Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

BBRC

Biochemical and Biophysical Research Communications 366 (2008) 401–407

www.elsevier.com/locate/ybbrc

Ischemia deteriorates the spike encoding of rat cerebellar Purkinje cells by raising intracellular Ca^{2+}

Shidi Zhao [a], Na Chen [b], Zhilai Yang [b], Li Huang [a], Yan Zhu [a], Sudong Guan [a], Qianfen Chen [a], Jin-Hui Wang [b,*]

[a] *Department of Physiology, Bengbu Medical College, Bengbu Anhui 233000, China*

[b] *State Key Lab for Macrobiomolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*

Received 15 November 2007
Available online 21 December 2007

Abstract

Ischemia-induced excitotoxicity at cerebellar Purkinje cells is presumably due to a persistent glutamate action. To the fact that they are more vulnerable to ischemia than other glutamate-innervated neurons, we studied whether additional mechanisms are present and whether cytoplasm Ca^{2+} plays a key role in their ischemic excitotoxicity. Ischemic changes in the excitability of Purkinje cells were measured by whole-cell recording in cerebellar slices of rats with less glutamate action. The role of cytoplasm Ca^{2+} was examined by two-photon cellular imaging and BAPTA infusion in Purkinje cells. Lowering perfusion rate to cerebellar slices deteriorated spike timing and raised spike capacity of Purkinje cells. These changes were associated with the reduction of spike refractory periods and threshold potentials, as well as the loss of their control to spike encoding. Ischemia-induced functional deterioration at Purkinje neurons was accompanied by cytoplasm Ca^{2+} rise and prevented by BAPTA infusion. Therefore, the ischemia destabilizes the spike encoding of Purkinje cells via raising cytoplasm Ca^{2+} without a need for glutamate, which subsequently causes their excitotoxic death.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Ischemia; Purkinje cells; Intracellular Ca^{2+}; Excitability; Two-photon imaging; Whole-cell recording

The ischemic death of cells due to their excitotoxicity in the brain is believed to be the increases of glutamate release, intracellular calcium, and free radicals in origin [1–6]. The efforts to correct these deficits have not substantially raised the impact to secure stroke patients [7,8]. It is likely that other mechanisms leading to neuronal excitotoxicity are involved [9].

Ataxia in patients recovering from global ischemic stroke is believed to be the loss of cerebellar Purkinje cells [10]. Excitotoxic death may result from the intensive innervation of glutamatergic climbing/parallel fibers onto Purkinje cells [10], deficiency of glutamate reuptake [6,11,12], and persistent activation of AMPA receptors [13–16]. As Purkinje cells are more vulnerable to the ischemia than other glutamate-innervated cells [6,10,17], we hypothesized that the intrinsic excitability of cerebellar Purkinje cells, independent of glutamate action, rises in the early stage of the ischemia, leading to their ischemic excitotoxicity.

We measured the intrinsic excitability of cerebellar Purkinje cells, and examined the role of cytoplasm Ca^{2+} in the early stage of ischemia with less glutamatergic action. Ischemia was simulated by reducing perfusion rate to cerebellar slices [9]. The intrinsic excitability of cells was measured as spike patterns, refractory periods, and threshold potentials [18]. The role of cytoplasm Ca^{2+} in ischemic excitotoxicity was tested by BAPTA infusion and two-photon Ca^{2+} imaging.

Methods and materials

Cerebellar slices. The sagittal slices of cerebellar vermis (400 μm) were prepared from Sprague–Dawley rats in postnatal days (PND) 17–21 (see

* Corresponding author.
E-mail address: jhw@sun5.ibp.sc.cn (J.-H. Wang).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.11.173

Ref. [19] for detail). A slice was transferred into a submersion chamber at 2 ml/min of perfusion rate with oxygenated ACSF (31 °C) for whole-cell recording [20] or two-photon cellular imaging. The selection of Purkinje cells were done under IR-DIC optics (Nikon E600FN), based on the criteria [19,21] for detail). The procedures are approved by Institutional Animal Care and Use Committee in Beijing, China.

In vitro ischemia with less glutamatergic inputs. To simulate artery occlusion and intracranial anastomotic circulation *in vivo* ischemic stroke, we reduced the perfusion rate to cerebellar slices from 2 to 0.2 ml/min [9]. We measured the intrinsic properties of sequential spikes and the imaging of Ca^{2+} levels in Purkinje cells before and 3 min after this procedure. To reduce the effect of glutamatergic inputs on our experiments, we used the sagittal sections of cerebellar vermis, in which parallel and climbing fibers to Purkinje cells were cut. Under this condition, we did not record spontaneous synaptic events. An addition of 6-cyano-7-nitroquinoxaline-2,3-(1H,4H)-dione (CNQX, 10 mM) in the perfusion solution removes the influence of ionotropic glutamate receptors.

