Anesthesiology 2004; 100:1151–9

Propofol Neuroprotection in Cerebral Ischemia and Its Effects on Low-molecular-weight Antioxidants and Skilled Motor Tasks

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Background: **Propofol is neuroprotective when administered immediately after stroke. The therapeutic window, duration of administration, and antioxidant mechanisms of propofol in neuroprotection are not known. The effects of propofol after stroke were examined in the conscious animal. The authors have previously shown that light propofol anesthesia (25 mg · kg^{-1} · h^{-1}) for a period of 4 h, even if delayed 1 h after the onset of ischemia, decreases infarct volume 3 days after the stroke.**

Methods: **Cerebral ischemia was induced in awake Wistar rats by a local intracerebral injection of the potent vasoconstrictor, endothelin (6 pmol in 3 μl) into the striatum. Propofol treatment after ischemia was delayed up to 4 h, and the infusion period shortened from 4 h to 1 h. Infarct volume was assessed 3 or 21 days after the stroke. Neurologic outcome was evaluated on days 14–21 after ischemia. Tissue ascorbate and glutathione concentrations were evaluated at 4 h and 3 days after ischemia.**

Results: **Infarct volumes were reduced 3 days after ischemia when propofol treatment (25 mg · kg^{-1} · h^{-1}) was delayed for 2 h (0.5 ± 0.3 mm^3) but not 4 h (2.0 ± 0.9 mm^3), compared with intralipid controls (2.4 ± 0.7 mm^3). The propofol infusion period of 3 h but not 1 h reduced infarct volume. Propofol treatment did not reduce infarct volume 21 days after the stroke, although motor function improvements (Montoya staircase test) were observed 14–21 days after the stroke. Propofol neuroprotection was independent of tissue ascorbate and glutathione concentrations.**

Conclusions: **Concurrent or delayed administration of propofol is neuroprotective 3 days after ischemia. Although there were no differences in infarct volume 21 days after ischemia, propofol-treated animals had functional improvements at this time.**

SEVERAL studies have investigated the neuroprotective effects of anesthetics against the detrimental effects of cerebral ischemia. Because cerebral ischemia is associated with the oxidation of endogenous antioxidants, compounds with antioxidant potential may be particularly beneficial to combat cerebral ischemia.

Propofol (2,6-diisopropylphenol) is a commonly used sedative and anesthetic. Like other anesthetic agents, it has properties that may protect the brain against focal cerebral ischemia. These properties include a reduction in cerebral metabolism, potentiation of γ-aminobutyric acid–mediated inhibition, and altered cerebral blood flow, which may beneficially redistribute flow.[1–4] However, unlike most other anesthetics, propofol has shown direct antioxidant activity conferred by the phenolic hydroxyl group in its structure.[5,6] This antioxidant property of propofol may make it a superior anesthetic agent to combat ischemia.

We have previously shown that a light depth of propofol anesthesia (25 mg · kg^{-1} · h^{-1}) for a period of 4 h, initiated concurrent with a stroke or delayed 1 h after ischemia, decreased infarct volume compared with the effect in control animals enduring the ischemic injury while awake.[7] We also showed that this effect of propofol was not mediated through changes in brain temperature or through inhibition of endothelin-induced vasoconstriction. The current series of experiments were performed using our awake stroke model, which induces an ischemic injury in the conscious rat after an intracerebral injection of the potent vasoconstrictor endothelin 1.

The following questions were addressed: (1) How long can the administration of propofol be delayed and still prove to be neuroprotective? (2) How much can the duration of propofol administration be shortened while still conferring protection? (3) Does propofol cause permanent long-term neuroprotection? (4) Does propofol improve motor deficits, learning deficits, or both after stroke? (5) Is propofol neuroprotection mediated through changes in endogenous antioxidants?

Materials and Methods

Surgical Preparations

The Animal Care Committee at the University of Western Ontario approved the experiments described below. Animals were kept on a 12-h light–dark cycle and given food and water *ad libitum* at all times. All surgical procedures were as previously described.[7] Briefly, a total of 162 adult male Wistar rats (weight, 300–350 g) were

This article is accompanied by an Editorial View. Please see: Patel P: No magic bullets: The ephemeral nature of anesthetic-mediated neuroprotection. ANESTHESIOLOGY 2004; 100:1049–51.

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Received from the Department of Anatomy and Cell Biology, University of Western Ontario, London, Ontario, Canada. Submitted for publication July 8, 2003. Accepted for publication October 28, 2003. Supported by grant No. T5000 from the Heart and Stroke Foundation of Ontario, Toronto, Ontario, Canada. Dr. Cechetto is a career investigator of the Heart and Stroke Foundation of Ontario. Presented at the 31st Annual Meeting of the Society for Neuroscience, San Diego, California, November 12, 2001, and the 32nd Annual Meeting of the Society for Neuroscience, Orlando, Florida, November 5, 2002.

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anesthetized with sodium pentobarbital (65 mg/kg intraperitoneal). Rectal temperature was maintained at 37°C using a heating pad throughout the surgical procedures. The right femoral vein was chronically cannulated for the subsequent infusion of propofol or intralipid (vehicle). A stainless steel guide cannula (23 gauge; 15.0 mm in length; 0.0 mm anterior and 3.0 mm lateral to bregma and 1.0 mm below the dura mater) was implanted into the brain and then fixed in place with dental acrylic. Immediately after the surgery, animals received an intramuscular injection of 0.3 ml buprenorphine (0.3 mg/ml Buprenex; Reckitt & Colman Pharmaceuticals Inc., Richmond, VA) and 0.03 ml of an antibacterial injectable solution (50 mg/ml enrofloxacin, Baytril; Bayer Inc., Etobicoke, Ontario, Canada). All wounds were closed, and the animals were allowed to recover for 4 days.

