The neuroprotective effect of propofol against brain ischemia mediated by the glutamatergic signaling pathway in rats

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Abstract Several mechanisms are involved in the neuroprotection of propofol against ischemia, but influences of propofol on the binding properties of glutamate receptors and the uptake of glutamate in brain ischemia are not known. The present study was undertaken to investigate these issues in rat global brain ischemic model using methods of neuropathological evaluation, radioligand binding assay with and uptake test for L-^{3}H-glutamate. It was shown that propofol used in anesthetic doses protected pyramidal neurons in the hippocampal CA1 subfield against delayed neuronal death normally induced by global brain ischemia. Simultaneously, the propofol decreased the value of maximal number of binding sites (Bmax), increased the value of equilibrium dissociation constant (Kd), and increased the glutamate uptake in the CA1 subfield. These findings indicate that it is, at least partly, via modulating the binding properties of glutamate receptors and the uptake of glutamate that propofol protects neurons against ischemic injury.

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Introduction

Much attention has been paid recently to the neuroprotective effect of propofol, a widely used clinic sedative and anesthetic. In vitro studies, propofol can protect cultured neurons against oxidative stress [1–3] or oxygen glucose deprivation exposure [4]. In vivo studies, propofol can also protect rat brain against ischemia [5–11]. Propofol is an agonist of Gamma aminobutyric acid (GABA$_A$) receptors [12]. Activation of the GABA$_A$ receptors leads to a variety of changes of neurons such as hyperpolarization of cellular membrane [10], calcium influx inhibition [13], etc., which benefit the survival of neurons subjected to ischemic insult, and contribute to the neuroprotective effects of propofol.

Glutamate is the most essential excitatory neurotransmitter in the brain and plays an important role in the maintenance of many brain functions. However, glutamate often over-accumulates in the extracellular fluid in brain ischemia. The over-accumulation of glutamate leads to over-activation of glutamate receptors, and then leads to a variety of subsequent cellular events such as calcium overload [14], cytotoxic edema [15], un-controlled activation of protein kinase, phospholipase or nitric oxide synthase [16], which are the main causes of neuronal degeneration and death after cerebral ischemia. Therefore, the over-accumulated glutamate is called an excitotoxin. In light of the important role of over-accumulated glutamate in ischemic neuronal injury, this study was devised to test the hypothesis that propofol protects neurons via modulating the binding properties of glutamate receptors and the uptake of glutamate in rats with cerebral ischemia.

Experimental procedure

Animal and grouping

Wistar rats weighing 280–320 g provided by The Experimental Animal Center of Hebei Medical University were used. Animal care and use followed guidelines for care and use of laboratory animals [17], and all efforts were made to minimize animal suffering and reduce the number of animals used in the present study.

Neuronal protection of propofol against global brain ischemia

Thirty male rats were randomly divided into the following three groups (n = 5 in each group or subgroup):

Sham group: Rats were subjected to a sham operation for global brain ischemia

Global brain ischemia group: Rats were exposed to global brain ischemic insult for 8 min

Propofol + global brain ischemia group: Rats were pretreated with propofol 3 h before global brain ischemia, when the animals had already woken from the anesthesia of propofol. In our preliminary experiments, it was shown that propofol administrated 3 h before a global ischemia could effectively protect the pyramidal neurons in the CA1 subfield of the hippocampus against global ischemic insult. To observe dose-dependency of propofol, this group was further divided into four sub-groups of 40, 50, 60 and 90 mg/kg, respectively. Propofol was administrated intravenously via the tail vein with a serial sequence consisting of an initial bolus (1/3 of the total dose) injected for 15 min and persistent infusion of the rest of the dose with a micro-pump for 1 h. The rats in each group were sacrificed on the seventh day after the cerebral ischemia for neuropathological evaluation.

Effects of propofol on the binding properties of glutamate receptors and the glutamate uptake in the brain

One hundred and ten male Wistar rats were randomly divided into four groups of Sham, global brain ischemia, propofol control (propofol + sham) and propofol + global brain ischemia groups. The treatments in each group were the same as those in above part, but only the effect of propofol at the largest dose of 90 mg/kg was examined. The measurements of the binding properties of glutamate receptors and the glutamate uptake were performed at time points of 0, 1 and 3 h after the re-perfusion of the brain in global ischemia group and propofol + global ischemia group. In order to correspond to the time points mentioned above, the measurements were performed at time points of

48, 49 and 51 h after sham operation in propofol control group, while the measurements were performed only 48 h after sham operation in sham group. Eleven rats were included in each group or subgroup, six of them were used for measuring the binding properties of glutamate receptors, and five for measuring the glutamate uptake in the brain.

