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VOLTAGE-DEPENDENT CALCIUM AND POTASSIUM CHANNELS IN SCHWANN CELLS CULTURED FROM DORSAL ROOT GANGLIA OF THE MOUSE

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SUMMARY

1. Whole-cell patch clamp studies were carried out on Schwann cells in organotypic cultures of dorsal root ganglia (DRG) from OF1 mice embryos (18–19 days).

2. In standard external solution, from a holding potential of −70 mV, two types of voltage-dependent K^+ currents were recorded: a fast transient current and a delayed sustained current. With a holding potential of −30 mV, only the delayed sustained current could be evoked.

3. Both K^+ currents were inhibited by tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) in a dose-dependent manner. For the transient current the half-maximal effective dose was 100 mM for TEA and 1·3 mM for 4-AP. For the delayed sustained current the half-maximal effective dose was 11 mM for TEA and 4 mM for 4-AP. Both currents were insensitive to external Ca^{2+}.

4. The delayed sustained current, isolated by use of a holding potential of −30 mV displayed a 'cumulative inactivation' which was removed by hyperpolarizing the membrane to −70 mV between each test pulse.

5. In K^+-free external and pipette solutions, with 10 mM-external Ca^{2+}, from a holding potential of −70 mV voltage-dependent Ca^{2+} channel currents were recorded. The threshold for activation was $-45{\cdot}3 \pm 5{\cdot}4$ mV (mean ± S.D., $n = 5$) and the current inactivated fully at the end of the test potential. The current was unaffected by 2 μM-tetrodotoxin (TTX) and totally blocked by 5 mM-Co^{2+}.

6. Equimolar replacement of external Ca^{2+} by Ba^{2+} did not significantly modify the voltage dependence (threshold for activation $-42{\cdot}8 \pm 6{\cdot}4$ mV, $n = 7$) or the magnitude of the inward current. Ca^{2+} and Ba^{2+} were equally permeant. The fully inactivating current was insensitive to both nifedipine and Bay K 8644 (1 μM each). Increasing the external Ba^{2+} concentration from 10 to 89 mM enhanced the Ba^{2+} current and shifted the voltage dependence of the current (threshold for activation, $-30{\cdot}5 \pm 7{\cdot}3$ mV, $n = 9$) along the voltage axis as expected for altered external surface potential.

7. In 89 mM-external Ba^{2+} solution, some cells displayed an additional slowly decaying current which was totally blocked by nifedipine (1 μM).

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8. Ca^{2+} channel currents were recorded only when DRG neurons were present in the culture, as excision of explants and subsequent axonal degeneration led to loss of detectable Ca^{2+} channel currents. This phenomenon was never observed for K^+ currents.

9. We conclude that mouse Schwann cells in organotypic culture possess voltage-dependent K^+ and Ca^{2+} channels. The fast transient K^+ current is similar to 'A' currents described in numerous neuronal cells. The delayed sustained current is similar to the delayed rectifier K^+ current widely distributed among excitable cells. Two types of Ca^{2+} channel currents are present on mouse Schwann cells: a fully inactivating current which resembles the T-type current more particularly described in neuronal cells and a slowly decaying current similar to the L-type current. DRG neurons appear necessary to the expression of functional Ca^{2+} channels in mouse Schwann cells.

INTRODUCTION

Schwann cells ensheath axons with myelin in the peripheral nervous system (PNS) and thereby increase conduction velocity. Until the end of the 1970s, like glial cells of the central nervous system (astrocytes and oligodendrocytes), Schwann cells were thought to be electrically passive with no detectable sign of excitability. The introduction of the patch clamp technique (Neher & Sakmann, 1976) by allowing voltage clamp recording on small cells has permitted demonstration of voltage-dependent Na^+ and K^+ channels in cultured Schwann cells from rabbit (Chiu, Shrager & Ritchie, 1984). Since then, numerous studies on cultured mammalian Schwann cells have demonstrated the presence of a variety of voltage-dependent ionic channels, i.e. Na^+ channels (Schrager, Chiu & Ritchie, 1985; Chiu, 1987, 1988), K^+ channels (Schrager *et al.* 1985; Howe & Ritchie, 1989; Konishi, 1989*a*, *b*) and Cl^- channels (Howe & Ritchie, 1989). If rabbit and rat Schwann cells display Na^+ and K^+ channels, up to now, only K^+ channels have been reported on mouse Schwann cells. Cl^- channels were recorded in rabbit Schwann cells. From all these studies, it can be seen that cultured mammalian Schwann cells express several different ionic channels, and that expression is dependent on the animal species. The physiological relevance of such channels, which are similar in many respects to those found in neuronal cells, remains unclear mainly because at the estimated resting membrane potential recorded in different vertebrate Schwann cells (-30 to -50 mV, Gray & Ritchie, 1985) most of these channels would be largely inactivated. For voltage-dependent K^+ channels, Konishi (1989*a*) concluded that they appeared to be involved in early stages of mouse Schwann cell proliferation. A relationship between Schwann cell proliferation and expression of voltage-dependent K^+ channels was also reported for rabbit Schwann cells after nerve transection (Chiu & Wilson, 1989). Recently, Konishi (1990) described a new type of voltage-dependent K^+ channels only present in mouse neonatal myelinating Schwann cells which, he suggested, might be involved in K^+ siphoning. Concerning the Na^+ channels, an attractive hypothesis made by Shrager *et al.* (1985) suggests that Schwann cells could act as a local factory of Na^+ channels for the axolemma of the axons they ensheath. From all these studies it can be seen that our knowledge of the electrical properties of cultured mammalian Schwann cells is very much scattered. Nevertheless, if such channels exist *in vivo*, it

is possible that mammalian Schwann cells might have other functions than simply the myelinization of the axons. The aim of this study was to investigate the electrophysiological properties of Schwann cells in an organotypic culture of mouse dorsal root ganglia (DRG) using the whole-cell configuration of the patch clamp technique. We report for the first time that cultured mouse Schwann cells possess not only voltage-dependent K^+ channels, but also voltage-dependent Ca^{2+} channels. Expression of these voltage-dependent Ca^{2+} channels depends on the presence of DRG neurons in the culture.

METHODS

Schwann cell preparation

Schwann cells were cultured from aseptically excised DRG from OF1 mice embryos (day 18 to 19 of gestation). Mice were killed by decapitation. DRGs were dissected and plated onto Petri dishes, 35 mm in diameter, coated with a double layer of rat tail collagen. Schwann cells were cultured in modified Eagle's medium (MEM, Gibco) containing nerve growth factor (NGF, 100 ng ml^{-1}), 5-fluoro-2′-deoxyuridine (10 μM), uridine (10 μM) and fetal calf serum (FCS, 10% v/v. Gibco). After 3–5 days, the medium was replaced by a Dulbecco's MEM–Ham nutrient mixture F12 (1:1) serum-free medium as described by Bottenstein & Sato (1979). NGF concentration was reduced to 20 ng ml^{-1}. In this culture medium, Schwann cells do not synthesize myelin and fibroblasts are few and non-proliferating (Bottenstein & Sato, 1979). Cells were kept in an incubator gassed with 95% O_2–5% CO_2 at 37 °C. The medium was changed twice a week. In some experiments, after 1 or 2 weeks of culture, explants were aseptically excised. Subsequent axonal degeneration was complete after 3–4 days.

Schwann cell identification

Schwann cells in this study were identified by established morphological criteria that we confirmed by immunostaining (Fig. 1; for review see Varon & Manthorpe, 1982): (i) the cells had small and spindle-shaped bodies with an oval nucleus; (ii) they had a bipolar shape with narrow tapered processes at each end; (iii) they tended to join end to end to form linear chains and align along neurites; (iv) some cells were associated physically with neurites; (v) under phase-contrast observation, Schwann cells appeared highly birefringent while contaminating fibroblasts were weakly birefringent and were flat and multipolar. Schwann cells were also identified by immunostaining (Fig. 1*B*). The cultures were washed in physiological bathing solution, fixed for 1 h in 2% paraformaldehyde at 4 °C and treated for 2 min with Triton X-100 (0·1%). The cultures were then incubated for 24 h with rabbit antiserum to bovine S 100 protein (Dako) at a 1:80 dilution, for 1 h with linking antibody at a 1:50 dilution (goat antiserum to rabbit immunoglobulins, Biosys), and finally for 1 h with peroxydase–goat antiperoxydase complex (PAP, Dako). Immunoreactivity was visualized with 3,3′-diaminobenzidine (DAB, 0·05% (w/v) in 0·05 M-Tris-HCl) and 0·01% H_2O_2.