Whole-cell recording. Electrical signals were recorded under current-clamp model by Axoclamp-2B and Multi-clamp 700B integrated with pClamp 9 (Axon Instrument Inc., Foster, CA, USA). The output bandwidth of amplifiers was 3 kHz. Spike patterns were evoked by depolarization pulses with various intensity and duration based on the aim of experiments. Pipettes were filled with standard solution (mM: 150 K-gluconate, 5 NaCl, 5 Hepes, 0.4 EGTA, 4 Mg–ATP, 0.5 Tris–GTP, and 5 phosphocreatine, pH 7.35, adjusted by 2 M KOH). The osmolarity of solution freshly made was 295–305 mOsmol. Pipette resistance was 5–6 MΩ.

The intrinsic properties of Purkinje cells in our study include threshold potentials for firing spikes and absolute refractory periods (ARP) after each spike. The definition and measurement of the parameters are given in the references [18,22]. Spike encoding (capacity and timing precision) is represented as inter-spike interval (ISI) and the standard deviation of spike timing (SDST).

Data were analyzed if resting membrane potentials at the recorded neurons were greater than −55 mV. The criteria for the acceptance of each experiment also included less than 5% changes in the resting membrane potentials, spike magnitude and input resistance throughout individual experiments. Vts, ARP, ISI, and SDST are presented as means ± SE. The comparisons under the control and ischemia were done by *t*-test.

Loading Ca^{2+} indicative dye into the neurons in slices. AM ester of Ca^{2+} indicative dye (Oregon Green BAPTA-AM) was dissolved in DMSO and 20% Pluronic F-127 (2 g Pluronic F-127 in 10 ml DMSO) to have its stock solution at 10 mM, which was diluted in ACSF to yield a final concentration of dye at 1 mM. The loading solution was added into the slice incubation chamber for 30 min, and washed out with oxygenated ACSF. A slice was transferred to a submersion chamber and perfused with the oxygenated ACSF at 2 ml/min for two-photon imaging experiments.

Two-photon cellular image. Ca^{2+} imaging in Purkinje neurons was taken by using a two-photon laser scanning microscope. Laser beam in the system (Mai Tai, Spectral Physics, Mountain View, CA, USA) was locked at 910 nm wavelength, <100 fs pulse width and 80 MHz repeat rate. Scanning system (Olympus FV-1000s, Tokyo, Japan) is mounted onto upright microscope (Olympus BX61WI) equipped with water immersion objectives (X40, 0.8NA). The average power delivered to brain slices was <20 mW. The parameters set for laser beam and photomultiplier tube were locked throughout experiments to have consistent comparison before and after the ischemia. Images were viewed and analyzed with Fluoviewer. Data are presented as the changes in the intensity of fluorescence.

Results

Ischemia deteriorates the excitatory states of cerebellar Purkinje cells

The ischemic changes in the intrinsic excitability of cerebellar Purkinje cells were shown in Fig. 1. Fig. 1A and B are the superimposed waveforms of spikes evoked by depolarization currents under the control and 3 min after reducing perfusion rate, respectively. Spike timing deteriorated and neurons were overexcited. To quantify the changes in spike capacity, we measured ISIs between spikes one and two (ISI_{1-2}) up to four and five (ISI_{4-5}). The values of ISI_{1-2} to ISI_{4-5} at Purkinje cells under the controls were 10 ± 0.52, 12.7 ± 0.63, 13.4 ± 0.62, and 14.2 ± 0.74 ms (filled circles in Fig. 1C, $n = 20$); and the values from ISI_{1-2} to ISI_{4-5} at those cells undergoing ischemia were 8.24 ± 0.74, 9.4 ± 1.0, 11 ± 1.0, and 11.5 ± 0.9 ms (open circles). ISI values for corresponding spikes are statistically lower under the ischemia than control ($p < 0.05$). The ischemia raises spike capacity, i.e., the overexcitation of cerebellar Purkinje cells.

In quantifying the changes of spike timing precision, we measured SDST of spike one ($SDST_1$) to five ($SDST_5$). The values of $SDST_1$ to $SDST_5$ at Purkinje cells under control were 0.72 ± 0.1, 1.2 ± 0.12, 1.5 ± 0.15, 1.85 ± 0.18, and 2.14 ± 0.2 ms (filled circles in Fig. 1D, $n = 20$); and the values from $SDST_1$ to $SDST_5$ at those cells during ischemia were 1.85 ± 0.37, 2.13 ± 0.32, 2.5 ± 0.3, 3.1 ± 0.47, and 3.42 ± 0.4 ms (open circles). SDST values for corresponding spikes are statistically higher under ischemia than control ($p < 0.01$). The ischemia deteriorates spike timing precision, i.e., the unstable encoding of cerebellar Purkinje cells, which leads to cerebellar dysfunction, such as ataxia.

We then studied the mechanisms underlying these ischemic changes. In terms of biophysical aspects, we examined spike refractory periods and threshold potentials during the ischemia. For biochemical mechanism, we mainly studied the essential role of cytoplasm Ca^{2+} in the ischemic dysfunction of Purkinje neurons.

