathing solution: The transient outward current was evoked by depolarizing the neuron between −75 and −25 mV. Voltage steps are shown in the inset. Note that the activation threshold shifted from about −45 to about −65 mV, the rising phase of the current was notably faster and the amplitude of the holding current increased. (*B*): Steady-state inactivation curves for the transient outward current were studied using the voltage protocol shown in the inset. The inactivation curves for the transient potassium current in control condition (in mM): (10 mM Ca^{2+}) (●), 2 Ca^{2+}-containing (♦), 20 Ca^{2+}-containing (▲) and 40 Ca^{2+}-containing Ringer (▼). The voltage record is shown in the inset. The smooth curves through these points are the best fits to the Boltzman distribution, where $V_{Half} = -60.41$ mV and $K = 3.81$ mV for control, $V_{Half} = -72.18$ mV and $K = 4.74$ mV for 2 mM $[Ca]_o$, $V_{Half} = -56.25$ and K = 4.9 for 20 mM $[Ca]_o$ and $V_{Half} = -46.54$ mV and $K = 4.85$ mV for 40 mM calcium-containing Ringer condition. Each data point is the mean from 5 experiments.

relationship and the steady-state inactivation curve moved in a hyperpolarizing direction by about 15 mV in 5 experiments (Fig. 1). Upon decreasing from normal $[Ca]_o$ (10 mM) to 2 mM, the inactivation curve shifted 11.5 mV in a hyperpolarizing direction, having a very similar effect on the current-voltage relationship (Fig. 1*B*). The holding current increased as in the case of 0 mM $[Ca]_o$. In both cases, the activation and inactivation processes of the current were speeded up (Fig. 1.*A*2).

On lowering $[Ca]_o$ from 10 to 2 mM, the activation threshold of the current shifted from −46.2 to −58.3 mV, whereas, when $[Ca]_o$ increased from 10 to 20 mM and to 40 mM, the threshold shifted towards depolarising potentials, −36.2 and −30.5 mV, respectively. Elevation of $[Ca]_o$ from the normal concentration of 10 to 20 and 30 mM caused the midpoint of the steady-state inactivation curve to shift in a depolarizing direction by 4.2 and 13.9 mV (Fig. 1*B*). As a result of these shifts, the current amplitude dependency on concentration and voltage markedly decreased, giving a greater reduction in the current amplitude at the higher concentrations, and suppressing to a larger extent at step potentials close to the activation threshold. It was also observed that when $[Ca]_o$ was increased, the rising and falling processes notably slowed down without changing the activation and inactivation fitting characteristics. These results indicate that the transient outward current in these soma membranes was markedly sensitive to modification of extracellular calcium concentration.

Effect of Low Ionic Strength on the Transient Outward Current

NaCl was replaced on an equimolar basis by mannitol to exclude the possibility that the results given above are due to an effect of nonspecific ionic strength or charge effect of the bathing solution rather than a specific effect of Ca^{2+}. The half inactivation voltage and the slope of the steady-state inactivation curve for the transient outward current was not changed, implying that the ionic charge on the divalent Ca^{2+} ion, and not the ionic strength in the bathing medium, is the major factor responsible for the shifts of activation and inactivation curves on the voltage axis.

Effect of Ionic and Organic Calcium Channel Blockers on the Transient Outward Current

In the presence of calcium ions, transient outward current was contaminated by calcium inward current and by other slow outward currents at potentials more depolarized than −20 mV. Therefore the effect of the calcium channel blockers on the transient outward current was investigated in the range between −50 and −20 mV, where this current can be investigated in isolation in sodium-free, calcium-containing Ringer. Replacement of $CaCl_2$ by $CoCl_2$ greatly reduced the amplitude of the transient outward current at step potentials close to the activation threshold (Fig. 2) ($N = 5$). The midpoint of the inactivation curve shifted by about 4 mV in a depolarizing direction with little effect on the slope. The current-voltage relationship also shifted to a similar extent.

