Calcium-dependent chloride current induced by axotomy in rat sympathetic neurons

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1. Seven to ten days after sectioning their axons, rat sympathetic neurons were studied using intracellular recording techniques in an *in vitro* preparation of the superior cervical ganglion.

2. In 75 % of axotomized cells, an after-depolarization (ADP) was observed following spike firing or depolarization with intracellular current pulses. Discontinuous single-electrode voltage-clamp techniques were employed to study the ADP. When the membrane potential was clamped at the resting level just after an action potential, a slow inward current was recorded in cells that showed an ADP.

3. In the presence of TTX and TEA, inward peaks and outward currents were recorded during depolarizing voltage jumps, followed by slowly decaying inward tail currents accompanied by large increases in membrane conductance. The inward peak and tail currents activated between −10 and −20 mV and reached maximum amplitudes around 0 mV. With depolarizing jumps to between +40 and +50 mV, net outward currents were recorded during the depolarizing jumps but inward tail currents were still activated.

4. In the presence of the Ca^{2+} channel blocker cadmium, or when Ca^{2+} was substituted by Mg^{2+}, the ADP disappeared. In voltage-clamped cells, cadmium blocked the inward tail currents. The reversal potential for the inward tail current was approximately −15 mV. Substitution of the extracellular NaCl by sucrose or sodium isethionate increased the amplitude of the inward tail current, and displaced its equilibrium potential to more positive values. Changes in extracellular $[K^+]$ did not appreciably affect the inward tail current amplitude or equilibrium potential. Niflumic acid, a blocker of chloride channels activated by Ca^{2+}, almost completely blocked the tail current.

5. No ADPs were observed in non-axotomized neurons, and when depolarizing pulses were applied while in voltage clamp no inward tail currents were evoked in these normal cells.

6. It is concluded that axotomy of sympathetic ganglion cells produces the appearance of a Ca^{2+}-dependent chloride current responsible for the ADP observed following spike firing.

Axotomy provokes several changes in the electrical properties of adult neurons indicating their potential for functional plasticity (see review by Titmus & Faber, 1990). In axotomized rat sympathetic ganglion cells, one or several spikes trigger an after-depolarization (ADP), instead of the after-hyperpolarization (AHP) invariably observed in control cells (Sánchez-Vives & Gallego, 1993*a*). This suggests that a new membrane conductance appeared after axotomy at or near the cell body, where the recording electrode was located. The purpose of the present work is to identify the conductance mechanism responsible for the ADP of axotomized ganglion cells. In normal rat sympathetic ganglion cells, calcium entry during the action potential activates calcium-dependent potassium currents that produce the long AHP typical of these neurons (McAfee & Yarowsky, 1979; Belluzzi & Sacchi, 1990). On the other hand, Cl^- (Owen, Segal & Barker, 1984; Mayer, 1985; Bader, Bertrand, & Schlichter, 1987; Akasu, Nishimura & Tokimasa, 1990; reviewed by Mayer, Owen & Barker, 1990) or cation currents (Partridge & Swandulla, 1988; Hasuo, Phelan, Twery & Gallagher, 1990) activated by calcium produce slow depolarizing after-potentials in several neuron types, but they have not been described in sympathetic ganglion cells. We have found that the ADP induced by axotomy is due to the activation of a Ca^{2+}-dependent Cl^- current. Part of this work has been presented in abstract form (Sánchez-Vives & Gallego, 1993*b*).

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METHODS

The methods used have been previously described (Sánchez-Vives & Gallego, 1993*a*). Briefly, 4- to 6-week-old Sprague–Dawley rats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, I.P.) and the main postganglionic branches of the right superior cervical ganglion were cut with aseptic precautions. Seven to ten days later, the animals were deeply anaesthetized and perfused through the heart with cold saline. The ganglion was taken from the rat and pinned to the Sylgard bottom of a chamber that was continuously superfused with saline (mM: NaCl, 128; KCl, 5; $CaCl_2$, 2·5; $MgCl_2$, 1; NaH_2PO_4, 1; $NaHCO_3$, 16; glucose, 5·5) equilibrated with 95% O_2–5% CO_2 (pH 7·4) at room temperature (22–25 °C). The low chloride solutions were made by replacing NaCl with either 256 mM sucrose or 128 mM sodium isethionate. The bath was grounded through an agar–KCl bridge. Potentials arising between the microelectrode and the control solution, and between this solution and the test solutions, were measured with respect to an Ag–AgCl electrode connected to the bath through an agar–KCl bridge. These potentials were usually smaller than 10 mV and were taken into account when plotting *I–V* curves. However, part of the changes in junction potentials developed at the tip of the microelectrode and were probably absent when it was inside the cell (e.g. Alvarez–Leefmans, Garmiño, Giraldez, & Noguerón, 1988). Therefore the values for the membrane potential while in the test solutions have to be taken only as approximate, especially when NaCl was substituted by sucrose, because microelectrode tip potentials are sensitive to changes in ionic strength of the solution (e.g. Agin & Holtzman, 1966).

Cells were impaled with microelectrodes filled with 3 M KCl or 3 M CsCl (60–100 MΩ) and data were collected only if the cell generated action potentials of at least 60 mV in amplitude. We considered that the neuron was axotomized if the synaptic potential evoked by supramaximal stimulation of the preganglionic trunk was smaller than 20 mV and the AHP shorter than 100 ms (Sánchez-Vives & Gallego, 1993*a*). It is unlikely that with these criteria non-axotomized cells were included in our sample, but we cannot exclude the possibility that some of the neurons that did not show ADPs (see below) were normal cells. The sampling frequency for discontinuous single-electrode voltage clamp was 4–8 kHz with a duty cycle of 30/70. Capacity compensation was continuously monitored and adjusted to ensure headstage settling. After filtering at 3 kHz, data were recorded on tape, digitized and stored in a computer for subsequent analysis using commercial software (Cambridge Electronic Design Limited, Cambridge, UK).