Electrophysiological recordings

Ionic currents were recorded between 1 day and 8 weeks in culture using the whole-cell configuration of the patch clamp technique (Hamill, Marty, Neher, Sakman & Sigworth, 1981) at room temperature (20–24 °C). In this study we used only Schwann cells aligned in the vicinity of the neurites. Cells were observed under a phase-contrast microscope (320×, Zeiss, Oberkochen, Germany). In standard conditions, patch pipettes had resistance between 5 and 10 MΩ and were filled with (mM): 120 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 11 ethyleneglycol-bis (β-aminoethylether)-*N,N′*-tetraacetic acid (EGTA), 11 glucose and 33 KOH (pH 7·4). In Ca^{2+} channel currents experiments, patch pipettes were filled with (mM): 120 CsCl, 2 $MgCl_2$, 10 HEPES, 11 EGTA, 11 glucose and 30 CsOH (pH 7·4). A continuous positive pressure was applied to the patch pipette and it was pressed to the membrane surface of the cell body. Then the patch pipette was tightly sealed to the cell membrane by applying a continuous negative pressure. Once the cell-attached configuration was obtained, the cell membrane was ruptured by suction through the pipette.

Immediately after the whole-cell configuration was establishing the zero-current potential was briefly measured in the current clamp mode. The voltage clamp was usually applied (unless otherwise mentioned) at a holding potential of −70 mV with the L/M-EPC-7 patch clamp system (List-electronic, Darmstadt, Germany). Signals were stored on a digital audio tape-recorder (DTR-

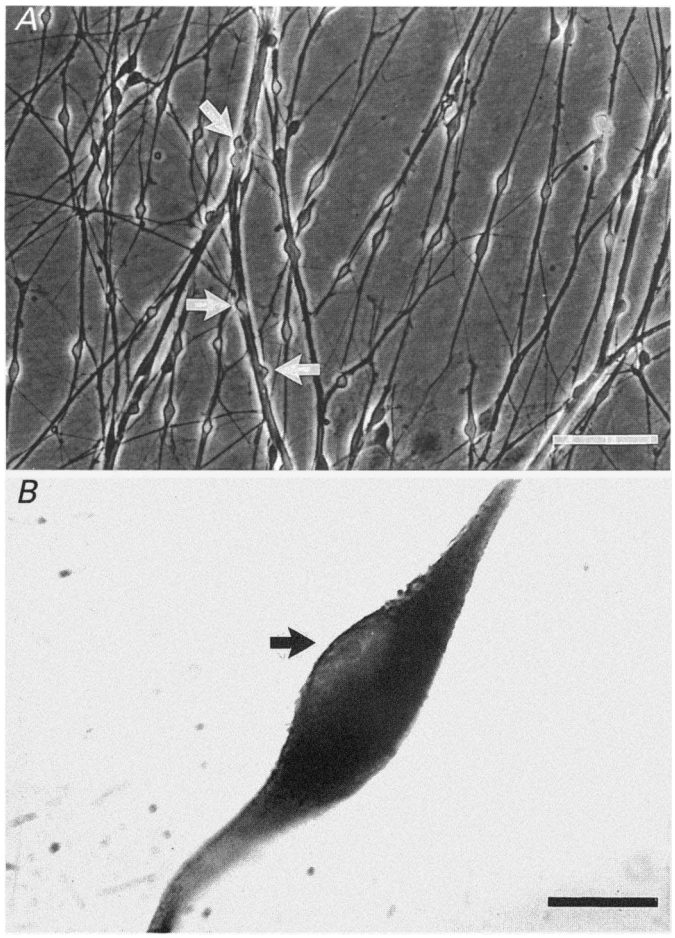

Fig. 1. Light micrographs of cultured mouse Schwann cells. *A*, phase-contrast micrograph of 8-day-old organotypic culture of Schwann cells. Some cells (arrow-heads) appeared to be associated physically with neurites. Most of the cells are aligned along neurites. *B*, light micrograph of an immunostained Schwann cell. Note the characteristic spindle-shaped body with an oval nucleus (arrow-head) and narrow tapered processes in opposite directions. Scale bar, 100 μm in *A*, 10 μm in *B*.

1200, Biologic, Grenoble, France) at a sampling frequency of 48 kHz. Whole-cell currents were low-pass filtered by an eight-pole Bessel filter (Frequency Devices, Haverhill, MA, USA) at 1 kHz before being displayed and sampled at 125 kHz by a TL-1 interface (Axon Instruments, Foster City, CA, USA) connected to a PC compatible computer system. Analysis was performed with pClamp 5·5 software (Axon Instruments). Leak and capacity currents were subtracted using averaged currents from hyperpolarizing voltage steps. Results in the text are expressed as means ± S.D. and significance was tested by means of Student's *t* test.

Voltage clamp fidelity

The Schwann cells in this study were bipolar with long processes and therefore part of the currents measured might have come from areas of membrane that were not well clamped. The patch clamp amplifier used in our experiments allowed for analog cancellation of capacitive currents both of patch pipette (with C_{fast} setting) and part of the cell membrane (with C_{slow} setting). Following the method used by Howe & Ritchie (1990), we first set C_{fast} and then C_{slow} for optimum compensation. This value of C_{slow} which we call C_1 corresponds to that part of the cell membrane with negligible access resistance (R_{acc}). The remainder of the cell was represented by a minimal circuit consisting of a capacitance C_2 in series with R_{acc} (note that our C_1 and C_2 correspond to C_{soma} and C_{dist} of Howe & Ritchie, 1990). The values were: $C_1 = 26{\cdot}4 \pm 5{\cdot}3$ pF ($n = 8$); $C_2 = 6{\cdot}5 \pm 1{\cdot}2$ pF ($n = 8$); $R_{acc} = 156 \pm 38$ M Ω ($n = 8$). If the specific membrane capacitance is 1 μF cm^{-2}, then C_1 must correspond to a membrane area of 2640 μm^2. The soma of a Schwann cell approximates a prolate spheroid with major and minor axes of $31 \pm 2{\cdot}6$ μm ($n = 18$) and $7{\cdot}8 \pm 0{\cdot}6$ μm ($n = 18$); its surface area is therefore approximately 612 μm^2. This is much smaller than the area corresponding to C_1 which means that C_1 represents not only the capacitance of the soma but also part of the distributed capacitance associated with the bipolar processes. We conclude that the membrane area studied was more from the processes than from the soma, but since the ratio of $C_1/C_2 = 4 > 1$, most of the membrane area through which the measured currents flowed was well clamped. Moreover the currents that we shall describe in this study appeared well behaved, they resembled those previously described in other cells of more amenable geometry, and they could be isolated pharmacologically.

External solutions

The standard external solution was (mM): 140 NaCl, 5 KCl, 5 $CaCl_2$, 10 HEPES, 11 glucose and 4 NaOH (pH 7·4). In some experiments, 4-aminopyridine (4-AP, Sigma) and tetraethylammonium chloride (TEA, Sigma) were added to the standard external solution. For high external TEA (10, 30 and 100 mM), external NaCl was reduced to 130, 110 and 40 mM respectively. In Ca^{2+} channel currents experiments, the external solution was (mM): 140 NaCl, 2 $MgCl_2$, 10 HEPES, 11 glucose and 4 NaOH (pH 7·4). Ca^{2+} or barium (Ba^{2+}) were used as charge carriers for Ca^{2+} channels. In some experiments nifedipine, Bay K 8644 and D600 (all from Sigma) were added to the external solution.

RESULTS

Membrane currents of mouse Schwann cells in standard conditions

From a holding potential of −70 mV, depolarizing steps to values more positive than −50 mV evoked large voltage-dependent outward currents (Fig. 2*A*). These outward currents displayed a fast transient component which inactivated rapidly and a delayed sustained component with slower inactivation kinetics. The mean amplitudes of the fast transient and the delayed sustained components at a test potential of +50 mV were respectively 2510 ± 682 and 1010 ± 348 pA ($n = 14$). With a cell membrane capacitance of $34{\cdot}4 \pm 4{\cdot}7$ pF ($n = 8$), the current densities of the fast transient and the delayed sustained components were respectively 73 and 29 pA pF^{-1}. The inactivation of outward currents could be fitted by two exponentials. The fast and slow time constants of inactivation were $70{\cdot}5 \pm 17{\cdot}5$ and 579 ± 359 ms ($n = 14$) for currents obtained at a test potential of +50 mV. The current–voltage relationship for the peak amplitude of the outward currents is shown in Fig. 2*B*. It can be seen that conductance increased upon membrane depolarization, i.e. the peak current was outwardly rectifying. The threshold for activation of the peak current ($-49{\cdot}9 \pm 5{\cdot}8$ mV, $n = 15$) was close to the zero-current potential ($-42{\cdot}3 \pm 9{\cdot}5$ mV, $n = 126$) determined in the current clamp mode (see Methods). In order to identify the ionic nature of the outward currents, we studied tail currents following repolarization

to different holding potentials. As shown in Fig. 2*C* and *D*, the tail current following a test pulse to 0 mV was clearly outward at −40 mV, zero at −70 mV and inward at −120 mV for both the transient (Fig. 2*C*) and the sustained (Fig. 2*D*) components. As the reversal potential of the outward currents estimated from those of the tail

