Alteration in rectification of potassium channels in perinatal hypoxia ischemia brain damage

Alteration in rectification of K+ channels

Author names and affiliations

Penghui Chen, PhD1, Liyan Wang, PhD2, Qiyue Deng, PhD1, Huaizhen Ruan, PhD1, and Wenqin Cai, PhD1

From Department of Neurobiology1, The Third Military Medical University, Chongqing 400038, China

Department of Pediatrics2, Xinqiao Hospital, The Third Military Medical University, Chongqing 400036, China

Corresponding author:

Penghui Chen Ph.D

Department of Neurobiology

The Third Military Medical University

Chongqing 400038, China

Tel: +86-23-6875-3935, +86-139-8300-9800

Email: chenpenghui@tmmu.edu.cn

GRANTS

This work was financially supported by National Natural Science Foundation of China (NSFC) 81070515, 30900467, Natural Science Foundation Project of CQ CSTC2011BA5007.

ACKNOWLEDGEMENT

We sincerely thank Prof. Wei Liu, Institute of Immunology, for English proof reading and manuscript corrections. We thank Prof. Ying Xiong for helpful comments on the manuscript.
ABSTRACT

Oligodendrocyte progenitor cells (OPCs) are susceptible to perinatal hypoxia ischemia brain damage (HIBD), which results in infant cerebral palsy due to the effects on myelination. The origin of OPC vulnerability in HIBD, however, remains controversial. In this study, we defined the HIBD punctate lesions by MRI diffuse excessive high signal intensity (DEHSI) in postnatal 7-day rats. The electrophysiological functional properties of OPCs in HIBD were recorded by patch clamp in acute cerebral cortex slices. The slices were intracellularly injected with Lucifer yellow and immunohistochemically labeled with NG2 antibody to identify local OPCs. Passive membrane properties and K⁺ channel functions in OPCs were analyzed to estimate the onset of vulnerability in HIBD. The resting membrane potential (RMP), membrane resistance (R_m) and membrane capacitance (C_m) of OPCs were increased both in the grey and white matter of the cerebral cortex. OPCs in both the grey and white matter exhibited voltage-dependent K⁺ currents, which consisted of the initiated rectified potassium currents (I_A) and the sustained rectified currents (I_K). The significant alternation in membrane resistance was influenced by the diversity of potassium channel kinetics. These findings suggest that the rectification of I_A and I_K channels may play a significant role in OPC vulnerability in HIBD.

KEYWORDS

oligodendrocyte progenitor cell (OPC); NG2; potassium channel; rectification; hypoxia ischemia brain damage (HIBD)

INTRODUCTION

Perinatal hypoxic-ischemic brain damage (HIBD) has been demonstrated to be a major contributor to developmental disabilities in children that leads to cerebral palsy (Miller and Ferriero 2009; Bonifacio et al. 2011). Numerous reports studying ischemia-induced neuronal damage were primarily focused on the general mechanisms of neuron death or survival. In the past two decades, much attention has concentrated on the morphology and function of oligodendrocyte progenitor cells (OPCs), which differentiate into myelin forming oligodendrocytes (Trotter et al. 2010). OPCs belong to the oligodendrocyte lineage but do not express the proteins with characteristics of mature myelinating oligodendrocytes (Stallcup 2002; Fiedorowicz et al. 2008). OPCs can be identified by NG2 chondroitin sulfate proteoglycan, and therefore are often referred to as NG2 glia in central nervous system (CNS). Defining the role of OPCs in vivo has remained elusive despite their phenotypic characterization as a part of the oligodendrocyte lineage (Richardson et al. 2011). A significant proportion of NG2 cells remains in the mature CNS and does not differentiate to oligodendrocytes (Rhodes et al. 2006). It is necessary to redefine the properties and functions of OPCs during HIBD. The observation that OPCs undergo characteristic changes during maturation suggests that these cells could be involved in regulation outgrowth, proliferation and the generation of synapses (Fiedorowicz et al. 2008). Pathological evidence indicates that OPCs are particularly susceptible to perinatal hypoxia ischemia, which results in decreased myelination and infant cerebral palsy. However, the precise mechanism remains unclear. Our previous electrophysiological recordings from OPCs have revealed that these cells possess unique membrane properties distinct from the traditional classes of glia and neuron (Chen et al. 2008). Potassium channels regulate neuronal excitability by sensing the voltage across
membrane and adjusting the flow of $K^+$ accordingly (Swayne and Wicki-Stordeur 2012). In contrast to
the abundance of potassium channels in glial cells, the function of potassium channels in OPCs
remains unknown.

