With the aging of the population and the increased number of elderly patients with co-existing conditions who undergo surgery, we met more and more patients who are at high risk for or suffered ischemic stroke pre-operatively. Therefore, choosing the proper anesthetics for these patients to preserve their compromised brain function is a major research endeavor for anesthetists.

Propofol (2,6-diisopropylphenol) is an intravenous sedative hypnotic agent. In case of low intracranial compliance, intracranial pressure can be decreased by propofol and increased by volatile anesthetics (Priebe 2007). Therefore, propofol may be more suitable for patients who have low intracranial compliance induced by pre-operative stroke. Additionally, Propofol post-conditioning promoted neurogenesis in the dentate gyrus of hippocampus, as measured by bromodeoxyuridine and neuron-specific nuclear protein immunofluorescence-double staining at day 28 after reperfusion. Finally, propofol post-conditioning increased the surface expression of AMPA receptor GluR2 subunit, thus inhibited the internalization of this part until 28 days after stroke. In conclusion, our data suggest that propofol post-conditioning provides long-term protection against focal cerebral ischemia/reperfusion injury in rats. Furthermore, we found that the inhibition of AMPA receptor GluR2 subunit internalization may contributed to this long-term neuroprotection.

**Keywords:** AMPA receptor, cerebral ischemia/reperfusion injury, GluR2 subunit, post-conditioning, propofol.

1994; Dingledine et al. 1999). In principal hippocampal neurons, AMPARs are primarily of the GluR1/GluR2 and GluR2/GluR3 configuration (Craig et al. 1993; Wenthold et al. 1996). Some studies showed that AMPAR lacking the edited GluR2 subunit are highly permeable to Ca$^{2+}$ (Verdoorn et al. 1991; Geiger et al. 1995), and GluR2-lacking AMPAR-mediated excitotoxicity is thought to play a critical role in CNS ischemic insults (Liu et al. 2006). Whereas considerable evidence underscores alterations in AMPAR subunit changes hours or days after focal cerebral ischemic injury, function of AMPARs changes at late times after ischemia remain unclear.

In this study, we estimated the long-term protection of propofol post-conditioning in rats subjected to focal ischemia/reperfusion generated by transient MCAO, and tested whether the AMPARs are involved in propofol post-conditioning.

**Materials and methods**

**Experimental animals and experimental design**

Adult male Sprague–Dawley rats (250–280 g) were cared for according to the Guide for the Care and Use of Laboratory Animals. The committee of experimental animals of Tianjin Medical University approved all the surgical procedures.

Rats were divided randomly into four groups: (i) sham-operated group $(n = 45)$, (ii) I/R group $(n = 58)$: 60 min MCAO followed by reperfusion, (iii) propofol Post-cond group $(n = 60)$: propofol 20 mg/kg/h was infused intravenously with syringe pump (Beijing Slgo Medical Technology Development Co., Ltd., Beijing, China) at the onset of reperfusion for 4 h, and (iv) Intralipid group $(n = 58)$: 10% Intralipid (Kabi Pharmacia, Clayton, NC) 2 mL/kg/h was infused intravenously with syringe pump at the onset of reperfusion for 4 h. In case of sham-operated and I/R group, equivalent dose of saline was administered in the same manner.

**Middle cerebral artery occlusion model**

Focal cerebral ischemia was induced by the intraluminal suture method (Longa et al. 1989). Briefly, the rats were anesthetized with Inactin (thiobutabarbital, 100 mg/kg), a nylon monofilament (diameter 0.26 mm) with a silicone-beaded tip was introduced into the right internal carotid artery through a nick made in the external carotid artery and advanced 17–20 mm distally from the right common carotid artery bifurcation to block the origin of right middle cerebral artery. After 1 h of occlusion, the filament was pulled out 10 mm from the sutured wound to allow reperfusion. Regional cerebral blood flow was monitored by laser-Doppler flowmeter (Periflux system 5000; Perimed Inc., Jarfalla, Sweden) with the use of a flexible probe placed over the skull (1 mm posterior to the bregma and 6 mm from the midline on the right side). When the MCA was occluded with insertion of the thread, rats that did not show a cerebral blood flow reduction of at least 70% were excluded from the experimental group (Xing et al. 2008). For sham-operated group, the filament was advanced less than 10 mm inside the internal carotid artery. Polyethylene catheters were inserted into the right femoral artery (for blood pressure measurement and sampling for plasma glucose and arterial blood gases) and the right femoral vein for drug administration. Body temperature was monitored with a rectal probe and maintained at 37 ± 0.5°C by warming blanket and lamps. Physiologic variables (mean arterial blood pressure, temperature, arterial blood gases and plasma glucose) before, during, and after ischemia were analyzed.

