啓明醫大學術誌 第21券 2號
Keimyung Med J
Vol. 21, No. 2, December, 2002

Electrophysiological Characterization of Voltage-Dependent Potassium Channels in Human Neural Stem Cells

Jae Hoon Bae, M.D.

Department of Physiology & Brain Research Institute,
Keimyung University School of Medicine, Taegu, Korea

Abstract : Neural stem cells maybe allowed using in development of transplantation for treatment of neuronal disorders. This study has characterized the profile of membrane ion channels in cultured human neural stem cells using the patch clamp technique in the whole cell mode. In voltage clamp mode, the cells expressed both outward and inward rectifying K^+ currents with no evidence for Na^+ current. The outward delayed rectifying K^+ current was activated by depolarization to potential more positive than -30 mV without inactivation and the reversal potential of this current was -54.1 mV, estimated from the observation of tail current. The inward rectifying K^+ current was activated by hyperpolarization to potential more negative than -60 mV and this current had two kinetics including a fast inactivating instantaneous current and a steady state current according to time course of hyperpolarizing pulse. Both the outward and the inward K^+ currents were blocked by 5 mM tetraethylammonium (TEA) in the same extent, but not by 2 mM 4-aminopyridine. It suggests that human neural stem cells have both outward and inward rectifying K^+ currents and the characteristics of TEA-sensitive outward rectifying K^+ currents without Na^+ currents are similar to those of glial precursor or progenitor cells.

Key Words : Electrophysiology, Neural stem cells, Voltage-dependent potassium channels

Introduction

Neural stem cells are primordial, uncommitted cells postulated to give rise to the array of more specialized cells of the central nervous system[1]. They are also defined as cells with the ability to proliferate, exhibit self-maintenance or renewal over the lifetime of the organism, generate a large number of clonally

related progeny, retain its multilineage potential over time, and produce new cells in response to injury or disease[2]. The human neural stem cells using this experiment were already reported as suitable cells defined by above-mentioned criteria[1].

The ion channel expression of the cell has been most commonly seen as having a function in electrical signal transmission[3]. In particular, expression of K^+ currents in cells is related to the level of cellular activity[4]. There are only a few studies reporting the electrophysiological characteristics of neural cell lines or glial progenitor cells. In the present, knowledge of the electrophysiological properties of multipotential or neuronal progenitor cells is very limited[5]. All cells had an outward K^+ current and a few cells had a transient outward current in multipotent neural cell lines of mice[6]. Many electrophysiological studies have reported various types of ion channels including voltage-dependent Na^+ current, inward and outward K^+ currents, Cl^- current and Ca^{2+} current in progenitor cells of the central nervous system[5,7]. The differences have been described in voltage dependence, kinetics and pharmacology of the channels according to the cell types and the conditions of culture or preparations. There is no report describing the electrophysiological properties of channels in human neural stem cells.

The present study have characterized ionic currents recorded from cultured human neural stem cells using whole cell patch clamp technique. The results suggest that human neural stem cells express at least two types of potassium currents, outward and inward rectifying, and both currents are sensitive to potassium channel blocker, tetraethylammonium, pharmacologically.

Materials & Methods

1. Cell culture

Primary dissociated cell cultures of embryonic human telencephalon tissues of 14 weeks gestation were prepared as described previously[8], and grown in T25 flasks or 25 mm glass coverslips in Dulbecco's modified Eagle medium (DMEM, Gibco-BRL, USA) with high glucose supplemented with 15% horse serum (Hyclone, USA), 20 μg/mL gentamicin (Gibco-BRL, USA), and 2.5 μg/μL amphotericin B (Gibco-BRL, USA).

Embryonic brain cell culture, after 7 -14 days *in vitro,* were infected with an amphotropic, replication-incompetent retroviral vector-containing *v-myc* as described previously[1,8]. Among the isolated several clones of human neural stem cells, H6 was expanded for this study. H6 cells express nestin and vimentin, both cell type specific markers for neural stem cells[1]. The cells were grown on 25 mm galss coverslips (Fisher, USA) previously coated with 10 μg/mL poly-L-lysin (Sigma, USA) and maintained in 10% fetal bovine serum (Hyclone, USA) containing DMEM or serum free medium consisting of high-glucose DMEM with insulin (10 μg/mL),

transferrin (10 μg/mL), sodium selenite (30 nM), hydrocortisone (50 nM), and triiodothyronine (0.3 nM)[8]. These chemicals were purchased from Sigma Co. (USA).

