J. Membrane Biol. 175, 25–33 (2000)

The Journal of
Membrane
Biology

Large Conductance Ca^{2+}-Activated K^+ Channels in Human Meningioma Cells

R. Kraft[1], **K. Benndorf**[2], **S. Patt**[1]

[1]Institute of Pathology (Neuropathology), Friedrich-Schiller University of Jena, Bachstr. 18, D-07740 Jena, Germany
[2]Institute of Physiology II, Friedrich-Schiller University of Jena, Goethestr. 1, D-07743 Jena, Germany

Received 18 June 1999/Revised: 18 January 2000

Abstract. Cells from ten human meningiomas were electrophysiologically characterized in both living tissue slices and primary cultures. In whole cells, depolarization to voltages higher than +80 mV evoked a large K^+ outward current, which could be blocked by iberiotoxin (100 nM) and TEA (half blocking concentration IC_{50} = 5.3 mM). Raising the internal Ca^{2+} from 10 nM to 2 mM shifted the voltage of half-maximum activation ($V_{1/2}$) of the K^+ current from +106 to +4 mV. Respective inside-out patch recordings showed a voltage- and Ca^{2+}-activated (BK_{Ca}) K^+ channel with a conductance of 296 pS (130 mM K^+ at both sides of the patch). $V_{1/2}$ of single-channel currents was +6, −12, −46, and −68 mV in the presence of 1, 10, 100, and 1000 μM Ca^{2+}, respectively, at the internal face of the patch. In cell-attached patches the open probability (P_o) of BK_{Ca} channels was nearly zero at potentials below +80 mV, matching the activation threshold for whole-cell K^+ currents with 10 nM Ca^{2+} in the pipette. Application of 20 μM cytochalasin D increased P_o of BK_{Ca} channels in cell-attached patches within minutes. These data suggest that the activation of BK_{Ca} channels in meningioma cells does not only depend on voltage and internal Ca^{2+} but is also controlled by the cytoskeleton.

Key words: Meningioma — Human — Patch clamp — BK_{Ca} channels — Cytoskeleton

Introduction

Large conductance (maxi) K^+ channels are voltage activated and show an additional Ca^{2+}-dependent gating (Blatz & Magleby, 1987). However, these BK_{Ca} channels differ in their Ca^{2+}-sensitivity to a high degree: Whereas BK_{Ca} channels from salivary glands open at −20 mV in the presence of only 10 nM internal Ca^{2+} (Petersen & Maruyama, 1984), those from skeletal muscle require micromolar Ca^{2+} concentrations (Barrett et al., 1982). Ca^{2+}-insensitive large conductance K^+ (BK) channels have been described in embryonic neurons (Mienville et al., 1996; Benz et al. 1998). Generally, BK_{Ca} channels are widely distributed in excitable and nonexcitable tissues. Excitable cells meet the requirements for the activation of these channels: strong depolarization and an increase of internal Ca^{2+} due to the activation of Ca^{2+} channels. Thus, BK_{Ca} channels contribute to the action potential repolarization and generate fast afterhyperpolarization in neurons (Lancaster & Nicoll, 1987). In many nonexcitable cells it is difficult to ascribe these channels a specific function. It has turned out, however, that the activity of BK_{Ca} channels is controlled by the cell cycle: Developmental changes are correlated with the channel density (Day et al., 1993), Ca^{2+}-sensitivity (Blair & Dionne, 1985) and gating kinetics (Bregestovski et al., 1988). On the other hand it has been shown that K^+ channels are required for the progression of cells through the cell cycle (Nilius & Wohlrab, 1992; Wonderlin & Strobl, 1996). Inhibition of BK_{Ca} channels with iberiotoxin, however, did not have an antiproliferative effect (Wondergem et al., 1998).

In human tissue, the highest levels of expression of the *hslo* gene, which encodes BK_{Ca} channels, were found in the brain (Tseng-Crank et al., 1994). Knowing that BK_{Ca} channels are present in a human astrocytoma cell line (Pallotta et al., 1987), we were interested in the incidence and functional role in other brain tumor cells. Herein, we studied BK_{Ca} channels in human meningiomas, which are frequent intracranial tumors with an incidence of 13 to 26% of all primary brain tumors (Kleihues & Cavenee, 1997). These tumors are derived from the arachnoidea and are characterized by a rela-

Correspondence to: S. Patt

tively slow proliferation. From the anatomy of the meninges it may be suggested that they act as a barrier or a regulatory interface between cerebrospinal fluid and the surface of the cerebral cortex. It is also noteworthy that cultured meningiomas have many functions in common with cells of normal leptomeninges (Feurer & Weller, 1991).

We describe electrophysiological properties of human meningioma cells in both tissue slices and primary cultures. BK_{Ca} channels are shown to generate a voltage-gated conductance of considerable magnitude. At physiological conditions the activation of this current has to meet one or more of the following requirements: membrane depolarization, increase of internal Ca^{2+}, and alteration of the cytoskeleton.

Materials and Methods

Patients and Tumors

Ten meningiomas obtained from surgical treatment were studied. Patients were between 45 and 79 years old (average: 62 years). The female:male ratio was 9:1. A predominance in woman (2:1 female: male) and a peak occurrence during the sixth and seventh decade of life has been reported (Kleihues & Cavenee, 1997). Surgery was performed at the Neurosurgical Departments of the Clinic Erfurt and the Clinic of the Friedrich-Schiller University Jena. During tumor resection, three specimens were taken from well-preserved parts of the tumor. One specimen was transferred to a sterile culture medium and stored at 4°C until cell culturing. The second specimen was immediately placed into an ice-cold carbonated bath solution for slice preparation. The third specimen was fixed for histological inspection. The tumor material was extirpated in neurosurgery which left the course of the operation unaffected. The protocol was approved by the ethical committee of the university.

Slice Preparation

Slices were prepared from six tumors. The tissue was cut into several 150 μm-thick slices using a vibratome (Campden Instruments, Loughborough, UK). Slices were stored at room temperature in a carbogenated (95% O_2, 5% CO_2) store-solution (ACSF). For patch-clamp experiments they were placed onto a coverslip and fixed with a U-shaped platinum grid with nylon mesh in the recording chamber (Edwards et al., 1989).

