**Neuroprotective Effects of Propofol in a Model of Ischemic Cortical Cell Cultures**

**Role of Glutamate and Its Transporters**

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**Background:** During cerebral ischemia, excess of glutamate release and dysfunction of its high-affinity transport induce an accumulation of extracellular glutamate, which plays an important role in neuronal death. The authors studied the relationship among propofol neuroprotection, glutamate extracellular concentrations, and glutamate transporter activity in a model of ischemic cortical cell cultures.

**Methods:** Thirteen-day-old primary cortical neuronal-glial cultures were exposed to a 90-min combined oxygen-glucose deprivation (OGD) followed by reoxygenation. Propofol was added only during the OGD period, and its effect was compared to that of the N-methyl-D-aspartate receptor antagonist dizocilpine (MK-801). Twenty-four hours after the injury, cell death was quantified by lactate dehydrogenase release and cell viability by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Extracellular concentrations of glutamate in culture supernatants and glutamate uptake were performed at the end of OGD period by high-performance liquid chromatography and incorporation of L-[3H]glutamate into cells, respectively.

**Results:** At clinically relevant concentrations (0.05–10 μM), propofol offered protection equivalent to that of MK-801. In particular, a decrease in glutamate extracellular concentrations and a decrease of glutamate uptake were observed. The inhibition of the glial GLT1 transporter by 3-methylglutamate did not further modify the effect of propofol on glutamate uptake, suggesting that GLT1 was not the major target of propofol.

**Conclusion:** Propofol showed a neuroprotective effect in this in vitro model of OGD, which was apparently mediated by a GLT1-independent restoration of the glutamate uptake impaired during the injury.

Propofol (2,6-disopropylphenol), is widely used as an intravenous anesthetic. Recent experiments suggest that propofol can also enhance neurologic outcome and decrease the infarct size in animal models of stroke.1-6 In vitro attempts to clarify the ways in which propofol could interface with experimental ischemia still remain unclear. Several hypotheses have been considered, such as reduction in cerebral metabolism,1 potentiation of γ-aminobutyric acid-mediated inhibition,4 antioxidant activity,6 or direct antieuxitoxic properties.7

Extracellular glutamate concentrations increase during ischemia, leading to excitotoxic neuronal death mainly due to overstimulation of glutamate N-methyl-D-aspartate (NMDA) receptors.8 In physiologic conditions, the extracellular concentrations of glutamate depends on release into the synaptic cleft and uptake into cells by high-affinity glutamate transporters located both on neurons (EAAC1) and glial cells (GLAST and GLT1). The role of glutamate uptake during ischemia has been recently revisited in view of an inhibition and even of an inversion of the glutamate transport,9,10 thus allowing the neurotransmitter to be released from the nervous cells into the extracellular space. Extracellular glutamate concentration initially was not considered as a major determinant of the neuroprotective effect of propofol on in vivo models of ischemia.11 However, it has been reported that propofol restored the glutamate uptake impaired by oxidative stress on cultured astrocytes, probably by scavenging free radicals formed during the injury.12,13 So, a possible protective effect of propofol on glutamate transporter activity during ischemia is likely to occur and requires further study.

The aim of this study was first to quantify the putative neuroprotective effect of propofol on primary mixed cultures of neurons and astrocytes from rat cerebral cortex submitted to a transient oxygen-glucose deprivation (OGD). Such an experimental protocol was developed to reproduce, at least partly, the whole sequence of events occurring during ischemia and reperfusion. The noncompetitive NMDA receptor antagonist dizocilpine (MK-801) was used as the reference neuroprotectant.14,15 Second, the putative involvement of the extracellular glutamate concentrations and glutamate uptake activity in the mechanism of the neuroprotective effect of propofol was explored.