Global brain ischemia

Global brain ischemia was produced by using the four vessels occlusion method [18]. First, the bilateral vertebral arteries were electrocauterized after chloral hydrate anesthesia (350 mg/kg) administrated through intraperitoneal injection. An incision of 1.5 cm in length was made on the skin directly overlying the first cervical vertebra on the dorsal side of the neck behind the occipital bone. The paraspinal muscles were separated from the middle, and the bilateral alar foramina of the first cervical vertebra were exposed. An electrocautery needle burned in advance was inserted into the alar foramen, and the bilateral vertebral arteries were electrocauterized permanently. Then, the bilateral common carotid arteries of the rats were exposed and snared with a silk suture which was snared by another silk suture conducted by a needle through the skin and knotted together on the ventral and posterior cervixes. The wound was sutured after the surgery. During the period of surgery, the body temperature of the rats was monitored with a rectal probe and maintained at about 37°C with a heating pad and head lamp until they recovered from the anesthesia, and then they were returned to cages, with each of them in a single cage.

Forty-eight hours after the surgery, global brain ischemia of rats was produced by tightening the silk sutures for 8 min under condition of no anesthesia. Changes in consciousness, righting reflex and diameter of pupils of the rats were observed to determine if brain ischemia was induced. Only the animals which lost their consciousness, righting reflex and their pupils dilated obviously after the tightening, were selected for further experiments. The brain was re-perfused by loosening and removing the silk sutures.

The rats in Sham group were subjected to a sham operation of the global brain ischemia, which consisted of all the surgical procedures except tightening the sutures snared on the common carotid arteries.

Neuropathological evaluation

Rats were sacrificed by decapitation at the determined time point and the brain was removed and fixed in 4% paraformaldehyde. Two days later, a 3 mm-thick brain slice including the bilateral dorsal hippocampus was excised coronally behind the optical chiasm, and embedded in

paraffin after dehydration with alcohol and xylene. Brain sections (6 μm thick) were mounted and stained with thionin. The delayed neuronal death (DND) in the hippocampal CA1 subfield was evaluated under a light microscope and scored by histological grade (HG) and neuronal density (ND) [19, 20]. HG was divided into four grades: 0: no neuron death; I: scattered single neuron death; II: mass neuron death; III: almost all neuron death. The ND 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 average number of pyramidal neurons in six areas of bilateral hippocampal CA1 subfield was calculated to establish the ND value.

Membrane binding assay

The experimental procedures were similar to the methods described by Meeker et al. [21].

Preparation of crude membrane of hippocampal CA1 subfield

Rats were sacrificed by decapitation at scheduled time points, and the brain was removed. The hippocampal CA1 subfield was quickly dissected out at 0–4°C normal saline from the brain and then stored at −80°C for the later assay.

The frozen hippocampal tissues were re-warmed at room temperature on the day of the assay. Two hippocampal tissues of two different rats were combined and homogenized using a homogenizer (the speed was regulated to 2,000 r/min for 1–2 min) in 1 mL of ice-cold Hepes buffer (containing 50 mmol/L of Hepes, 96 mmol/L of NaCl, 1.2 mmol/L of $CaCl_2$, 1 mmol/L of $MgCl_2$, 0.32 mol/L of sucrose, PH value is 7.35–7.45 at 4°C). The homogenate was centrifuged for 10 min at 1,000g at 4°C. The supernatant was collected carefully and centrifuged for 25 min at 25,000g again. The resultant pellet was re-suspended in Hepes buffer (containing 50 mmol/L of Hepes, 96 mmol/L of NaCl, 1.2 mmol/L of $CaCl_2$, 1 mmol/L of $MgCl_2$, PH value is 7.35–7.45 at 4°C) and centrifuged for 25 min at 25,000g for three times to reduce non-specific binding. The final pellet was re-suspended in 400 μL Hepes buffer to give a protein concentration of approximately 2 mg/mL, as determined by Coomassie Brilliant Blue G250. The whole process of the operation was done under the condition of 4°C.

### Procedures of radioligand binding assay

The specimen of the membrane (50 μL; final concentration of protein of 1 mg/mL) and L-^{3}H-glutamate (80 nmol/L; volume of 20, 40, 60, 120, 240 and 320 μL,

respectively; final concentration ranging from 3.2 to 51.2 nmol/L) were incubated in a total volume 500 μL Hepes buffer for 90 min at 4°C to determine the total binding. Non-specific binding was determined by incubating them in the presence of 25 μL of glutamate (1 mmol/L). At the end of incubation, the reaction was terminated by ice-bath and rapid vacuum filtration through glass fibre filter (Whatman GF/B). The filters were washed three times with 5 mL ice-cold Hepes buffer in order to remove free L-^{3}H-glutamate. The filters were dried at 80°C for 30 min. Then the filters were transferred to liquid scintillation vials, and 5 mL cocktail (1.4-Di-[2,(5′-phenyloxazol)]-benene] POPOP 0.2 g, 2.5-Diphenyloxazole PPO 2 g, 500 mL xylene) was added in, and the count per minute (cpm) of each vial was measured with a scintillation spectrometer (262 state-operated workshop, China). The specific binding was obtained by subtracting the non-specific binding from the total binding. In addition, the same volume L-^{3}H-glutamate solution was dripped on the blank filter to calculate the total radioactivity. The saturation curve fitting and regression of scatchard plot were done by using the Prism 4.0 software. The binding properties of the glutamate receptors were evaluated with the values of Bmax and Kd, which represented the number and affinity of the receptors respectively, and were calculated using Prism 4.0 software.