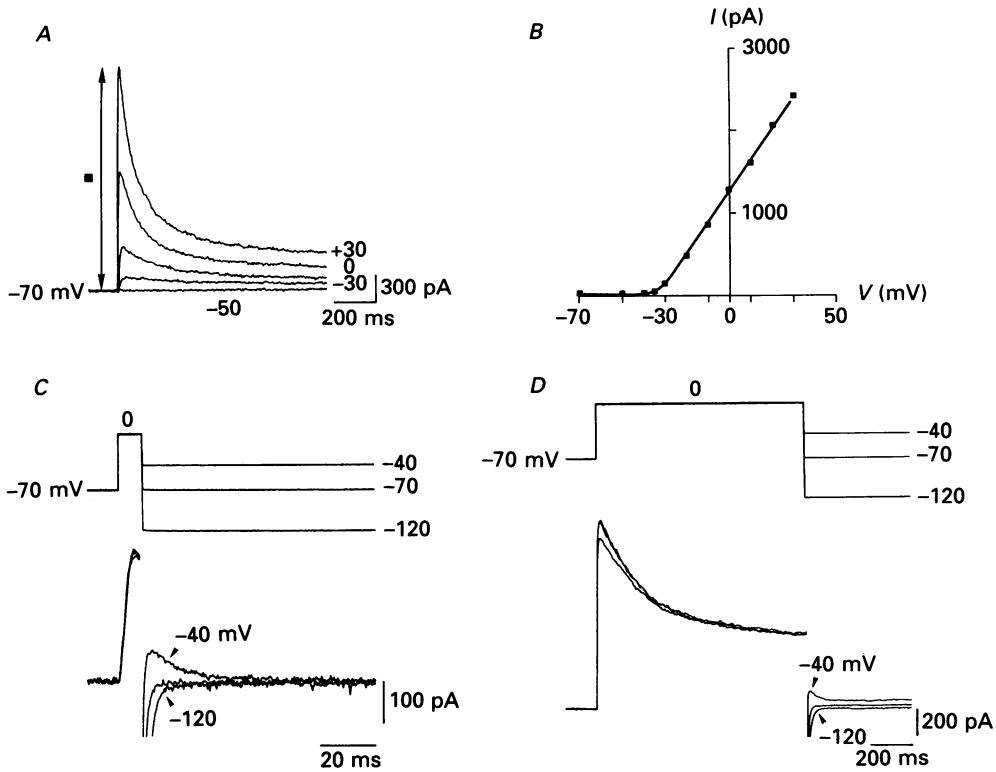

Fig. 2. Voltage-dependent K^+ currents in cultured mouse Schwann cells. *A*, family of current traces obtained by stepping from a holding potential of −70 to −50, −30, −20, 0 and +30 mV. Note the presence of a fast transient outward component followed by a delayed sustained outward component. *B*, current–voltage relationship of the peak outward current (■) of the cell shown in *A*. *C* and *D*, reversal potential of the peak outward current (*C*) and of the sustained outward current (*D*) estimated from those of the tail currents. The external bathing solution contained 5 mM-Ca^{2+}.

currents (about −70 mV) was close to the calculated value for the potassium equilibrium potential (E_K, about −86 mV in our conditions) these results suggest that the outward currents are predominantly carried by K^+.

Evidence for two types of K^+ current

As already shown, depolarization from a holding potential of −70 mV up to 0 mV evoked a fast transient outward component followed by a delayed sustained component (Fig. 3*A*). On the same cell, from a holding potential of −30 mV depolarization to the same test potential evoked only a delayed sustained current

almost equal to the sustained component obtained with a holding potential of −70 mV (Fig. 3*A*). This current inactivated very slowly, if it all, during a maintained depolarization (Fig. 3*B*). The current–voltage relationships for these two currents are shown in Fig. 3*C*. The delayed sustained K^+ current, obtained from a holding

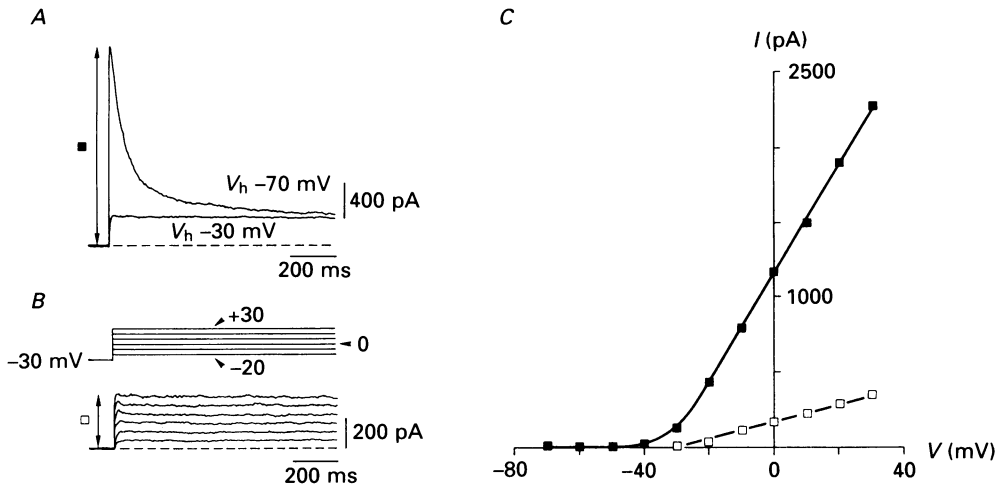

Fig. 3. Evidence for two types of K^+ currents in cultured mouse Schwann cells. *A*, outward currents elicited by stepping from two different holding potentials (−70 and −30 mV) to 0 mV. Note the disappearance of the transient component for the current obtained from −30 mV. *B*, family of current traces obtained from −30 mV on the same cell as shown in *A*. *C*, current–voltage relationships of the peak outward current (■) and the delayed sustained outward current (□). The external bathing solution contained 5 mM-Ca^{2+}.

potential of −30 mV, displayed an inactivation process, called 'cumulative inactivation', similar to those described in molluscan nerve cell bodies (Aldrich, Getting & Thompson, 1979), in human T lymphocytes (DeCoursey, Chandy, Gupta & Cahalan, 1984) and in mouse Schwann cells cultured from sciatic nerves (Konishi, 1989*a*). In Fig. 4*A*, voltage pulses from −30 to 0 mV were separated by 15 s. The current decreased at each pulse to reach a steady-state level in about 3 min. The degree of 'cumulative inactivation' was independent of the rate of pulse delivery over the range tested (one every 5 s to one every 30 s, not shown). Repolarization to −70 mV between two pulses immediately and durably restored the amplitude of the initial current. The current–voltage relationship obtained by stepping to −70 mV between each test pulse is plotted in Fig. 4*C*.

Taking advantage of the steady-state inactivation of the transient K^+ current at −30 mV (see Fig. 3*A*), we also used a double-pulse protocol to isolate the sustained delayed K^+ current directly from a holding potential of −70 mV which avoided the 'cumulative inactivation' phenomenon (Fig. 4*B*). A test pulse to 0 mV activated an outward current composed of the fast transient and the delayed sustained K^+ currents. Then a pre-pulse to −20 mV was applied to inactivate the fast transient K^+ current. The pre- and test pulses were separated by 50 ms, which was short enough

to avoid recovery from inactivation for the fast transient K^+ current. With this protocol, the outward current recorded during the test pulse was clearly only sustained (Fig. 4*B*). The current–voltage relationship obtained with the double-pulse protocol is also plotted in Fig. 4*C*. It can be seen that no significant differences in the