**Materials and Methods**

The experimental protocols, involving animals and their care, strictly conformed to the guidelines of the French Agriculture and Forestry Ministry (decree 87-848), authorized researcher P. Pisano, authorization No. 1359,
GLUTAMATE UPTAKE PROTECTION BY PROPOFOL DURING OGD

Preparation of Cell Cultures

Mixed cortical neuronal-glial cell cultures were prepared from cortex of 18-day-old Wistar rat fetuses. Briefly, after removal of meningeal, striatal, and hippocampal tissues and olfactory bulbs, cerebral cortices were pooled and maintained in ice-cold phosphate-buffered saline solution (PBS; Bio-Whittaker, Emerainville, France) supplemented with glucose (33 mM). Cells were mechanically dissociated from forebrains in dissociation medium by trituration through a fire-polished 9-in Pasteur pipette. After centrifugation (400 rpm for 10 min), the pellets were resuspended in Minimum Essential Medium with Earle’s salts, l-glutamine free (Life Technologies, Cergy-Pontoise, France), supplemented with 15 mM glucose (final concentration 20 mM), 5% fetal bovine serum, 5% horse serum (Sigma, Saint-Quentin Fallavier, France), 50 U/ml penicillin, and 50 μg/ml streptomycin and were plated to achieve a confluent monolayer (10^5 cells/cm²) on plastic 12-well culture plates (Costar, Brumath, France) previously coated with polyornithine (10 μg/ml). Culture dishes were incubated at 37°C in a humidified 6.0% CO₂ and 94.0% air atmosphere. Experiments were performed on mature cultures, after synaptogenesis, in a serum-free medium, at 13 days in vitro. Cell types at 13 days in vitro were determined to be 66.9 ± 4.5% neurons, 20.3 ± 5.7% astrocytes, and 12.8 ± 2.7% undetermined cells, using immunohistochemical staining (see Immunohistochemistry section).

Oxygen–Glucose Deprivation followed by Reoxygenation

Mixed cultures were exposed to a transient OGD as described by Goldberg and Choi with little modification. Cells were placed in an anaerobic chamber (partial pressure of oxygen [PO₂] maintained at < 2 mmHg), and the medium was exchanged three times with a glucose-free balanced salt solution (BSS₀–O₂: 116 mM NaCl, 5.4 mM KCl, 0.8 mM MgSO₄, 1.0 mM Na₂HPO₄, 1.8 mM CaCl₂, 26.2 mM NaHCO₃, 0.025 mM phenol red, 20 mM sucrose) bubbled with an anaerobic gas mix (95% N₂, 5% CO₂) for 30 min to remove residual oxygen. Cell cultures submitted to OGD were called control cells and were incubated in this solution at 37°C for a 90-min period to produce lethal oxygen deprivation. This OGD duration produced injury selectively limited to neurons, with no injury to the glial layer (see Results). Sham wash cell cultures, not submitted to OGD, were placed in BSS₀–O₂ containing 20 mM glucose and aerated for 10 min with an aerobic gas mix (95% air, 5% CO₂). OGD was terminated by removing cultures from the chamber, replacing the exposure solution with oxygenated Minimum Essential Media supplemented with 20 mM glucose, and returning the multiwells to the incubator under normoxic conditions. Drugs (propofol, MK-801) were added at the start of OGD and were removed by washout during the reoxygenation process. Twenty-four hours after the injury, assessment of neuronal damage was qualitatively performed by phase-contrast microscopy and quantitatively by two biochemical tests measuring the release of cytoplasmic lactate dehydrogenase (LDH) and the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and by counting of the immunolabeled neurons. Moreover, at the end of the OGD period, before reoxygenation, extracellular glutamate concentrations and glutamate uptake activity were determined.

Phase Contrast Microscopy

Neuronal injury was estimated by examination of cell morphology using a phase-contrast microscope at ×20, 24 h after the injury. A normal neuron was characterized by a soma with round and smooth shape, and processes that were uniform and smooth in appearance. Neurons were considered nonviable if they exhibited a rough appearance, irregular soma, or fragmented process.

