Spontaneous Neuronal Activity in Organotypic Cultures of Mouse Dorsal Root Ganglion Leads to Upregulation of Calcium Channel Expression on Remote Schwann Cells

CLAIRE BEAUDU-LANGE,[1] AURORE COLOMAR,[1] JEAN-MARC ISRAEL,[2] JONATHAN A. COLES,[1,3] AND THIERRY AMÉDÉE[1]*

[1]*INSERM U394, Bordeaux Cedex, France*
[2]*INSERM U378, Bordeaux Cedex, France*
[3]*INSERM U438, CHU, Grenoble Cedex, France*

KEY WORDS glia; peripheral nervous system; tetrodotoxin; action potential; voltage-dependent channel

ABSTRACT It is well established that neurons regulate the properties of both central and peripheral glial cells. Some of these neuro-glial interactions are modulated by the pattern of neuronal electrical activity. In the present work, we asked whether blocking the electrical activity of dorsal root ganglion (DRG) neurons in vitro by a chronic treatment with tetrodotoxin (TTX) would modulate the expression of the T-type Ca^{2+} channel by mouse Schwann cells. When recorded in their culture medium, about one-half of the DRG neurons spontaneously fired action potentials (APs). Treatment for 4 days with 1 µM TTX abolished both spontaneous and evoked APs in DRG neurons and in parallel significantly reduced the percentage of Schwann cells expressing Ca^{2+} channel currents. On the fraction of Schwann cells still expressing Ca^{2+} channel currents, these currents had electrophysiological parameters (mean amplitude, mean inactivation time constant, steady-state inactivation curve) similar to those of control cultures. Co-treatment for 4 days with 1 µM TTX and 2 mM CPT-cAMP, a cAMP analogue that induces the expression de novo of Ca^{2+} channel currents in Schwann cells deprived of neurons, maintained the percentage of Schwann cells expressing Ca^{2+} channel currents, showing that TTX does not directly affect the expression of Ca^{2+} channel currents by Schwann cell. We conclude that blocking spontaneous activity of DRG neurons in vitro downregulates Ca^{2+} channel expression by Schwann cells. These results strongly suggest that DRG neurons upregulate Ca^{2+} channel expression by Schwann cells via the release of a diffusible factor whose secretion is dependent on electrical activity. *GLIA 29:281–287, 2000.* © 2000 Wiley-Liss, Inc.

INTRODUCTION

Neurons regulate central and peripheral glial cells properties through different mechanisms, involving adhesion signals or diffusible molecules. Some of these neuro-glial interactions are modulated by the pattern of the electrical activity fired by the neurons. For example, the expression of functional inwardly rectifying K^+ channels ($K_{IR}{}^+$) in nonmyelinating Schwann cells of sural and sympathetic nerves in mice is upregulated by electrical activity (Konishi, 1994). Myelination of CNS neurons by oligodendrocytes is inhibited when action potential (AP) propagation is blocked with tetrodotoxin (TTX) both in vitro and in vivo, whereas stimulating neuronal electrical activity with α-scorpion toxin

*Correspondence to: Thierry Amédée, INSERM U394, IFM Rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France.
E-mail: thierry.amedee@bordeaux.inserm.fr

Received 12 July 1999; Accepted 17 September 1999

© 2000 Wiley-Liss, Inc.

(α-ScTX) increases the myelination rate in vitro (Demerens et al., 1996). Modulating the frequency of the electrical activity of dorsal root ganglion (DRG) neurons in vitro differentially regulated N-cadherin, N-CAM and L1 mRNAs and consequently the rate of adhesion of Schwann cells to their neurites (Itoh et al., 1995, 1997).

Schwann cells in organotypic culture of mouse DRG express the T-type Ca^{2+} channel current (Amédée et al., 1991). This current is present on about one-half of Schwann cells, both those that are apposed to neurites and those that are isolated, but it disappears from almost all Schwann cells within 2 days when the neurons are removed and the neurites degenerate. We have shown that the current was maintained by the presence of neurons on the *trans* side of a porous membrane above the Schwann cell culture, but that daily transfer of medium from a neuronal culture to a Schwann cell culture did not. We therefore suggested that the diffusible factor released by the neurons is rapidly degraded, which may make identification more difficult (Beaudu-Lange et al., 1998). The subject of the present study is the mechanism of release of the factor by the neurons.