Ischemia lowers spike refractory periods and threshold potentials

Absolute refractory periods (ARP) of sequential spikes at cerebellar Purkinje cells appear shorter under the ischemia than control (Fig. 2A). Fig. 2B shows a comparison of ARP at Purkinje cells under these two conditions. The values of ARP_1 to ARP_4 were 3.75 ± 0.11, 3.87 ± 0.17, 4.0 ± 0.16, and 4.28 ± 0.19 ms under control (filled symbols, $n = 16$); and the values were 3.3 ± 0.13, 3.25 ± 0.14, 3.3 ± 0.13, and 3.5 ± 0.21 ms during ischemia (open symbols). ARP values for corresponding spikes are significantly lower during the ischemia than under the control ($p < 0.01$).

Threshold potentials are calculated as the difference between resting membrane potential (Vr) and threshold potential (Vts). The use of Vts–Vr, instead of Vts alone, is based on the following facts. The values of Vr and Vts vary among the CNS neurons [22]; Vts–Vr is an energy barrier to raise Vr toward Vts; and Vts–Vr represents how synaptic inputs easily drive neurons to fire spikes. Fig. 2D shows the comparison of Vr–Vts values at Purkinje cells under the control and ischemia. Vts–Vr values for spikes 1 up to 5 are

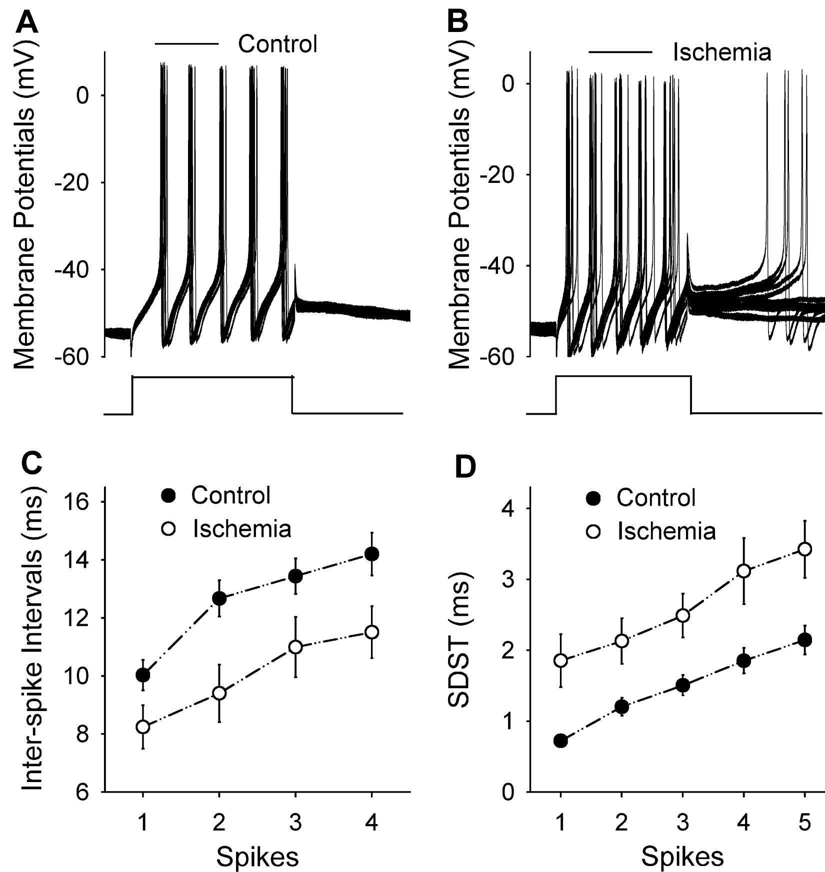

Fig. 1. The simulated ischemia raises the excitability and deteriorates the spike timing of cerebellar Purkinje neurons. (A) The superimposed waveforms of sequential spikes are evoked by depolarization current pulses (0.3 nA, 100 ms) at CPC under the control condition. (B) The superimposed waveforms of sequential spikes are evoked by depolarization currents (0.3 nA, 80 ms) 3 min after reducing perfusion rate. (C) The comparisons of inter-spike intervals for sequential spikes between control (filled symbols, $n = 20$) and ischemia (open symbols, $n = 20$). (D) The comparisons of the standard deviation of spike timing between control (filled symbols) and ischemia (open symbols).

13.5 ± 0.89, 15.76 ± 0.9, 17.2 ± 0.86, 18.2 ± 0.82, and 18.96 ± 0.84 ms under the control (filled symbols, $n = 20$); and the values are 10.2 ± 0.97, 13.1 ± 1.0, 14.6 ± 0.98, 15.5 ± 1.1, and 15.9 ± 1.03 ms during ischemia (opens). Vts–Vr values for corresponding spikes under these two conditions are statistically different ($p < 0.05$). Threshold potentials were lowered during the ischemia.