Glutamate uptake

The measure of glutamate uptake was similar to the previous reports [22–27]. In brief, rats were sacrificed by decapitation and the brain was removed. The hippocampal CA1 subfield was quickly dissected from the hippocampus and put into ice-cold D-Hanks solution (Hanks free of Ca^{2+}) containing 0.25% trypsin, and then cut into small pieces of 1 mm^3 for digestion. After 10 min of digestion at 37°C, the samples were transferred to Hanks solution via a measuring pipette to terminate the digestion, and sucked up and down 40–60 times with the transfer pipettor, and then kept standing for 10 min. The supernatant was collected and centrifuged at 1,000 rpm for 10 min at 4°C. Then, the supernatant was discarded, and the cell pellets were suspended in 250 μL Hanks solution for further uptake analysis. The protein concentration of the cells was determined by the Coomassie assay. After protein concentration analysis, the samples were divided into four parts, of which two were used for total glutamate uptake analysis, and the others were for non-specific glutamate uptake. Total glutamate uptake was determined by incubating the cells (in 50 μg protein content) with L-^{3}H-glutamate solution (80 nmol/L 100 μL, GE Healthcare, Batch 67) at 37°C for 15 min. Non-specific glutamate uptake was determined by incubating the cells with L-^{3}H-glutamate

Fig. 1 The neuroprotective effect of propofol against delay neuronal death (DND) induced by global brain ischemia. The *upper* are representative thionin-stained photomicrographs (*Scale bar* = 25 μm). The *lower dot and bar graphs* are quantitative presentation of the neuroprotective effect of propofol with histological grade and neuronal density. Each *dot* represents one animal assigned to that grade. *$P < 0.05$ versus sham group. #$P < 0.05$ versus ischemia group. It could be found that DND of pyramidal neurons in the hippocampal CA1 subfield induced by brain ischemia could be effectively and dose-dependently prevented by propofol in doses no *lower* than 50 mg kg^{-1}
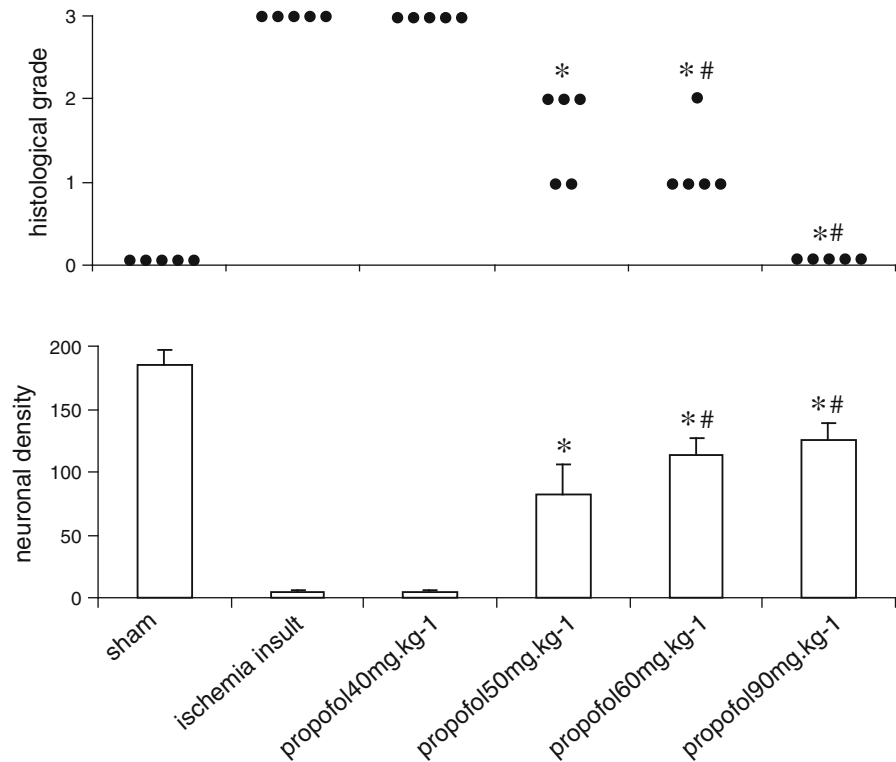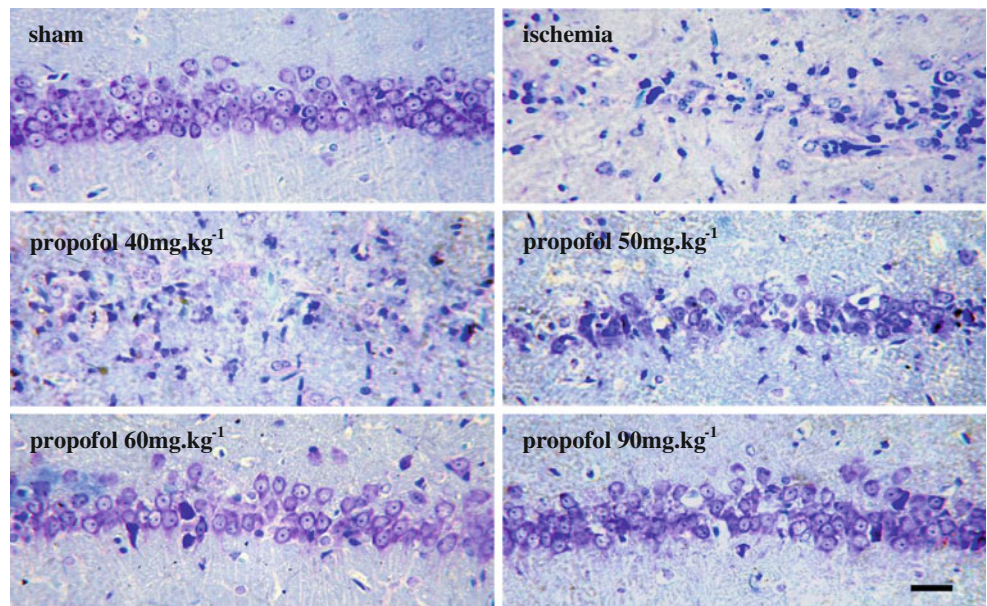
solution in the same concentration as above and glutamate (100 μmol/L 80 μL). The reaction was terminated by ice-bath and the samples were centrifuged at 1,000 rpm for 10 min. The cell pellets were suspended in 1 mL of Hanks solution and were centrifuged at 1,000 rpm for 10 min again. Then 100 μL of NaOH (0.3 M) were added to the cell pellets for cell schizolysis. After 20 min, this final 100 μL of solution was added onto a Whatman (UK) filter,

