Research report

Intracerebroventricular propofol is neuroprotective against transient global ischemia in rats: extracellular glutamate level is not a major determinant

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Abstract

Excessive glutamate accumulation in extracellular space due to ischemia in the central nervous system (CNS) is believed to initiate the cascade toward irreversible neuronal damage. An intravenous general anesthetic, propofol (2,6-diisopropylphenol) has been implicated to be neuroprotective against cerebral ischemia. The purpose of this study was to test the hypothesis that intracerebroventricular propofol produced a reduction in extracellular glutamate level during global ischemia and the resultant neuroprotection. Adult male Wistar rats were anesthetized with halothane in nitrous oxide/oxygen and mechanically ventilated. Propofol (3 or 10 mg/kg), Intralipid as a vehicle for propofol, or artificial cerebrospinal fluid (aCSF) was administered into the cerebral ventricles 15 min prior to a 10-min forebrain ischemia elicited by the four-vessel occlusion. Extracellular glutamate concentration in the hippocampal CA1 was continuously monitored during the peri-ischemic period with a microdialysis biosensor. Neuronal cell loss in the hippocampal CA1 was evaluated by cresyl-violet staining of sections 7 days later. Propofol (3 and 10 mg/kg) and Intralipid, compared with aCSF, similarly reduced the extracellular glutamate accumulation during the peri-ischemic period (P<0.05), indicating that the extracellular glutamate reduction that was seen primarily reflects the effect of Intralipid. The number of intact neurons in the hippocampal CA1 in propofol 10 mg/kg-treated rats was significantly higher than that in rats treated with propofol 3 mg/kg, Intralipid, or aCSF (P<0.05). We conclude that intracerebroventricular propofol exhibits neuroprotection against transient global forebrain ischemia; however, the extracellular glutamate level during ischemia is not a major determinant of this neuroprotection. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Propofol (2,6-diisopropylphenol) in a lipid emulsion is used clinically as an intravenous general anesthetic and as a sedative for critically ill patients. Propofol depresses electroencephalographic activity [14] and decreases the cerebral metabolic rate for oxygen (CMRO₂) [39,21] and the cerebral blood flow [21,41]. Propofol also has antioxidant effects [24]. These features of propofol may provide protection against ischemia of the central nervous system (CNS). To date, however, a neuroprotective effect of intravenous or intraperitoneal propofol on global and focal cerebral ischemia in animal models is still controversial [2,45,19,43,30,38,42].

Glutamate, the major excitatory neurotransmitter in the mammalian CNS, activates post-synaptic receptors when released from the pre-synapse. Excessive activation of post-synaptic glutamate receptors has been implicated in initiating the cascade that leads to neuronal cell death [31,10]. Extracellular glutamate concentrations in the CNS are normally low, but increase during ischemia [18,3]. The extracellular glutamate level depends on glutamate release into the extracellular space and its uptake into cells [5,4]. For this reason, regulation of glutamate release and of its re-uptake during the peri-ischemic period could be a promising strategy for alleviating neuronal injury. The
effect of propofol on the extracellular glutamate level during cerebral ischemia remains to be elucidated. In this study, we tested the hypothesis that propofol delivered directly to the CNS produced a reduction in extracellular glutamate level during global ischemia and the resultant neuroprotection in the hippocampal CA1 region of the rat.

2. Materials and methods

2.1. Reagents

Glutamate oxidase, propofol, and Intralipid® were obtained from Yamasa (Chiba, Japan), Aldrich (Milwaukee, WI, USA), and Pharmacia AB (Stockholm, Sweden), respectively. All other reagents were purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Subjects, preparation, and ischemia