We ask whether altering the electrical activity of DRG neurons in vitro would modulate the functional expression of Ca^{2+} conductances by isolated Schwann cells. We report that chronic treatment with TTX downregulated Ca^{2+} channel current expression by Schwann cells in vitro. We show that TTX was acting neither via a long-term neuronal toxic effect, nor directly on the Schwann cell Ca^{2+} conductances, but specifically by blocking spontaneous electrical activity of DRG neurons. It is therefore proposed that the neuronal diffusible signal that causes upregulation of Ca^{2+} channel current expression by Schwann is released by neurons in an electrically dependent way.

MATERIALS AND METHODS

Organotypic Cultures of Dorsal Root Ganglia

DRG were cultured from OF1 E19 mouse embryos as described in detail by Amédée et al. (1991) and Beaudu-Lange et al. (1998). Ganglia were cultured in α-modified Eagle's medium (αMEM; Gibco, Cergy-Pontoise, France) containing nerve growth factor (NGF; 20 ng/ml, Chemicon, Temecula) and complemented with $NaHCO_3$, 26 mM; Hepes, 15 mM; glucose, 28 mM; and N2 (insulin, 5 mg/L; transferrin, 100 mg/L; putrescine, 16 mg/L; progesterone, 6 μg/L; and sodium selenite, 8 μg/ml, all from Sigma).

Electrophysiology

Currents were recorded from cells cultured for 2–8 weeks by using the whole cell configuration of the patch-clamp technique at room temperature (20–24°C). The standard external solution was 140 mM NaCl, 10 mM $CaCl_2$, or 10 mM $BaCl_2$, 2 $MgCl_2$, 10 mM HEPES, 11 mM glucose, and 4 mM NaOH (pH 7.4). Patch pipettes were pulled from borosilicate glass capillaries (GF 150 TF-10, Clark Electromedical Instruments, Pangbourne, UK) and filled with 120 mM CsCl, 2 mM $MgCl_2$, 10 mM Hepes, 11 mM ethylene glycol-bis(β-aminoethylether)-N,N′-tetraacetic acid (EGTA), 11 mM glucose, and 30 CsOH (pH 7.4). In control conditions, patch pipettes had resistances of 5–10 MΩ. Voltage-clamp protocols were applied from a holding potential of −70 mV, using a L/M-EPC-7 patch-clamp system (List-Electronic, Darmstadt, Germany). Signals were stored on a digital audio tape recorder (DTR-1200, Biologic, Grenoble, France) at a sampling frequency of 48 kHz and displayed on a chart recorder. Whole cell currents were sampled at 125 kHz by a TL-1 interface (Axon Instruments, Foster City, CA). The time constant of inactivation of the Ca^{2+} channel currents was calculated by fitting an exponential using the least-squares method of the Clampfit program of p-Clamp 5.5 (Axon Instruments).

Intracellular potentials were recorded through single microelectrodes using an Axoclamp-2A (Axon Instruments). Recording microelectrodes were pulled from borosilicate glass capillaries (GS 150F-10, Clark Electromedical Instruments) and filled with 3 M KCl. In reference conditions, microelectrodes had resistance within the range 80–100 MΩ. Experiments were carried out at 37°C and signals were digitized (Neurodata) and stored on a videotape recorder and chart recorder. Neurons whose resting potential was more positive than −45 mV and/or whose membrane resistance was lower than 50 MΩ were discarded from the analysis. Results in the text are expressed as means ±s.d. and significance between mean values in different conditions was tested by means of Student's *t*-test. The χ^2-test was used to compare the number of cells displaying Ca^{2+} channel currents between control and TTX-treated cultures. For each set of experiments, different organotypic cultures have been used in order to minimize possible batch artifacts. Electrical activity of DGR neurons recorded in standard external solution, three cultures; recorded in culture medium, two cultures. Electrical activity of DRG neurons in TTX-treated cultures, two cultures. Ca^{2+} channel currents of Schwann cells in TTX-treated cultures, four cultures.