2. Electrophysiology

Cultured human neural stem cells plated on coverslip (2 to 5 days) were placed on the stage of an inverted microscope (Nikon TMS, Nikon, Japan). Patch pipettes were fabricated from borosilicate capillaries (Corning glass #7052, Corning, USA) and had resistances in the range 3 to 5 MΩ when filled with intracellular solution. The capacitance and series resistance of pipette were manually compensated and the 85% compensation of series resistance was routinely achieved to minimize voltage errors. Membrane currents were measured with patch-clamp technique in the wholecell recording configuration. The holding potential was -60 mV in all experiments. Macroscopic currents were recorded using an Axopatch 200A amplifier (Axon Instruments, USA) and anlayzed by pClamp 6.0 software (Axon Instruments, USA). Current signals were filtered at 1 kHz and sampled at 5 kHz.

3. Recording solutions

The standard bath solution contained (in mM) NaCl, 140; KCl, 5; CaCl2, 1; MgCl2, 1; glucose, 10 and HEPES, 10. The pH was adjusted with NaOH to 7.3. In some experiments 5 mM tetraethylammonium (TEA) was added into bath solution to block potassium currents. Application of TEA was by perfusion of bath solution onto the cells from a gravity-fed system[9]. The pipette solution contained (in mM) KCl, 140; NaCl, 10; $CaCl_2$, 1; EGTA, 11; HEPES, 10 and ATP, 1 and the pH was adjusted with KOH to 7.3. All chemicals were purchased from Sigma Co.

4. Statistics

Data were expressed as means ± SEM. Statistical significance was evaluated by Student's *t*-test. P-value less than 0.05 was considered significant change.

Results

1. Outward K^+ current

The protocol included application of single depolarizing step for 400 msec, from a holding potential of -60 mV to +40 mV, to activate outward current was used. In ten of neural stem cells, an outward current was observed (Fig. 1A). It was a delayed rectifying, voltage dependent outward current without inactivation during depolarizing pulse, more frequently observed in round-shaped (n=7) than in processed cell (n=3). The peak amplitude of this current was 1.29 ± 0.38 nA. The round-shaped cells highly expressed the outward currents in 5 cells cultured with 15% horse serum-containing medium compared with those in 2 cells cultured with serum-free medium. Regardless of cell morphology, the expression of outward current did not

show any difference between cells cultured in serum-containing medium (n=6) and cells cultured in serum-free medium (n=4).

To determine the properties of the outward current, the current reversal potential was measured using a tail current protocol. The membrane was clamped to +40 mV to activate the outward current and consecutively stepped to a potential in the range of -30 mV to -90 mV with a 10 mV decrement. A current-voltage plot of tail current was constructed in Fig. 1B and the average data were collected from seven experiments. The current amplitude was measured at 3 msec after voltage change to less depolarization. The reversal potential of this current was -54.1 ± 2.17 mV and was close to the potassium (K^+) equilibrium potential (E_K=-80 mV).

To clarify the pharmacological properties of this outward K^+ current, the classic K^+ channel blockers, TEA and 4-aminopyridine were added into bath solution. The outward current was blocked by 5 mM TEA in all tested cells (Fig. 2, n=4, P<0.01). Peak current (after leak subtraction) at +40 mV was declined by TEA to 29.9 ± 7.38% of the control value within 5 min after application. The current was also easily recoverable to 65.1 ± 15.93% of the control value within 5 min after washing with bath solution. The almost complete blockade of currents in the presence of TEA indicated that the current was carried by K^+. This current was insensitive to external 2 mM 4-aminopyridine (data not shown).

