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Satellite Glial Cells *In Situ* within Mammalian Prevertebral Ganglia Express K^+ Channels Active at Rest Potential

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Abstract. Patch-clamp experiments were performed on satellite glial cells wrapped around sympathetic neurons in the rabbit coeliac ganglion. With the cleaning method used, the glial cells could be kept in place and were directly accessible to the patch-clamp pipettes. Whole-cell recordings showed that glial cells had almost ohmic properties. Their resting potential (-79.1 ± 1.2 mV) was found to be very nearly the same as the K^+ reversal potential and $\approx$20 mV more negative than that of the neurons they encapsulated. Unitary currents from ionic channels present in the glial membrane were recorded in the cell-attached configuration with pipettes filled with various amounts of K^+, Na^+ and gluconate. Only K^+-selective channels with slight inwardly rectifying properties (in the presence of 150 mM $[K^+]_o$) were detected. These channels were active ($P_o = 0.7$–0.8) at the cell resting potential. The channel conductance, but not its opening probability, was dependent on the $[K^+]$ in the pipette. Cl^--selective channels (outwardly rectifying and large conductance channels) were detected in excised patches.

The properties of the K^+ channels (increased inward current with $[K^+]$ and detectable outward current at low $[K^+]$) are well suited for siphoning the K^+ released by active neurons.

Key words: Satellite glial cells — Sympathetic neurons — Inward rectification — K^+ channels

Introduction

Since their discovery in 1846, glial cells have been investigated in many studies but little is known so far about their functional role (Somjen, 1988). The hypotheses most commonly put forward can be summarized as follows: mechanical support and electrical isolation of neurons, secretory role, nutritive function, modulation of the action of neuromediators, regulatory action on the ionic composition of the extracellular space (Kuffler, 1967). Concerning the last of these hypotheses, Orkand, Nicholls and Kuffler (1966) discovered that glial cells had a high potassium conductance. These authors suggested that potassium ions driven in the extracellular space by the neuronal activity could enter glial cells, thus preventing the neurons from depolarizing.

The patch-clamp technique has made it possible to record potassium channel activity in glial cells, and many studies have been devoted to determining whether their characteristics might fit the concept of potassium uptake by the glial cells (*see reviews* by Barres, Chun & Corey, 1990; Ritchie, 1992). It should be pointed out that these studies have been mainly performed on cultured or acutely isolated glial cells. Although the channels detected in cells examined just after isolation are surely present *in situ*, this is not necessarily the case for those collected on cultured glial cells. For this reason, "the danger exists that glial cell physiology is developing into a physiology of cultured cells" (Walz & Mac Vicar, 1988). A few studies dealing with the characteristics of unitary and whole-cell currents have been carried out in glial cells kept in place in the frog optic nerve (Marrero et al., 1989, 1991) and mammalian brain slices (Berger, Schnitzer & Kettenmann, 1991; Berger et al., 1992; Steinhäuser et al., 1992).

A successful attempt was recently made to apply patch-clamp techniques to nondissociated neurons in mammalian sympathetic ganglia (Gola et al., 1992). With this technique it was also possible to

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record the activity of glial cells *in situ*, that is satellite glial cells wrapped around the nerve cell body of ganglionic neurons.

The present study was performed on the rabbit coeliac ganglion using this original version of the patch-clamp technique. The aim here was twofold: (i) to assess the electrical properties of the glial cells *in situ*, and (ii) to record the activity of potassium channels.

Preliminary accounts of some of these results have been published elsewhere (Gola & Niel, 1990).

Materials and Methods

Experiments were performed on coeliac ganglia from 62 rabbits. The ganglia were dissected out and pinned in a Sylgard-coated petri dish continuously perfused (at 5 ml/min) with a modified Krebs saline having the following composition (in mM): 120 NaCl, 5 KCl, 1 NaH_2PO_4, 2.5 $CaCl_2$, 1 $MgSO_4$, 25 $NaHCO_3$ and 11 glucose. The solution was bubbled with a 95% O_2/5% CO_2 gas. The connective tissue covering the ganglia was softened with proteolytic enzymes (5 mg/ml proteases type XIV from Sigma) for 5 min. A selected ganglionic region was then mechanically cleaned with a gentle stream of Ringer saline for 1.5–2 hr. The cleaning procedure has been previously described (Gola et al., 1992). The ganglion was transilluminated with optic fibers and examined with an Olympus SZ stereomicroscope (magnification: 120–150×).

Electrophysiological recordings were obtained using patch-clamp electrodes in either the cell-attached or whole-cell configurations. Patch pipettes were pulled in 3–4 steps with a P-87 Flaming-Brown puller (Sutter Instrument, Novato, CA). When filled with a KCl-rich (150 mM) saline, they had tip resistances of 4–10 MΩ. They were moved to the cells using a hydraulic micromanipulator (Narishige model). Voltage and current were recorded with a List EPC7 amplifier. Most of the experiments were carried out at room temperature (20–22°C) and a few (n = 7) at 35–36°C. All the illustrations are from experiments performed at 20–22°C.

Pipettes were filled with a KCl-rich saline containing 150 mM KCl, 1 mM $MgCl_2$, and 5 mM Tris (pH 7.5). The Ca content corresponded to that of either the bath saline (2.5 mM) for recording in the cell-attached configuration, or the intracellular milieu (5 mM EGTA, 5 mM $CaCl_2$; pCa ≈ 7) for whole-cell recording. In some experiments, KCl was replaced (in equimolar amounts) by either NaCl or gluconate (K salt).

In a previous paper (Gola & Niel, 1993) we demonstrated that a +9 mV junction potential was created when KCl-filled pipettes were used in whole-cell recordings. The intracellular recordings were therefore corrected by positively shifting the zero voltage base line (by 9 mV).

