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ORIGINAL ARTICLE

Cerebral ischemic preconditioning reduces glutamate excitotoxicity by up-regulating the uptake activity of GLT-1 in rats

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Abstract Our previous study has shown that cerebral ischemic preconditioning (CIP) can up-regulate the expression of glial glutamate transporter-1 (GLT-1) during the induction of brain ischemic tolerance in rats. The present study was undertaken to further explore the uptake activity of GLT-1 in the process by observing the changes in the concentration of extracellular glutamate with cerebral microdialysis and high-performance liquid chromatography. The results showed that a significant pulse of glutamate concentration reached the peak value of sevenfold of the basal level after lethal ischemic insult, which was associated with delayed neuronal death in the CA1 hippocampus. When the rats were pretreated 2 days before the lethal ischemic insult with CIP which protected the pyramidal neurons against delayed neuronal death, the peak value of glutamate concentration decreased to 3.9 fold of the basal level. Furthermore, pre-administration of dihydrokainate, an inhibitor of GLT-1, prevented the protective effect of CIP on ischemia-induced CA1 cell death. At the same time, compared with the CIP + Ischemia group, the peak value of glutamate concentration significantly increased and reached sixfold of the basal level. These results indicate that CIP induced brain ischemic tolerance via up-regulating GLT-1 uptake activity for glutamate and then decreasing the excitotoxicity of glutamate.

Keywords Cerebral ischemic preconditioning · GLT-1 · Glutamate · Microdialysis · HPLC · Dihydrokainate

Introduction

Glutamate is the major excitatory amino acid neurotransmitter in the central nervous system, but excessive extracellular levels of glutamate induced by brain ischemia lead to excitotoxicity and cell delayed death (Choi and Rothman 1990). The extracellular glutamate homeostasis in the central nervous system is mainly regulated by the uptake activity of excitatory amino acid transporters (EAATs), because there is no enzyme to decompose glutamate in extracellular fluid (Danbolt 2001). Five distinct high-affinity, sodium-dependent EAATs are identified. It is generally accepted that in many brain regions the dominant glutamate transporter is EAAT2, which is mainly distributed in astrocytes and also named as glial glutamate transporter-1 (GLT-1) (Mitani and Tanaka 2003; Rothstein et al. 1996; Rao et al. 2001; Tanaka et al. 1997).

It is well known that cerebral ischemic preconditioning (CIP), transient sublethal cerebral ischemia, can protect hippocampal neurons against delayed neuronal death normally induced by lethal ischemic insult (Chen et al. 1996; Kitagawa et al. 1990; Kirino 2002). Our previous study has shown that CIP protected pyramidal neurons against delayed neuronal death induced normally by lethal global brain ischemia, and simultaneously up-regulated the

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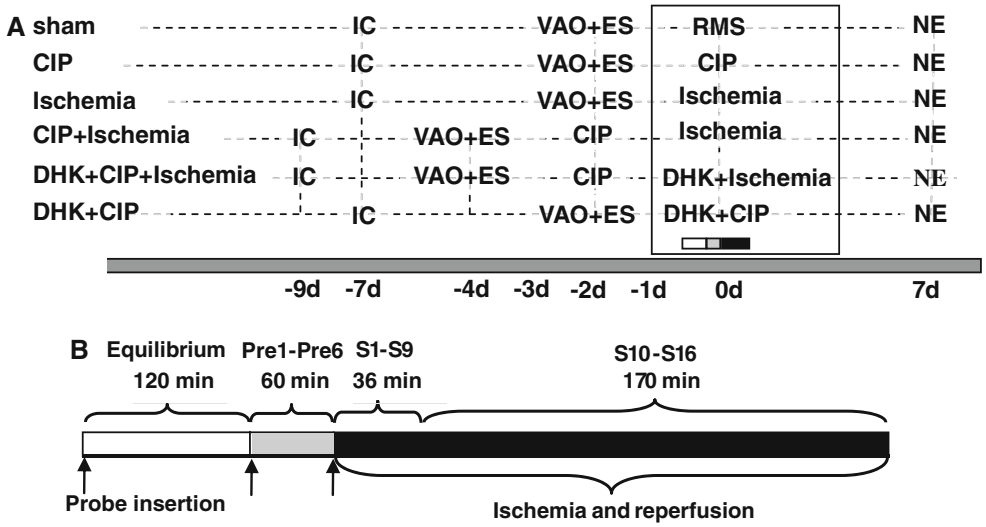