**Neurological evaluation**

Animals were examined for neurological deficits 24 h after MCAO by a blinder to the identity of the groups using a 5-point neurological function score that was described by Longa et al. (1989): 0, no deficit; 1, failure to extend left forepaw fully; 2, circling to the left; 3, failing to the left; 4, no spontaneous walking with a depressed level of consciousness. Only animals those showed no or incomplete forelimb placing with rotational asymmetry 24 h after MCAO were included in the subsequent analysis (Thored et al. 2007).

**Behavioral assessment by Morris Water Maze test**

On day 9 and 23 after MCAO, rats $(n = 4–5/group)$ were trained using Morris Water Maze (MWM) according to a protocol reported by Vorhees and Williams (2006). Briefly, the maze (150 cm diameter, 50 cm high; DMS-2, Chinese Academy of Sciences, China) was filled with water of 22 ± 2°C (Morris et al. 1982; Morris 1984). A target platform (10 cm diameter) was hidden 2 cm below the surface of water in a fixed southwest location halfway between the wall and center of the maze. The MWM training consisted of spatial acquisition and reference memory probe trials. For spatial acquisition, rats were trained to rely on visual distal cues to locate a submerged escape platform. Latency (time to reach the platform) and swim speed were recorded with a computerized tracking system (Ethovision 3.0; Noldus Information Technology, Wageningen, the Netherlands). Four trials from four different random start positions at north, east, southeast, and northwest were tested daily (each lasted 2 min with 30-s intervals) for 5 days. Rats that failed to find the platform within 2 min were guided to it by an experimenter, and their maximum latency score was recorded as 120 s.

At 24 h after the last training day, rats were tested for reference memory. For this, the hidden platform was first removed. Rats started at a northeast position (NE), 180° from the original hidden platform position, and were allowed to swim for 60 s. The time period (s) when a rat stayed in the goal quadrant, where the hidden platform was previously located, was recorded by the computerized swimming tracking system and expressed as a percent of time in the 60-s total swimming period. The swim speed was also recorded and used, together with swimming speed measured during spatial acquisition, to exclude potential changes that were caused by MCAO-induced movement impairment. In this study, swimming time and distance within only a 30-cm circular zone around the previous platform, that is, not in the whole quadrant, were recorded (Yun et al. 2007).

**Measurement of infarct volume**

On day 1, 14 and 28 after MCAO, Brains $(n = 4–5/group)$ for each time point, 13–15 in each group) was collected after reperfusion and five coronal slices of 2-mm thickness were obtained. The brain slices were stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, St Louis, MO, USA) in the dark at 37°C for 30 min and fixed by 4% paraformaldehyde (Sigma) overnight. The posterior surface of each slice was photographed and analyzed by using Image Pro Plus 5.1 (Media Cybernetics, Bethesda, MD, USA).
lesion volume was calculated by multiplying the area by the thickness of slices. We adopted a method previously described by Belayev et al. (1996) to eliminate the contribution of edema to the ischemic lesion as following formula: Corrected Infarct Volume = Contralateral Hemisphere Volume – (Ipsilateral Hemisphere Volume – Measured Infarct Volume). The infarct volume was presented as the percent of infarct area to the total brain regions (Yang et al. 1998) by an observer naive to the treatment group.

