

Anesthetic Concentrations of Propofol Protect against Oxidative Stress in Primary Astrocyte Cultures

Comparison with Hypothermia

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Background: **The extracellular concentration of glutamate in the brain increases after oxidative damage. This increase may be caused, in part, by changes in glutamate transport by astrocytes. The authors hypothesized that propofol and hypothermia mitigate the effects on astrocytes of oxidative stress.**

Methods: **Primary cultures of rat cerebral astrocytes were subjected to oxidative stress by incubation with *tert*-butyl hydroperoxide for 30 min, followed by a 30–90-min washout period. The effects of prophylactic (simultaneous with *tert*-butyl hydroperoxide application) and delayed (administered 30 min after the oxidant) propofol or hypothermia were determined by measuring the uptake of glutamate as well as the release of preloaded D-aspartate (a nonmetabolizable analog of glutamate) and endogenous lactate dehydrogenase (a cytosolic marker).**

Results: **Delayed administration of an anesthetic concentration of propofol (1–3 μM) prevented the inhibition of high-affinity glutamate uptake, stimulation of D-aspartate release, and increase in lactate dehydrogenase release caused by *tert*-butyl hydroperoxide (1 mM, 37°C). The protective effect of propofol (EC_{50} = 2 μM) on glutamate uptake was 20-fold more potent than that of α-tocopherol (EC_{50} = 40 μM). Prophylactic hypothermia (28 and 33°C) also protected astrocytes from *tert*-butyl hydroperoxide. Delayed hypothermia was not protective but did not compromise rescue by propofol.**

Conclusions: **Clinical levels of propofol and hypothermia mitigate the effects of oxidative stress on astrocytic uptake and retention of glutamate, with propofol having a relatively larger therapeutic window. The ability of these treatments to normalize cell transport systems may attenuate the pathologic increase in extracellular glutamate at synapses and thus prevent excitotoxic neuronal death.**

ANESTHETIC concentrations of propofol (2,6-diisopropylphenol) protect against ischemic brain injury in animal models.[1–6] The structure of propofol is unlike other hypnotic sedatives but resembles the native antioxidant α-tocopherol (vitamin E) in containing a phenolic OH-group. This anesthetic scavenges free radicals, reduces disulfide bonds in proteins, and inhibits lipid peroxidation.[7–11] Mild or moderate hypothermia, typically at 28–33°C, also confers cerebral protection in animals during global or focal ischemia and appears to improve outcome in patients during cerebral aneurysm surgery.[12–17] A contributing factor may be that suppression of oxidative metabolism by propofol[1] and hypothermia[16,18,19] slows the production of reactive oxygen species on reperfusion. Furthermore, both propofol[11] and hypothermia[20,21] oppose oxidative modification of cell proteins and lipids. Brain cooling also attenuates the brain edema and the elevation of intracranial pressure and extracellular L-glutamate concentration caused by experimental stroke.[18,19,22]

Astrocytes play a dominant role in sequestering synaptically released glutamate. This clearance mechanism is essential for normal glutamatergic transmission and protects neurons from excitotoxic injury.[23–26] Elevation of extracellular glutamate concentration after ischemia-reperfusion in brain may result from impaired uptake and accelerated release of glutamate from oxidatively stressed astrocytes. High-affinity uptake of glutamate occurs through a Na^+-dependent mechanism of secondary active transport that may be more sensitive to oxidative injury than are facilitative transport systems, such as those that mediate glucose uptake. Astrocytes also release glutamate through volume-sensitive organic anion channels (VSOAC; also named volume expansion-sensing outward rectifier anion channels) that become activated when these cells swell after ischemic brain injury.[27] Oxidative stress in cultured astrocytes causes a dysregulation of osmotic control that leads to activation of VSOAC.[28] The glutamate release mechanism can be studied in cultured astrocytes preloaded with radiolabeled D-aspartate, which is a nonmetabolizable analog of glutamate.

In vitro studies are appropriate for studying mechanism and time of action at the cellular level. They can show unambiguously which cell types are susceptible to specific interventions. In particular, experiments with primary astrocyte cultures can distinguish readily between changes in glutamate uptake and release, two processes that are difficult to resolve *in situ*. Hypothermia often occurs in patients during propofol anesthesia.[29] The purpose of the present study was to compare the effects of propofol and hypothermia on astrocytic glutamate uptake and release after oxidative stress. We hypothesized that clinically relevant levels of propofol and hypothermia protect astrocytes from injury. Furthermore, we probed the mechanism of action of propofol by comparing it to drugs that modulate lipid peroxida-

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tion (α-tocopherol), γ-aminobutyric acid $(GABA)_A$ receptors (thiopental, midazolam), and glutamate receptors (ketamine).