It is noted that the concentrations of potassium ions change much more slowly than the voltage. They
can integrate and respond to the recent pattern of cellular activation (Yasuda et al. 2008). Therefore,
the aim of this study was to investigate the diversity and rectification of potassium channels in OPCs
and determine their role in hypoxia ischemic brain damage by whole-cell patch clamp methods in acute
rat brain cortical slices. The patch clamp recordings were measured in OPCs that were intracellularly
injected with Lucifer yellow and labeled with NG2. Only a single cell was injected and recorded in each
slice. The passive membrane electrical properties of the perinatal OPCs were compared with the
samples suffering from hypoxia ischemic brain damage.

We found that OPCs located in the grey but not the white matter were slightly excitable and did
express functional voltage-gated $Na^+$ channels. OPCs in both grey and white matter, however,
exhibited voltage-dependent $K^+$ currents with same dynamics as inwardly rectifying $K^+$ (Kir) channel.
These $K^+$ channels could have different rectifications in OPCs according to their electrical properties.
The initiated rectified $K^+$ currents ($I_A$) and the sustained rectified $K^+$ currents ($I_K$) were present in OPCs
in slices from different regions. We also investigated the role of these $K^+$ channels in the vulnerability of
OPCs during HIBD. The membrane potential was depolarized by the permeability of the $K^+$ channels,
which resulted in transmembrane cation flowing activation and edema. The rectification of the
potassium channels fell off, indicating a decreased contribution of the channels to membrane function
in HIBD. As a result, the failed rectification of the potassium channels and right shift of the
current-voltage curve might cause irreversible metabolism disorders in OPCs. Our findings suggest that
rectification of $I_A$ and $I_K$ channels may play a significant role in OPC vulnerability in HIBD.

MATERIALS AND METHODS

Animal model of hypoxia-ischemic brain damage

A majority of these experimental studies used a model of unilateral hypoxic-ischemic brain damage in
the immature rat (Vannucci et al. 1999; Vannucci and Vannucci 2005). This methodology consists of
unilateral common carotid artery ligation followed by a period of systemic hypoxia produced by the
inhalation of 8 % oxygen balanced with nitrogen. Postnatal 7-day SD rats of both genders
corresponding to a brain development of 34-36 weeks gestational age in the human fetus (Romijn et al.
1991) were purchased from Experimental Animal Central of Third Military Medical University (TMMU)
with their birth mothers. All research was done according to a protocol approved by the Animal Care
and Use Committee of TMMU. The rats were anesthetized by chloral hydrate. Their left common
carotid artery was ligated using an 8-0 polypropylene suture. The entire surgical procedure was
completed within 3 min under a stereomicroscope. Then the pups were allowed to recover and nurse
for 2 h with their dams. The environmental temperature during the surgery and recovery was kept at
28 °C. The rats were then placed for 60 min in a tightly sealed hypoxia chamber with a constant flow
(350 mL/min) of humidified 8% $O_2$ balanced with 92% $N_2$. The hypoxia chamber was kept in a
temperature-controlled water bath to maintain the chamber temperature at 37 °C. Following the hypoxic exposure, the pups were returned back to their mothers for recovery. The hypoxic-ischemic injury degree of animal brains was estimated after 2 h using a scoring system as previously described (Sheldon et al. 1998; Björkman et al. 2010).

