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Single Potassium Channels of Human Glioma Cells

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Summary

Single potassium channels in the membrane of human malignant glioma cells U-118MG were studied using the technique of patch clamp in cell-attached and inside-out configurations. Three types of potassium channels were found which differed from each other under conditions close to physiological in their conductance and gating characteristics. The lowest-conductance channel (20 pS near the reversal potential) showed a mild outward rectification up to 45 pS at positive voltages and spontaneous modes of high and low activity. At extreme values of potentials its activity was generally low. The intermediate conductance channel had an S-shaped I-V curve, giving a conductance of 63 pS at reversal, and a low and voltage independent opening probability. The high-conductance (215 pS) channel was found to be activated by both membrane potential and Ca^{2+} ions and blocked by internal sodium at high voltages. The current-voltage curves of all three channel types displayed saturation.

Key words
Glial cells – Potassium channels – Patch clamp

Introduction

A well-recognized function of glial cells in the central nervous system is to manage the extracellular concentration of potassium ions and so to keep or modulate the activity of neurones (Orkand 1977, 1987). In accordance with this function, potassium conductance was found to be dominant in glial cells (Kettenmann *et al.* 1983, Bevan and Raff 1985, Brew *et al.* 1986, Newman 1986, 1988, Brismar and Collins 1988), although other types of ionic channels were also identified (MacVicar 1984, Bevan *et al.* 1984, 1985, Shrager *et al.* 1985, Gray and Ritchie 1985, Chiu 1987, Ritchie 1987).

Currently, the picture of single channel K^+ currents in glial cells is far from being definite and studies on cells of different origin are required. Because of the complex geometry and small dimensions of glial cells, the technique of patch clamp (Sakmann and Neher 1983) is of great value in their study. Potassium channels varying in their conductance, pharmacology, voltage- and calcium-dependence were identified using both single-channel (Kettenmann *et al.* 1984, Geletyuk and Kazachenko 1984, Leech 1986, Brew *et al.* 1986, Sonnhof and Schachner 1986, Quandt and MacVicar 1986, Nowak *et al.* 1987, Nilius and Reichenbach 1988, Leech and Moreton 1988) and whole-cell measurements (Shrager *et al.* 1985, Bevan and Raff 1985, Nowak *et al.* 1987, Brismar and Collins 1988).

The aim of this paper is to identify potassium currents at the single channel level in the membrane of human glioma cells.

Methods

Experiments were performed using line U-118MG of glial cells originating from a human malignant tumour (Pontén 1975). The cells were cultivated in the Eagle's minimal essential medium (USOL, Czechoslovakia, see "MEM" in Tab.1) on glass coverslips. Both, cells attached to the coverslip, and a fresh suspension of cells (obtained using trypsin and versen) were used. Experimental solutions are listed in Tab. 1. Experiments were carried out at room temperature (25 °C).

The patch clamp technique (Hamill *et al.* 1981) was used for single channel measurements. Pipettes for patch clamping were prepared from borosilicate glass capillaries with internal fibres by two-step pulling. Heat-polished pipettes had a resistance of about 20 MΩ. After formation of a tight seal, patch resistance was usually around 10 GΩ. Single channel

currents were measured using EPC-7 (List Medical, FRG) equipment and filtered at 1 or 2 kHz by a 4-pole Bessel filter. Current records were digitized by means of a 12-bit A/D converter of a SM 4-20 minicomputer (Datasystém, Czechoslovakia) at 5 kHz and analyzed using an interactive set of programs for single-channel analysis (Zahradníková 1987).