LDH Release

Neuronal injury was quantitatively assessed by the measurement of LDH release into the bathing medium, immediately and 24 h after the OGD. This cytosolic enzyme is released from damaged or destroyed cells, and it has previously been established that LDH release correlates linearly with the number of damaged or dying neurons after both excitotoxic and apoptotic injuries. We used the Cytoxicity Detection Kit (LDH) from Roche Diagnostic (Meylan, France). Background LDH concentrations (12%) were determined in sham wash sister cultures and subtracted from experimental values to yield the LDH activity specific to the experimental injury. Results obtained immediately and 24 h after the injury were pooled and expressed as percent of the maximal LDH concentration corresponding to a near complete neuronal death without glial cell death (= 100), determined by assaying sister cell cultures exposed to 300 μM NMDA and 1 μM glycine (Tocris; Fisher Bioblock Scientific, Illkirch, France) for a full 24 h.

MTT Reduction Test

Neuronal viability was quantitatively evaluated by the MTT reduction test. This quantifies the formation of a dark blue formazan product formed by the reduction of the tetrazolium ring of MTT by the mitochondrial succinate dehydrogenase in living cells. Twenty-four hours after exposure to OGD, cultures were incubated with MTT (250 μg/ml) at 37°C in culture medium for 3 h. Cultures were then washed and incubated in 0.08 N HCl/isopropanol to dissolve the blue formazan product. Cell viability corresponded to the value of the optical density read at 570 nm with background subtraction at 630 nm. Values obtained from cultures exposed to 300 μM NMDA and 1 μM glycine during the 24 h after the

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OGD (100% neuronal death) were subtracted from those measured in all experimental conditions. Results were expressed as percent of the optical density measured in sham wash cells.

**Immunohistochemistry**

To determine the number of astrocytes and neurons present in the culture, cells were plated on polyornithine-coated coverslips instead of 12-well plates and fixed, 24 h after the injury, with 4% (w/v) paraformaldehyde for 10 min. After washing with PBS, cells were pre-incubated (30 min at room temperature) in 18.3 mg/ml lysine (Sigma) and then for 10 min in PBS containing 3% (w/v) sheep serum (Sigma) and 0.1% (w/v) Triton X-100. For double immunochemical staining, cells were incubated (1 h at room temperature) using two primary antibodies. Neurons were labeled with mouse monoclonal antimicrotubule-associated protein (MAP2a; 1:450 dilution; Roche Diagnostics), and astrocytes were labeled with rabbit monoclonal anti-gliafibrillary acidic protein (GFAP; 1:500 dilution; Dako, Trappes, France). Following extensive rinsing with PBS, coverslips were exposed for 1 h at room temperature to secondary antibodies from Molecular Probes (Interchim, Montluçon, France): Alexa Fluor 488 goat antirabbit immunoglobulin G (H+L) and Alexa Fluor 546 goat antimouse immunoglobulin G (H+L) diluted 1:250 in PBS containing 3% (w/v) sheep serum and 0.1% (w/v) Triton X-100. Nuclear counterstaining was performed with 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI; 1:1,000 dilution; Sigma). Finally, the slides were mounted in Fluorsave (Calbiochem, Merck Eurolab, Strasbourg, France) before examination with a fluorescence microscope (Leica Microsystems, Marseille, France). The numeration was performed 24 h after the OGD exposure, with or without propofol. Results were expressed as percent of the total number of nuclei counterstained.

**Measurement of Extracellular Amino Acids**

Extracellular concentrations of glutamate in the culture supernatants were measured in duplicate at the end of the OGD period by high-performance liquid chromatography. An aliquot of 250 μl culture supernatant was deproteinized by 250 μl perchloric acid, 0.5 N, mixed and centrifuged (30,000 rpm for 10 min) at 4°C. The supernatant was then stored at −80°C until high-performance liquid chromatography analysis. All samples were analyzed for glutamate content using high-performance liquid chromatography along with a fluorometric detection (scanning fluorescence detector 474; Waters Corporation, Saint Quentin en Yvelines, France) after precolumn derivatization with o-phthalaldehyde using a modified version of the method described by Geddes and Wood.