Some ganglia were conventionally processed (*see* Gola et al., 1992) for examination with a Jeol scanning electron microscope and a Siemens Elmiskop 101 electron microscope.

Results

Electrophysiological Identification of Glial Cells

Patch-clamp experiments were performed on visually identifiable superficial neurons that seemed to be cleared of connective tissue. Once seals in the gigaohm range had been achieved, the patch membrane was ruptured by applying either suction or a large (150–180 mV) hyperpolarizing pulse to the pipette. In about 30% of the trials, the pipette entered a structure characterized by a large resting potential and the absence of spikes in response to depolarizing current pulses. The original experiment that led us to assume that the patch pipette had entered glial cells enveloping ganglionic neurons is described in Fig. 1.

After the membrane patch rupture, a large negative pressure was applied to the voltage recording pipette. This resulted first in the transient return of the recording towards the zero baseline and then to voltage changes that were undoubtedly generated by neurons. The duration of the recording close to the zero baseline level was highly variable. In most cases, it was immediately followed by the neuronal signals. To account for this sequence, it was assumed that the electrode became sealed on and then entered a glial cell, crossed the neuron-glial space, and finally penetrated a neuron.

The ganglia on which most of the patches were a priori performed in glial cells were examined with the electron microscope. In these ganglia, the superficial neurons observed with a scanning microscope had a granular appearance (Fig. 2*A*), which contrasted with the smooth aspect of neurons that were directly accessible by the patch electrodes (*see* Fig. 1 in Gola et al., 1992). Observation with a transmitted electron microscope showed that the granular surface was due to the presence of glial cells characterized by their large, electron-dense nucleus. The glial cells gave off thin cytoplasmic layers (≈1 μm thick) which covered the neuron. The glial cell and neuron membranes were either intimately associated or separated by lacunar spaces. The shift to the zero baseline that occurred upon applying strong suction to the patch pipette in Fig. 1, presumably corresponded to the transient passage through these lacunar intercellular spaces.

Comparative Membrane Properties of Ganglionic Neurons and Glial Cells

The patch electrodes sealed easily and firmly to glial cells. The sealing occurred either spontaneously upon releasing the pressure applied to the pipette or with the aid of a slight suction. The seal resistance was routinely >20 GΩ. Sealing to the neuronal membrane required larger negative pressures and had a lower electrical resistance.

Data concerning the passive properties of both cell types are illustrated in Fig. 3. They were ob-

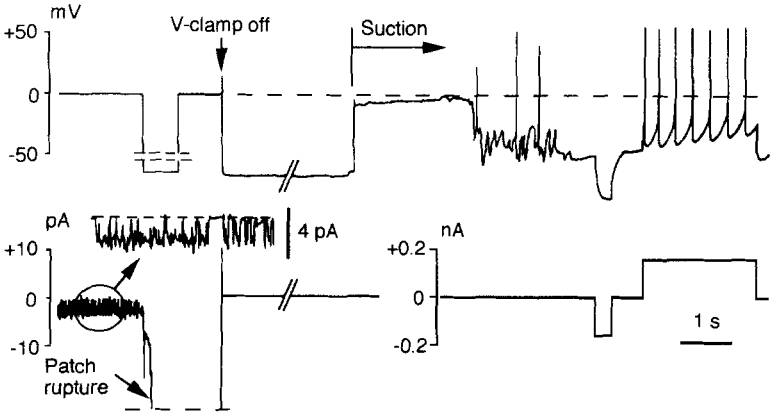

Fig. 1. Electrophysiological tests demonstrating the presence of glial cells. At the beginning of the experiment, the patch electrode (filled with 150 mM KCl) was sealed to a membrane containing one channel. Upper trace: patch potential; lower trace: patch current. In the voltage-clamp mode (first part of the recording), voltages are expressed as the inverse values of the patch pipette voltage. The membrane patch was then ruptured by applying a large (>150 mV) hyperpolarizing pulse. The electrode recorded the resting potential (−69 mV) from an inexcitable cell. A strong persistent suction was then applied to the pipette. The pipette crossed the glial cell and then entered the neuron.