Intracerebral Injections of Endothelin 1

All intracerebral injections of endothelin were made into awake animals. Four days after the surgery, a stainless steel injection cannula (30 gauge; 19.0 mm in length) was inserted into the guide cannula, with the tip 4.0 mm below the end of the guide cannula, allowing for an intracerebral injection approximately into the center of the striatum. An injection of 6.0 pmol endothelin 1 (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada) in 3 μl phosphate-buffered saline was made into the striatum at a constant rate over a 2-min period using a Hamilton microinjector (CR-700-20; Hamilton Co., Reno, NV). In a separate series of experiments, 60 pmol endothelin 1 in 3 μl phosphate-buffered saline was injected to determine whether this higher concentration of endothelin would lead to a larger volume of infarction. The injection cannula was left in place after the endothelin injection for a period of 5 min to prevent leakage of the endothelin up the injection cannula tract.

Infusions

After the endothelin-1 injections, paired rats of similar mass were randomly assigned to undergo infusion with either propofol (1%; Abbott Laboratories Ltd., Saint-Laurent, Quebec, Canada) or intralipid vehicle alone (10%; Pharmacia & Upjohn Inc., Mississauga, Ontario, Canada). Propofol was infused at a rate of 25 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} for a period of 4 h starting 2 or 4 h after the onset of ischemia. In a separate series of experiments, the infusion period for propofol (25 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) or intralipid was decreased to either 3 or 1 h, initiated immediately after the endothelin-1 injection.

In the final series, animals received the injection of endothelin at the higher dose (60 pmol in 3 μl), and then infusions of either propofol (25 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) or intralipid were initiated immediately after the endothelin injections and continued for a period of 4 h. Body temperature in all propofol-treated animals was monitored continuously throughout the infusion period with a rectal thermometer and maintained at 37°–37.5°C using a heating pad and an overhead light. We have previously demonstrated that maintaining body temperature at 37°–37.5°C in animals treated with propofol at a dose of 25 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} results in no differences in brain temperature during the infusion period or in the next 3 days of survival compared with intralipid controls.[7]

Infarct Volume Assessment

Three or 21 days after the stroke, the animals were transcardially perfused with phosphate-buffered saline, followed by 4% paraformaldehyde under a deep pentobarbital anesthesia (100 mg/kg intraperitoneal). The brains were cryoprotected in 30% sucrose for 2 days before sectioning. Coronal sections of the brains (40 μm) were cut on a cryostat and stained with thionine. Sections were collected every 160 μm, throughout the entire extent of the striatum, encompassing the entire infarct. Typically, approximately 10 sections were evaluated to estimate infarct volume. The number of sections evaluated varied between animals depending on infarct size.

The sections were then examined under a light microscope (Leitz Diaplan; Leica Canada, Willowdale, Ontario, Canada), and the areas of infarcted tissue were measured (SigmaScan Pro 5.0; SPSS Inc., Chicago, IL). In addition, the hemispheric areas of each tissue section were measured to account for any brain swelling that might have occurred after the stroke. Infarct size for each section was calculated as the ratio of the contralateral to the ipsilateral hemisphere multiplied by the area of the infarct. Area measurements were made by an investigator blinded to the identity of the brain sections. The volume of the infarct was calculated in cubic millimeters by integrating the infarct sizes for each of the tissue sections that contained infarcted tissue.

Montoya Staircase Test

In a separate series of animals, the Montoya staircase test was used to examine motor control after the stroke. The training, testing procedures, and apparatus have been previously described.[8] A total of 27 rats were randomly assigned to three groups: sham–intralipid, stroke–intralipid, and stroke–propofol. Rats underwent surgical preparations as described before. In the animals that were randomly assigned to the sham–intralipid group, a guide cannula was fixed in place above the skull but was not implanted into the brain, and care was taken not to damage or rupture the dura mater. After the 4 days of recovery after the initial surgical preparations, animals were trained for 8 days (days −8 to −1) in the Montoya staircase apparatus. The rats were placed into the apparatus once daily at the same time each day during the training period. Three Noyes precision food pellets (Research Diets Inc., New Brunswick, NJ; PFA/100045) were placed in each of the 14 wells (7 per side). The

numbers of pellets eaten from each well was recorded at the conclusion of the 20-min testing session. Initially, rats were deprived of food for 1 day (day −9) before the start of the training period. During the training period (days −8 to −1), rats were fed 10 g normal chow at the conclusion of each testing session to maintain body weight at approximately 85% of its initial value. After the training period (day 0), these animals received the intracerebral injection of endothelin (6 pmol in 3 μl), followed immediately by infusions with propofol or intralipid (vehicle) for a period of 4 h as described in the Infusions section. Rats assigned to the sham–intralipid group did not receive the initial intracerebral injection of endothelin and were only infused with intralipid for 4 h. Retesting trials on the Montoya staircase test were performed on days 14–21 after the stroke, in the same manner as the training trials. At the conclusion of the retesting period (day 21), infarct volume was determined as described in the Infarct Volume Assessment section.