Methods

Materials

L-[^{3}H]Glutamic acid (38–46 Ci/mmol), D-[^{3}H]aspartic acid (20 Ci/mmol), and 2-deoxy-D-[^{3}H]glucose (26 Ci/mmol) were purchased from Amersham Canada (Oakville, Ontario, Canada). Ketamine was purchased from Warner-Lambert Canada (Scarborough, Ontario, Canada), midazolam from Hoffmann–La Roche Canada (Mississauga, Ontario, Canada), and thiopental from Abbott Lab Limited Canada (Montreal, Quebec, Canada). D-Aspartate, 2-deoxy-D-glucose, 1,9-dideoxyforskolin, L-glutamate, *tert*-butyl hydroperoxide (t-BOOH), and the lactate dehydrogenase (LDH) assay kit (pyruvate start procedure) were obtained from Sigma Chemical Company (St. Louis, MO). Horse serum was purchased from Gibco Laboratories (Burlington, Ontario, Canada). Propofol was purchased from Aldrich Chemical Company (Oakville, Ontario, Canada), and Intralipid from Clintec Nutrition Company (Mississauga, Ontario, Canada). Propofol was dissolved in either ethanol or Intralipid. α-Tocopherol, thiopental, midazolam, and ketamine were dissolved in ethanol, and 1,9-dideoxyforskolin was dissolved in dimethylsulfoxide. Control cultures received the same concentration of each vehicle (Intralipid 0.2 μl/ml, ethanol 3 μl/ml, or dimethylsulfoxide 3 μl/ml) as did the drug-treated cultures.

Cell Cultures

The experimental protocols were approved by the University of Western Ontario Council on Animal Care. One-day-old Wistar rats were decapitated, and the neopallium was used to prepare primary cultures of astrocytes, according to our published procedure.[30] The method used depends on differential maturation of glial and neuronal cells. The neuronal population is relatively well differentiated, and therefore neurons tend not to survive the mechanical dissociation and culture conditions. Furthermore, the use of serum favors growth of type-1 astrocytes instead of oligodendrocytes. The astrocyte cultures were grown in horse serum–supplemented, minimum essential medium (MEM). They reached confluence after 2 weeks, when each 60-mm dish contained approximately 3 million cells. The cultures were nearly homogenous for cells that express the astrocyte markers, glial fibrillary acidic protein, and connexin43 gap junction protein.[31,32] These cultures were used after 14–22 days in culture.

Experimental Procedures

To evaluate the effects of oxidative injury on the uptake systems for glutamate and glucose, the astrocytes first were incubated for 3 h in serum-free MEM (pH 7.3, equilibrated with 5% CO_2:95% air; 37°C). Next, the cells were incubated for 30 min in transport medium (containing 134 mM NaCl, 5.2 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 10 mM glucose, and 20 mM HEPES; 300 mOsm; pH 7.3, equilibrated with air). The organic peroxide, t-BOOH (1 μl/ml; 1 mM final concentration), was added to produce oxidative stress, while aqueous vehicle was added to control cultures. Subsequently, the cells were washed and incubated for another 30 min in transport medium that did not contain t-BOOH. The effects of prophylactic (simultaneous with the application of t-BOOH) and delayed (administered 30 min after t-BOOH) administration of propofol or changes in temperature (28–37°C), as well as delayed treatment with α-tocopherol, thiopental (100 μM), midazolam (5 μM), ketamine (100 μM), or the VSOAC blocker dideoxyforskolin (100 μM),[33] were determined. Thermostats maintained the incubators and water baths at the appropriate temperatures during the experiments.

To assess secondary active transport of glutamate, the initial rate of Na^+-dependent glutamate uptake was measured as described previously.[34] Briefly, the astrocytes were washed in HEPES buffered transport medium (pH 7.3, 37°C) and then were incubated for 1 min with [^{3}H]glutamate (100 μM, 10 mCi/mmol) in the same medium. To assess facilitated transport of glucose, the initial rate of 2-deoxyglucose uptake was measured in glucose-free transport medium according to our published procedure.[30] The astrocytes were washed in HEPES buffered transport medium (pH 7.3, 23°C) and then incubated for 1 min with 2-deoxy-D-[^{3}H]glucose (60 μM, 3 mCi/mmol) in the same medium. At the end of the uptake periods, cells were washed three to five times in ice-cold Tris-sucrose buffer (pH 7.3) to halt radiotracer uptake and were then scrape-harvested in 1 ml of ice-cold water. Aliquots of media and cell harvests were combined with scintillation cocktail, and their radioactive contents were analyzed by liquid scintillation counting. Uptake rates were expressed per milligram cell protein, which was measured by the Lowry method.

The rate of release of preloaded D-aspartate (a nonmetabolizable analog of L-glutamate) from astrocytes was measured using a modification of the procedure described previously.[34] In our previous study, we loaded astrocytes overnight with D-aspartate in serum-supplemented MEM. For the present experiments, astrocytes were rendered more sensitive to t-BOOH by incubation in serum-free MEM (pH 7.3, equilibrated with 5% CO_2: 95% air; 37°C) for the overnight loading with D-[^{3}H]aspartate (10 μM, 0.5 μCi/ml). Subsequently, the cells were washed (time zero of efflux) and incubated for 30 min in HEPES-buffered transport medium with or without 1 mM t-BOOH. Next, the cells were incubated for a further 90 min in transport medium that lacked t-BOOH but did contain propofol or propofol vehicle. The efflux media

were sampled every 30 min throughout the experiment by removing 1-ml aliquots and replacing them with an equivalent volume of fresh incubation medium. Finally, D-[^{3}H]aspartate was measured in media aliquots and scrape-harvested cells by liquid scintillation counting. Efflux rates were corrected for the small volume of radioactive loading medium that was not removed by washing at time zero of efflux (1 μl/mg cell protein).[35] Cumulative efflux rates are expressed as percentages of the D-[^{3}H]aspartate present in the cells at the beginning of the efflux period.

Cell injury also was assessed by monitoring the release of the cytosolic marker, LDH, and by observing astrocyte morphology with video microscopy. LDH release was expressed as a percentage of the total LDH present in the control cells. During video recording, the temperature of the medium was maintained at 37°C using a temperature probe present in the incubation medium that was connected to a thermostat and heat lamp.