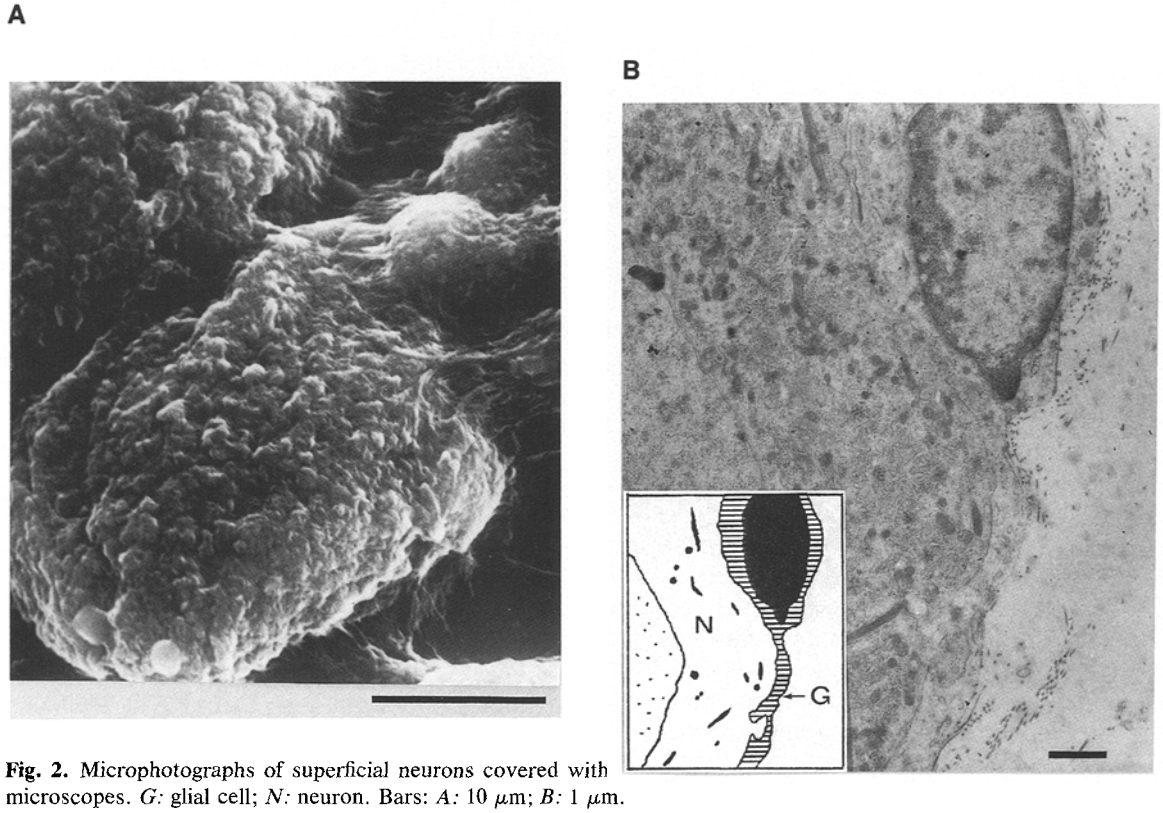

Fig. 2. Microphotographs of superficial neurons covered with [illegible]) electron microscopes. *G:* glial cell; *N:* neuron. Bars: *A:* 10 μm; *B:* 1 μm.

tained from whole-cell recordings performed either on glial cells or on neurons superficially devoid of glial cells. These neurons were either silent or spontaneously firing (Gola & Niel, 1993). The data reported below were restricted to silent neurons. In addition to the absence of action potentials, glial cells differed from neurons in two main respects: glial cells had a high resting potential and a comparatively low input resistance. Recordings performed in cells of the two types are illustrated in Fig. 3*A*.

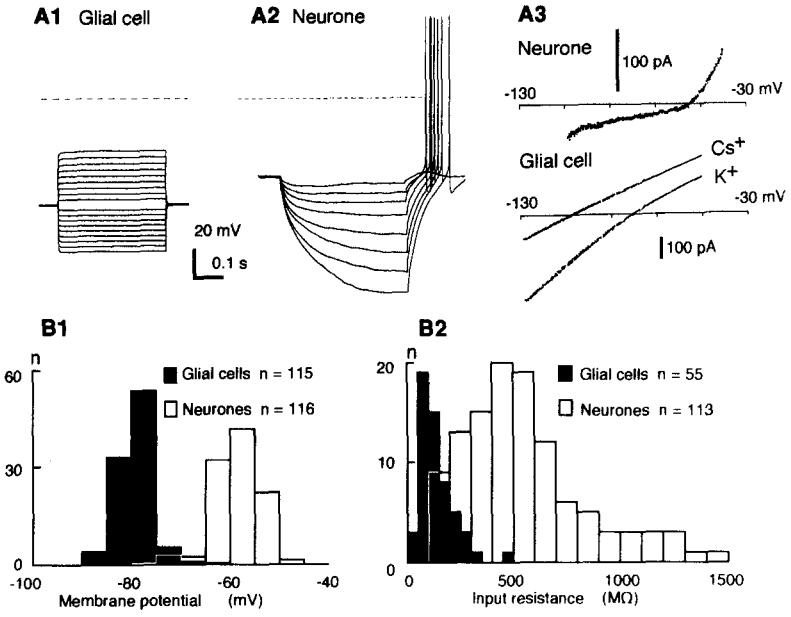

Fig. 3. Comparative membrane properties of sympathetic neurons and their satellite glial cells. (*A*) Whole-cell recordings from a glial cell (*A1*) and a ganglionic neuron (*A2*). Dashed line: 0 mV. Resting potential: glial cell: −78 mV; neuron: −57 mV. Voltage changes were induced by current pulses: +360 to −360 pA (40 pA step) in *A1;* −20 to −160 pA (20 pA step) in *A2*. (*A3*) Quasi steady-state current-voltage curves obtained by slowly depolarizing (10 mV/sec) the cells in the voltage clamp mode from −110 to −40 mV (neuron) and from −130 to −50 mV (glial cell). The glial cell recordings were performed with 5 mM K^+ in the bath (K^+) and after substituting 5 mM Cs^+ for K^+ (Cs^+). (*B*) Distribution of resting potentials (*B1*) and input resistances (*B2*) of neurons and glial cells. Bin: *B1:* 5 mV; *B2:* 50 and 100 MΩ for glial cells and neurons, respectively.

Under whole-cell current clamp conditions, glial cells responded to current pulses by showing square-shaped voltage changes (Fig. 3*A1*), whereas neurons displayed slow capacitive changes (Fig. 3*A2*). The steady-state current-voltage relationship was obtained by applying slowly rising ramps in the voltage-clamp mode (Fig. 3*A3*). Glial cells had an *I-V* curve which displayed a moderate inward rectification (rectification ratio, 1.2–1.9), whereas that of neurons displayed both inward and outward rectifications. The inward rectification in glial cells was abolished when Cs^+ (5 mM) was either added to the bath or substituted for K^+ in the bath saline. The K^+-free Cs^+-containing saline hyperpolarized the glial cells by 25–30 mV.

