Electrophysiological Properties of Human Astrocytic Tumor Cells In Situ: Enigma of Spiking Glial Cells

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Boréy, Angélique and Harald Sontheimer. Electrophysiological properties of human astrocytic tumor cells in situ: enigma of spiking glial cells. J. Neurophysiol. 79: 2782–2793, 1998. To better understand physiological changes that accompany the neoplastic transition of astrocytes to become astrocytoma cells, we studied biopsies of low-grade, pilocytic astrocytomas. This group of tumors is most prevalent in children and the tumor cells maintain most antigenic features typical of astrocytes. Astrocytoma cells were studied with the use of whole cell patch-clamp recordings in acute biopsy slices from 4-mo- to 14-yr-old pediatric patients. Recordings from 53 cells in six cases of low-grade astrocytomas were compared to either noncancerous peritumoral astrocytes or astrocytes obtained from other surgeries. Astrocytoma cells almost exclusively displayed slowly activating, sustained, tetrodotoxin (TTX)-sensitive outward potassium currents (delayed rectifying potassium currents; $I_{\text{DR}}$) and transient, tetrodotoxin (TTX)-sensitive sodium currents ($I_{\text{Na}}$). By contrast, comparison glial cells from peritumoral regions or other surgeries showed $I_{\text{DR}}$ and $I_{\text{Na}}$, but in addition these cells also expressed transient "$A$"- and $B$-type $K^+$ currents and inwardly rectifying $K^+$ currents ($I_{\text{IR}}$), both of which were absent in astrocytoma cells. $I_{\text{Na}}$ constituted the predominant conductance in comparison astrocytes and was responsible for a high-resting $K^+$ conductance in these cells. Voltage-activated $Na^+$ currents were observed in 37 of 53 astrocytoma cells. $Na^+$ current densities in astrocytoma cells, on average, were three- to fivefold larger than in comparison astrocytes. Astrocytoma cells expressing $I_{\text{IR}}$ could be induced to generate slow action potential-like responses (spikes) by current injections. The threshold for generating such spikes was $–34 \text{ mV}$ (from a holding potential of $–70 \text{ mV}$). The spike amplitude and time width were $52.5 \text{ mV}$ and $12 \text{ ms}$, respectively. No spikes could be elicited in comparison astrocytes, although some of them expressed $Na^+$ currents of similar size. Comparison of astrocytes to astrocytoma cells suggests that the apparent lack of $I_{\text{IR}}$, which leads to high-input resistance ($>500 \text{ M\Omega}$), allows glioma cells to be sufficiently depolarized to generate $Na^+$ spikes, whereas the high resting $K^+$ conductance in astrocytes prevents their depolarization and thus generation of spikes. Consistent with this notion, $Na^+$ spikes could be induced in spinal cord astrocytes in culture when $I_{\text{Na}}$ was experimentally blocked by $10 \mu\text{M} \text{ Ba}^{2+}$, suggesting that the absence of $I_{\text{IR}}$ in astrocytoma cells is primarily responsible for the unusual spiking behavior seen in these glial tumor cells. It is unlikely that such glial spikes ever occur in vivo.

INTRODUCTION

Unlike most neurons, glial cells retain the ability to divide postnatally (Gensert and Goldman 1996; Korr 1986). Glial proliferation accompanies reactive gliosis, the brain’s response to injury (Reier 1986) and the malignant transformation of glial cells. Glial-derived tumors are collectively termed gliomas and include astrocytic, oligodendroglial, ependymal, and glial-neuronal tumors (Kleihues et al. 1993a,b). The majority of gliomas fall into the astrocytic lineage (astrocytomas) and presumably derive from astrocytes. They still share some common features with astrocytes, including variable expression of glial fibrillary acidic protein (GFAP), $S100$, and growth factor receptors (Bigner et al. 1981; Collins 1995; Kleihues et al. 1993a,b). This is particularly true for low-grade astrocytomas, the most prevalent primary brain tumor in children. Factors that induce the transition from glia to glioma are not well understood. However, it is evident that this transition is accompanied by a number of genetic alterations, gene deletions, and gene amplifications (Collins 1995; Westmark et al. 1995) that are also typical of cancer cells outside the nervous system.

Attempts to understand mechanisms that underlie cell growth and differentiation have largely focused on studying cancers of the body. Several of these studies suggest that alterations in ion channel complement are associated with and possibly are essential to allow cell proliferation to occur (Sontheimer 1995). For example, in lymphocytes entry into the cell cycle is associated with the up-regulation of $Ca^{2+}$-activated $K^+$ channels (Cahalan et al. 1991; DeCoursey et al. 1984) and blockade of these channels retards cell proliferation. Pharmacological block of $K^+$ channels also inhibits proliferation of brown fat cells (Pappone and Ortiz-Miranda 1993), melanoma cells (Nilius and Wohlrab 1992), human breast cancer cells (Woodfork et al. 1995), lung small cancer cells (Pancrazio et al. 1993), retinal glial cells (Puro et al. 1989), Schwann cells (Wilson and Chiu 1993), O2-A progenitor cells (Gallo et al. 1996), and astrocytes (Pappone et al. 1994). Mechanisms by which ion channel complement affects cell proliferation are largely unknown. Some recent studies have begun to shed light on interactions between tumor suppressor genes or oncogenes and ion channel function. For example, transfection of fibroblasts with ras21 or exposure of fibroblasts to epidermal growth factor or platelet-derived growth factor leads to the induction of a novel $Ca^{2+}$-activated $K^+$ channel that is essential for cell cycle progression in these cells (Huang and Rane 1994). In Droso phila, the tumor suppressor gene $dlg$ interacts with "$A$"-type $K^+$ channels leading to channel clustering (Tejedor et al. 1997).