The depolarizing steps from -60 mV to

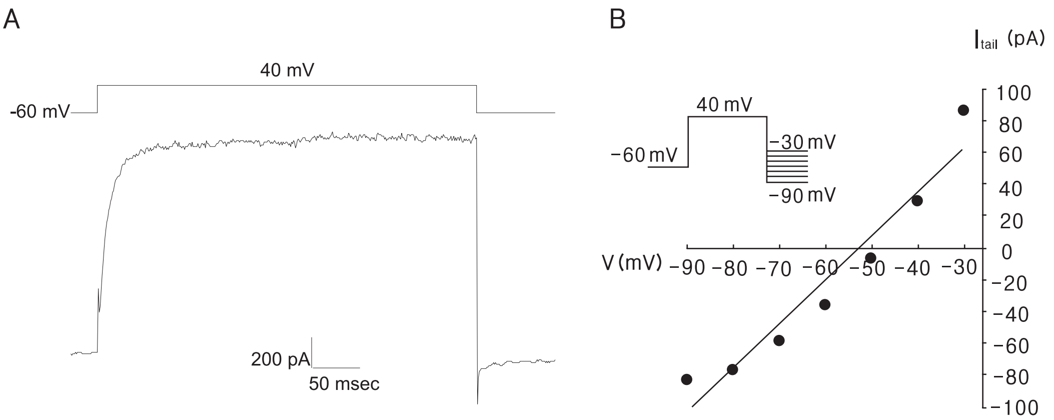

Fig. 1. Identification of outward K^+ current from human neural stem cells. (A) A typical recording of outward K^+ current. Current was recorded by single depolarization step for 400 msec from holding potential of -60 mV to +40 mV. This current was a delayed rectifying and a voltage-dependent outward current without inactivation. (B) Current-voltage plot of tail currents. Tail current protocol was used to determine the reversal potential of the outward K^+ current. The membrane was clamped to +40 mV to activate the outward current and subsequently stepped to a potential in the range of -30 mV to -90 mV with a 10 mV decrement. The reversal potential of the current was -54.1 mV (n=7).

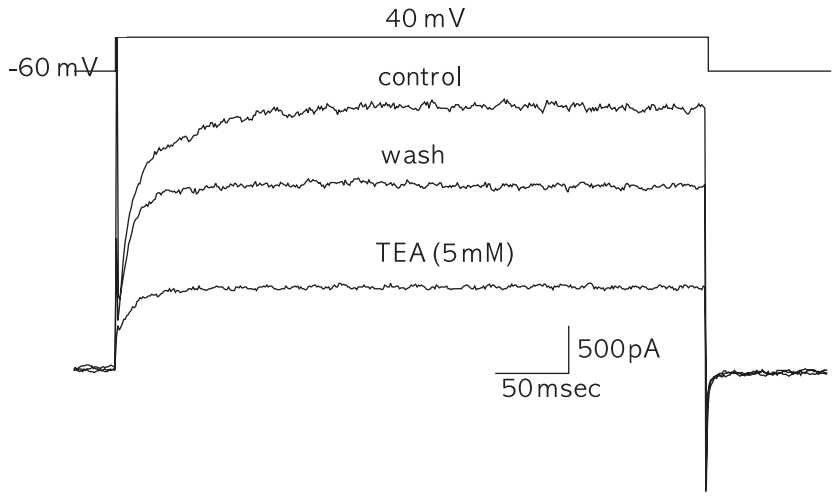

Fig. 2. Outward current responses to tetraethylammonium (TEA) in human neural stem cells. Outward K^+ current was recorded with a depolarization to +40 mV (same protocol as for Fig. 1A) in absence of TEA (control), at 5 min after application of TEA (5 mM), and at 5 min after washing with bath solution. Outward K^+ current was almost completely blocked by TEA and was easily recoverable. This indicated that the current was carried by K^+.

+50 mV with a 10 mV increment leaded to the generation of step-like enhancements of outward K^+ current of control cells in the range above -30 mV (Fig. 3A). Application of TEA also reduced the amplitude of peak current at +50 mV to 22.5% of the control value (n=4). A current-voltage plot with data averaged from four cells is presented in Fig. 3B. The conductance of control cells, estimated from the slop from -20 mV to +50 mV, was 20.6 ± 8.83 pS and was reduced to 4.2 ± 2.01 pS by TEA.