The lowered threshold potentials make cells more sensitive to excitatory inputs. The shortened refractory periods permit cells firing spikes at a high rate [18]. Therefore, the ischemia-induced reduction of Vts–Vr and ARP at Purkinje neurons underlies their ischemic overexcitation.

Noteworthy, the linear correlations between intrinsic properties and spike patterns denote that ARP and Vts control spike encoding [18]. We examined whether the ischemic deterioration of spike encoding is due to the failed control of neuronal intrinsic properties to the spiking, i.e., a loss of linear correlations during the ischemia. As shown in supplementary, linear correlation between ARP and ISI under the control ($r^2 = 0.78$, filled symbols) was converted to non-linear during the ischemia ($r^2 = 0.46$, open symbols; $p < 0.01$); and linear correlation between ARP and SDST under the control ($r^2 = 0.94$, filled symbols) was converted into non-linear during the ischemia ($r^2 = 0.57$, open symbols; $p < 0.01$). These results indicate that the ischemia declines the role of refractory periods in controlling spike programming at cerebellar Purkinje cells.

Cytoplasm Ca^{2+} is essential to ischemic overexcitation in Purkinje neurons

We examined the role of cytoplasm Ca^{2+} in the ischemia-induced overexcitation of Purkinje cells by measuring or lowering intracellular Ca^{2+} levels under the condition of less glutamatergic action (see Methods). If Ca^{2+} increases in Purkinje cells during ischemia, Ca^{2+} is associated with their ischemic overexcitation. If lowering cytoplasm Ca^{2+} prevents their ischemic overexcitation, Ca^{2+} must be required.

Ca^{2+} imaging in Purkinje cells of cerebellar slices was performed with two-photon laser scanning microscope to test whether intracellular Ca^{2+} rises during the ischemia. After preloaded Oregon Green BAPTA-AM, a cerebellar slice was transferred into the submersion chamber with the perfusion of the oxygenated ACSF at a rate of 2 ml/min. 30 min after this control, we measured Ca^{2+} levels