Table 1
Composition of the experimental solutions

No.	Name	K^+	Na^+	Mg^{2+}	Ca^{2+}	Cl^-	EGTA	HEPES
1	BS	5	140	2	5	159	0	10
2	MEM*	5.4	133	0.8	1.8	125	0	0
3	5K135Na	5	135	1	0	142	1	10
4	140K	140	0	1	1	142	1	10
5	140K1Ca	140	0	1	1	142	0	10

Concentration is given in mmol/l. All solutions were set to pH = 7.4 with Tris.
*MEM (Eagle's minimal essential medium) was supplemented with 10 % foetal calf serum, 100 IU/ml of penicillin, 100 μg/ml of streptomycin, and 100 μg/ml of kanamycin. Sulphate, phosphate, and carbonate anions were present in 0.8, 1, and 15 mmol/l, respectively.
HEPES: N-2-Hydroxyethylpiperazine-N'-2-ethanesulphonic acid.
Tris: Tris-(hydroxymethyl)-aminomethane.
EGTA: Ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid.

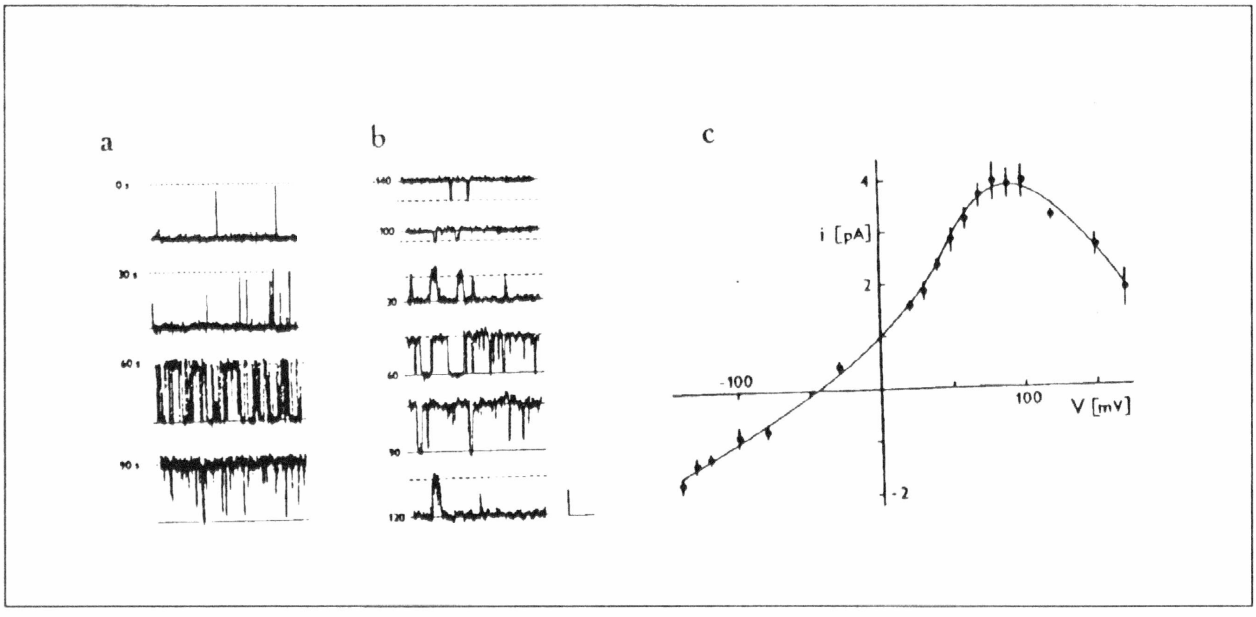

Fig. 1
The low conductance potassium channel. a) High- and low-duty cycles of the 20 pS channel. The first openings (upper left record, denoted as t = 0 s) were observed after a 8-min sojourn of the channel in the closed state. Other selected records were taken 30, 60 and 90 seconds later, the corresponding open state probabilities were 0.01, 0.05, 0.50, and 0.95. Full lines indicate zero current level and dashed lines the open channel current (3.9 pA). Holding potential +85 mV relative to the resting potential. Filter setting 1 kHz. b) Unitary currents at different voltages. Recording conditions as in a). Membrane potentials are given with the traces. Calibration bars 2 pA and 20 ms. Filtering at 1 kHz. c) Single channel current-voltage curve of the 20 pS channel. Individual points are from the experiments shown in b). The bars show ± S.D. of current amplitudes. The line between the points was drawn by eye.