Evaluation of Endogenous Antioxidants In Vivo

A total of 33 rats were used in this separate series of experiments. Rats received an injection of 60 pmol endothelin followed by the previously described infusions of propofol (25 $mg \cdot kg^{-1} \cdot h^{-1}$) or intralipid vehicle for a period of 4 h. These animals were decapitated either immediately or 3 days after the conclusion of the propofol–intralipid infusion period. A series of sham nonischemic animals received the control infusion of intralipid for a period of 4 h before decapitation. The brain was immediately removed, and tissue samples of the ipsilateral striatum, ipsilateral cortex, contralateral striatum, and contralateral cortex were quickly dissected on ice. Tissue samples were placed in 1.5-ml tubes and then frozen in dry ice. Samples were stored in a −80°C freezer until antioxidant analysis was completed.

Tissue glutathione concentration was determined as previously described.[9] This method uses sodium borohydride as the reducing agent and monobromobimane as the thiol-specific fluorochrome, followed by high-performance liquid chromatography with fluorescence detection. A Shimadzu fluorescence detector (Shimadzu RF-535; Shimadzu Corporation, Kyoto, Japan) was used, and the excitation and emission wavelengths were set at 390 and 480 nm, respectively.

Ascorbic acid was assayed by high-performance liquid chromatography with electrochemical detection, according to the procedure of Behrens and Madere[10] as modified by Wilson.[11,12] Tissues were combined with 0.85% metaphosphoric acid containing 500 μM 3,4-dihydroxybenzyl-amine as the internal standard (1 ml/0.2 g tissue). They were then homogenized on ice and centrifuged (14,000 rpm for 3 min at 4°C). Aliquots of supernatant were passed though a 45-μm Millex filter (Waters Limited, Mississauga, Ontario, Canada) and injected into the high-performance liquid chromatography with electrochemical detection system. Separation of ascorbic acid and 3,4-dihydroxybenzyl-amine was achieved with a Resolve C18 reverse-phase column. The mobile phase consisted of 80 mM sodium acetate buffer containing 1 mM n-octylamine, 15% methanol, and 0.015% metaphosphoric acid. The final pH of the mobile phase was 4.6. The elution volumes of ascorbic acid and 3,4-dihydroxybenzyl-amine were determined by electrochemical detection with a Water 464 amperometric detector (Waters Limited). Tissue ascorbic acid concentration was determined by interpolation on an external standard curve and corrected for percent recovery of the internal standard.

In Vitro *Antioxidant Assessment*

Primary cultures of cerebral astrocytes were prepared from the neopallium of 1-day-old Wistar rats according to the protocol of Dixon and Wilson.[13] These were grown to confluence in horse serum (10%) supplemented with minimum essential medium in 60-mm dishes. Astrocytes were used for experiments after 17–26 days in culture.

Glutathione Assay

Astrocytes were initially incubated at 37°C for 3 h in serum-free minimum essential medium, and either propofol (8–40 μM, 1.4–7.1 μg/ml) or intralipid was added to the incubation medium for the final hour. Next, the cells were incubated for an additional 1 h in HEPES-buffered physiologic saline including propofol (or intralipid) with or without the oxidant tert-butyl hydroperoxide (t-BOOH; 1 mM). Cultures were harvested in ice-cold (4°C) phosphate-buffered saline with 0.2% Triton X-100 and 2.5% sulfosalicylic acid (pH 7.4). The intracellular concentration of glutathione was measured according to the method described by Siushansian *et al.*[14]

Ascorbic Acid Assay

Astrocytes were incubated for 3 h at 37°C in serum-free minimum essential medium containing ascorbic acid (200 μM), and either intralipid (vehicle) or propofol (40 μM) was added to the medium for the final hour. Next, the cells were incubated for an additional hour in HEPES-buffered physiologic saline containing intralipid or propofol with or without t-BOOH (1 mM). Lastly, the cells were washed with Tris-sucrose buffer (4°C) and assayed for ascorbic acid. Ascorbic acid concentrations were determined by acidic extraction and high-performance liquid chromatography with electrochemical detection.[12]

Data Analysis

The volumes of the infarct for each of the groups were subjected to an analysis of variance and the Dunnett multiple comparison test. Tissue ascorbic acid and glutathione concentrations from all brain regions were first

subjected to a two-way analysis of variance. The values from all treatments in specific brain regions were then subjected to a one-way analysis of variance and the Tukey test to determine differences between treatments. Differences between mean values in the number of pellets eaten and *in vitro* ascorbic acid and glutathione concentrations between treatments were evaluated by a one-way analysis of variance followed by the Tukey test. A P value of less than 0.05 was considered significant. *Post hoc* tests were only performed if the preceding analysis of variance was significant. Data are expressed as mean ± SD of the mean.

Results

Infarct Volumes

It was observed that the endothelin caused an infarct ipsilateral to the injection and restricted to the subcortical caudate putamen in all animals when evaluated 3 or 21 days after ischemia. There was no observable lesion on the contralateral side in any animal. In intralipid-treated animals, the infarcts were oval shaped, extending in the rostral–caudal and the medial–lateral directions as previously reported.[7] Some infarcts seemed to extend in the dorsal–ventral direction, which may be attributed to endothelin leaking up the injection cannula tract into cortical regions.

The infarct volumes for the intralipid controls (pair-matched to the animals that received the 25-mg · kg^{-1} · h^{-1} propofol dose) delayed for various times after the onset of ischemia were 2.6 ± 0.7 mm^3, delayed 2 h (n = 8), and 2.3 ± 0.6 mm^3, delayed 4 h (n = 10). The infarct volumes in both intralipid series were not significantly different from each other and therefore were pooled (fig. 1A). The hemispheric size in the intralipid and propofol groups at all time points did not differ significantly from one side to the other, and therefore, no correction for hemispheric swelling was required.