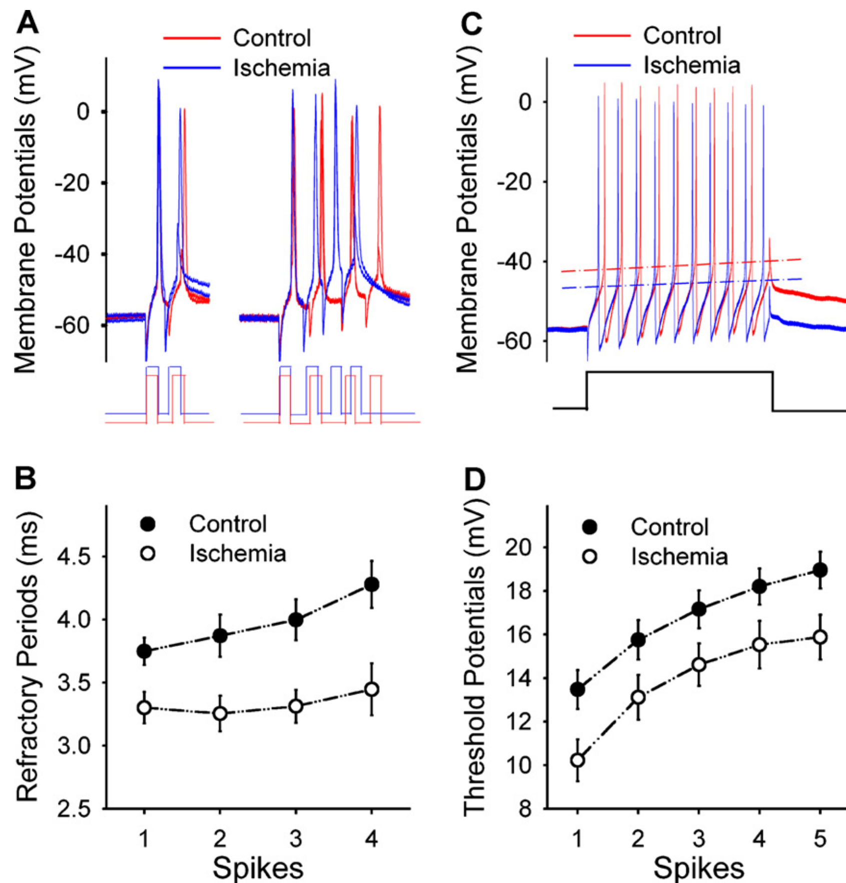

Fig. 2. The ischemia reduces the refractory period (ARP) and threshold potentials (Vts) of sequential spikes at Purkinje neurons. (A) The superimposed waveforms show the measurement of ARP1 (left) and ARP3 (right) induced by depolarization current pulses (3 ms). Red lines show control; blue lines show ischemia. (B) The comparisons of ARP1–4 of sequential spikes under the control (filled symbols, $n = 16$) and 3 min after reducing perfusion rate (open symbols, $n = 16$). (C) The superimposed waveforms show the measurement of Vts induced by depolarization current pulse (160 ms). Red lines show control; and blue lines show ischemia. (D) The comparisons of Vts of sequential spikes under control (filled symbols, $n = 20$) and 3 min after reducing perfusion rate (open symbols, $n = 20$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in Purkinje cells. The perfusion rate to this slice was reduced to 0.2 ml/min. After this treatment for 3 min, we measured Ca^{2+} levels in those Purkinje cells under the same conditions as the control. Fig. 3 shows the change of Ca^{2+} in Purkinje cells under the control (left panels) and ischemia (right panels) from one of six experiments. The levels of Ca^{2+} in those Purkinje cells with low activities rise substantially during the ischemia.

To examine if the rise of Ca^{2+} is required for the ischemia-induced overexcitation of Purkinje cells, we loaded BAPTA (a chelator of Ca^{2+}) into these cells via recording pipettes, and measured their spike encoding under BAPTA infusion before and after the ischemia. Fig. 4 shows the changes in spike parameters of Purkinje cells under the control (filled symbols) and ischemia (open symbols). The values of ISI_{1-2} to ISI_{4-5} were 26.66 ± 1.87, 27.77 ± 1.84, 27.99 ± 1.8, and 28.46 ± 1.68 ms under the control; and the values were 25.95 ± 1.81, 26.71 ± 1.61, 26.79 ± 1.73, and 27.92 ± 1.68 ms under the ischemia (Fig. 4C). The values for $SDST_1$ up to $SDST_5$ were 1.76 ± 0.22, 2.95 ± 0.26, 3.2 ± 0.28, 3.62 ± 0.26, and 4.1 ± 0.3 ms under the control; and the values were 1.61 ± 0.25, 2.79 ± 0.4, 3.52 ± 0.32, 3.95 ± 0.43, and 4.24 ± 0.41 ms under the ischemia (Fig. 4D). The values for corresponding spikes under the two conditions are not statistically different ($n = 11$, $p > 0.20$). The preventive effect of BAPTA on ischemia-induced overexcitation indicates the requirement of rising cytoplasm Ca^{2+} for the ischemic excitotoxicity of cerebellar Purkinje cells.

Discussion

We found that the ischemia raises cellular excitability and worsens spike timing precision under the condition of less glutamatergic action, which causes Purkinje cells dysfunction, excitotoxic death, and behavioral changes, such as post-ischemic ataxia. In terms of the biophysical mechanism, the ischemia reduces spike refractory periods and threshold potentials, as well as breaks down the control of ARP to spike encoding. The increase of cytoplasm Ca^{2+} plays an essential role of in such ischemic changes