Each sample was diluted 1:20 in 0.15 M sodium borate buffer, and 10 μl of the dilution was directly injected into a C-18 column (5 μm Spherisorb; Richard Scientific, Novato, CA; ODS2 150 × 3 mm). The mobile phase consisted of 0.1 m potassium acetate (pH 5.8) in methanol (v/v, 1/7). Elution was performed with a methanol gradient ranging from 12.5 to 56%. Glutamate concentrations in the culture supernatants were calculated with reference to a standard mixture (Millenium32 version 3.05; Waters Corporation, Milford, MA). Results were expressed in micromolars.

**Uptake Experiments**

Uptake experiments were performed at the end of the injury on sham wash and on control cells, treated or untreated with propofol. In experiments performed to test the potential direct effect of propofol on basal glutamate uptake, the compound was added on sham wash cells 90 min before uptake measurements. After two washes of the cells with the uptake buffer (10 μM glucose, 5 mM KCl, 127 mM NaCl, 2.5 mM CaCl2, 0.2 mM MgSO4, 0.3 mM NaH2PO4, and 10 mM HEPES; pH 7.4), uptake assays were performed as previously described.

Briefly, the reaction started by adding L-[3H]glutamate (specific activity 15–25 Ci/mmol NEN, Paris, France) at 10−6 M final concentration, diluted in the buffered physiologic medium. Incubations were performed at 37°C for 2 min. The reaction was stopped by rapidly adding 1 ml cold, sodium-free buffer, followed by two washes with the same cold medium. To dissolve the cells, 1 N NaOH was added to the culture dishes, and the radioactivity was assessed by liquid scintillation counting at least 12 h later. To determine the part of radioactivity not due to sodium-dependent glutamate transport, L-[3H]glutamate uptake was assessed in physiologic medium in which sodium was omitted and replaced by choline. Glutamate transport was calculated as glutamate uptake rate in the presence of sodium minus glutamate uptake in the absence of sodium. Protein content was determined by the method developed by Lowry et al. with bovine serum albumin as the standard. To estimate the role of the glial glutamate transporter 1 (GLT1) in the measured uptake rates, some incubations were performed in the presence of threo-3-methyl glutamate (3-MG; Tocris; Fisher Bioblock Scientific), an inhibitor of this carrier, which was dissolved in water and added at the time of the uptake experiments. Results (pmol glutamate/mg protein/min) were expressed as percent of the value obtained in sham wash cells.

**Statistical Analysis**

Statistical analysis was performed for each parameter, investigated by one-way analysis of variance followed by post hoc Tukey test for multiple comparisons (SAS version 8.1; SAS Institute Inc., Cary, NC). Values are reported as mean ± SD. Differences were considered significant for P < 0.05.
2,6-Diisopropylphenol (pure propofol) was supplied by AstraZeneca (Rueil Malmaison, France) and was dissolved in dimethylsulfoxide shortly before application to obtain final concentrations of 0.005–10 μM in the culture medium. Propofol was added at the beginning of the OGD period, and final dimethylsulfoxide concentration never exceeded 0.1%. The vehicle control experiments were performed by using the same amounts of dimethylsulfoxide and showed that the vehicle alone had no effect on the activities measured (data not shown). Dizocilpine maleate (MK-801; Tocris; Fisher Bioblock Scientific) a powerful noncompetitive NMDA glutamate receptor antagonist, was dissolved in water and added at 10 μM final concentration to the culture medium in a similar fashion to propofol for each experiment. Threo-3-methyl glutamate, a relatively specific inhibitor of the glial glutamate transporter GLT1,22 was dissolved in water and added at 300 μM final concentration in the uptake medium.