Whole cell patch clamp recordings

For the preparation of acute brain tissue slices, the forebrains were dissected in ice-cold artificial cerebrospinal fluid (ACSF) containing 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 1.3 mM MgSO₄, 26 mM NaHCO₃ and 10 mM glucose, pH 7.4. Cerebral cortex slices (400 μm) were cut using a vibroslicer (752 M, Campden Instruments), and recovered in oxygenated ACSF at 30 °C for at least 30 min. The slices were then transferred to a submersion-style recording chamber, held in place with a nylon wired platinum grid, and continuously superfused (4-6 mL/min) with oxygenated ACSF at 29-31 °C. Whole cell patch clamp recording area within hydropsia lesions was defined according to the MRI experiment. The membrane current and voltage were recorded using the whole cell patch clamp technique and a Multiclamp 700B patch clamp amplifier (Molecular Devices). The patch electrodes were pulled from thick walled borosilicate glass capillaries with resistances of 5-6 MΩ when filled with an intracellular solution containing 5 mM NaCl, 145 mM KCl, and 10 mM HEPES, pH 7.2, and 0.1% Lucifer yellow for the intracellular injection. The cells were maintained in physiological saline solution containing 140 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES, pH 7.4. All experiments were performed at 20-24 °C (room temperature). The data were obtained at 10 kHz using a DigiData 1322 interface (Molecular Devices) linked to a personal computer controlled by pClamp 9.2 (Inchauspe et al. 2012). Whole-cell capacitance (Cₘ) was compensated, and P/4 was subtracted from the leak currents during the voltage clamp recordings for the K⁺ channel currents. Cₘ was measured by integrating the capacitive transient evoked during a 10 mV depolarizing step from a holding potential of -70 mV (Bakiri et al. 2011). Resting membrane potential (RMP) and input resistance (Rᵢₜ) were determined from voltage responses to a hyperpolarizing current pulse of 0-200 pA. To evoke active reactions, the depolarizing current (100 pA) was injected at the resting membrane potential. Cells having a maximal inward whole-cell current exceeding 1.5 nA or recording typical action potentials (AP) were all ignored.

Intracellular injection and immunohistochemical staining

Lucifer yellow in the pipette solution was injected into the cytoplasm of the recorded cells on the slices using a large hyperpolarizing current. The slices were fixed with 4% paraformaldehyde for 30 min soon after patch clamp recording. After washing with 0.01M phosphate buffered saline (PBS), the slices were blocked with PBS containing 5% normal goat serum (NGS, Sigma-Aldrich) and 0.1% Triton X-100 for 25 min and then incubated overnight at 4 °C with anti-NG2 (Chemicon) 1:200 diluted in PBS containing 10% NGS and 0.1% Triton X-100 (Sigma-Aldrich). After washing, the slices were incubated with goat anti-rabbit IgG conjugated with Alexa Fluor 568 (Invitrogen) 1:1000 diluted in PBS containing
0.1% Triton X-100 for 30 min at 37 °C. Following counterstaining of the nucleus with 4', 6 - diamidino - 2 - phenylindole (DAPI, Invitrogen), the slices were washed and mounted on a glass slide with fluorescent mounting medium (Dako Cytomation). The immunostained sections were observed using a laser scanning confocal microscope (FV1000-ASW, Olympus).

**Magnetic resonance imaging (MRI)**

Rats were inhaled with ether anesthesia and placed in a Bruker3T small animal MRI (BrukerBiospin, Switzerland). MR imaging was performed 2 h post HIBD as described by Obenaus et al (2011). The respiratory rate was monitored throughout the procedure, and the body temperature was maintained at 37 ± 0.5 °C. A T2-weighted image set was recorded according to the following parameters: rapid acquisition relaxation enhancement factor 8, repetition time 5357 ms, echo time 15.50ms with an in-plane resolution of 0.086 0.172 0.35mm with 39 contiguous slices. All MR imaging examinations for brain hydropsia were performed using a 3-T imager (Philips MedicalSystems) according to the standard protocol for newborn infant brain imaging (Weisenfeld and Warfield 2009; de Bruïne et al. 2011). A separate group of pups were used as control infants for brain MR scanning. Diffuse excessive high signal intensity (DEHSI) range in cerebral cortex defined by MRI was used as the electrophysiology recording area for patch clamp.