Bromodeoxyuridine labeling and immunofluorescence-double staining
Bromodeoxyuridine (BrdU), a thymidine analog which replaces thymidine in newly synthesized DNA, was used to label endogenous proliferating cells. BrdU (100 mg/kg) was injected IP on days 7 to 9 after MCAO. After 28 days, the animals were deeply anesthetized and transcardially perfused with 100 mL saline 0.9%, followed by 50 mL 4% in 0.2 M phosphate buffer. The brains were removed, post-fixed for 24 h in paraformaldehyde–phosphate buffer and 50 mL 4% in 0.2 M phosphate buffer. The brains were removed, post-fixed for 24 h in paraformaldehyde–phosphate buffer and placed for 48 h in 30% sucrose. The 33 mm coronal sections of brain were prepared at the level of bregma –3.3 ± 0.2 mm.

Sections (n = 4–5/group) with immunofluorescence-double staining were used to calculate the ratio between BrdU + neuron-specific nuclear protein (NeuN)-positive cells and the total amount of BrdU positive cells. Briefly, the sections were then incubated overnight at 4°C with the primary rat anti-BrdU-antibody (1 : 500, sheep-polyclonal IgG; Abcam Biotechnology, Cambridge, UK) and the mouse anti-NeuN-antibody (anti-neuron-specific nuclear protein, 1 : 250, cat-monoclonal IgG; Chemicon International, Temecula, CA, USA). After washing with tris-buffered saline for 20 min, the sections were incubated in a secondary antibody mix (1 : 500; fluorescein-conjugated donkey anti-rat IgG, Jackson ImmunoResearch Laboratories West Grove, PA, USA and rhodamine red-X-conjugated IgG, donkey anti-mouse, Jackson ImmunoResearch Laboratories) for 2 h. Sections were rinsed in tris-buffered saline and then mounted on glass slides. The analysis of the sections was performed using a fluorescence microscope (NIKON ECLIPSE-80i, Nikon, Japan). The counts of cells were made by an investigator who was unaware of the treatment conditions. For each animal, 50 BrdU-positive cells in the ipsilateral dentate gyrus (DG) were analyzed for coexpression of BrdU and NeuN to determine the ratio of newly generated neurons (BrdU + NeuN) to the total amount of newborn cells (BrdU). Positive and negative controls and tests for excluding cross-reactions for the two secondary antibodies were performed.

Surface receptor cross-linking with bis (sulfosuccinimidyl) suberate
Surface and intracellular AMPA receptor GluR2 levels were determined with a protein cross-linking assay (Boudreau and Wolf 2005). Briefly, on days 1, 14 and 28 after MCAO, rats (n = 4–5/group for each time point, 13–15 in each group) were decapitated and bilateral hippocampus was isolated on an ice-cold platform. Hippocampus from each rat were chopped into 400 μm slices and then incubated with 2 mM bis (sulfosuccinimidyl) suberate (BS²⁺; Pierce Biotechnology, Rockford, IL, USA) in ice-cold artificial cerebrospinal fluid for 15 min at 4°C. Cross-linking was terminated by adding 100 mM glycine (10 min at 4°C). The slices were centrifuged and pellets were resuspended in ice-cold lysis buffer containing protease and phosphatase inhibitors as Boudreau and Wolf (2005) suggested. A brief centrifugation was performed, total protein concentration of the supernatant was determined by the BCA Protein Assay reagent kit (Pierce). Samples were aliquoted (~15 aliquots per rat) and stored at ~80°C for future analysis.

Western blotting
Protein (30 μg) was separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes and probed with primary antibodies for AMPAR subunits GluR2 (N terminus, 1 : 1000, Millipore, Billerica, MA, USA) at 4°C overnight. Blots were washed and then incubated with goat anti-mouse IgG secondary antibody conjugated to horseradish peroxidase (1 : 5000; Millipore) for 1 h at 25°C and signals were visualized by ECL (Amersham, Buckinghamshire, UK). Densities of surface and intercellular bands were determined using Total Lab (Nonlinear Dynamics, Newcastle, UK). Protein was determined by staining membranes with Ponceau S (Sigma-Aldrich). A potential concern is that cross-linking might interfere with immunodetection of the surface band, preventing accurate determination of the surface/total (S/T) ratio. We ruled this out by comparing identical amounts of cross-linked and non-cross-linked tissue probed with antibodies to GluR2. The sum of the density of surface and intracellular bands in the cross-linked tissue was equal to the density of the single subunit band in the non-cross-linked tissue. This demonstrates that GluR2 in the tissue was detected after cross-linking. We used Quantity One Analyzer 4.5 (Bio-Rad Laboratories, Hercules, CA, USA) to measure the band densities in blinded fashion. T (Total) protein level = surface (cross-linked) protein + intercellular (non-cross-linked) protein; S/T ratio = surface protein / (surface protein + intercellular protein).