**Results**

**Protective Effects of Propofol against OGD-induced Neuronal Injury**

Neuronal damage was first assessed by light microscopic observations of either sham wash cells (fig. 1, Aa) or oxygen-glucose-deprived cells 24 h after a 90-min injury (fig. 1, Ab). As soon as the cells were removed from the anaerobic chamber, an osmotic swelling of the cell bodies was observed, followed by their shrinkage and disintegration of processes or irregular borders cell bodies, during the first hours after the reoxygenation. Finally, OGD elicited marked cell injury and accumulation of cellular debris 24 h after the injury (fig. 1, Ab). Propofol, 10 μM (fig. 1, Ac), and MK-801, 10 μM (fig. 1, Ad), added during OGD markedly decreased ischemic damages. Scale bar, 20 μm. (B) Compared to sham wash cultures (Ba), OGD elicited a dramatic and significant decrease of the number of neurons (MAP2a; red) in control cultures (Bb). The number of underlying glial cells (GFAP; green) was not affected by the OGD, but the morphologic characteristics of astrocytes shifted from a stellar to a flat configuration. Degenerative cells were only labeled by DAPI (blue). Propofol (Bc) and MK-801 (Bd) exhibited a neuroprotective effect against ischemic injury. Scale bar, 20 μm.
Twenty-four hours after the oxygen–glucose deprivation (OGD) period, cell counting was performed after staining with mouse monoclonal antimicrotubule-associated protein (MAP2a) for neurons and rabbit monoclonal antiglial fibrillary acidic protein (GFAP) for astrocytes. Nuclear counterstaining was performed with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) to estimate the total amount of cells. OGD elicited a dramatic and significant decrease of the neuronal count in control cultures compared to sham wash cultures. Propofol partially reversed this decrease in a more potent fashion than MK-801. The number of the GFAP/DAPI-positive cells (underlying glial cells) was not affected by the injury. The numeration was performed on three independent cultures, and four distinct fields (approximately 150 cells) were counted per culture. Cell counts were expressed as mean ± SD (% of the total number of cells estimated by the DAPI staining.

* P < 0.05 versus sham wash. † P < 0.05 versus control. ‡ P < 0.05 versus propofol.

Propofol Inhibited the OGD-evoked Increase of the Glutamate Extracellular Concentration

At the end of the 90-min OGD exposure, before reoxygenation, glutamate extracellular concentrations were significantly increased compared to those measured in sham wash cells (fig. 3; control: 17.5 ± 5.4 μM vs. sham wash: 1.3 ± 0.8 μM). Propofol (10 μM) did not modify the glutamate extracellular concentration in sham wash cells. However, when propofol (concentrations at least equal to 0.05 μM) was added during the injury, it counteracted the OGD-induced elevation of glutamate concentrations. Moreover, propofol (0.5–10 μM) brought the extracellular glutamate concentration to sham wash amounts.
Propofol Partially Restored the Excitatory Amino Acid Uptake Rate after OGD Exposure: Role of Pharmacological Blockade of GLT1

At the end of the 90-min OGD, before reoxygenation, we observed a 40.6 ± 13.3% impairment of the uptake rate measured in sham wash cells (fig. 4). Propofol did not exhibit any effect on basal excitatory amino acid uptake rate measured on sham wash cells. However, after the OGD, propofol (0.005–10 μM) counteracted in a dose-dependent manner the OGD-induced decrease of the apparent glutamate uptake rate. Moreover, 10 μM propofol maintained the glutamate uptake rate at the level measured in sham wash cells. To clarify the involvement of the glial glutamate transporter GLT1 in the uptake activity measured at the end of OGD with or without propofol, experiments were also performed in the presence of 300 μM 3-MG, which preferentially inhibits this carrier (fig. 5). 3-MG induced a 42.4 ± 14.1% decrease of the glutamate uptake rate measured in sham wash cells but did not further affect this activity after a 90-min OGD exposure (percent sham wash; control: 59.4 ± 13.3% vs. OGD + 3-MG: 53.4 ± 10.3%). Another GLT1 inhibitor, dihydrokainate (500 μM), exhibited the same effects (data not shown). Moreover, the glutamate uptake rate measured in OGD cells treated with 0.5 μM propofol did not differ significantly in the presence or in the absence of 3-MG.