All animals receiving propofol at 25 mg · kg^{-1} · h^{-1} seemed heavily sedated or anesthetized. They did not exhibit any response to the eye-blink reflex elicited by touching the cornea or any withdrawal reflex resulting from pinching the hind paw. In these animals, the infarct volumes were 0.5 ± 0.3 mm^3, delayed 2 h (n = 9), and 2.0 ± 0.9 mm^3, delayed 4 h (n = 9) after ischemia. Propofol delayed 2 h after the onset of ischemia significantly reduced infarct volume compared with the corresponding intralipid treatment (fig. 1A). When propofol administration was delayed 4 h after ischemia, infarct volume was not significantly different from the corresponding intralipid control treatment. Moreover, the shape of the infarct in these animals was similar in appearance to that of the animals treated with intralipid at the same time point (4 h after ischemia).

The infarct volumes for the animals in which the propofol infusion (25 mg · kg^{-1} · h^{-1}) period was initiated immediately after the onset of ischemia, but

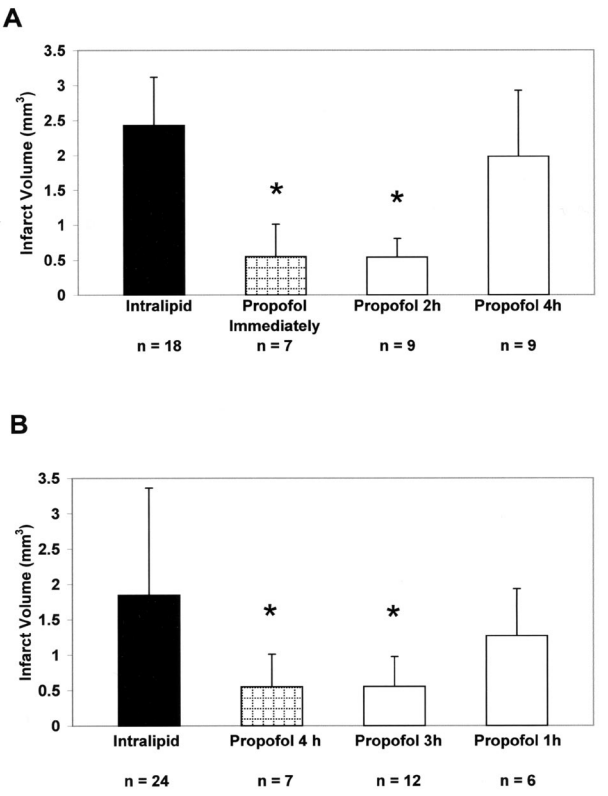

Fig. 1. Bar graph illustrating infarct volume assessed 3 days after ischemia. In the first series (*A*), the rats were treated with intralipid or propofol (25 mg · kg^{-1} · h^{-1}) for a period of 4 h, initiated either 2 or 4 h after the onset of endothelin-induced ischemia (6 pmol in 3 μl). In a separate series of animals (*B*), the infusion period of intralipid or propofol (25 mg · kg^{-1} · h^{-1}) was shortened to either 3 or 1 h, initiated immediately after the onset of ischemia. Each propofol treatment was matched to an intralipid group in which animals were infused during the same time course. There were no significant differences between intralipid groups from the various time points within each series of experiments, and therefore, they were pooled. The *dashed bar* in both panels represents the infarct volume in animals treated with propofol immediately after the onset of endothelin-induced ischemia for a period of 4 h as previously reported.[7] * Significant difference from control (P < 0.05).

for shortened periods, were 0.6 ± 0.4 mm^3 for the 3-h infusion period (n = 12) and 1.3 ± 0.7 mm^3 for the 1-h infusion period (n = 6). In the corresponding intralipid-treated animals, the infarct volumes were 1.7 ± 1.6 mm^3 for the 3-h infusion period (n = 17) and 2.3 ± 1.0 mm^3 for the 1-h infusion period (n = 7). The infarct volumes in these intralipid series were not significantly different from each other and therefore were pooled (fig. 1B). Propofol-treated rats showed a significant reduction in infarct volume compared with the corresponding intralipid treatment for the 3-h infusion period initiated immediately after ischemia. However, when the infusion period was shortened to 1 h, there were no significant differences in infarct volumes between propofol- and intralipid-treated animals (fig. 1B).

Using the hemispheric area as a correction factor for normalization of the infarct area did not change the

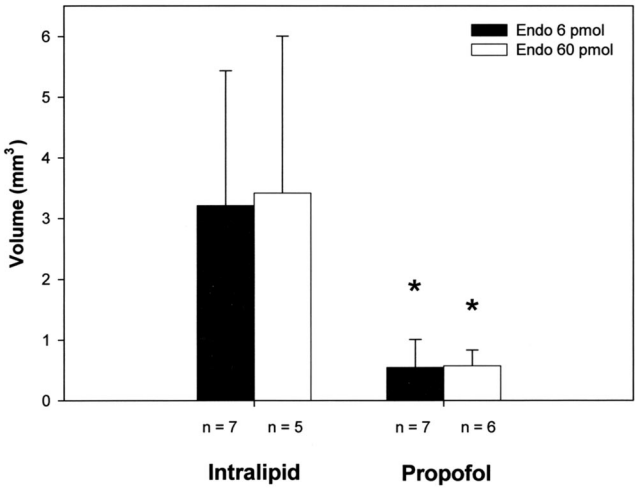

Fig. 2. Bar graphs illustrating the infarct volumes assessed 3 days after ischemia in animals treated with propofol (25 mg · kg^{-1} · h^{-1}) or intralipid for a period of 4 h, initiated immediately after the injection of either a low (6 pmol in 3 μl) or a high (60 pmol in 3 μl) dose of endothelin 1 (Endo) into the striatum. * Significant difference from the corresponding intralipid group ($P < 0.05$).

significance of the differences in infarct volumes between propofol and intralipid groups.