Preparation of Cell Cultures and Proliferation Assay

After removal of blood and blood vessels, the tumor tissue was mechanically dissected using scalpels. Subsequent trypsinization (0.025% trypsin in calcium and magnesium free phosphate buffered saline) was performed for 15 min at 37°C. Trypsin was removed by centrifugation and resuspension in DMEM medium. Cells were dissociated by mechanical trituation to obtain a suspension. The cells were plated onto uncoated glass coverslips and then cultured at 37°C in a humidified 5% CO_2/95% air incubator (Forma Scientific, Marjetta). DMEM medium, containing 10% fetal calf serum (FCS) and 1% antibiotics (penicillin, streptomycin), was changed twice per week. For proliferation experiments, cells were plated in a 24-well tissue culture plate at a density of 10^4 cells/well. DMEM medium, containing K^+ channel blockers, was added two days after seeding. All values were normalized to controls treated with DMEM medium containing 10% FCS and 1% antibiotics only. On day 6, cells were resuspended in phosphate buffered saline after trypsinization. Using a Fuchs-Rosenthal chamber, cells were counted from 4 wells at each inhibitor concentration.

Patch-Clamp Experiments

Membrane currents were measured in the whole-cell, cell-attached or inside-out configuration of the patch-clamp technique (Hamill et al., 1981). The recording chamber, mounted on an upright microscope (Axioskop FS, Zeiss, FRG), was continuously perfused (5 ml/min) with salt solution. Cells were visible when using water immersion optics (× 40). For experiments in slices the bath solution was carbogenated (95% O_2, 5% CO_2). Pipettes were pulled from borosilicate glass capillaries (Hilgenberg, Malsfeld, FRG). Pipette resistances in Ringer's saline averaged 4–7 MΩ for both whole-cell and single-channel recordings. The signals were filtered at 2 or 5 kHz (Axopatch 200B, Axon Instrument, Foster City, CA) and digitized with a sampling rate of 10 or 20 kHz. Pulsing, recording and determination of input resistance and cell capacitance was performed with the ISO2 software (MFK, Niedernhausen, FRG). Leak and capacitive currents were removed by subtracting averaged current traces when stepping from −60 to −80 and −40 mV. At these voltages no extra current was observed. If not otherwise noted, whole-cell currents were elicited from a holding potential of −60 mV by pulses to voltages between 180 and −180 mV (20 mV increment, pulse duration 50 or 500 msec). In cell-attached and inside-out patches pulses of 30 or 100 msec duration were applied. Patches were clamped to V_c from a holding potential of 0 mV. In cell-attached patches V_m was calculated from V_c by adding the mean V_{rest} measured in slices or in cultures. Statistical data are presented as mean ± SEM.

Solutions and Chemicals

The store-solution for slices (ACSF) had the following composition (in mM): NaCl 124.0, $NaHCO_3$ 24.0, NaH_2PO_4 1.2, KCl 5.0, $CaCl_2$ 1.8, $MgCl_2$ 1.2. The bath solution contained (in mM): NaCl 150.0, KCl 5.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, HEPES 5.0, pH 7.4. In most of the whole-cell experiments, pipettes were filled with a pipette solution composed of (in mM): KCl 130.0, $CaCl_2$ 1.0, $MgCl_2$ 2.0, K_2ATP 3.0, EGTA 10.0, HEPES 10.0, pH 7.4. The concentration of free Ca^{2+} was calculated as 10 nM. In some experiments, pipette solution contained no EGTA and 2 mM $CaCl_2$. The internal bath solution used for inside-out patches contained (in mM): KCl 130.0, $MgCl_2$ 1.0, HEPES 10.0, pH 7.4. These solutions containing 1000, 100 and 10 μM Ca^{2+} were obtained by adding 1000, 100 and 10 μM $CaCl_2$, respectively, directly to the solution (Barrett et al., 1982). 1 μM free Ca^{2+} was obtained by addition of 100 μM $CaCl_2$ and 107 μM EGTA. Solutions containing 10 nM and 1 μM Ca^{2+} were calculated with the computer program BAD4 (Brooks & Storey, 1992). For all single-channel experiments pipettes were filled with an external solution containing (in mM): KCl 130.0 or K-gluconate 130.0, $CaCl_2$ 1.0, $MgCl_2$ 2.0, EGTA 10.0, HEPES 10.0, pH 7.4. The bath solution for cell-attached patches was similar to those used for whole-cell recordings. Cytochalasin D was dissolved in dimethylsulfoxide (DMSO). Appropriate amounts of this stock solution were diluted in the bath solution to give a final concentration of 20 μM. The corresponding DMSO content in the bath solution was 1%. Cell culture media were purchased from Gibco. Iberiotoxin, charybdotoxin, cytochalasin D and all other chemicals were obtained from Sigma.

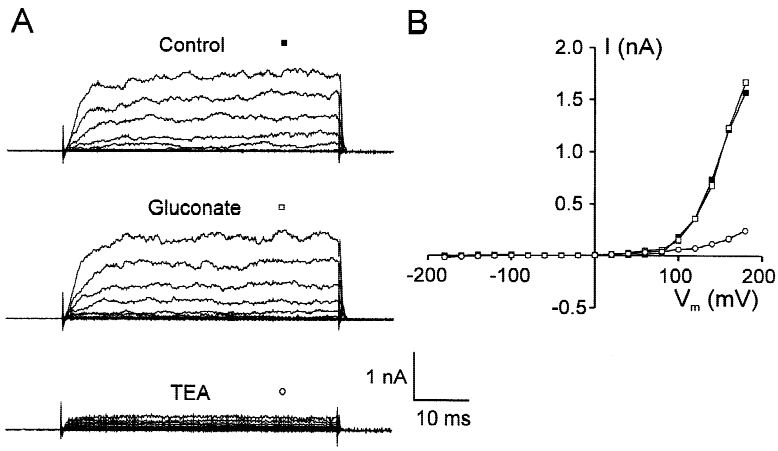

Fig. 1. Voltage-activated whole-cell currents in meningioma slices. (*A*) Time-dependent outward currents were activated at depolarization > +80 mV before (control) and after replacement of 100 mM external Cl^- by gluconate. Application of 20 mM TEA blocked the current. All currents were leak-subtracted. (*B*) Steady-state current amplitudes shown in *A* with 161 mM (solid squares) and 61 mM (open squares) external Cl^- were equal. TEA decreased these currents by 80%.

