Neuroprotective Effects of Propofol in Models of Cerebral Ischemia

Inhibition of Mitochondrial Swelling as a Possible Mechanism

Chiara Adembri, M.D., Ph.D.,* Luna Ventura, M.D.,† Alessia Tani, B.Sc.,‡ Alberto Chiarugi, M.D., Ph.D.,§ Elena Gramigni, M.D.,† Andrea Cozzi, B.Sc.,# Tristano Pancani, B.Sc.,** Raffaele A. De Gaudio, M.D., †† Domenico E. Pellegrini-Giampietro, M.D., Ph.D.§

*Assistant Professor, † Resident in Anesthesiology, †† Professor. Dipartimento di Area Critica Medico Chirurgica, Sezione di Anestesiologia e Terapia Intensiva, ‡ Research Technician, Dipartimento di Anatomia, Istologia e Medicina Legale, § Associate Professor, # Research Assistant, ** Research Assistant, Dipartimento di Farmacologia Preclinica e Clinica, Università di Firenze.


Address reprint requests to Dr. Adembri. Dipartimento di Area Critica Medico Chirurgica, Università di Firenze, Viale Morgagni 85, 50134 Firenze, Italy. Address electronic mail to: chiara.adembri@unifi.it. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

**Background:** Propofol (2,6-diisopropylphenol) has been shown to attenuate neuronal injury in a number of experimental conditions, but studies in models of cerebral ischemia have yielded conflicting results. Moreover, the mechanisms involved in its neuroprotective effects are yet unclear.

**Methods:** The authors evaluated the neuroprotective effects of propofol in rat organotypic hippocampal slices exposed to oxygen-glucose deprivation, an in vitro model of cerebral ischemia. To investigate its possible mechanism of action, the authors then examined whether propofol could reduce Ca2+-induced rat brain mitochondrial swelling, an index of mitochondrial membrane permeability, as well as the mitochondrial swelling evoked by oxygen-glucose deprivation in CA1 pyramidal cells by transmission electron microscopy. Finally, they evaluated whether propofol could attenuate the infarct size and improve the neurobehavioral outcome in rats subjected to permanent middle cerebral artery occlusion in vivo.

**Results:** When present in the incubation medium during oxygen-glucose deprivation and the subsequent 24 h recovery period, propofol (10–100 µM) attenuated CA1 injury in hippocampal slices in vitro. Ca2+-induced brain mitochondrial swelling was prevented by 30–100 µM propofol, and so were the ultrastructural mitochondrial changes in CA1 pyramidal cells exposed to oxygen-glucose deprivation. Twenty-four hours after permanent middle cerebral artery occlusion, propofol (100 mg/kg, intraperitoneal) reduced the infarct size by approximately 30% when administered immediately after and up to 30 min after the occlusion. Finally, propofol administered within 30 min after middle cerebral artery occlusion was unable to affect the global neurobehavioral score but significantly preserved spontaneous activity in ischemic rats.

**Conclusions:** These results show that propofol, at clinically relevant concentrations, is neuroprotective in models of cerebral ischemia in vitro and in vivo and that it may act by preventing the increase in neuronal mitochondrial swelling.

PROPOFOL (2,6-diisopropylphenol) is an intravenous sedative–hypnotic agent commonly used in anesthesia and intensive care that has been tested as a neuroprotective agent in models of cerebral ischemia. Both positive1–4 and negative5–7 results have emerged from in vitro studies. Similarly, in vivo studies have yielded conflicting results: Propofol seems to be able to reduce the extent of posts ischemic damage in models of transient8–12 but not permanent13 focal ischemia. A number of mechanisms have been claimed to explain the neuroprotective effects of propofol, including reduction of the cerebral metabolic rate for oxygen,14 antioxidant activity toward both lipophilic and hydrophilic radicals,1,14,15 activation of γ-aminobutyric acid type A receptors,16,17 inhibition of glutamate receptors,18,19 and reduction of extracellular glutamate concentrations via inhibition of Na+ channel–dependent glutamate release20 or enhancement of glutamate uptake.1,3

Mitochondria are involved in the energetic supply and are implicated in ischemic dysfunction in all living cells, especially in neurons.21 The oxidative phosphorylation activity of neuronal mitochondria rapidly deteriorates under ischemic conditions, leading to energetic failure and cell death. Moreover, as a consequence of ischemic oxidative stress and mitochondrial Ca2+ overload, the mitochondrial permeability transition pore (MPTP) opens, allowing the free movement of small-molecular-weight solutes but not of proteins.22,23 This results in mitochondrial swelling and eventually in rupture of the outer membrane and release of proapoptotic factors, such as cytochrome C—which can also be released in an MPTP-independent manner24—and the apoptotic inducible factor, that are normally sequestered and maintained inactivated in the space between the inner and outer mitochondrial membranes.21 Accordingly, drugs capable of preventing increases in mitochondrial membrane permeability, such as cyclosporine, protect cerebral tissue against ischemia-induced damage.25 Propofol has been shown to enhance posts ischemic functional recovery in the heart by inhibiting the opening of the MPTP,26,27 but no studies have yet been conducted in the brain.

In the current study, we evaluated the neuroprotective effects of propofol, at clinically relevant concentrations, in rat organotypic hippocampal slices exposed to oxygen-glucose deprivation (OGD), an in vitro model of cerebral ischemia we have been extensively using in our laboratory.28 Then, to elucidate its mechanism of action, we examined whether propofol could act by reducing Ca2+-induced rat brain mitochondrial swelling, an index
of increased mitochondrial membrane permeability, and whether it could prevent the ultrastructural mitochondrial changes evoked by OGD in CA1 pyramidal cells. Finally, we examined the effects of propofol on the extent of the infarct and the neurobehavioral outcome in rats subjected to permanent middle cerebral artery occlusion (MCAO), a severe model of focal ischemia in vivo.