Discussion

The neuroprotective effect of propofol on in vivo models of focal1–5 or global ischemia6–24 seems to be well established. Numerous in vitro studies have attempted to elucidate the mechanisms of this propofol-mediated neuroprotection, but most of the experiments were performed on models that only mimicked some of the damage observed during ischemia such as NMDA toxicity25,26 or oxidative stress on cultured rat cortical astrocytes.12,13

In this study, using a transient OGD on primary mixed cultures of neurons and astrocytes from rat cerebral cortex, we have shown that propofol partially reversed the OGD-induced neuronal death 24 h after the injury, as well as the increase in extracellular glutamate concentrations and the decrease of glutamate uptake activity both observed at the end of the ischemic injury. Moreover, we also reported that the glial glutamate transporter GLT1 did not seem to be a major target site for the protective effect of propofol.

The propofol concentrations used were chosen to be as close as possible to the concentrations of free propofol measured in the extracellular fluid of the somatosensory cortex in rats anesthetized with propofol (1 μM),27 and to the propofol concentrations which scavenged peroxynitrite in human hepatic microsomes (2 μM).28

Fig. 4. Propofol curtailed the oxygen–glucose deprivation (OGD)–induced decrease of the glutamate uptake rate. Uptake experiments were performed at the end of the injury, before reoxygenation. A 90-min OGD elicited a 40.6 ± 13.3% impairment of the glutamate uptake compared to the transport activity measured in sham wash (SW) cells. Propofol counteracted, in a dose-dependent manner, this decrease of the glutamate uptake rate. Moreover, 10 μM propofol maintained the glutamate uptake rate at the level measured in sham wash cells. *P < 0.05 versus control. n = 6 cultures per condition pooled from three experiments.

Fig. 5. Protective effect of propofol on glutamate transporters did not involve the astroglial glutamate transporter (GLT1). Uptake experiments were performed at the end of the injury, before reoxygenation. Experiments were performed in the presence or absence of 300 μM threo-3-methyl glutamate (3-MG), a rather selective inhibitor of GLT1. 3-MG induced a 42.4 ± 14.1% decrease of the glutamate uptake rate measured in sham wash cells but did not further affect this activity after a 90-min oxygen–glucose deprivation. Moreover, the glutamate uptake rate measured in oxygen-glucose–deprived cells treated with 0.5 μM propofol did not differ significantly in the presence or in the absence of 3-MG. *P < 0.05 versus without 3-MG. n = 12 cultures per condition pooled from three experiments.
induced an allosteric inhibition of NMDA receptors and protected cultured hippocampal neurons against NMDA neurotoxicity. On the other hand, propofol exhibited either a lack of protection against NMDA excitotoxicity in primary brain cell cultures or a worsening of the NMDA-induced neuronal damage on hippocampal slices. Thus, an NMDA receptor-mediated neuroprotective effect of propofol could not be ruled out, but this did not seem to be the main mechanism of its antiischemic properties.