which was transferred to a vial containing 5 mL of scintillation mixture (500 mL of xylene mixed with 0.2 g of POPOP and 2 g of PPO, Solarbio, Beijing, China). Cpm measured by a liquid scintillation counter (262 state-operated workshop, China) was used to reflect glutamate uptake. The glutamate uptake was obtained by subtracting the non-specific glutamate uptake from the total glutamate uptake.

Fig. 2 A sample of the saturation binding (**a**) and scatchard plot (**b**) of ^{3}H-Glutamate to the hippocampal CA1 subfield cellular membranes in sham group. Abbreviations: *TB* total binding, *SB* specific binding, *NSB* non-specific binding, *glu* glutamate, *cpm* count per minute. The SB is obtained by subtracting the NSB from the TB. Data are presented in mean ± SD (n = 6)
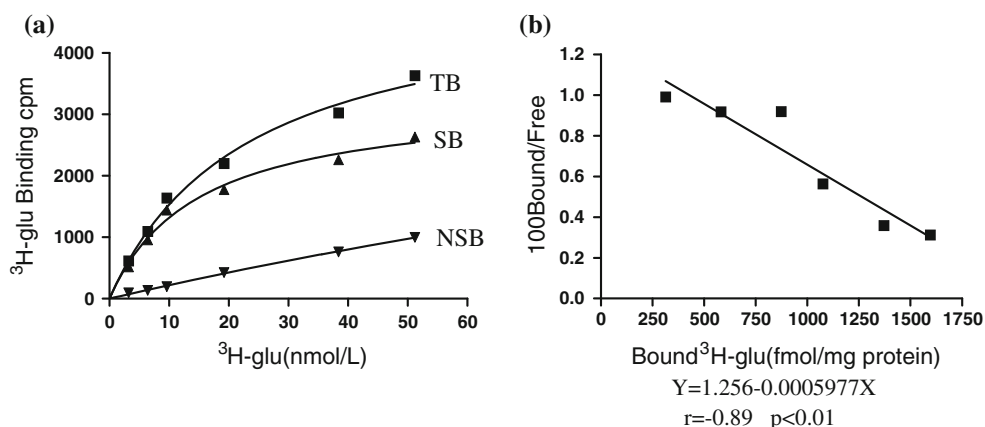
Statistical analysis

Statistical analysis was performed by Kruskal–Wallis analysis of variance (ANOVA) on ranks together with Dunn's test as multiple comparisons in HG between groups. Other data were expressed as mean ± SD (standard deviation) and assessed by one-way ANOVA combined with Students-Newman-Keuls test as multiple comparison method. *P* values <0.05 were considered statistically significant.