Drug Treatment

TTX (Sigma, Saint Quentin Fallavier, France) was first dissolved at 1 mM in distilled water as a stock solution and kept at −20°C. The day of the experiment, aliquots of stock solution were added to culture medium to a final concentration of 1 μM (for recording neuronal electrical activity) or in the standard external solution at a final concentration of 2 μM (for recording Ca^{2+} channel currents in Schwann cells) for acute treatment.

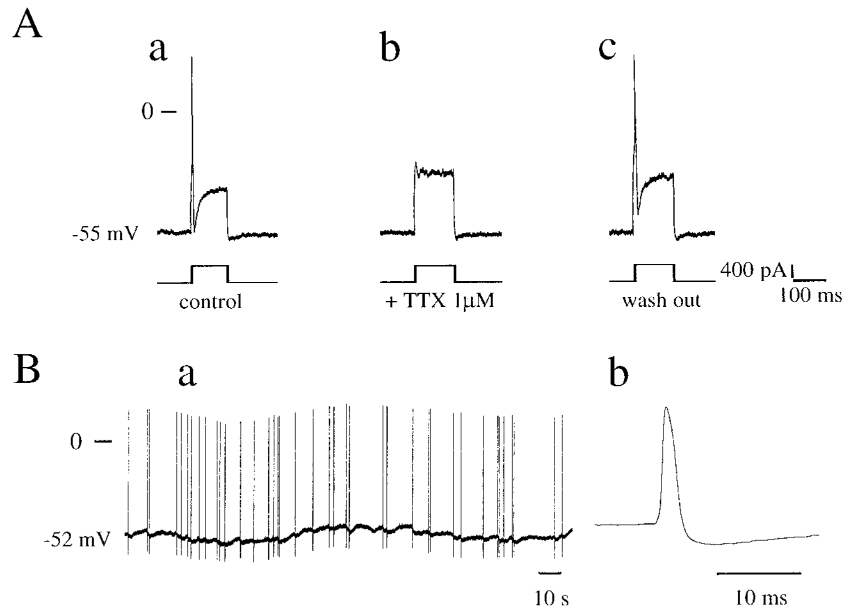

Fig. 1. Electrical activity of mouse dorsal root ganglion (DRG) neurons. **A:** (a) An action potential (AP) is triggered by a depolarizing current pulse (400 pA, 100 ms) from a membrane potential of −55 mV (b) the AP is blocked by tetrodotoxin (TTX) (1 μM) (c) the blockade is reversible on wash out of TTX (10 min). **B:** (a) spontaneous electrical activity recorded from a membrane potential of −52 mV. The mean firing frequency is 0.6 Hz; (b) single spontaneous AP on an expanded time scale. All records are made in culture medium at 37°C.

For chronic treatment, TTX was added to the culture medium at a final concentration of 1 μM and replaced daily.

RESULTS

Electrical Activity of DRG Neurons in Organotypic Cultures

In a first series of experiments, DRG neurons were recorded in the standard external solution used for electrophysiological experiments (see under Materials and Methods). The mean resting membrane potential was −55.5 ±1.2 mV (n = 51). About 60% of the recorded neurons did not give successful impalements for more than 5 min and were therefore discarded from this study. On the remaining 40 % (21 neurons), APs with overshoots ranging between +20 and +30 mV were triggered by depolarizing current pulses in almost all neurons. Only five neurons spontaneously fired APs from a threshold of around −50 mV. The frequency of this spontaneous firing was low and irregular, ranging from 0.1 to 1 Hz (mean value: 0.3 ± 0.2 Hz, n = 5).

When neurons were recorded in their culture medium instead of the standard external solution, the mean resting membrane potential was not significantly modified (−58.1 ± 3.1 mV, n = 14). Depolarizing current pulses triggered APs (Fig. 1Aa), which were blocked in five of six neurons tested by 1 μM TTX, a specific antagonist of TTX-sensitive Na^+ channels (Fig. 1Ab), and the remaining neuron displayed purely TTX-resistant APs. Of the five neurons displaying TTX-sensitive APs one neuron displayed also TTX-resistant APs, which were blocked by 1 mM Cd^{2+} and therefore involved Ca^{2+}-dependent regenerative responses. The wash out of TTX for 1–3 min allowed the recovery of evoked APs in five of six neurons (Fig. 1Ac). The occurrence of spontaneous activity was encountered much more frequently when recordings were made in culture medium compared with the standard external solution and was observed in about 55% (6 of 11) neurons. Figure 1B shows a typical spontaneous electrical activity in a DRG neuron recorded in culture medium. The firing frequency ranged from 0.1 to 2 Hz (mean value 0.6 ± 0.3 Hz, n = 6). We noticed that stable recordings (i.e., when the membrane potential or the amplitude of the AP remained stable for more than 5 min of impalement) were obtained more often when neurons were recorded in their culture medium. Therefore, we used these experimental conditions in the rest of this study.