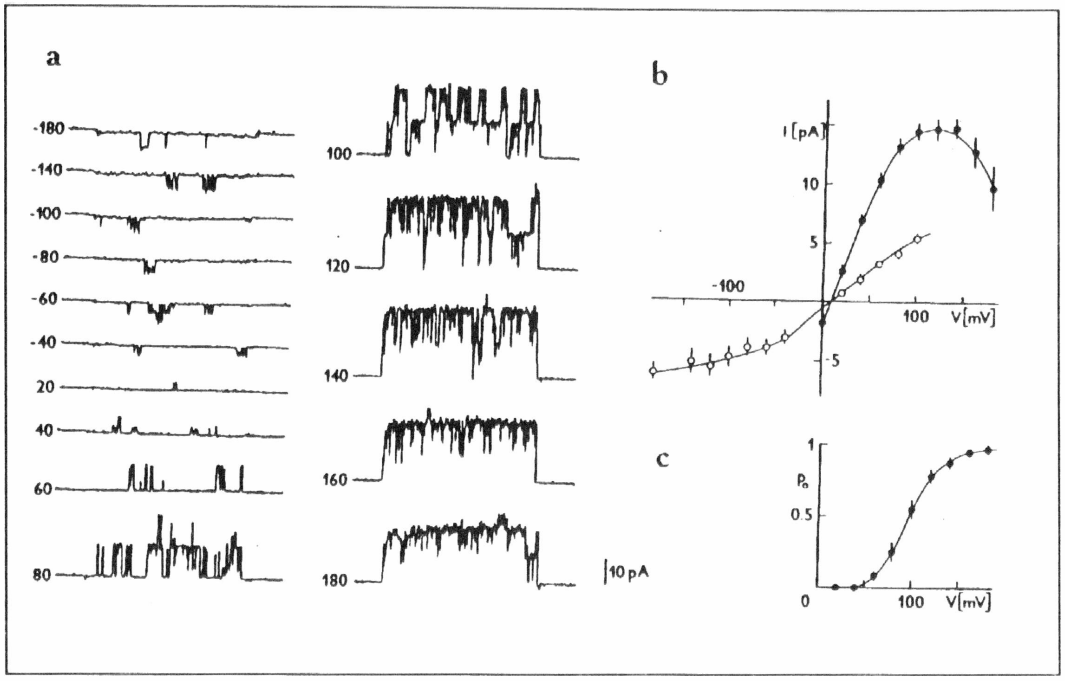

Fig. 2

Unitary potassium currents in a depolarized cell. a) Single channel records at different voltages. Numbers at the records are amplitudes of 150 ms voltage pulses relative to the resting potential. Capacitive and leakage currents were digitally subtracted. At negative voltages only the voltage-independent activity of the 63 pS channel could be observed. At positive potentials voltage-dependent activation of two high-conductance channels (215 pS) is obvious. At +40 and +60 mV, separate openings of both channel types can be seen. b) Current-voltage curves of single channels from a depolarized cell. Full symbols are for voltage-dependent unitary current giving maximal slope conductance of 215 pS between 0 mV and +50 mV. Open symbols are for voltage-independent openings. Slope conductance around reversal potential is 63 pS. Reversal potential for both I-V curves is +7 mV. The lines were drawn by eye. Vertical bars are ± S.D. c) Voltage-dependence of the open state probability of the high-conductance channel. The simultaneous presence of two channels in the patch was taken into consideration. Vertical bars represent ± S.D. At 20 and 40 mV pulses p_O was around 0.001.