Results

Propofol protected the pyramidal neurons in the hippocampal CA1 subfield against global ischemia

As shown in Fig. 1, pyramidal neurons in the hippocampal CA1 subfield in sham group were orderly arranged in 2–3 cell layers. The outline of the neurons was intact, nucleus was full and nucleolus was clear. The HG of all the rats was 0 and the ND was 184.5 ± 11.1. In ischemia group, almost all pyramidal neurons in the hippocampal CA1 subfield died. The HG significantly increased and the ND significantly decreased compared with the sham group. In propofol treatment groups (except 40 mg/kg), most of the pyramidal neurons in the hippocampal CA1 subfield survived, the HG was decreased and the ND was increased in a propofol dose-dependent manner. The dose was too low in the subgroup of 40 mg/kg to show any neuroprotective effect. The results indicated that propofol used in anesthetic doses could protect pyramidal neurons of the hippocampal CA1 subfield against global brain ischemia.

Propofol decreased the binding of glutamate to its receptors in the hippocampal CA1 subfield during global ischemia

Saturation of the binding

Figure 2 shows a sample of the saturation binding curve and scatchard plot of L-^{3}H-Glutamate binding to the hippocampal CA1 subfield cellular membranes in sham group. The non-specific binding increased linearly with the increase of the concentration of L-^{3}H-glutamate. Although the total binding curve was characterized by a tendency of saturation, it did not reach saturation at the examined concentrations of L-^{3}H-glutamate. The specific binding showed a clear curve approaching saturation.

Changes in the value of Bmax

The value of Bmax of glutamate receptors increased significantly after the global brain ischemia compared with the sham group. This increase began 1 h after the brain re-perfusion, and peaked at the third hour during the 3 h we observed (Fig. 3a). Bmax was down-regulated in propofol alone or propofol + global brain ischemia group compared with sham or ischemic group, respectively. This response was most obvious at the first hour and return to the sham level at the third hour of the re-perfusion (Fig. 3a).

Changes in the value of Kd

Kd has no significant changes after the global brain ischemic insult in each time point observed. Propofol treatment increased Kd at the time point of 1 h and the response returned to sham level at time point of 3 h (Fig. 3b).
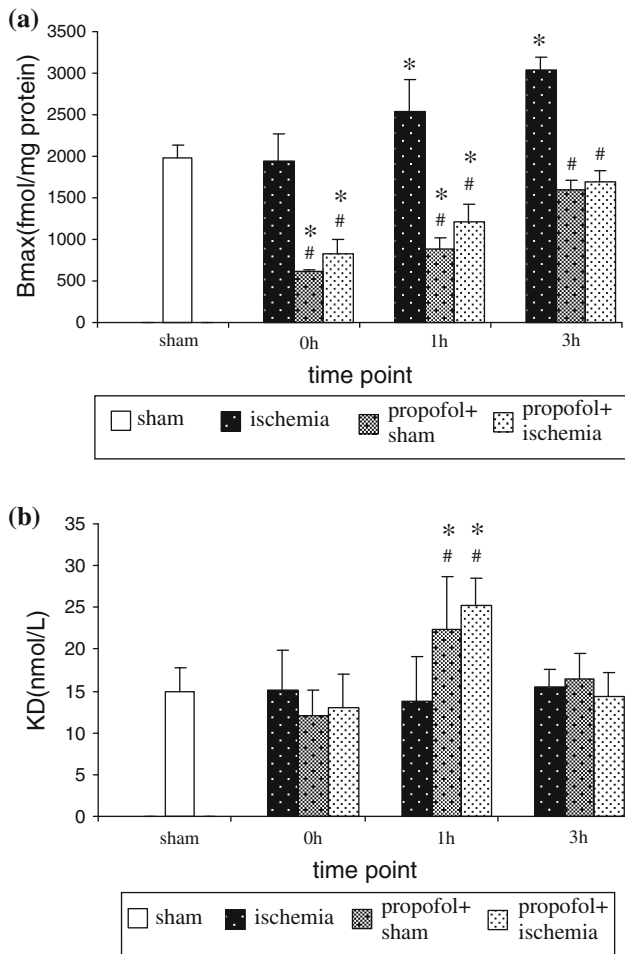
(a)

(b)