All animal care procedures in this study were performed according to the Guidelines for Animal Experiments of the Kumamoto University School of Medicine. The animal care and use committee for our institute approved the study protocol. We used adult male Wistar rats weighing between 230 and 290 g. The animals were given free access to food and water. At the time of the experiment, they were anesthetized with 4% halothane in nitrous oxide/oxygen (F1O2; 0.33), after which their tracheas were intubated and they were connected to a rodent ventilator (7025; Ugo Basile, Camerio, Italy). They were mechanically ventilated (tidal volume, 10 ml/kg; frequency, 60/min) with 1% halothane in nitrous oxide/oxygen. The pericranial temperature was continuously monitored with a tissue implantable thermocouple microprobe (IT-14; Physitemp Instruments Inc., Clifton, NJ, USA) and maintained at 37.0±0.2°C during surgery, brain ischemia, and recovery before the initiation of ischemia, and perfused with PBS containing cumulated concentrations of L-glutamate. The animals were given free access to food and water. At the time of the experiment, they were anesthetized with 4% halothane in nitrous oxide/oxygen (F1O2; 0.33), after which their tracheas were intubated and they were connected to a rodent ventilator (7025; Ugo Basile, Camerio, Italy). They were mechanically ventilated (tidal volume, 10 ml/kg; frequency, 60/min) with 1% halothane in nitrous oxide/oxygen. The pericranial temperature was continuously monitored with a tissue implantable thermocouple microprobe (IT-14; Physitemp Instruments Inc., Clifton, NJ, USA) and maintained at 37.0±0.2°C during surgery, brain ischemia, and recovery from anesthesia with the use of a temperature controller (TCAT-1A; Physitemp Instruments Inc.) and a radiant heating lamp. Electroencephalogram (EEG) from bilateral areas of the brain was continuously monitored and recorded. The tail artery was cannulated for monitoring of arterial pressure and analysis of arterial blood gases, glucose, and hematocrit. Global forebrain ischemia was achieved by the four-vessel occlusion method originally described by Pulsinelli and Brierley [27]. In brief, vertebral arteries were coagulated at the alar foramina of the first cervical vertebra, and then each common carotid artery was snared and tightened with a 3-0 silk suture by applying a weight of 15 g. Forebrain ischemia was confirmed by isoelectric EEG. Reperfusion was initiated by cutting the carotid sutures.

2.3. Microdialysis biosensor

We used a microdialysis biosensor (General 20-10-2-2; Sycopel International, Ltd., Boldon, Tyne and Wear, UK) for continuous measurement of extracellular glutamate concentration as described previously in detail [3,26]. In brief, the microdialysis biosensor was made up of a platinum working electrode, a silver/silver chloride counter electrode, a silver reference electrode, and two vitreous silica tubes for changing the solution inside the biosensor, all of which were set inside a hollow semi-permeable membrane, 230 μm in outside diameter. The biosensor was filled and perfused with phosphate buffered saline (PBS) pH 7.4 (mM: NaCl, 146; KCl, 2.7; Na2HPO4·2H2O, 4.3; KH2PO4, 1.4; CaCl2, 2.4) by a perfusion pump (EP60; Eicom, Kyoto, Japan). O-phenylenediamine was electropolymerized onto the platinum electrode in PBS bubbled with 100% nitrogen and stirred to avoid interference from electroactive molecules such as ascorbate. The current from hydrogen peroxide produced by glutamate oxidation was detected amperometrically on the platinum electrode at +650 mV by an electrochemical detector (EPS 800, Eicom) and recorded on a polygraph in real time. After stabilization of the current at +650 mV, the biosensor was perfused with glutamate oxidase (0.05 U/μl) dissolved in PBS at a rate of 0.2 μl/min. The calibration in vitro was performed by placing the tips in the PBS containing cumulated concentrations of L-glutamate. The current that developed on the platinum electrode increased linearly with L-glutamate concentrations up to at least 300 μM. The sensitivity and response time of the microdialysis biosensors used were around 0.15 nA/μM and within 20 s for a 90% steady state response, respectively. The microdialysis biosensors were also calibrated for L-glutamate in different concentrations (10 and 30%) of Intralipid, which was a vehicle for propofol in this study (see Experimental design), to see whether Intralipid influenced on the calibration curve. The biosensor was implanted stereotaxically on the left side of the hippocampal CA1 area 75 min before the initiation of ischemia, and perfused with PBS containing glutamate oxidase at a rate of 0.2 μl/min to initiate in vivo real-time measurement of glutamate. The area under the curve defined as integrated increments of glutamate concentration from the pre-ischemic baseline value between the beginning and 20 min after the end of ischemia was calculated from the actual trace using NIH Image version 1.61 software.

