BK Channels in Human Glioma Cells

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Received 19 June 2000; accepted in final form 13 October 2000

Ransom, Christopher B. and Harald Sontheimer. BK channels in human glioma cells. J Neurophysiol 85: 790–803, 2001. Ion channels in excitable cells are involved in proliferation and volume regulation. Glioma cells robustly proliferate and undergo shape and volume changes during invasive migration. We investigated ion channel expression in two human glioma cell lines (D54MG and STTG-1). With low [Ca$^{2+}$]$_i$, both cell types displayed voltage-dependent currents that activated at positive voltages (more than +50 mV). Current density was sensitive to intracellular cation replacement with the following rank order: K$^+$ > Cs$^+$ = Li$^+$ > Na$^+$. Currents were >80% inhibited by iberiotoxin (33 nM), charybdotoxin (50 nM), quinine (1 mM), tetrahydrate (30 µM), and tetraethylammonium ion (TEA; 1 mM). Extracellular phloretin (100 µM), an activator of BK(Ca$^{2+}$) channels, and elevated intracellular Ca$^{2+}$ negatively shifted the I-V curve of whole cell currents. With 0, 0.1, and 1 µM [Ca$^{2+}$], the half-maximal voltages, $V_{1/2}$, for whole cell current activation were +150, +65, and +12 mV, respectively. Elevating [K$^+$], potentiated whole cell currents in a fashion proportional to the square-root of [K$^+$]. Recording from cell-attached patches revealed large conductance channels (150–200 pS) with similar voltage dependence and activation kinetics as whole cell currents. These data indicate that human glioma cells express large-conductance, Ca$^{2+}$-activated K$^+$ (BK) channels. In amphotericin-perforated patches bradykinin (1 µM) activated TEA-sensitive currents that were abolished by preincubation with bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid-AM (BAPTA-AM). The BK channels described here may influence the responses of glioma cells to stimuli that increase [Ca$^{2+}$].

INTRODUCTION

The vast majority of primary brain tumors in adult humans arise from glial cells. These neoplasms carry a very poor prognosis due to their invasive migration that renders surgical treatment untenable (Cotran et al. 1994). Ion channels may contribute to this invasive behavior by influencing salt and water movements between intracellular and extracellular compartments during shape and volume changes associated with migration through the tortuous extracellular space of brain tissue (Soroceanu et al. 1999). In addition, ion channels in glia and other excitable cell types have been shown by many laboratories to be functionally involved in proliferation (Brismar et al. 2000; Chin et al. 1997; DeCourcey et al. 1984; Dubois and Rouzaire-Dubois 1992; Nilius and Wohlrab 1992; Pappas et al. 1994; Puro et al. 1989; Rouzaire-Dubois and Dubois 1990, 1998; Schlichter et al. 1996; Wiecha et al. 1998; Wilson and Chiu 1993). Thus there is good reason to believe that ion channels in glioma cells could contribute to the malignant behavior of these cells (i.e., invasive migration and uncontrolled proliferation). Moreover, ion channels expressed by glioma cells may represent novel therapeutic targets in the treatment of this deadly disease.

Human glioma cells express a variety of ion channels. These include voltage-gated K$^+$ currents (Chin et al. 1997), voltage-gated Na$^+$ currents (Brismar and Collins 1989), Ca$^{2+}$-activated K$^+$ currents (Brismar and Collins 1989; Pallotta et al. 1987), voltage-gated Cl$^-$ currents (Ulrich and Sontheimer 1996), and volume-regulated Cl$^-$ currents (Bakhramov et al. 1995) (for review, see Brismar 1995). The expression of large-conductance, Ca$^{2+}$-activated K$^+$ channels (BK) by glioma cells is of particular interest because these channels are related to the degree of differentiation and proliferative state of retinal glial (Muller) cells (Bringmann et al. 2000). Specifically, Muller cells lose their BK channels during development and regain them when cells proliferate in response to injury or disease. These results suggest dedifferentiated/proliferating glial cells, glioma cells representing the most extreme case, revert to a developmental biophysical phenotype that includes BK channel expression (Bordey and Sontheimer 1997; Bringmann et al. 2000; MacFarlane and Sontheimer 1997). We examined ion channel expression in two human glioma cell lines and found that both cell types highly express large-conductance, Ca$^{2+}$-activated K$^+$ channels (BK channels). Our study confirms that BK channel expression is a common feature of human glioma cells (Brismar 1995). In addition, we provide additional descriptions of the pharmacology, Ca$^{2+}$-dependence, and [K$^+$]-dependence of these currents in glioma cells. This detailed biophysical thumbprint is necessary for critical evaluation of the functions of BK channels in human glioma cells.

METHODS

Cell culture

All experiments were performed on the glioma cell lines STTG-1 (anaplastic astrocytoma, WHO grade III) and D54-MG (glioblastoma multiforme, WHO grade IV). STTG-1 cells were obtained from American Type Tissue Collection (Rockville, MD), and D54-MG cells were a gift from Dr. D. Bigner (Duke University). We received D54MG cells at passage 515 and STTG1 cells at passage 14. Vials of cells arrived frozen and were thawed and resuspended in culture medium (see following text). These cells were plated on four large culture flasks (Becton Dickinson, Lincoln Park, NJ) and grown to confluency. Cells were detached from the flasks with a 1- to 2-min exposure to culture media supplemented with trypsin (1.5 mg/ml).
This suspension was added to an equal volume of culture medium and spun at 1,200 g for 5 min in a centrifuge (Lab-Line Instruments Inc., Melrose Park, IL). We aspirated the supernatant and resuspended the pellet from three flasks in 90 ml of a freezing solution (culture medium with 5% DMSO). This suspension was divided into 180 0.5-ml aliquots and stored in liquid nitrogen for later use. Some cells were plated directly onto glass coverslips in 24-well plates (Becton Dickinson) for experiments and into a culture flask (Becton Dickinson) for future passage. Data in this paper were obtained from cells passed ≤100 times. However, no appreciable difference in membrane currents were observed in cells passed ≤300 times.