Because rat sympathetic ganglion cells have dendrites, it is possible that the voltage was not completely controlled in all the membrane, especially during large depolarizing jumps. However, most of our analysis is of slow tail currents and should be less affected by this problem. Likewise, the temporal limitation of the single electrode clamp at the frequencies used, does not impede the study of slow currents like the one underlying the ADP.

RESULTS

After-depolarization in axotomized cells

In thirteen out of thirty-three axotomized cells the action potential was followed by an ADP (Fig. 1*A a*) which was never observed in control cells (Sánchez-Vives & Gallego, 1993*a*). During the ADP there was a marked increase in input conductance, as demonstrated by the decreased voltage response to small hyperpolarizing current pulses (not shown).

In 60% of the axotomized cells in which the action potential was followed by an AHP, after-depolarizations were evoked by trains of spikes (Sánchez-Vives & Gallego, 1993*a*). In these cells, the AHP was replaced by an ADP if the action potential was evoked shortly after another action potential or after a train of spikes (Fig. 1*B b*). Because the conductance change generating the ADP is calcium dependent (see below), this result suggests that calcium concentration is elevated for several hundred milliseconds following spike firing, thus allowing the activation of the ADP conductance by the entry of calcium during the second action potential. In fact, it has been shown that the recovery of basal calcium levels after electrical activity is slowed in axotomized sympathetic neurons (Sánchez-Vives, Valdeolmillos & Gallego, 1993).

We have previously shown that the ADPs recorded in axotomized neurons with KCl-filled microelectrodes reached an average peak potential of −43 mV (Sánchez-Vives & Gallego, 1993*a*). Although in that work, and in the present experiments, we used high-resistance microelectrodes, it is probable that there was some leakage of Cl^- into the cells. Therefore, in eleven axotomized cells from two ganglia, we measured the amplitude of the ADPs that followed single action potentials using microelectrodes filled with 3 M potassium acetate. Under this condition, the ADP ranged from 1 to 12 mV, with a mean value of 5 mV (S.D. = 3·2). These neurons had an average resting potential of −55 mV; therefore the absolute potential reached during the ADP was −50 mV. This result suggests that the equilibrium potential for the conductance responsible for the ADP was displaced towards positive values by the Cl^- leaked from the KCl-filled microelectrodes.

Voltage clamp of the after-depolarization

In axotomized cells showing a marked ADP it was possible to record the current underlying this after-potential by electronically switching to voltage-clamp mode at the end of the spike repolarization (Fig. 1*A*). An inward current with slow activation and decay was observed (Fig. 1*A b*), but the analysis was complicated by the small amplitude of this current and the simultaneous activation of calcium-dependent K^+ currents (Sánchez-Vives & Gallego, 1993*a*). Therefore, TTX (1 μM) and TEA (10–20 mM) were added to the control solution to block sodium and potassium currents. Also, in most instances we used microelectrodes filled with 3 M CsCl. Under these conditions, depolarizing pulses while in current clamp evoked in most axotomized cells regenerative responses followed by long ADPs (Fig. 2*Aa*). In voltage clamp, inward peaks and large outward currents were recorded during voltage jumps from

−50 or −60 mV to near 0 mV, followed by slowly decaying inward tail currents (Fig. 2*Ab*). The tail current increased as the duration of the depolarizing pulse increased, reaching a maximum amplitude at about 400 ms (Fig. 2*B*). As shown below, the tail current appears to be a calcium-dependent chloride current and will be referred to as I_{ADP}. We have used depolarizing voltage jumps with durations from 50 to 500 ms to evoke the I_{ADP}.

Figure 3*A* shows examples of the currents evoked in one cell by depolarizing voltage jumps of increasing amplitude. The current–voltage plots for the peak inward current during the depolarizing jump (Fig. 3*B*) and for the peak tail I_{ADP} as a function of the membrane potential during the pulse (Fig. 3*C*) indicate that both currents activated (between −20 and −10 mV) and reached maximum values at similar voltages (about 0 mV), but depolarizing jumps positive to +20 mV produced a net outward current during the pulse whereas at that voltage the tail current was still inward. This indicates activation of an outward current at membrane potentials positive to 0 mV during the pulse. No TTX-resistant sodium currents have been found in rat superior cervical ganglion cells (Belluzzi & Sacchi, 1986; Schofield & Ikeda, 1989), therefore these results suggest that the inward current is carried by calcium and that the calcium entry triggers the I_{ADP}, which appears as an outward current during the pulse and as an inward tail current upon repolarization (see also Owen *et al.* 1984; Mayer, 1985; Akasu *et al.* 1990).

Calcium dependence of the I_{ADP}

When the inorganic calcium channel blocker cadmium was applied to the bath (1 mM, $n = 4$) a near complete suppression of the I_{ADP} (Fig. 4), as well as of the inward current during the pulse, was observed. While in current clamp, the ADP observed after the action potential or evoked by current pulses or spike trains disappeared when cadmium was applied ($n = 4$) or when calcium was substituted by magnesium ($n = 9$).