Despite our expanding knowledge of ion channel expression in normal rat or mouse glial cells, little is known about properties of glial-derived tumor cells. The few studies that have investigated glial cell-derived tumors have largely focused on studying several well-established human cell lines. These transformed glial cells possess a plethora of ion channels (Brismarr 1995; Matute et al. 1992). However, culturing...
cells and passing them on for generations may alter their phenotypes as has been demonstrated in other cells, including glial cells (Bigner et al. 1981; Kennedy et al. 1987; Noble et al. 1995). We recently reported that ion channel complement of astrocytes in situ differs from the channel complement of cultured astrocytes (Bordey and Sontheimer 1997). Our understanding of possible biophysical changes underlying malignant transformation of glial cells is further hampered by the fact that properties of ‘normal’ human glial cells are largely unknown.

We set out to investigate properties of astrocyte-derived tumors as compared with noncancerous control astrocytes in patient biopsies. We focused on a comparison between the lowest grade astrocyte tumors, the pilocytic astrocytomas, because unlike higher grade gliomas, these astrocytoma cells maintain several biochemical and morphological features with astrocytes and may thus be most relevant in understanding changes that underlie the transition from glia to glioma. Our data suggest that astrocytoma cells lose expression of inwardly rectifying K⁺ channels, which are abundantly expressed in astrocytes. These channels are believed to be instrumental in aiding K⁺ buffering in the brain. They also endow astrocytes with a relatively negative and stable resting potential. Because of the absence of \( I_K \) in conjunction with the prominent expression of \( I_Na \) in astrocytoma cells, these cells could be induced to fire slow, action potential-like responses not seen in normal astrocytes. The latter, however, could be induced to exhibit spikes when \( I_Na \) was pharmacologically inhibited suggesting that the lack of \( I_Na \) in astrocytoma cells is principally responsible for the unusual spiking behavior.

**METHODS**

**Patient selection**

Human brain specimens were obtained from six pediatric patients who underwent resections of primary central nervous system neoplasms. Clinically these cases did not present with seizures. Parallel recordings were performed in comparison tissue also obtained from three pediatric patients. These tissues were resected outside the tumor margins of a choroid plexus carcinoma and a hypotalamalic pilocytic astrocytoma, respectively. One additional comparison biopsy was obtained from the cortex of a patient who underwent a hemispherectomy to surgically treat Rasmussen’s encephalitis. Histopathological examination showed that none of these comparison tissues contained neoplastic cells. We refer to these tissues throughout as comparison tissue, acknowledging the fact that cells in these tissues may also have altered properties.

**Slice preparation**

Methods for preparing slices are described in detail in Ullrich et al. (1997). Human tissue was obtained during surgery and collected in the operating room where it was immediately placed in ice-cold (4°C) calcium-free artificial cerebrospinal fluid (ACSF–Ca-free) containing (in mM) 116 NaCl, 4.5 KCl, 0.8 MgCl₂, 26.2 NaHCO₃, 11.1 glucose, and 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), oxygenated with 95% O₂–5% CO₂. Because of its fragile and mixed consistency, tissue was embedded in agar and then glued (cyanoacrylate glue) to the stage of a Vibratome. Slices of 150–300 μm were cut in cold oxygenated ACSF–Ca-free and transferred to a beaker filled with ACSF–Ca-free at room temperature. After a recovery period of ≥2 h in ACSF–Ca-free, slices were placed in a flow-through chamber continuously perfused with oxygenated ACSF containing 1.8 mM CaCl₂ at room temperature. The chamber was mounted on the stage of an upright microscope (Nikon Optiphot2) equipped with a ×40 (2-mm working distance) water immersion objective and Nomarski optics.