Stimulating either preganglionic splanchnic or postganglionic efferent nerves with single shock (10–20 V, 0.5 msec) did not modify the glial cell resting potential. Repetitive nerve stimulation (10 Hz, 5–10 sec) resulted in 2–4 mV slow depolarizations in 20% of the glial cells tested. The stimulation-induced activity of the underlying neuron was occasionally recorded by the intraglial pipette as 1–2 mV fast capacitive transients.

The resting potential of glial cells was -79.1 ± 1.2 mV ($n = 115$, Fig. 3*B*). That of silent ganglionic neurons was -59.2 ± 1.5 mV ($n = 116$). The input resistance deduced from the slope of the *I-V* curves was 137 ± 12 MΩ ($n = 55$) in the case of glial cells and 547 ± 27 MΩ ($n = 113$) in that of neurons. The glial cell input resistance at 35–36°C was 106 ± 10 MΩ, which gave a temperature sensitivity coefficient of $Q_{10} = 1.2$. The mean time constant of the neuron voltage change in response to hyperpolarizing current pulses was 125 msec. As mentioned above, glial cells had very fast voltage changes, whose time constant was probably less than 5 msec. From the input resistances (R_{in}) and time constants (τ), we calculated that the membrane capacity ($C = \tau \cdot R_{in}^{-1}$) of neurons was 240 pF and that of glial cells less than 40 pF. Assuming that both cell types had a specific membrane capacity of 1 $\mu F\ cm^{-2}$ (10^{-2} pF μm^{-2}), the corresponding surface (S) was: glial cells: $S = 4\ 10^3\ \mu m^2$; neurons: $S = 24\ 10^3\ \mu m^2$. The specific membrane resistance ($S \cdot R_{in}$) would therefore be $\approx 10^5\ \Omega\ cm^2$ in the case of neurons and $\approx 5\ 10^3\ \Omega\ cm^2$ in that of glial cells. Glial cells therefore appeared to have a low specific membrane resistance. This finding is in line with the data showing that they are densely endowed with K^+-selective channels (*see below*).

Glial K^+-Selective Channels

The channels present in the membrane of glial cells were identified as described in Fig. 1, i.e., by first recording the currents from cell-attached patches and by then identifying the cell upon patch rupture. Since we were primarily interested in the properties of K^+-selective channels, most of the experiments were performed with patch electrodes containing isotonic KCl.

Glial cells were found to have several types of K^+ channels. The channel most commonly observed is illustrated in Fig. 4*A*. This channel had a charac-

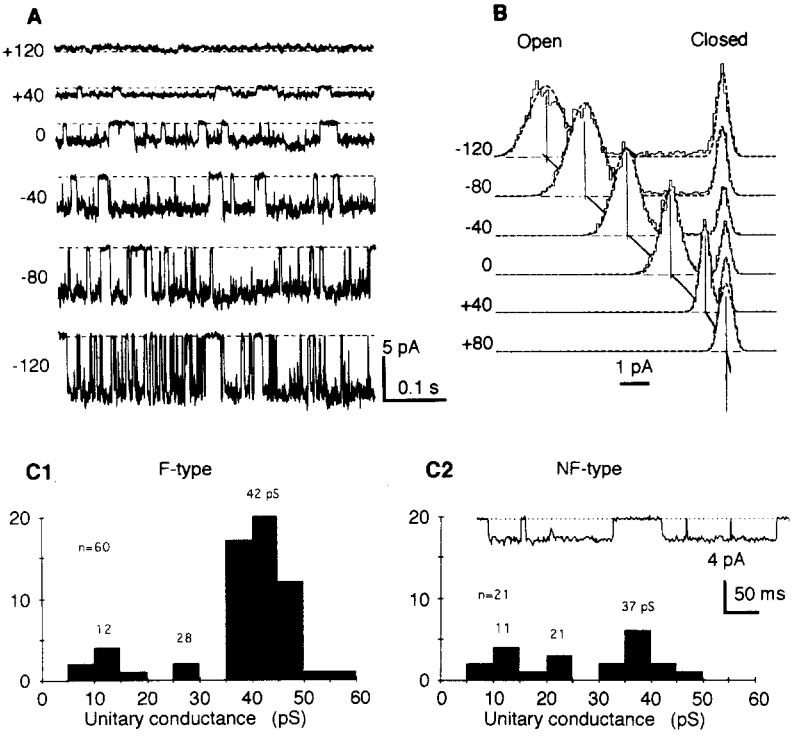

Fig. 4. Cell-attached K^+ channels in glial cells. (*A*) Unitary currents from a cell-attached patch. Pipette filled with 150 mM KCl. Voltages are expressed as changes from the cell resting potential (inverted voltage applied to the pipette). Closed states are indicated by dashed lines. (*B*) Amplitude histograms from 10 sec recordings. Same experiment as in *A*. The sharp peaks corresponding to the closed state were aligned. Histograms were spaced vertically according to their voltage. Distributions were fitted to Gaussian functions (dashed lines superimposed on the experimental traces). Bin: 0.1 pA. The open state displayed a characteristic Gaussian noise, particularly at large hyperpolarizations. The unitary channel conductance at negative voltages was 42 pS. (*C*) Distribution of unitary conductances (measured at negative potentials) of inwardly rectifying channels with (F-type) or without (NF-type) fast flickering. The recording in C2 was obtained on a 37 pS NF-type channel. Data from experiments with pipettes filled with 150 mM KCl.

teristic noise in the open state. The fact that it was never observed in neurons helped to immediately identify the glial membrane. The channel current at negative potentials corresponded to a unitary conductance of 42 pS (slope of the linear part of the current-voltage relation). The amplitude histograms in Fig. 4*B* show that the channel was open at the cell resting potential (0 mV applied to the pipette) and that the opening probability was slightly voltage dependent, with a pronounced fast flickering at negative voltages. The histograms also show the large Gaussian noise that occurred when the channel opened. Reversal of the current direction occurred at 80 mV patch depolarization, which corresponded almost to the cell resting potential. In addition, large depolarizations induced tiny outward currents (slope conductance: 12–14 pS), i.e., the channel was inwardly rectifying (rectification ratio ≈3).