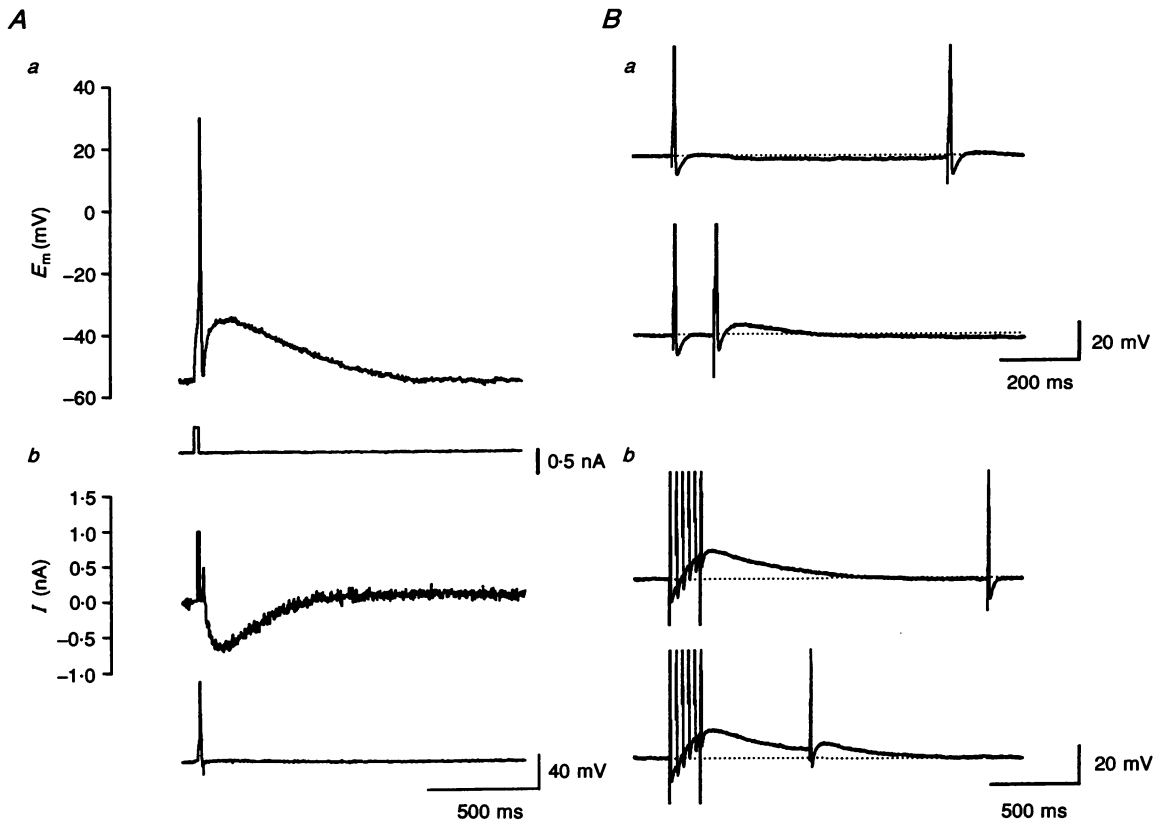

Figure 1. After-depolarizations recorded in axotomized ganglion cells
A a, the action potential (upper trace) evoked by an intracellular current pulse (lower trace) is followed by an ADP. E_m, membrane potential. *A b*, in the same cell, the membrane potential (lower trace) was clamped at the resting level at the end of the repolarization phase of an action potential evoked by an intracellular current pulse. An inward current was recorded (upper trace) which corresponds to the ADP. *Ba*, the action potential evoked by a short intracellular current pulse was not followed by an ADP in this neuron (upper trace), but a second spike generated shortly after the first one was followed by a clear ADP (lower trace). Notice the slow AHP in both traces. *B b*, in the same cell, a depolarizing potential built up during repetitive stimulation. An action potential evoked 600 ms after the train was followed by an ADP. The dotted line corresponds to the resting membrane potential (−51 mV).

Conductance change and reversal potential for the I_{ADP}

Changes in membrane conductance during the I_{ADP} were measured using hyperpolarizing step commands of 20 ms superimposed on the voltage protocol employed to activate the I_{ADP} (Mayer, 1985). As seen in Fig. 5*A*, membrane conductance was markedly increased during the I_{ADP}; for three cells the mean conductance increase at the peak of the tail current was 71 nS. An approximately linear relationship was observed between the conductance change and the amplitude of the I_{ADP} (Fig. 5*B*), although the points deviated from linearity during the first 100 ms. This could be due to contamination with other currents activated by the depolarizing jump, like the M-current, or to incomplete block of other K^+ currents.

The reversal potential of the I_{ADP} was estimated in twelve cells with a two-pulse protocol, as shown in Fig. 6*A*. The current–voltage relation for the current at the peak of the tail current was linear in the range −100 to −50 mV (Fig. 6*B*), giving extrapolated reversal potentials between −2 and −32 mV, with an average of −16 mV (S.D., 8 mV), which is similar to that found in parasympathetic ganglion cells of the rabbit impaled with Cl^--filled microelectrodes (Akasu *et al.* 1990). A direct assessment of the tail current reversal potential was not possible because it lies within the potential range for activation of the conductance, but in other neurons this extrapolation method gives values which agree with those obtained with more direct measurements (Mayer, 1985; Akasu *et al.* 1990).

The reversal potential for the I_{ADP} determined in solution without TTX and TEA was -25 ± 6 mV ($n = 5$), which indicates that in this situation potassium currents and the I_{ADP} were activated simultaneously, shifting the equilibrium potential towards more hyperpolarized values.

Ionic dependence of the I_{ADP}

In other neurons, inward calcium-dependent tail currents are carried by chloride ions (Mayer, 1985; Owen, Segal & Barker, 1986; Bader *et al.* 1987; Akasu *et al.* 1990). To test