Statistics

Data are presented as mean ± SD values from n number of experiments, with duplicate or triplicate replications (*i.e.*, two or three culture dishes per treatment) in each experiment. The *t* test (two-tailed) was used to compare mean values based on a single level of treatment. One-way analysis of variance or repeated-measures analysis of variance and the Tukey-Kramer multiple comparison test were used to evaluate the effects of treatments. *P* less than 0.05 was considered significant.

Results

Effects of t-BOOH and Propofol at 37°C

Exposure to organic peroxide (t-BOOH) inhibited the rate of astrocytic glutamate uptake and both prophylactic and delayed administration of an anesthetic concentration of propofol (1 μM) attenuated this effect (table 1). Propofol was dissolved in ethanol for comparison with other drugs dissolved in this vehicle (α-tocopherol, thiopental, midazolam, and ketamine; table 1 and fig. 1), but it was dissolved in Intralipid for later experiments to resemble more closely the commercial preparations of the anesthetic that are administered to patients. Propofol 1 μM had the same effect on glutamate transport rate in oxidatively stressed astrocytes whether the anesthetic was delivered in ethanol of Intralipid vehicle (data not shown). The potency of propofol (EC_{50} = 2 ± 1 μM) was significantly greater than that of α-tocopherol (44 ± 1 μM; $P < 0.05$; fig. 1), whereas the intravenous anesthetics thiopental, midazolam, and ketamine were ineffective (table 1).

In contrast to Na^+-dependent glutamate uptake, facilitated glucose transport was not affected by identical exposures to t-BOOH and propofol. Thus, initial rates of 2-deoxyglucose uptake for astrocytes exposed to 1 mM t-BOOH and Intralipid seriatim (40 ± 6 μmol · g protein^{-1} · min^{-1}) did not differ from cells exposed to t-BOOH and 1 μM propofol seriatim (38 ± 5 μmol · g protein^{-1} · min^{-1}) or from those exposed to aqueous vehicle and Intralipid seriatim (39 ± 4 μmol · g protein^{-1} · min^{-1}) (n = 3 independent experiments with triplicate determinations in each). These normal rates of passive transport indicate that, with or without propofol, the plasma membrane remained intact 60 min after t-BOOH exposure began.

Table 1. Comparison of the Effects of Intravenous Anesthetics and α-Tocopherol on Glutamate Uptake in Astrocytes Exposed to t-BOOH

Treatment	Glutamate Uptake Rate (μmol · g protein^{-1} · min^{-1})
Prophylactic drug treatment during t-BOOH exposure	
Control	33 ± 9
t-BOOH	2 ± 1*
t-BOOH and propofol (1 μM)	21 ± 3†
t-BOOH and propofol (8 μM)	28 ± 9†
Delayed drug treatment after t-BOOH exposure	
Control	36 ± 8
t-BOOH, then vehicle	5 ± 4*
t-BOOH, then propofol (1 μM)	16 ± 8*†
t-BOOH, then propofol (8 μM)	21 ± 5*†
t-BOOH, then ketamine (100 μM)	9 ± 6*
t-BOOH, then thiopental (100 μM)	4 ± 3*
t-BOOH, then midazolam (5 μM)	2 ± 1*

Astrocytes were incubated for 3 h in serum-free minimum essential medium (pH 7.3, equilibrated with 5% CO_2:95% air; 37°C). They next were incubated for 30 min in HEPES-buffered medium, with 1 mM *tert*-butyl hydroperoxide (t-BOOH) or without this organic peroxide (control). A 30-min recovery period followed before the initial rate of glutamate uptake was measured in 1-min transport assays. Drugs were administered in ethanol vehicle, either simultaneously with t-BOOH (prophylactic drug treatment) or after the incubation with t-BOOH had ended (delayed drug treatment). In both cases, the drugs were present for the remainder of the experiments.

* $P < 0.05$ compared with control. † $P < 0.05$ compared with t-BOOH-treated cells (n = 3–13 independent experiments with triplicate determinations in each).

The ability of astrocytes to retain excitatory amino acids was investigated in astrocytes that had been preloaded overnight with D-[^{3}H]aspartate in serum-free MEM. t-BOOH (1 mM, 30 min) stimulated D-aspartate release after a latent period of 60 min, and 3 μM propofol prevented this effect (fig. 2). Because oxidative stress leads to activation of VSOAC in astrocytes,[28] we investigated if the VSOAC blocker dideoxyforskolin (100 μM) could inhibit the propofol-sensitive component of excitatory amino acid efflux. We observed that dideoxyforskolin inhibited partially the efflux of D-aspartate caused by t-BOOH (fig. 3).