Our culture medium was Dulbecco’s modified essential medium (Life Technologies, Grand Island, NY) with 10% fetal calf serum (HyClone, Logan, UT). Cells were kept in an incubator (Lab-Line Instruments) at 37°C in a 90% O₂-10% CO₂ humidified environment. This resulted in a pHₐ of 7.4.

**Electrophysiology**

Standard patch-clamp techniques were used to record whole cell and single-channel membrane currents (Hamill et al. 1981). Patch pipettes were pulled on an upright puller (PP-83, Narishige Instruments, Tokyo) from thin-walled, glass capillary tubing with filament (MTW150F-4, WPI, Sarasota, FL) and had resistances of 3–5 MΩ. For experiments with amphotericin B (Sigma, St. Louis, MO) perforated patches, we closely followed the procedures of Rae et al. (1991). Briefly, amphotericin was dissolved and thoroughly triturated in DMSO (final concentration of 65 μM). This stock was added to our standard pipette solution (final amphotericin concentration of 0.3 μM). Pipettes used for amphotericin-perforated-patch recording were flame-polished on a microforge (MF-83, Narishige Instruments) and had resistances of 1–3 MΩ. Inclusion of Lucifer yellow (Sigma) in our pipette solutions for amphotericin-perforated patch recordings allowed us to distinguish perforated-patch recordings from whole cell recordings (fluorescence rapidly appeared in cells following break-through). We used an Axopatch 200B amplifier (Axon Instruments, Redwood City, CA) controlled by a PC-compatible microcomputer (Dell Computers, Dallas, TX) running Axon instruments software (pClamp7). Data were stored directly to disk using a Digidata 1200 A-D interface (Axon Instruments). Data were acquired at 10 kHz and filtered at 1 and 2 kHz for patch and whole cell recordings, respectively. Capacitance and series resistance, Rₛ, compensation was performed with the Axopatch amplifier. Rₛ was compensated ≤80%. No post hoc correction of Rₛ was performed; because the currents under study were quite large, we simply note that the voltage error associated with Rₛ will underestimate the steepness of the I-V curve. Experiments were not performed on cells with a Rₛ >10 MΩ (except with amphotericin-perforated patches). Cells were visualized with an inverted microscope (Nikon, Melville, NY). A three-axis micromanipulator (Newport, Irvine, CA; mounted onto a custom frame fitted to the microscope) held the preamplifier headstage and pipette holder. The recording chamber had a volume of ~300 μl and was constantly superfused with control extracellular solution at a rate of ~0.5 ml/min. A triple-barreled microperfusion device with a stepper motor (SP-77B perfusion fast-step, Warner Instruments, Hamden, CT) was used to apply test solutions directly to cells. Two barrels were fed by 2-to-1 manifolds, and one barrel was fed by a 4-to-1 manifold. Control solutions were continuously flowing in each barrel between applications of the five test solutions. The microperfusion flow pipes and stepper motor were mounted on a manual micromanipulator (MX-110, Soma Scientific Instruments, Irvine, CA) attached to our isolation table (Micro-g, Peabody, MA) with a magnetic base. Grounding the recording chamber via an agar salt bridge (4% agar, 1 M KCl) minimized liquid junction potentials produced by test solutions.

**Solutions**

Our standard bath solution contained the following (in mM): 5 KCl, 135 NaCl, 1.6 Na₂HPO₄, 0.4 NaH₂PO₄, 1 MgSO₄, 10 glucose, and 32.5 HEPES (acid). pH was adjusted to 7.4 with NaOH. The osmolality was ~300 mOsm. In experiments with elevated [K+]o, KCl was substituted with an equimolar amount of NaCl. Drugs were added directly to this solution. Our standard pipette solution contained (in mM): 145 KCl, 1 MgCl₂, 10 HEPES (acid), and 10 EGTA. pH was adjusted to 7.25 with Tris-base, and Ca²⁺ was added from a stock solution to achieve a target free Ca²⁺ concentration of 20 nM. We calculated the calcium to add to our pipette solution in experiments with elevated free calcium concentrations with a software program based on equations provided in Marks and Maxfield (1991). This program takes into account ionic strength and pH. We corrected for EGTA purity. For target free Ca²⁺ concentrations of 0.1 and 1 μM, we added 4.3 and 8.6 mM Ca²⁺, respectively. To inhibit rises of [Ca²⁺], we loaded cells with the acetoxyethyl ester form of 1,2-bis(2-aminophenoxy)ethane,N,N,N',N'-tetraacetic acid (BAPTA-AM; Molecular Probes, Eugene, OR). BAPTA-AM was dissolved in DMSO and added to our culture media at a final concentration of 100 μM. Cells were incubated for 20–30 min before recording. All chemicals were purchased from Sigma unless otherwise noted. Scorpion toxins (charybdotoxin and iberiotoxin) were purchased from Alomone Labs (Jerusalem, Israel).