Electrical Activity of DRG Neurons in TTX-Treated Organotypic Cultures

TTX (1 μM) was added to the culture medium for 4 days. Such chronic treatment did not noticeably alter the morphology of the ganglia, neurites, and Schwann cells under phase-contrast microscopy (not shown). We then looked at the effects of TTX treatment on the electrical activity of DRG neurons. The mean resting membrane potential recorded in the culture medium was −58.1 ± 1.6 mV (n = 12). Depolarizing current pulses failed to trigger APs in all neurons tested (n = 12), and none of these neurons showed spontaneous electrical activity. After wash out of TTX for 1 h, evoked APs were recorded in five of six neurons; recovery of spontaneous electrical activity was observed in one neuron.

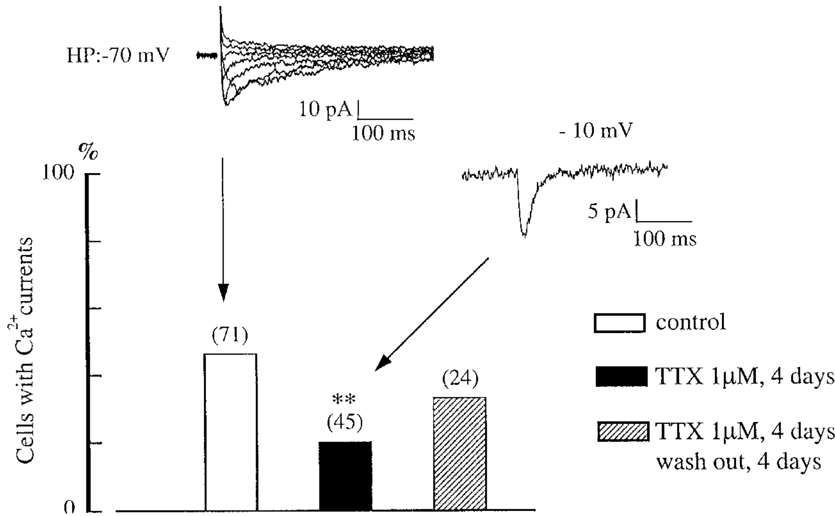

Fig. 2. Ca^{2+} channel currents in mouse Schwann cells. Histogram showing the percentage of Schwann cells expressing Ca^{2+} channel currents in control cultures (white column, n = 71), in tetrodotoxin (TTX) -treated cultures (black column) (4 days treatment, n = 45), and after wash out for 4 days of the drug (hatched column n = 24). $^{**}P < 0.01$ χ^2 test. The insets show representative currents elicited by voltage steps from a holding potential of −70 mV. The currents in control conditions correspond to steps to −30, −20, −10, 0, 10, 20, and 30 mV. The current in a TTX-treated culture was elicited by step to −20 mV.