Results

Morphology of Meningioma Cells

Using phase-contrast optics, the slices showed a concentric arrangement of tumor cells and a large amount of collagen material. The majority of cells in living vibratome slices was small and rod shaped. The soma diameter was about 10 μm.

Primary cultures were established from 9 tumors. Cultured cells from 5 tumors grew in whorls on some coverslips. Two types of cells could be observed: elongated cells with either two or three long processes, and polygonal cells, displaying syncytial sheets of epitheliumlike character. Nuclei of all cells were round or oval and had 5–7 μm in diameter. Dye-coupling between confluent cells was observed by means of the fluorescence dye Lucifer Yellow. Electrophysiological measurements were performed at nonconfluent cells between day 5 and 30 after plating.

Meningioma Cells Develop a K^+ Outward Current at Strong Depolarizations

The resting membrane potential was measured under current clamp conditions in cells of both slices ($n = 9$) and cultures ($n = 36$) to be -59 ± 6 mV and -46 ± 4 mV, respectively. After switching to voltage-clamp, the input resistance and the capacitance of the cells were determined at −60 mV to be 666 ± 97 MΩ and 34 ± 3 pF, respectively.

In cells of slices, depolarization from −60 mV to voltages >+80 mV elicited a time-dependent, noninactivating outward current (Fig. 1*A*). The activation time course was clearly resolved. The current noise increased noticeably in proportion to the amplitude of this current, indicative for underlying single channel currents of large amplitude. To identify the nature of this current, we first replaced 100 mM of the external Cl^- by either gluconate ($n = 2$) (Fig. 1*A*) or glutamate ($n = 2$). None of these interventions altered the currents. However, outward currents were depressed when the pipettes were filled with 130 mM Cs^+ instead of K^+ ($n = 2$). This shows that K^+ ions, but not Cl^- ions, carry the voltage-activated outward current. Furthermore 20 mM of the K^+ channel inhibitor tetraethylammonium (TEA) blocked most of the time-dependent current ($n = 3$) (Fig. 1*B*). Altogether, the results in Fig. 1 show that the voltage-activated currents in meningioma slices are generated by voltage-dependent K^+ channels.

The observed currents were also identified in cells of primary cultures. In addition to TEA ($n = 21$) we tested iberiotoxin (IbTx) ($n = 5$), a specific blocker of BK_{Ca} channels (Galvez et al., 1990). Figure 2*A* illustrates the inhibitory effect of IbTx (100 nM) and TEA (10 mM). The control and blocked currents (Fig. 2*B*) are similar to those recorded in the slices. The amplitude of the tail currents following the depolarizing pulses clearly correlated with the amplitude of the current during the pulses. Furthermore, they were similarly sensitive to IbTx and TEA. We therefore conclude that the tail currents and the currents at the depolarizing pulse are generated by the same K^+ channels. Despite this correlation, in cultured cells we observed repeatedly larger tail currents than the corresponding steady-state currents during the pulses (*see* Fig. 2*A*). We attribute this phenomenon to worse space clamp conditions in the cultured cells compared to the cells in the slices (*see* Morphology of meningioma cells). This phenomenon is therefore not further discussed. We determined the dose-response relation of the TEA-induced reduction of outward currents in cultured meningioma cells. The data points illustrated in Fig. 2*C* are means ($n = 3$) of steady-state whole-cell current amplitudes after a voltage-step from −60 to 180 mV. TEA was applied in the sequence of the concentrations 1, 5, 10, 20 and 50 mM. Fit of the data yielded a