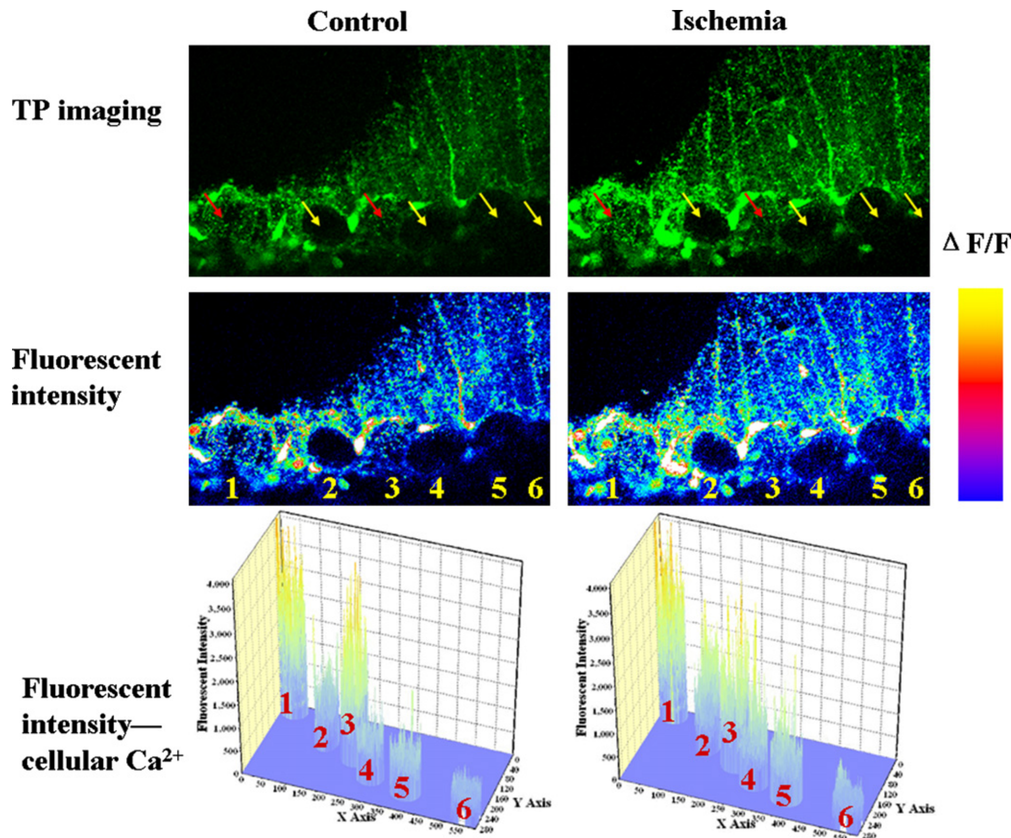

Fig. 3. The increase in Ca^{2+} levels during ischemia. Top panels are Ca^{2+} imaging of cerebellar Purkinje cells two-photon (TP) laser scanner confocal microscope (laser beam 910 nm, PMT scanner FTIC). Middle panels show the analyses of Ca^{2+} levels, in which blue-red-yellow indicates Ca^{2+} levels from low to high. Bottom panels show the analyses of Ca^{2+} levels based on the column height of fluorescent intensity. Left panels show Purkinje cell imaging under control; and right panels show imaging of the same Purkinje cells after reducing perfusion rate for 3 min. Ca^{2+} levels rise in Purkinje neurons with low (yellow arrows) and high (red arrows) basal Ca^{2+} activity (top panels), especially Ca^{2+} increase in cells with low basal activity (cells 2, 4, 5, and 6, see middle and bottom panels). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in Purkinje cells, in which the pathway independent of glutamatergic action is involved.

In addition to our study showing glutamate-independent excitotoxicity, other data imply that the glutamate action is not a sole mechanism of ischemic cell death. For example, cerebellar Purkinje cells are more vulnerable to ischemic excitotoxicity than other glutamate-innervated cells [6,10,17]. Although the ischemic cell death may result from glutamate-dependent neuronal excitotoxicity [1–3,5,6,10], the methods used to correct such deficits have not substantially improved the outcome of stroke patients [7,8]. These facts support that the mechanisms beyond glutamatergic action are involved in the ischemic excitotoxicity of cerebellar Purkinje cells. Thus, new therapeutic strategies, other than anti-glutamate, remain to be developed to prevent ischemic stroke.

We ascertain an essential role of cytoplasm Ca^{2+} in the ischemic overexcitation of Purkinje neurons with compelling strategies. Two-photon imaging shows cytoplasm Ca^{2+} rise in Purkinje cells during the ischemia, indicating that Ca^{2+} is associated with their overexcitation. The prevention of ischemic overexcitation by lowering cytoplasm Ca^{2+} grants Ca^{2+} requirement. It is noteworthy that the mediate steps from ischemia to glutamate-independent rise of cytoplasm Ca^{2+} remain to be addressed.

We measured the changes in the intrinsic excitability of cerebellar Purkinje cells during ischemia, such as spike capacity and timing precision. Our finding, ischemia-induced destabilization of excitation and spike encoding, provides the first quantitative description for the functional deterioration of Purkinje cells during ischemia, as well as the complement for the previous studies that were focused on addressing ischemic changes in the morphology and numerical loss of Purkinje cells.

In studying the mechanisms of Ca^{2+} downstream for ischemic overexcitation, we found that spike refractory periods were shortened, which allows an increase in spike capacity, and that threshold potentials were lowered, which makes Purkinje neurons more sensitive to excitatory inputs firing spikes. Moreover, the disappearance of linear correlation between refractory periods and spike encoding shows that cellular intrinsic properties lose their control over spike encoding during the ischemia, leading to the deterioration of spike timing precision at cerebellar Purkinje cells, and the dysfunction of the cerebellum.