In addition to the 42 pS channel, several other K^+ channels were observed. All these channels had inwardly rectifying properties. They differed in the size of the unitary conductance (measured at negative voltages) and in the presence (F-type such as the 42 pS channel) or absence (NF-type) of fast flickering in response to hyperpolarization. The unitary conductance distribution of both F-type and NF-type channels (Fig. 4*C*) suggested that they might constitute the same population working under different conditions. The main point was that all these channels had a large opening probability at the cell resting potential.

Several 42 pS F-type channels were generally present in most patches, so that they accounted for >90% of the K^+ current. A detailed analysis of the dynamic properties of the channel was performed on eight patches that contained only one channel. The results are given in Fig. 5. The open times distribution was fitted to a single exponential, whose time constant was reduced by hyperpolarization (from 18 msec at 0 mV to 12 msec at −120 mV). The closed time also had a single exponential distribution; its time constant (7–11 msec) was found to be slightly voltage dependent. In this analysis we have omitted periods during which the channels remained quiescent. Long-lasting closures occurred in some patches; they did not appear to obey a random process.

The change in the mean open time upon hyperpolarization resulted in an increase in the number of openings and closings per second (flickering appearance) and in a decrease in the opening probability. At the cell resting potential, the opening probability (P_o) was 0.73 ± 0.06. The $P_o(V)$ curve was fitted to Boltzmann equations with a slope factor of 40–50 mV. With 150 mM KCl-filled pipettes, no data on P_o at depolarized levels were available owing to the smallness of the corresponding unitary current. Pipettes filled with low [K^+] (20 mM KCl, 130 mM NaCl) increased the size of the outward currents (*see* Fig. 6), from which it was possible to extend the $P_o(V)$ curve to positive potentials. The results showed that P_o remained at a high level (0.7–0.8,

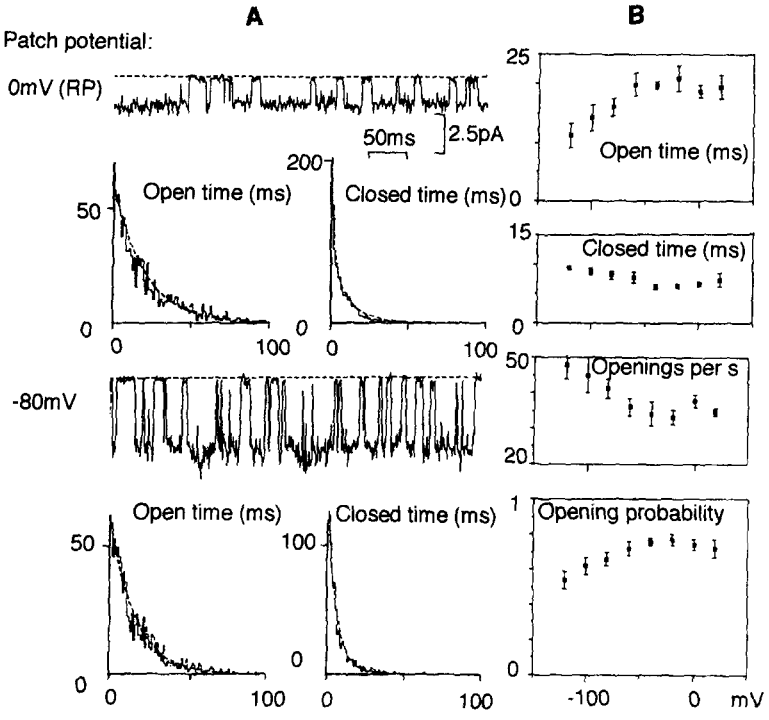

Fig. 5. Dynamic properties of the 42 pS K^+ channel. (*A*) Distribution of open times and closed times at 0 mV and −80 mV. The open times and closed times were fitted to single exponential functions having the respective time constants, 19.6 and 11 msec at 0 mV, 15 and 7 msec at −80 mV. Bin: 1 msec; 1123 events in 44 sec at 0 mV; 944 events in 24 sec at −80 mV. (*B*) Voltage dependence of the mean open time, mean closed time, number of openings/closings per sec and opening probability. Data pooled from eight experiments except for the opening probability (n = 15). Voltage referred to the cell resting potential.