Fig. 3 Changes in the value of Bmax (**a**) and Kd (**b**) of glutamate receptors, which indicates the amount and the affinity of glutamate receptors bound to glutamate, respectively, in the hippocampal CA1 subfield after propofol infusion assayed with radio-ligand binding assay. Data are presented in mean ± SD (n = 6 in each group). *$P < 0.05$ versus sham group; #$P < 0.05$ versus ischemia groups at the same time point. It is shown in (**a**) that the amount of glutamate receptors increased at 1 and 3 h after the cerebral ischemic insult compared with that in sham group, while propofol infusion significantly reduced the amount either in propofol control (propofol + sham) or propofol + ischemia group. In (**b**), it is shown that the affinity of glutamate receptors was significantly reduced by propofol infusion both in propofol control (propofol + sham) and propofol + ischemia groups at 1 h after cerebral ischemic insult compared with sham and ischemia groups, respectively

Propofol increased the uptake of glutamate in the hippocampal CA1 subfield during global brain ischemia

Global brain ischemia had no effect on the glutamate uptake during the observed period from the time point of 0–3 h after re-perfusion of the brain. However, the glutamate uptake increased at the first hour and returned to the sham level at the third hour after propofol treatment in both
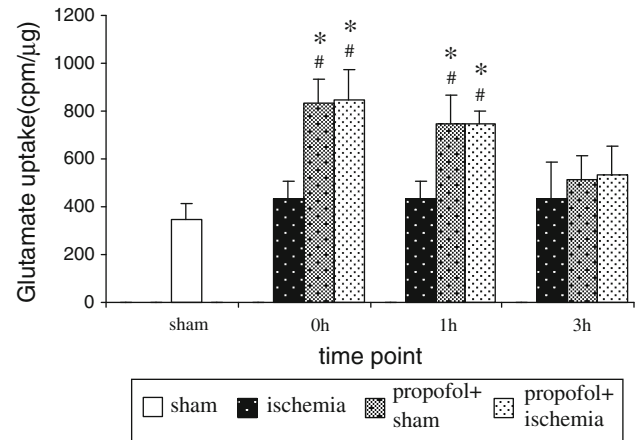

Fig. 4 Changes in glutamate uptake of hippocampus CA1 subfield. Data are presented in mean ± SD (n = 5 in each group). *$P < 0.05$ versus sham group; #$P < 0.05$ versus ischemia groups at the same time point. It is showed that the glutamate uptake was significantly increased by propofol infusion in propofol control (propofol + sham) and propofol + ischemia groups at 0 and 1 h after cerebral ischemic insult compared with sham and ischemia groups, respectively, at the same time point

of the propofol alone group and the propofol + global brain ischemia group (Fig. 4).

Discussion

The aim of the present study is to test the hypothesis that the neuroprotection of propofol against cerebral ischemia is, at least partly, via modulating the binding properties of glutamate receptors and the uptake of glutamate. Firstly, we found that pre-treatment with propofol at anesthetic doses attenuated the DND of pyramidal neurons in the rat hippocampal CA1 subfield induced by global brain ischemia, confirming the neuronal protection of propofol, which are supported by the previous reports either in vitro [1–4] or in vivo [5–11].

Propofol is thought to be an agonist of GABA$_A$ receptor. Many studies have shown a variety of the mechanisms involved in the neuronal protection of propofol against brain ischemia, such as reducing the cerebral metabolic rate of oxygen [28, 29], clearing away oxygen free radicals and lipid peroxide [30, 31], reducing the release of glutamate [32, 33] and so on. In the present study, we first provide evidence that propofol can exert neuronal protection against global brain ischemia in vivo by modulating the binding properties of glutamate receptors and the uptake of glutamate.

First, we found that the number of glutamate receptors bound by glutamate reflected by the value of Bmax in hippocampal CA1 subfield increased significantly at all the time points observed after global brain ischemia, although

the affinity of glutamate receptors reflected by the value of Kd did not change. Global ischemia-induced increase in the extracellular fluid glutamate has been demonstrated by many studies [34–36]. This may be responsible for the increase in the number of the glutamate receptors, although detailed mechanisms involved in them remain to be clarified. The increased number of glutamate receptors and glutamate accumulation in the extracellular fluid induced by global ischemia cause excessive opening of receptor channels, plenty of calcium influx and then neuronal injuries even DND.

Second, we found that infusion of propofol down-regulated the number of glutamate receptors bound compared with sham or global brain ischemic rats at all the time points observed. Moreover, the infusion of propofol also down-regulated the affinity of the glutamate receptors reflected by the increase in the value of Kd at the time point of 1 h observed. These findings indicated that propofol down-regulated the binding properties of glutamate receptors, which leads to a decrease of the binding and activation of glutamate receptors by glutamate, and then might decrease the subsequent events such as over opening of calcium channel, over-influx of calcium and the neuronal injuries resulting from the over-load of calcium. While it is hard to speculate which receptor subtype plays the main role in the glutamate binding properties, since only the overall changes in the binding of glutamate receptors were examined in the current study. Our future study will focus on clarifying the subtype using receptor subtype specific ligands. Furthermore, some studies have shown that the propofol binding site is GABA$_A$ receptors rather than glutamate receptors [12]. Thus, we presume that propofol modulating the binding properties of glutamate receptors in hippocampal CA1 subfield may depend on "cross talk" mechanisms between different kinds of receptors [37]. Considerable evidences show that the second-messenger systems or direct protein–protein interaction may mediate the "cross-talk" between these receptor systems [38].