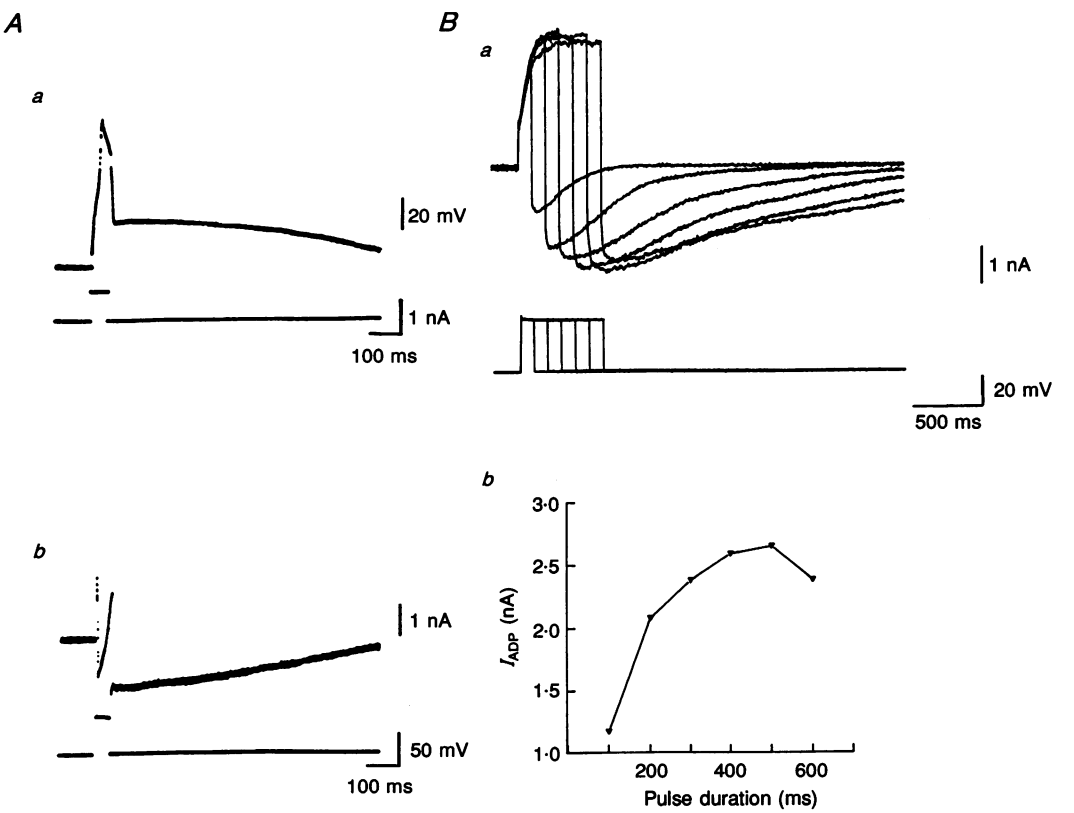

Figure 2. Inward currents triggered by depolarizing voltage jumps in axotomized neurons
A a, in the presence of TTX and TEA a depolarizing current pulse (lower trace) generated a slow action potential followed by a prolonged ADP. *A b*, when the membrane potential of the same cell was clamped at −60 mV, a depolarizing jump to 0 mV (lower trace) evoked an inward current followed by an outward relaxation and a slowly decaying inward tail current (upper trace, leak current has not been subtracted). *Ba*, in another cell, depolarizing jumps of increasing duration, to −10 mV from a holding potential of −50 mV, generated progressively larger and longer inward tail currents (leak currents were not subtracted and were large enough to impede the observation of the inward peak at the beginning of the pulse). *Bb*, relationship between pulse duration and maximum amplitude of the inward tail current (I_{ADP}) for the cell shown in *Ba*. In these and subsequent voltage-clamp records the bathing solution contained TTX and TEA.

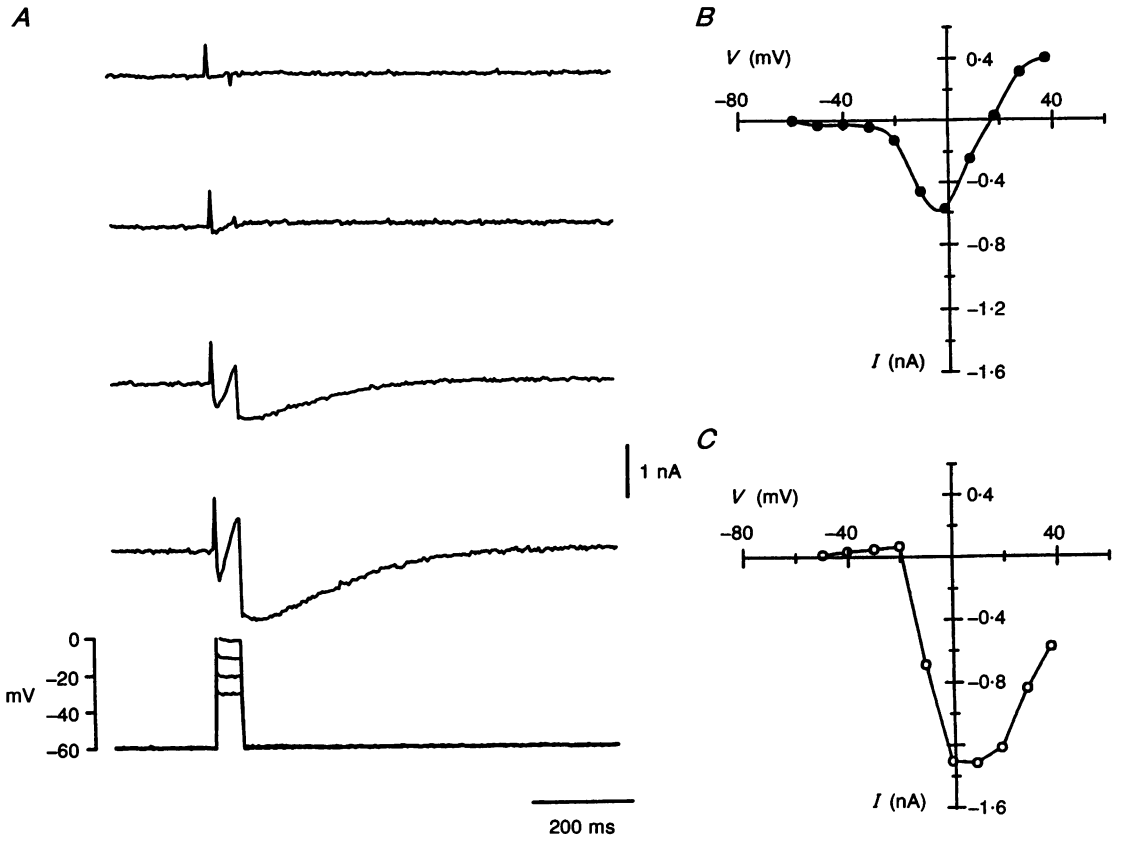

Figure 3. Current–voltage and tail current availability relationships
A, responses recorded during and after voltage jumps from a holding potential of −60 mV. The two larger pulses evoked inward peaks followed by slowly developing outward currents and on repolarization to −60 mV slow inward tail currents were recorded (leak currents were subtracted from all records). *B*, *I–V* plot for the inward current measured 15 ms after the beginning of the pulse. *C*, plot of the tail current amplitude measured 35 ms after repolarization to the holding potential of −60 mV, following voltage jumps to the values indicated in the voltage axis.