The protein content of cell-attached astrocytes did not change during the first 60 min after addition of t-BOOH to the cultures (data not shown). After 120 min, the cell protein content in cultures exposed to t-BOOH (362 ± 109 μg protein–culture) also did not change significantly

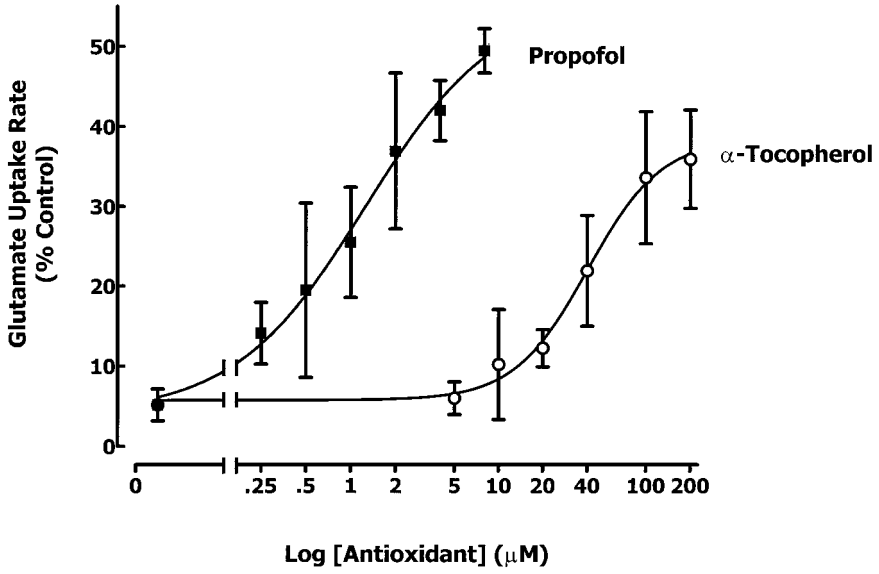

Fig. 1. Propofol and α-tocopherol protect glutamate uptake with different potencies. Astrocytes cultures were incubated with *tert*-butyl hydroperoxide (1 mM) or aqueous vehicle for 30 min and then were incubated with propofol (0.2–8.0 μM), α-tocopherol (5–200 μM), or ethanol vehicle for another 30 min. Subsequently, the rate of glutamate uptake was measured (100 μM, 1 min) at 37°C. Plotted are the mean ± SD values expressed as percent control for n = 3 experiments (with triplicate determinations in each).

($P < 0.05$) compared with either control or (300 ± 103 μg protein–culture) or those cultures exposed to t-BOOH and propofol seriatim (325 ± 80 μg protein–culture; n = 7 experiments). However, microscopic examination of the astrocytes showed that blebbing of the plasma membrane occurred after t-BOOH and preceded cell lysis. LDH efflux rate was measured to evaluate further the integrity of the plasma membrane. No LDH efflux was detectable from control cells during the 120-min incubation period. Astrocytes that were incubated with t-BOOH and 3 μM propofol seriatim had significantly less LDH release at 120 min (1.0 ± 0.5%) than did those incubated with t-BOOH and Intralipid (28.3 ± 9.3%; $P < 0.05$; n = 3 independent experiments). Thus, delayed administration of propofol prevented oxidative disruption of the astrocyte plasma membrane at 37°C.

Effects of Hypothermia on Astrocytes Exposed to t-BOOH

The initial rate of glutamate uptake was slowed by cooling astrocytes during the 1-min transport assay to 33°C (31 ± 8 $\mu mol \cdot g\ protein^{-1} \cdot min^{-1}$) or 28°C (25 ± 6 $\mu mol \cdot g\ protein^{-1} \cdot min^{-1}$), compared with the 37°C control (44 ± 13 $\mu mol \cdot g\ protein^{-1} \cdot min^{-1}$) ($P < 0.05$; n = 4 independent experiments with triplicate replications in each). This demonstrated a direct inhibitory effect of mild and moderate hypothermia on the secondary active transport of glutamate. However, this effect was completely reversible because the glutamate uptake rate returned to control values when astrocytes that had been cooled to 33 or 28°C for 60 min were rewarmed to 37°C for a 1-min transport assay (fig. 4).

To test whether prophylactic hypothermia alters injury

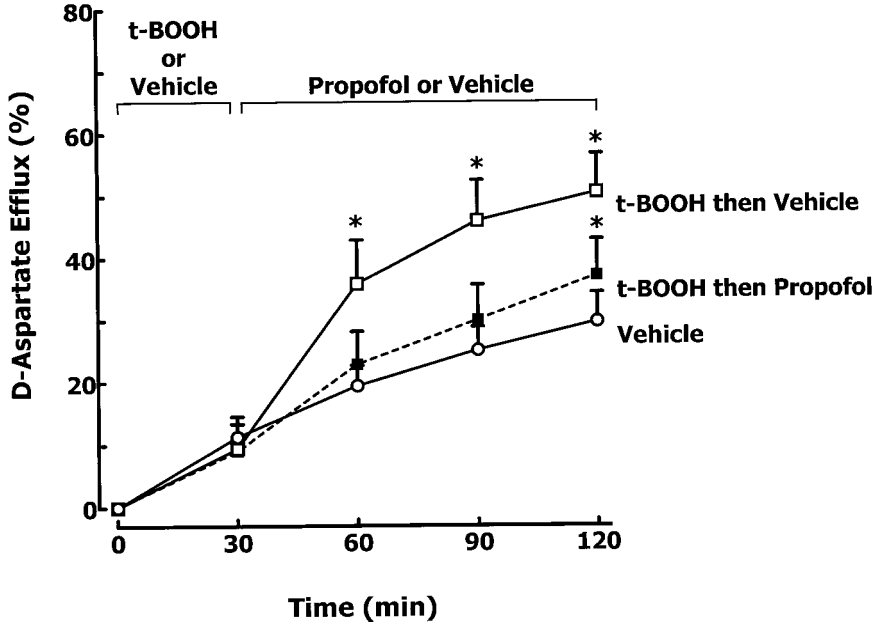

Fig. 2. Time course of D-aspartate efflux from astrocytes exposed to organic peroxide and propofol seriatim. Astrocytes were preloaded with D-[^{3}H]aspartate (10 μM) overnight. Subsequently, beginning at time zero of efflux, they were incubated with *tert*-butyl hydroperoxide (t-BOOH; 1 mM) or aqueous vehicle for 30 min, followed by incubation with propofol (1 μM) or Intralipid vehicle for an additional 90-min period (37°C). Cumulative D-aspartate efflux rates are expressed as percentages of the D-aspartate present in the cells initially. Plotted are the mean ± SD values for seven experiments with triplicate determinations in each. **P* less than 0.05 compared with vehicle control that did not receive t-BOOH.