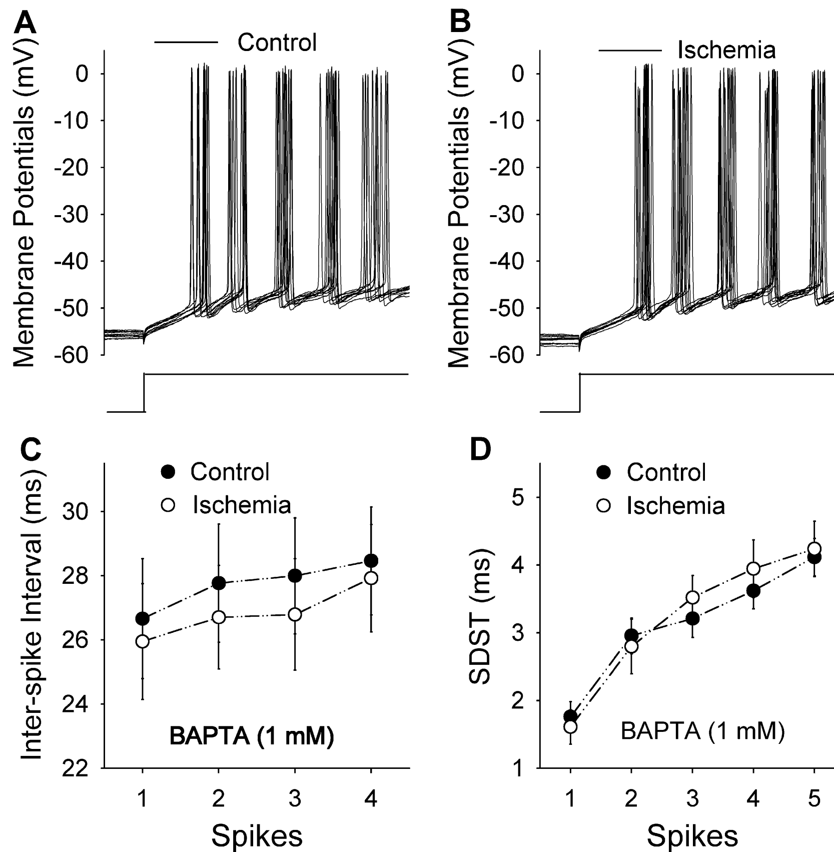

Fig. 4. The infusion of BAPTA into Purkinje neurons prevents ischemia-induced overexcitation. (A) The superimposed waveforms of sequential spikes are evoked by depolarization pulses (0.3 nA) under the control. (B) The superimposed waveforms of sequential spikes are evoked by depolarization currents (0.3 nA) 3 min after reducing perfusion rate. (C) The comparisons of inter-spike intervals for sequential spikes between control (filled symbols, $n = 11$) and ischemia (open symbols, $n = 11$) while infusing BAPTA. (D) The comparisons of the standard deviation of spike timing between control (filled symbols) and ischemia (open symbols) while infusing BAPTA.

The refractory periods and threshold potentials of sequential spikes are controlled by voltage-gated sodium channels [21,23]. The ischemic reduction of ARP and Vts is likely due to the facilitation of VGSC activation. A recent study demonstrated the neuroprotective effect of sodium channel blockers on cerebral ischemia [24], consistent to our prediction. It is noteworthy that a rise in VGSC-mediated excitability facilitates the activation of voltage-gated calcium channels and the accumulation of sodium ions in cells. Both strengthen Ca^{2+} increase in Purkinje cells.

We propose a model for the mechanisms of rising cytoplasm Ca^{2+} and Purkinje cell excitation during ischemia. The ischemia or hypoxia lowers the level of adenosine triphosphate (ATP), which causes a deficiency of glutamate transporter in presynaptic terminal (pathway one). Glutamate accumulation in synaptic cleft prolongs activation of its receptors and in turn raises intracellular Ca^{2+} level. This pathway is consistent with the previous theory [6,11–16,25]. In addition, ATP consumption during the ischemia lowers the function of the primary active transporters (Ca^{2+} and Na–K pumps) and subsequently secondary active one (Na–Ca exchanger), leading to their inability to pump Ca^{2+} out of cytoplasm (pathway two). Together these with Ca^{2+}-induced Ca^{2+} release, cytoplasm Ca^{2+} in Purkinje cells rises via glutamate-independent manners. The rise of cytoplasm Ca^{2+} is essential to ischemia-induced overexcitation in Purkinje cells, which further raises cytoplasm Ca^{2+} by activating more voltage-gated calcium channels. This positive feedback leads eventually to the excitotoxicity of cerebellar Purkinje cells.

Acknowledgments

We thank Dr. Sarah Perrett for critical reading before manuscript submission. This study is granted by National Awards for Outstanding Young Scientist (30325021), NSFC (30470362, 30621130077), CAS Knowledge Innovation Program (KSCX2-YWR-39), and National Basic Research Program (2006CB500804, 2006CB911003) to J.H.W.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.11.173.

References

[1] F. Block, Global ischemia and behavioural deficits, Prog. Neurobiol. 58 (1999) 279–295.

[2] D.W. Choi, Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage, Trends Neurosci. 11 (10) (1988) 465–469.

[3] P. Lipton, Ischemic cell death in brain neurons, Physiol. Rev. 79 (4) (1999) 1431–1568.

[4] R.D. Schwartz-Bloom, R. Sah, r-Aminobutyric acid A neurotransmission and cerebral ischemia, J. Neurochem. 77 (2001) 353–371.

[5] B.C. White, J.M. Sullivan, D.J. DeGracia, B.J. O'Neil, R.W. Neumar, L.I. Grossman, J.A. Rafols, G.S. Krause, Brain ischemia and reperfusion: molecular mechanisms of neuronal injury, J. Neurol. Sci. 179 (2000) 1–33.

[6] J.P. Welsh, G. Yuen, D.G. Placantonkis, T.Q. Yu, F. Haiss, E. O'Heaen, M.E. Molliver, S.A. Aicher, Why do Purkinje cells die so easily after global brain ischemia? Aldolase C, EAAT4, and the cerebellar contribution to posthypoxic myoclonus, Adv. Neurol. 89 (2002) 331–359.