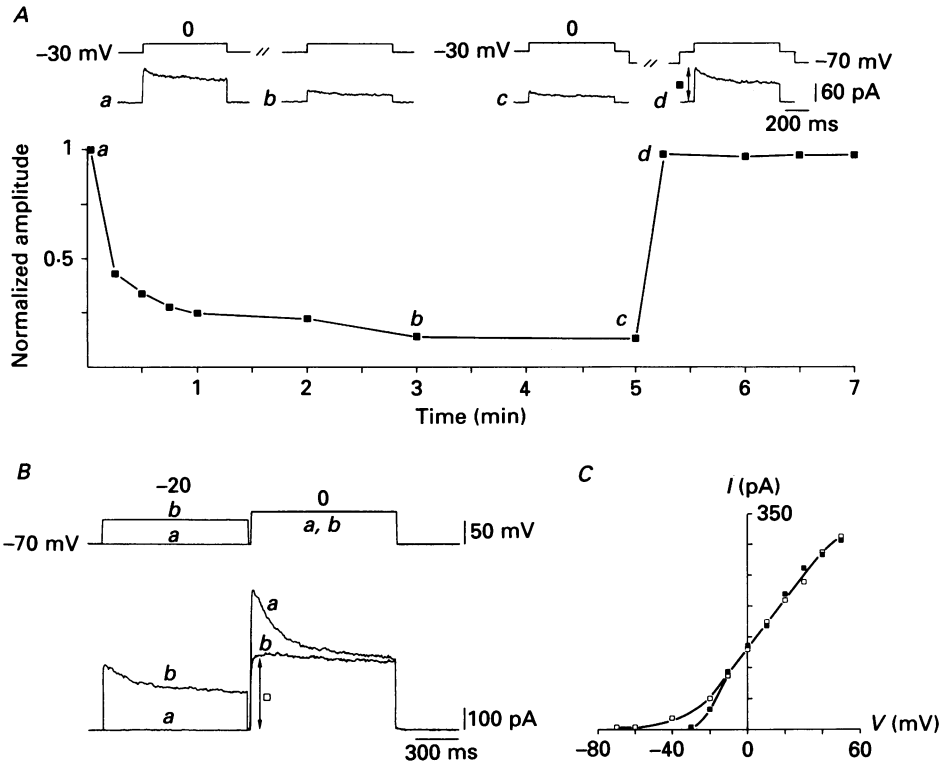

Fig. 4. Cumulative inactivation of the delayed sustained K^+ current during repetitive depolarizations from −30 mV, and isolation by a double-pulse protocol of the delayed sustained K^+ current from −70 mV. *A*, test potentials (1 s pulses from −30 to 0 mV) were delivered at a rate of one each 15 s (*a*, control current). Note the progressive decrease in the amplitude of the current until a steady-state level was reached in about 3 min (*b* and *c*). Hyperpolarization to −70 mV between each pulse from −30 to 0 mV restored immediately and durably the control amplitude of the delayed sustained K^+ current (*d*). *B*, a pre-pulse from −70 to −20 mV (*b*) was used to inactivate the fast transient K^+ current, and was followed by a test pulse to 0 mV. Pulses were separated by 50 ms. Note that the current obtained during the test pulse (*b*) was clearly only sustained and equal to the sustained current obtained without pre-pulse (*a*) by stepping from −70 to 0 mV. *C*, current–voltage relationships of the delayed sustained K^+ current obtained with the two different protocols. The external bathing solution contained 5 mM-Ca^{2+}.

current–voltage curves were observed. The potential at which 50% of the transient current was inactivated was $-35{\cdot}6 \pm 3{\cdot}0$ mV ($n = 5$) and the steady-state inactivation was complete at 0 mV. The potential at which 50% of the transient current was activated was $-14{\cdot}1 \pm 3{\cdot}6$ mV ($n = 11$).

Pharmacology of the two types of K^+ current

We tested first the possible presence of a Ca^{2+}-dependent K^+ component in the outward currents. In a first set of experiments, Ca^{2+} was omitted from the standard external solution and Mg^{2+} concentration was increased to 10 mM in order to reduce

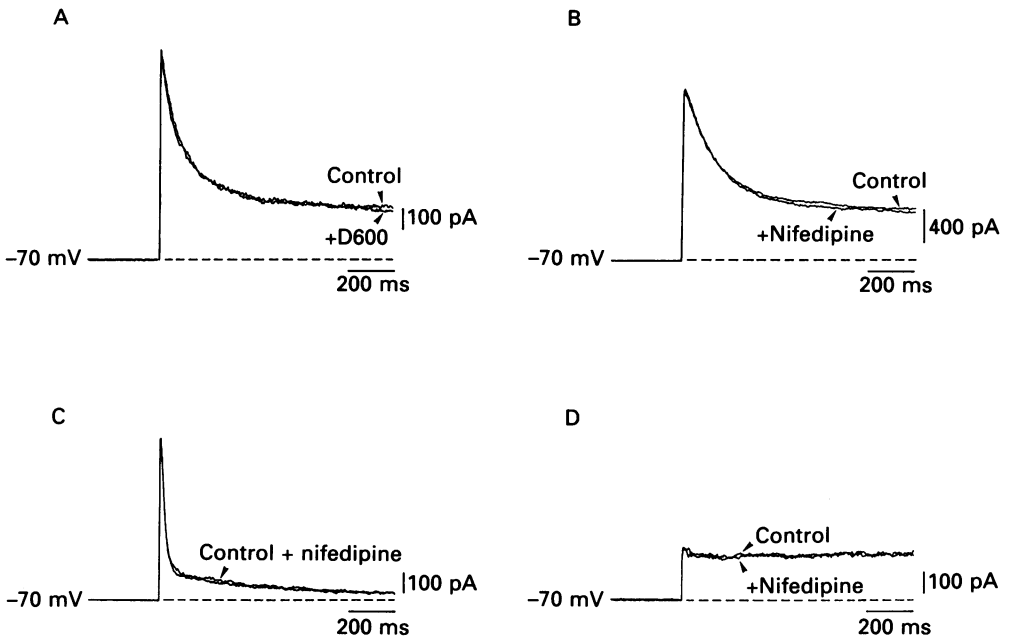

Fig. 5. Lack of effect of D600 and nifedipine on the two types of K^+ current in cultured mouse Schwann cells. D600 and nifedipine were added to the external bathing solution. *A*, D600 (1 μM) was ineffective either on the fast transient K^+ current or on the delayed sustained K^+ current. *B*, the same result was obtained for nifedipine (1 μM). *C* and *D*, nifedipine (1 μM) was ineffective on a cell displaying spontaneously either a mainly transient K^+ current (*C*) or a mainly sustained K^+ current (*D*). The external bathing solution contained 5 mM-Ca^{2+}.

alteration of the surface membrane potential. A shift in the voltage dependence was still apparent (see Discussion), but the time courses of the outward currents were almost identical, whatever the voltage pulse (not shown). In a second set of experiments we used two organic Ca^{2+} channel blockers. As shown in Fig. 5*A*, 1 μM-D600 had no effect on either the transient or the sustained K^+ currents. The same results were obtained for the dihydropyridine (DHP) Ca^{2+} antagonist nifedipine at 1 μM (Fig. 5*B*). Therefore, we conclude that external Ca^{2+} does not seem to participate in increasing the K^+ conductance. In a few cells ($< 5\%$ of the total population) depolarization from a holding potential of -70 mV induced either mainly transient K^+ currents (Fig. 5*C*) or mainly sustained K^+ currents (Fig. 5*D*). Nifedipine (1 μM) was also ineffective in blocking these two types of K^+ current.

To characterize further the outward K^+ currents, we looked for effects of two K^+ channel blockers, TEA and 4-AP. The transient and sustained currents in the presence of the blocker were normalized to the control currents recorded before the

addition of the blocker. Both the transient and the sustained currents were inhibited by TEA and 4-AP in a dose-dependent manner. The inhibitions were fully reversible in 3–5 min. With TEA the sustained K^+ current was more affected than the transient K^+ current (Fig. 6*A*) while the opposite was true for 4-AP (Fig. 8*A*). The

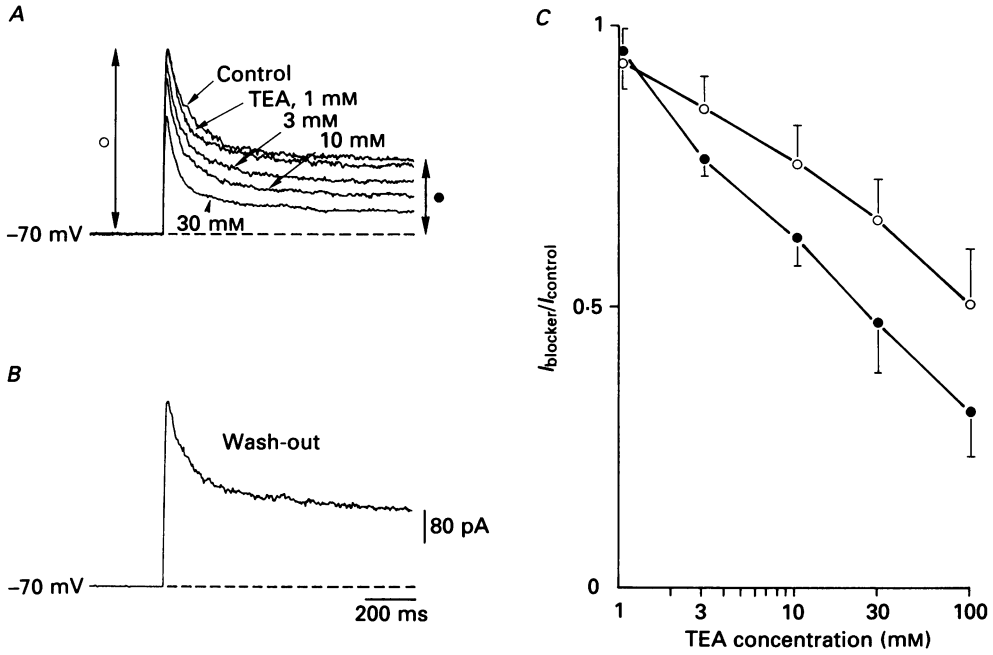

Fig. 6. Dose–response curves of the effects of TEA on the peak transient K^+ current and the delayed sustained K^+ current in cultured mouse Schwann cells. *A*, a cumulative dose–response curve was obtained on each cell by stepping from −70 to 0 mV. Peak (○) and sustained (●) currents in the presence of TEA were normalized to the control current recorded before the addition of TEA. Between each cumulative dose 4 min elapsed which allowed a steady-state inhibition of the K^+ currents. *B*, recovery after washing out TEA. *C*, dose–response curves for the peak (○)and the sustained (●) currents. Vertical bars indicate standard deviations for different cells (6 cells for each dose). Note the logarithmic scale for the abscissa. The half-maximal dose was 100 mM for the peak current and 24 mM for the sustained current. The external bathing solution contained 5 mM-Ca^{2+}.