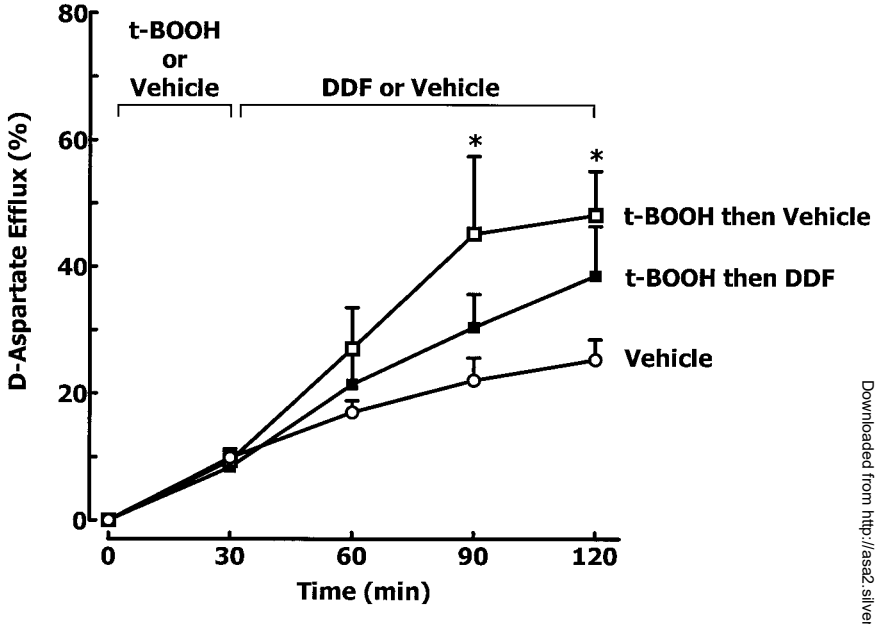

Fig. 3. Dideoxyforskolin decreases D-aspartate release from astrocytes after *tert*-butyl hydroperoxide (t-BOOH)–induced oxidative stress. Astrocytes were preloaded with D-[^{3}H]aspartate (10 μM) overnight. Subsequently, beginning at time zero of efflux, they were incubated with t-BOOH (1 mM) or aqueous vehicle for 30 min at 37°C. Next, they were incubated with 1,9-dideoxyforskolin (DDF; 100 μM) or drug vehicle (dimethylsulfoxide) for an additional 90-min period at 37°C. Plotted are cumulative efflux rates after 60 min for four experiments. **P* less than 0.05 compared with the vehicle control that did not receive t-BOOH.

by t-BOOH, astrocytes were exposed to the organic peroxide for 30 min at either 33 or 28°C, maintained at the same temperature during a subsequent 30-min incubation with propofol or its vehicle, and finally returned to 37°C for the 1-min glutamate uptake assay. Figure 4 shows that prophylactic cooling lessened significantly the inhibition by t-BOOH of glutamate uptake. The combination of prophylactic hypothermia with delayed administration of 1 μM propofol had the same effect as hypothermia alone (fig. 4).

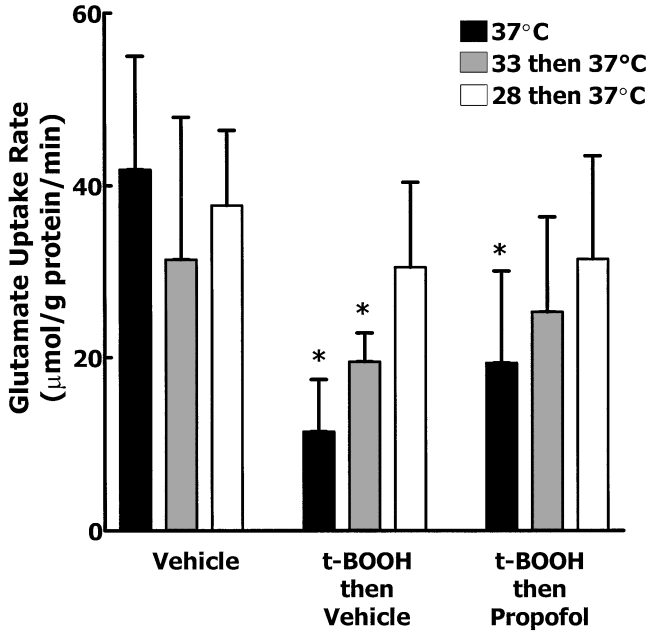

Fig. 4. Prophylactic hypothermia protects the glutamate uptake system from organic peroxide. Astrocytes were incubated at 28, 33, or 37°C with *tert*-butyl hydroperoxide (t-BOOH; 1 mM) or aqueous vehicle for 30 min. They were then incubated with propofol (1 μM) or Intralipid vehicle for another 30 min. Finally, the rate of glutamate uptake was measured (100 μM, 1 min) at 37°C. Plotted are the mean ± SD values for four to eight experiments. **P* less than 0.05 compared with the 37°C vehicle control that did not receive t-BOOH.