**Whole cell recordings and data analysis**

Whole cell patch-clamp recordings were obtained as previously described for hippocampal slices (Bordey and Sontheimer 1997; Edwards et al. 1989). Patch pipettes were pulled from thin-walled borosilicate glass (1.55 mm od; 1.2 mm id; TW150F-40, WPI) on a PP-83 puller (Narishige, Japan). Pipettes had resistances of 4–6 MΩ when filled with the following solution (in mM): 145 KCl, 0.2 CaCl₂, 1.0 MgCl₂, 10 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), and 10 HEPES (sodium salt), pH adjusted to 7.2 with tris(hydroxymethyl)aminomethane (Tris). To label cells for later morphological and antigenic identification, 0.1% Lucifer yellow (LY; dilithium salt) was added to the pipette solution. Voltage-clamp recordings were performed with an Axopatch-200A amplifier (Axon Instruments). Current signals were low-pass filtered at 5 kHz and digitized on-line at 25–100 kHz by using a Digidata 1200 digitizing board (Axon Instruments) interfaced with an IBM-compatible computer system. Data acquisition, storage, and analysis were done with the use of pClamp version 6 (Axon Instruments). For all measurements, capacitance and series resistance compensation (60–80%) were used to minimize voltage errors. Settings were determined by compensating the transients of a small (5 mV) 10-ms hyperpolarizing pulse as to minimize voltage errors. Settings were determined by compensating the transients of a small (5 mV) 10-ms hyperpolarizing voltage step; the capacitance reading of the amplifier was used as value for the whole cell capacitance. After compensation, series resistances ranged from 6 to 10 MΩ. Membrane resistance (\( R_m \)) was determined from the average response to 50 hyperpolarizing (10 mV) current pulses (20 ms). The resulting membrane current charging curves were fitted either to a single or double exponential.

Unless indicated otherwise, capacitive and leak conductances were subtracted on-line by a modified P/5 protocol. This P/5 protocol consisted of five hyperpolarized subpulses of \(-\frac{1}{5}\) amplitude executed before each episode, summed and added to the current trace of interest. Subpulses holding potential was \(-80\) mV. Peak currents were determined by using Clampfit (Axon Instruments) and statistical values (mean ± SE in the text, ±SD in the table; \( n \) = number of cells tested) were evaluated with a statistical graphing and curve-fitting program (Origin, MicroCal). Chemicals were purchased from Sigma unless otherwise noted.

**Steady-state parameters for activation and inactivation of sodium current**

To establish steady-state activation or inactivation curves, the peak current \( I \) was measured at each potential and the corresponding conductance \( G \) was calculated by using the following equation

\[
G = \frac{I}{V - V_i}
\]

where \( V \) is the membrane command potential and \( V_i \) is the predicted equilibrium (Nernst) potential for the ion (i) under consideration. At 20°C, calculated equilibrium potentials for potassium and sodium were \(-87.7\) and 67.0 mV, respectively. The measured peak amplitudes and the calculated peak conductance were then normalized and plotted as a function of the membrane potential during the test pulse. The resulting inactivation and activation curves were fit to the Boltzmann equation

\[
G_i/G_{i(max)} = 1/(1 + \exp(V/V_{1/2} - V_k))
\]

where \( G_{i(max)} \) is the maximum ionic conductance at peak current, \( V_{1/2} \) is the voltage where \( G_i \) is one-half of \( G_{i(max)} \), and \( k \), the slope factor, determines the voltage-dependent behavior of \( G_i \).
RESULTS

Whole cell recordings were obtained from acute tissue slices of nine patient biopsies and included 53 glioma cells and 9 nonneoplastic astrocytes for comparison. Although comparison tissue was also derived from patients, these tissues did not contain neoplastic cells as confirmed by histopathological examinations. Hence, despite the possibility that cells in these tissues may also have altered properties, they represent the best possible comparison tissue for purposes of this study. All tissues were histopathologically characterized and classified with the use of criteria set forth by the World Health Organization (WHO) (Kleihues et al. 1993a,b). They included four cases of pilocytic astrocytomas (WHO grade I) and one desmoplastic and one giant cell astrocytoma, also considered to be WHO grade I tumors. These tumors were resected from children between 4-mo and 14-yr old (mean age 5 yr). Comparison tissue (3 cases) was cortical tissue associated with a choroid plexus carcinoma and a hypotalphalamic pilocytic astrocytoma and cortical tissue from a patient with Rasmussen’s disease (3-yr-old patient).

Tumor tissue consisted of densely packed, round tumor cells (Fig. 1A; black arrows point out round cells) and their processes. Cell processes stained positive for GFAP (Bordey and Sontheimer 1997; Kharbanda et al. 1993). Tumor tissue could be readily distinguished from surrounding normal peritumoral tissue microscopically (Fig. 1C). The tumor tissue was fragile and showed thinner sections after cutting. Figure 2 shows two representative cells, one from a pilocytic astrocytoma cell from the hypothalamus and one from a nonneoplastic comparison astrocyte from peritumoral tissue from a choroid plexus carcinoma. These cells were each filled with LY during whole cell recording to allow morphological and antigenic characterization. The comparison astrocyte was GFAP positive and its morphology resembled that of stellate astrocytes in rat hippocampus in situ (Bordey and Sontheimer 1997) with 2–3 major processes and many smaller processes of ~50–60 μm (Fig. 2A). The pilocytic astrocytoma cells had a round cell body and extended only 2–3 major processes (Fig. 2B). This cell, like many others, showed some LY coupling to adjacent cells. Typically, dye spread to less than five adjacent cells.