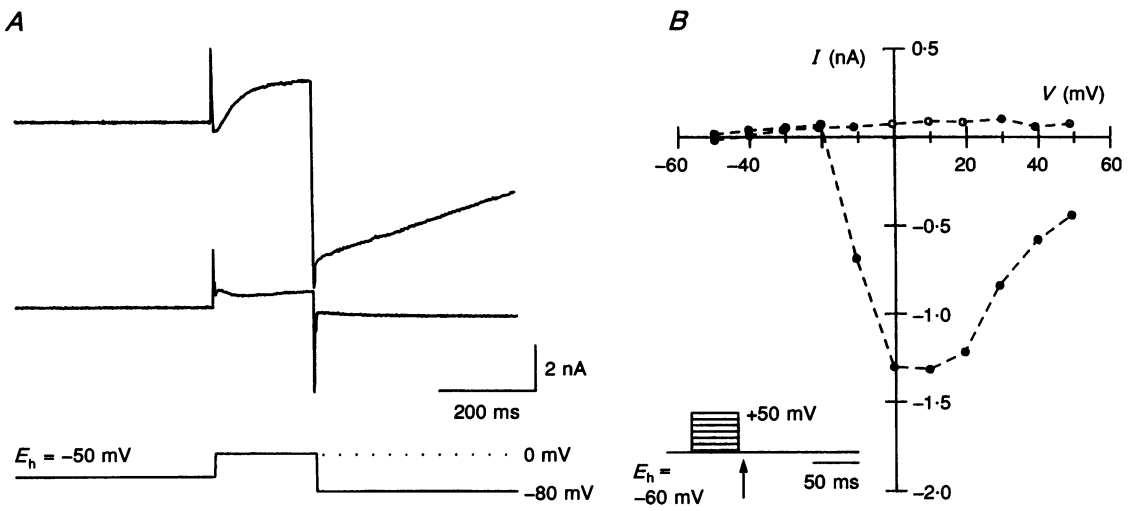

Figure 4. Effect of 1 mM cadmium on the I_{ADP}
A, currents recorded in control solution (upper trace) and in solution with 1 mM cadmium (middle trace), in response to the pulse protocol shown in the lower trace. The membrane potential was repolarized to −80 mV after the pulse to increase the amplitude of the I_{ADP} (leak currents have been subtracted). *B*, different cell, *I–V* relationships for the tail currents evoked by the protocol shown in the inset, in control solution (●) and in solution with 1 mM cadmium (○). The I_{ADP} was completely blocked in the cadmium solution. E_h, holding potential.

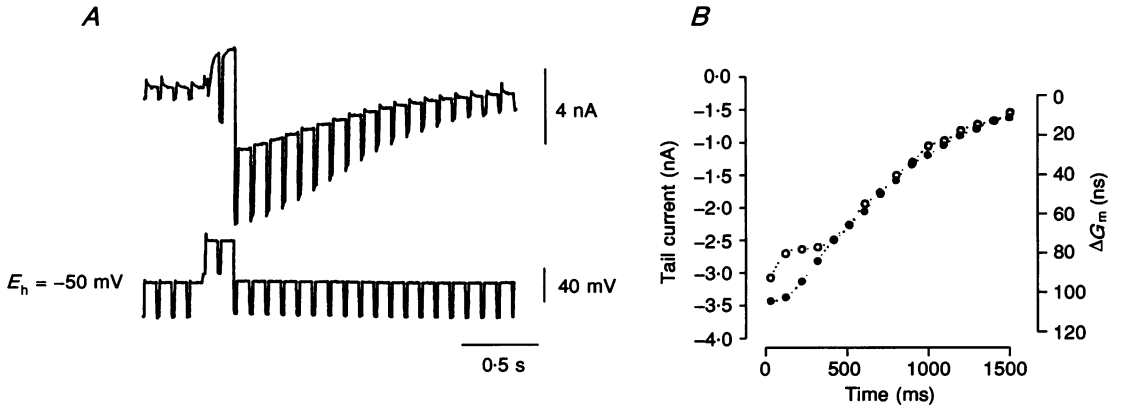

Figure 5. Membrane conductance (G_m) increase during the I_{ADP}
A, the inward tail current was evoked by a depolarizing jump to 0 mV and hyperpolarizing command pulses (40 mV, 20 ms) were superimposed at a rate of 10 Hz. A marked increase in membrane conductance is observed during and after the pulse. *B*, tail current amplitude (●) and increase in membrane conductance (○) plotted against time following repolarization to −50 mV.

for this possibility we decreased the chloride concentration of the bathing medium and estimated the change in the reversal potential of the I_{ADP} using a pulse protocol similar to that shown in Fig. 6. When the NaCl of the solution was substituted by sucrose (final chloride concentration 22 mM) the amplitude of the I_{ADP} increased markedly (Fig. 7*A*) and the reversal potential shifted to more depolarized values (Fig. 7*B*; +44 and +55 mV in two cells). Similar results were obtained by substituting the NaCl with sodium isethionate (Fig. 7*B*), although in this case the increase in amplitude was generally smaller and the reversal potential shifted to +11 ± 5 mV ($n = 4$). The Nernst equation predicts a reversal potential of +33 mV in the low Cl^- solution. These discrepancies could be due to partial permeability of chloride channels to other ions (Hussy, 1992) or to isethionate (Schlichter, Grygorczyk, Pahapill & Grygorczyk, 1990) and to uncertainties on the assessment of junction potentials, especially in the sucrose solution (see Methods). Nevertheless, the results agree with the I_{ADP} being carried by Cl^- ions.