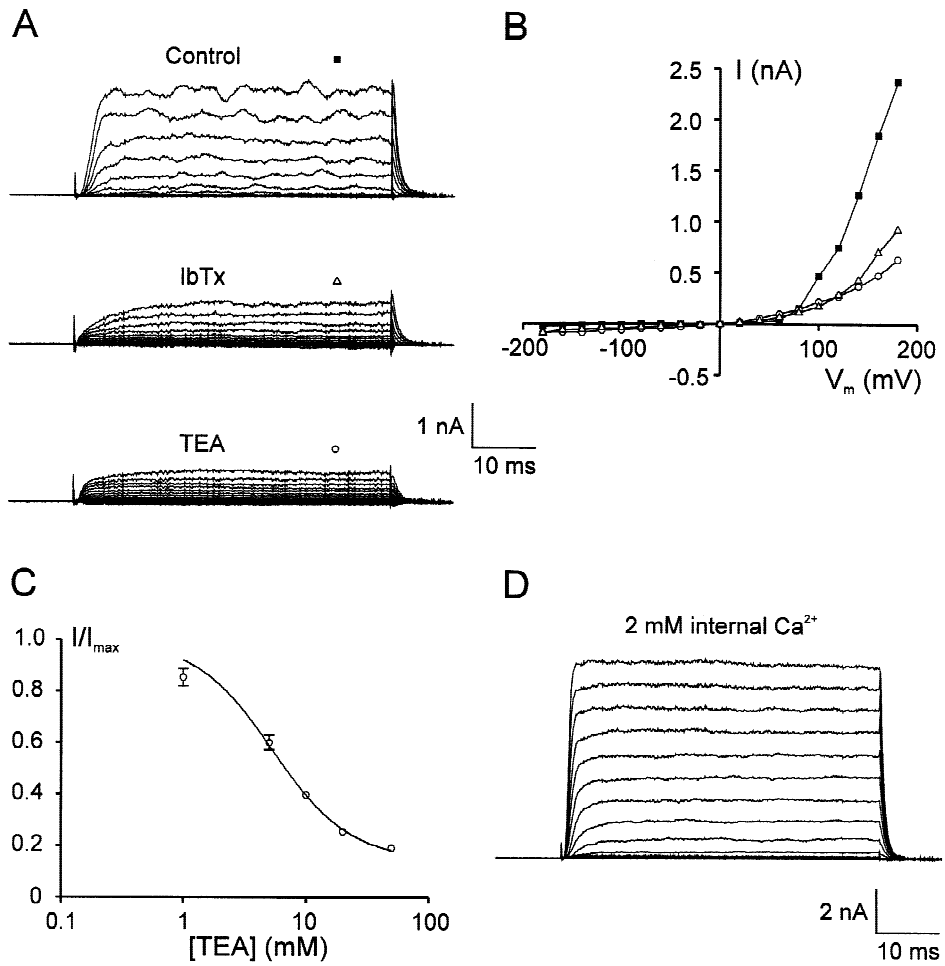

Fig. 2. Basic properties of outward K^+ currents in cultured meningioma cells. (*A*) Outward currents were inhibited by 100 nM of the specific BK_{Ca} channel blocker iberiotoxin (IbTx) and after washout by 10 mM TEA. The cell capacitance was 22 pF. (*B*) IbTx and TEA blocked the steady-state currents shown in *A* by about 60 and 70%, respectively. (*C*) Whole-cell currents of three other cells were recorded in the presence of 0, 1, 5, 10, 20, and 50 mM TEA. The data points were fitted with the Hill equation $IC_{50}/(IC_{50} + C)$, where IC_{50} was 5.2 ± 0.3 mM. (*D*) Large outward currents were activated at depolarization > −20 mV when the pipette solution contained 2 mM Ca^{2+}. The cell capacitance was 28 pF.

half-blocking concentration IC_{50} = 5.2 mM. Additionally, we measured currents with the high Ca^{2+} concentration of 2 mM and no EGTA in the pipette (n = 6). Recordings started later than one min after the formation of the whole-cell configuration for a reasonable perfusion of Ca^{2+} into the cell. The resulting currents were of larger amplitude and lower noise (Fig. 2*D*) than with 10 nM internal Ca^{2+} (Fig. 2*A*). K^+ currents with 2 mM internal Ca^{2+} were also sensitive to TEA and IbTx. Their threshold of activation was shifted to less positive voltages. Outward currents of similar amplitude were recorded at +180 mV and 10 nM internal Ca^{2+} (2.6 ± 0.4 nA; n = 6) and +80 mV and 2 mM internal Ca^{2+} (2.4 ± 0.2 nA; n = 6). Figure 3*A* illustrates that 2 mM internal Ca^{2+} shifted the *IV*-relationship to less positive voltages by about 100 mV in comparison to 10 nM Ca^{2+}. To quantify the influence of internal Ca^{2+} concentrations on the activation of the K^+ current, we plotted the normalized conductance G/G_{max} as function of voltage and fitted the data points with a Boltzmann function (Fig. 3*B*) yielding the voltage of half-maximal activation ($V_{1/2}$) and the slope parameter (s) indicating the voltage changing the K^+ conductance e-fold. The increase of internal Ca^{2+} shifted $V_{1/2}$ to less positive potentials by 102 mV whereas s was about 15 mV at both Ca^{2+} concentrations.

A possible effect on tumor cell proliferation was investigated using the K^+ channel blockers TEA, IbTx and charybdotoxin. The number of cultured meningioma cells was significantly decreased at > 5 mM TEA and became about 20% of the control value at 20 and 50 mM TEA. It should be stressed, however, that the typical BK_{Ca} channel blockers iberiotoxin and charybdotoxin had no decreasing effect on proliferation at concentrations of 100 nM.

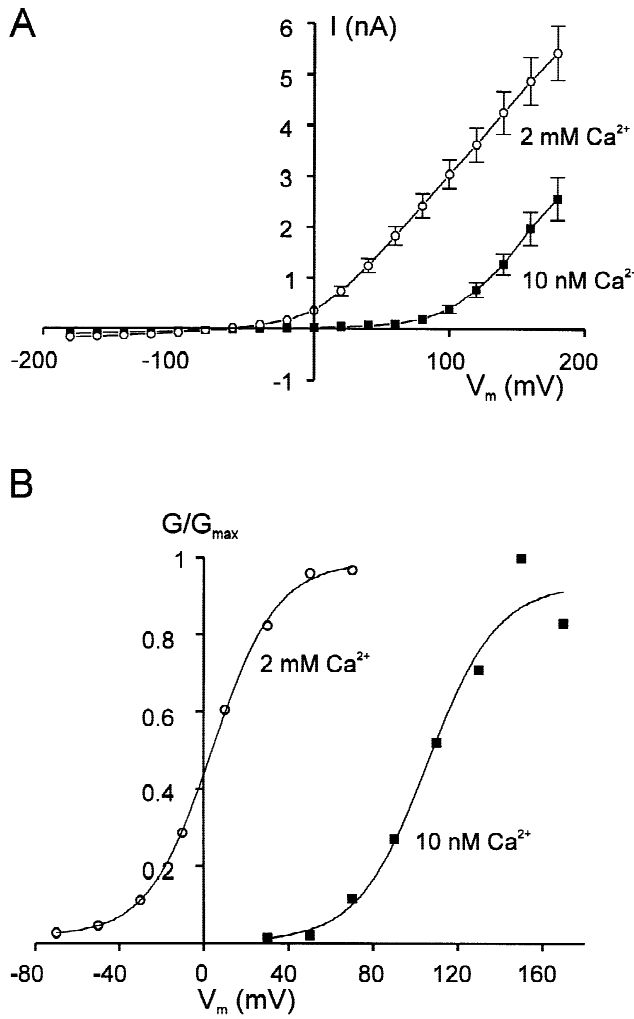

Fig. 3. Effect of internal Ca^{2+} on outward K^+ current of cultured meningioma cells. (*A*) IV-relation of K^+ current amplitudes at Ca^{2+} concentrations of 10 nM (n = 6) and 2 mM (*n* = 6). (*B*) Voltage dependence of the normalized conductance at the two Ca^{2+} concentrations calculated from the data in *A*. The individual data points of the conductance *G* represent the current difference of two neighbored current amplitudes in *A*. G_{max} is the maximum *G* value at each Ca^{2+} concentration. The error bars have been omitted. The continuous lines are fits with the Boltzmann function $1/[1 + exp(V_{1/2} - V)/s]$. The fits yielded for 10 nM Ca^{2+}: $V_{1/2}$ = +105.8 mV ± 6.2 mV, s = 16.6 mV ± 6.1 mV; for 2 mM Ca^{2+}: $V_{1/2}$ = +4.2 mV ± 0.9 mV, s = 15.1 mV ± 0.9 mV.