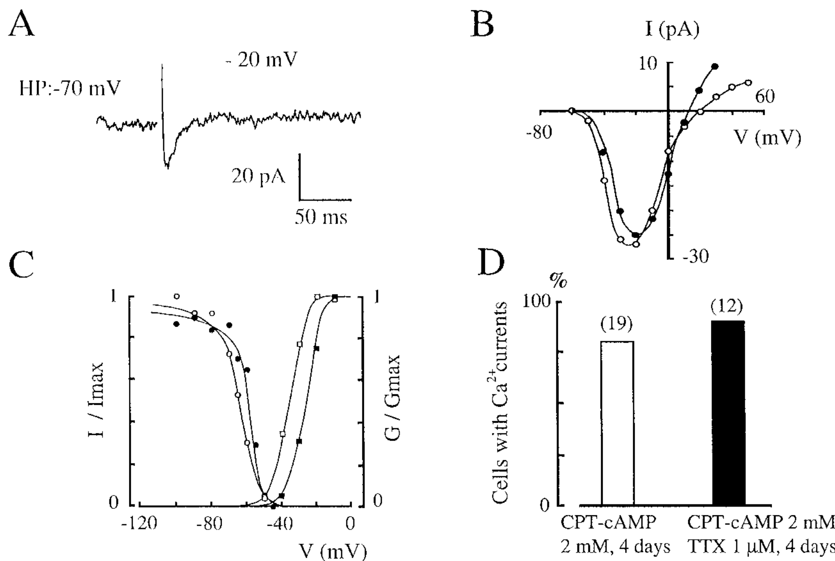

Fig. 3. Lack of direct effect of TTX on Ca^{2+} currents in mouse Schwann cells. **A:** representative Ca^{2+} current recorded from a Schwann cell treated for 4 days with tetrodotoxin (TTX) (1 µM). The holding potential was −70 mV, and the cell membrane potential was stepped to −20 mV for 200 ms. **B:** Current-voltage relationships of the cell shown in A (white circles) and a control cell shown in Fig. 2 (black circles). Note that both curves have similar shapes. **C:** Activation and steady-state inactivation curves of representative Ca^{2+} channel currents in control conditions (black symbols) and in TTX-treated Schwann cells (white symbols). **D:** Histogram showing the percentage of Schwann cells expressing Ca^{2+} channel currents cultured for 4 days in the absence of neurons either in the presence of 2 mM CPT-cAMP for 4 days, (white column, n = 19) or in the presence of 2 mM CPT-cAMP and 1 µM TTX (black column, n = 12).

Ca^{2+} Channel Currents of Schwann Cells in TTX-Treated Organotypic Cultures

Ca^{2+} channel currents were recorded from isolated Schwann cells, i.e. those not contacting neurites, in K^+-free solutions using Ca^{2+} or Ba^{2+} as a charge carrier as in previous work (Amédée et al., 1991; Beaudu-Lange et al., 1998). In control organotypic cultures, T-type Ca^{2+} channel currents of at least 5 pA in amplitude were expressed by 46.5% (n = 71) of isolated Schwann cells (Fig. 2). Chronic treatment of organotypic cultures for 4 days with 1 µM TTX significantly reduced the percentage of Schwann cells expressing T-type Ca^{2+} channel currents to 20% (n = 45, $P < 0.01$) (Fig. 2). Four days after wash out of TTX, the percentage of Schwann cells expressing T-type Ca^{2+} channel currents had increased again to 34% (n = 24, Fig. 2).

On the fraction of Schwann cells still expressing T-type Ca^{2+} channel currents (Fig. 3A), the mean amplitude of the currents (10.9 ± 2.1 pA, n = 9) was similar to that (9.8 ± 1.1 pA, n = 10) in control conditions. The time constants of the currents were not significantly affected by TTX treatment (mean: 20.2 ± 2.8 ms, n = 5 compared with 16.5 ± 2.2 ms, n = 8 in control conditions). Moreover, activation and steady state inactivation curves were similar in TTX-treated Schwann cells and Schwann cells in control conditions (Fig. 3C).

TTX Does Not Act Directly on Ca^{2+} Channel Currents in Schwann Cells

We tested first the possibility of an acute direct effect of TTX on T-type Ca^{2+} channel currents in organotypic cultures of Schwann cells. The acute addition of TTX (2 µM) to the standard extracellular solution did not change the percentage of Schwann cells expressing T-type Ca^{2+} channel currents (54.5%, n = 77). Moreover, the current-voltage (I/V) curve in the presence of

TTX was not different from an I/V curve obtained in control conditions (Fig. 3B).

We then tested whether TTX interfered in some way with the genomic expression of Ca^{2+} channels in Schwann cells. When cultured in the absence of neurons, only a small percentage of mouse Schwann cells express Ca^{2+} channel currents, but their expression can be induced by treatment for 4 days with CPT-cAMP (2 mM), a nonhydrolyzable cAMP analogue, (Beaudu-Lange et al., 1998). When this was done, it was found that the presence of 1 μM TTX did not alter the percentage of Schwann cells expressing de novo Ca^{2+} channel currents (Fig. 3D).