**Analysis**

Data were analyzed off-line with the software package Origin (v0.5.0, MicroCal Software, Northampton, MA). All curve-fitting was performed using a least-squares curve-fitting routine provided by the software. Inhibition curves were fit with the following equation

$$III_{max} = I/I_0 + [(drug)/IC_{50}]^n$$

where $III_{max}$ is the fractional remaining current, $IC_{50}$ is the half-maximal inhibitory concentration, and $n$ is the Hill slope. To quantify the voltage dependence of currents under different conditions, we fit normalized currents to a Boltzmann equation of the following form (Weiss and Magleby 1990)

$$\frac{III}{III_{max}} = \frac{1}{[1 + \exp(-q(V - V_n)/kT)]}$$

where $III_{max}$ is normalized current, $g$ is the effective gating charge, $V_{0.5}$ is the half-maximal voltage, $k$ is the Boltzmann constant, and $T$ is temperature in Kelvin. Under our conditions, the term $kT$ was ~25.6. We calculated conductance as follows

$$g = I/(V_m - E_K)$$

$V_m$ is membrane potential and $E_K$ is the potassium equilibrium potential determined with the Nernst equation. Statistical analysis was performed with Excel (Microsoft, Bellevue, WA). We used a paired, one-tailed t-test to evaluate data for statistical significance with an alpha value of $P < 0.05$.

**Results**

**Whole cell recordings from glioma cells**

Under typical whole cell recording conditions (i.e., low intracellular Ca²⁺), the human glioma cells studied (D54MG and STTG-1) had stereotypical I-V relationships. Voltage-dependent currents were seen that activated only at positive potentials (more than +50 mV; see Fig. 1). Due to the voltage dependence and rapid deactivation of this current (see Single-channel recordings), tail-current analysis of the reversal potential was not a feasible approach to determine the charge-carrying species. We therefore opted to examine the effect of
intracellular cation replacement on whole cell current density (pA/pF). Figure 1 shows whole cell currents in a representative STTG-1 cell that was serially patched with pipettes containing KCl- or CsCl-based pipette solutions. Current density (pA/pF) was reversibly reduced by intracellular cations other than K+ with the following rank order: K+ > Cs+ > Li+ > Na+ (see Fig. 1C). These data suggest the currents are largely carried by K+ ions. Data from singly and serially patched cells are included in Fig. 1C. These data were obtained from a single passage of STTG-1 cells. Unless otherwise stated, the voltage protocol we used to elicit currents was to step the membrane potential from -120 to +180 mV for 40–80 ms in 20-mV increments from a holding potential of -40 mV (see Fig. 1A, inset). With KCl-based pipette solutions, the average resting membrane potential (measured as the 0 current potential) of 16 STTG-1 astrocytoma cells was -43 ± 13 mV (mean ± SD for all subsequent values, range = -9 to -64 mV), much smaller than typically seen in “normal” rodent glia (Bordey and Sontheimer 1997).

**Pharmacology of the voltage-dependent currents**

The voltage-dependent currents in glioma cells were sensitive to several well-known K+ channel blockers. These included the organic compounds tetraethylammonium ion (TEA) and quinine and the scorpion venom peptides charybdotoxin and iberiotoxin. Because we wanted to quantitatively assess the effects of these drugs on the voltage-dependent currents, online leak-subtraction was performed for these experiments. Charybdotoxin was effective at 50 nM and inhibition was voltage-dependent; block was reduced as the membrane potential was made more positive (at 50 nM, I_{drug}/I_{control} was ≈0.05 at +80 mV and ≈0.5 at +180 mV; data not shown). Iberiotoxin, a selective inhibitor of BK channels (Galvez et al. 1990), inhibited currents in a voltage-independent fashion with an apparent half-maximal concentration (IC_{50}) of ≈10 nM (see METHODS for details and Fig. 2). In a subset of experiments, we tested iberiotoxin at 10 nM and found it was effective at this concentration (I/I_{control} = 0.88 at +140 mV). TEA inhibition of voltage-dependent currents had an IC_{50} of ≈250 μM (see Fig. 3). In a subset of experiments, we tested 10 μM TEA on currents at +140 mV and found no effect.

Tetrandrine is an inhibitor of BK currents that has different effects on channels with and without auxiliary β subunits. At 3 μM, tetrandrine has negligible effects on channels composed of α subunits alone but causes >50% inhibition of channels associated with β subunits (Dworetzky et al. 1996). On average, the voltage-dependent currents in glioma cells were inhibited by 63 and 91% by 3 and 30 μM tetrandrine, respectively (n = 5, see Fig. 4). These results are consistent with the presence of a β subunit. The illustrated currents are “TEA-sensitive” currents obtained by subtracting the average current evoked with a voltage step to +120 mV in the presence of 10 mM TEA from each trace. No time-dependent current component remained in the presence of 10 mM TEA. The effects of all of these drugs were completely reversible.

The pharmacological profile [inhibition by low concentrations of TEA (IC_{50} < 0.5 mM), charybdotoxin, and iberiotoxin] of the voltage-dependent currents is consistent with them representing the activity of BK channels. To pursue this further, we extracellularly applied phloretin, a plant molecule that activates BK channels (Gribkoff et al. 1997), during recordings to observe its effects on whole cell currents. Extracellular phloretin (0.1 mM) increased current amplitude and negatively shifted the activation potential (see Fig. 5). The apparent half-maximal voltages, V_{0.5}, determined from Boltz-
mann fits to the mean currents elicited with ramp voltages (normalized to the peak value seen with phloretin), were +177 and +65 mV under control conditions and in the presence of phloretin, respectively (n = 4, Fig. 5B). Off-line leak subtraction was performed on the ramp currents comprising the data illustrated in Fig. 5B (using the linear portion of these data between −80 and −20 mV). Phloretin-induced currents were reduced by >50% with low concentrations of TEA (0.5 mM) as would be predicted if they represent the activity of the same channel population underlying the voltage-dependent current (Fig. 5C).