The recordings from these two cells (Fig. 2, A and B, right panels) are representative examples for each tissue group, and the traces show raw data without leak subtraction elicited by 50-ms voltage steps to potentials ranging from −160 to 60 mV (10-mV increments). These recordings show prominent expression of outward potassium currents in both glioma and comparison astrocytes. However, comparison cells always expressed large inward K+ currents in addition to outward K+ currents. Inward K+ currents were absent in glioma cells. This difference in rectification is more apparent in the corresponding current-voltage curves (I-V) plotted in Fig. 2C. Current amplitudes were determined 4 ms (●, ■) and 35 ms (○, □) after the beginning of the recording and plotted as a function of applied potentials. I-V curves of astrocytoma cells (■, □) were always outwardly rectifying, whereas I-V curves of comparison astrocytes (●, ○) were either inwardly rectifying or linear at potentials near the resting potential. We previously reported expression of outwardly rectifying glioma-associated chloride currents (GCC) that are activated by very positive voltages (e.g., >45 mV (Ullrich et al. 1997)). To eliminate possible contamination of current traces with GCC, we restricted our analysis of peak currents to potentials <30 mV, where Cl− currents were not activated. A more detailed dissection of inward and outward currents is discussed below and shown in Figs. 3–4.

Data from all recorded cells are summarized in Table 1, which in addition to densities of current and conductance of the different current subtypes also gives mean values (±SD) of resting membrane potential (Vr), membrane capacitance (Cm), and resting membrane resistance (Rm, measured at −80 mV). For consistent comparison, all biophysical parameters (e.g., Cm, Vr, and Rm) were determined in the first 3 min of whole cell recordings. Conductance densities were used as measures that allow us to compare the relative expression of current components independent of cell size. Conductance of outward currents was determined at 30 mV and conductance of If at −150 mV. Kinetic and pharmacological analysis of the outward and inward currents in tumor cells were performed in cells having series resistances <10 MΩ and with the use of leak-subtraction protocols.

Astrocytoma cells had significantly more depolarized membrane potentials than comparison astrocytes whose potential was similar to that reported previously for rat astrocytes (Bordey and Sontheimer 1997) (P < 0.001). However, mean membrane capacitance values were similar, suggesting that cells were of approximately equal size. This is consistent with our morphological observations (Fig. 2).

Sodium current, INa

Transient, fast, inward currents were observed in 37 of 53 (70%) glioma cells. These currents were almost completely blocked by 100 nM TTX (Fig. 3A; n = 10 cells, 3 cases). TTX-block was partially reversible and did not significantly alter the resting potential of the cell. Currents were activated with voltage steps more positive than −50 mV that peaked within <3 ms and inactivated rapidly (Fig. 3A, left panel). Current amplitudes were voltage-dependent, peaked at −30 mV, and decreased with more depolarized voltage steps. I-V curves were generated either in the presence of TEA (10 mM) to block K+ currents (Fig. 3B, ●) or used the TTX-sensitive current component after subtraction (Fig. 3B, ○). This analysis included 14 cells from five cases (every biopsy except giant cell astrocytoma) and mean values were plotted in Fig. 3B. The extrapolated current reversal potential was 64.7 ± 1.7 (SE) mV (n = 14), which is in good agreement with the predicted equilibrium potential for Na+ ions (ENa = +68 mV) under the imposed ionic gradients suggesting that these fast, transient, inward currents were carried by Na+ ions. Steady-state activation and inactivation curves (Fig. 3D) were constructed according to Eqs. 1 and 2 (see METHODS) and again included 14 cells from the same five cases. Boltzmann fits of the activation curves gave values of −20.0 ± 2.5 mV for V1/2 and 6.9 ± 0.7 mV−1 for k (n = 7). Sodium currents demonstrated typical steady-state inacti-
FIG. 1. Human astrocytoma biopsies. A: high power photograph (×400) of a live section of a pilocytic astrocytoma. Cells are densely packed and tend to have round cell bodies (arrows point to representative tumor cells). B: glial fibrillary acidic protein (GFAP) staining of the same slice as in A; primarily processes traversing the slice stain positive for GFAP (white arrows). Cell bodies are only weakly stained. C: low power photograph (×100) of same slice as in A shows tumor margins delineated by a dashed white line.

vation (Fig. 3, C and D). The Boltzmann fits of the inactivation curves gave an e-fold change per 8.0 ± 0.4 mV⁻¹ (n = 18) and a mean V_{1/2} of −59.2 ± 1.9 mV (n = 18). Parameters for the activation and inactivation curves in comparison astrocytes were similar with values for V_{1/2} of −21.6 and −57.4 mV and 8.7 and 7.5 mV⁻¹ for k, respectively (data not shown).

To compare the relative Na⁺ channel expression in astrocytoma cells to comparison astrocytes, we determined sodium conductance density, G_{Na} (sodium conductance divided by the membrane capacitance), for each cell and pooled data from each patient. Astrocytoma cells in the four pilocytic and the desmoplastic tumors consistently expressed conductance densities that were approximately three- to fivefold higher than in comparison cells (Table 1).