half-maximal effective doses of TEA (Fig. 6*C*) and 4-AP (Fig. 8*C*) were 100 and 1·3 mM for the transient, and 24 and 4 mM for the sustained current. As already shown in Fig. 4, two protocols could be used to isolate the sustained current, and we used these to test more accurately the effect of TEA on the sustained current. In Fig. 7*A*, the sustained current was isolated from a holding potential of −30 mV, by repolarizing the cell to −70 mV between each pulse. In Fig. 7*B*, on the same cell, the sustained current was isolated at −70 mV by inactivating the transient current during a pre-pulse to −20 mV. Both protocols gave similar results for TEA sensitivity of the sustained current (Fig. 7*C*). The half-maximal effective dose of TEA was 11 mM. We did not attempt to study 4-AP sensitivity of the sustained current with the protocol shown in Fig. 7*A*, as 4-AP block is known to be voltage dependent (Meves & Pichon, 1977; Howe & Ritchie, 1988).

Lack of Na^+ current in mouse Schwann cells

We tested for the presence of an inward Na^+ current by using a K^+-free external solution containing 5 mM-Co^{2+} instead of 5 mM-Ca^{2+} in order to maintain a high enough concentration of divalent cations to allow a tight seal. Potassium was

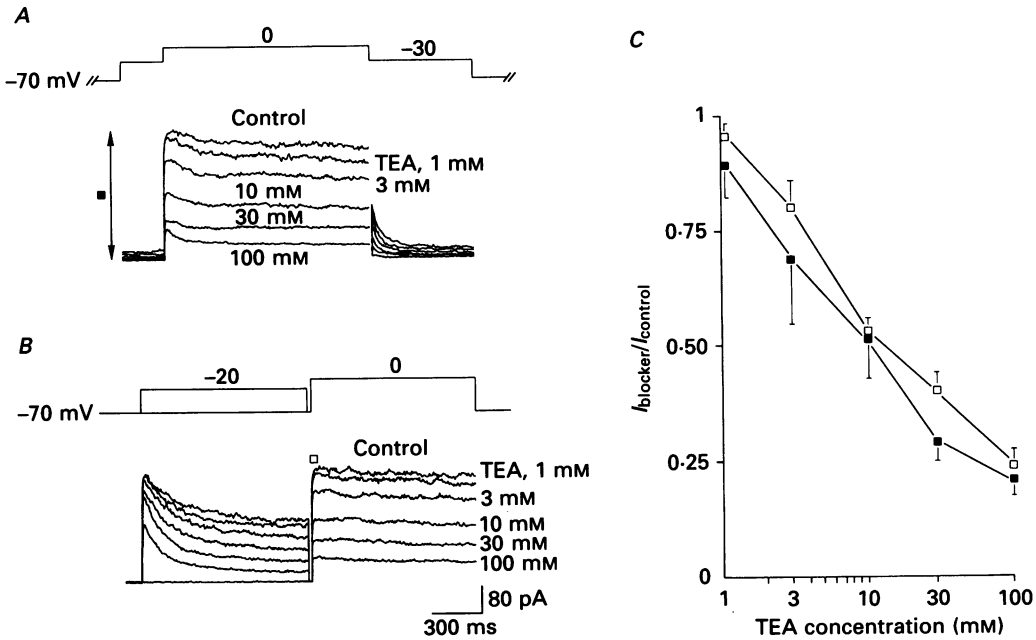

Fig. 7. Dose–response curves of the effects of TEA on the delayed sustained K^+ current in cultured mouse Schwann cells. *A*, cumulative inactivation of the current was removed by hyperpolarizing to −70 mV between each test pulse (see Fig. 4*A*). *B*, isolation of the delayed sustained K^+ current by a double-pulse protocol (see Fig. 4*B*). Sustained K^+ current in the presence of TEA was normalized to the control current recorded before the addition of TEA (*A* and *B*). *C*, dose–response curves for the delayed sustained K^+ current obtained with the two different protocols. Vertical bars indicate standard deviations from different cells (3 cells for each dose). Note that for each dose, values obtained with the two protocols were not significantly different. The half-maximal dose was 11 mM. The external bathing solution contained 5 mM-Ca^{2+}.

replaced in the pipette solution by caesium (Cs^+). In these conditions, depolarizing or hyperpolarizing steps from a holding potential of −70 mV evoked only leakage currents (Fig. 9*A*). In order to remove any possible Na^+ channel inactivation, the membrane was hyperpolarized to −120 mV for 5 s before stepping to −40, −20 and 0 mV (Fig. 9*B*). Contrary to results in rabbit and rat cultured Schwann cells (Howe & Ritchie, 1989) and similarly to Hoppe, Lux, Schachner & Kettermann, (1989) in the same preparation as ours, no sign of an inward Na^+ current was found with this protocol in any of the twenty-five cells tested.

Ca^{2+} channel currents in mouse Schwann cells

When K^+ was removed from the external solution, and replaced in the pipette solution by Cs^+, with 10 mM-Ca^{2+} in the bathing solution, depolarizing steps from a holding potential of −70 mV evoked voltage-dependent inward currents (Fig. 10*A*).

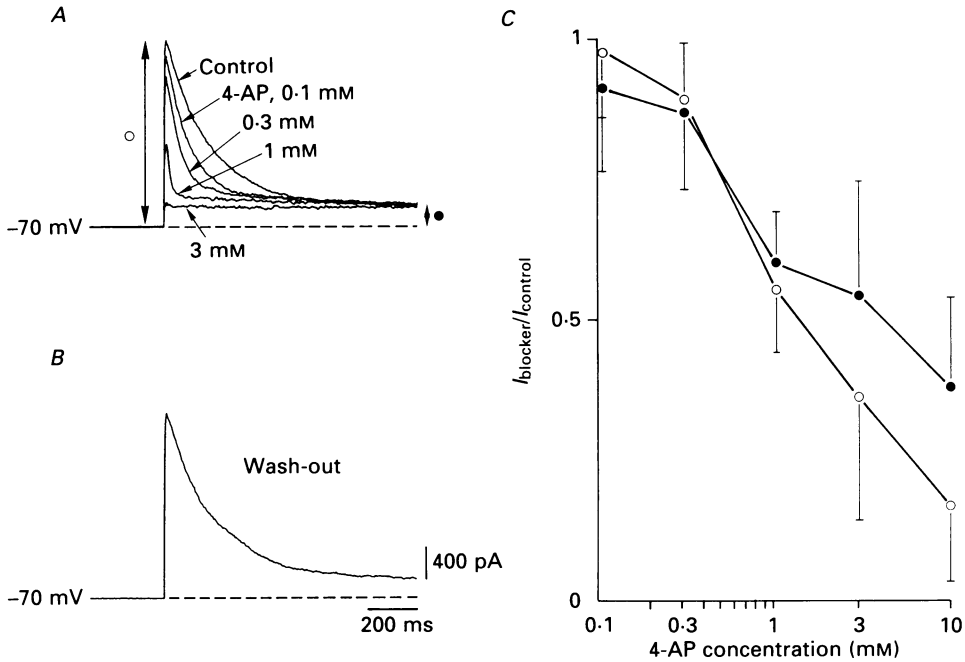

Fig. 8. Dose–response curve of the effects of 4-AP on the peak transient and the delayed sustained K^+ currents in cultured mouse Schwann cells. *A*, cumulative dose–response curve (for protocol see Fig. 6*A*). Note in this cell the complete inhibition of the fast transient K^+ current without any noticeable effect on the delayed sustained K^+ current with 3 mM-4-AP. *B*, recovery after wash-out of 4-AP. *C*, dose–response curves for the peak (○) and the sustained (●) currents. Vertical bars indicate standard deviations from different cells (5 cells for each dose). The half-maximal dose was 1·3 mM for the peak current and 4 mM for the sustained current. The external bathing solution contained 5 mM-Ca^{2+}.