Infarct Volumes with High-dose Endothelin-1 Injections

Figure 2 shows the infarct volumes in animals injected with 6 or 60 pmol endothelin followed by immediate treatment with propofol (25 mg · kg^{-1} · h^{-1}) or the control infusion of intralipid for a period of 4 h. The infarct sizes for the high-dose endothelin were 3.4 ± 2.5 mm^3 for the intralipid group (n = 5) and 0.6 ± 0.3 mm^3 for the propofol group (n = 6).

The corresponding infarct volumes for the lower dose of endothelin (6 pmol) were 3.2 ± 2.2 mm^3 for the intralipid-treated rats (n = 7) and 0.5 ± 0.4 mm^3 for the propofol-treated rats (n = 7). Although propofol significantly reduced the infarct for both endothelin doses compared with intralipid, there were no significant differences in infarct volume between low-dose (6 pmol in 3 μl) and high-dose (60 pmol in 3 μl) endothelin injections for either the propofol-treated or the intralipid-treated group (fig. 2).

Montoya Staircase Test

All the rats showed a similar pattern of pellet consumption before the induction of the stroke. Initially, rats retrieved few pellets (< 5). However, by the end of the training phase (day −1), rats retrieved between 20 and 25 pellets. The total number of pellets available onthe apparatus was 42 (21 per side). Figure 3 shows the average number of pellets eaten on days 14–21 after the stroke. In the paw contralateral to the stroke, intralipid-treated animals showed a significant difference ($P <$

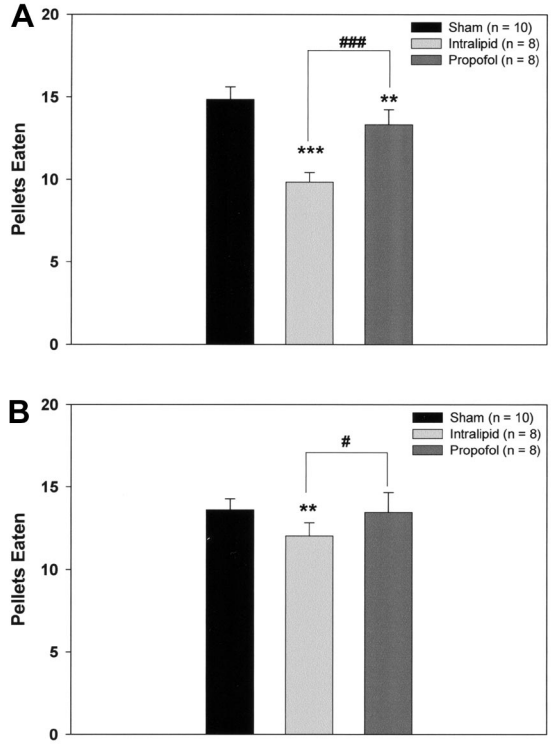

Fig. 3. Bar graph depicting the effects of ischemia and propofol on the number of pellets eaten. A series of sham nonischemic animals (Sham) received the control infusion of intralipid for a period of 4 h without the injection of endothelin. In the two other series, animals were treated with intralipid or propofol (25 mg · kg^{-1} · h^{-1}) for a period of 4 h, initiated immediately after the onset of endothelin-induced ischemia (6 pmol in 3 μl). Plotted is the mean number of pellets eaten on days 14–21 after ischemia with the paw either contralateral (*A*) or ipsilateral (*B*) to the stroke. ** $P < 0.01$, *** $P < 0.001$ compared with sham. # $P < 0.05$, ### $P < 0.001$ for the difference between propofol and intralipid treatments.

0.001) in the number of pellets eaten compared with sham controls. Propofol-treated animals showed a smaller difference ($P < 0.01$) compared with sham controls; moreover, a strong significant difference ($P <$ 0.001) between the propofol and intralipid groups was also observed. In the paw ipsilateral to the stroke, intralipid-treated animals showed a significant reduction in the number of pellets eaten compared with both sham and propofol-treated animals.

In the animals used for the Montoya staircase test, infarct volume was assessed 21 days after ischemia. The infarct volumes were 1.6 ± 0.9 mm^3 for the intralipid-treated rats (n = 8) and 1.1 ± 0.4 mm^3 for the propofol-treated rats (n = 8). There were no significant differences in infarct volumes between the propofol and intralipid treatments when evaluated 21 days after the stroke ($P = 0.15$).