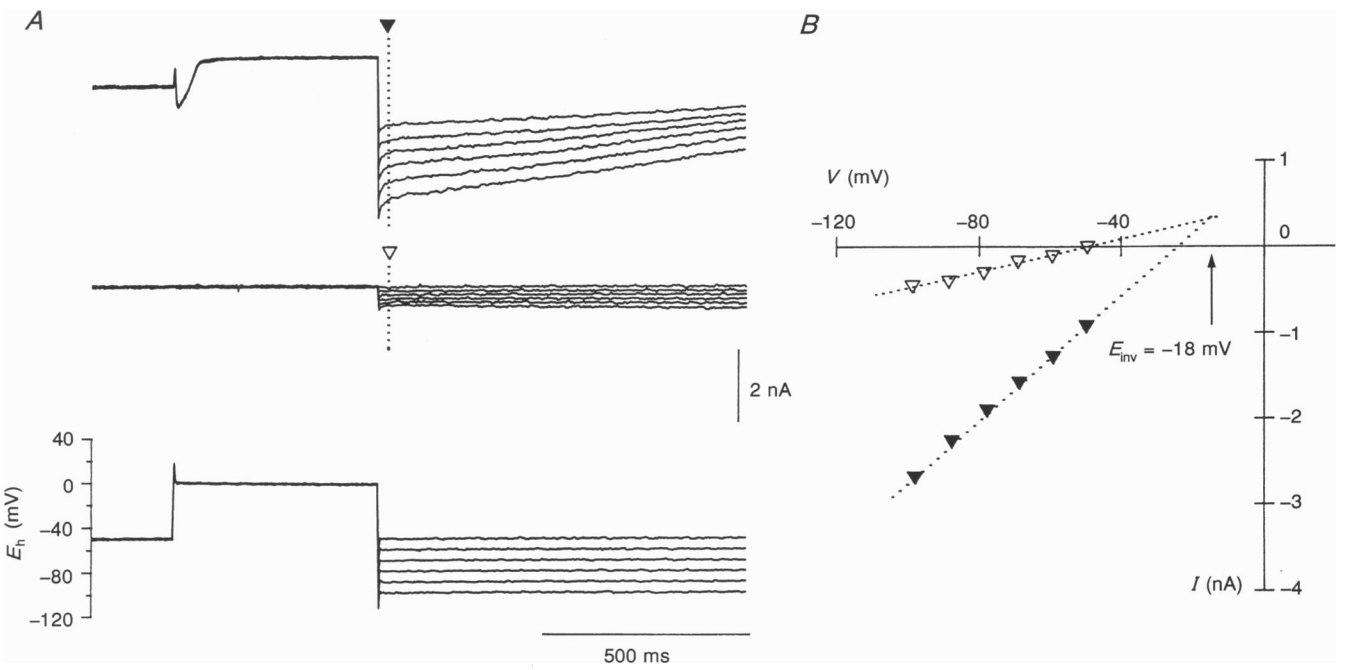

Figure 6. Extrapolated reversal potential for the I_{ADP}
A, inward tail currents (upper trace) were evoked by depolarizing jumps to 0 mV and the membrane was repolarized to different potentials (lower trace). When no depolarizing pulse was applied no tail currents were observed (middle trace). *B*, *I–V* relationships for the tail current measured at the points marked in *A*. The plots are linear and the intersection corresponds to the extrapolated reversal potential (E_{inv}) of −18 mV.

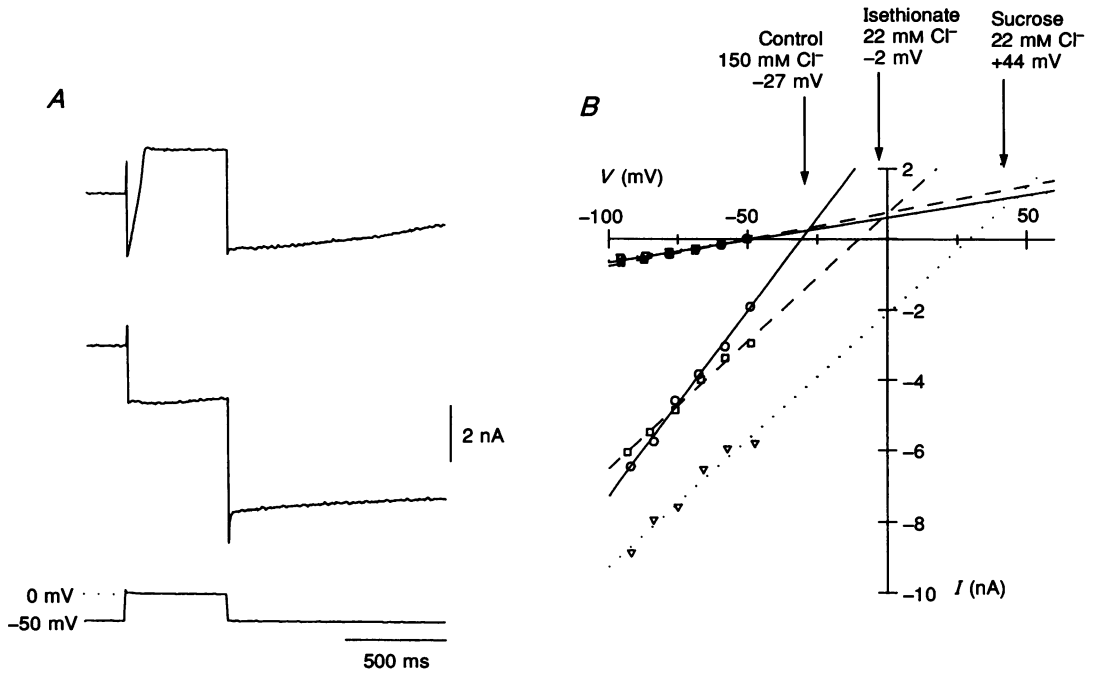

Figure 7. Effect of low Cl^- solution on the I_{ADP}
A, currents evoked in control solution (upper trace) and when the NaCl was substituted by sucrose (middle trace) by the protocol shown in the lower trace. In sucrose the current was inward during the pulse to 0 mV because the chloride reversal potential (E_{Cl}) was positive, and the amplitude of the tail current increased markedly (leak currents have been subtracted). *B*, in the same cell, the reversal potential for the I_{ADP} was estimated with the pulse protocol shown in Fig. 6 in control solution (○), when the NaCl was substituted by sodium isethionate (□), and when the NaCl was substituted by sucrose (△). The final Cl^- concentration and the extrapolated reversal potentials are indicated in the figure.