**Statistical analysis**

Data analysis and statistics were performed using Clampfit (Molecular Devices), SPSS (Statistics 13, Apache Software), and Prism (GraphPad Software). All data are presented as means ± S.E.M. For a comparison between two datasets, Independent-Samples T-test of SPSS was performed. Differences were regarded as significant when the P value was below 0.05.

**RESULTS**

**Visualization of the hypoxia-ischemic region by magnetic resonance imaging (MRI)**

DEHSI is defined as the regions showing high signal intensity on the T2-weighted images but low intensity on the T1-weighted images in the periventricular frontal and parieto-occipital area (Hart et al. 2010). In this study, the high signal intensity regions on T2- weighted images were assessed for lesions in the P7 rats and evaluated for the morphological and electrophysiological analyses (Fig. 1 A and B). The hydropsia area in the HIBD rats was 1.21±0.16 mm² on the coronal reconstructions (Fig. 1 C) and 3.37 ± 0.18 mm² on the sagittal reconstructions (Fig. 1 D). The measured relative optical density (ROD) on the coronal reconstructions in the HIBD rats was 91.79 ± 11.66, dramatically greater than the sham-operated rats (52.17 ± 5.63, p = 0.006), while those on the sagittal reconstructions were 76.89 ± 20.63 vs 54.71 ± 7.95, p = 0.033. The DEHSI of the cerebral cortex represents a premature developmental phenomenon rather than white matter injury.

**Passive membrane properties of OPCs and neuron during HIBD**
The membrane electrical properties of OPCs were initially recorded in whole-cell membrane test mode. The resting membrane potential (RMP) of OPCs was -68.16 ± 8.80 mV in the grey matter and -73.8 ± 5.71 mV in the white matter. RMP of OPCs in both grey and white matter decreased significantly 2h after HIBD (Fig. 2A). OPCs had a relatively low input resistance (R_in) value of 132.16 ± 49.99 MΩ in grey matter and 160.02 ± 40.63 MΩ in white matter, which are comparable to the values reported for NG2-expressing cells in the hippocampal slices (Prüss et al. 2011). R_in of OPCs in the white matter was increased from 160.02 ± 40.63 MΩ to 589.15 ± 50.33 MΩ within 2 h of HIBD (Fig. 2B). Mean cell capacitance (C_m) of OPCs was 24.89 ± 7.31 pF in the grey matter and 16.98 ± 4.95 pF in the white matter of cerebral cortex slice, with slight increase in the process of HIBD without statistically significant differences (Fig. 2C). However, the passive membrane parameters (RMP, R_in and C_m) of neuron were not significantly altered during HIBD (Fig. 2A-C). Therefore, we reason that OPCs are more vulnerable than neuron in HIBD because of sensitive change of passive membrane properties. It was noteworthy that the significant alternation of the membrane permeability (R_in) could be influenced by the kinetics of ion channel.

To remark the cell types present in patch clamp whole cell recording, the recorded slices were intracellularly injected with Lucifer yellow. The recorded cells, which were merged with NG2 immunopositive expression, can be identified as OPCs in the developmental stage. OPCs in vivo exhibited a stellate morphology and contained small cell bodies with processes projecting in many directions (Fig. 3). OPCs were scatteredly localized in the cerebral cortex. Although OPCs represented a phenotypically homogeneous population, heterogeneity in morphology was apparent in the white and grey matter of the cerebral cortex. The morphology of OPCs was relatively complex in the grey matter, with small and elongated soma from which numerous long, fine, and multibranching processes extended in all directions. Moreover, OPCs in the white matter contained a small soma surface area with processes extending in two opposite directions.