Finally, we found that propofol increased the glutamate uptake in global brain ischemic rats. The effect of propofol on glutamate uptake has been paid attention to for the mechanisms underlying the neuroprotection of propofol against brain ischemia [1–4]. However, only in vitro models such as co-cultured neurons and glial cells are investigated in these studies. The present study first confirmed that propofol indeed increased the glutamate uptake in global brain ischemic rats. This increase was especially obvious in the early stage of time points 0 h and 1 h observed after re-perfusion of brain. The time course of the changes in the capacity of glutamate uptake was similar to that in the binding properties of glutamate receptors. Studies have shown that the glial glutamate transporter-1

plays a predominant role in removing the extra-cellular glutamate [39, 40]. GABA$_A$ receptors, which can be activated by propofol, are distributed widely on glial cells throughout the central nervous system [41, 42]. Thus, we presume that modulating glutamate uptake of glial cells might be an important mechanism in the neuroprotection of propofol against brain ischemia.

In conclusion, propofol played a neuroprotective role against global brain ischemia in vivo, at least partly, by modulating the binding properties of glutamate receptors and the uptake of glutamate.

References

1. Sitar SM, Hanifi Moghaddam P, Gelb A et al (1999) Propofol prevents peroxide-induced inhibition of glutamate transport in cultured astrocytes. Anesthesiology 90:1446–1453
2. Peters CE, Korcok J, Gelb AW et al (2001) Anesthetic concentrations of propofol protect against oxidative stress in primary astrocyte cultures: comparison with hypothermia. Anesthesiology 94:313–321
3. Daskalopoulos R, Korcok J, Farhangkhgoee P et al (2001) Propofol protection of sodium-hydrogen exchange activity sustains glutamate uptake during oxidative stress. Anesth Analg 93:1199–1204
4. Velly LJ, Guillet BA, Masmejean FM et al (2003) Neuroprotective effects of propofol in a model of ischemic cortical cell cultures: role of glutamate and its transporters. Anesthesiology 99:368–375
5. Wang J, Yang X, Camporesi CV et al (2002) Propofol reduces infarct size and striatal dopamine accumulation following transient middle cerebral artery occlusion: a microdialysis study. Eur J Pharmacol 452:303–308
6. Bayona NA, Gelb AW, Jiang Z et al (2004) Propofol neuroprotection in cerebral ischemia and its effects on low-molecular-weight antioxidants and skilled motor tasks. Anesthesiology 100:1151–1159
7. Gelb AW, Bayona NA, Wilson JX et al (2002) Propofol anesthesia compared to awake reduces infarct size in rats. Anesthesiology 96:1183–1190
8. Adembri C, Venturi L, Tani A et al (2006) Neuroprotective effects of propofol in models of cerebral ischemia: inhibition of mitochondrial swelling as a possible mechanism. Anesthesiology 104:80–89
9. Ergün R, Akdemir G, Sen S et al (2002) Neuroprotective effects of propofol following global cerebral ischemia in rats. Neurosurg Rev 25:95–98
10. Ito H, Watanabe Y, Isshiki A et al (1999) Neuroprotective properties of propofol and midazolam, but not pentobarbital, on neuronal damage induced by forebrain ischemia, based on the GABA$_A$ receptors. Acta Anaesthesiol Scand 43:153–162
11. Yano T, Nakayama R, Ushijima K (2000) Intracerebroventricular propofol is neuroprotective against transient global ischemia in rats: extracellular glutamate level is not a major determinant. Brain Res 883:69–76

12. Orser BA, Wang LY, Pennefather PS et al (1994) Propofol modulates activation and desensitization of GABAA receptors in cultured murine hippocampal neurons. J Neurosci 14:7747–7760

13. Lingamaneni R, Hemmings HC Jr (2003) Differential interaction of anaesthetics and antiepileptic drugs with neuronal Na + channels, Ca2 + channels, and GABA(A) receptors. Br J Anaesth 90:199–211

14. Benveniste H, Jørgensen MB, Diemer NH et al (1988) Calcium accumulation by glutamate receptor activation is involved in hippocampal cell damage after ischemia. Acta Neurol Scand 78:529–536

15. Shinohara Y, Yamamoto M, Haida M et al (1990) Effect of glutamate and its antagonist on shift of water from extra- to intracellular space after cerebral ischaemia. Acta Neurochir Suppl (Wien) 51:198–200

16. Szydlowska K, Tymianski M (2010) Calcium, ischemia and excitotoxicity. Cell Calcium 47:122–129

17. Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109–110

18. Pulsinelli WA, Brierley JB (1979) A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 10:267–272

19. Kitagawa K, Matsumoto M, Tagaya M et al (1990) 'Ischemic tolerance' phenomenon found in the brain. Brain Res 528:21–24