Among the different criteria proposed by Hagiwara & Byerly (1981) for identifying voltage-dependent Ca^{2+} channels, are listed: persistence of the signal when Ca^{2+} in the external solution is replaced by Ba^{2+} or strontium (Sr^{2+}), insensitivity of the signal to high concentrations of tetrodotoxin (TTX) and blockage of the signal by Co^{2+}. Indeed, voltage-dependent inward currents persisted when external Ca^{2+} was equimolarly replaced by Ba^{2+} (Fig. 10*C*). The current–voltage relationships showed that the threshold of activation for the Ca^{2+} current was about −40 mV ($-45{\cdot}3 \pm 5{\cdot}4$ mV, $n = 5$) and the current reached a maximum near −10 mV (Fig. 10*B*). For Ba^{2+} currents, the threshold of activation was also about −40 mV ($-42{\cdot}8 \pm 6{\cdot}4$ mV, $n = 7$), and maximum amplitude at about −10 mV (Fig. 10*D*): neither the voltage dependence of the inward currents nor their overall amplitude

were significantly modified. Inward Ca^{2+} or Ba^{2+} currents were totally insensitive to external addition of 2 μM-TTX (not shown). Numerous studies on excitable cells, especially neuronal cells (for review, see Miller, 1987) have demonstrated a multiplicity of voltage-dependent Ca^{2+} channel types each with its own set of

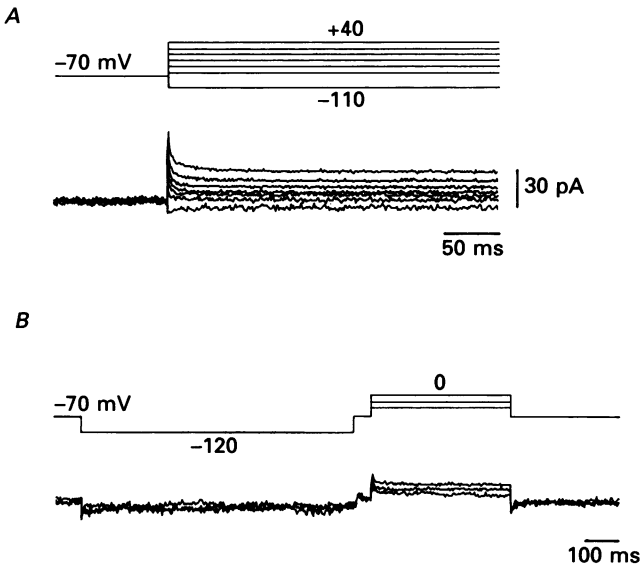

Fig. 9. Lack of Na^+ current in cultured mouse Schwann cells. *A*, family of current traces obtained by stepping from a holding potential of −70 to −110 mV and to depolarizing values with 20 mV increments up to 40 mV. *B*, the cell was held at −70 mV, stepped for 5 s to −120 mV and then with a 50 ms delay stepped from −70 mV to different test potentials (−40, −20 and 0 mV). Note in both cases the absence of any detectable inward current. K^+ -free external bathing and pipette solutions were used (see Methods).

electrical and pharmacological properties. Figure 11*A* shows that the fully inactivating current was not significantly affected by either 1 μM-Bay K 8644 (DHP, a Ca^{2+} agonist) or 1 μM-nifedipine, both compounds being highly selective for the L-type Ca^{2+} channel (Kamp & Miller, 1987). The steady-state inactivation curve is shown in Fig. 11*B*. The steady-state inactivation was complete at −40 mV and half-inactivation was obtained at −60 mV. Increasing the external Ba^{2+} concentration from 10 to 89 mM enhanced the inward Ba^{2+} current (Fig. 11*C*). The inward Ba^{2+} current was totally blocked by 5 mM-Co^{2+} (Fig. 11*C*). From the current–voltage relationship (Fig. 11*D*), it can be seen that the voltage dependence of the current is shifted along the voltage axis as expected for altered external surface potential (Ohmori & Yoshii, 1977). The thresholds for activation with 10 and 89 mM-Ba^{2+} were $-42{\cdot}8 \pm 6{\cdot}4$ ($n = 7$) and $-30{\cdot}5 \pm 7{\cdot}3$ mV ($n = 9$) respectively. The shift of the threshold for activation was statistically significant ($P = 0{\cdot}003$). All these characteristics of the fully inactivating current are similar to those of T-type Ca^{2+} channel currents.

Evidence for two types of Ca^{2+} channel current in mouse Schwann cell

Up to now, the co-existence of two types of Ca^{2+} channels in single glial cell has been reported only in rat cortical astrocytes (Barres, Chun & Corey, 1989). With Ba^{2+} as a charge carrier for Ca^{2+} channels, whatever the test potential, most of the

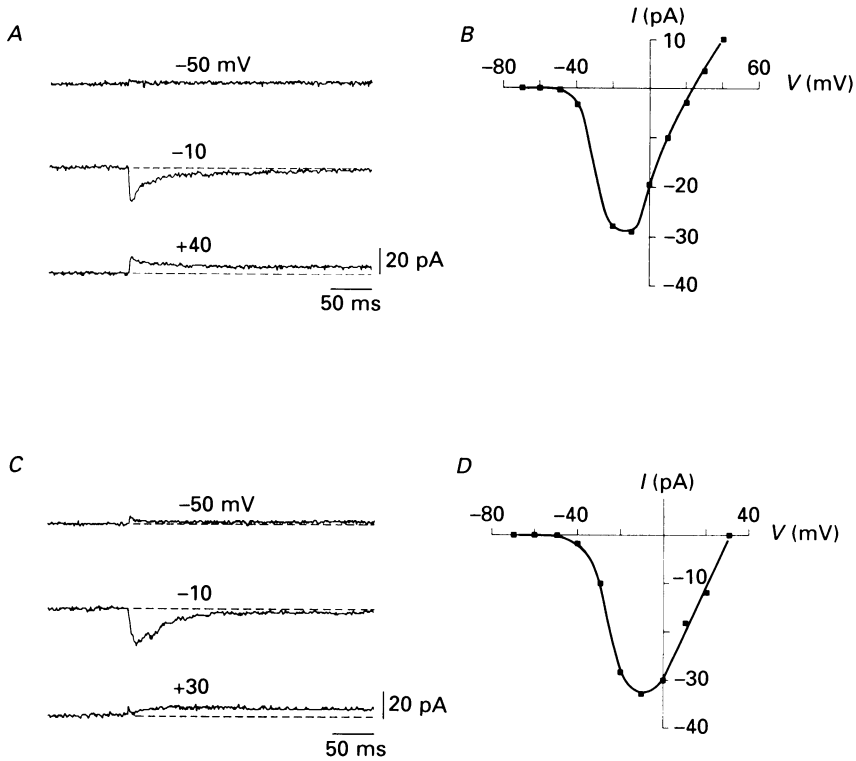

Fig. 10. Calcium channel currents in cultured mouse Schwann cells. *A*, family of current traces obtained by stepping from holding potential of −70 mV to different test potentials (value indicated above each current trace) in 10 mM-external Ca^{2+} solution. *B*, current–voltage relationship of the peak inward current (■) on the same cell as shown in *A*. *C*, family of current traces obtained in 10 mM-external Ba^{2+} solution. *D*, current–voltage relationship of the current (■) on the same cell as shown in *C*. K^+-free external bathing and pipette solutions were used.

Schwann cells we tested displayed the fully inactivating inward currents just described. However, in some cells, the fully inactivating current evoked by small depolarizations (Fig. 12*Aa*) was supplemented during stronger depolarizations by a slowly decaying current with a sustained component at the end of a 350 ms test potential (Fig. 12*Ab*). The current–voltage relationships of the peak and the sustained currents are shown in Fig. 12*B*. External addition of 1 μM-nifedipine, progressively reduced the Ba^{2+} current. After 4–5 min a steady state was reached in which only a fully inactivating inward current remained (Fig. 12*C*). These results are consistent with the existence of two types of Ca^{2+} channel currents. The sustained

current, because of its high threshold of activation (about −20 mV), its long-lasting time course and its sensitivity to nifedipine could be described as an L-type Ca^{2+} channel current.