DISCUSSION

In this article, we show that DRG neurons in organotypic cultures fired spontaneous APs with low and irregular frequencies. Both evoked and spontaneous APs were blocked by micromolar concentrations of TTX. In parallel, a chronic treatment of organotypic cultures with TTX significantly reduced the percentage of Schwann cells expressing detectable Ca^{2+} channel currents. TTX downregulates the expression of Ca^{2+} channel currents by Schwann cells only in organotypic cultures (i.e., in the presence of DRG neurons), while in the absence of neurons it has no acute or long-term effect on Ca^{2+} channel currents. Taken together, these results strongly suggest that TTX downregulates the expression of Ca^{2+} channel currents by Schwann cells by acting on neuronal activity.

Effects of TTX on the Electrical Activity of Mouse DRG Neurons

Acute application of TTX in the micromolar range blocked APs in the vast majority of DRG neurons and chronic treatment blocked both spontaneous and triggered APs in all neurons tested. However, few DRG neurons displayed APs which were resistant to TTX. The expression of TTX-resistant Na^+ channels have been reported in mouse DRG neurons (Yoshida et al., 1978; Matsuda et al., 1978). These channels are mainly expressed by small (<25 μm) and medium (25–40 μm) DRG neurons, while TTX-sensitive channels prevailed in large (>40 μm) DRG neurons (Scholz et al., 1998). Because of the limited access to neurons within the ganglion, mostly largest neurons have been impaled in this work and this could explained the apparent low proportion of TTX-resistant APs that we have recorded .

Spontaneous electrical activity of DRG neurons has been reported in vivo (Fitzgerald, 1987; Miletic and Lu, 1993) but usually is not reported in vitro (Varon and Raiborn, 1971; Yoshida et al., 1978; Matsuda et al., 1978; Fields et al., 1992). In our organotypic culture, DRG neurons fired spontaneously and the occurrence of this activity was favored when DRG neurons were recorded in their culture medium at 37°C. We have no firm explanation for such discrepancies in vitro, but we believe that differences in cell dissociation procedure, bathing solution (e.g., standard salt solution versus culture medium) and embryonic stage of development (see below) could well be involved.

TTX Does Not Act Directly on Schwann Cells

TTX has been reported to block Ca^{2+} channels on human atrial cells (Lemaire et al., 1995). However, acute application of TTX did not block Ca^{2+} channel currents displayed by Schwann cells. We also investigated possible long term effects of TTX on Ca^{2+} channel currents in Schwann cells by looking at some basic electrophysiological parameters (mean amplitude, activation and inactivation curves, time constant of inactivation) of T-type Ca^{2+} channel currents. None of these parameters was altered by a 4-day treatment with TTX on the fraction of Schwann cells still expressing Ca^{2+} channel currents.

Schwann cells in organotypic cultures have been reported to express Na^+ channels (Beaudu-Lange et al., 1998), so we considered the possibility of an action of TTX on Schwann cells properties by its binding on Na^+ channels. However, we consider this hypothesis very unlikely because Na^+ channels are expressed by less than 3% of Schwann cells in our mouse organotypic cultures , and moreover are only weakly sensitive to TTX (see Fig. 9B, Beaudu-Lange et al., 1998). Therefore, although we cannot rule out completely that DRG neurons in some way induced functional TTX binding sites on Schwann cells, a much simpler explanation is that TTX acted only on neurons.

TTX Does Not Induce Neuronal Death in Organotypic Cultures

As Ca^{2+} channel currents expressed by Schwann cells disappeared within two days when neurons were caused to degenerate (Beaudu-Lange et al., 1998), we also asked whether TTX was not simply acting on Schwann cells through a long term toxic effect on neurons. We consider it highly unlikely, as neurons after a 4-day treatment with TTX were still able to fire APs upon stimulation after wash out for 1 h of TTX. However, we failed to record spontaneous electrical activity in these conditions suggesting that the cellular mechanisms responsible for the regenerative activity were more sensitive to chronic treatment by TTX than triggered APs. However, a much longer wash out of TTX (4 days) seemed to allow a partial recovery of the downregulation of Ca^{2+} currents in Schwann cells. A simple explanation of this phenomenon would be that a partial recovery of neuronal spontaneous activity would induce de novo the expression of Ca^{2+} channels by mouse Schwann cells.