The Voltage-Dependent K^+ Outward Current is Generated by BK_{Ca} Channels

Cell-attached patches were studied in meningioma cells of both slices and cultures. Single channel currents recorded from a cell in tumor slices are plotted in Fig. 4*A*. Such large-conductance channels were found in > 90% of the patches. The number of channels in these patches was estimated to be between one and ten from the maximum superimposition of the unitary events. At voltage pulses higher than +50 mV channel openings were undetectable, when patches contained between one and five channels. The *iV*-relation is shown in Fig. 4*B*. For the following it is important to stress that the actual membrane potential V_m over the cell-attached patch is the sum of the clamped potential V_c and the resting potential V_{rest}. From the linear part of the *iV*-relation we extrapolated a reversal potential V_{rev} at V_c = +67 mV which corresponds to V_m = +8 mV if adding the measured resting potential (in slices) of −59 mV. V_{rev} was equal with either K-gluconate or KCl in the pipette. This suggests that the K^+ ions but not the Cl^- ions are the charge carrier. The value V_m = +8 mV indicates that in our cell the cytosolic K^+ concentration is below +130 mM used in the pipette. The slope conductance was calculated to be 276 pS. At V_m > +70 mV the slope conductance decreases and at higher voltages it becomes negative (Fig. 4*B*). One explanation for such a negative slope conductance could be a fast voltage-dependent block by an internal cation. Averaged currents at V_m = +111 mV and +121 mV are shown in Fig. 4*C*. At +121 mV the amplitude of the ensemble average current is larger and the kinetics of activation is faster compared to the current at +111 mV, quite as observed in the whole-cell currents (Fig. 1*A*). It is therefore concluded that the whole-cell currents described in the previous sections are generated by large-conductance K^+ (BK_{Ca}) channels.

Ca^{2+}-Sensitivity and Cytoskeletal Control of BK_{Ca} Channels

We further verified the Ca^{2+}-sensitivity of single BK_{Ca} channels in cultured meningioma cells. A patch containing only one active BK_{Ca} channel was clamped in the cell-attached configuration and excised subsequently. Both *iV*-relation and open probability over a wide range of internal Ca^{2+} are plotted in Fig. 5. The slope conductance was calculated to be 296 pS in the inside-out configuration with symmetric 130 mM KCl and 10 μM internal Ca^{2+}. At very positive potentials (V_m > +70 mV), which were necessary to open the channel in the cell-attached patch, the slope conductance of the single-channel current was negative. In all experiments (*n* = 3) excision of the patched membrane and subsequent application of elevated internal Ca^{2+} led to an activation of BK_{Ca} channels at lower voltages. The P_oV-relation shown in Fig. 5*B* was obtained from the channel activity during 100 pulses of 100 ms duration at each Ca^{2+} concentration. Fitting of the data points with the Boltzmann equation (*see* legend to Fig. 5) gave $V_{1/2}$ values of +7, −12, −46, and −68 mV for 1, 10, 100, and 1000 μM internal Ca^{2+}, respectively. Thus, a tenfold increase in Ca^{2+} concentration produced a negative shift of the ac-

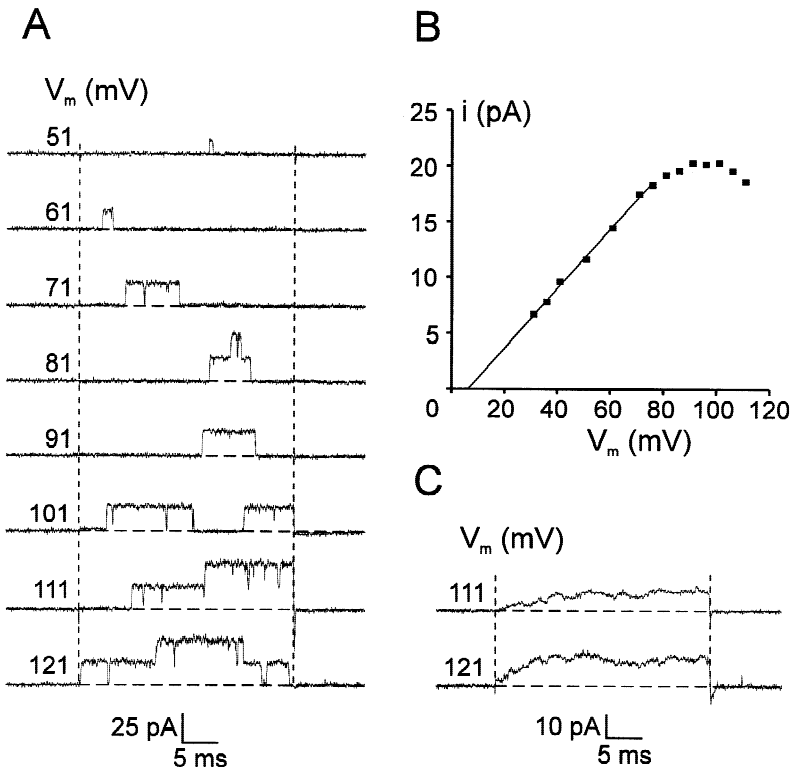

Fig. 4. Single BK_{Ca} channels in a cell from a living meningioma slice. (*A*) The pipette solution contained 130 mM KCl. The traces are leak subtracted. At least two independent channels could be observed in this patch. The dashed line marks the closed level. (*B*) The amplitude of the single channel currents shown in *A* was calculated from 10 traces at each voltage by means of amplitude histograms and a fit of Gaussian functions. V_m was determined by adding the mean $V_{rest} = -59$ mV (in slices) to the clamp voltage (V_c). The slope conductance calculated for the linear range of the *iV*-relationship was 276 pS. (*C*) Ensemble average currents at $V_m = +111$ mV and +121 mV calculated from 30 individual traces each. All traces are leak subtracted.

tivation curve by approximately 20 mV. For cell-attached patch recording $V_{1/2}$ (related to V_m) was +106 ± 4 mV if adding the mean value of $V_{rest} = -46$ mV. The slope parameter (s) of the Boltzmann fits at each Ca^{2+} concentration was between 10 and 13 mV.