Decline of Ca^{2+} channel currents with time following DRG excision

When Schwann cells were cultured with DRG neurons, in the very first days of culture (day 1 to day 3), 84% ($n = 19$) of cells displayed detectable Ca^{2+} channel

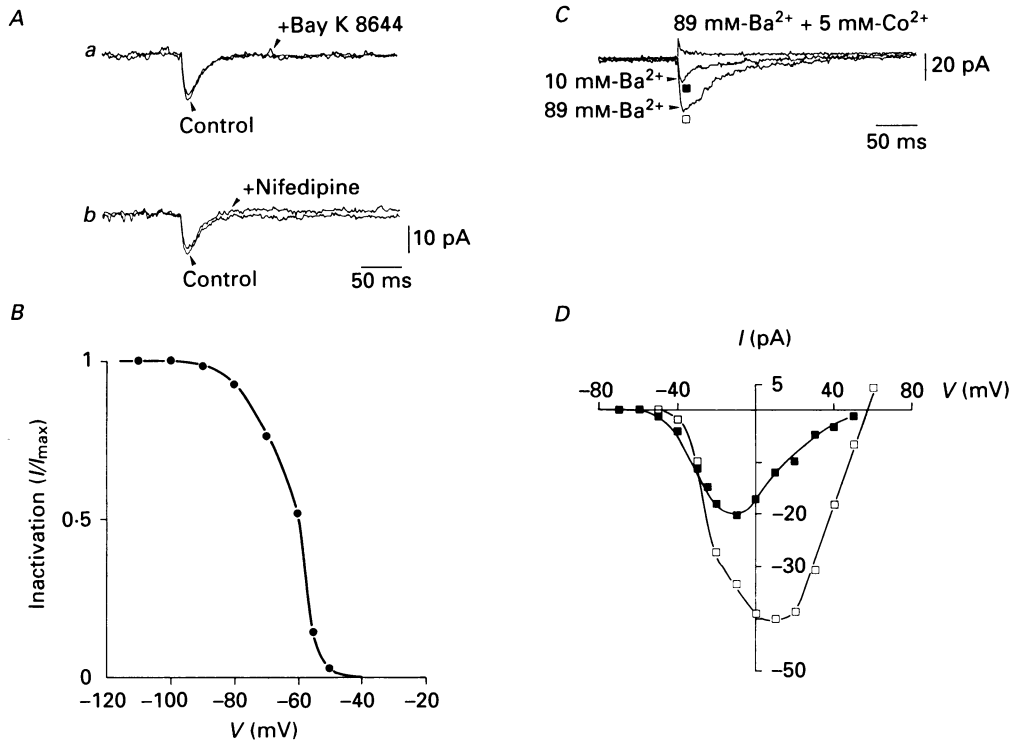

Fig. 11. Evidence for a T-like Ca^{2+} channel current in cultured mouse Schwann cells. *A*, insensitivity of the fully inactivating Ba^{2+} current to 1 μM-Bay K 8644 (*Aa*) and 1 μM-nifedipine (*Ab*). *B*, steady-state inactivation curve plotted from the same cell as shown in *A*. Half-inactivation of the current was obtained at −60 mV, and the inactivation was complete a −40 mV (curve fitted by eye). *C*, dependence of the fully inactivating current on the external Ba^{2+} concentration and blockade of the current by 5 mM-Co^{2+}. *D*, current–voltage relationship of the peak inward current in 10 mM (■) and 89 mM (□) external Ba^{2+} solution. Note the shift of the threshold for activation from −53 mV in 10 mM-external Ba2+ to −40 mV in 89 mM-external Ba^{2+}. K^+-free external bathing and pipette solutions were used.

currents (amplitude ⩾ 5 pA). After longer times in culture, the percentage of Schwann cells displaying Ca^{2+} channel currents decreased to reach a constant level, between 2 and 9 weeks, of about 30% ($n = 70$). This spontaneous decrease was never observed for either transient or delayed K^+ currents. In order to examine whether or not expression of Ca^{2+} channel currents was linked to the presence of DRG neurons,

we excised the explants in some cultures. Three to four days later, when the axons had degenerated, only one out of fifteen Schwann cells displayed detectable Ca^{2+} channel currents. This lability was not observed for K^+ currents.

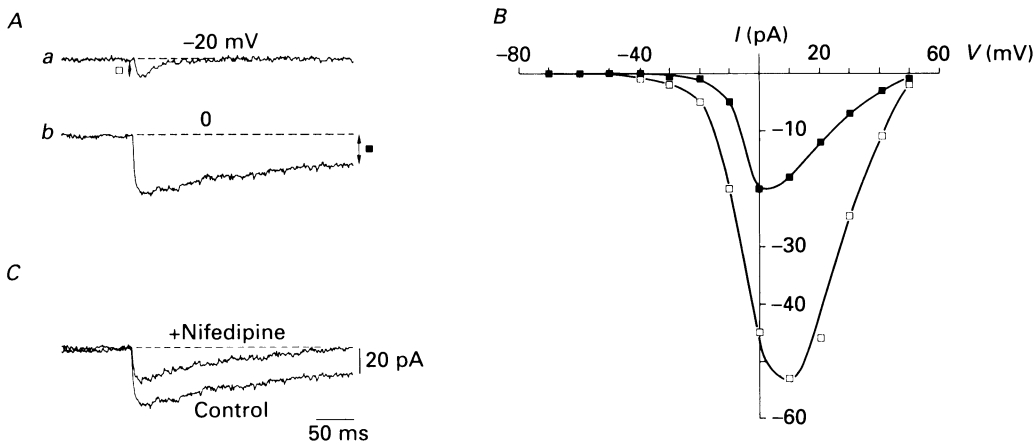

Fig. 12. Evidence for an L-type Ca^{2+} channel current in cultured mouse Schwann cells. *A*, current traces obtained by stepping from a holding potential of −70 to −20 (*a*) and 0 mV (*b*). Note the presence of a slowly decaying current at the end of a 300 ms pulse (*b*). *B*, current–voltage relationship of the peak inward (■) and the slowly decaying current (□) on the same cell as shown in *A*. *C*, suppression of the slowly decaying current by 1 μM-nifedipine on the same cell as shown in *A*. K^+-free external bathing and pipette solutions were used. The external Ba^{2+} concentration was 89 mM.

DISCUSSION

Under our culture conditions mouse Schwann cells possessed voltage-dependent K^+ and Ca^{2+} channels. In standard external solution, the zero-current potential was close to −40 mV. It cannot be assumed immediately that this value is the resting membrane potential (RMP) but it provides an estimate of the actual RMP (Push & Neher, 1987). Similar values have been reported in Schwann cells dissociated from sciatic nerves of newborn mice (around −40 mV, Konishi, 1989*a*) or newborn rabbits (between −30 and −40 mV, Chiu *et al.* 1984) and Schwann cells dissociated from DRG of embryonic mice (−41 mV, Hoppe *et al.* 1989).

K^+ channel currents

In standard external solution, mouse Schwann cells display prominent voltage-dependent outward K^+ currents. K^+ currents have been reported in Schwann cells isolated from sciatic nerves of rats (Shrager *et al.* 1985), rabbits (Chiu *et al.* 1984) and mice (Konishi, 1989*a*). In the same preparation as ours, Hoppe *et al.* (1989) reported the presence of voltage-dependent and pH-sensitive K^+ currents. According to differences in voltage dependence, inactivation kinetics and pharmacological properties, we conclude that mouse Schwann cells in our cultures display two types of K^+ currents. The transient K^+ current is similar in many respects to the 'A' current described in numerous neuronal cells (Rudy, 1988). Its threshold for

activation is around −50 mV, i.e. negative to the estimated RMP, and the conductance increases upon membrane depolarization. It is half-inactivated at the estimated RMP. At −30 mV, the transient current is inactivated by 85–100%. It inactivates rapidly (70–100 ms at room temperature). The transient current is far more sensitive to 4-AP (half-maximal effective dose, 1·3 mM) than TEA (half-maximal effective dose, 100 mM). Bevan, Chiu, Gray & Ritchie (1986) reported the existence of a similar inactivating K^+ current in type 2 astrocytes from rat optic nerve. The delayed sustained K^+ current inactivates very slowly (1 s at room temperature). It is blocked by both TEA and 4-AP in the same concentration range (half-maximal effective dose, 11 and 4 mM respectively). Not surprisingly, we found a small discrepancy with the half-maximal effective dose obtained with TEA on the delayed sustained current in the presence of the transient K^+ current (24 mM). This difference is probably due to some contamination of the delayed sustained K^+ current by the transient one which is less sensitive to TEA. Like the transient K^+ current, the delayed sustained K^+ current is outwardly rectifying. Therefore, the delayed sustained K^+ current recorded from cultured mouse Schwann cells is similar to the delayed rectifier K^+ current widely distributed among excitable cells. However, this delayed K^+ current shows a striking property reported in murine T lymphocytes (Lewis & Cahalan, 1988), human T lymphocytes (DeCoursey *et al.* 1984; Cahalan, Chandy, DeCoursey & Gupta, 1985), and more recently on Schwann cells isolated from sciatic nerves of mice (Konishi, 1989*a*). As the depolarizing pulses are repeatedly applied, the current becomes smaller and smaller. This inactivation process, called 'cumulative inactivation' was only observed at −30 mV (cf. Konishi, 1989*a* who reported it at −70 mV). We could prevent this phenomenon either by hyperpolarizing the membrane to −70 mV between each test pulse from −30 mV, or by inactivating the transient current by a pre-pulse. Both K^+ currents were insensitive to external Ca^{2+}. As reported by Fukushima, Hagiwara & Henkart, (1984), total replacement of the external Ca^{2+} by Mg^{2+} shifted the current–voltage relationship of the K^+ currents in the negative direction along the voltage axis. We endeavoured to overcome this problem, probably due to a change in the surface potential of the cell membrane, by increasing external Mg^{2+} concentration to 10 mM. The shift was still noticeable, but the time courses of the K^+ currents were almost identical for all the voltage range. Therefore we conclude that external Ca^{2+} has no significant direct effect on K^+ outward currents. These findings were reinforced by using two organic Ca^{2+} channel blockers, nifedipine and D600. Even at a relatively high concentration (1 μM) nifedipine was ineffective in altering either the transient or the delayed rectifier K^+ currents. D600, a less specific Ca^{2+} channel blocker, was also totally ineffective. Some cells displayed spontaneously mainly transient or mainly delayed rectifier K^+ currents, which reinforces the idea of the existence of two different types of K^+ channels and not simply two interconvertible states of one type of K^+ channel.