20. Kato H, Liu Y, Araki T et al (1991) Temporal profile of the effects of pretreatment with brief cerebral ischemia on the neuronal damage following secondary ischemic insult in the gerbil: cumulative damage and protective effects. Brain Res 553:238–242

21. Meeker RB, Greenwood RS, Hayward JN (1994) Glutamate receptors in the rat hypothalamus and pituitary. Endocrinology 134:621–629

22. Vesce S, Bezzi P, Rossi D et al (1997) HIV-1 gp120 glycoprotein astrocyte control of extracellular glutamate by both inhibiting the uptake and stimulating the release of the amino acid. FEBS lett 411:107–109

23. Tasca CI, Santos TG, Tavares RG et al (2004) Guanine derivatives modulate l- glutamate uptake into rat brain synaptic vesicles. Neurochem Int 44:423–431

24. dos Santos AQ, Nardin P, Funchal C et al (2006) Resveratrol increases glutamate uptake and glutamine synthetase activity in C6 glioma cells. Arch Biochem Biophys 453:161–167

25. Sakai T, Yoshitoshi T, Nagai Y et al (2006) Increased glutamate uptake and GLAST expression by cyclic AMP in retinal glial cells. Graefes Arch Clin Exp Ophthalmol 244:359–363

26. Moretto MB, Thomazi AP, Godinho G et al (2007) Ebselen and dilrg- anyl chalcogenides decrease in vitro glutamate uptake by rat brain slices: Prevention by DTT and GSH. Toxicol In Vitro 21:639–645

27. Fernández-Tomé P, Brera B, Arévalo MA et al (2004) β-Amyloid25–35 inhibits glutamate uptake in cultured neurons and astrocytes: modulation of uptake as a survival mechanism. Neurobiol Dis 15:580–589

28. Oshima T, Karasawa F, Satoh T (2002) Effects of propofol on cerebral blood flow and the metabolic rate of oxygen in humans. Acta Anaesthesiol Scand 46:831–835

29. Newman MF, Murkin JM, Roach G et al (1995) Cerebral physiologic effects of burst suppression doses of propofol during nonpulsatile cardiopulmonary bypass. Anesth Analg 81:452–457

30. De La Cruz JP, Sedeño G, Carmona JA et al (1998) The in vitro effects of propofol on tissular oxidative stress in the rat. Anesth Analg 87:1141–1146

31. Grasshoff C, Gillessen T (2002) The effect of propofol on increased superoxide concentration in cultured rat cerebrocortical neurons after stimulation of N-methyl-D-aspartate receptors. Anesth Analg 95:920–922

32. Lingamaneni R, Birch ML, Hemmings HC Jr (2001) Widespread inhibition of sodium channel-dependent glutamate release from isolated nerve terminals by isoflurane and propofol. Anesthesiology 95:1460–1466

33. Ratnakumari L, Hemmings HC Jr (1997) Effects of propofol on sodium channel-dependent sodium influx and glutamate release in rat cerebrocortical synaptosomes. Anesthesiology 86:428–439

34. Benveniste H, Drejer J, Schousboe A et al (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem 43:1369–1374

35. Shimada N, Graf R, Rosner G et al (1993) Ischemia-induced accumulation of extracellular amino acids in cerebral cortex, white matter, and cerebrospinal fluid. J Neurochem 60:66–71

36. Uchiyama-Tsuyuki Y, Araki H, Yae T et al (1994) Changes in the extracellular concentrations of amino acids in the rat striatum during transient focal cerebral ischemia. J Neurochem 62:1074–1078

37. Vernier P, Cardinaud B, Valdenaire O et al (1995) An evolutionary view of drug-receptor interaction: the bioamine receptor family. Trends Pharmacol Sci 16:375–381

38. Lee FJ, Wang YT, Liu F (2005) Direct receptor cross-talk can mediate the modulation of excitatory and inhibitory neurotransmission by dopamine. J Mol Neurosci 26:245–252

39. Danbolt NC (1994) The high affinity uptake system for excitatory amino acids in the brain. Prog Neurobiol 44:377–396

40. Schousboe A (1981) Transport and metabolism of glutamate and GABA in neurons are glial cells. Int Rev Neurobiol 22:1–45

41. Fraser DD, Duffy S, Angelides KJ et al (1995) GABAA/benzodiazepine receptors in acutely isolated hippocampal astrocytes. J Neurosci 15:2720–2732

42. Bovolin P, Santi MR, Puia G et al (1992) Expression patterns of gamma- aminobutyric acid type A receptor subunit mRNAs in primary cultures of granule neurons and astrocytes from neonatal rat cerebella. Proc Natl Acad Sci USA 89:9344–9348