Fig. 1 The graphical presentations of experimental protocol (**a**) and sample collection protocol of microdialysis in CA1 hippocampus (**b**). *CIP* cerebral ischemic preconditioning for 3 min, *DHK* dihydrokainate, *ES* embedding nylon monofilaments and sutures looping the bilateral common carotid arteries, *IC* implanting cannula(s), *NE* neuropathological evaluation, *Pre* samples were collected before ischemia, *RMS* removing monofilaments and sutures, *S* sample, *VAO* vertebral artery occlusion. The graph **a** shows the experimental protocols for each group. At first, a guide cannula was implanted into hippocampal CA1 subfield for microdialysis in rats of each group. Another guide cannula was implanted into lateral cerebral ventricle for DHK administration in rats of DHK + CIP + Ischemia group and DHK + CIP group. After a 5-day recovery, the rats were subjected to VAO and ES. RMS or CIP or ischemia was performed respectively 2 days after ES. Microdialysis was performed 3 h before the last treatment in each group. At last, neuropathological evaluation was performed 7 days after the microdialysis. The graph **b** shows the sample collection protocol of microdialysis in CA1 hippocampus. The *left*, *middle* and *right upward arrows* indicate the onset of the equilibrium, sample collection, and ischemia or the sham operation in each group

expression of GLT-1 within the hippocampal CA1 subfield in rats (Zhang et al. 2007). Furthermore, GLT-1 antisense oligodeoxynucleotides (AS-ODNs) could dose-dependently inhibit neuroprotective effects of CIP by regulating glutamate uptake activity via GLT-1 (Geng et al. 2008; Liu et al. 2011).

To provide direct evidence for the involvement of CIP in preconditioning neuroprotection by preventing the excitotoxicity, the present study was undertaken to detect changes in glutamate concentration in the brain during the induction of brain ischemic tolerance by intra-cerebral microdialysis and high-performance liquid chromatography (HPLC). In addition, the concentration of aspartate was also detected because it also has an excitotoxic effect in brain ischemia.

Materials and methods

Animal and grouping

Thirty-six adult male *Wistar* rats (280–320 g in weight, 10-week old) were provided by The Experimental Animal Center of Hebei Medical University. Animals were housed on a 12-h light/12-h dark cycle, with access to food and water ad libitum. All experiments were approved by the Ethics Committee of Hebei Medical University.

The rats were randomly divided into six groups ($n = 6$ in each group): (1) Sham group: rats were subjected to the sham operation for global brain ischemia; (2) CIP group: rats were subjected to global brain ischemia for 3 min; (3) Ischemia group: rats were subjected to global brain ischemia for 8 min; (4) CIP + Ischemia group: rats were first subjected to a CIP, and then were subjected to 8-min global brain ischemia 2 days after the CIP; (5) dihydrokainate (DHK, a selective inhibitor of GLT-1) + CIP + Ischemia group: rats were intracerebroventricularly injected with DHK 30 min before global brain ischemia for 8 min, other procedures were the same as those in the CIP + Ischemia group. (6) DHK + CIP group: rats were intracerebroventricularly injected with DHK 30 min before global brain ischemia for 3 min, other procedures were the same as those in the CIP group. The experimental protocols for each group are showed in Fig. 1a.

Implanting guide cannula into hippocampal CA1 subfield and lateral cerebral ventricle

Rats under anesthesia with chloral hydrate (350 mg/kg, i.p.) were secured in a stereoscopic frame. Two guide

cannulas were implanted according to Atlas. One (MD-2251, BAS, USA) was implanted into the CA1 hippocampus (AP: 4.16 mm, ML: 2.8 mm, DV: 2.61 mm) with a 40° gradient from the lateral to medial side to localize it along the pyramidal cell layer for microdialysis. The other (Reward, China) was implanted into the lateral cerebral ventricle (AP: 0.8 mm, ML: 1.5 mm, DV: 3.8 mm) for DHK administration. Three stainless steel screws and dental cement were used to fix the guide cannulas to the skull. After a 5-day recovery, the rats were used for further experiments.