To test for an influence of the cytoskeleton on the BK_{Ca} channel activity we added 20 μM cytochalasin D to the bath solution during cell-attached patch experiments. We examined patches containing between five and ten active channels and an initial open probability $NP_o < 0.5$ at $V_m = +74$ mV (Fig. 6*Aa*). We continuously recorded current traces of 200 msec duration from a holding potential of 0 mV (corresponding to $V_{rest} = -46$ mV) and +120 mV (corresponding to $V_m = +74$ mV). NP_o was calculated for each trace from the mean current at $V_m = +74$ mV divided by the unitary current. In two control experiments with 1% DMSO but in the bath solution but without cytochalasin D, NP_o was <0.5 over a measuring interval of 30 min. In three other patches the channel activity increased after addition of cytochalasin D. Three minutes after application of cytochalasin D the channel activity raised dramatically (maximum $NP_o = 6$ at $V_m = +74$ mV; Fig. 6*Ab*). The increase of channel activity was transient and NP_o decreased to values < 1 within the following minute (Fig. 6*Ac*). At $V_m = +144$ mV and before the treatment with cytochalasin D, the patch shown in Fig. 6 contained at least 10 channels. The unitary conductance of channels was 17 pA at $V_m = +74$ mV. Thus the maximum open probability P_o was ~0.6 at $V_m = +74$ mV. Figure 6*B* illustrates the time course of the whole experiment.

Discussion

This study describes BK_{Ca} channels in human meningioma cells. The single-channel conductance was 276 and 296 pS at 130 mM external K^+ in the cell-attached and inside-out patch configuration, respectively. Whole cells developed a corresponding large delayed rectifier K^+ current only when they were depolarized to extremely positive voltages ($V_m > +80$ mV). Perfusion of the cells via the patch pipette with 2 mM Ca^{2+} shifted the voltage of half-maximum activation to +4 mV. $V_{1/2}$ at physiologically cytosolic Ca^{2+} concentrations was +106 mV. This value matches the $V_{1/2}$ value of +102 mV in whole cells with 10 nM Ca^{2+}. In cytochalasin D experiments activation of BK_{Ca} channels at physiological Ca^{2+} concentration und membrane potential indicated control by the cytoskeleton.

It has been reported that BK_{Ca} channels in the brain are not only expressed in neuronal tissues. They were also found in the epithelium of the choroid plexus (Christensen & Zeuthen, 1987). The choroid plexus secretes cerebrospinal fluid into the ventricles of the brain. The low open probability of BK_{Ca} channels in cell-attached patches from this preparation ($P_o \sim 0.5$ at $V_m \sim +75$ mV) resembles that in the channels in meningioma cells. At

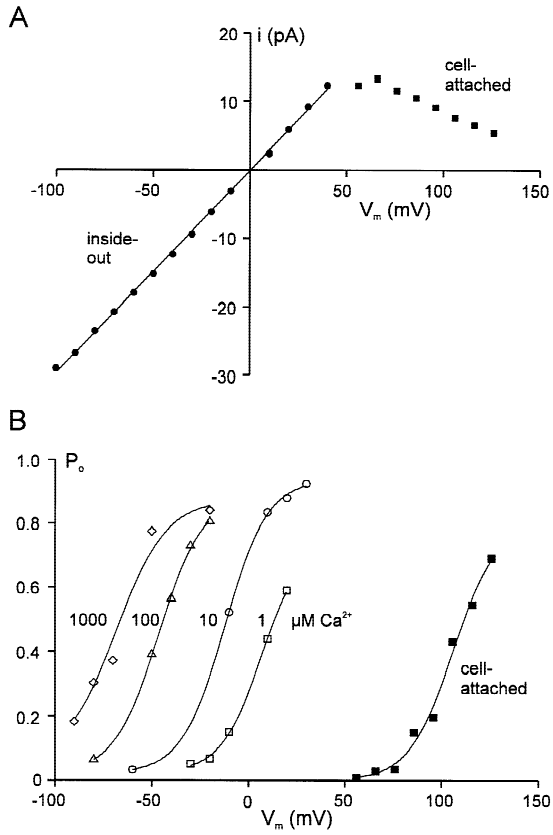

Fig. 5. Conductance and activation of a BK_{Ca} channel in a cultured meningioma cell. Current amplitudes from a single channel recorded before and after excision of the patch. (*A*) In the cell-attached patch the amplitude of the single channel current decreased at $V_m > +70$ mV. V_m was determined by adding the mean $V_{rest} = -44$ mV (in cultures) to the voltage (V_c). Hence the *iV*-relation during the inside-out recording shows a wide range of linearity with a slope conductance of 296 pS. (*B*) Ca^{2+}- and voltage dependence of the same channel. The absolute P_o as a function of V_m is shown for the cell-attached patch configuration and at different Ca^{2+} concentrations after excision. The points were fitted with the Boltzmann equation $1/[1 + exp(V_{1/2} - V)/s]$, where $V_{1/2} = +106 \pm 4$ mV and $s = 11$ mV for the cell-attached patch configuration, $V_{1/2} = +7 \pm 2$ mV and $s = 10$ mV for 1 µM Ca^{2+}, -12 ± 1 mV and $s = 11$ mV for 10 µM Ca^{2+}, -46 ± 1 mV and $s = 11$ mV for 100 µM Ca^{2+} and −68 mV and $s = 13$ mV for 1 mM Ca^{2+}.