Nickel (4 mM) decreased the amplitude of the transient outward current. Cadmium (200 μM) resulted in a further reduction in the amplitude of the current (Fig. 3 *B*1) ($N = 5$). The reduction of the transient outward current by these calcium channel blockers seemed more effective in blocking at potentials close to the transient outward current threshold than at more depolarizing potentials, implying that there might be a shift of the voltage dependency of the steady-state activation and inactivation curves. This is supported by the findings that the transient outward current was evoked from a holding potential of −25 mV, when the cell was bathed in 200 μM cadmium and 4 mM nickel, or 200 μM lanthanum.

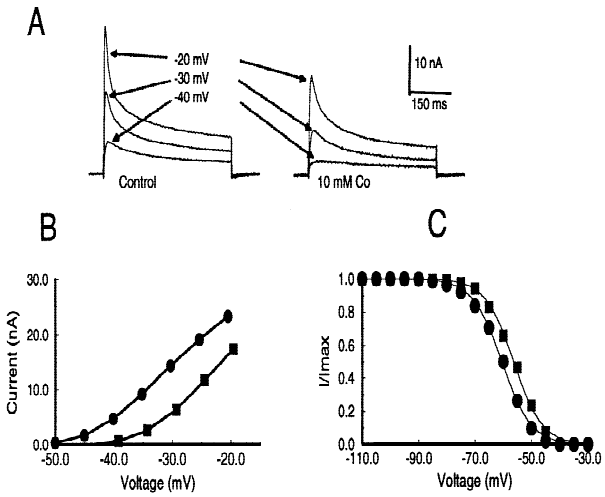

Fig. 2. Effect of cobalt on the transient outward current. The transient outward current was evoked by depolarizing the neuron to −40, −30 and −20 mV from −100 mV holding potential in control condition (*A*, left hand side) and in 10 mM $CoCl_2$ containing Ca-free Ringer (*A*, right hand side). (*B*) Current voltage relationship (*I-V*) of the transient outward current of the same experiment. (*C*) Steady-state inactivation curve for the transient outward current. The half inactivation voltage and slope (V_{Half}) were −60.5 mV and $K = 5.2$ mV in control (●) and −56.32 mV and $K = 5.06$ mV in cobalt containing Ringer (■), respectively.

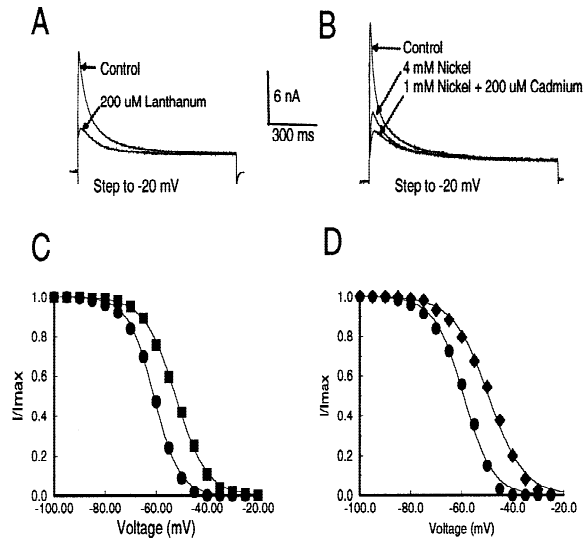

Fig. 3. Effect of lanthanum, nickel and cadmium on the transient outward current. (*A*) The transient outward outward current was evoked by depolarizing the neuron to −20 from −100 mV holding potential. In panel *A*, 200 μM lanthanum and in panel *B*, 4 mM nickel and 200 μM cadmium were added to the Ringer solution. (*C*) The steady-state inactivation curve for the transient outward current has a half inactivation membrane potential and slope of −60.9 and 5 mV in control Ringer (●) and −52 and 6 mV in 200 μM lanthanum containing Ringer (■), respectively. (*D*) The steady-state inactivation curves for the transient outward current have half inactivation membrane potential and slopes of −59.2 and 5.7 mV in control Ringer (●) and −49.5 and 7 mV in 4 mM nickel and 200 μM cadmium containing Ringer (♦), respectively.