2.4. Experimental design

Rats were randomly assigned to one of four groups to be treated with: (a) propofol 3 mg/kg (n=9); (b) propofol 10 mg/kg (n=10); (c) Intralipid (n=10); or (d) artificial cerebrospinal fluid (aCSF; mM: NaCl, 126.5; KCl, 2.4; KH2PO4, 0.5; CaCl2, 1.1; MgCl2, 1.1; Na2SO4, 0.5) (n=9). Propofol was dissolved in Intralipid, a fat emulsion composed of 10% soya bean oil, 1.2% egg yolk lecithin and 2.5% glycerol, since it is poorly water-soluble. These agents, 20 μl in volume and warmed at 37°C, were
stereotaxically infused for 1 min into the bilateral cerebral ventricles (10 μl into each ventricle) through burr holes 15 min prior to the initiation of ischemia. The glutamate analysis and anesthesia were terminated 30 min after the initiation of reperfusion. Animals were directly monitored until they fully woke from the anesthesia, then their tracheas were extubated.

2.5. Histology

The rats surviving for 7 days were sacrificed under pentobarbital anesthesia, then perfused transcardially with heparinized normal saline followed by 10% neutral buffered formalin. The brains were removed and fixed in 10% neutral buffered formalin, processed and embedded in paraffin. Five-micrometer-thick coronal sections were taken from the dorsal hippocampus and stained with cresyl violet acetate. The number of histologically intact pyramidal cells with a distinct nucleus and nucleolus in a 1-mm length of the middle portion of the CA1 subfield was counted on the right hippocampus by an inspector blinded to intervention using a microscope at ×400 magnification. The location of the microdialysis electrode was ascertained in each 5 μm-thick coronal section stained with hematoxylin and eosin. If the track of the electrode was outside the CA1 region, the glutamate data was excluded.

2.6. Statistics

Values were expressed as the mean±S.D. and data were analyzed by a one-way analysis of variance or repeated-measures analysis of variance. The Student–Newman–Keuls multiple comparison procedure was then used to determine which pairs of means differed. A chi-square test was used to identify differences in the survival rates. Statistical significance was defined as a P value <0.05.

3. Results

Fig. 1 demonstrates a representative calibration curve of the glutamate biosensor that we used in different concentrations of Intralipid. Without Intralipid, the current developed by glutamate oxidation was on a linear line up to 300 μM of glutamate and the sensitivity of this biosensor was 0.16 nA/μM. Neither 10 nor 30% of Intralipid had any marked interference with the linearity and sensitivity of the glutamate biosensor.

The physiological data are presented in Table 1. No significant differences were observed in all variables among the aCSF-, Intralipid-, 3 mg/kg propofol-, and 10 mg/kg propofol-treated groups. Any treatment did not influence on the baseline values of mean arterial pressure and pericranial temperature. The transient ischemia had no effects on blood gases and pericranial temperature in all treatment groups. The mean arterial pressure during ischemia was higher than those were at the baseline and after the intracerebroventricular administration in all treatment groups (P<0.05).

Neither aCSF nor Intralipid administration into the cerebral ventricles influenced the EEG before ischemia. A pattern of burst suppression with an interval reaching approximately 10 s appeared within a few minutes after the intracerebroventricular injection in all rats given 3 or 10 mg/kg of propofol. Thereafter, the EEG was gradually restored to the same pattern as that seen before propofol administration, and the pattern of burst suppression was no longer observed in any of the propofol-treated rats by 15 min after the injection (just prior to initiation of ischemia) (Fig. 2).