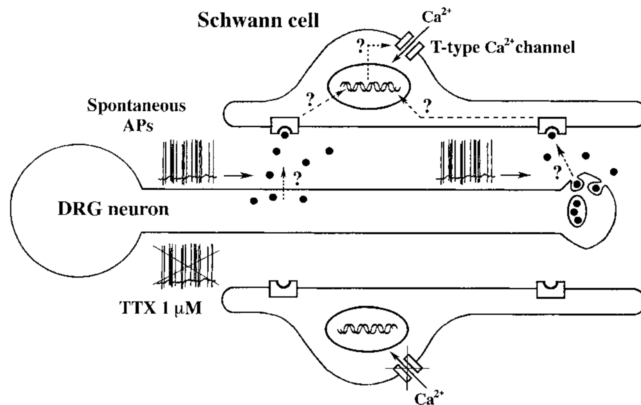

Fig. 4. Model of neuronal regulation of Ca^{2+} channel expression by Schwann cells. Diagram illustrating the upregulation of Ca^{2+} channel expression by a Schwann cell induced by a spontaneously active dorsal root ganglion (DRG) neuron (upper) and the downregulation in the presence of tetrodotoxin (TTX) (lower). It is hypothesized that spontaneous APs trigger the release of a diffusible factor (●) either along the axon or at the axonal tip, or both. This factor will bind on Schwann cell membrane receptors and will upregulate the genomic expression of the T-type Ca^{2+} channel. The intracellular pathways that link the membrane receptor and the genomic expression are unknown. When the spontaneous electrical activity is blocked by TTX, the factor is not released, and this pathway is not activated.

Model of Neuronal Regulation of Ca^{2+} Channel Expression by Schwann Cells

On the basis of our results, we propose the following scheme (Fig. 4). The spontaneous electrical activity fired by DRG neurons in vitro is responsible for the release of a diffusible factor which acts at a distance on Schwann cells. Our results do not identify the nature of the diffusible factor that upregulates Ca^{2+} channel expression by Schwann cells. Nevertheless, it is conceivable that it could be a neurotransmitter or a neuropeptide, whose secretion and or synthesis is regulated by electrical activity. The localization of its release could be along the axon and/or at the axonal tip. By acting on Schwann cell membrane receptors, the neuronal factor would upregulate Ca^{2+} channel expression by Schwann cells. Altering the neuronal electrical activity by TTX would suppress or strongly decrease the release of the factor and would lead to the downregulation of the genomic expression of Ca^{2+} channels.

Physiological Relevance of the Neuronal Regulation of Ca^{2+} Channel Expression by Schwann Cells

Spontaneous electrical activity of rat embryonic DRG neurons activity starts in vivo when the axon terminals reach the periphery and its occurrence is developmentally regulated, being recorded in 33% of DRG neurons at E17, 58% at E18, 37% at E19, and 21% at E20 (Fitzgerald, 1987). The frequency of the activity was low and irregular (<0.5 Hz) at early stages and increased with later stages of development (1–10 Hz at E19) (Fitzgerald and Fulton, 1992). Hence, spontaneous electrical activity described in vivo is similar to the activity we have recorded in vitro.

Low frequencies of electrical activity are known to be effective in regulating the expression of different genes and ion channels in DRG neurons in vitro. For example, stimulation at 0.5 Hz for 24 h causes prolonged downregulation of T-type Ca^{2+} channel currents in DRG neurons (Li et al., 1996). Furthermore, regulation of the expression of glial voltage-dependent ionic channels by neuronal activity has been recently reported. The expression of K_{IR}^{+} is upregulated by neuronal activity in satellite glial cells in mouse superior cervical ganglion (Konishi, 1996) and in nonmyelinating Schwann cells of sural and sympathetic nerves (Konishi, 1994). The author suggests that this regulation is linked to the clearance of K^{+} released in the extracellular space during neuronal activity.