To further investigate whether these suppressing effects of the ionic calcium channel blockers on the transient outward current are results of the shifts in the activation and the inactivation curves in depolarizing directions or of a dependency of the current on calcium influx, further experiments were carried out in low calcium-containing (1 mM) Ringer and in the presence of 10 μM amiloride and 1 μM nifedipine. These are reported to block Ca^{2+} influx, and it is thus possible to study the action of these ionic calcium channel blockers on the transient outward current in isolation from calcium currents over a wider range of potentials with the knowledge that calcium influx is blocked by these organic calcium channel blockers. Calcium influx was suppressed by reducing the $[Ca]_o$ to 1 mM and adding nifedipine (1 μM) and amiloride (10 μM) to the Ringer. When switching to 4 mM nickel and 200 μM cadmium (Fig. 4*A*), or to 200 μM lanthanum containing Ringer from control Ringer, the amplitude of the transient potassium current decreased in a voltage-dependent manner. The inactivation curve shifted by about 23 mV in a positive direction on the voltage axis upon the application of nickel and cadmium ($N = 5$) (Fig. 4*B*) and by 27 mV upon application of 200 μM lanthanum ($N = 5$).

A shift was observed in the activation curve for the transient outward current the mid-activation potential moved from −6.24 mV in control to 19.24 mV in nickel and cadmium containing Ringer (*see* Fig. 4*C*). Lanthanum caused the activation curve to shift to a similar extent. The reduction of the transient outward current amplitude in calcium inward current-eliminated condition by the inorganic calcium channel blockers can be largely, if not exclusively, accounted for by the shifts of the activation and the inactivation curves by these ions.

It was of interest to investigate the effects of the external application of the organic calcium channel blockers, amiloride and nifedipine, since they have been reported to block specifically T-type and L-type calcium channels respectively (Janahmadi, 1996; Fig. 5*A*), and given their structures and concentration, they are unlikely to have a surface charge effect. Therefore these blockers were useful to test whether the effect of ionic calcium channel blockers is due to a change in a surface charge or to Ca^{2+}-dependency. Nifedipine (1 μM) and amiloride (10 μM) did not significantly reduce the amplitude of the transient outward current ($N = 6$). The half inactivation membrane potential in control conditions was not significantly different from that in amiloride and nifedipine containing Ringer ($N = 6$).

Finally, the blocking actions of amiloride, nifedipine, nickel and cadmium on calcium currents were confirmed. Outward currents were suppressed by adding 4-aminopyridine (5 mM) and tetraethylammonium (50

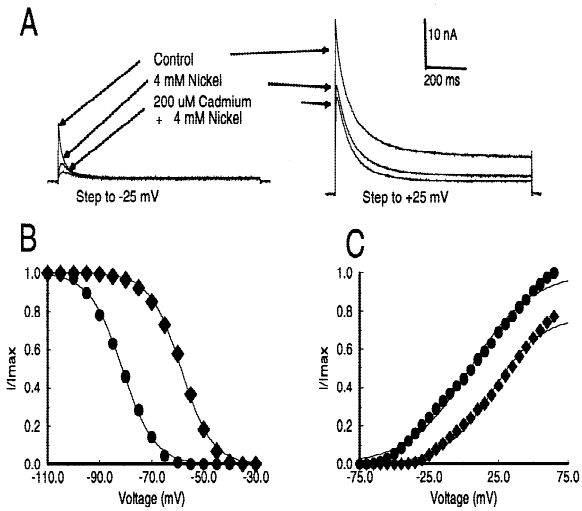

Fig. 4. Effect of nickel and cadmium on the inactivation and the activation curves for the transient outward current in nifedipine (1 μM) and amiloride (10 μM) and low calcium containing Ringer. (*A*) The transient outward currents were evoked by depolarizing the neuron to −25 and +25 mV from −100 mV holding potential. (*B*) The steady-state inactivation curves for the transient outward current have half inactivation potentials and slopes of −81.3 and 6.2 mV in control (●) and −58.7 and 5.9 mV in 200 μM cadmium and 4 mM nickel containing Ringer (♦). (*C*) The activation curves for the transient outward current have half activation potentials and slopes of +4.5 and 22.3 mV in control (●) and +21 and 17.6 mV in 200 μM cadmium and 4 mM nickel containing Ringer (♦). Each data point represents five experiments.