**Active membrane properties of OPCs during HIBD**

We further investigated the active membrane electrical properties of OPCs. Whole cell voltage-gated currents were recorded and off-line analyzed at holding potential -90 mV, followed by voltage stimulation from -50 mV to +50 mV with step of 10 mV. Sodium and potassium channels were activated at different holding potentials. OPCs in the grey matter of the cerebral cortex exhibited large outward potassium currents and small inward sodium currents (Fig. 4A and B), which agrees well with previous studies in the hippocampus (Chen et al. 2008). The inward Na⁺ current was activated in command -10 mV and reached maximum current amplitude at -0.51 ± 0.13 nA (N=12) in command +20 mV (Fig. 4A and C). Considering the effects of the outward K⁺ currents, the sodium to potassium conductance ratio (g_Na/g_K, SPCR) was calculated at different membrane commands. The maximal SPCR was 1.27 ± 0.13 (N=12) at a command potential of 0 mV. The experimental data shown here significantly differ from the corresponding parameters measured in our previous study in pyramidal neurons of hippocampus (Chen et al. 2008). OPCs in the white matter of the cerebral cortex, however, exhibited no typical inward sodium currents even when the same type of potassium currents was recorded. Notably, the SPCR and activation of Na⁺ current in grey matter obviously changed in HIBD (Fig. 4B) compared with the control (Fig. 4A). The inward Na⁺ current was activated at the slightly depolarized command of -40 mV and reached the maximum current (-1.05 ± 0.28 nA, N=7) at -20 mV. The maximal SPCR was 6.46 ± 1.08 (N = 7) at a command of -20 mV (Fig. 4B and D). The inward Na⁺ current amplitudes of OPCs significantly increased in HIBD. Besides, OPCs also produced inward currents and reached the
maximal SPCR in a relatively small membrane depolarization command. Conclusively, all these data observed in our experiments indicate an up-shifted excitability of the OPC membrane after HIBD.

**Different rectified potassium channels in OPCs in HIBD**

Whole-cell K⁺ currents were recorded at a holding potential of -40 mV. The currents showed time and voltage dependent inactivation at potentials more negative than -60 mV. The current-voltage (I-V) relationship obtained from the voltage protocol was used to evaluate the rectified properties of the various potassium channels. As shown in Figure 5, OPCs in the cerebral cortex contained different rectified potassium channels. The sustained K⁺ currents (Iₖ, Fig. 5 A) activated from the depolarized pre-stimulated potential at -50 mV were slowly activating and non-inactivating, which is similar to the shape that has been previously described for Kᵥ channels in neurons (Kilic et al. 1996). Another type of K⁺ current (I₄) activated from the hyperpolarized pre-stimulated pulse at -110 mV was fast activating, and was inactivated at a voltage of -40 mV (Fig. 5 B).

The pure outward potassium current can be estimated by subtracting the outward K⁺ currents obtained at Vₜ -50 mV (Iₖ) from the currents obtained at -110 mV (I₄). If this is done for I₄ - Iₖ, the 'subtracted' current showed similar amplitude and shape with Iₖ, suggesting that the pure current passing through the K⁺ channels could be analyzed using a mathematical method (Fig. 5 C and D). These types of potassium currents had approximately the same reversal potential of -70mV, in consistence with the equilibrium potential of potassium concentration. In addition, I₄ currents in OPCs displayed outward rectified properties. We also tested these parameters in the white matter of the cerebral slices, and got similar results (data not shown).