Correlation between infarct size and the results of probe test in MWM
To know whether there was a correlation between the infarct size and the performance of MWM testing induced by post-conditioning, we chose two rats with the same age and similar weight as a pair (five pairs in each group) and used them for testing the two parameters separately. The First parameter we recorded was the infarct size after TTC staining, the second one was the time spent in the target quadrant of the last cycle of probe test. The time point we chose was 28 days after MCAO.

Correlation between neurogenesis and infarct size
To test weather neurogenesis may provide neuroprotection for rats after transient MCAO, we did another correlation analysis between neurogenesis in the DG and infarct size. Because rats in sham group were not underwent brain ischemia, almost none of neurogenesis was found in this group. Therefore we only did the analysis for the other three groups. Briefly, we chose two rats with the same age and similar weight as a pair in I/R, Post-cond and Intralipid group (five pairs in each group) and used them for neurogenesis and infarct size measurement separately. The two parameters of each pair were used to conduct the correlation analysis. The time point we chose was 28 days after MCAO.

Statistical analysis
Data are presented as mean ± SEM. Water maze data, infarct volume and western blot data were analyzed using the general linear
models repeated measures ANOVA (SPSS, version 16.0; SPSS Science, Inc., Chicago, IL, USA), followed by post hoc LSD test was used to analyze group differences of the data collected at different time points. Total number of newly generated neurons and the volume of the DG were analyzed by one-way ANOVA and followed by post hoc LSD test. Correlation analysis was performed using Pearson’s correlation coefficient. All were considered statistically significant for p-values below 0.05.

Results

Physiological data
No statistical differences were observed in physiologic parameters in experimental groups (data not shown).

Propofol post-conditioning attenuated long-term spatial memory deficiencies after transient MCAO
To evaluate the ability for spatial acquisition, we tested all rats by MWM, which measures the ability of a rat to navigate from a start location in a water maze to a submerged escape platform (Morris et al. 1982; Morris 1984). As expected, latency (the time to reach platform) and path length were significantly shortened during the two trails of 5-day acquisition period, suggesting that spatial acquisition had developed. Rats in propofol Post-cond group required less time to find the platform than those in I/R and Intralipid groups, although they spent more time than those in sham-operated group (p < 0.05, Fig. 1a and b). Analysis of escape latency revealed significant differences between groups (p < 0.05), but there were no significant differences in the swimming speed among these groups (data not shown). In the two sessions (14 and 28 days after reperfusion) of probe test, rats in propofol Post-cond group spent significantly more time to find the platform than the other groups (I/R, Intralipid) in the quadrant where the platform had been, but the time in target quadrant in that group was still shorter than the time in sham-operated group (p < 0.05, Fig. 1c). These data indicate that 1 h of ischemia and subsequent reperfusion impaired learning and memory, but propofol post-conditioning reduced this insult induced learning and memory deficit, and the effect sustained to 28 days after transient MCAO.

Propofol post-conditioning provided long-term reduction of infarct volume
TTC staining of brain slices analyzed to determine the percentage of infarct area in the whole brain. Representative coronal brain sections from the four groups were showed (Fig. 2a). In I/R group, an extensive lesion was developed in ipsilateral striatum and lateral cortex. Infarct volume at 1, 14, and 28 days after reperfusion decreased significantly in propofol Post-cond group compared with I/R group (p < 0.05). There was no significant reduction in infarct volume in Intralipid group compared with I/R group at different time points (Fig. 2a and b). No evidence of
An infarction was observed on the TTC slices in sham-operated rats (Fig. 2a and b), indicating the CNS integrity. We also found the decreased infarction area in propofol Post-cond group confined to limited cortical layer, with distinct boundary and slight swelling. Thus, it was confirmed that propofol post-conditioning reduced the infarct area in ipsilateral striatum until 28 day after transient MCAO.