Cell and Tissue Antioxidants

Incubation of ascorbic acid–loaded astrocyte cultures with a clinical concentration of propofol (40 μM) had no significant effect on cell ascorbic acid concentration. In

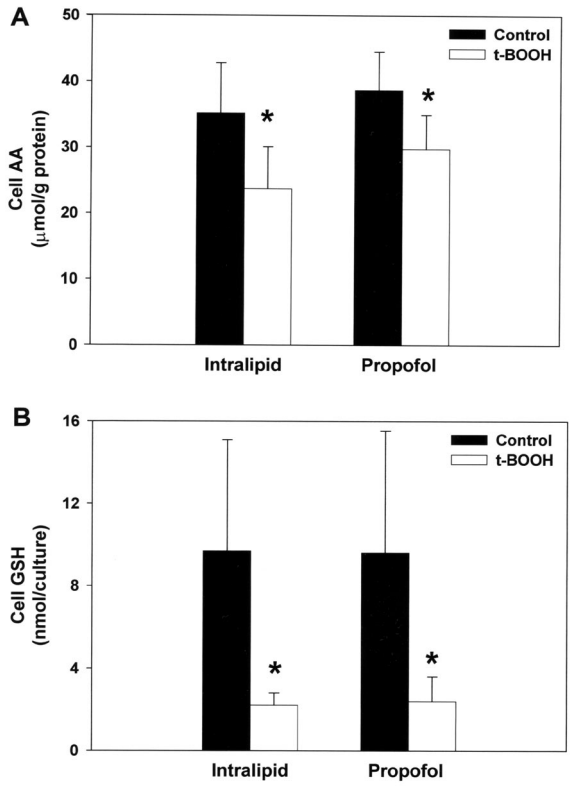

Fig. 4. Propofol does not prevent the loss of intracellular ascorbic acid (AA) and glutathione (GSH) in primary cultures of astrocytes after tert-butyl hydroperoxide (t-BOOH) treatment. Shown are the results from four independent experiments assaying AA (*A*) or GSH (*B*), with triplicate replication in each. * Significant effect of t-BOOH compared with aqueous control ($P < 0.05$).

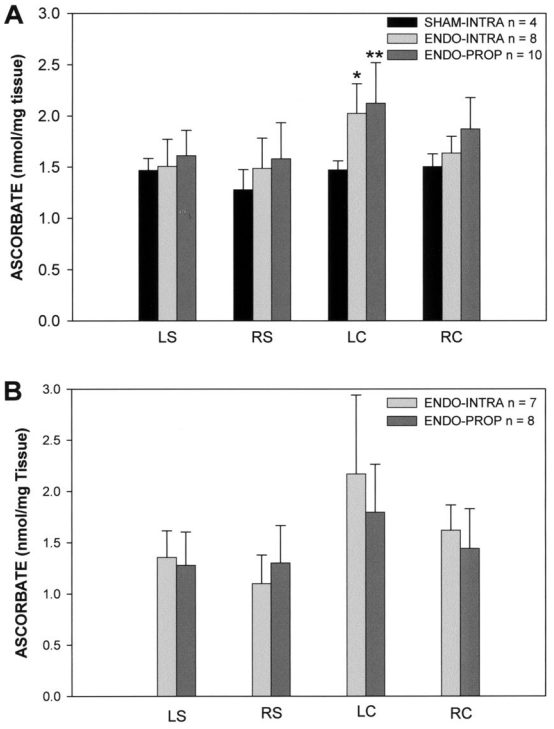

Fig. 5. Bar graphs illustrating ascorbic acid concentrations in the right striatum (RS), left striatum (LS), right cortex (RC), and left cortex (LC) after ischemia in the RS by the injection of endothelin (60 pmol in 3 μl). Animals were treated with intralipid (ENDO-INTRA) or 25 mg · kg^{-1} · h^{-1} propofol (ENDO-PROP) for 4 h. Tissue samples were harvested at the end of the infusion period (*A*) or 3 days after ischemia (*B*) and assessed for ascorbic acid content using high-performance liquid chromatography. For *A*, a series of sham nonischemic animals (SHAM-INTRA) received the control infusion of intralipid for a period of 4 h. * $P < 0.05$, ** $P < 0.01$ compared with sham.

addition, propofol did not prevent the decrease in cell ascorbic acid concentration induced by the oxidant t-BOOH (fig. 4A). Similarly, propofol (40 μM) failed to prevent the loss of glutathione produced by t-BOOH (fig. 4B).

In vivo treatment of rats with propofol (25 mg · kg^{-1} · h^{-1} for 4 h) immediately after the onset of ischemia had no significant effect on tissue ascorbic acid concentration compared with intralipid treatment at either 4 h (fig. 5A) or 3 days (fig. 5B) after ischemia. Nevertheless, both intralipid- and propofol-treated animals showed a significant increase in ascorbic acid concentration in the cortex contralateral to the infarct compared with sham-treated animals (fig. 5A). Propofol (25 mg · kg^{-1} · h^{-1} for 4 h) failed to alter significantly the total tissue glutathione concentration compared with intralipid (vehicle) at either 4 h (fig. 6A) or 3 days (fig. 6B) after ischemia.

Discussion

We previously demonstrated in rats that a light depth of propofol anesthesia (25 mg · kg^{-1} · h^{-1}) for a period of 4 h, when initiated concurrent with ischemia or delayed 1 h after its onset, decreases the size of the infarct volume measured 3 days after the stroke.[7] The current experiments extend our previous findings and demonstrate that propofol administration can be delayed up to 2 h after the onset of a stroke and can improve neurologic outcome compared with rats that experienced ischemia in the conscious state. However, when treatment was delayed 4 h after the onset of ischemia, propofol was ineffective in reducing infarct volume compared with controls. This evidence suggests that propofol has at least a 2-h therapeutic window after mild transient focal cerebral ischemia in rats.