It is therefore of interest to examine what could be the physiological relevance of a neuronal modulation of the expression of Ca^{2+} channels by Schwann cells. The functional relevance of Ca^{2+} channels in Schwann cells remains unclear but, assuming that their expression is regulated in vivo by DRG neurons, one function of these channels could be the role of a "sensor" of changes of neuronal electrical activity that occurs during embryonic development. The expression of Ca^{2+} channels by Schwann cells would permit significant elevation of intracellular calcium resulting in the initiation of myelination (Kirishuk et al., 1995, Demerens et al., 1996) or release of excitatory amino acids (Jeftinija and Jeftinija , 1998).

REFERENCES

Amédée T, Ellie E, Dupouy B, Vincent JD. 1991. Voltage-dependent calcium and potassium channels in Schwann cells cultured from dorsal root ganglia of the mouse. J Physiol (Lond) 441:35–56.

Beaudu-Lange C, Despeyroux S, Marcaggi P, Coles JA, Amédée T. 1998. Functional Ca and Na channels on mouse Schwann cells cultured in serum-free medium: regulation by a diffusible factor from neurons and cAMP. Eur J Neurosci 10:1796–1809.

Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C. 1996. Induction of myelination in the central nervous system by electrical activity. Proc Natl Acad Sci USA 93:9887–9892.

Fields RD, Yu C, Neale EA, Nelson PG. 1992. Chronic electrical stimulation of multicompartment cell cultures. In: Kettenmann H, Grantyn R, editors. Practical electrophysiological methods. New York: Wiley-Liss. p 67–76.

Fitzgerald M. 1987. Spontaneous and evoked activity of fetal primary afferents in vivo. Nature 326:603–605.

Fitzgerald M, Fulton PB. 1992. The physiological properties of developing sensory neurons. In: Scott SA, editor. Sensory neurons. New York: Oxford University Press. p. 287–306.

Itoh K, Stevens B, Schachner M, Fields RD. 1995. Regulated expression of the neural cell adhesion molecule L1 by specific patterns of neural impulses. Science 270:1369–1372.

Itoh K, Ozaki M, Stevens B, Fields RD. 1997. Activity-dependent regulation of N-cadherin in DRG neurons: differential regulation of N-cadherin, NCAM, and L1 by distinct patterns of action potentials. J Neurobiol 33:735–748.

Jeftinija SD, Jeftinija KV. 1998. ATP stimulates release of excitatory amino acids from cultured Schwann cells. Neurosci 82:927–934.

Kirischuk S, Scherer J, Kettenmann H, Verkhratsky A. 1995. Activation of P_2-purinoreceptors triggered Ca^{2+} release from $InsP_3$-sensitive internal stores in mammalian oligodendrocytes. J Physiol (Lond) 483.1:41–57.

Konishi T. 1994. Activity-dependent regulation of inwardly rectifying potassium currents in non-myelinating Schwann cells in mice. J Physiol (Lond) 474.2:193–202.

Konishi T. 1996. Developmental and activity-dependent changes in K^{+} currents in satellite glial cells in mouse superior cervical ganglion. Brain Res 708:7–15.

Lemaire S, Piot C, Seguin J, Nargeot J, Richard S. 1995. Tetrodotoxin-sensitive Ca^{2+} and Ba^{2+} currents in human atrial cells. Receptors Channels 3:71–81.

Li M, Jia M, Fields RD, Nelson PG. 1996. Modulation of calcium currents by electrical activity. J Neurophysiol 76:2595–2607.

Matsuda Y, Yoshida S, Yonezawa T. 1978. Tetrodotoxin sensitivity and Ca component of action potentials of mouse dorsal root ganglion cells cultured in vitro. Brain Res 154:69–82.

Miletic V, Lu GW. 1993. Characteristics of action potentials recorded from cat spinal ganglion neurons in vivo. Brain Res Bull 31:531–538.

Scholz A, Appel N, Vogel W. 1998. Two types of TTX-resistant and one TTX-sensitive Na^{+} channel in rat dorsal root ganglion neurons and their blockade by halothane. Eur J Neurosci 10:2547–2556.

Varon S, Raiborn C. 1971. Excitability and conduction in neurons of dissociated ganglionic cell cultures. Brain Res 30:83–98.

Yoshida S, Matsuda Y, Samejima A. 1978. Tetrodotoxin-resistant sodium and calcium components of action potentials in dorsal root ganglion cells of the adult mouse. J Neurophysiol 41:1096–1106.