Fig. 3 exhibits changes in extracellular glutamate concentration during the peri-ischemic period in various treatment groups. The baseline values of extracellular glutamate concentration in the hippocampal CA1 region were 27±9, 20±9, 25±14, and 27±16 μM in aCSF-, Intralipid-, 3 mg/kg of propofol-, and 10 mg/kg of propofol-treated groups, respectively, and there were no significant differences among the groups. The baseline values were not affected by the intracerebroventricular administration of any compound tested before the ischemic period. At 4 min after the initiation of ischemia, the glutamate concentration in aCSF group reached to a higher level than Intralipid and 3 mg/kg propofol groups (P<0.05). The glutamate levels during 6–10 min of ischemic period in aCSF group were significantly greater than those in Intralipid and 3 mg/kg and 10 mg/kg propofol groups (P<0.05). However, no significant differences were ob-
Table 1
Physiologic data*

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<td>n</td>
<td>9</td>
<td>10</td>
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<td>Body weight (g)</td>
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<td>266±19</td>
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<td>Hematocrit (%)</td>
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<td>Blood glucose (mg/dl)</td>
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Mean arterial pressure (mmHg)

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<th>5 min before reperfusion</th>
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<td>aCSF</td>
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<td>76±9</td>
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<td>3 mg / kg propofol</td>
<td>74±13</td>
<td>69±11</td>
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<td>10 mg / kg propofol</td>
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Arterial pH

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$PaCO_2$ (mmHg)

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$PaO_2$ (mmHg)

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<td>147±14</td>
<td>152±15</td>
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<tr>
<td>Intralipid</td>
<td>140±29</td>
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<td>151±31</td>
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<tr>
<td>3 mg / kg propofol</td>
<td>129±18</td>
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Pericranial temperature (°C)

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<th>5 min before reperfusion</th>
<th>30 min after reperfusion</th>
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<tr>
<td>Intralipid</td>
<td>37.0±0.1</td>
<td>37.0±0.1</td>
<td>37.0±0.1</td>
<td>37.0±0.1</td>
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<tr>
<td>3 mg / kg propofol</td>
<td>37.0±0.1</td>
<td>37.0±0.1</td>
<td>37.0±0.1</td>
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</table>

*Values are expressed as the mean±S.D. *P<0.05 vs. baseline, † P<0.05 vs. 15 min after treatment, ‡ P<0.05 vs. 30 min after reperfusion.

served in the time course of extracellular glutamate among Intralipid and 3 and 10 mg / kg propofol treatments. The area under the curve in aCSF-treated animals was greater than those were in Intralipid and propofol-treated animals (P<0.05). There were no significant differences in the area under the curve among Intralipid and 3 and 10 mg / kg propofol-treated animals (Fig. 4). All aCSF-treated rats survived for the 7-day observation period. Two of 10, one of 9, and one of 10 rats died in the Intralipid, 3 and 10 mg / kg propofol treatment groups, respectively, during the 7-day reperfusion. No significant differences in mortality rate were observed among the treatment groups.

The number of intact pyramidal cells in the hippocampal CA1 was greater in the 10 mg / kg propofol group than in the aCSF, Intralipid, or 3 mg / kg propofol group (P<0.05, Fig. 5). However, no statistical differences were observed in the number of intact pyramidal cells among groups treated with aCSF, Intralipid, and 3 mg / kg propofol, respectively (Fig. 5B).

4. Discussion

This work demonstrates that propofol (10 mg / kg) administered into the cerebral ventricles mitigates ischemic
neuronal death when assessed at 7 days. Notably, propofol (3 and 10 mg/kg) and Intralipid similarly reduced the extracellular glutamate accumulation in the hippocampal CA1 region exposed to transient global forebrain ischemia.

Although propofol is lipophilic and easily crosses the blood–brain barrier [32], we administered propofol into the cerebral ventricles to clarify its neuroprotective property, which remains controversial when administered intravenously or intraperitoneally in the wake of several studies using different ischemic models and animals [2,45,19,43,30,38,42]. The intracerebroventricular administration in the present study required a considerably lower dose of propofol to elicit electroencephalographic burst-suppression and neuroprotection against global ischemia as compared with the amount required via intravenous or intraperitoneal administration reported previously [2,19].
The administration of propofol used in this study also prevented the systemic hypotension usually observed in both humans [13,12] and animals [22,17] by intravenous administration of propofol. Hypotension may influence the neuroprotective effects of propofol due to a reduction of cerebral blood flow. Thus, intracerebroventricular administration could minimize the hemodynamic effects of propofol and still produce CNS effects such as that seen after systemic administration.