We have also demonstrated that the duration of propofol treatment immediately after ischemia can be shortened to 3 h and still confer protection. On the other hand, when the propofol treatment period was limited to 1 h, no significant improvements were noted with propofol-treated rats compared with the intralipid control rats. This evidence suggests that the propofol treatment must last approximately 3 h to be effective. We have previously demonstrated that the endothelin-induced vasoconstriction used in these experiments is

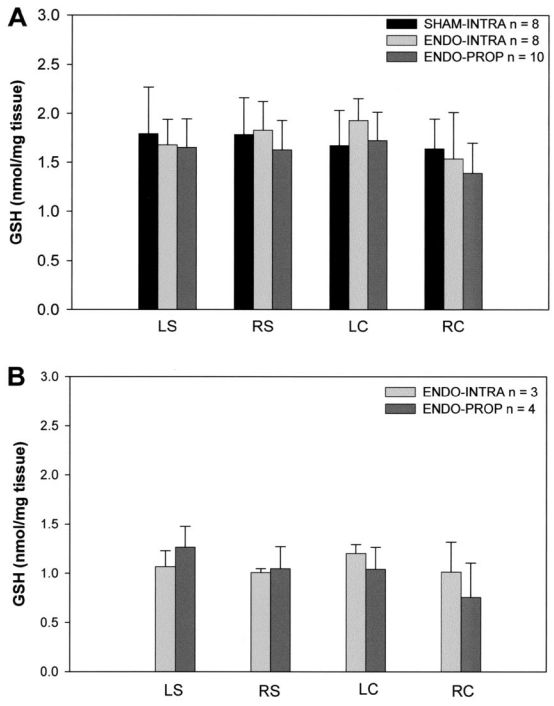

Fig. 6. Bar graphs illustrating glutathione (GSH) concentrations in the right striatum (RS), left striatum (LS), right cortex (RC), and left cortex (LC) after ischemia in the RS by the injection of endothelin (60 pmol in 3 μl). Animals were treated with intralipid (ENDO-INTRA) or 25 mg · kg^{-1} · h^{-1} propofol (ENDO-PROP) for a period of 4 h. Tissue samples were harvested at the end of the infusion period (*A*) or 3 days after ischemia (*B*) and assessed for GSH content. For *A*, a series of sham nonischemic animals (SHAM-INTRA) received the control infusion of intralipid for a period of 4 h.

reversible and lasts approximately 1 h.[7] Therefore, it is not surprising that the administration of propofol anesthesia for a period of 1 h initiated immediately after the onset of ischemia failed to show significant reductions in infarct volume compared with control because this would allow the animal to endure the entire reperfusion period in the absence of propofol. The antioxidant effects of propofol would be most beneficial during reperfusion when free radicals markedly increase. Antioxidant defense by propofol may contribute to the improved outcome observed when propofol was administered for 3 h. In the case where propofol was limited to 1 h, the increasing concentrations of free radicals at 1 h after ischemia would coincide with decreasing concentrations of propofol, which may account for the failure of 1-h propofol infusion to improve outcome.

Propofol was not effective in reducing infarct volume compared with awake controls when outcome was assessed 21 days after ischemia. Similarly, isoflurane delays but does not permanently prevent cerebral infarction in rats subjected to cerebral ischemia.[15] The lack of improvement with propofol anesthesia might be attributed to smaller lesions 21 days after stroke in the control animals compared with the infarcts detected 3 days after ischemia. A possible decrease in edema or inflammation might be responsible for a reduction in infarct volume after 21 days in the intralipid control rats. However, in the current study, we have also found that improvements in motor behavior were observed during days 14–21 after the stroke in propofol-treated animals. Therefore, if changes in brain morphology underlie the behavioral improvements, these were too subtle to be detected.

We also investigated whether a higher dose of endothelin would increase the extent of infarction. Injections of 6 or 60 pmol endothelin resulted in similar size infarcts in these experiments. Saturation of striatal endothelin receptors may have been achieved with 6 pmol endothelin, resulting in no additional activation when 60 pmol endothelin was used. It should also be noted that although the concentrations of endothelin differed, the volume of injection remained constant (3 μl). Therefore, it is possible that an increase in the volume rather than in concentration would have resulted in increased infarct volume because the vasoconstrictor could presumably diffuse over a larger volume and thereby cause in increase in infarction. Nonetheless, the current studies suggest that 6 pmol endothelin in 3 μl is an optimal dose to induce transient focal cerebral ischemia in the rat striatum.

Intralipid is an intravenous lipid emulsion that has been used as a nutrient supply for patients in the intensive care unit.[16] It has also been used as a vehicle for lipid-soluble compounds, such as propofol. In this investigation, intralipid was used in all experiments as a control to ensure that any neuroprotective effects after propofol treatment were due to the anesthetic and not the vehicle. Intralipid does not interact with γ-aminobutyric acid type A receptors, whereas propofol is believed to exert anesthetic and possibly neuroprotective effects through its interactions with these receptors.[17]

We have demonstrated previously that when a dose of 25 mg · kg^{-1} · h^{-1} of propofol is used and body temperature is maintained at 37°C, this results in no differences in brain temperature during the infusion period or in the next 3 days of survival between propofol- and intralipid-treated animals; furthermore, we showed that propofol had no effect on the magnitude or time course of the endothelin-induced vasoconstriction compared with intralipid-treated animals.[7] Therefore, the neuroprotective effects of propofol anesthesia cannot be attributed to changes in brain temperature or to a direct inhibition of the vasoconstriction caused by endothelin. To elucidate the possible mechanisms that mediate propofol neuroprotection, we examined the effects of propofol both *in vivo* and *in vitro* on endogenous low-molecular-weight antioxidants. We chose to investigate ascorbic acid and glutathione because they are the most abundant low-molecular-weight antioxidants in the central nervous system.[18]