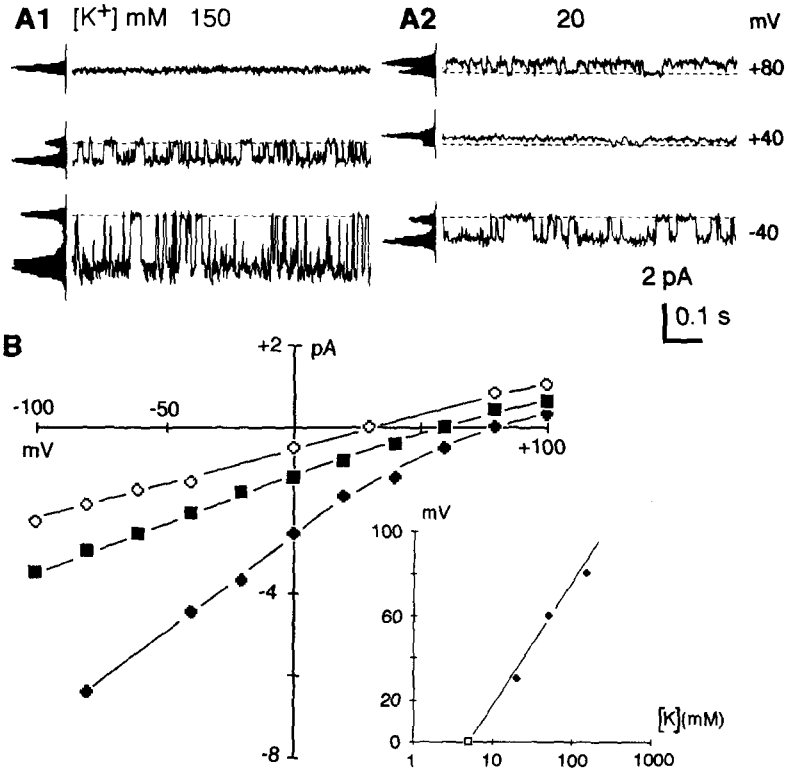

Fig. 6. K^+ selectivity of the 42 pS channel. (*A*) Cell-attached channel recordings. Patch pipette filled with 150 mM KCl (*A1*) and 20 mM KCl, 130 mM NaCl (*A2*). Amplitude histograms are attached to the current recordings. The current reversed direction at +80 mV in *A1* and +32 mV in A2. (*B*) Unitary current-voltage relationships. Patch performed successively on the same glial cells. Pipettes contained 150 mM KCl (filled diamonds), 50 mM KCl (filled squares) and 20 mM KCl (open diamonds); change in osmolarity compensated for with NaCl. Inset: current reversal potential as a function of $[K^+]$ in the pipette. The reversal potential was expressed relative to the cell resting potential (−81 mV, open square at $[K^+]$ = 5 mM).

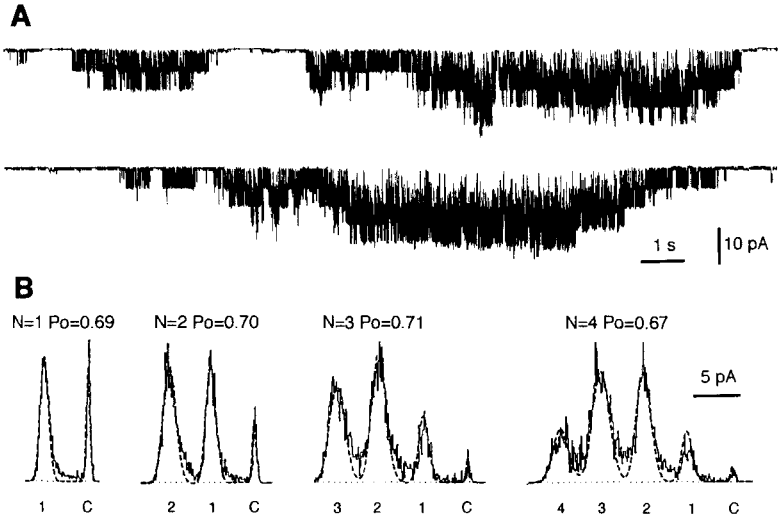

Fig. 7. Synchronous opening of clustered cell-attached K^+ channels. (*A*) Continuous recording (75 sec) from a cell-attached patch containing four 42 pS K^+ channels. Patch potential: −40 mV. Pipette filled with isotonic KCl. (*B*) Amplitude histograms from periods with 1–4 simultaneous channel openings. The experimental histograms were fitted to the distribution (dotted curves) predicted for N (1–4) independent channels having the individual opening probability P_o indicated. (*C*) Closed state; 1–4: simultaneous opening of 1–4 channels. Bin: 0.1 pA.

$n = 7$) at the cell resting potential as well as upon depolarization (up to 120 mV).

The K^+ selectivity of the channels was assessed by changing the K^+ content of the pipette saline. The two series of recordings in Fig. 6*A* were obtained in the same glial layer with a pipette filled with 150 mM KCl (Fig. 6*A1*) and 20 mM KCl–130 mM NaCl (Fig. 6*A2*). In this experiment, 14 successive glial patches were obtained with pipettes containing either 20, or 50, or 150 mM KCl. The corresponding *I-V* curves are given in Fig. 6*B*. The reversal potential of the unitary current shifted by the predictable amount when dealing with K^+-selective channels (inset in Fig. 6*B*). In three experiments gluconate was used instead of chloride, which did not affect the current reversal potential.

The unitary current at negative voltages decreased with the K^+ content of the pipette. With 20 mM potassium, the unitary conductance of the 42 pS F-type channel decreased to 15 pS. The overall effect of low $[K^+]$ was therefore to consistently reduce the inward rectification. Although the channels had intrinsic rectifying properties (as revealed under symmetrical KCl conditions), the current behaved almost linearly under physiological conditions. This behavior reflected the fact that glial cells have almost ohmic whole-cell properties in spite of the high density of their inwardly rectifying K^+ channels.

The inset in Fig. 6*B* shows the change in the unitary current reversal potential (relative to the cell resting potential) with the K^+ content of the pipette. The straight line through experimental points had a slope of 58 mV for a tenfold change in $[K^+]$. These results indicated that the channels were highly selective to K^+ ions (as compared with Na^+ and anions). That the cell resting potential (0 mV at $[K^+]$ = 5 mM) was located on the regression line showed that the glial cell membrane was highly and almost exclusively permeant to K^+ ions and that the cell was therefore clamped at a level close to the K^+ equilibrium potential.