Propofol post-conditioning stimulated neurogenesis in the DG at 28 days

Studies have demonstrated that ischemic or traumatic brain injury can induce neurogenesis in regions of the brain that are normally non-neurogenic (Emsley et al. 2005). In this study, neurogenesis in the DG of hippocampus was assessed by injecting the cell proliferation marker BrdU. Because BrdU is known to exert cytotoxic effects on proliferating lymphocytes (Reome et al. 2000), it was administered only on days 7 to 9 after MCAO. Rats were killed 28 days after MCAO, and neurogenesis was evaluated by staining of coronal hippocampal sections with antibodies against BrdU and NeuN (a marker of post-mitotic neurons). We counted the newly formed BrdU + NeuN positive cells in the granular cell layer of the hippocampal DG in different groups (Fig. 3a and b).

After transient MCAO, the amount of BrdU + NeuN positive neurons increased 131% in the DG of propofol Post-cond group as compared with I/R group (p < 0.05). Ischemia/reperfusion injury stimulated neurogenesis in the DG (272% of sham, p < 0.05). There was no significant difference of neurogenesis in the DG between I/R and Intralipid groups (Fig. 3a and b). The average volume of the ipsilateral DG for all groups is displayed in Fig. 3c. The volume of the DG was similar for all groups, independent of the drug usage or cerebral ischemia.

Propofol postconditioning inhibited the internalization of AMPA receptor GluR2 subunit in hippocampus

Surface (S) and intracellular (I) AMPA receptor GluR2 levels were determined with a protein cross-linking assay (Boudreau and Wolf 2005). A measure of total receptor subunit protein (T) is obtained by summing S+I. Rats in the I/R and Intralipid groups had significantly decreased GluR2 S/T values compared with sham-operated rats (Fig. 4a and b; p < 0.05), indicating that focal cerebral ischemia/reperfusion challenge produced GluR2 internalization in hippocampus. We observed that rats in propofol Post-cond group had higher GluR2 S/T value than those in the I/R and Intra groups (p < 0.05), indicating the restriction of GluR2-containing AMPARs in cell surface, and this trend was kept until 28 days after reperfusion (Fig. 4a and b). However, saline challenge did not alter GluR2 distribution in sham-operated rats. There was no difference in total subunit protein expression among the four groups on days 1, 14 and 28 after transient MCAO (Fig. 4c).

Correlation between infarct size and the results of probe test in MWM

Correlation analysis between infarct size and the performance in probe test of MWM at 28 days after MCAO revealed a strong negative correlation in the four groups with the Pearson correlation coefficient was −0.96 (p = 0.0001, Fig. 5).

Correlation between neurogenesis and infarct size

The correlation coefficient between the results of neurogenesis and infarct size was −0.921 (p = 0.0001, Fig. 6).

Discussion

In this study, we examined the long-term effect of propofol post-conditioning. We demonstrated that propofol 20 mg/kg/h infused intravenously at the onset of reperfusion (post-
conditioning) for 4 h not only reduced infarction, but also reduced escape latency and increased the time spent during the probe test in the quadrant where the platform had been. There was a negative correlation ($r = -0.96$, $p = 0.0001$) between infarct size and the performance of probe test in MWM at 28 days after MCAO. We also showed that propofol post-conditioning promoted neurogenesis in the DG, this negatively correlated with infarct size ($r = -0.912$, $p = 0.0001$). Additionally, propofol post-conditioning inhibited the internalization of AMPAR GluR2 subunit in hippocampus, indicating a critical protective role of maintain AMPAR GluR2 subunit surface expression in propofol induced post-conditioning. The neuroprotective effect sustained to 28 days after focal cerebral ischemia in rats, suggesting long-term protection.