Ca^{2+} channel currents

This is the first report which describes Ca^{2+} channel currents in mammalian Schwann cells. Up to now, glial Ca^{2+} channels have been only reported in rat cortical astrocytes (MacVicar, 1984; MacVicar & Tse, 1988), type 2 rat cortical astrocytes

(Barres *et al.* 1989) and retinal glial cells (Newman, 1985). The present study indicates clearly not only that cultured mouse Schwann cells from DRG express Ca^{2+} channel currents, but also that maintenance of this expression is dependent on the presence of DRG neurons in culture. Three lines of evidence demonstrate the presence of Ca^{2+} channel currents. First the inward current persisted when external Ca^{2+} was equimolarly replaced by Ba^{2+}, another charge carrier for voltage-dependent Ca^{2+} channels. In addition, increasing the external Ba^{2+} concentration from 10 to 89 mM enhanced the overall amplitude of the inward current and induced a significant shift of both the threshold for activation and the potential at which the maximal amplitude of the current was obtained. This shift can be attributed to modification of the surface membrane potential (Ohmori & Yoshii, 1977; Wilson, Morimoto, Tsuda & Brown, 1983; Byerly, Chase & Stimers, 1985). Second, the inward current was totally insensitive to 2 μM-TTX, a very potent blocker of Na^+ channels in excitable cells. It should be noted that in itself this result is necessary but not sufficient since TTX-resistant Na^+ channels have been found in numerous excitable tissues including snail neurons (Kostyuk, Kristhal & Doroshenko, 1974), denervated or non-innervated mammalian skeletal muscle cells (Frelin, Vigne, Schweitz & Lazdunski, 1984), rat cardiac cells (Renaud, Fosset, Schweitz & Lazdunski, 1986) and myometrial smooth muscle cells (Amédée, Renaud, Jmari, Lombet, Mironneau & Lazdunski, 1986). Last, the inward Ca^{2+} or Ba^{2+} currents were totally blocked by 5 mM-Co^{2+}, a well-known voltage-dependent Ca^{2+} channel blocker (for review, see Hagiwara & Byerly, 1981). Two types of Ca^{2+} channel currents were found in our cells. The majority of the mouse Schwann cells displayed a fully inactivating Ca^{2+} channel current. This current was DHP insensitive, to both agonists and antagonists. With 10 mM-external Ba^{2+} the current was half-inactivated at about -60 mV and its threshold for activation was in the range -50 to -40 mV. Ca^{2+} and Ba^{2+} currents had roughly the same amplitude which means that the Ca^{2+} channel has the same relative permeability to Ca^{2+} or Ba^{2+}. Therefore this current resembles the T-type current. With 89 mM-external Ba^{2+}, some cells displayed an additional slowly decaying current, which was nifedipine sensitive and with a more positive threshold for activation (-30 to -20 mV). This current is similar to the L-type Ca^{2+} channel. One could argue that there was only one type of Ca^{2+} channel current, the L-type current, and that the fully inactivating current recorded in some cells could be due to Ca^{2+}-dependent inactivation of the L-type current. We feel that this is highly unlikely as (i) Ba^{2+} was used as a charge carrier, (ii) 10 mM-EGTA was present in the pipette solution.

Loss of Ca^{2+} channel currents following excision of the explants

The present study strongly suggests that expression of Ca^{2+} channel currents in the Schwann cells was dependent on the presence of DRG neurons in the culture. Once explants had been excised, and after axonal degeneration, mouse Schwann cells did not display Ca^{2+} channel currents. All the experiments reported in this study have been done on Schwann cells aligned near but not touching neurites which suggests that if DRG neurons do modulate the expression of Ca^{2+} channel currents they can do so at a distance. Modulation of the expression of ionic channels by axons has been

also reported for Na^+ channels on adult rabbit Schwann cells associated with non-myelinated axons of sciatic nerves (Chiu, 1988).

Physiological relevance of K^+ and Ca^{2+} channels

Cultured mouse Schwann cells possess classical voltage-dependent K^+ and Ca^{2+} channels. What role could these channels play *in vivo* if they exist? First of all, as for Na^+ channels on rabbit Schwann cells (Shrager *et al.* 1985) it is difficult to assert that the transient K^+ current and the T-like Ca^{2+} channel current could play a role in any excitability of the Schwann cells since at the estimated RMP (about -40 mV) half of the transient K^+ current and almost all of the T-like Ca^{2+} channel current would be inactivated. One possibility could be that, even within a few seconds of establishing the whole-cell configuration, the measured value ($-42{\cdot}3 \pm 9{\cdot}5$ mV) may have been artificially depolarized because of perturbation by the seal. Therefore transient K^+ currents and T-like Ca^{2+} channel currents might be more available than estimated and could play a role in excitability. The vast majority of Schwann cells in this study that displayed both Ca^{2+} channel currents were found with 89 mM-external Ba^{2+} and we did not study inactivation curves in physiological external Ca^{2+} solution. Unfortunately, steady-state inactivation parameters have not been studied either in rat cortical astrocytes (MacVicar, 1984; MacVicar & Tse, 1988; Barres *et al.* 1989) or in retinal glial cells (Newman, 1985), but L-type currents on DRG neurons with 2–10 mM-external Ca^{2+} are available from 50% (Fox, Nowycky & Tsien, 1987), 66% (Fedulova, Kostyuk & Veselovsky, 1985) to 100% (Taylor, 1988) at -40 mV. Therefore by analogy, it is conceivable that the L-type current in mouse Schwann cells is at least partially available around -40 mV. Second, it has been shown that several neurotransmitters modify neuronal activity by modulating both K^+ and Ca^{2+} channel activities. For example, acetylcholine inhibits the fast transient K^+ current of cultured hippocampal neurons (Nakajima, Nakajima, Leonard & Yamaguchi, 1986). Identification of membrane receptors on mammalian Schwann cells is still lacking but numerous studies on Schwann cells of invertebrates have reported the existence of receptors for acetylcholine (nicotinic), glutamine and peptides (for review see Villegas, Evans & Reale, 1988). It is possible that at least some of these receptors are present on mammalian Schwann cells and therefore could modulate the K^+ and Ca^{2+} channel activities. For example, elevated intracellular cyclic AMP enhances L-type Ca^{2+} channel currents in cultured astrocytes from rat cerebral cortex (MacVicar & Tse, 1988). Therefore cyclic AMP-dependent modulation of glial Ca^{2+} channels could mediate secretory processes and morphological differentiation (MacVicar, 1987) although this later point remains controversial (Barres *et al.* 1989) and motility.

It has been speculated (Gray & Ritchie, 1985) that Schwann cells might act as a local factory of ionic channels (Na^+ and perhaps K^+ channels) for axons they ensheath. This hypothesis in Schwann cells was based on several observations: (i) Schwann cell Na^+ and K^+ channels appear in many respects quite similar to those present in neuronal membrane, (ii) Na^+ channels have an average lifetime of about 3 days (Ritchie, 1988) and as protein synthesis occurs only in the soma of the neurons, in a long motoneuron the axonal transport could be too slow to replenish

the axonal membrane, (iii) it has been shown that large molecules can be transferred from Schwann cell to axons (Tytell & Lasek, 1984). Mouse DRG neurons possess T-, N- and L-type channels (Kostyuk, Shuba & Savchenko, 1988) and the present results show that T- and L-type Ca^{2+} channels, at least, are present in the Schwann cells. Therefore it is tenable that Schwann cells could supply some of them.

In summary, cultured mouse Schwann cells possess voltage-dependent K^+ and Ca^{2+} channels. Their physiological relevance remains to be elucidated. Expression of Ca^{2+} channel currents on Schwann cells in the vicinity of neurites is dependent on the presence of DRG neurons. This observation suggests that even at short range Schwann cells and DRG neurons have co-ordinated functions and that messages are exchanged between them. Dynamic glial–axon interaction has been reported recently (Marrero, Astion, Coles & Orkand, 1989) in the optic nerve of the frog where nerve activity facilitated voltage-dependent inward glial currents. These authors concluded that the facilitation was probably due to the release of an unidentified substance from the axons. In this respect, existence and modulation by neurotransmitters and/or intracellular second messengers of voltage-dependent ionic channels on Schwann cells could interact with neuronal activity.

Note added in proof. Corvalan, Cole, de Vellis & Hagiwara (1990) have reported the existence of an L-type calcium current and a transient type calcium current in cultured rat astrocytes. The expression of both currents was dependent on neuron-astrocyte interactions, since calcium currents were recorded only from astrocytes cocultured with neurons.

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