Other indirect antiexcitotoxic mechanisms could be involved in the neuroprotective effects of propofol during ischemia, such as the inhibition of the efflux of glutamate. Accumulation of glutamate in the extracellular space after in vivo or in vitro experimental ischemia is now a well-known phenomenon depending on an excessive release coupled to a decreased or reversed glutamate uptake process. At the end of the 90-min injury, we also described an OGD-induced increase in extracellular glutamate concentrations, which was similar to that observed in in vivo and in vitro models of cerebral ischemia. We reported here that propofol, although lacking an effect on basal extracellular glutamate concentrations in sham wash cells, counteracted the OGD-induced increase in extracellular glutamate concentrations. This effect is probably not due to the inhibition of glutamate release because Bickler et al. reported a lack of effect of propofol on hypoxia-evoked glutamate release on cortical brain slices. The maintenance of nonexcitotoxic glutamate concentrations during ischemic conditions in the presence of propofol could be due to a decrease of glutamate release dependent on presynaptic depolarization or on oxidative stress-induced astrocytic efflux but did not seem to be related to an effect on the reversal of the glutamate uptake process. We rather suggested that propofol could lower extracellular glutamate concentrations after OGD at least in part via a drug-induced preservation of the glutamate transport activity. Propofol was inactive by itself on the glutamate uptake rate of the sham wash cultures in normoxic conditions. This lack of effect of propofol on glutamate uptake has also been described on synaptosomes from various rat brain regions and on cultured astrocytes. However, we reported here for the first time that, at clinically relevant concentrations, propofol curtailed the glutamate uptake rate inhibition in cultures exposed to OGD. This protective effect of propofol on the glutamate uptake may be due to several phenomena. First, reactive oxygen species, formed during and after ischemia, may induce an inhibition of the glutamate uptake either directly by oxidation of the sulfhydryl groups of the glutamate carriers or indirectly via the acidification of intracellular pH due to the sodium-hydrogen exchanger oxidation. Propofol’s specific structure, including a phenolic hydroxyl group, confers free radical scavenging and therefore antioxidant properties leading to the protection of glutamate transporters and sodium-hydrogen exchanger after oxidative injury of primary astrocytes cultures by tert-butyl-hydroperoxide. So, in a similar fashion, in our in vitro model of OGD, propofol may limit the OGD-induced impairment of glutamate uptake via its antioxidant properties.

Second, another possibility could be that propofol may protect the transport activity via protein kinase C activation. We have previously shown that this kinase up-regulated the glutamate uptake rates on synaptosomes and nervous cell cultures. Do et al. also reported that the propofol-mediated enhancement of the activity of the neuronal glutamate transporter EAAC1 in Xenopus oocytes required the activation of protein kinase C.

Whatever the mechanism involved in the propofol-mediated protection of the glutamate transporters during OGD, the residual transport activity measured in control cells treated by this drug did not seem to reflect a GLT1-dependent process, since 3-MG, a rather specific GLT1 inhibitor, did not further inhibit glutamate uptake during OGD. However, 3-MG was able to dramatically reduce the uptake rate of the sham wash cells, indicating that the glutamate transport process in our normoxic experimental conditions relied for a great part on GLT1. The fact that 3-MG did not further decrease the glutamate transport activity during OGD may suggest that the GLT1-mediated glutamate uptake was totally inhibited by ischemic conditions. Similar findings have been reported during ischemia on hippocampal slices in the presence of dihydrokainate, another specific GLT1 blocker. Moreover, the effect of propofol on the glutamate uptake during OGD was not affected by 3-MG, indicating that propofol protected a transport mechanism which probably relied on glutamate transporters other than GLT1—the glial glutamate aspartate transporter or the neuronal excitatory amino acid carrier 1, both expressed in our experimental conditions.

In conclusion, this study confirmed a neuroprotective effect of propofol, at clinically relevant concentrations, in an in vitro model of OGD. It could be assumed that this beneficial effect of propofol on neuronal viability is mediated, at least in part, by a restoration of the glutamate transport activity, which is dramatically impaired by the OGD, thus allowing extracellular glutamate concentrations to be maintained below excitotoxic concentrations. Finally, our results also indicate that propofol protects a GLT1-independent transport mechanism, which could be mediated by the glial glutamate aspartate transporter or the neuronal excitatory amino acid carrier 1. Further research is needed to clarify the mechanism of the effect of propofol on glutamate transporter activity (increase in the number of membrane transporters or increase in the affinity of the transporters for glutamate).
References