Establishment of global brain ischemic model

Global brain ischemia was induced using four-vessel occlusion method reformed from Pulsinelli and Brierley (1979). First, the bilateral vertebral arteries were permanently occluded by electro-cauterization under chloral hydrate anesthesia (i.p., 350 mg/kg). Then, the bilateral common carotid arteries (BCCAs) were exposed and looped with nylon monofilament. Another two sutures were cut through each side of the loops to remove them. All monofilaments and sutures were conducted through the skin and fixed on the posterior cervixes. The wounds were sutured after the operation. During surgery, the rat body temperature was monitored with a rectal probe and maintained at about 37 °C with heating pad and lamp until they recovered from the anesthesia. Two days after the surgery, global brain ischemia was produced by occluding the blood flow within the BCCAs by pulling the monofilaments around the BCCAs without anesthesia. An occlusion in a short period of 3 min was normally used as CIP, or a relatively long 8 min ischemia was used as ischemic insult, which is lethal for CA1 pyramidal neurons and usually results in delayed neuronal death. When the CIP was followed by an 8-min ischemic insult (the CIP + Ischemia group), the interval between them was 2 days. Global brain ischemia was considered to be successful in cases of animals in which the pupils were enlarged, the reflex to light disappeared, and the righting reflex disappeared during the four-vessel occlusion.

Rats in the sham group were subjected to all treatments for global brain ischemia except the occlusion of the BCCAs. In other words, the sham operation refers to removing monofilaments and sutures (RMS) without occluding the blood flow within the BCCAs.

Neuropathological evaluation

At the determined time points, rats were anesthetized with chloral hydrate and perfused through the ascending aorta with normal saline followed by 4 % paraformaldehyde. A 3-mm-thick brain slice including the bilateral dorsal hippocampus was excised coronally and fixed in 4 % paraformaldehyde. The slices were embedded in paraffin after post-fixation overnight with the same fixative, dehydration with alcohol, and transparence with xylene. The paraffin-embedded brain tissues were sectioned in 6 mm thickness. The sections were stained with thionin. The delayed neuronal death of pyramidal neurons in the CA1 hippocampus was evaluated under light microscope and scored by neuronal density (Kato et al. 1991; Kitagawa et al. 1990). The neuronal density of the hippocampal CA1 subfield was determined by counting the number of surviving pyramidal neurons with intact cell membrane, full nucleus, and clear nucleolus within 1 mm linear length of the CA1. The intact pyramidal cells were counted by investigators without any knowledge of group using an attached transparent graticule in the eye lens. The average numbers of pyramidal neurons in three areas of the CA1 hippocampus were calculated to establish the neuronal density value.

Preparation of the perfusate solution

The perfusate solution is artificial cerebrospinal fluid (ACSF) (pH 7.4), which was prepared according to methods described by Thümen et al. (2002), which contained 125 mM NaCl, 4 mM KCl, 2 mM ($CaCl_2 \cdot 2H_2O$), 1.14 mM ($MgSO_4 \cdot 7H_2O$), 1.29 mM KH_2PO_4, and 25 mM $NaHCO_3$. The ACSF was stored at 4 °C. Before using, 5.3 mg glucose was added in every 5 ml ACSF.

Brain microdialysis

After the recovery rate of the probe (MD-2200, BAS, USA) was tested in vitro before each microdialysis to normalize the concentration in the microdialysate for each rat, in vivo microdialysis was carried out in awake and freely moving animals. The detail of microdialysis protocol as shown in Fig. 1b was as follows. Three hours before the last treatment in each group, the probe was inserted into the guide cannula. After 2-h stabilization, samples of the microdialysate were collected at 4 °C using syringe pump and refrigerated fraction collector (MF-9096, BAS, USA). Samples were collected every 10 min for 1 h as baselines before the ischemia. During the initial 36 min from the onset of ischemia or the sham operation, samples were collected every 4 min and a total of nine samples were consecutively collected. Afterward, samples were collected every 10 min, and a total of 17 samples were consecutively collected over the following 170 min. The position of the probe was determined by neuropathological evaluation 7 days after the microdialysis. Only samples collected from rats, in which the probe was verified as being located in the CA1 hippocampus, were used for analysis.

High-performance liquid chromatography (HPLC)

Preparation of *O*-phthaldialdehyde (OPA): Five milligram OPA (Sigma Company, USA) was dissolved in 150 μl methanol, followed by adding 5 μl mercaptoethanol and 1.1 ml boric acid buffer (0.4 M, pH 9.6). The OPA solution was saved away from light at 4 °C and added 2 μl mercaptoethanol every day. The OPA solution was used not more than one week.

Preparation of the reference solution: The internal reference was norvaline (Nor, Sigma Company, USA) solution (250 μM). Solitary references of each amino acid solution were prepared in a concentration of 10 mM glutamate (Sigma Company, USA) and 5 mM aspartate (Sigma Company, USA), respectively. The solvent of all the reference solutions was filtered by 0.45 μm filter membrane. Each solution was subpackaged and kept at −80 °C for future use.