The MWM task is thought to test spatial learning capability and reference memory (Morris 1984). Of the neighboring regions undergoing delayed cell death in response to ischemic insults induced by MCAO, the most vulnerable are found in the hippocampus, which plays a major role in learning and memory (Butler et al. 2002). However, the reduction of neuronal damage in the hippocampus rather than in the striatum is known to be the basis for the improvement of memory performance in the MWM (Block et al. 1997; Lee et al. 2003). Similar to the previous studies (Borlongan et al. 2005; Rönnbäck et al. 2005), ischemic animals exhibited mean speed, movement time, and rest time that were not significantly different from sham animals, indicating that any abnormalities revealed by this task could not be due to motor impairment.

The notion that the adult mammalian central nervous system is incapable of significant self-repair or regeneration was revisited after the detection of self-renewing and multipotent neuronal stem cells. By cell division, stem cells produce progenitor cells which then rapidly generate new neurons, astrocytes, and oligodendrocytes in the adult brain (Taupin and Gage 2002). The subgranule layer of the DG seems to be one of the most important regenerative centers (Kuhn et al. 1996). Cells derived from the subgranular layer of the DG move into the granular layer of the DG and sprout...
dendrites to the CA3 region of the hippocampus (Hastings and Gould 1999). In the current study, there were only a few newly generated neurons in the DG of the brain 28 days after cerebral ischemia. In contrast, experiments in young adult Sprague-Dawley rats, 4 h of 20 mg/kg/h propofol infused increased the number of newborn neurons to 131% (of I/R group) at 28 days after cerebral ischemia. This suggests that ischemia induce neurogenesis could be promoted by propofol post-conditioning. However, about 80% of the initially proliferated cells disappear within 4 wk, which explains the small increase in new neurons after 28 days (Takasawa et al. 2002). The long observation period was chosen because it was expected that after 28 days only those new generated neurons survive, which are integrated into the DG. Clinically, only the long-term surviving neurons are of interest, in another word, could become functional neurons and provide neuroprotection in the future. As the current study showed, at 28 days after MCAO, with the neurogenesis increased by propofol post-conditioning, the infarct size of brain decreased.

Muth-Köhne et al. (2010) found that AMPAR GluR2 expression to be highly correlated with neuronal differentiation, as previous reports indicate a predominance of GluR2-containing, Ca^{2+}-impermeable receptors involved in synaptic transmission (Seeburg et al. 1998; Lu et al. 2009). N-methyl-D-aspartate receptors (NMDARs) significantly con-

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Fig. 4 Effect of propofol post-conditioning on expression of AMPAR GluR2 subunit. (a) Western blot analysis showed BS^3 cross-linked surface and intracellular pools of AMPAR GluR2 subunit and the expression at days 1, 14 and 28 after reperfusion. (b) Quantification of surface/total GluR2 subunit expression at days 1, 14 and 28 after reperfusion. (c) Quantification of total GluR2 subunit expression at days 1, 14 and 28 in each group. Bar represent mean ± SE (n = 4–5/group), *p < 0.05 versus sham-operated group. #p < 0.05 versus I/R group. Sham, sham-operated group; I/R, I/R group; Pro, propofol Post-cond group; Intra, Intralipid group.
tribute to the overall activity of developing circuits (Khazipov et al. 1995; Ben-Ari et al. 1997; Pickard et al. 2000; Ben-Ari 2002). As NMDARs are blocked by Mg²⁺ at negative resting membrane potentials, they need the coactivation of AMPARs in mature neurons which serves to release the NMDARs Mg²⁺ block by pre-depolarization of the membrane. However, in immature neurons and neuroblasts, the required pre-depolarization can also be provided through the activation of gamma-aminobutyric acid A receptors (GABAARs) (Durand et al. 1996; Pickard et al. 2000). Therefore, we speculated that NMDARs and GABA-ARs are required in the genesis of endogenous neural stem cells, and Ca²⁺-impermeable AMPARs allows differentiation and discrimination of neural stem cells. Propofol is an intravenous anesthetic drug commonly used on patients today. It has been established that propofol potentiates (Concas et al. 1990; Hales and Lambert 1991) and, in higher concentrations, directly activates (Krasowski et al. 1998; Björnström et al. 2002) the GABAARs. It is therefore possible that propofol post-conditioning activated GABA-ARs and promotes the genesis of neural stem cells in the early stage after ischemia. Then the inhibition of the internalization of AMPAR GluR2 subunit induced by propofol post-conditioning, allowed the differentiation and discrimination of neural stem cells, promote these cells becoming neurons.