[7] T. Dalkara, M.A. Moskowitz, Recent developments in the experimental stroke, Neurosci. News 2 (1999) 20–27.

[8] A. Devasenapathy, V.C. Hachinski, Current treatment of acute ischemic stroke, Neurosci. News 2 (1999) 4–13.

[9] J.-H. Wang, Short-term cerebral ischemia causes the dysfunction of interneurons and more excitation of pyramidal neurons, Brain Res. Bull. 60 (1–2) (2003) 53–58.

[10] J.R. Sanar, R. Hawkes, Patterned Purkinje cell death in the cerebellum, Prog. Neurobiol. 70 (2003) 473–507.

[11] Y.W. Inage, M. Itoh, K. Wada, S. Takashima, Expression of two glutamate transporters, GLAST and EAAT4, in the human cerebellum: their correlation in development and neonatal hypoxia-ischemic damage, J. Neuropathol. Exp. Neurol. 57 (6) (1998) 554–562.

[12] A. Yamashita, K. Makita, T. Kuroiwa, K. Tanaka, Glutamate transporters GLAST and EAAT4 regulate postischemic Purkinje cell death: an in vivo study using a cardiac arrest model in mice lacking GLAST or EAAT4, Neurosci. Res. 55 (3) (2006) 264–270.

[13] J.R. Brorson, P.A. Manzolillo, S.J. Gibbons, R.J. Miller, AMPA receptor desensitization predicts the selective vulnerability of cerebellar Purkinje cells to excitotoxicity, J. Neurosci. 15 (6) (1995) 4515–4524.

[14] M. Hamann, D.J. Rossi, C. Mohr, A.L. Andrade, D. Attwell, The electrical response of cerebellar Purkinje neurons to simulated ischemia, Brain 128 (2005) 2408–2420.

[15] M. Tomiyama, J.M. Palacios, R. Cortes, G. Mengod, Flip and flop variants of AMPA receptor subunits in the human cerebellum: implication for the selective vulnerability of Purkinje cells, Synapse 31 (2) (1999) 163–167.

[16] S. Zheng, Z. Zuo, Isoflurane preconditioning reduces Purkinje cell death in an invitro model of rat cerebellar ischemia, Neuroscience 118 (1) (2003) 99–106.

[17] F. Fonnum, E.A. Lock, Cerebellum as a target for toxic substances, Toxicol. Lett. 112–113 (2000) 9–16.

[18] N. Chen, S.L. Chen, Y.L. Wu, J.H. Wang, The measurement of threshold potentials and refractory periods of sequential spikes by whole-cell recordings, Biochem. Biophys. Res. Commun. 340 (2006) 151–157.

[19] S. Guan, S. Ma, Z. Yan, R. Ge, Q. Wang, J.H. Wang, The intrinsic mechanisms underlying the maturation of programming sequential spikes at cerebellar Purkinje cells, Biochem. Biophys. Res. Commun. 345 (2006) 175–180.

[20] J.-H. Wang, P.T. Kelly, Ca2+/CaM signaling pathway up-regulates glutamatergic synaptic function in non-pyramidal fast-spiking neurons of hippocampal CA1, J. Physiol. (Lond) 533 (2) (2001) 407–422.

[21] N. Chen, Y. Zhu, X. Gao, S. Guan, J.-H. Wang, Sodium channel-mediated intrinsic mechanisms underlying the differences of spike programming among GABAergic neurons, Biochem. Biophys. Res. Commun. 346 (2006) 281–287.

[22] M. Zhang, F. Hung, Y. Zhu, Z. Xie, J. Wang, Calcium signal-dependent plasticity of neuronal excitability developed postnatally, J. Neurobiol. 61 (2004) 277–287.

[23] N. Chen, X. Chen, J. Yu, J.-H. Wang, After-hyperpolarization improves spike programming through lowering threshold potentials and refractory periods mediated by voltage-gated sodium channels, Biochem. Biophys. Res. Commun. 346 (2006) 938–945.

[24] M.J. O'Neill, C.A. Hicks, M.A. Ward, D.J. Osborne, G. Wishart, K.S. Mathews, D.P. MclLaughllin, J.A. Stamford, D.R. McCarty, K.E. Patrick, C. Roman, J.H. Fleisch, J. Gilmore, J.R. Boot, LY393615, a novel neuronal Ca2+ and Na+ channel blocker with neuroprotective effects in models of in vitro and in vivo cerebral ischemia, Brain Res. 888 (1) (2001) 138–149.

[25] A. Mitani, H. Yanase, S. Namba, M. Shudo, K. Kataoka, In vitro ischemia-induced intracellular Ca2+ elevation in cerebellar slices: a comparative study with the values found in hippocampal slices, Acta Neuropathol. (Berl.) 89 (1) (1995) 2–7.