The pharmacology of the voltage-dependent currents in glioma cells strongly suggests that they are mediated by BK channels.

$[Ca^{2+}]_i$ dependence

If the voltage-dependent currents in human glioma cells are mediated by BK channels, elevation of the intracellular Ca$^{2+}$ concentration, $[Ca^{2+}]_i$, would be predicted to shift the $I-V$ curve toward more negative potentials. To evaluate this, we made whole cell recordings from STTG-1 cells with pipette solutions with 10 EGTA/zero-added Ca$^{2+}$ and free Ca$^{2+}$ concentrations of 0.1 and 1 mM (see Fig. 6). Increasing $[Ca^{2+}]_i$ resulted in modest increases in current density at negative potentials (likely due to linear Cl$^{-}$ currents) but dramatically increased current densities across the range of 0 to +120 mV (see Fig. 6B). The half-maximal inhibitory concentration, IC$_{50}$, and Hill coefficient are indicated in the figure.

D: IC$_{50}$ values (determined as in C) as a function of $V_m$.
FIG. 5. Effects of extracellular phloretin (0.1 mM) on whole cell currents. A: whole-cell currents evoked with voltage ramps under the indicated conditions. Voltage ramps were applied from -60 to +180 mV at a rate of 1 mV/ms. B: summary of phloretin effects on ramp currents. Data points are means ± SD (n = 4) of ramp currents (normalized to the maximum value seen with phloretin). The apparent half-maximal voltage, $V_{0.5}$, was -177 mV under control conditions and -165 mV with phloretin. C: inhibition of control and phloretin currents by 0.5 mM TEA as a function of voltage.

FIG. 4. Inhibition of voltage-dependent currents by tetrandrine. A: whole-cell currents evoked with voltage steps to +120 mV under the indicated conditions. Illustrated currents are TEA-sensitive currents (see text). B: time course of the experiment illustrated in A. C: means ± SD of fractional remaining current at +120 mV with 3 and 30 μM tetrandrine. Data are from 5 D54MG cells.

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Single-channel recordings

To identify the ion channels underlying the Ca2+-activated K+ currents, we made cell-attached recordings from these cells. Of primary interest were the single-channel conductance, voltage dependence, and the activation kinetics of these channels in cell-attached patches. The latter two parameters could be compared with whole cell currents to identify channels as those underlying the whole cell currents.

The activation kinetics of large-conductance channels in cell-attached patches resembled those of whole cell currents. We constructed ensemble-average currents (average of 200–300 voltage steps to an activating test potential) and compared the kinetics of the ensemble average to whole cell currents at or near the same membrane potential (see Fig. 7). The accuracy of these comparisons was limited by our ability to measure the resting membrane potential of a cell after obtaining a whole cell recording because this value was used to determine the transmembrane potential during the cell-attached phase of the experiment. We measured the resting potential as the zero-current potential seen immediately following breakthrough. Figure 7A illustrates one such experiment in which the transmembrane potential produced by the test potential was judged to be +101 mV ($V_{\text{pip,test}}$ was −120 mV and the measured $V_m$ was −19 mV). It was clear by inspection that the ensemble average strongly resembled the whole cell current (Fig. 7C), and the time constants of activation ($\tau$, determined from single exponential fits of the data; Fig. 7B, dashed line) were also very similar ($\tau_{\text{ensemble}} = 10$ ms and $\tau_{\text{whole-cell}} = 8$ ms). In four ensemble/whole cell pairs at $\approx +120$ mV, $\tau_{\text{ensemble}}$ was 4 ± 1 ms and $\tau_{\text{whole-cell}}$ was 5 ± 2 ms (Fig. 7D). The slightly faster $\tau$ of ensemble-average currents is expected because these channels were exposed to 135 mM [K+]o (concentration of K+ in our pipette solution) while whole cell currents were recorded with 5 mM [K+]o and the $\tau$ of whole cell currents was slightly reduced by elevated [K+]o (see following text). Inward currents through these channels were observed infrequently following an activating voltage step (see Fig. 7A). The tail current in the ensemble average in Fig. 7B has completely deactivated in <2.0 ms. These last findings support our initial approach to study the ionic selectivity of these currents (intracellular cation replacement). Under conditions of low [Ca2+]o, the voltage dependence and rapid deactivation of these BK channels limits the ability to measure reversal potentials with a tail-current protocol.

Activation of channels in cell-attached patches required positive potentials, similar to whole cell currents with low [Ca2+]o. Channels in the cell-attached patch illustrated in Fig. 7A were only seen at transmembrane potentials greater than +40 mV (see Fig. 7E). The unitary current of channels was determined from Gaussian fits of amplitude frequency histograms (see Fig. 7E, inset). With standard pipette solution (KCl-based), the slope of the unitary current-$V$ plot for channels activating at positive potentials suggested an average single-channel conductance of $\approx 150$ pS. However, a wide range of single-channel conductances was observed (range = 120–220 pS, $n = 32$), similar to previous studies on BK channels (Reinhart et al.
1989). The unitary conductance for the channels illustrated in Fig. 7A was 194 pS (see Fig. 7E). Consistent with the high current density of whole cell currents (80 pA/pF at +120 mV), only 2/32 cell-attached patches did not display multi-channel activity. In light of this and the fact that [Ca$^{2+}$]$_{i}$ (an unknown value during cell-attached recordings) modulates the voltage dependence of the channel, we elected not to undertake a more detailed analysis of the voltage dependence of channels in cell-attached patches.