2. Inward K^+ current

To determine the specificity of the inward current in 23 human neural stem cells, hyperpolarizing protocols from a holding potential of -60 mV to -120 mV in a 10 mV decrement were tested. Hyperpolarization to potentials more negative than -60 mV activated an inward rectifying current (Fig. 4A). This current was expressed at the same population (n=5) in both round and processed cells regardless of serum. In approximately one-fourth, the round cells cultured in serum-containing medium did not show this inward current. Although the round cells in serum-containing medium showed the inward current as same population as the round cells in serum-free medium, the amplitudes of this current in cells of serum medium were significantly ($P<0.05$) decreased compared to those of both an

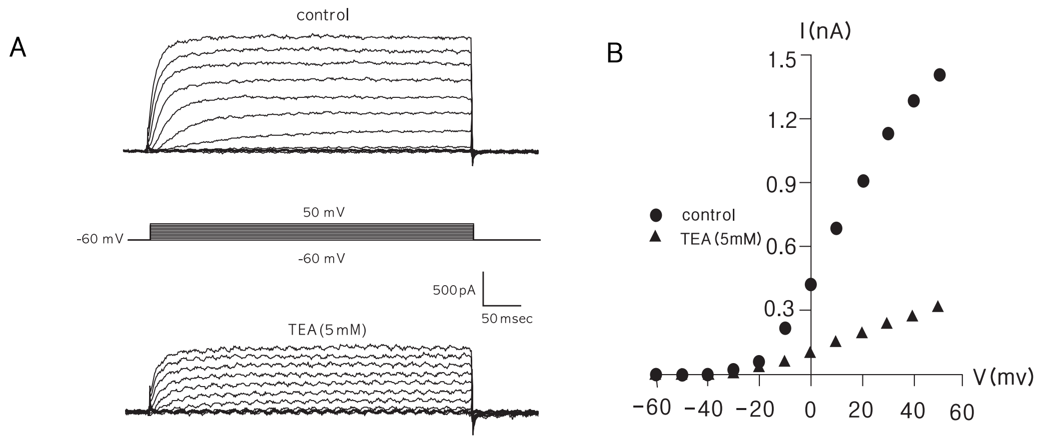

Fig. 3. Properties of outward rectifying K^+ current in human neural stem cells. (A) Outward rectifying K^+ current. Typical voltage-dependent outward K^+ currents were recorded by step pulses between -60 and +50 mV from holding potential of -60 mV with 10 mV increment before (control) and after application of TEA (5 mM). TEA decreased the amplitude of outward currents to 22.5% of control (n=4). (B) Current-voltage plot of outward K^+ currents. TEA (5 mM) decreased the current conductance estimated from the slop between -20 and +50 mV. The current conductance was 20.6 pS in control and 4.2 pS after TEA application (n=4).

instantaneous and a steady state in serum-free medium. The processed cells also showed the similar trend in amplitude of inward current, but there was no significant difference between the cells in serum-containing and the cells in serum-free media.

This inward current exhibited two kinds of kinetic behavior. The current amplitudes at 5 msec and 70 msec after voltage change from holding potential were determined as an instantaneous and a steady state, respectively. The amplitude of steady state inward current of control cells was -269.7 ± 136.26 pA at -120 mV (n=3). After application of TEA, the averaged amplitude of inward current was decreased to 57.1% of the control. The blockade of this current by TEA indicated that this inward current was also carried by K^+.