The concentrations of glutamate and aspartate in the microdialysate were measured by OPA-β-mercaptoethanol precolumn derivatization HPLC (G1311A, Agilent, USA) with fluorescence detection. Ten microliters of microdialysate, 2 μL Na_2CO_3 (0.1 M, pH 10.0), 10 μL OPA solution, and 2 μL Norvaline (internal standard, 250 μM) were mixed and reacted for 2 min at room temperature. Then a 24-μL aliquot of the reaction mixture was injected into the HPLC system. The separation was performed on a Dikma C18 column (5 μm, 150 × 4.6 mm) with the mobile phases of 0.1 M sodium acetate buffer (pH 6.3, including 0.5 % tetrahydrofuran, phase A) and methanol (phase B). The analysis was performed with linear gradient from A:B (100:0) to 35 % B within 6 min; 60 % B within 17 min; 80 % B within 18 min; then eluted with 100 % B for 5 min to elute other components. The flow rate was set to 1.0 mL/min. The excitation and emission wave lengths were 340 and 450 nm, respectively.

Statistical analysis

All data were presented as mean ± SD, and were tested by one-way ANOVA, combined with a LSD test as a multiple comparison method to test for differences between groups. $P < 0.05$ was considered to be significant.

Results

DHK inhibited the effect of CIP on the concentration of glutamate during the induction of brain ischemic tolerance

Glutamate, aspartate, and norvaline (internal reference) were separated clearly. The standard curves for glutamate and aspartate were shown in Fig. 2.

There were no significant differences in the baseline concentrations of glutamate among the groups except a slight increase in the baseline in DHK + CIP + Ischemia group and DHK + CIP group (Fig. 3). In the sham group, there was no obvious change in the concentration of glutamate pre-, during and post-sham operation. In the CIP group, an increase in glutamate concentration of 1.7 ± 0.2 fold of the basal level was observed after CIP for 3 min. In the DHK + CIP group, the maximum increase in glutamate concentration was 2.0 ± 0.3 fold of the basal level, which was similar to that of the CIP group. In the Ischemia group, the 8-min global brain ischemia induced a significant pulse of glutamate concentration, which began at the onset of the ischemia and rapidly reached the peak value of 7 ± 0.8 fold of the basal level at the end of ischemia. The increased concentration of glutamate returned to the baseline level at the time point of 4 min after reperfusion. In the CIP + Ischemia group, the peak value of glutamate concentration was 3.9 ± 0.4 fold of the basal level, which

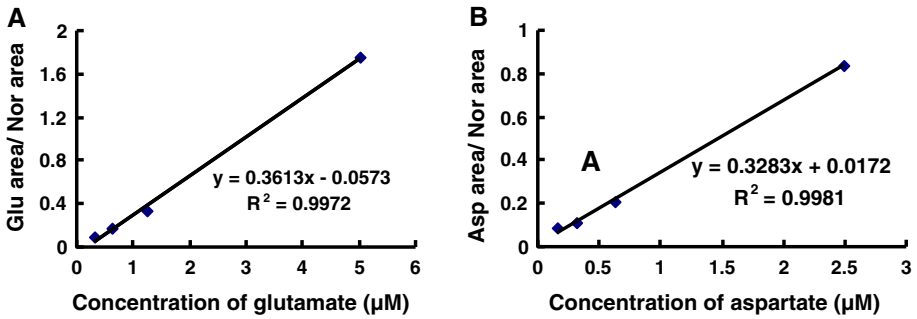

Fig. 2 The standard curves for glutamate (**a**) and aspartate (**b**). *X*-axis is the concentration of glutamate or aspartate, and *Y*-axis is the ratio of the area of glutamate or aspartate to the area of internal reference (norvaline) in the chromatogram. *Asp* aspartate, *Glu* glutamate, *Nor* norvaline. *R* is the regression coefficient. $R^2 > 0.99$ is acceptable