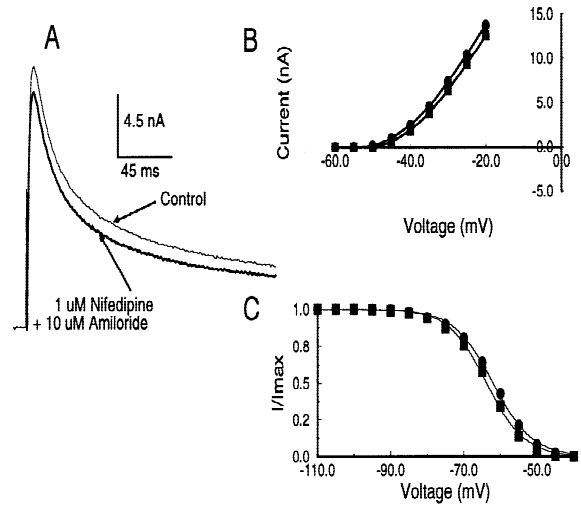

Fig. 5. Effect of amiloride and nifedipine on the transient outward current. The transient outward current was evoked by depolarizing the neuron to −20 mV from a holding potential of −100 mV. The transient outward currents were recorded in control (thin line), and 10 μM amiloride and 1 μM nifedipine containing Ringer (thick line). Note that only the onset part of current traces are shown. (*B*) The current-voltage (*I-V*) relationships for the transient outward outward current in control (●), and 10 μM amiloride and 1 μM nifedipine containing Ringer (■). (*C*) The inactivation curves were fitted by the Boltzman equation: half inactivation membrane potentials (V_{Half}) and slope factors (K) were −62 and 5.4 mV in control, −63.8 and 5.1 mV in nifedipine and amiloride containing Ringer respectively. In panel *B* and *C* each data point is the mean from 6 experiments.

mM substituted for sodium) to the Ringer. Sodium inward current was blocked with tetrodotoxin (1 μM). The inward current response to step depolarizations from a holding potential of −90 mV revealed a large inward current (Fig. 6). This was reduced by approximately 55% in the presence of amiloride (10 μM) and the remaining current was blocked by nifedipine (1 μM), confirming the existence of two types of calcium inward current (Fig. 6*A*). Similarly, the addition of cadmium (100 μM) to control Ringer reduced the inward current by about 60% and the remaining current was abolished by addition of nickel (4 mM).

Discussion

The model used here allows a thorough investigation of the soma membranes of identified neurones. The use of sharp electrodes reduces the risk of alterations of the cell's internal environment due to leakage from larger tipped whole cell clamp electrodes. This is important when considering a possible role for calcium.

The major findings of this work are that the voltage dependency of the steady-state activation and inactivation curves for the transient outward current in soma membranes of isolated F76 and D1 neurons of *Helix aspersa* were shifted to different extents in a depolarizing direction on the voltage axis by di- and trivalent ionic calcium channel blockers, Co^{2+}, Cd^{2+}, Ni^{2+} and La^{3+} and by increased $[Ca]_o$ and were shifted in a hyperpolarizing direction along the voltage axis by decreased $[Ca]_o$. The magnitude of these shifts were so large that it is unlikely that they are due to a surface charge effect. There was no shift when the specific calcium channel blockers, nifedipine and amiloride were applied. It is thus apparent that the effect of di- and trivalent cations on the transient outward current is similar to that produced by an increase in $[Ca]_o$. However the effect is not dependent on an influx of calcium into the cell since the specific calcium channel blockers had no effect.