An inward current-voltage plot with data averaged from three cells is presented in Fig. 4B. The instantaneous current-voltage plot was basically showed the linear relationship in both control and TEA treated cells. The slope conductance was 9.3 ± 3.17 pS in control and 4.6 ± 1.32 pS in TEA treated cells. Due to the strong inactivation of inward current, the relationship of steady state exhibited a marked region of negative slope conductance at below -100 mV. The current at steady state showed the characteristic of inward rectifying current in both control and TEA treated cells and reduced by TEA. This current was also insensitive to external 2 mM of 4-aminopyridine (data not shown). All cells recorded in this experiments did not show sodium current in solutions

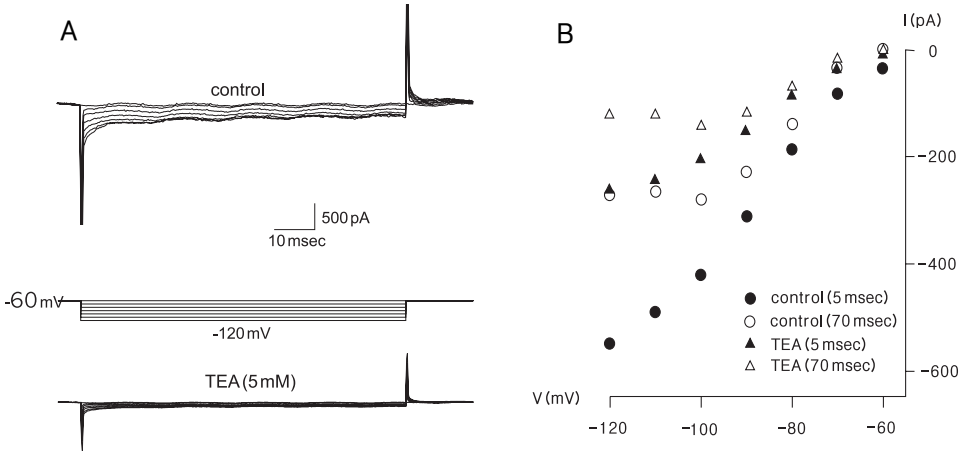

Fig. 4. Properties of inward rectifying K^+ current in human neural stem cell. (A) Inward rectifying current. Typical voltage-dependent inward K^+ currents were recorded by hyperpolarizing steps from a holding potential of -60 mV to -120 mV in a 10 mV decrement. TEA (5 mM) also blocked the inward rectifying K^+ current. (B) Current-voltage plot of inward current. The current exhibited two kinds of kinetic behavior. The current at instantaneous (open symbols) and at steady state (filled symbols) was determined at 5 msec and 70 msec after voltage change, respectively. The instantaneous plot showed the linear relationship in both control (circle) and TEA treated (triangle) cells (n=3). In steady state plot, The negative slope conductance was exhibited at below -100 mV. TEA (5 mM) blocked this inward rectifying currents that were carried by K^+.

designed to block the potassium currents with TEA (5 mM) or 4-aminopyridine (2 mM).

Discussion

Voltage-activated K^+ currents are primarily responsible for the repolarization of the action potential in almost all excitable cells, this is also abundantly expressed in non-excitable cells including microglia[9], astrocytes[10], and oligodendrocytes[11] in the central nervous system.

We observed the kinetics of outward K^+ channels which showed a characteristic outward rectification. It also had no decay which had similar electrophysiological properties with the glial precursor cells of early stage in the postnatal corpus callosum slice[7]. Like as this experiment, the multipotent neural cell lines produced by retroviral transfection of avian *myc* oncogene in mice expressed the delayed rectifying K^+ currents and did not show Na^+ currents[6]. In this study, the reversal potential estimated from observation of tail current was at -54 mV, and this was similar with -60 mV in glial precursor cell[7], -40 mV in cultured human microglia[9], -65 mV in adult oligodendrocyte progenitors[11]. These data suggest that the outward K^+ current of human neural stem cells were similar to those of glial or glial precursor cells.

This study also tested the pharmacological properties of this outward current by perfusion of 5 mM TEA into bath solution to block K^+ current. This current was only depressed by TEA and was insensitive to 4-aminopyridine. Neuronal outward K^+ current are typically more sensitive to TEA than 4-aminopyridine[12], whereas this current is equally sensitive to both TEA and 4-aminopyridine in astrocytes[7] and in microglia[13]. Gial precursor cells did not express Na^+ currents which consistent with the present study. In addition, multipotent mouse neural stem cell lines showed a delayed rectifying K^+ current but did not express the Na^+ current[6]. Therefore, the presence of voltage-dependent K^+ currents without Na^+ currents might be one of the characteristic prosperities of neural stem cells[6].