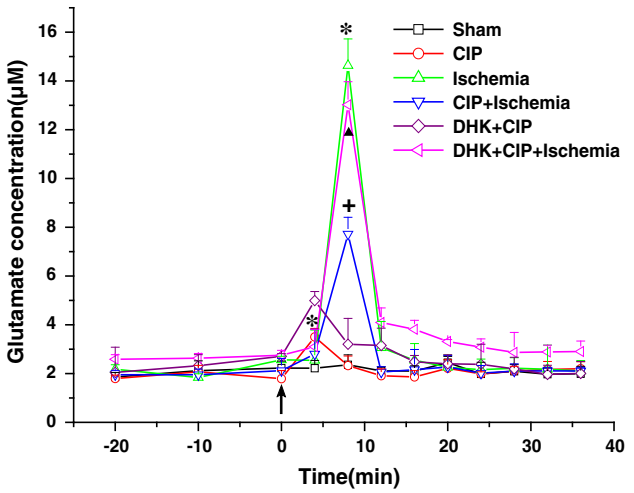

Fig. 3 Changes in the concentrations of glutamate in microdialysate of the CA1 hippocampus in each group. Abbreviations are the same as those in Fig. 1. The upward arrow indicates the onset of ischemia or corresponding time point in each group. $^{*}P < 0.05$ vs sham group, $^{+}P<0.05$ vs Ischemia group, $^{\blacktriangle}P < 0.05$ vs CIP + Ischemia group. The glutamate concentration increased immediately after insult ischemia for 8 min, and the increase was significantly inhibited by CIP in CIP + Ischemia group. The DHK pre-administration significantly blocked the inhibiting effect of CIP on extracellular glutamate concentration in DHK + CIP + Ischemia group

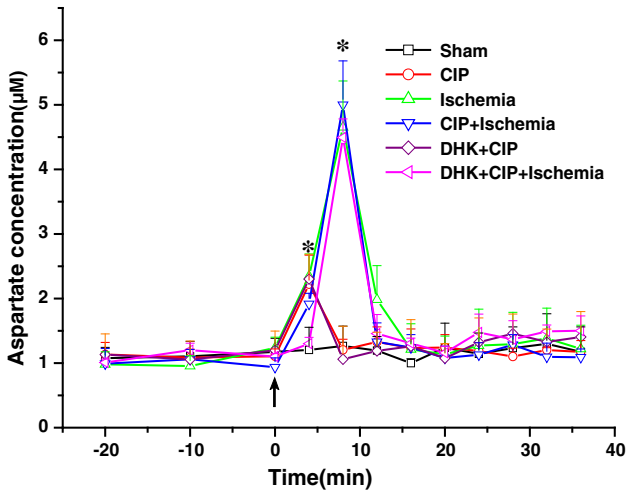

Fig. 4 Changes in the concentrations of aspartate in microdialysate of the CA1 hippocampus in each group. Abbreviations are the same as those in Fig. 1. The upward arrow indicates the onset of ischemia or corresponding time point in each group. $^{*}P < 0.05$ vs sham group. The aspartate concentration increased immediately after insult ischemia for 8 min. Compared with Ischemia group, neither CIP 2 days before the ischemic insult nor the administration of DHK had effects on the aspartate concentrations induced by ischemic insult

indicated that the increase in the glutamate concentration induced by global brain ischemia for 8 min was significantly inhibited by CIP. In the DHK + CIP + Ischemia group, the maximum increase in glutamate concentration was 6 ± 0.7 fold of the basal level, which indicated that pre-administration of DHK, a selective inhibitor of GLT-1, blocked the inhibitory effect of CIP on the increase in glutamate concentration induced by ischemic insult (Fig. 3).

DHK had no effect on the concentration of aspartate during the induction of brain ischemic tolerance

In the sham group, there was no obvious change in the concentration of aspartate pre-, during and post-sham operation (Fig. 4). The tendency of changes in concentration of aspartate in the CIP, DHK + CIP, and Ischemia groups was similar to that of glutamate in the corresponding group. The peak values of concentration of aspartate in the CIP, DHK + CIP, and Ischemia group were 2 ± 0.7, 2.2 ± 0.8, and 4.8 ± 1.0 fold of the basal level, respectively. However, in the CIP + Ischemia group, the peak value of concentration of aspartate was 5 ± 1.1 fold of the basal level, and no significant change was found compared with ischemia group. Furthermore, in the DHK + CIP + Ischemia group, the peak value of concentration of aspartate was 4.5 ± 0.5 fold of the basal level, and no significant change was found compared with CIP + Ischemia group. This result indicated that neither CIP nor the administration of DHK had effects on the aspartate concentration induced by ischemic insult.