Channels in outside-out patches displayed all the properties of whole cell currents, including activation at positive potentials, block by low concentrations of TEA and iberiotoxin, and dependence on intracellular K$^{+}$. It was difficult to measure unitary current in outside-out patches due to the large number of channels in our patches. In outside-out patches with sufficiently low numbers of channels to resolve single-channel currents over a range of potentials, the unitary-current slope conductance was 92 pS ($n = 4$) in standard bath solution (low K$^{+}$, high Na$^{+}$). The lower unitary conductance in outside-out patches compared with cell-attached patches is likely due to the higher [K$^{+}$]$_{o}$ experienced by channels during cell-attached recording (see following text, Fig. 9D). The unitary current-V curve of channels in outside-out patches developed a pronounced negative slope at potentials greater than +60 mV (20 nM [Ca$^{2+}$]$_{i}$).

[K$^{+}$]$_{o}$-dependence of whole cell currents

Our ability to measure the reversal potential of the voltage-dependent currents was previously limited by their high voltage dependence in the absence of intracellular Ca$^{2+}$. With elevated [Ca$^{2+}$]$_{i}$ (1 μM), clear shifts in reversal potential were observed when [K$^{+}$]$_{o}$ was raised from 5 to 135 mM (see Fig. 8). With 135 mM [K$^{+}$]$_{o}$ currents reversed near E$_{K}$ (−2 mV under these conditions). These experiments confirm the results of our intracellular cation replacements. Despite the 84 mV decrease in driving force for K$^{+}$ ions, current amplitudes above +30 mV were potentiated by elevating [K$^{+}$]$_{o}$ to 135 mM. For the D54-MG cell illustrated in Fig. 8, this potentiation translated into a three- to six-fold increase in whole cell conductance across a range of potentials (see Fig. 8C). Elevated [K$^{+}$]$_{o}$ had insignificant effects on voltage dependence, using V$_{0.5}$ as an index, in experiments with or without [Ca$^{2+}$]$_{i}$ (see Fig. 8C, inset).

The potentiation by [K$^{+}$]$_{o}$ allowed us to detect BK current activation by 1 μM [Ca$^{2+}$]$_{i}$ near typical resting membrane potentials.
potentials (−40 mV; see Fig. 8D). We made rapid applications of 135 mM $[K^+]_o$ to cells. High $[K^+]_o$ resulted in large inward currents at −40 mV in cells with elevated $[Ca^{2+}]_i$. The TEA sensitivity of $[K^+]_o$-induced currents was consistent with BK currents. The current remaining in 10 mM TEA is likely a $K^+$-dependent leak current (note the lack of increased noise in Fig. 8D). The latency from application of high $[K^+]_o$ solution to the response may reflect the speed of our stepper motor and the wash-on and -off times of the high $[K^+]_o$ solution.

Because glioma cells in the interior of a tumor mass are likely to experience elevated $[K^+]_o$, especially near the necrotic center, we wished to determine the range of $[K^+]_o$ over which glioma BK currents are potentiated. In 6/6 cells tested, current amplitudes were increased by 25 mM $[K^+]_o$, (see Fig. 9, A and B). In Fig. 9C, the mean specific $K^+$ conductance (nS/pF) of six cells was plotted as a function of $[K^+]_o$. Conductance increased proportional to the $\sqrt{[K^+]_o}$ as has been described for $[K^+]_o$ potentiation of the conductance of inwardly rectifying $K^+$ currents (Newman 1993; Ransom and Sontheimer 1995), with an ≈5-fold increase in specific conductance going from 5 to 135 mM $[K^+]_o$. Specific conductances were 0.44 ± 0.1, 0.86 ± 0.3, 1.71 ± 0.4, and 2.12 ± 0.7 nS/pF ($n = 6$) in 5, 25, 83, and 135 mM $[K^+]_o$, respectively. Elevated $[K^+]_o$ slightly decreased the time constant of activation (at +120 mV, $\tau_m = 4.8 \pm 0.5$ ms in control and $\tau_m = 3.9 \pm 0.4$ ms in 135 mM $K^+$). The potentiation of currents by $K^+$ was not due to reduction of $[Na^+]_o$ per se because $Na^+$ substitution with choline$^+$ or $N$-methyl-$D$-glucamine$^+$ (NMDG, data not shown) did not increase glioma BK currents.

Consistent with previous studies (Hurley et al. 1999; Lerche et al. 1995; Wann and Richards 1994), the unitary current was increased by elevating $[K^+]_o$. Figure 9D illustrates the effect of increasing $[K^+]_o$, on unitary current amplitude in a representative outside-out patch. At a constant voltage (+70 mV), the unitary current amplitude was increased from +6.7 pA in 5 mM $[K^+]_o$ to +13.6 pA in 135 mM $[K^+]_o$ (see Fig. 9E).