The other K^+ current recorded in this experiment was an inward rectifying current which plays a dominant role in setting the resting membrane potential of the cells[14]. This current is characterized by a large open probability close to and negative of resting membrane potential, whereas it is nearly closed at the depolarized potentials. The inactivating phenomenon of this current results from a blocking action of extracellular Na^+[15]. Although the kinetic behavior and current -voltage relationship for this inward current were very similar to those observed in rat O-2A glial cell progenitors[16] and microglia[3], the conductance was peak at more positive potential than the conductance in cultured mouse motor neurons[17]. The pharmacological properties of this inward current was sensitive to TEA, but it did not respond to 4-aminopyridine. External Cs^+ and Ba^{2+} depolarized the cells and nearly abolished the conductance of inward rectifying K^+ channel[18]. The similar characteristics were also observed in neurons[19], microglia[13], and adult oligodendrocyte progenitor cells[11], raising the possibility that this current might be a physiological marker for the neural stem or progenitor cells[6].

Potassium currents may modulate the differentiation of oligodendrocytes[20] and astrocytes[21]. As maturation is accompanied by stereotypic changes in ionic expression patterns, therefore channel modulation can influence differentiation. For example, K^+ channel modulator TEA also inhibited the proliferation of oligodendrocyte progenitor proliferation[22]. It is also possible that a different developmental or differentiation stage of cells reflect different sets of ion channel expressions[8]. This experiment could observe that 3 of 13 round cells cultured in serum-containing medium did not show the inward current. The round cells were found in acutely dissociated or within 2 to 3 days after plating neural stem cells, thereafter these cells were morphologically changed into processed cells. The different morphologies represented different states of activation of the cells[4]. In cultured oligodendrocytes, outward K^+ current developed within 1 to 2 days after plating, while inward currents did not appear until several days

later[23]. This condition of culture or preparation may also affect to the expression of inward K^+ current[18,23]. Because serum may affect to delay the expression of inward current, serum can alter the ion channel phenotype or density of currents expressed in round cells especially after enzymatic preparation[18,21]. To determine the effect of serum on expression of inward K^+ current, it is needed to compare the further electrophysiological study in conjunction with immunocytochemical study.

In conclusion, this study suggests that the two types of K^+ currents of human neural stem cells have electrophysiological properties of both glia and neuron, but the characteristics of outward rectifying K^+ current are more similar to those of glial precursor or progenitor cells than neuronal cells in the central nervous system.

Summary

This study has characterized the profile of membrane ion channels in cultured human neural stem cells using the patch clamp technique in the whole cell mode. In voltage clamp mode, the cells expressed both outward and inward rectifying K^+ currents with no evidence for Na^+ current. The outward delayed rectifying K^+ current was activated by depolarization to potential more positive than -30 mV with no inactivation and the reversal potential of this current was -54.1 mV estimated from the observation of tail current. The inward rectifying K^+ current was activated by heperpolarization to potentials more negative than -60 mV and this current had two kinetics including fast inactivating instantaneous current and steady state current according to time course of hyperpolarizing pulse. Both the outward and the inward K^+ currents were blocked by 5 mM tetraethylammonium (TEA) in the same extent, but not by 2 mM 4-aminopyridine. It suggests that human neural stem cells have both outward and inward rectifying K^+ currents and the characteristics of TEA-sensitive outward rectifying K^+ currents without Na^+ currents are similar to those of glial precursor or progenitor cells.

References

1. Flax JD, Aurora S, Yang C, Simonin C, Wills AM, Billinghurst LL, *et al.* Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. *Nat Biotechnol* 1998;**16**:1033-9.
2. Weiss S, Reynolds BA, Vescovi AL, Morshead C, Craig CG, van der Kooy D. Is there a neural stem cell in the mammalian forebrain? *Trends Neurosci* 1996;**19**:387-93.
3. Kettenmann H, Banati R, Walz W. Electrophysiological behavior of microglia. *Glia* 1993;**7**:93-101.
4. Korotzer A, Cotman CW. Voltage-gated currents expressed by rat microglia in culture. *Glia* 1992;**6**:81-8.
5. Feldman DH, Thinschmidt JS, Peel AL, Papke RL, Reier PJ. Differentiation of ionic currents in CNS progenitor cells: dependence upon substrate attachment and epidermal growth factor. *Exp Neurol* 1996;**140**:206-17.