DHK inhibited brain ischemic tolerance induced by CIP

Finally, we re-confirmed the inhibitory effect of DHK on brain ischemic tolerance induced by CIP in order to elucidate the significance of DHK on the glutamate concentration. In the hippocampal CA1 subfield of the sham group, the pyramidal neurons were healthy with normal morphology (Fig. 5a, b), the neuronal density was 212 ± 7.2 mm^{-1} (Fig. 5h). No significant neuronal damage was observed after CIP (Fig. 5c). Obvious neuronal death and loss were observed after the lethal ischemic insult for 8 min in the Ischemia group (Fig. 5d). Compared with sham group, the neuronal density was significantly decreased (Fig. 5h). In the CIP + Ischemia group, the neuronal death and loss normally induced by lethal ischemic insult were prevented by the CIP 2 days before the lethal ischemic insult (Fig. 5e). Compared with the CIP + Ischemia group, significant neuronal death and loss were observed in the DHK + CIP + Ischemia group (Fig. 5f), while no obvious neuronal damage was observed in the DHK + CIP group (Fig. 5g). These results indicated that CIP successfully induced brain ischemic tolerance and DHK could block the brain ischemic tolerance induced by CIP.

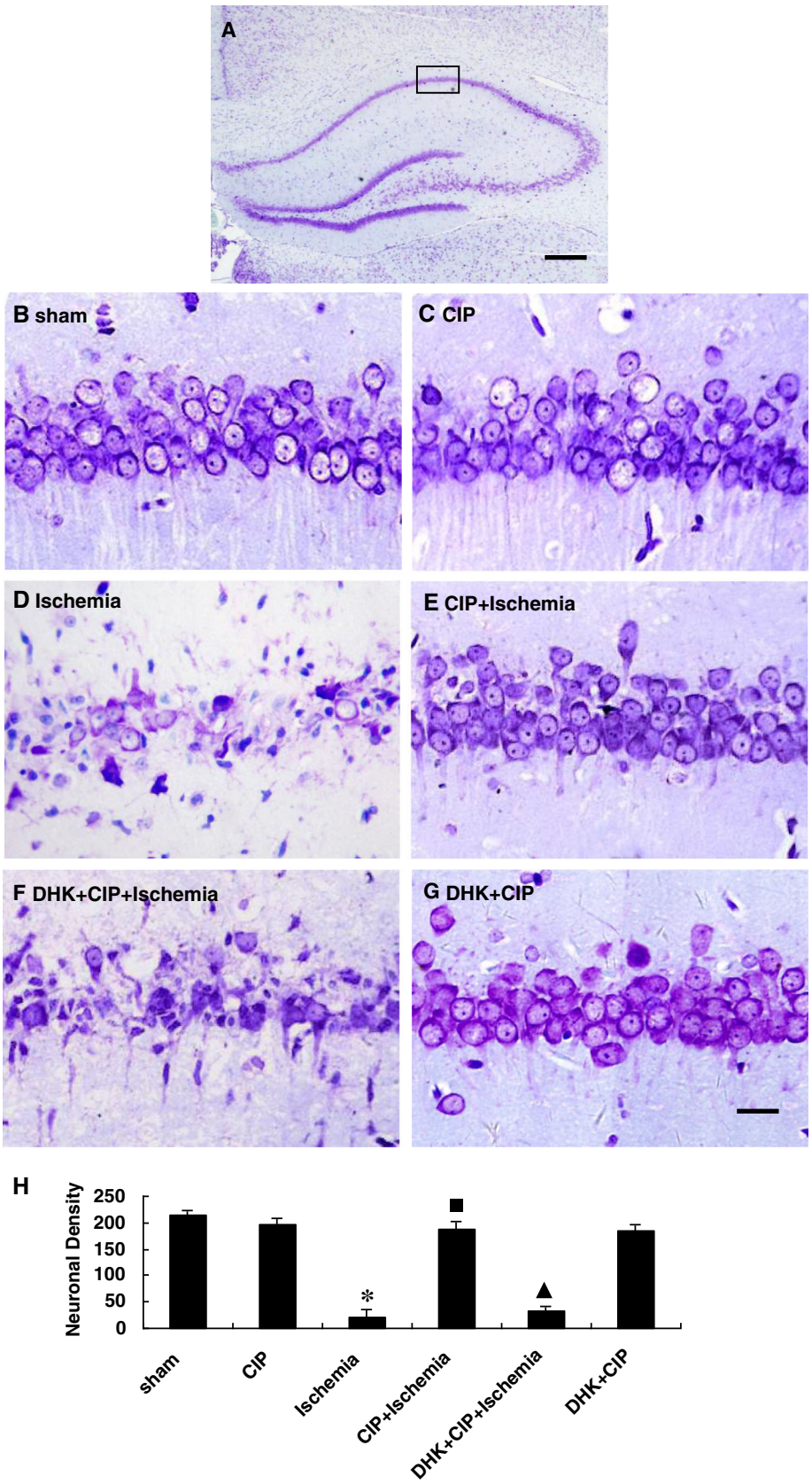

Fig. 5 Dihydrokainate inhibited the brain ischemic tolerance induced by CIP (thionin staining) (*Scale bar* is 400 μm in **a** and 20 μm in **g** for **b**–**g**). Abbreviations are the same as those in Fig. 1. $^{*}P < 0.05$ vs sham group, $^{\blacksquare}P < 0.05$ vs Ischemia group, $^{\blacktriangle}P < 0.05$ vs CIP + Ischemia group. Dihydrokainate inhibited the neuroprotective role of CIP against delayed neuronal death in the CA1 hippocampus normally induced by lethal brain ischemia. Figure 1 the graphical presentations of experimental protocol (**a**) and sample collection protocol of microdialysis in CA1 hippocampus (**b**). *CIP* cerebral ischemic preconditioning for 3 min, *DHK* dihydrokainate, *ES* embedding nylon monofilaments and sutures looping the bilateral common carotid arteries, *IC* implanting cannula(s), *NE* neuropathological evaluation, *Pre* samples were collected before ischemia, *RMS* removing monofilaments and sutures, *S* sample, *VAO* vertebral artery occlusion

Discussion

Glutamate is an important excitatory neurotransmitter in the central nervous system. After cerebral ischemia, the neurotoxicity induced by excessive glutamate is one of the major causes for neuronal injury (Benveniste et al. 1984; Hinzman et al. 2012; Romera et al. 2007). Cerebral microdialysis is a well-known method to study the concentration of extracellular glutamate (Choi et al. 2011; Douen et al. 2000; Lang et al. 2011; Mdzinarishvili et al. 2012; Yang et al. 2012). Therefore, we investigated the changes in the concentration of extracellular glutamate by cerebral microdialysis and HPLC to explore the alteration in the uptake activity of GLT-1 during the induction of brain ischemic tolerance by CIP, since the concentration of extracellular glutamate is the direct and objective index for evaluating glutamate excitotoxicity (Jellish et al. 2005; Ritz et al. 2004).