Transient Outward Current is Modulated by $[Ca]_o$ and Ionic Calcium Channel Blockers

The increase in the holding current when extracellular calcium was removed indicates the important role of calcium in the gating mechanism and this is different from the sense of involvement of Ca^{2+} in production of Ca-dependent components such as $I_{K(C)}$ (Armstrong & Lopez-Barneo, 1987) (*see* Fig. 1). The presence of the transient outward current in Ca-free and low-calcium

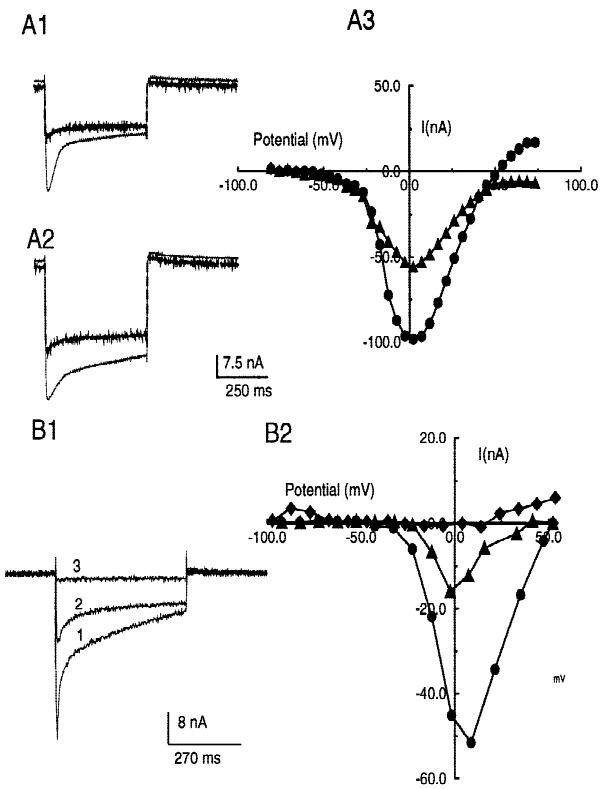

Fig. 6. Effect of amiloride and nifedipine, nickel and cadmium on calcium inward currents. Outward currents were supressed with extracellular application of 4-AP (5 mM), TEA (50 mM, substituted for Na^+) and TTX (1 μM) (*see* Janahmadi (1996) for the detailed isolation protocol). The calcium currents were elicited with depolarizing steps to potentials greater than −40 mV from a holding potential of −90 mV, revealing both T- and L-type. (*A*1) The current traces were evoked by stepping to −10 mV (*A*1) and +10 mV (*A*2) from −90 mV holding potential before and after amiloride (10 μM). (*A*3) Amiloride reduced the peak current amplitude by about 55% (▲) and the remaining current was blocked by nifedipine (1 μM). (*B*1) The calcium inward current traces were elicited from −90 mV holding potential before (1) and after bath application of Cd^{2+} (2) and Ni^{2+} (3). (*B*2) *I/V* relationship of peak inward calcium current before drug application (●), in the presence of 100 μM Cd^{2+} (▲) and 4 mM Ni^{2+} (♦). Peak calcium current was reduced about 60% by 100 μM Cd^{2+}. The residual current in the presence of 100 μM Cd^{2+} was eliminated by 4 mM Ni^{2+}. (Data presented as mean, $n = 5$).

Ringer suggests that a complete Ca-dependency is unlikely. On the contrary, there were increases in the amplitude of the current in response to a given depolarizing voltage step. If it was Ca^{2+}-dependent, then the current should have been abolished or at least reduced when extracellular calcium was removed, as reported in rat cerebellar granule cells (Carignani et al., 1991). The increase in the amplitude of the current can probably be explained by a hyperpolarizing shift of the voltage-dependent activation and inactivation parameters for the transient outward current. On the other hand, the amplitude of the current decreased when $[Ca]_o$ was increased; this is not a typical observation for a Ca^{2+}-dependent current component. For example, in the case of a Ca^{2+}-dependent K current in *Aplysia*, it was reported that when $[Ca]_o$ is increased, the amplitude of the current increased (Junge, 1985). The decrease of the current amplitude seen in the present study when $[Ca]_o$ was increased, can be accounted by the depolarizing shifts of current-voltage relationship and the steady-state inactivation curves.

The reductions of the transient outward current by di- and trivalent cations were consistently voltage-dependent, being more inhibited at step potentials close to the activation threshold of the current, showing the marked depolarizing shifts of the current-voltage relationships and the inactivation curves for this current (*see* Figs. 2 and 3). Even when calcium inward currents were suppressed by the organic calcium channel blockers, amiloride and nifedipine, these ionic calcium channel blockers reduce the amplitude of the current, shifting the activation and inactivation curves in depolarizing directions (Fig. 4).