*Endogenous activation of BK channels*

Large-conductance channels in cell-attached patches were generally only observed at positive potentials. However, there were examples of patches in which we detected large-conduc-
brane channels at the resting potential \( V_{\text{pip}} = 0 \text{ mV} \) during cell-attached recording (see Fig. 10). In Fig. 10A, the current response of a cell-attached patch to ramping the pipette potential from +100 to −100 mV from a holding potential of 0 mV is illustrated (STTG-1 cell). The currents show a strong voltage dependence. Single-channel currents could be resolved across a range of potentials and the unitary slope conductance of the channels illustrated in Fig. 10B was 226 pS (see Fig. 10C). The large conductance and voltage dependence is consistent with these channels being BK channels.

**Bradykinin activation of glioma BK currents**

The activation of glioma BK channels at negative potentials requires a rise in \([\text{Ca}^{2+}]_i\). Cellular signals increasing \([\text{Ca}^{2+}]_i\) are expected to activate these currents. Bradykinin is one such mediator that increases \([\text{Ca}^{2+}]_i\) in many cell types, including human glioma cells (T. Manning and H. Sontheimer, unpublished observations). We used amphotericin perforated-patch recordings to demonstrate activation of glioma BK currents by bradykinin (see Fig. 11). In our amphotericin-perforated patch experiments, we gained electrical access within 3 min after establishing a giga seal with series resistances of 12–35 MΩ (see Fig. 11A). Because we were interested in bradykinin effects on the voltage dependence of glioma BK currents, we applied voltage ramps from −120 to +120 mV from a holding potential of −40 mV every 5 s (rate of change was 1 mV/ms). In 9/11 D54MG cells and 5/5 STTG-1 cells, application of bradykinin (1 μM) resulted in a rapid (≤5 s) and transient activation of currents with a strong voltage dependence (see
This activation was manifested as both an increase in current amplitude and, most importantly, a negative shift in the activation potential. The data in Fig. 11B are displayed on an expanded scale in the inset to demonstrate that bradykinin resulted in current activation at negative potentials. This shift was much larger in some cells (down to 250 mV). The negative shift in current activation can also be appreciated with the time course of current change at 140 mV illustrated in Fig. 11C. Because bradykinin effects were transient and we acquired data at 5-s intervals, it is possible that we missed the peak response to bradykinin (and therefore the maximum negative shift in activation). The bradykinin-induced currents were sensitive to 1 mM TEA as expected for BK currents, although these experiments were complicated by the transient nature of bradykinin effects and the fact that we could only elicit a single response in cells (even 15 min after the initial application). To evaluate whether the activation of currents by bradykinin was due to elevation of \([\text{Ca}^{2+}]_i\), we incubated cells with the membrane-permeant, fast calcium-chelator BAPTA-AM (100 \(\mu\)M) for 20–30 min. No responses to bradykinin were seen in 4/4 D54MG cells and 2/2 STTG1 cells incubated with BAPTA-AM during perforated-patch recording (see Fig. 11, D and E). In addition, no response to bradykinin was seen during conventional whole cell recording. These data indicate that bradykinin elevates \([\text{Ca}^{2+}]_i\), of human glioma cells and leads to a transient activation of BK currents at typical resting potentials.

**DISCUSSION**

Our study demonstrates that two human glioma cell lines (STTG-1 and D54MG) express BK channels. We have obtained data from another glioma cell line frequently used by others (U373MG) and confirmed BK expression by these cells as well. The identification of these currents as BK currents is based on the \([\text{K}^+]_i\)-dependence, pharmacological profile, \(\text{Ca}^{2+}\) sensitivity of whole cell currents, and the similar voltage dependence and kinetics of large-conductance channels in cell-attached patches to whole cell currents. In addition to confirming that BK channel expression is a common feature of many human glioma cells (Brismar 1995), our study provides additional descriptions of the pharmacology, \(\text{Ca}^{2+}\) sensitivity, \([\text{K}^+]_o\) dependence of the BK channels in these cells, and one example of an endogenous ligand (bradykinin) that activates them. The expression of BK channels by human gliomas is intriguing because, as pointed out by Tseng-Crank et al. (1994), the single gene for these channels is located on chromosome 10, which is affected in many tumors including 60% of glioblastomas (Kleihues et al. 1995). Our laboratory has recently PCR cloned a novel BK \(\alpha\)-subunit from glioma cells using specific primers for BK channels, confirming BK expression in these cells (X. Liu and H. Sontheimer, unpublished observations).

The glioma cells studied here expressed high levels of BK channels. We routinely recorded very large (>10 nA) currents from these cells. The mean current density of five STTG-1 cells at 60 mV with 0.1 \(\mu\)M \([\text{Ca}^{2+}]_i\) was \(\approx70\) pA/pF. Using
this value and a single-channel current of \( \approx 9 \) pA at \(+60\) mV (from 4 outside-out patches with normal bath solution, i.e., high Na\(^+\), low K\(^+\)), one can calculate a lower limit for channel density of seven to eight channels/\( \mu \text{m}^2 \) [using a specific membrane capacitance value of \(1 \) pF/\( \mu \text{m}^2\) (Hille 1992)]. Given this density, it is not surprising that our outside-out patches had large numbers of channels. Our channel density estimate is a lower limit because open probability is unlikely to be unity under these conditions and the single-channel conductance (determined with \([\text{Ca}^{2+}]_i\)) if anything would be reduced by elevations of \([\text{Ca}^{2+}]_i\) (Marty 1981).

Previous studies on these cell lines showed that a substantial proportion of the voltage-gated outward currents were inhibited by \([\text{Cl}^-]_o\) removal (Ullrich and Sontheimer 1996). However, under our experimental conditions we found that the majority of the voltage-dependent outward currents were sensitive to intracellular cation replacement and iberiotoxin. We suspect these differences may relate to the variations in the relative expression of K\(^+\) and Cl\(^-\) currents in these cells (unpublished observations).