Synchronous Opening of Clustered K^+ Channels

Most of the patches contained several K^+ channels generally of the same type. We observed that clustered K^+ channels did not behave independently. Simultaneous openings occurred in bursts (Fig. 7*A*), which suggests that there existed either a common gating mechanism or a positive cooperativity between channels. Analysis of the opening probability when several channels were active failed to reveal the existence of any cooperativity in channel opening. The amplitude histograms in Fig. 7*B* were obtained during periods at which 1–4 channels from a 4-channel-containing patch opened simultaneously. They were fitted to the distribution predicted for N (N = 1–4) independent identical channels. The result showed that the individual opening probability remained unaltered when the number of active channels varied and that the active channels behaved independently. The burst process, therefore, did not depend on the properties of the closed-open transition or on the existence of interactions between channels. Rather, it probably involved a common mechanism, which may be metabolically regulated, that switches the channels from the silent to the active state. Once in the active state, the channels may open and close independently at a rate governed by the cell polarization.

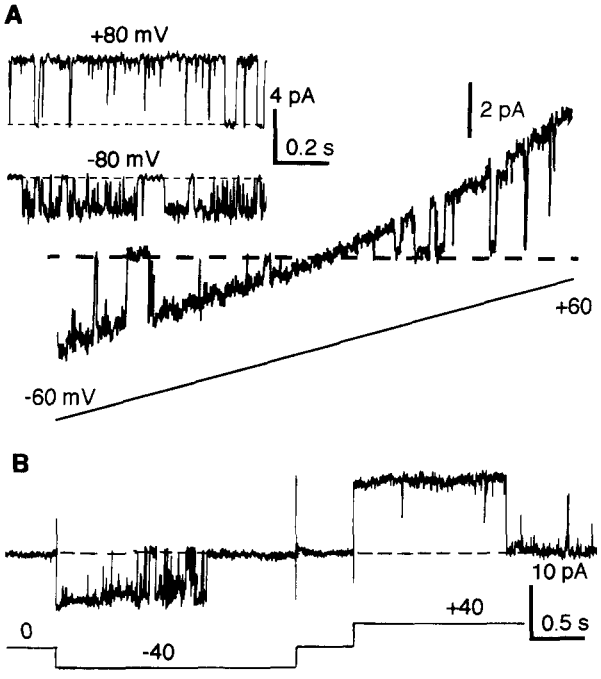

Fig. 8. Anion channels in excised outside-out patches. (*A*) Outwardly rectifying anion channel. Unitary current in response to a slowly changing voltage (20 mV/sec). The current reversed direction at 0 mV. Inset: current recordings at +80 and −80 mV. (*B*) Large conductance anion channel. The channel closed on shifting the patch to either positive or negative voltages. Its unitary conductance was 320 pS. In *A* and *B* the patch pipette was filled with the KCl-rich saline and the patch was bathed in physiological saline.

Excised Patches

Our attempts to excise channels in the inside-out configuration were mostly unsuccessful. Shortly after excision, the unitary currents became saw-shaped, which indicated that a small membrane vesicle had formed at the tip of the pipette. Several passages at the air-bath interface failed to disrupt the vesicle and finally destroyed the patch. This behavior, together with the ease with which the pipette could be sealed to glial cells, indicated that the bilayer had its own dynamics. This was a consistent impediment, however, to studying the channel properties and regulation.

Outside-out patches were easier to obtain from the whole-cell configuration. None of the K^+ channels observed in the cell-attached mode remained active after excision, which confirmed the dependence of the channels on intracellular components. Outside-out patches were found to contain anionic channels. Whatever the cationic content (K^+ or Na^+) of the pipette and bath saline, these channels produced currents that reversed at 0 mV. The reversal potential shifted positively when the bath chloride was changed to gluconate.

Two anionic channels were present: an outwardly rectifying channel and a large conductance (320–350 pS) channel. The outwardly rectifying channel gradually became visible after excision. Its unitary conductance was 89 ± 6 and 43 ± 5 pS (n = 9) at positive and negative potentials, respectively (Fig. 8*A*). The channel opening probability was not found to depend on the voltage, although flickering was induced on hyperpolarization. The large conductance anionic channel was observed after stepping the patch to large polarizations. It displayed erratic behavior, apparently characterizing the occurrence of numerous substates. Once activated, the channel had a high opening probability at voltages of almost 0 mV. Step changes of 30–40 mV in either direction inactivated the channel; the inactivation was readily relieved on returning to 0 mV.

Discussion

This study demonstrates the properties of ion channels in satellite glial cells *in situ* within mammalian prevertebral sympathetic ganglia.

Three major cell types are to be found in prevertebral ganglia: the sympathetic neurons, the chromaffin SIF (small intensively fluorescent) cells and the satellite glial cells. Satellite cells encapsulate the neurons and SIF cells and the processes of both cell types. The electrophysiological properties of the neurons have been extensively characterized. Much less is known in comparison about the SIF and satellite cells. Concerning the glial cells, in studies on ganglionic neurons, glial cells were occasionally impaled with intracellular microelectrodes (Crowcroft & Szurszewski, 1971).

It has been recognized that satellite cells have a high resting potential, are inexcitable and do not exhibit synaptic potentials in response to ganglionic nerve stimulation. Our whole-cell patch-clamp study confirms and extends these findings. The resting potential of the satellite cells occurred at −79 mV, a value which is very similar to the K^+ equilibrium potential as determined from the reversal potential of K^+-selective channels present in their membrane. There existed a 20 mV difference between satellite cells and the neurons they encapsulate. Unlike neurons, glial cells have almost ohmic input resistance.