**Outward potassium currents**

To study outward currents, depolarizing voltage steps were applied from a holding potential of −70 mV. This protocol activated sodium currents followed by sustained outward currents. The outward currents recorded in 38 of 53 (72%) tumor cells showed delayed and slow inactivation (Fig. 4A). The I-V relationship of outward currents derived from mean data of eight cells from two pilocytic astrocytomas suggested an activation threshold of approximately −50 mV (Fig. 4B) as compared with approximately −30 mV for two comparison astrocytes. The voltage-dependence of the sustained current showed kinetics reminiscent of delayed rectifying potassium currents (I_{DR}) described in numerous other preparations (Rudy 1988). Bath application of 10 mM TEA reduced I_{DR} by 32% (n = 4; Fig. 4C). Tail current analysis yielded a reversal potential of −85 mV closed to the predicted Nernst reversal potential for K⁺ (E_K = −88 mV; Fig. 4, D and E).

If currents were activated after applying a conditioning 200-ms prepulse to −110 mV (a protocol used to activate transient A-type K⁺ currents), no additional transient out-
FIG. 2. Astrocytoma cells are electrophysiologically distinct from comparison astrocytes. Left panel: characteristic morphology of Lucifer yellow±filled cells; right panel: representative whole cell currents after stepping the cell membrane from −160 to +80 mV from a holding potential of −80 mV of the corresponding recorded cell. (A: comparison nontumorous astrocyte; B: pilocytic astrocytoma cell; C: corresponding current-voltage relationships of the displayed traces from A and B.) Current amplitudes were determined 4 ms (●, ●) and 35 ms (○, ○) after the beginning of the recording and plotted as a function of applied potentials. I-V curve of the pilocytic astrocytoma cell (●, ○) was outwardly rectifying, whereas I-V curve of the comparison astrocyte (●, ○) was linear.

ward potassium current could be activated in 89% of all studied cells (data not shown). In the remaining six pilocytic astrocytoma cells, which were all from one tumor case (posterior fossa), transient A-currents with conductance densities of 391.6 ± 148.7 pS/pF could be identified and example recordings are shown in Fig. 5. These currents could be isolated by activation from a prepulse potential of −110 mV (Fig. 5A) and subsequent subtraction of currents activated from a prepulse potential of −70 mV (Fig. 5B) yielding a transient current component that activates at potentials greater than −60 mV (Fig. 5C). This is more readily visible if peak currents are plotted as a function of applied potential (Fig. 5D). Steady-state inactivation of the transient current was analyzed by varying the prepulse potential from −180 to −20 mV. The subtracted transient current (from protocols with and without prepulse; Fig. 5E) was plotted as a function of applied prepulse potential in Fig. 5D (○) for five tumor cells and showed a $V_{1/2}$ (for inactivation) of −84.4 ± 1.7 mV ($n = 5$). This value was more hyperpolarized than a $V_{1/2}$ of −60.3 mV in a comparison astrocyte (data not shown). Moreover, at the resting membrane potential of pilocytic astrocytoma cells (−43.4 ± 4.7; $n = 6$), this transient outward potassium current displayed no overlap between steady-state activation and inactivation curves (Fig. 5D), suggesting that currents were not available for activation at the cells resting potential.

Whole cell recordings were also obtained in which the membrane was stepped from a prepulse potential of 0 mV to more negative potentials ranging from −180 to 0 mV (data not shown). This protocol was applied to facilitate isolation of $I_{IR}$ (Ransom and Sontheimer 1995). This type of current was found only in 2 of 11 cells of one pilocytic astrocytoma and was absent in 96% of all studied cells (see Table for conductance values). The inward current showed a marked current inactivation at potentials more negative than −130 mV. In addition, the I-V curve showed inward rectification typical of cesium- and barium-sensitive inwardly rectifying K⁺ channels.

Spikes induced by current injections in astrocytoma cells

Current injection into some tumor cells induced transient overshooting responses reminiscent of slow action potentials
(Fig. 5, A and B, at different timescales). Only single spikes could be induced with each current injection; thus we consider these spikes not regenerative. In four cells held at −70 mV, an average of 10 action potentials gave a mean spike amplitude of 52.5 ± 3.5 mV. The threshold was −33.9 ± 1.0 mV. The mean rise time (beginning of the pulse to the peak spike value) was 17.1 ± 2.8 ms and the duration of the spike was 11.8 ± 0.6 ms. Because generation of action potentials depends on the ratio of inward to outward current, we calculated conductance densities (conductance obtained by Eq. 1, METHODS, divided by the membrane capacitance) mediated by potassium channels (G_K, corresponding to the total potassium density of conductance in these cells) and sodium channels (G_Na) to obtain the ratio G_K:G_Na. The ratio G_K:G_Na in astrocytoma cells was 2.7, a value that is significantly lower (P < 0.001) than a ratio of 11.8 ± 5.6 (n = 8) in nontumor astrocytes (Table 1).