Results

Under conditions close to physiological, i.e. cells adhering to the coverslip with MEM in the bath, cell-attached configuration, and patch pipette filled with the 5 mmol/l KCl and 135 mmol/l NaCl solution, activity of a low-conductance (20 pS) channel was dominating. Its openings were observed in practically every patch. The channel used to be silent for long periods of time (hundreds of seconds), independently of the holding potential (Fig. 1a). During periods of high activity transitions between open and closed states seemed to be dependent on the membrane potential, being most frequent between +30 and +90 mV relative to the resting potential (Fig. 1b).

The corresponding current-voltage curve is shown in Fig. 1c. Between -140 and +20 mV the curve is slightly nonlinear, with single-channel conductance of 20 pS around the reversal potential. Between +20 and +70 mV the conductance increases up to 45 pS. At stronger depolarizations, the amplitude of unitary currents attains maximum (around +90 mV) and above +100 mV even decreases with voltage.

The value of reversal potential (-43 mV relative to the resting potential) indicates that the channel is permeable mainly for potassium ions. The selectivity of the channel was evaluated in another kind of experiments with excised membrane patch (not shown), when the external side of the membrane was exposed to a 140 mmol/l KCl solution and internal side bathed with low-K, high-Na (BS solution, see Table 1), unitary currents reversed their direction at a transmembrane potential of +70 mV. From this value a permeability ratio P_{Na}/P_K = 0.025 was calculated using the Goldman-Hodgkin-Katz equation. The conductance of the channel was again close to 20 pS.

Openings of channels with higher conductance were occasionally also observed under the above mentioned conditions or in cells spontaneously detached from the coverslip. Conditions more favourable for their activity were obtained if cells adhering to the coverslips were depolarized by

perfusion of the experimental chamber with a high-potassium solution (140 mmol/l KCl and 1 mmol/l $CaCl_2$ in Table 1). Results of the cell-attached experiment, with patch-pipette filled with 140 mmol/l KCl and 1 mmol/l $CaCl_2$ solution, are shown in Fig. 2. Measurements were done using 150 ms voltage pulses from a zero holding potential. Activity of two types of K channels can be distinguished. At negative voltages, only the channel with a conductance of 63 pS (around the reversal, see Fig. 2b) was active. Its activity was independent of the membrane potential and generally was very low. The probability of staying in the open state during the pulse was between 0 and 0.08.

At positive membrane potentials the activity of a high-conductance channel was dominating (Fig. 2a). Its openings could be occasionally seen at 0 mV. The open state probability p_o has clear signs of voltage dependence (Fig. 2c). The threshold is at +40 mV, half-maximal activation at +95 mV, and a maximal value of 0.96 is reached above +160 mV.

Current-voltage curves of both channel types are shown in Fig. 2b. The voltage-independent channel has a conductance of 63 pS between -40 to +60 mV. At negative voltages the conductance decreases and at potentials below -120 mV the single-channel current goes to saturation. The reversal potential of +7 mV is the same for both channels. Conductance of the high-conductance channel equals 215 pS and is constant from 0 to +50 mV. At more positive membrane potentials the channel conductance decreases. The determination of unitary currents at very positive voltages was complicated by interference of other channel types and by increased channel flickering.

At negative voltages, I-V relationship of the 215 pS channel could not be measured under above mentioned conditions, as the probability of opening was practically zero. If this channel is Ca-dependent (as was supposed), its open state probability should be increased by increasing intracellular Ca^{2+} concentration. This may be achieved simply by excising the membrane patch and forming an inside-out configuration. An example of such an experiment is given in Fig. 3a, where the intracellular side of the excised membrane was exposed to BS (high Na, high Ca) solution and the extracellular side to 140 mol/l KCl solution. According to expectations, activity of the high-conductance channel appeared after patch excision and persisted even at negative pulse voltages. The probability of finding the channel in the open state was around 0.5 and was virtually independent of the membrane potential. We did not succeed in detecting well-resolved open-to-closed transitions at voltages positive enough to elicit outward currents. At these voltages, records were very noisy (about 1 pA peak-to-peak), obviously because of simultaneous activity of the above described low-conductance channels.