Hypothermic temperatures did not alter the rate of D-aspartate release from control astrocytes that were not exposed to t-BOOH (fig. 5A). However, cooling astrocytes to either 33 or 28°C during exposure to this oxidant prevented stimulation of D-aspartate release for 60 min (fig. 5A). This protective effect of prophylactic hypothermia was transient and disappeared after 90 min (fig. 5B).

Hypothermia and propofol differed with respect to therapeutic windows. There was no protection of glutamate transport by delayed hypothermia (33 or 28°C), which was begun after a 30-min period of normothermic exposure to t-BOOH (fig. 6). In contrast, propofol was effective at this time, regardless of whether the temperature was 37, 33, or 28°C (fig. 6). Delayed cooling also failed to decrease the effects of t-BOOH on D-aspartate efflux, whereas rescue by propofol was observed at all three temperatures (fig. 7).

Discussion

The extracellular glutamate concentration in brain increases to excitotoxic levels after trauma, ischemia, and other pathologies characterized by oxidative stress and swelling of astrocytes.[14,15,27,36–38] Astrocytes are the most abundant cells in brain, and dysfunction of these non-neuronal cells may be an important cause of the failure of injured brain to regulate extracellular glutamate concentration. This is evident from the observation that suppression of neuronal activity by barbiturate coma (*i.e.*, burst–suppression with thiopental) often fails to normalize extracellular glutamate concentration in

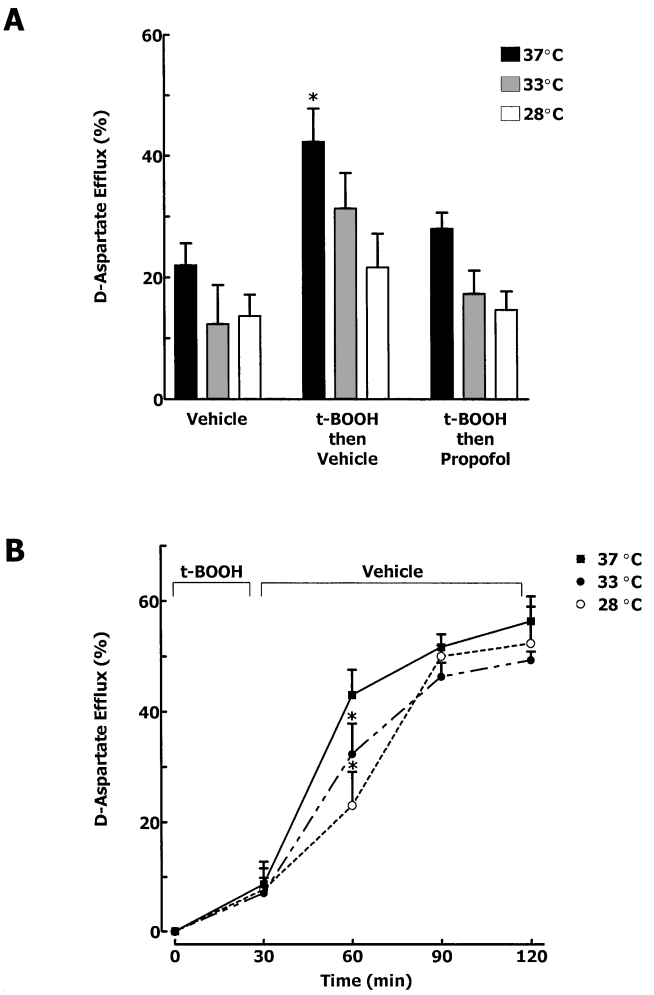

Fig. 5. Prophylactic hypothermia and delayed propofol independently prevent the stimulation of D-aspartate efflux caused by organic peroxide. Astrocytes were preloaded with D-[^{3}H]aspartate (10 μM) overnight. Subsequently, beginning at time zero of efflux, they were incubated for 30 min with *tert*-butyl hydroperoxide (t-BOOH; 1 mM) or aqueous vehicle at 28, 33, or 37°C. Finally, they were incubated with propofol (1 μM) or Intralipid vehicle for an additional 90 min at the same temperatures. (*A*) Cumulative efflux rates after 60 min for three experiments. **P* less than 0.05 compared with the 37°C vehicle control that did not receive t-BOOH. (*B*) Transient protective effect of hypothermia against t-BOOH during the 20-min efflux period in the same experiments. **P* less than 0.05 compared with the 37°C treatment.