In the present study, lethal global brain ischemic insult for 8 min caused glutamate to increase to sevenfold of baseline. This characteristic is consistent with several reports which have shown that the extracellular concentration of excitatory amino acids significantly increases during cerebral ischemic insult (Rothstein et al. 1996; Namura et al. 2002). The increases may result from increased release, decreased uptake by astrocytes and neurons, decreased metabolism in astrocytes, and even reversal transport of glutamate by GLT-1 (Baker et al. 2002; Hinzman et al. 2012; Kosugi and Kawahara 2006; Mitani and Tanaka 2003; Nyitrai et al. 2006; Tauskela et al. 2012; Zhang et al. 2008). For example, Hinzman et al. (2012) observed that disruptions in calcium-mediated glutamate release and glial regulation of extracellular glutamate contribute to increased extracellular glutamate in the striatum 2 days after diffuse brain injury. Oxygen/glucose deprivation-induced reversal of GLT-1 is crucial to excitotoxic death of neurons, but contributes to the survival of astrocytes themselves (Kosugi and Kawahara 2006). Our previous study indicated that the GLT-1 expression was significantly down-regulated after lethal ischemic insult, which might cause a decrease in glutamate uptake (Zhang et al. 2007; Liu et al. 2011). Furthermore, the increase in the concentration of extracellular glutamate was followed by significant delayed neuronal death of pyramidal neurons in the hippocampal CA1 subfield after the lethal brain ischemia for 8 min. These results further confirmed the critical role of excitotoxicity of glutamate after lethal ischemic insult.

The CIP for 3 min 2 days before the lethal ischemic insult was shown to play a protective role on the pyramidal neurons in the CA1 hippocampus against delayed neuronal death normally induced by the lethal ischemic insult (Zhang et al. 2007). Herein we found that CIP significantly attenuated the increase in the concentration of glutamate normally evoked by lethal ischemic insult, and histological evaluation showed significant protection of CIP on pyramidal neurons of the CA1. Our previous study indicated that the down-regulation of GLT-1 protein induced by lethal brain ischemic insult was prevented by the CIP (Zhang et al. 2007). These findings further support that the protection of CIP may be due to the up-regulation of GLT-1. This observation coincided with previous reports that have shown the neuroprotective effect resulted from the increased uptake activity of GLT-1 (Jellish et al. 2005; Mimura et al. 2011; Ritz et al. 2004; Romera et al. 2007; Yang et al. 2012). For example, Yang et al. (2012) demonstrated that pre-ischemic treadmill training up-regulates GLT-1 expression, decreases extracellular glutamate concentration, reduces cerebral infarct volume, and improves neurobehavioral score in rats which suffered middle cerebral artery occlusion. The above data suggested that the protection of CIP on neurons may be through preventing the excitotoxicity of glutamate by regulating the uptake activity of GLT-1.