The current-voltage relationship from this patch is shown in Fig. 3b. At negative potentials the I-V curve is approximately linear and gives a slope conductance of 150 pS. At positive voltages the conductance decreases. Extrapolation to the zero current gives a reversal potential of approximately +80 mV from which a value of $P_{Na}/P_K < 0.01$ was calculated. Despite the low accuracy in determination of the reversal, it is clear that the channel is a potassium-selective one.

Discussion

Using the cell-attached and inside-out configurations of the patch clamp, we have identified three distinct types of potassium channels in the membrane of human glioma cells. The channels are highly selective for potassium over chloride and sodium, but can be clearly distinguished from each other by their conductance and voltage- and Ca^{2+}-dependence. Each of them has some special feature which may be useful for the glial cell in different physiological situations. Their activity may be regulated, in principle, through metabolical state of the cell (Stanfield 1987) or through membrane receptors (Dunlap *et al.* 1987).

All three described channel types show a saturation of the current-voltage curve and some of them even a decrease of current amplitude at potentials above +100 mV. This was already observed for the high-conductance Ca^{2+}-activated potassium channel in other cell types and ascribed to a strongly voltage-dependent block by internal sodium ions (Singer and Walsh 1984). As glial cells have relatively high concentrations of Na^+ in their cytoplasm (Orkand *et al.* 1985) this mechanism is very likely and, as our observations show, may be valid for K channels more generally.

The small conductance channel can be distinguished by a conductance of about 20 pS, periods of high and low activity, and low p_o at extreme potentials. Channels of similar conductance and selectivity, but with gating properties of a delayed rectifier, were observed in Schwann cells (Shrager *et al.* 1985). Potassium channels of molluscan glial cells were reported to have 20 pS conductance at negative voltages and to show outward rectification (Geletyuk and Kazachenko 1984). Multiple conductance levels ranging from 6 to 125 pS were observed in oligodendrocytes from the mouse spinal cord (Kettenmann *et al.* 1984), the occurrence of about 20 pS channels being the most frequent. Similar results were obtained in glial cells from the cockroach CNS (Leech 1986). A 20 pS noninactivating potassium channel was reported in mouse astrocytes; its properties were not characterized further (Nowak *et al.* 1987). Periods of high and low activity were reported