The I-V relationship for the different types of K⁺ channel currents was shifted in HIBD in both the grey and white matter. I₄ and Iₖ in OPCs in the grey matter contained low rectified properties in HIBD (Fig. 6 A and B). Reversal potential of I₄ and Iₖ was also decreased in HIBD. By comparison, no significant change in the reversal potential of I₄ and Iₖ were observed in the white matter. However, The I-V relationship of I₄ and Iₖ both shifted to the right although I₄ activated slowly while Iₖ activated fast during depolarization (Fig. 6 C and D). Taken together, the shift of I-V relationship observed in both the grey and white matter kept good consistence with the K⁺ permeability induced membrane resistance in HIBD.

**DISCUSSION**

Hypoxic-ischemic brain damage, ranging from selective neuronal death to infarction or a combination of both, is a near universal finding in the ligated rat pups surviving a 2-3 h exposure to hypoxia (Bonifacio et al. 2011). The damage is usually restricted to the hemisphere ipsilateral to the ligation and is primarily observed in the cerebral cortex, periventricular white matter and hippocampus (Miller and Ferriero 2009). Such neuropathological damage is rarely seen in the contralateral hemisphere and never seen in pups rendered hypoxic without carotid artery ligation (Vannucci and Vannucci 2005). An understanding of the pathophysiology of perinatal hypoxic-ischemic brain damage is essential to design effective interventions (Cuaycong et al. 2011).

MRI is difficult in small animals but can be easily performed in infant rats using a special coil. T1 and T2 scans were performed to determine the regions of edema and injury in the juvenile rats with HIBD.
Diffuse injury generally occurs in infants and is responsible for the high incidence of mental and behavioral disorders (Vottier et al. 2011). In this study, diffuse and subtle form of grey and white matter injury in postnatal 7-day rats 2 h after HIBD were reflected by DEHSI and punctuate cerebral cortex lesions (Fig. 1), albeit other methods, e.g. alterations in brain volumes, are also thought to reflect diffuse cerebral cortex injury and probably associated with early adverse neurodevelopmental outcome (Lapilover et al. 2012). Overall, the coronal and sagittal T2-weighted MRI demonstrated a rough area of cerebral pathological malformation in HIBD.

To demonstrate the depolarized response in OPCs, a small inward Na$^+$ channel current was observed in OPCs upon applying a depolarizing voltage step (Fig. 4). The persistent small sodium current probably plays a crucial role in the generation of excitability. The increase of maximal inward current amplitude in HIBD suggests that OPCs attain large active reacts during depolarization. The active command of the inward current shifted from -10 mV to -40 mV indicates that OPCs are more excitable in HIBD. The increase of maximal SPCR from 1.27 ± 0.13 (control) to 6.46 ± 1.08 (HIBD) shows the cumulative effects of the sodium and potassium channels on OPC activation. In parallel, downshift of the active command of maximal SPCR in HIBD suggests that small amounts of membrane depolarization could result in a large SPCR shift. Therefore, during HIBD, these electrophysiological properties may represent a significant increase of OPC excitability.

Interestingly, we observed two classes of potassium channels showing different properties (Fig. 5), the first transiently opening from the inactivated state (I$\text{A}$), while the second displaying markedly delayed inactivation (I$\text{K}$). The phenotype of the I-V relationship curve of the potassium channels changed in HIBD. K$^+$ currents delayed the onset of myelin protein re-expression, but later there was a beneficial effect via an increase of proliferating OPCs and mature oligodendrocytes (Hawkins and Butt 2013). Our observations on both persistent and transient current are consistent with previous reports in terms of voltage sensitivity and susceptibility to inactivation (Bielefeldt et al. 1992; Dufour et al. 2011). Moreover, we examined the relationship between the RMP of OPCs and reversal potential of different potassium channels, and the change in the membrane potential agrees well with that calculated from the Nernst equation. Taken together, these results indicate that OPCs in the cerebral cortex may express at least two distinct K$^+$ channels, I$\text{K}$ and I$\text{A}$. I$\text{K}$ channels appear to play a critical role in establishing a hyperpolarized RMP (Cressman et al. 2009). Outward I$\text{K}$ currents were dramatically reduced in HIBD primarily resulting from a decrease in the sustained component.