In STTG-1 astrocytoma cells, we found a half-maximal voltage for whole cell current activation, \(V_{0.5}\), of \(+12\) mV with...
1 μM [Ca\(^{2+}\)]. The \(V_{0.5}\) for BK channels in human smooth muscle was +35 mV with 1 μM [Ca\(^{2+}\)], (Hurley et al. 1998) and in human skeletal muscle the \(V_{0.5}\) was +50 mV with 0.5 μM [Ca\(^{2+}\)], (extrapolated \(V_{0.5}\) of +55 mV at 1 μM [Ca\(^{2+}\)]). (Lerche et al. 1995). Two BK channels cloned from human brain had \(V_{0.5}\) of +18 mV with 10 μM [Ca\(^{2+}\)], (bbr5) and +9 mV with 24 μM [Ca\(^{2+}\)], (bbr3) (Tseng-Crank et al. 1994). In native human macrophages, BK channels had a \(V_{0.5}\) of ∼+22 mV with 3 μM [Ca\(^{2+}\)], (Gallin 1984). The Ca\(^{2+}\) sensitivity of BK channels in a leukemic human macrophage cell line was the closest to that reported here for BK channels in glioma cells; \(V_{0.5}\) was −7.5 mV with 3 μM Ca\(^{2+}\) (DeCoursey et al. 1996). Thus, glioma BK channels have an equal or greater Ca\(^{2+}\) sensitivity than those described in many other human preparations. An enhanced Ca\(^{2+}\) sensitivity would presumably allow BK channels in human glioma cells to gate in response to smaller [Ca\(^{2+}\)], rises than is required in other cells. Clear activation of channels was seen near typical resting potentials (∼−40 mV) with 1 μM [Ca\(^{2+}\)], (see Fig. 8D). The tetrandrine sensitivity of glioma BK currents was consistent with the presence of a β subunit, and this would contribute to the Ca\(^{2+}\) sensitivity (Dworetzky et al. 1996; McManus et al. 1995). The possibility exists that our data (obtained from whole cell recordings) may underestimate the true Ca\(^{2+}\) sensitivity of these channels. If [Ca\(^{2+}\)], regulatory mechanisms are still intact during whole cell recordings, the steady-state [Ca\(^{2+}\)], may not equal [Ca\(^{2+}\)]_{pipette} particularly at distal parts of cells (Mathias et al. 1990).

The high voltage dependence of BK currents in the absence of Ca\(^{2+}\) raises questions about their functional role. Gating at the resting potential is requisite for a meaningful role of BK channels in glioma biology. We were able to demonstrate endogenous activation of these channels in cell-attached patches and bradykinin stimulation of cells during amphotericin-perforated patch recording shifted activation of BK currents into the range of typical resting membrane potentials. We suggest that bradykinin is only one example of a mediator that could lead to BK activation via elevation of [Ca\(^{2+}\)].

The [K\(^{-}\)]_o dependence of the BK currents in glioma cells is a feature of these channels with functional implications. The square-root relationship of conductance and [K\(^{-}\)]_o makes the relative modulation of BK channels by [K\(^{-}\)]_o, the greatest in the physiologic range of [K\(^{-}\)]_o. The potentiation of BK currents by [K\(^{-}\)]_o suggests that the functions of glioma BK channels would be augmented under conditions of elevated [K\(^{-}\)]_o, such as may exist near the center of a rapidly-growing tumor. Inhibition of BK channels reduced Muller cell proliferation only in the presence of 20 mM [K\(^{-}\)]_o (Bringmann et al. 2000). This was suggested to be due to effects on membrane potential but could also relate to BK potentiation by K\(^{-}\). Our data suggest that the whole cell BK conductance would be increased approximately twofold under these conditions. BK current enhancement by elevated [K\(^{-}\)]_o, is due in part to an increased single-channel conductance, in line with other studies (Hurley et al. 1999; Lerche et al. 1995; Wann and Richards 1994). This may be a result of allosteric actions of K\(^{-}\) ions themselves on the channel or altered permeation properties with high [K\(^{-}\)]_o. Our data are suggestive of an allosteric action of K\(^{-}\) because the observed potentiation was opposite to that predicted by driving force considerations or single-file pore models of permeation. No potentiation was seen during Na\(^{+}\) substitution with choline\(^{+}\) or NMDG\(^{+}\), indicating that the potentiation was not due to Na\(^{+}\) removal per se. However, we cannot rule out complicated ion-ion interactions between Na\(^{+}\) and K\(^{+}\) within the pore of the channel as giving rise to the increased unitary currents in high [K\(^{+}\)]o.