The tissue concentrations of ascorbic acid reported

here are consistent with previous reports in the prefrontal cortex, hippocampus, and cerebellum.[19] These regions have the highest concentrations of ascorbic acid and glutathione in the rat central nervous system.[18] In the current experiments, basal concentrations of ascorbic acid were 1.5–2.0 nmol/mg in the cortex and approximately 1.5 nmol/mg in the striatum. *In vivo*, we failed to show any significant difference in ascorbic acid content between intralipid and propofol treatments, suggesting that the reduction in infarct volume observed in propofol-treated animals is independent of tissue ascorbate concentrations. *In vitro*, t-BOOH, an alkyl peroxide that enters cells to induce lipid peroxidation, oxidizes intracellular ascorbic acid and glutathione.[20–22] We observed that prophylactic treatment with propofol failed to prevent the decrease in intracellular ascorbic acid caused by t-BOOH. The fact that propofol is lipophilic and thus accumulates within membranes may explain its failure to preserve hydrophilic ascorbic acid.

Tissue concentrations of glutathione in the current study were approximately 1.5 nmol/mg in the cortex and striatum, in accord with previous reports.[19,23] The assay used to determine *in vivo* concentrations in the current studies measures total glutathione by reducing any oxidized glutathione (glutathione disulphide [GSSG]) back to glutathione before analysis. Therefore, even if the concentrations of GSSG were altered in the current experiments, they would have no effect on total glutathione concentrations measured by our assay. Under normal conditions, the ratio of glutathione to GSSG is extremely large, because only minute amounts of GSSG are present.[23] During ischemia, GSSG concentrations are thought to increase as glutathione becomes oxidized by reactive radical species.[23] However, even during ischemia, GSSH concentrations comprise only a very small proportion of the total glutathione.[23] If the neuroprotective effects of propofol were mediated through glutathione, one would expect significant differences in glutathione concentrations between propofol- and intralipid-treated animals. However, the results presented here failed to show any significant differences in glutathione concentrations between intralipid and propofol treatments at either 4 h or 3 days after ischemia. *In vitro*, propofol was unable to prevent glutathione depletion by t-BOOH. Similar to ascorbic acid, the majority of glutathione is localized in the cytosol and may be inaccessible to propofol, which accumulates in membranes.

The current studies indicated that there was no apparent effect *in vivo* of ischemia on glutathione or ascorbic acid concentrations compared with sham (figs. 5 and 6). Others have reported significant reductions in glutathione concentrations after a much more severe ischemic challenge to the brain by decapitation ischemia,[19] bilateral carotid artery occlusion,[23,24] four-vessel occlusion ischemia,[25] or middle cerebral artery occlusion.[26] The transient focal ischemic model used in the current studies is a relatively mild ischemic injury in comparison with these other challenges. Therefore, it is likely that our model of ischemia is not severe enough to cause marked disruptions in glutathione or ascorbic acid tissue contents.

Propofol has been reported to directly scavenge free radicals and decrease lipid peroxidation.[27–29] In ascorbate-depleted astrocytes exposed to peroxyl radicals, glutamate uptake was restored by propofol.[30] Therefore, the decrease in infarct volume observed in the current studies might be a reflection of direct scavenging actions of propofol against reactive oxygen species generated during the ischemic injury. However, both the *in vivo* and the *in vitro* experiments in the current study failed to show any changes in low-molecular-weight antioxidants caused by propofol. This suggests that the neuroprotection in propofol-treated animals is not directly dependent on ascorbic acid or glutathione concentrations.

We used the Montoya staircase test to determine whether motor deficits were present after the stroke. This test has clear advantages for our unilateral model of focal cerebral ischemia because it measures motor activity in each paw, thereby allowing for the investigation of unilateral deficits. The unilateral injection of endothelin into the striatum resulted in a significant reduction in the paw contralateral to the stroke in the number of pellets eaten from day 14 to day 21 after ischemia in both the intralipid and propofol groups compared with sham animals. However, we also observed that propofol-treated animals performed significantly better than controls in terms of the number of pellets eaten. The decrease in the number of pellets eaten described above is indicative of motor deficits due to the stroke.

In the paw ipsilateral to the stroke, we also discovered a small but significant reduction in the number of pellets eaten by the intralipid-treated animals from day 14 to day 21 after ischemia compared with sham animals. However, this effect was absent in propofol-treated animals. Moreover, propofol-treated animals showed a significant improvement compared with intralipid-treated animals. Although one might only expect motor deficits in the paw contralateral to the stroke, with no changes in the ipsilateral paw, the large deficit observed in the contralateral paw may lead to parallel but smaller deficits in the ipsilateral paw. The large deficit observed in the contralateral paw may also hinder performance in the ipsilateral paw. The latter may be the result of the need for postural stability, as the animal might make use of the ipsilateral paw more for stability while performing the task rather than for the retrieval of food pellets, because of the motor deficits experienced on the contralateral paw.

In summary, our data indicate that light propofol anesthesia for a period of 4 h can be used as a treatment for stroke in rats to provide functional improvements. Our

data further demonstrate that propofol treatment can be delayed up to 2 h after the onset of ischemia and the duration of treatment can be decreased to 3 h while still retaining its neuroprotective effects, which are observed at 3 days after ischemia. Furthermore, we have demonstrated that the beneficial effect of propofol is not due to an effect on low-molecular-weight antioxidants.

The authors thank Sandra M. Sitar, M.Sc. (Department of Physiology and Pharmacology, the University of Western Ontario, London, Ontario, Canada), for assistance.

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