The shift of the reversal potential in a depolarizing direction and the increased amplitude of the I_{ADP} when NaCl was substituted by sucrose suggest that the current is not carried by cations as in other calcium-dependent conductances (Partridge & Swandulla, 1988). This is supported by the lack of effect of high (10 mM) or low (2·5 mM) potassium concentrations on the amplitude or reversal potential of the I_{ADP} ($n = 2$, data not shown).

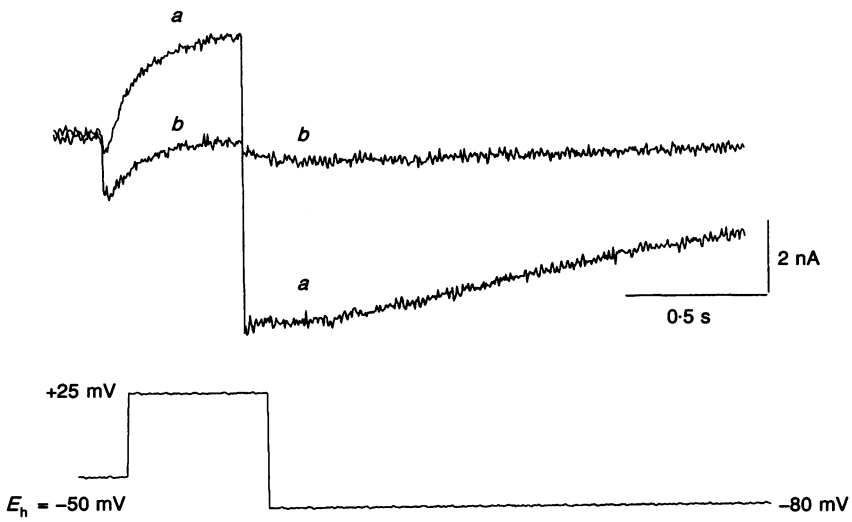

Figure 8. Block by niflumic acid of the I_{ADP}
The pulse protocol shown in the lower trace was applied and the currents recorded in control solution (*a*) and in a solution with 125 μM niflumic acid (*b*). The outward relaxation during the pulse and the inward tail current were almost completely blocked by the drug (leak currents have been subtracted).

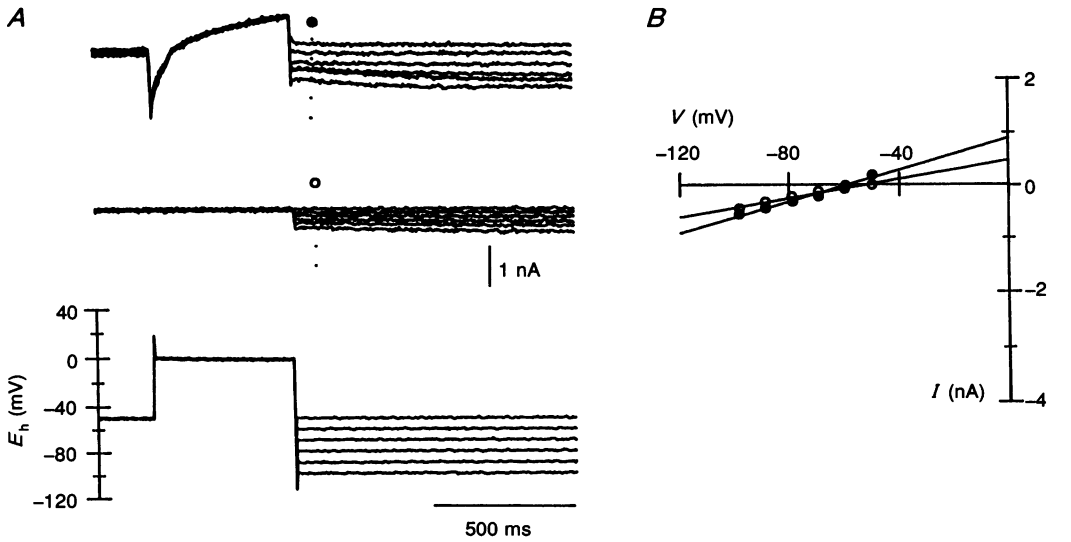

Figure 9. Lack of inward tail currents in a non-axotomized ganglion cell
A, the pulse protocol shown in the lower trace was used in an attempt to evoke the I_{ADP}. The currents recorded during the repolarization to different potentials were similar whether the depolarizing pulse was applied (upper trace) or not (middle trace). Leak currents have not been subtracted. *B*, *I–V* relationships for the currents measured at the points marked in *A*. There were probably small residual K^+ currents not blocked by the TEA, but the inward tail currents were absent. Compare with Fig. 6.

Furthermore, the ADP recorded after an action potential while in current clamp increased in amplitude when NaCl was substituted by sucrose ($n = 4$, data not shown).

Effect of niflumic acid on the I_{ADP}

Niflumic acid blocks calcium-activated chloride currents in *Xenopus* oocytes by acting on the anion channel (White & Aylwin, 1990). Figure 8 shows that bath application of 125 μM niflumic acid almost completely blocked the tail current ($n = 3$). Another blocker of calcium-activated chloride currents, flufenamic acid (White & Aylwin, 1990), was applied in one case (100 μM) producing a 50 % decrease in the current. Ethanol (1/1000, v/v), used to dissolve these channel blockers, did not affect the I_{ADP}.