Experimental spike induction in spinal cord astrocytes in vitro

Spinal cord astrocytes were shown to possess significant Na^+ channel densities in vitro, allowing action potential-like response induced by current injection (Sontheimer and Waxman 1992). This appears largely because of a lack of inhibition by neurons whose presence suppresses Na^+ channel expression in these cells (Thio et al. 1993) and is probably an artifact of cell culture. However, these cells present a good model system to study whether our hypothesis, namely that the relative expression of I_{IR} and I_{Na} determines whether spikes can be induced, is correct. At 7–10 days in vitro, these cells express prominent inwardly rectifying potassium currents in addition to I_{IR} (Fig. 7A, left panel) (see also Ransom and Sontheimer 1995). The potassium current was blocked by 10 μM barium (Fig. 7A, right panel) as previously reported (Ransom and Sontheimer 1995). Current injection under current clamp could not elicit a spiking response from these cells (Fig. 7B). Blockade of I_{IR} by 10 μM Ba^2+ transforms these “passive” cells into spiking cells (Fig. 7B, right panel). Figure 7B illustrated the progressive transformation due to the Ba^2+ application in current-clamp recordings during the wash in of the drug. A current injection of 200 pA was applied every 5 s and the respective potential values (resting potential and potential in response to the current injection) were plotted against time (Fig. 7B, right panel). These data clearly suggest that expression of inwardly rectifying K^+ channels prevents the spike generation by current injection.

DISCUSSION

Our results suggest that marked differences exist between astrocytoma cells and comparison astrocytes, most strikingly the loss of I_{IR} and A-type outward potassium currents (I_A) in transformed glial cells that appeared to exclusively express delayed rectifier potassium channels. In addition, astrocytoma cells showed an up-regulation of sodium currents and were able to generate current-induced spikes that were absent in comparison tissue. Blocking I_{IR} in normal rat glial cells in culture allowed these cells to generate current-induced
spikes, suggesting that expression of $I_{IR}$ prevents depolarization and spike generation in normal astrocytes.

*Delayed rectifier potassium channels*

Astrocytoma cells and comparison astrocytes consistently expressed $I_{DR}$. Indeed, these channels were ubiquitously expressed in nonexcitable cells (Rudy 1988) and were implicated in the control of proliferation of various cell types (Gallo et al. 1996; Nilius and Wohlrab 1992; Pancrazio et al. 1993; Pappas et al. 1994; Pappone and Ortiz-Miranda 1993; Puro et al. 1989; Wilson and Chiu 1993; Woodfork et al. 1995). We recently reported an increase in the conductance of delayed rectifying $K^+$ channels in gliotic proliferative astrocytes in an in vitro model of spinal cord injury (MacFarlane and Sontheimer 1997). A striking result from our study is the hyperpolarized shift in the activation threshold for $I_{DR}$ to values close to $-50 \, \text{mV}$ instead of $-20$ or $-30 \, \text{mV}$ in other cell types, including rat hippocampal astrocytes, in situ (Bordey and Sontheimer 1997). It is possible that this change is due to some intracellular modulation affecting delayed rectifying $K^+$ channels (Chung and Schlichter 1997; Cole et al. 1996; Koh et al. 1996; Perozo and Bezanilla 1990). $I_{DR}$ were also present in comparison human cells in our study and in normal rat hippocampal astrocytes in situ.

*Inwardly rectifying potassium channels*

Our observation that astrocytoma cells lack $I_{IR}$ is in contrast with a previous report that studied glioma cell lines (Brismar 1995). The reason for this discrepancy is unclear. It is possible that cell lines (in vitro) display features that differ from cells in situ or in vivo. Moreover, we studied low-grade gliomas, whereas glioma cell lines used by others were derived from high-grade gliomas (glioblastoma). Studies of rat C6 astrocytoma cells, believed to be more astrocyte-like and highly GFAP positive, also lack inwardly rectifying $K^+$ channels. The absence of $I_{IR}$ in pilocytic astrocytoma is consistent with other proliferating cell types that
TABLE 1. Membrane properties and conductance densities in glioma cells and comparison astrocytes