6. Ryder EF, Snyder EY, Cepko CL. Establishment and characterization of multipotent neural cell lines using retrovirus vector-mediated oncogene transfer. *J Neurobiol* 1990;**21**:356-75.
7. Berger T, Schnitzer J, Ketenmann H. Developmental changes in the membrane current pattern, K^+ buffer capacity, and morphology of glial cells in the corpus callosum slice. *J Neurosci* 1991;**11**:3008-24.
8. Choi T, Bae JH, Choi HB, Kim SS, McLarnon JG, Suh-Kim H, *et al.* Human neural stem cells: electrophysiological properties of voltage-gated ion channels. *Neuroreport* 2002;**7**:1447-52.
9. McLarnon JG, Xu R, Lee YB, Kim SU. Ion channels of human microglia in culture. *Neuroscience* 1997;**78**:1217-28.
10. Tse FW, Fraser DD, Duffy S, MacVicar BA. Voltage-activated K^+ channels in acutely isolated hippocampal astrocytes. *J Neurosci* 1992;**12**:1781-8.
11. Borges K, Wolswijk G, Ohlemeyer C, Kettenmann H. Adult rat optic nerve oligodendrocyte progenitor cells express a distant repertoire of voltage- and ligand-gated ion channels. *J Neurosci Res* 1995;**40**:591-605.
12. Martina M, Schultz JH, Ehmke H, Monyer H, Jonas P. Functional and molecular differences between voltage-gated K^+ channels of fast-spiking interneurons and pyramidal neurons of rat hippocampus. *J Neurosci* 1998;**18**:8111-25.
13. Norenberg W, Gebicke-Haerter PJ, Illes P. Voltage-dependent potassium channels in activated rat microglia. *J Physiol* 1994;**475**:15-32.
14. Duffy S, Fraser DD, MacVicar BA. Potassium channels. In: Kettenmann H, Ransom BR, editors. *Neuroglia.* New York: Oxford UP; 1995, p.185-201.
15. Standen NB, Stanfield PR. Potassium depletion and sodium block of potassium currents under hyperpolarization in frog sartorius muscle. *J Physiol* 1979;**294**:497-520.
16. Williamson AV, Compston DAS, Randall AD. Analysis of the ion channel complement of the rat oligodendrocyte progenitor in a commonly studied in vitro preparation. *Eur J Neurosci* 1997;**9**:706-20.
17. McLarnon JG, Kim SU, Michigawa M, Xu R. Properties of inward and outward potassium currents in cultured mouse motor neurons. *Neuroscience* 1995;**64**:139-51.
18. Barres BA, Koroshetz WJ, Swaetz KJ, Chun LLY, Corey DP. Ion channel expression by white matter glia: the O-2A glial progenitor cell. *Neuron* 1990;**4**:507-24.
19. Rudy B. Diversity and ubiquity of K^+ channels. *Neuroscience* 1998;**25**:729-49.
20. Sontheimer H, Trotter J, Schachner M, Lettenmann H. Channel expression correlates with differentiation stage during development of oligodendrocytes from their precusor cells in culture. *Neuron* 1989;**2**:1135-45.
21. Barres BA, Chun LLY, Corey DP. Ion channels in vertebrate glia. *Annu Rev Neurosci* 1990;**13**:441-74.
22. Yuan X, Eissen AM, McBain CJ, Gallo V. A role for glutamate and its receptors in the regulation of oligodendrocyte development in cerebellar tissue slices. *Development* 1998;**125**:2901-14.
23. Soliven B, Szutchet S, Arnason BGW, Nelson DJ. Forskolin and phorbol esters decrease the same K^+ conductance in cultured oligodendrocytes. *J Mem Biol* 1988;**105**:177-86.