Glial K^+ Channels

All the cell-attached channels we identified in glial cell membranes were selective to K^+ and displayed

inwardly rectifying properties with 150 mM KCl in the pipette. We did not attempt to identify the other channel types. Nevertheless, the experiments performed with pipettes containing various amounts of KCl or NaCl failed to reveal the presence of voltage-gated K^+ channels of the delayed rectifier type, or that of Na^+ and Cl^- channels.

The K^+ channels present in glial cells have been thoroughly documented (*review by* Barres et al., 1990; Chiu, 1991; Ritchie, 1992). These channels have been detected in either whole-cell currents or at the single level in cultured or acutely dissociated cells: astrocytes (Barres, Chun & Corey, 1988), oligodendrocytes (Kettenmann et al., 1982; Kettenmann, Orkand & Lux, 1984; Barres et al., 1988, Soliven et al., 1988), Schwann cells (Wilson & Chiu, 1990) and retinal Müller cells (Brew et al., 1986; Nilius & Reichenbach, 1988).

With 150 mM KCl, the most representative K^+ channel we observed had a unitary conductance of 42 pS and a high opening probability at the cell resting potential. These data raise two questions that are directly relevant to the role this channel may play in glial function: (i) why, despite the unique presence of inward rectifiers, do glial cells display only slight inward rectification under whole-cell voltage clamp conditions? and (ii) are the inward rectifiers open at normal $[K^+]$?

Two facts contribute to giving glial cells *in situ* their almost linear characteristics. (i) Extensive electrical coupling exists between most glial cells (except Schwann cells), which reduces the apparent cell input resistance by allowing the injected current to spread into the glial syncytium (Kuffler, Nicholls & Orkand, 1966). The inward whole-cell current that persisted when Cs^+ was substituted for K^+ in the bath (*see* Fig. 3) presumably flowed into the neighboring glial cells. Dye coupling has been observed in autonomic ganglia (Hanani et al., 1989). Acutely dissociated glial cells from various nervous structures have input resistances which are one order of magnitude greater than those we measured (*see* Barres et al., 1990). (ii) The rectification ratio through the inwardly rectifying channels was found to be consistently reduced by lowering the K^+ content of the electrode. The rectification was almost suppressed at $[K^+] = 20$ mM.

The 42 pS channel had a high opening probability at the cell resting potential in the presence of isotonic KCl. The voltage dependence of a classical inward rectifier depends strongly, however, on $[K^+]_o$, which raises questions about the state of the channel in the presence of physiological $[K^+]$. Several lines of evidence indicate that the 42 pS channel is not a classical inward rectifier. The voltage dependence of its opening was rather low, increasing *e*-fold for 40–50 mV depolarization; it did not shift when $[K^+]$ was raised from 20 to 150 mM. At the cell resting potential, the opening probability was not sensitive to $[K^+]_o$. These properties contrast with those of a classical inward rectifier whose opening probability decreased (instead of increasing) *e*-fold for 5–10 mV depolarization.

The 42 pS channel had a number of properties in common with the K^+ channels characterized by inwardly rectifying properties detected in retinal glial cells (Brew et al., 1986), in cultured oligodendrocytes (Soliven et al., 1988; McLarnon & Kim, 1989) and in mammalian Schwann cells (Chiu, 1991): high opening probability at the cell resting potential, moderate voltage dependence of the opening, flickering at hyperpolarized levels and channel conductance dependence on $[K^+]_o$.

Functional Aspects

It has long been suggested that glial cells do take up K^+ released by active neurons into the intercellular space. Subsequent to the "spatial buffering" model by Orkand et al. (1966), several mechanisms have been put forward (*reviewed by* Barres et al., 1990, Ritchie, 1992). The discovery of inwardly rectifying K^+ channels in astrocytes and oligodendrocytes has substantiated the idea that K^+ after accumulating in the intercellular space passively enters the glial cells (McLarnon & Kim, 1989). It was suggested that this entry might be counterbalanced by either potassium exit from remote areas or chloride entry through Cl^--selective channels.

The excised chloride channels we have identified were silent in cell-attached patches. The excision-activation of the large Cl^- channel and the outwardly rectifying Cl^- channel is a basic property which is common to various cell types including the glial ones (Gray, Bevan & Ritchie, 1984; Gray & Ritchie, 1986; Barres et al., 1988), muscle cells and epithelia. It has been hypothesized that silent Cl^- channels in oligodendrocytes may be activated by a signal emanating from the active nerve cell. We were unable to activate cell-attached Cl^- channels by applying repetitive nerve stimulation that proved to efficiently fire the ganglionic neurons both synaptically and antidromically.

The properties of the 42 pS channel suggest that the same channel may be involved in both the entry and exit of K^+ ions. During neuronal activity, the channels located in front of the neuron work in the presence of elevated $[K^+]_o$, which increases the inward rectification ratio. The increase in the $[K^+]_o$ would make the local K^+ equilibrium potential more positive than the glial resting potential. Both events

may drive extracellular K^+ into the glial cell down its electrochemical gradient. In the opposite membrane, the K^+ channels are bathed in low $[K^+]_o$. Under these conditions, the inward rectification is almost suppressed and since the channel also has an outward current conductance, K^+ ions can leave the glial cell.

The rationale for this hypothesis is based on only one type of K^+ channels being present here and on the channel individual properties. The channels were detected in the external glial membrane, however. The possibility cannot be ruled out that segregation of channels may also occur in satellite cells as previously demonstrated in Müller cells (Newman, 1984; Brew et al., 1986).

One of the key questions about the function of glial channels concerns their mode of regulation by the underlying neurons. The occurrence of the synchronous openings we observed in multi-channel patches suggests that the K^+ channels are subject to metabolic control. Further studies on on-neuron glial cells should reveal whether or not these regulations are correlated with the neuronal activity.

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