The transient A-type K$^+$ (I$\text{A}$) channel current has been shown to be ubiquitous in various central neurons (Rogawski 1986; Pedarzani and Stocker 2008) and type-2 astrocytes (Barres et al. 1988; Adermark and Lovinger 2008), and thought to be negligible in comparison with the delayed rectifying K$^+$ (I$\text{K}$) channel current. Intriguingly, our experimental results showed that I$\text{A}$ was far from being negligible (Fig. 5 A and B). Our observation is consistent with previous reports studying an immortalized human neural stem cell line (Cho et al. 2002) and type-1 astrocytes (Barres et al. 1990; Ullah et al. 2009). Previous electrophysiological studies of development have considered the outward current could be divided into transient A-type and persistent delayed rectifier (DR) components (Bahrey and Moody 2003; Zhou and Hablitz 1996) or separate current components based on inactivation kinetics (Foehring and Surmeier 1993; Locke and Nerbonne 1997). Little is known, however, about the current components identified by molecular composition.

An electronics parameter of rectification was adopted to identify the I$\text{K}$ and I$\text{A}$ channel isoforms that predominantly mediate the K$^+$ channel currents in OPCs. Rectification is the electrical and physical
process that converts alternating current to direct current (Santiago and Birchenough 2005), which has been commonly used as an electrolytic rectifier (Geddes 1997; Geddes et al. 1987). Potassium channels display high permeability and carry out single direct (outward) current in most of cell types (Keep et al. 2012; Raz-Prag et al. 2010). It has been confirmed that potassium channels exert a powerful influence on the rectification of neurons (Goh et al. 2011; Hori and Takahashi 2009). Comparisons of rectify of $I_K$ and $I_A$ in OPCs in the grey and white matter revealed a novel coupled system of potassium currents, the components of which are widely distributed throughout the brain (Reyes-Haro et al. 2005; Bordey and Sontheimer 1999), but its existence and importance has not been intensively studied during HIBD (Swayne and Wicki-Stordeur 2012). Although potassium channels are known to exist in many cell types, the role of $K^+$ in rectifying the membrane electrophysiology remains unclear (Jensen et al. 2011).

This study indicated that the rectified properties of $I_K$ and $I_A$ channels present in OPCs were changed in HIBD, with considerably faster $K^+$ current activation. Opening this potassium channel could raise the threshold for action potential generation (Ikematsu et al. 2011). Since these two types of $K^+$ channels have significantly different properties, it seems likely that they play different functional roles in HIBD (Yuan et al. 2011; Lee et al. 2008). It is difficult to account for these divergent results, but one possible explanation is that there are differences among $K^+$ channels studied in slices, which could be sampled unequally depending on intact or dissociated preparation. RMP of OPCs in both grey and white matter decreased significantly in HIBD, which suggested intracellular potassium flowed out of membrane. The polarized state of membrane potential was consequently disturbed. As a protective mechanism, rectification of potassium channel could be activated to reintegrate transmembrane $K^+$ concentration gradient, and further reestablish the rest membrane potential and maintain the stable state of OPCs. In this study, rectification of potassium channels fell off in HIBD, leading to decreased contribution to membrane functions. As a result, failed rectification of the potassium channels and depolarized RMP of OPCs might primary cause irreversible metabolism disorders in HIBD.

CONCLUSIONS

Potassium channels play important roles in OPCs during the early stage of HIBD. The membrane potential was depolarized due to the change of $K^+$ channel permeability, which resulted in transmembrane cation flow activation and edema. The failed rectification of the potassium channels might be primary cause for OPC instability. It is worth to further study the relationship between electrophysiological integration and OPC sensitivity to HIBD.