<table>
<thead>
<tr>
<th></th>
<th>Astrocytoma (6 cases)</th>
<th>4 Pilocytic astrocytoma</th>
<th>Desmoplastic astrocytoma</th>
<th>Giant cell astrocytoma</th>
<th>Comparison astrocytes (3 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_r, \text{ mV} )</td>
<td>-35.5 ± 18.5*** (53)</td>
<td>-32.6 ± 20.3*** (35)</td>
<td>-49.2 ± 18.9 (6)</td>
<td>-41.9 ± 16.1** (12)</td>
<td>-63.9 ± 12.5 (9)</td>
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<tr>
<td>( R_m, \text{ M}\Omega )</td>
<td>822.3 ± 921.5 (40)</td>
<td>838.2 ± 630.0* (40 to 1886) (23)</td>
<td>960.6 ± 742.0* (6)</td>
<td>421.3 ± 465.3 (11)</td>
<td>288.0 ± 188.3 (8)</td>
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<tr>
<td>( C_m, \text{ pF} )</td>
<td>50.1 ± 33.6 (53)</td>
<td>36.4 ± 29.7 (35)</td>
<td>19.0 ± 14.8* (6)</td>
<td>84.0 ± 21.2*** (12)</td>
<td>44.7 ± 18.4 (8)</td>
</tr>
<tr>
<td>( I_{Na}/C_m, \text{ pA/pF} )</td>
<td>-21.9 ± 20.8* (37)</td>
<td>-27.8 ± 19.9** (24)</td>
<td>-34.0 ± 18.9** (4)</td>
<td>-0.8 ± 0.3 (9)</td>
<td>-4.4 ± 5.9 (8)</td>
</tr>
<tr>
<td>( G_{Na}/C_m, \text{ pS/pF} )</td>
<td>315.4 ± 294.6 (37)</td>
<td>394.4 ± 276.0 (24)</td>
<td>525.3 ± 267.6 (4)</td>
<td>11.5 ± 4.7 (9)</td>
<td>81.1 ± 89.4 (8)</td>
</tr>
<tr>
<td>( I_{K}/C_m, \text{ pA/pF} )</td>
<td>25.3 ± 22.2 (38)</td>
<td>32.3 ± 22.9* (22)</td>
<td>37.5 ± 20.9* (5)</td>
<td>7.0 ± 4.1* (11)</td>
<td>14.4 ± 7.3 (8)</td>
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<tr>
<td>( G_{K}/C_m, \text{ pS/pF} )</td>
<td>220.5 ± 193.6 (38)</td>
<td>281.5 ± 199.5 (22)</td>
<td>326.8 ± 182.1 (5)</td>
<td>61.2 ± 36.0 (11)</td>
<td>111.5 ± 65.9 (8)</td>
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<td>( G_{A}/C_m, \text{ pS/pF} )</td>
<td>743.1 ± 195.6</td>
<td>1 pilocytic: 6/8</td>
<td>248.5 ± 197.0 (5)</td>
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<tr>
<td>( G_{IR}/C_m, \text{ pS/pF} )</td>
<td>391.6 ± 148.7</td>
<td>1 pilocytic: 6/8</td>
<td>248.5 ± 197.0 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( G_{IR}/G_{Na} )</td>
<td>1.2 ± 1.3*** (18)</td>
<td>0.9 ± 0.4** (4)</td>
<td>5.18 ± 2.6** (9)</td>
<td>11.8 ± 5.6 (8)</td>
<td>7.3 ± 7.7 (8)</td>
</tr>
<tr>
<td>( G_{IR}/G_{Na} )</td>
<td>2.3 ± 2.5**</td>
<td>(0.04 to 9.0) (31)</td>
<td>(0.04 to 9.0) (31)</td>
<td></td>
<td></td>
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</table>

Values are means ± SD; number of cells tested within parentheses. \( G_{A}/C_m \), transient outward potassium conductance; \( I_{Na}/C_m \), inwardly rectifying potassium current divided by the membrane capacitance; \( G_{IR}/C_m \) and \( G_{IR}/C_m \), delayed rectifying potassium current and conductance divided by the membrane capacitance, respectively. * \( P < 0.05 \), significant; ** \( P < 0.01 \), very significant; *** \( P < 0.001 \), extremely significant.

also lack \( I_{IR} \), for example, leukemia cells (Missiaen et al. 1997), neuroblastoma cells (Arcangeli et al. 1995), early mouse embryos (Day et al. 1993), and HeLa cells (Takahashi et al. 1994). In addition, injury induced proliferation of spinal cord astrocytes in situ correlates with a down-regulation of \( I_{IR} \) (MacFarlane and Sontheimer 1997). In hippocampal slices \( I_{IR} \) channel expression is developmentally regulated and its expression appears to characterize primarily

FIG. 5. Transient current recordings in pilocytic astrocytoma cells. Transient currents were isolated by sequentially activating currents from a prepulse potential of \(-110 \text{ mV} \) (A) and subsequent subtraction of currents activated from a prepulse potential of \(-70 \text{ mV} \) (B), yielding a transient current component that activates at potentials \( \approx -70 \text{ mV} \) (C). Mean values of peak currents were plotted as a function of applied potential (D) for 5 cells. Steady-state inactivation of the transient current was analyzed by varying the prepulse potential from \(-180 \text{ to } -20 \text{ mV} \) (E). Mean values of the subtracted transient currents from 5 cells were plotted as a function of applied prepulse potential in Fig. 5D (f).
differentiated nonproliferating astrocytes (Bordey and Sontheimer 1997).

The lack of $I_{IR}$ currents in astrocytoma cells is due either to a modulation of the channel protein or to the down-regulation of its expression. Our detection methods do not allow to discriminate between these possibilities and, for purposes of our study, this difference is not germane. However, the loss of functional $I_{IR}$ currents may have important physiological consequences. This channel class is known to participate in the potassium homeostasis in the brain (Amedee et al. 1997; Newman and Reichenbach 1996) and one would predict that in the absence of this channel, potassium buffering would be impaired in tumors. In addition, these potassium channels, in conjunction with chloride channels, are involved in cell-volume regulation and, consequently, volume regulation may also be compromised in tumor cells. Interestingly, glioma cells had a more rounded-up morphology and appeared swollen (phase image). Inwardly rectifying K$^+$ channels are thought to establish the hyperpolarized resting potential typical of most astrocytes. Its absence in glioma thus explains the depolarized resting potential of tumor cells. Depolarized membrane potentials are considered to be a characteristic of tumor cells (Cone 1970).