To clarify the above suggestion, we further investigated the effect of DHK on glutamate concentration, and found that DHK pre-administration inhibited the protection of CIP against the increase in extracellular glutamate concentration induced by lethal ischemic insult in DHK + CIP + Ischemia group. Because DHK is a well-known specific inhibitor of GLT-1 (Seki et al. 1999; Verma et al. 2010), our present study suggests that CIP by triggering GLT-1 expression could improve glutamate uptake activity of GLT-1 and preventing the over accumulation of glutamate, and then play the neuroprotective effect against brain ischemia. In the same lane, others have previously shown that the neuroprotective effect resulted from the increased uptake activity and/or the expression of GLT-1. For example, Beta-lactam antibiotics such as ceftriaxone confer neuroprotection in cerebral ischemia/reperfusion injury by up-regulation of GLT-1 protein expression and activity (Mimura et al. 2011; Rothstein et al. 2005; Verma et al. 2010). Preconditioning with sublethal forebrain ischemia or intermittent normobaric hyperoxia or normobaric hyperoxia induces ischemic tolerance and up-regulation the expression of glutamate transporters (Bigdeli et al. 2008, 2009).

It is well known that the concentration of glutamate in synaptic cleft is dependent on the glutamate release from the presynaptic terminals and the uptake by glial cells and neurons. Some studies showed that preconditioning with cortical spreading depression or 4-aminopyridine with bicuculline decreased glutamate release and led to tolerance to ischemia in brain or cultured neurons (Douen et al. 2000; Tauskela et al. 2008, 2012). Our previous study investigated the functional changes of GLT-1 during the

induction of the brain ischemic tolerance and found that CIP up-regulated the binding properties of GLT-1 including maximum binding and affinity for glutamate and glutamate uptake by L-^{3}H-glutamate assay (Liu et al. 2011). These findings indicate that up-regulation of GLT-1 uptake activity for glutamate is another mechanism to relieve the excitotoxicity of glutamate in the induction of brain ischemic tolerance in our experimental system. The present study further indicated that CIP significantly attenuated the increase in the concentration of glutamate normally evoked by lethal ischemic insult.

The glutamate concentration under DHK + CIP + Ischemia treatment is not greater than that under Ischemia alone treatment. The glutamate concentration after lethal ischemic insult alone may result from increased release, decreased uptake, and even reversal transport of glutamate by GLT-1. In the CIP + Ischemia group, CIP induced brain ischemic tolerance, and significantly attenuated the increase in the concentration of glutamate after the lethal ischemic insult. We presume that the reason may be related to the decreased release, increased uptake, and without reversal transport. On this basis, DHK administration inhibited the uptake ability of GLT-1, without altering the release. So, the glutamate concentration under DHK + CIP + Ischemia treatment is not greater than that of Ischemia group. The glutamate concentration under DHK + CIP treatment is greater than that under CIP alone treatment. In the CIP group, an increase in glutamate concentration of 1.7 ± 0.2 fold of the basal level was observed after CIP for 3 min. We presume the increase after CIP alone maybe related to increased release, and the partial decrease in glutamate uptake. In the DHK + CIP group, DHK inhibited the rest uptake activity of GLT-1. Therefore, the glutamate concentration in the DHK + CIP group is higher than that of the CIP group.

Aspartate is another excitatory neurotransmitter and activates some glutamate receptors (McDonald and Johnston 1990). In the present study, the changes in aspartate concentrations in the sham, CIP, and Ischemia groups were similar to those of glutamate in the corresponding group and coincidence with other report (Dohmen et al. 2005). However, CIP had no effect on the increase in the aspartate concentration induced by lethal ischemic insult. Administration of DHK also had no effect on the concentration of aspartate. It has been reported that aspartate is neither a substrate for cloned vesicular transporters that package glutamate into vesicles (Bellocchio et al. 2000) nor is it a substrate for the vesicular uptake measured in brain tissue (Naito and Ueda 1985), which could explain the low affinity to GLT-1 and lack of effect.

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Conflict of interest The authors declare no conflict of interest.

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