Absence of depolarizing potentials in non-axotomized cells

In thirty-seven non-axotomized cells from unoperated ganglia we did not observe ADPs following a single spike or a train of action potentials, nor were we able to evoke depolarizing potentials with large current pulses (Sánchez-Vives & Gallego, 1993*a*). Even in the presence of apamin (20 nM, $n = 4$), which blocks the slow AHP in these cells (Kawai & Watanabe, 1986), no ADPs were revealed by trains of spikes or depolarizing pulses (not shown).

We tried to evoke the I_{ADP} in normal cells ($n = 5$) in the same conditions that we used to study the current in axotomized neurons: in the presence of TTX and TEA to block sodium and potassium currents. Figure 9 shows the lack of inward tail currents following a depolarizing pulse in a control cell (compare with Fig. 6). Even in low-chloride (22 mM) solution, similar pulse protocols failed to evoke inward tail currents in control cells.

DISCUSSION

We have characterized a calcium-activated chloride current in axotomized rat sympathetic ganglion cells. This current shares features with calcium-dependent chloride currents described in other neurons (Owen *et al.* 1984; Mayer, 1985; Owen *et al.* 1986; Bader *et al.* 1987; Akasu *et al.* 1990; Mayer *et al.* 1990), but is observed only after axonal section, underlying the spike after-depolarization present in these axotomized neurons (Sánchez-Vives & Gallego, 1993*a*).

Non-axotomized rat sympathetic ganglion cells do not show ADPs (McAfee & Yarowsky, 1979; Kawai & Watanabe, 1986; Kawai & Watanabe, 1989; Sánchez-Vives & Gallego, 1993*a*), which is in accordance with the lack of inward tail currents in control cells bathed in TTX and TEA, even after increasing the current driving force by lowering the external Cl^- concentration. Therefore, as discussed by Sánchez-Vives & Gallego (1993*a*), a new conductance appears at or near the cell body after axotomy. It is not known whether this reflects redistribution of ion channels present in other parts of the neuron (i.e. distal dendrites), incorporation of new channels or changes in the properties of channels existing in the normal cell.

The results show that a calcium-dependent chloride current is the main current responsible for the ADP in axotomized sympathetic neurons. It is unlikely that Ca^{2+}-activated cation currents (Partridge & Swandulla, 1988) play a significant role in the ADP, because the reversal potential became more depolarized when NaCl was substituted by sucrose and changes in external K^+ did not significantly affect the I_{ADP}. The properties of the I_{ADP} are

similar to those of Ca^{2+}-activated Cl^- currents described in other neurons (Owen *et al.* 1984; Mayer, 1985; Owen *et al.* 1986; Bader *et al.* 1987; Akasu *et al.* 1990; Mayer *et al.* 1990), namely slow activation on depolarization, dependence on Ca^{2+}, sustained activation at depolarized potentials and slow turn-off on repolarization evidenced by the prolonged time course of the tail current. The ADP that follows one or several action potentials in axotomized ganglion cells shares these characteristics, including the production of plateau-like responses of several hundred milliseconds when TTX and TEA are added to the bathing solution (Sánchez-Vives, 1992).

The finding that the ADP reaches more positive values when the microelectrodes are filled with KCl than when potassium acetate is used, suggests that the intracellular Cl^- concentration is increased by leakage from the microelectrode. Therefore, the calculated chloride equilibrium potential (E_{Cl}) is probably more positive than that corresponding to the intact cell. In rat sympathetic ganglion cells, chloride equilibrium potentials positive to the resting membrane level have been calculated on the basis of intracellular chloride concentration measurements with ion-sensitive microelectrodes ($E_{Cl} = -27$ mV; Ballanyi, Grafe, Reddy & ten Bruggencate, 1984) or with an electron microprobe ($E_{Cl} = -34$ mV; Galvan, Dörge, Beck & Rick, 1984). Therefore, if the intracellular chloride concentration does not decrease markedly after axotomy, activation of the I_{ADP} should produce depolarization of the cell membrane. Our data obtained with potassium acetate electrodes indicate that this is probably the case when cells fire one or several spikes *in vivo*.

The physiological significance of the ADP of axotomized ganglion cells is unknown. During the ADP the cells did not fire repetitively (Sánchez-Vives, 1992), possibly due to inactivation of sodium currents and to the large increase in membrane conductance produced by the simultaneous activation of calcium-dependent Cl^- and K^+ currents. Thus, it is unlikely that the ADP increases the firing rate of the neurons. Rather it will contribute to the depressing effect of the calcium-activated K^+ currents. We have suggested that the channels responsible for the ADP may be present in non-axotomized cells but located on the dendrites, far away from the cell body (Sánchez-Vives & Gallego, 1993*a*). If this is the case and if local calcium entry is produced by synaptic activity, the Cl^- current could help to maintain Ca^{2+} entry by avoiding collapse of transmembrane potential, as proposed in non-excitable cells (Penner, Matthews & Neher, 1988). In agreement with this idea, the recovery of basal intracellular calcium levels increased by electrical activity is markedly slowed in axotomized sympathetic ganglion cells (Sánchez-Vives *et al.* 1993).

Finally, the main function of the calcium-activated Cl^- conductance described in this work may not be related to electrical signalling. Chloride channels participate in the volume recovery response which follows osmotically induced increases in cell volume, although the role of calcium in activating such channels is controversial (Hoffmann & Simonsen, 1989; Sarkadi & Parker, 1991; McCarty & O'Neil, 1992). If, as some reports indicate (see review by Lieberman, 1971), there is a phase of cell swelling after axotomy, the channels responsible for the I_{ADP} could be involved in the cell's response to recover its normal volume. In this view, the possible location of the channels in the dendrites of normal cells would be related to the more stringent conditions for regulation of cell volume derived from the larger surface-to-volume ratio.

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