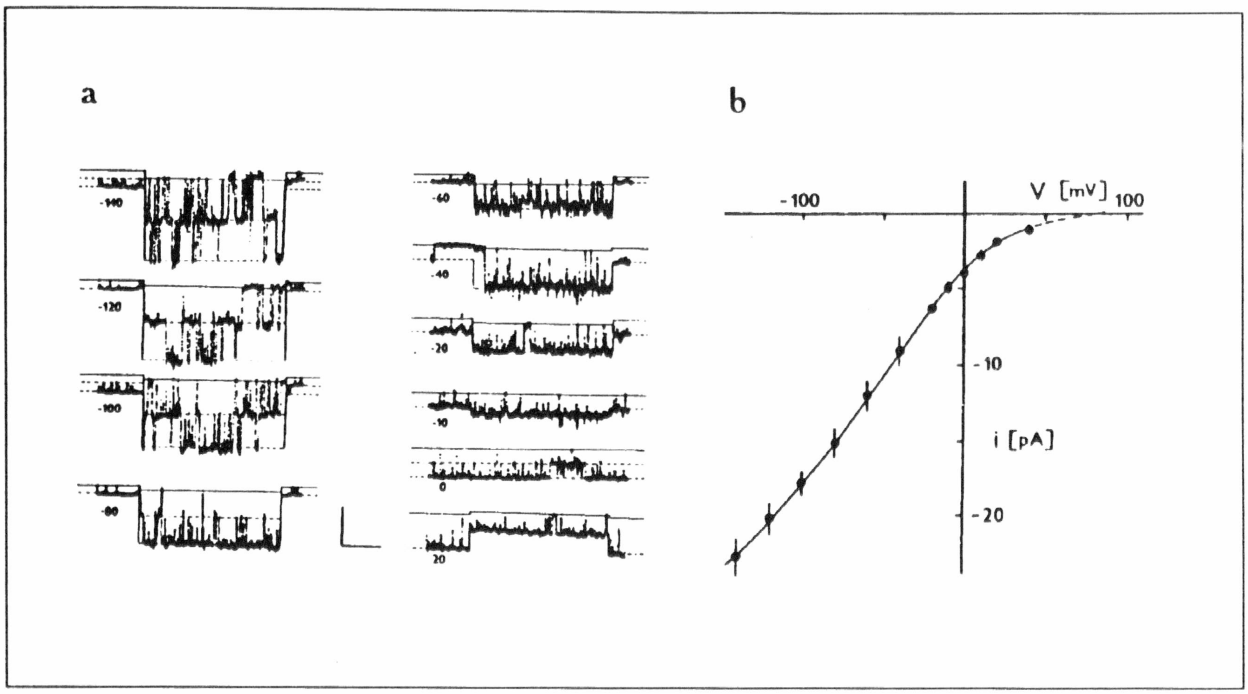

Fig. 3

The high conductance K channel in excised membrane patch. a) Unitary currents during 150 ms voltage steps from a 0 mV holding potential. Inside-out configuration. Pipette solution 140 mmol/l KCl. Internal side of the membrane exposed to BS solution (high Na, high Ca). Before patch excision, no openings of this channel were observed. Closed levels are marked by full line, open channel levels by dashed lines. Two channels were present in the patch. Vertical calibration bar is 20 pA for -140 mV to -60 mV pulses; 10 pA for -40 mV to 0 mV pulses; and 4 pA for +20 mV pulse. Horizontal calibration is 50 ms for all records. Filter setting 2 kHz. b) Current-voltage curve from the experiment shown in a). Vertical bars denote ± S.D. The line extrapolated to the zero current level gives a reversal potential of about +80 mV.

for channels having 45-90 pS conductance in cultured astrocytes (Jalonen and Holopainen 1989).

The medium-conductance channel found in our preparation is the least characterized one. Its characteristic properties are the conductance of about 65 pS and saturation of the single-channel current at hyperpolarized and depolarized voltages as well. Although potassium channels of intermediate conductance were already reported in cells of glial origin (Kettenmann *et al.* 1984, Leech 1986, Leech and Moreton 1988), in only one case such a saturation was reported (Jalonen and Holopainen 1989). The 65 pS channels in U-118MG cells differ from other intermediate conductance channels in their opening probability, which is very low (cf. values of p_o = 0.8-0.9 reported by Leech and Moreton (1988) and Jalonen and Holopainen (1989).

The high conductance channel can be identified with the "BK" Ca^{2+}-activated K channel that was already reported in a variety of preparations (for a review see Petersen and Maruyama 1984) including astrocytes (Nowak *et al.* 1987). A high-conductance voltage-dependent K channel reported in astrocytes (Sonnhof and Schachner 1986) and in retinal Müller glial cells (Nilius and Reichenbach 1988) has also very similar properties. Under conditions close to physiological the channel is strongly voltage-dependent, however, at millimolar Ca_i the voltage dependence disappears (Benham *et al.* 1986). Another interesting property of the high-conductance channel which may be of great importance to glial cells is that its conductance substantially decreases in asymmetric Na/K ionic conditions (Na^+ ions present from one side). A similar conductance decrease was reported for "BK" channels also in other cell types (Singer and Walsh 1984).

We have observed signs of several other channel types in our preparation, which would need closer inspection. Nevertheless, it was the reason why we started to work with high resistance pipettes. The density of channels and especially of potassium ones was still very high and observations with really a single channel in the patch were rare. Therefore, more exact classification of ionic channels in glioma cells will need more refined techniques of channel dissection and testing for drug sensitivity as well. The presented mosaic of K channels is in agreement with the generally accepted opinion about their role in the function of glial cells, but also of other cell types (Zachar *et al.* 1978).

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