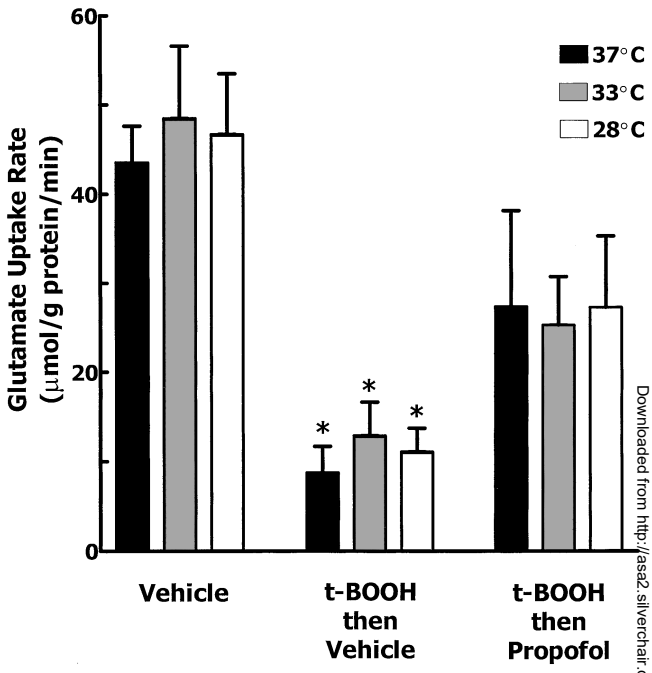

Fig. 6. Hypothermia applied 30 min after organic peroxide fails to protect glutamate transport but does not inhibit rescue by propofol. Astrocytes were exposed to *tert*-butyl hydroperoxide (t-BOOH; 1 mM) or aqueous vehicle for 30 min at 37°C. Next they were incubated with propofol (1 μM) or Intralipid vehicle for an additional 30-min period at 28, 33, or 37°C. Subsequently, the initial rate of glutamate uptake (100 μM, 1 min) was measured at 37°C. Plotted are mean ± SD values for three experiments. **P* less than 0.05 compared with vehicle control that did not receive t-BOOH.

the brain of postischemic patients.[39] The present study used a cell-permeant organic peroxide (t-BOOH) and primary astrocyte cultures to model the effects of oxidative insult. t-BOOH (1 mM, 37°C) inhibited astrocytic glutamate uptake and increased D-aspartate efflux within 60 min. Plasma membrane disruption, reflected in the release of cytosolic LDH into the medium, was significantly increased after 120 min of t-BOOH exposure. Propofol and hypothermia curtailed oxidative injury, with propofol demonstrating a larger therapeutic window. Delayed cooling neither protected against oxidative injury nor compromised rescue by propofol. Experiments with astrocytes that had not been stressed showed that, unlike propofol, hypothermia reversibly slowed glutamate uptake in undamaged cells.

Propofol differed from other commonly used intravenous anesthetics in being able to normalize astrocytic glutamate uptake and D-aspartate efflux rates after t-BOOH. Relatively high concentrations of thiopental, midazolam, or ketamine did not rescue glutamate uptake after t-BOOH. Failure of thiopental and midazolam excludes mediation by $GABA_A$ receptor activation, while failure of ketamine excludes NMDA receptor inhibition for this action of propofol. On the other hand, we have compared propofol to an established inhibitor of lipid peroxidation, α-tocopherol (vitamin E). Delayed administration of α-tocopherol (EC_{50} = 44 μM) was effective in arresting the inhibition by t-BOOH of glutamate uptake, although it was less potent than propofol (EC_{50} = 2 μM). The effect of α-tocopherol indicates that lipid peroxidation is involved in the inhibition of glutamate transport by t-BOOH. Propofol resembles α-tocopherol in possessing a phenolic OH-group. This moiety allows the anesthetic to inhibit lipid peroxidation at concentrations as low as 2 μM in microsomal suspensions.[11] Because propofol concentrations sufficient to restore glutamate transport (fig. 1) also inhibit lipid peroxidation,[11] it seems probable that the antioxidant activity of propofol

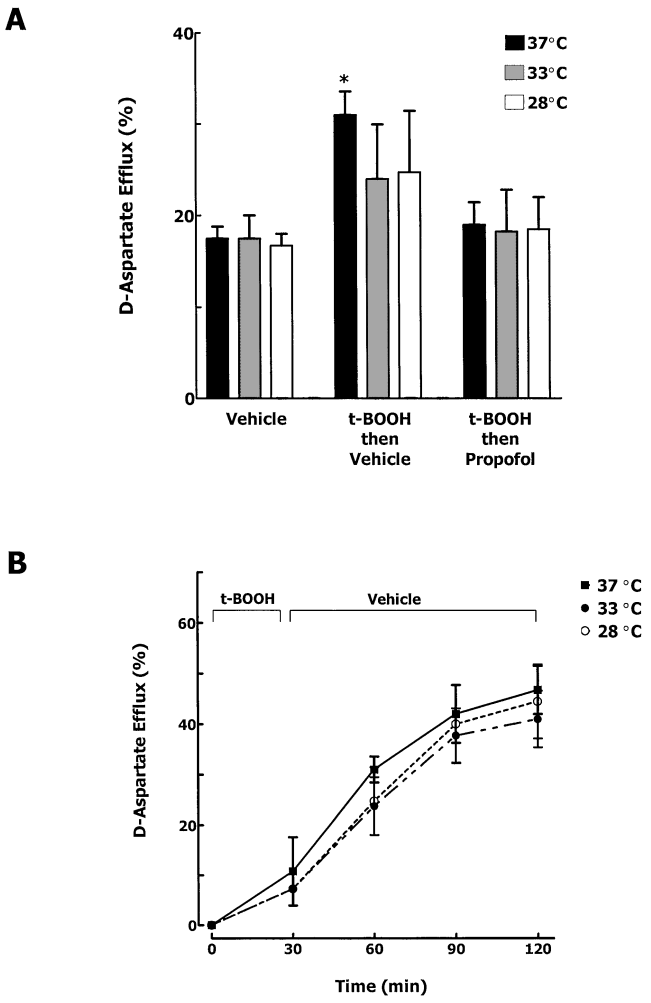

Fig. 7. Hypothermia applied 30 min after organic peroxide fails to prevent D-aspartate efflux but does not inhibit rescue by propofol. Astrocytes were preloaded with D-[^{3}H]aspartate (10 μM) overnight. Subsequently, beginning at time zero of efflux, they were exposed to *tert*-butyl hydroperoxide (t-BOOH; 1 mM) or aqueous vehicle for 30 min at 37°C. Next, they were incubated with propofol (1 μM) or Intralipid vehicle for an additional 30-min period at 28, 33, or 37°C. (*A*) Cumulative efflux rates after 60 min. **P* less than 0.05 compared with the 37°C vehicle control that did not receive t-BOOH. (*B*) Delayed hypothermia does not have a significant effect on D-aspartate release from the t-BOOH–injured astrocytes when the data from the entire 120-min efflux period are analyzed simultaneously by repeated-measures analysis of variance (four experiments).