REFERENCES


Barres BA, Chun LL, Corey DP. Ion channel expression by white matter glia: I. Type 2 astrocytes and


Richardson WD, Young KM, Tripathi RB, McKenzie I. NG2-glia as multipotent neural stem cells: fact or fantasy? *Neuron* 70: 661-673, 2011.


FIGURE LEGENDS

Fig. 1. T2-weighted MR image in postnatal 7-day rats on coronal reconstructions (A) and sagittal reconstructions (B). The image shows DEHSI in the parietal and occipital areas (dotted lines) of the cerebral cortex on coronal (C) and sagittal reconstructions (D) in rats 2 hrs post HIBD.

Fig. 2. Passive membrane properties of OPCs and neuron were recorded in membrane-test mode. It showed that the resting membrane potential (RMP) of OPCs in grey matter (OPC/GM) and white matter (OPC/WM) decreased significantly after HIBD (A). Input resistance ($R_{in}$) of OPCs in grey matter (OPC/GM) and white matter (OPC/WM) increased significantly after HIBD (B). Mean cell capacitance ($C_m$) of OPCs exhibited slightly increase with no significant differences. However, the passive membrane parameters (RMP, $R_{in}$ and $C_m$) of neuron were not altered significantly after 2hrs HIBD.*
Fig. 3. Slices were intracellularly injected with Lucifer yellow in the grey matter of the cerebral cortex in control (A) and HIBD rats (B). Slices were intracellularly injected with Lucifer yellow in the white matter of the cerebral cortex in control (C) and HIBD rats (D). Only one recorded cell was injected in every slice. The slice was fixed with paraformaldehyde and stained with an NG2 primary antibody coupled with Alexa Fluor 568. The nuclei were stained with DAPI. The immunostained sections were observed using a laser scanning confocal microscope in 5 μm layers. The injected cells that were NG2 immunopositive were identified as OPCs. The white arrow indicates the detection point by the patch clamp pipette with Lucifer yellow. Bar 25 μm.

Fig. 4. Whole cell voltage gated channel currents in OPCs in the grey matter in control (A) and in HIBD 2hrs (B). The inward Na\(^+\) current in the OPCs in the grey matter was activated in command -10 mV and reached the maximum current (-0.51 ± 0.13 nA) in command of +20 mV. The maximal SPCR was 1.27 ± 0.13 in 0 mV (C). The inward Na\(^+\) current in OPCs in the grey matter 2 h post HIBD was activated in the depolarized command of -40 mV and reached the maximum current (-1.05 ± 0.28 nA) in command of -20 mV. The maximal SPCR was 6.46 ± 1.08 in command of -20 mV (D).

Fig. 5. Whole-cell recordings of the K\(^+\) channel currents from OPCs in the holding potential -80 mV. A: K\(^+\) currents activated from a depolarized pre-stimulated -50 mV and -40 mV to 110 mV with 10 mV step waveform were slowly activating and noninactivating (I\(_{\text{Kr}}\)). B: K\(^+\) currents activated from a more hyperpolarized pre-stimulated potential of -110 mV with a combination of rapidly activating, fast inactivating currents (I\(_{\text{A}}\)). C: The pure outward flowing potassium currents (green square) were calculated by the arithmetic function of Clampfit 9.2. D: Current-voltage relationship curve of I\(_{\text{K}}\) (red triangle) shows inward rectification. I-V of I\(_{\text{A}}\) (blue dot) shows outward rectification.

Fig. 6. I\(_{\text{A}}\) and I\(_{\text{K}}\) of OPCs in the grey matter exhibited low rectification in HIBD (A and B). The reversal potential of I\(_{\text{A}}\) and I\(_{\text{K}}\) was also decreased in HIBD. In the white matter, the I-V relationship of I\(_{\text{A}}\) shifted to the right and activated slowly during depolarization (C and D). I\(_{\text{K}}\) activated fast during depolarization with a steep I-V relationship. There was no significant change in the reversal potential of I\(_{\text{A}}\) and I\(_{\text{K}}\) in the white matter.