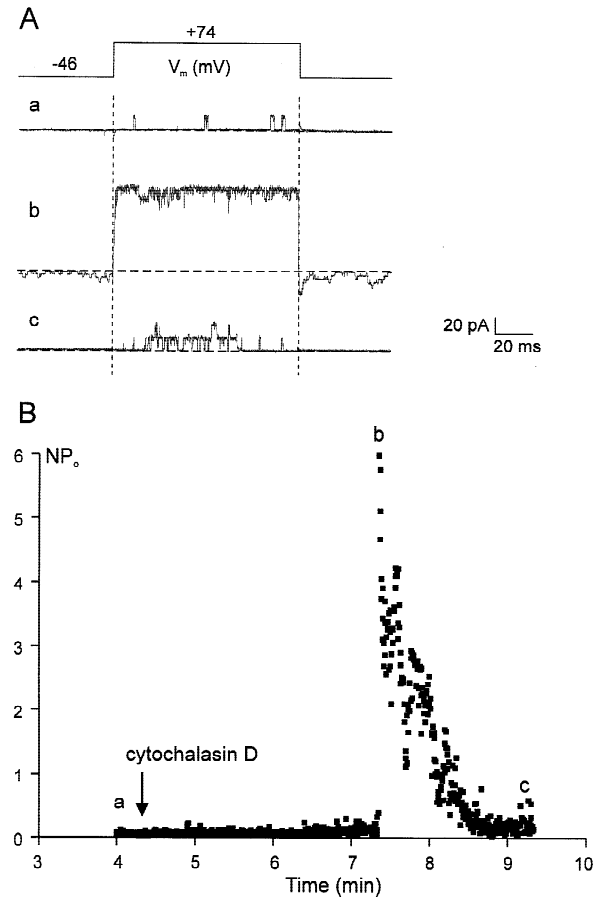

Fig. 6. Effect of cytochalasin D on the BK_{Ca} channel activity in a cell-attached experiment. The patch contained at least 10 channels. (*A*) Single channel recordings before (a), three minutes (b) and five minutes (c) after the application of 20 µM cytochalasin D. (*B*) Time-course of the NP_o at $V_m = +74$ mV. Cytochalasin D was added 4:20 minutes after formation of the patch.

10 µM Ca^{2+}, $V_{1/2}$ was about −10 mV in both cell types. Because these channels are only open at strong depolarization and micromolar Ca^{2+}, it appears that they usually do not produce large K^+ conductance at V_{rest}. Thus a role in secretion or another regulatory function could not be ascribed to the BK_{Ca} channels in choroid plexus cells. We also do not expect a contribution of these channels to the K^+ flux across the membrane of meningeal cells. Moreover, at physiological voltages the ion conductance of meningioma cells was generally low. This is in common with the idea that meninges function as a barrier between cerebrospinal fluid and the surface of the cerebral cortex (Feurer & Weller, 1991).

In cultured meningioma cells we investigated the role of BK_{Ca} channels in proliferation. We found a dose-dependent reduction of the relative cell number at TEA concentrations >5 mM and a 80% reduction at >20 mM TEA. We furthermore tested the influence of 100 nM iberiotoxin and 100 nM charybdotoxin on tumor growth. A decrease of cell number could not be observed. It is known, however, that TEA inhibits proliferation by an relatively unspecific block of K^+ channels, even in millimolar concentrations (Wonderlin & Strobl, 1996). Therefore it is likely that in our cells TEA has affected a K^+ channel different from BK_{Ca} channels. It is also possible that the antiproliferative effect of TEA is not due to an inhibition of K^+ currents. A failure of iberiotoxin to inhibit proliferation was also found in human bladder tumor (HTB-9) cells (Wondergem et al., 1998), which also express BK_{Ca} channels to a high degree (Monen et al., 1998).

We obtained nearly the same $V_{1/2}$ for whole-cell currents at 2 mM Ca^{2+} as for single channel currents at a 2000-fold lower Ca^{2+}-concentration in inside-out patches. This suggests that application of Ca^{2+} to the internal side of inside-out patches activated BK_{Ca} channels more effectively than intracellular perfusion. From Ca^{2+}-insensitive BK channels it is known that they may switch to a high activity mode when they are isolated from the cytosolic environment (Khan et al., 1993). These channels seem to be under effective cytoskeletal control. This was concluded from the finding that activation of Ca^{2+}-insensitive BK channels was observed after application of cytochalasin B and colchicine in smooth muscle cells (Ehrhardt et al., 1996) and hippocampal neurons (Benz et al., 1998). In contrast to BK channels, BK_{Ca} channels showed an unchanged activity in cell-attached and inside-out patches after the treatment with the Ca^{2+} ionophore ionomycin as shown in astrocytoma cells (Pallotta et al., 1987). In these cells elevated Ca^{2+} concentration alone could effectively open the BK_{Ca} channels. Our data suggest that large internal Ca^{2+} concentrations are not sufficient for a full activation of BK_{Ca} channels. An increase of whole-cell conductance could not be observed during perfusion with 2 mM Ca^{2+} indicating fast buffering or a loss of the Ca^{2+} sensitivity of the channels. The great difference between channel activity in whole-cell and inside-out measurements favor a regulatory mechanism that is associated with the cell interior. One possible determinant is the cytoskeleton of which actin is one main component because it has been shown that the inhibition of the actin polymerization by cytochalasins affect different species of ion channels. For example, renal low conductance K^+ channels respond with deactivation (Wang et al., 1994) and amiloride-sensitive Na^+ channels become activated (Cantiello et al., 1991). Besides the cytoskeleton other cytosolic factors might influence the channel activity. As shown for BK channels in excised patches, a brain tissue extract applied cytosolically effectively depresses P_o (Benz et al., 1998). This finding is discussed that an inhibitory molecule is associated with the internal membrane surface. It should be stressed, however, that the BK_{Ca} channels investigated in the present study are controlled by several mechanisms. More specific interpretations of the channel activation are therefore limited. Further dissection of these individual mechanism will be necessary to improve the understanding of the function of BK_{Ca} channels in nonexcitable cells, and possibly also to improve the understanding of the physiology of tumor cells.