The basal levels of extracellular glutamate in the rat hippocampal CA1 region reported here are similar to those in the rat striatum measured by using the microdialysis electrodes in Zhao et al. [46]. The change in extracellular glutamate in the hippocampal CA1 region was qualitatively similar to that monitored in the same region subjected to transient ischemia by using the glutamate microdialysis electrode in Terada et al. [37].

Glutamate is released from nerve endings (vesicular release), and its post-synaptic action is terminated by its uptake into neurons and glia by glutamate transporters [35]. Therefore, the extracellular glutamate concentration depends on the glutamate release and its uptake (glutamate dynamics). In ischemic conditions, an accelerated glutamate release by an increase of vesicular release and a reversed uptake of glutamate transporters causes extracellular glutamate accumulation, which is a major cause of neuronal cell death because glutamate receptor antagonists reduce the number of neurons killed [6]. Several compounds inhibiting glutamate release (e.g. lamotrigine) or stimulating its uptake (e.g. (R)-(-)-5-methyl-1-nicotinoyl-2-pyrazoline) represent a cerebroprotective effect against ischemia [36,34]. Propofol may have a potential to modify glutamate dynamics during cerebral ischemia. It has been reported that propofol inhibits glutamate release by blocking current through sodium channels or activating GABA<sub>₆</sub> receptors at clinically relevant concentrations [28,29,9]. Propofol also has been shown to prevent the inhibition of glutamate uptake in cultured astrocytes exposed to peroxide in pharmacologic models of brain trauma and reperfusion injury [35]. However, we could not clearly describe that propofol itself modulated the glutamate dynamics because two concentrations of propofol plus Intralipid and Intralipid alone similarly reduced the extracellular glutamate concentration in the late phase of ischemic period in comparison with the aCSF-treated control. Moreover, the areas under the curve representing net glutamate amount released into the extracellular space were also similar in propofol plus Intralipid and Intralipid alone treatment groups. Thus, it seems likely that the extracellular glutamate reduction seen during ischemic period primarily reflects the effect of Intralipid.

The systemic (intravenous or intraperitoneal) administration of propofol dissolved in Intralipid would result in the delivery of very high concentrations of propofol to the CNS, but relatively little Intralipid may penetrate the blood–brain barrier. The intracerebroventricular administration of Intralipid might have a clearer pharmacological effect, rather than the systemic administration, on brain cells governing the glutamate release and its re-uptake. Intracerebroventricular Intralipid with and without propofol lowered the extracellular glutamate concentration during ischemia to the similar level (≈100 μM in Fig. 3). However, Intralipid alone did not contribute to the neuronal survival. The extracellular glutamate level above 100 μM for more than a few minutes triggers neuronal cell death [11]. The reduced glutamate level during ischemia seen in the present study would have been still capable of inducing neuronal death in the hippocampal CA1.

The mechanism by which the intracerebroventricular Intralipid reduced extracellular glutamate accumulation due to cerebral ischemia is unclear as yet. As demonstrated in Fig. 1, 30% or less of Intralipid did not interfere with the glutamate detection by the microdialysis biosensor. It is unlikely that 20 μl of Intralipid administered into the cerebral ventricles of adult rats reflected an extracellular Intralipid concentration higher than 30% in the hippocampus. In addition, glutamate levels were unchanged after the intracerebroventricular administration of Intralipid. Glutamate concentrations determined in the animals given Intralipid intracerebroventricularly would not have been underestimated. Amorim et al. reported that propofol and Intralipid similarly attenuated the decrease in ATP content of rat hippocampal slices during anoxia, which, given that Intralipid contains lipids, suggests that Intralipid is an energy source [1]. The glutamate gradient across the plasma membrane of brain cells, which is markedly higher within cells than in the extracellular space, is maintained by energy-dependent carrier-mediated glutamate uptake into neurons and glia [25]. In the hippocampus under energy deprivation, net glutamate uptake was decreased within 2–3 min and later the reversed glutamate uptake was promoted [20]. One possible explanation is that Intralipid reduced ATP depletion, resulting in the attenuation of extracellular glutamate elevation during ischemia.