**Transient outward potassium channels**

In glioma cells, transient A-type K$^+$ channels are largely absent. Like the loss of $I_{IR}$, the absence of transient outward potassium currents appears to be an early feature that accompanies the transformation of a normal astrocyte to become a tumor cell. The role of these channels in glial cells is not known. In neurons they modulate frequency of action potential discharge (Rudy 1988). Interestingly, A-currents were recently implicated in the extension of neurites (Wu and Barish 1996). Glioma cells displayed fewer processes than normal glial cells and show numerous alterations in extracellular matrix (McKeever et al. 1997) and membrane architecture (Kharbanda et al. 1993; Nagano et al. 1993). In analogy to the role of A-currents in neurite extension, a loss of A-currents in glioma cells might be a result of perturbations in extracellular matrix.

**Na$^+$ channels**

Our data suggest a two- to fivefold up-regulation of Na$^+$ channel density in astrocytoma cells without any significant biophysical or pharmacological alterations. This could have been due to a functional modulation of sodium channels or an increase in channel expression. Considering the second possibility and assuming a single-channel conductance of 20 pS and an open probability of 0.25 (Barres et al. 1989), mean sodium channel densities in pilocytic astrocytoma and desmoplastic astrocytoma had mean values of 0.8 ± 0.5 channels/$\mu$m$^2$ (ranging from 0.1 to 1.75) and 1.0 ± 0.5 channels/$\mu$m$^2$, respectively. These values are significantly higher than values of 0.16 ± 0.18 channels/$\mu$m$^2$ (0.02 to 0.4) in comparison human glial cells. This increase in $I_{Na}$ in conjunction with the suppression of $I_{IR}$ accounted for the usual spiking behavior of astrocytoma cells after current injection.

**Biophysical “spikes” are a consequence of altered channel complement**

Our data suggest that the lack of $I_{IR}$ allows induction of spikes by current injection. Our experiments with spinal cord astrocytes in vitro corroborate this. These cells have the adequate ion channel complement, both Na$^+$ channels and inwardly rectifying K$^+$ channels. Blocking $I_{IR}$ by an application of low concentrations of barium turned “passive” glial cells into spiking glial cells, suggesting that the activity of inwardly rectifying K$^+$ channels prevented such spikes in Na$^+$ channel-bearing glial cells.

**Functional consequences and implications**

The role of these slow glial spikes is an enigma. We believe that they probably never occur in vivo. However, others (Labrakakis et al. 1997) suggested that the genesis of tumor (glioblastoma multiform) -associated epileptic seizure may be due to spiking glioma cells. However, at the resting potential of glioma cells, Na$^+$ channels are essentially fully inactivated, curtailing activation. The cases studied here did not present with seizure symptoms.

Much has been speculated about the potential role of glial...
FIG. 7. Glial spikes can be induced in normal astrocytes after pharmacological blockade of $I_{IR}$. A, top panel: under voltage-clamp configuration currents recorded in control (left) and in the presence of bath application of 10 $\mu$M Ba$^{2+}$ (right). Holding potential was $-80$ mV. Currents were activated after depolarizing cell to 0 mV for 100 ms, before application of hyperpolarizing voltage step from $-180$ to $-10$ mV for 40 ms. Bottom panel: under current-clamp configuration, membrane potentials recorded from the same cell as in top panel in control (left) and in presence of 10 $\mu$M Ba$^{2+}$ (right). Ba$^{2+}$ blocked the $I_{IR}$ and allowed recorded cell to spike in response to a current injection (100-pA step). B: under current clamp, a 200-pA current was injected every 5 s from a membrane potential of $-70$ mV (resting potential of the recorded cell). Ba$^{2+}$ was applied at trace number 6, depolarized the cell, and turned its square passive response into a spiking response (left). Right panel: resting potential of the cell (●) and peak amplitude of the current-injected responses (○) measured between 10 and 20 ms were measured every 5 s and plotted against time of recording. This graph illustrates the depolarizing and spiking-induced effects of Ba$^{2+}$ onto the cell.

Na$^+$ channels (Ritchie 1992). These speculations include roles in fueling the Na$^+$/K$^+$-ATPase (Sontheimer et al. 1994), ephaptic coupling to neurons (Chao et al. 1994), or channel biosynthesis and transfer to neurons (Bevan et al. 1987). We do not want to expand on these suggestions. Instead, we are compelled to speculate that the most intriguing finding is the loss of $I_{IR}$ amplifying the relative contribution of Na$^+$ currents to the whole cell conductance. The latter, we believe, is an important aspect in cell differentiation. We speculate that inwardly rectifying K$^+$ channels are expressed in differentiated, nondividing astrocytes, whereas they are down-regulated in dividing cells for unknown reasons. Indeed, this was observed in numerous cell types, including astrocytes, and our recent study showed the rapid loss of $I_{IR}$ in conjunction with gliotic proliferation (MacFarlane and Sontheimer 1997).

Thus the observed changes are consistent with enhanced proliferation of transformed glial cells. Glial spikes can be
experimentally induced as a consequence of these changes in channel complement. However, they are a biophysical exercise with probably little physiological significance.

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