The electrophysiology of the glioma cells studied here is greatly different from that of “normal” rodent glia (Bordey and Sontheimer 1997; Sontheimer 1994). Specifically, mature astrocytes have low input resistances, inwardly rectifying K\(^{+}\) currents, and large resting potentials. The glioma cells studied here had larger input resistances, outwardly rectifying I-V curves, and small resting potentials. In addition, there are no descriptions of large-conductance, Ca\(^{2+}\)-activated K\(^{+}\) channels in astrocytes (but see Nowak et al. 1987; Quandt and MacVicar 1986). Furthermore, astrocytes express voltage-gated delayed-rectifier and A-type K\(^{+}\) currents that were absent from the glioma cells under investigation here (Bordey and Sontheimer 1997). Thus the biophysics of glioma cells are distinct from “normal” glia. In normal rodent glia, K\(^{+}\) channel expression is functionally involved in proliferation (Bordey and Sontheimer 1997; MacFarlane and Sontheimer 1997, 2000a,b). In rat astrocytes, inwardly rectifying K\(^{+}\) channels (K<sub>ir</sub>) are associated with a quiescent proliferative state and selective inhibition of K<sub>ir</sub> increases proliferation (MacFarlane and Sontheimer 2000a). Given the role of K\(^{+}\) channels in the proliferation of normal glia, it is reasonable to suggest that the altered biophysics of glioma cells, in particular BK expression, could contribute to their malignant behavior. The relevance of studies on rodent astrocytes to human glioma cells is supported by the observations that only STTG-1 cells expressed Ba\(^{2+}\)-sensitive inwardly rectifying K\(^{+}\) currents, and these cells grew to confluence approximately threefold slower than D54MG cells when plated at the same density (unpublished observations).

There are several types of Ca\(^{2+}\)-activated K\(^{+}\) channels [K(Ca)]. In addition to the voltage-dependent large-conductance Ca\(^{2+}\)-activated K\(^{+}\) channels (the type described in this study), there are small- and intermediate-conductance Ca\(^{2+}\)-activated K\(^{+}\) channels that are voltage independent. In excitable cells, the varieties of Ca\(^{2+}\)-activated K\(^{+}\) channels likely perform similar roles, namely to oppose depolarizations accompanied by rises in [Ca\(^{2+}\)]. In inexcitable cells, the different classes of Ca\(^{2+}\)-activated K\(^{+}\) channels may underlie different functions in different cell types. Retinal glial (Muller) cells from diseased retina (proliferative retinopathy) show increased expression of BK channels, suggesting a correlation between BK channel expression and proliferative retinopathy (Bringmann et al. 2000). The link between BK channels and a proliferative/undifferentiated state is substantiated by the observations that normal Muller cells lose their BK channels postnatally with a time course paralleling the maturation of these cells and BK channel inhibition reduced proliferation of rabbit Muller cells and human endothelial cells (Bringmann et al. 2000; Wiecha et al. 1998). Likewise, intermediate-conductance Ca\(^{2+}\)-activated K\(^{+}\) channels are up-regulated in transformed fibroblasts, suggestive of a role in proliferation (Pena and Rane 1999; Rane 1991). In human THP-1 monocytes, however, BK channels were only observed in PMA-differentiated cells that are nondividing (DeCoursey et al. 1996). Other studies on astrocytoma cells and meningioma cells found no effects of charybdotoxin on cell growth (Chin et al. 1997; Kraft et al. 2000), suggesting K(Ca) channels do not contribute to the proliferative
response of these tumor cells, in contrast to data from Muller cells, endothelial cells, and fibroblasts. We have performed $[H^+]$-thymidine incorporation assays on our D54MG and STTG1 cells and found no effect of TEA on $[H^+]$-thymidine incorporation (unpublished observations), similar to the results in other astrocytoma cells and meningioma cells (Chin et al. 1997; Kraft et al. 2000).

Alternatively, K(Ca) channels have been shown to participate in volume regulation and migration (Pasantes-Morales et al. 1994; Schwab et al. 1999). Potassium channels are postulated to participate in migration by affecting net salt fluxes, in conjunction with Cl⁻ channels. These salt fluxes, with their accompanying water, result in volume/shape changes permissive to migration through narrow spaces and promote the movement of cytosol into the expanding leading edge. In human glioma cells, TEA (at concentrations that selectively inhibit BK channels, i.e., $<1$ mM) reduced migration by $\sim$40% (Sorocoeau et al. 1999). In contrast, other studies on human glioma cells have suggested that BK activation completely stops migration (Bordey et al. 2000). The precise role of BK channels in glioma cells remains to be determined.

In summary, we have shown that the human glioma cell lines we studied are well endowed with BK-type K⁺ channels. Taken together with the work of others, our study indicates that BK channels are a common feature of human glioma cells. The BK channels in glioma cells were active at typical resting potentials with $[Ca^{2+}]_i$, near 1 μM. The $[K^+]_o$ dependence of BK channels in glioma cells is an attribute of these channels with functional implications for glioma biology. We propose that these channels will contribute to the response of glioma cells to stimuli that increase $[Ca^{2+}]_i$, such as bradykinin.

The authors appreciate the discussions and comments of Drs. Robin Lester, David Weiss, and Zucheng Ye.

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-36972 and American Cancer Society Grant RPG-97-083-01CDD to H. Sontheimer and by a Medical Scientist Training Program scholarship to C.B. Ransom.

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Bordey A, Sontheimer H, and Troussard J. Muscarinic activation of BK channels in glioma cells and meningioma cells (Chin et al. 1997; Kraft et al. 2000). Alternatively, K(Ca) channels have been shown to participate in volume regulation and migration (Pasantes-Morales et al. 1994; Schwab et al. 1999). Potassium channels are postulated to participate in migration by affecting net salt fluxes, in conjunction with Cl⁻ channels. These salt fluxes, with their accompanying water, result in volume/shape changes permissive to migration through narrow spaces and promote the movement of cytosol into the expanding leading edge. In human glioma cells, TEA (at concentrations that selectively inhibit BK channels, i.e., $<1$ mM) reduced migration by $\sim$40% (Sorocoeau et al. 1999). In contrast, other studies on human glioma cells have suggested that BK activation completely stops migration (Bordey et al. 2000). The precise role of BK channels in glioma cells remains to be determined.

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