AMPAR-mediated excitotoxicity is thought to play a critical role in CNS ischemic insults (Liu et al. 2006). During cerebral ischemia, A rise in [Ca²⁺], is thought to initiate a cascade of events leading to cell death, including activation of proteases and endonucleases, generation of free radicals that destroy cell membranes by lipid peroxidation, and induction of apoptosis (Rothman and Olney 1986; Choi 1990, 1995; Puttfarcken et al. 1993; Bredesen 1995; Mel- drum 1995).

Although AMPARs were initially thought to be relatively impermeable to Ca²⁺, it is now clear that there is also AMPAR exhibiting considerable Ca²⁺ permeability. AMPARs containing the GluR2 subunit exhibit low Ca²⁺ permeability, whereas AMPARs lacking GluR2 are much more Ca²⁺ permeable (Hollmann et al. 1991; Hume et al. 1991; Burnashev 1996). In the adult brain, nearly 100% of the mRNA encoding GluR2 is edited at the Q/R site corresponding to residue 607, where the genomic glutamine (Q607) codon is converted to an arginine (R) codon (Geiger et al. 1995; Jonas and Burnashev 1995; ). Edited GluR2 (R) subunits form Ca²⁺-impermeable channels, whereas unedited GluR2 (Q) channels are permeable to Ca²⁺ flow. Therefore, AMPARs in most principal neurons of adult hippocampus are heteromeric, contain GluR2, and have a low permeability to Ca²⁺ (Bochet et al. 1994; Jonas et al. 1994; Geiger et al. 1995). Global ischemia reduces GluR2 mRNA levels in hippocampal CA1 neurons (Gorter et al. 1997). This observation led to the ‘GluR2 hypothesis’, which postulates that reduced GluR2 surface expression allows Ca²⁺ entry through AMPAR channels and determines vulnerability of neurons in cerebral ischemia (Peng et al. 2006). We showed that AMPAR GluR2 subunit in hippocampal neurons redistribute to the cell surface during propofol post-conditioning and this effect sustained to 28 days post-ischemia. However, when ischemic rats were challenged with saline or Intralipid, the AMPAR GluR2 subunit S/T ratio decreased, indicating a reduction in cell surface expression. Therefore, the inhibition of AMPAR GluR2 internalization may be one of the most important mechanisms in long-term neuroprotection induced by propofol post-conditioning.

Propofol showed sustained (28 days) neuroprotection in a rat model of incomplete cerebral ischemia and reperfusion (clip-occlusion of the right common carotid artery in combination with hemorrhagic hypotension for 45 min).
(Engelhard et al. 2004). But in this study, propofol was administrated 45 min before cerebral ischemia, indicating the neuroprotection induced by propofol is pre-conditioning effect. In reality, the onset of brain ischemia in patients with stroke and brain trauma often occurs outside the hospital and is not predictable. Therefore, post-conditioning is a relatively novel concept against stroke (Zhao et al. 2006). Our previous studies have showed that propofol (20 mg/kg/h) post-conditioning provided acute (up to 24 h) neuroprotection in rats with transient MCAO (Wang et al. 2009). The current study demonstrated the long-term neuroprotection (up to 28 days) induced by propofol post-conditioning. This finding could be ideal for propofol in many applications for patients with ischemic brain injury, such as before, during, and after surgery, neuroradiology, and intensive care.

In conclusion, propofol post-conditioning (20 mg/kg/h, infused at the onset of reperfusion for 4 h) provided long-term protection against cerebral ischemia by reducing lesion size, improving spatial learning and memory ability and promoting neurogenesis in the DG in a rat focal ischemia model. Propofol post-conditioning’s ability to inhibit the internalization of AMPAR GluR2 subunit during cerebral ischemia may contribute to this protection.

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Disclosure of potential conflicts of interests

The authors declare no conflicts of interests related to this work.

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