This work was supported by the Thüringer Ministerium für Wissenschaft, Forschung und Kultur (Nr.: B 301-96085, FKZ 01ZZ9602). We thank Professor Rolf Kalff (Clinic of Neurosurgery, Friedrich-Schiller University Jena) and Peter Möller (Clinic of Neurosurgery, Klinikum Erfurt GmbH) for their collaboration in providing tumor material, Dr. Gunter Neupert and Helga Raabe (Institute of Pathology, Friedrich-Schiller University Jena) for cell cultures, and Renate Klupsch (Neuropathology) for excellent technical assistance.

References

Barrett, J.N., Magleby, K.L., Pallotta, B.S. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. *J. Physiol.* **331:**85–100

Benz, I., Meyer, D.K., Kohlhardt, M. 1998. Properties and the cytoskeletal control of Ca^{2+}-independent large conductance K^+ channels in neonatal rat hippocampal neurons. *J. Membrane Biol.* **161:**275–286

Blair, L.A., Dionne, V.E. 1985. Developmental acquisition of Ca^{2+}-sensitivity by K^+ channels in spinal neurones. *Nature* **315:**329–331

Blatz, A.L., Magleby, K.L. 1987. Calcium-activated potassium channels. *Trends Neurosci.* **10:**463–468

Bregestovski, P.D., Printseva, O.Y., Serebryakov, V., Stinnakre, J., Turmin, A., Zamoyski, V. 1988. Comparison of Ca^{2+}-dependent K^+ channels in the membrane of smooth muscle cells isolated from adult and fetal human aorta. *Pfluegers Arch.* **413:**8–13

Brooks, S.P.J., Storey, K.B. 1992. Bound and Determined: A computer program for making buffers of defined ion concentrations. *Anal. Biochem.* **201:**119–126

Cantiello, H.F., Stow, J., Prat, A.G., Ausiello, D.A. 1991. Actin filaments control epithelial Na channel activity. *Am. J. Physiol.* **261:**C882–C888

Christensen, O., Zeuthen, T. 1987. Maxi K^+ channels in leaky epithelia are regulated by intracellular Ca^{2+}, pH and membrane potential. *Pfluegers Arch.* **408:**249–259

Day, M.L., Pickering, S.J., Johnson, M.H., Cook, D.I. 1993. Cell-cycle control of a large-conductance K^+ channel in mouse early embryos. *Nature* **365:**560–562

Edwards, F.A., Konnerth, A., Sakmann, B., Takashaki, T. 1989. A thin slice preparation for patch clamp recordings from neurons of the mammalian central nervous system. *Pfluegers Arch.* **414:**600–612

Ehrhardt, A.G., Frankish, N., Isenberg, G. 1996. A large-conductance K^+ channel that is inhibited by the cytoskeleton in the smooth muscle cell line DDT_1 MF-2. *J. Physiol.* **496:**663–676

Feurer, D.J., Weller, R.O. 1991. Barrier functions of the leptomeninges: a study of normal meninges and meningiomas in tissue culture. *Neuropathol. Appl. Neurobiol.* **17:**391–405

Galvez, A., Gimenez-Gallego, G., Reuben, J.P., Roy-Contancin, L., Feigenbaum, P., Kaczorowski, G.J., Garcia, M.L. 1990. Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion buthus tamulus. *J. Biol. Chem.* **265:**11083–11090

Hamill, O.P., Marty, A., Neher, E., Sackmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Khan, R.N., Smith, S.K., Morrison, J.J., Ashford, M.L.J. 1993. Properties of large-conductance K^+ channels in human myometrium during pregnancy and labour. *Proc. R. Soc. Lond.* **251:**9–15

Kleihues, P., Cavenee, W.K. 1997. Pathology and Genetics of Tumours of the Nervous System. International Agency for Research on Cancer, Lyon

Lancaster, B., Nicoll, R.A. 1987. Properties of two calcium-activated hyperpolarizations in rat hippocampal neurons. *J. Physiol.* **389:**187–203

Mienville, J.M., Barker, J.L. 1996. Immature properties of large-

conductance calcium-activated potassium channels in rat neuroepithelium. *Pfluegers Arch.* **431:**763–770

Monen, S.H., Schmidt, P.H., Wondergem, R. 1998. Membrane potassium channels and human bladder tumor cells. I. Electrical properties. *J. Membrane Biol.* **161:**247–256

Nilius, B., Wohlrab, W. 1992. Potassium channels and regulation of proliferation of human melanoma cells. *J. Physiol.* **445:**537–548

Pallotta, B.S., Hepler, J.R., Oglesby, S.A., Harden, T.K. 1987. A comparison of calcium-activated potassium channel currents in cell-attached and excised patches. *J. Gen. Physiol.* **89:**985–997

Petersen, O.H., Maruyama, Y. 1984. Calcium-activated potassium channels and their role in secretion. *Nature* **307:**693–696

Tseng-Crank, J., Foster, C.D., Krause, J.D., Mertz, R., Godinot, N., DiChiara, T.J., Reinhart, P.H. 1994. Cloning, expression, and distribution of functionally distinct Ca^{2+}-activated K^+ channel isoforms from human brain. *Neuron* **13:**1315–1330

Wang, W.H., Cassola, A., Giebisch, G. 1994. Involvement of actin cytoskeleton in modulation of apical K^+ channel activity in rat collecting duct. *Am. J. Physiol.* **267:**F592–F598

Wondergem, R., Cregan, M., Strickler, L., Miller, R., Suttles, J. 1998. Membrane potassium channels and human bladder tumor cells. II. Growth properties. *J. Membrane Biol.* **161:**257–262

Wonderlin, W.F., Strobl, J.S. 1996. Potassium channels, proliferation and G1 progression. *J. Membrane Biol.* **154:**91–107