Electroencephalographic burst suppression is regarded as a clinical endpoint for titration of CMRO<sub>₂</sub>, reduction by anesthetics [40]. Yamasaki et al. reported that a dose sufficient to induce a pattern of burst suppression was required for neuroprotection against incomplete global cerebral ischemia in rats undergoing intravenous propofol infusion [44]. In contrast, Warner et al. demonstrated that pentobarbital, which has effects similar to those of propofol on brain electrical activity, CMRO<sub>₂</sub>, and cerebral blood flow, showed a maximal neuroprotective effect against rat focal cerebral ischemia when infused intravenously at a dose corresponding to an active EEG [41]. Although the type of ischemia differed between these studies, Yamasaki et al. suggested dissimilar neuroprotective mechanisms between propofol and pentobarbital [44]. In our study, 3 and 10 mg/kg of propofol were respectively administered by single bolus injection into the cerebral ventricle, producing a similar but transient burst-suppres-
sion pattern before the initiation of ischemia. However, only 10 mg/kg propofol showed a neuroprotective effect. These results suggest that CMRO₂ suppression when ischemia was induced was minimal for neuroprotection by intracerebroventricular propofol under the conditions used here.

In rodent models of transient forebrain ischemia specifically, propofol demonstrates neuroprotection in terms of hippocampal CA1 damage [2,19,43]. The pyramidal cells of the hippocampal CA1 are selectively vulnerable to brief ischemia, and the specific pattern of their death, known as delayed neuronal death, occurs 3–4 days following the ischemic event [43]. Several mechanisms have been implicated in neuroprotection by propofol against delayed neuronal death. Propofol has been shown to inhibit the activation of NMDA-glutamate receptors of hippocampal neurons [15] and the subsequent massive increase in intracellular Ca²⁺ [8]; to reduce hippocampal neuronal damage induced by kainic acid [23]; and to enhance GABAergic function as effectively as midazolam, a neuroprotective benzodiazepine [19], thus providing a counteractive effect on glutamate neurotoxicity. Moreover, propofol possesses an antioxidative property because it contains a phenolic hydroxyl group that donates electrons to the free radicals generated during ischemia and reperfusion [24,32]. These beneficial characteristics may explain the neuroprotection afforded by intracerebroventricular propofol at the higher dose.

In the present study, all rats were subjected to cerebral ischemia under halothane/nitrous oxide anesthesia. In a previous study by Harp et al. [16], halothane anesthesia at a concentration of 0.6 and 2% reduced CMRO₂ by 20–30% and 50%, respectively. Saltzman et al. reported that halothane was neuroprotective against spinal ischemic injury in the rat [33]. On the other hand, nitrous oxide has been shown to worsen the neurological outcome following cerebral ischemia [7]. It is thus possible that the presently observed neuroprotective action of 10 mg/kg propofol was derived from a synergistic effect of propofol and halothane.

We have demonstrated by using a microdialysis biosensor that: (1) propofol and Intralipid similarly reduce extracellular glutamate increase in the hippocampal CA1; and (2) 10 mg/kg of propofol, though not Intralipid or 3 mg/kg of propofol, alleviates the delayed CA1 neuronal cell loss when administered intracerebroventricularly in a rat model of transient global forebrain ischemia. Together, these data indicate that intracerebroventricular propofol has a neuroprotective property that is not determined primarily by extracellular glutamate level during ischemia.

Acknowledgements

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References

increase in vascular capacitance is due to inhibition of sympathetic vasoconstrictive activity, Anesthesiology 89 (1998) 1495–1500.