is responsible for the restoration of glutamate transport observed after delayed administration of this anesthetic.

The antioxidant properties of propofol also may explain its inhibition of excitatory amino acid release from astrocytes exposed to t-BOOH. Oxidative stress in cultured astrocytes causes a dysregulation of osmotic control that leads to activation of VSOAC that are permeant to excitatory amino acids.[28] Dideoxyforskolin inhibits VSOAC activity in astrocytes without affecting high-affinity transporters of excitatory amino acids.[33,40] Our observation that this VSOAC blocker partially inhibited D-aspartate efflux from t-BOOH pretreated astrocytes indicates that these channels mediate a large component of the excitatory amino acid efflux. Ischemia-induced glutamate release also can be inhibited by a VSOAC blocker (4,4'-dinitrostilben-2,2'-disulfonic acid) *in situ*.[27] The remaining dideoxyforskolin-insensitive component of D-aspartate release after t-BOOH is attributable to simple diffusion through the disrupted plasma membrane, because it was accompanied by release of cytosolic LDH. Therefore, our results indicate that propofol decreases the release of excitatory amino acids after oxidative stress by inhibiting activation of VSOAC in moderately stressed astrocytes and by preventing membrane lysis in the most severely injured cells.

The concentration of free propofol (not bound to protein) during anesthesia is approximately 1 μM in plasma,[41] and this most lipophilic anesthetic is known to concentrate into brain.[42] Thus, the propofol concentrations that we observed to defend astrocytes in primary culture from oxidative stress are similar to those that occur in brain during anesthesia and improve outcome from experimental cerebral ischemia.[1–6]

Whether hypothermia is beneficial for brain function may depend, at least in part, on how it modifies glutamate uptake and release. With regard to possible adverse consequences, we observed a direct effect of temperature when we compared glutamate uptake rates at 28, 33, and 37°C in astrocytes that had not been exposed to t-BOOH. Cooling slowed the glutamate uptake system of these undamaged astrocytes. These findings indicate a mechanism by which mild and moderate hypothermia may retard clearance of extracellular glutamate and thereby elevate glutamate concentration. An advantage of propofol over hypothermia may arise because, as shown in the present experiments, this anesthetic does not slow glutamate uptake.

Another possible problem with hypothermic therapy is that rewarming after selective brain cooling may worsen reperfusion injury.[43] Of particular concern is that fast rewarming from deep hypothermia increases the extracellular concentration of glutamate in brain.[44] However, we did not observe any deleterious effects of rewarming on cultured astrocytes from 28°C to normal temperature.

Our study also elucidates molecular mechanisms through which hypothermia may benefit ischemic brain. Prophylactic application of mild hypothermia lessens the increase in extracellular glutamate concentration caused by ischemia–reperfusion in the brain of rat,[15,37,38] gerbil,[36] and swine.[14] An important reason for this is that cooling prevents inhibition of glutamate uptake during reperfusion of brain.[17,44] Because the present *in vitro* experiments have shown that the direct effect of hypothermia is to slow glutamate uptake, the enhancement of postischemic glutamate uptake observed *in situ*[17,44] must be caused by an indirect action. The present investigation identifies one such indirect action as the preven-

tion by hypothermia of damage to glutamate transport by oxidants.

A contributing factor in cerebral protection by both hypothermia[18,19] and propofol[1] may be that they suppress oxidative metabolism. This may slow production of reactive oxygen species. For example, hypothermia decreases the levels of oxygen-based free radicals measured by electron paramagnetic resonance after ischemic insult in gerbil brain.[16] Furthermore, both propofol[11] and hypothermia[20,21] oppose oxidative modification of cell lipids and proteins.

However, there is also evidence of a limited therapeutic window for hypothermia. When cultured astrocytes are exposed to ischemic conditions, intra-ischemic hypothermia (32°C) mitigates cell death, whereas postischemic hypothermia does not.[45] The present experiments found that hypothermia could not protect oxidatively stressed astrocytes when delayed 30 min. Our results are consistent with the *in situ* observations that prophylactic hypothermia is superior to delayed cooling for suppressing the postischemic increase in extracellular glutamate concentration[38] and infarct size.[13] Although our data indicate that hypothermia has only transient effects on excitatory amino acid fluxes, it may defend brain cells for longer periods by other mechanisms. Indeed, hypothermia has been found to be protective against ischemia even when the extracellular glutamate concentration is elevated by intracerebral infusion of this excitatory amino acid.[46]

In conclusion, clinical levels of propofol and hypothermia mitigate the effects of oxidative stress on astrocytic uptake and retention of excitatory amino acids, with propofol having a relatively larger temporal window. These experimental results provide support for observations of the beneficial effects of these interventions made in the clinical setting.

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