Therefore, taken together, when interpreting the effect of inorganic calcium channel blockers on currents, the changes to the steady-state inactivation and activation curve shifts have to be taken into account. Depolarizing shifts in activation and inactivation for the transient potassium outward current by the ionic calcium channel blockers, Co^{2+}, La^{3+}, Ni^{2+} and Cd^{2+} were strikingly parallel and the potency was different, being least with Co^{2+} and most with La^{3+} (Fig. 2). These results appear to rule out the possibility of total or partial dependency of the transient outward current on calcium entry into the cell.

Possible Mechanisms for the Effect of $[Ca]_o$ and Ionic Calcium Blockers

Since the increases in $[Ca]_o$ resulted in very similar effects to those induced by extracellular application of di- and trivalent organic calcium blockers, it may be possible that the effects are caused by the same or similar mechanisms. In squid giant axon, it has long been known that divalent cations cause the voltage-dependent gating parameters of the Na and delayed rectifier K channels to shift in depolarizing directions (Frankenhaeuser & Hodgkin, 1957). These authors postulated a surface-potential theory whereby adsorption of calcium ions onto the negatively charged particles on the surface of membrane creates a larger potential difference across the membrane, thus changing the electric field across the voltage sensor. This in turn results in a depolarizing shift of the voltage parameters of the current.

This effect was considered to be related to the screening of the negative charge at the membrane surface in a number of preparations (Watkins & Mathie, 1994;

Talukder & Harrison, 1995; Davidson & Kehl, 1995). In snail neurons, this theory appears to have some validity, but it has some shortcomings. Upon application of di- and trivalent ions and changes in $[Ca]_o$, the steady-state inactivation curves shifted in parallel fashions as expected according to this theory. However upon detailed investigation, all the gating parameters such as the time constant of activation are predicted to move equally on the voltage axis without causing any other changes according to the Hodgkin and Huxley model. But this was not the case; both in the case of decreased $[Ca]_o$ to 2 mM and in Ca^{2+}-free Ringer, the activation and the inactivation processes speeded up dramatically (Fig. 1). Secondly and more importantly, decreasing the ionic strength clearly does not have any effect on the gating of the transient potassium outward current channels. But according to the model, lowering extracellular ionic strength should, in fact, change the gating parameters of the transient potassium current. Taken together, the modulation of the gating parameters by $[Ca]_o$ and by the inorganic Ca^{2+} channel blockers can not easily and exclusively be attributed to the simple surface potential theory.

One of the important findings was that various cations have different abilities to modulate the transient potassium current. For example, 10 mM $CoCl_2$ substitution for 10 mM $CaCl_2$ resulted in depolarizing shift of the steady-state inactivation and activation curves by 4.2 and 8 mV, respectively. They were equal in concentration and they have equal charges. Another example was that in low calcium concentration (1 mM) and in the presence of amiloride and nifedipine, application of La^{3+} (200 μM) or Ni^{2+} (4 mM) and Cd^{2+} (200 μM) caused the steady-state inactivation curves to shift by about 27 and 23 mV in a depolarizing direction, respectively. These cations not only cancelled out the hyperpolarizing shift of the steady-state activation curves that were exerted when $[Ca]_o$ was reduced from 10 to 1 mM, but also caused further depolarizing shifts. These shifts are very large and seem to be much larger than the surface potential theory could account for. For example, the addition of La^{3+} (200 μM) can not account for the reversal of the effect of removing calcium (9 mM) if it were working by a surface charge effect.

Therefore these results appear to favor an alternative mechanism suggested by Mayer and Sagiyama (1988). This model involves a direct interaction between di- and trivalent cations and the gating particles of the channel protein.

Whatever else, many of the events described in this paper occur at voltages close to the threshold for action potential firing. There is thus the possibility that changes in calcium concentration close to the soma, perhaps brought about by neighboring glial cells, can modify the firing properties of these neurones.

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