Materials and Methods

The experimental protocol was conducted according to the Italian guidelines for animal care (DL 116/92) in application of the European Communities Council Directive (86/609/EEC) and was formally approved by the Animal Care Committee of the Department of Pharmacology of the University of Florence (Florence, Italy).

Materials

Propofol (2,6-diisopropylphenol) in an intralipid emulsion (Diprivan) was supplied by Astra-Zeneca (Basilglio, Milano, Italy). The lipid vehicle (Intralipid) was purchased from Fresenius Kabi (Verona, Italy). For in vitro experiments, pure propofol (Sigma-Aldrich, Milano, Italy) was used and dissolved in dimethylsulfoxide (0.1% final concentration). Propidium iodide (PI) was purchased from Molecular Probes (Leiden, The Netherlands), 2,3,5-triphenyl-2H-tetrazolium chloride (tetrazolium red) from Sigma-Aldrich. Tissue culture reagents were obtained from Gibco-BRL (San Giuliano Milanese, Milano, Italy) and ICN Pharmaceuticals (Opera, Milano, Italy).

OGD and Assessment of CA1 Pyramidal Cell Injury in Organotypic Rat Hippocampal Slices

Organotypic hippocampal slice cultures were prepared as previously reported.29 The hippocampi were removed from the brains of 8-day-old Wistar rats (Harlan-Nossan, Udine, Italy), and transverse slices (420 μm) were prepared using a McIlwain tissue chopper in a sterile environment. Isolated slices were first placed into ice-cold Hanks’ balanced salt solution supplemented with 5 mg/ml glucose and 1.5% Fungizone (GIBCO BRL) and then transferred to humidified semipermeable membranes (30 mm Millicell-CM 0.4-μm tissue culture plate inserts; Millipore, Rome, Italy; four per membrane). These were placed in six-well tissue culture plates containing 1.2 ml medium containing 50% Eagle’s minimal essential medium, 25% heat-inactivated horse serum, 24% Hanks’ balanced solution, 5 mg/ml glucose, 1 mM glutamine, and 1.5% Fungizone. Slices were maintained at 37°C and 100% humidity in a 95% air–5% CO2 atmosphere, and the medium was changed every 4 days. Experiments were conducted after 14 days in vitro.

Oxygen-glucose deprivation was induced as previously described.29 Organotypic hippocampal slices were preincubated for 30 min in serum-free medium and then subjected to OGD by exposing them to a serum-free medium devoid of glucose and previously saturated with 95% N2–5% CO2. After 30 min of incubation at 37°C in an airtight anoxic chamber (PROOM model 110; Biospherix, Redfield, NY), the cultures were transferred to oxygenated serum-free medium containing 5 mg/ml glucose and 5 μg/ml PI and returned to the incubator under normoxic conditions until neuronal injury was evaluated 24 h later. Under these experimental conditions, 30 min of OGD induces a CA1 pyramidal cell damage that is approximately 70% of the maximal damage achieved by exposing the slices for 24 h to 10 mM glutamate (see Pellegrini-Giampietro et al.29). Propofol (1, 10, 30, and 100 μM) was added to the incubation medium during the 30-min OGD injury and the subsequent 24-h recovery period. Cell injury was assessed using PI, a polar dye that enters the cells only if the membrane is damaged and becomes fluorescent after binding to DNA. PI fluorescence was viewed using an Intracellular Imaging Inc. (Cincinnati, OH) imaging system, consisting of a xenon-arc lamp, a Nikon TMS-F inverted microscope equipped with a fluorescence optics module for epi-illumination, a low-power objective (4×), and a rhodamine filter set. To quantify cell death, the CA1 hippocampal subfield was identified and encompassed by a frame using the drawing function in the image software, and the intensity of PI fluorescence (i.e., the mean of the fluorescence intensity values of each pixel in the area defined by the frame) was collected (for details, see Pellegrini-Giampietro et al.29). Background PI fluorescence was determined in control cultures not exposed to OGD and was subtracted from all experimental values. There was a linear correlation between PI fluorescence and the number of injured CA1 pyramidal cells as detected by morphological criteria (see Pellegrini-Giampietro et al.29).

Isolation of Brain Mitochondria and Measurement of Mitochondrial Swelling

Swelling of isolated mitochondria is a recognized index of increased mitochondrial membrane permeability.30–32 Brain mitochondria were isolated by differential centrifugation, according to Lindenmayer et al.33 Briefly, brains of adult Sprague-Dawley rats (Harlan-Nossan, Udine, Italy) (weight, 280–310 g) were quickly removed and homogenized by Ultra-Turrax in 5 ml of an ice-cold medium containing 300 mM sucrose, 5 mM/ml bovine serum albumin, 2 mM EDTA, and 10 mM TRIS-HCl (pH 7.4). The whole homogenate was centrifuged for 5 min at 3,000 g to pellet nuclei and unbroken cells. Subsequently, the supernatant was spun at 12,000 g for 5 min to sediment mitochondria. The pellet was washed and
then resuspended in 1 ml ice-cold bovine serum albumin–free and EDTA-free sucrose buffer (300 mM sucrose, 10 mM TRIS-HCl, pH 7.4) to give a mitochondrial protein content of 0.25 mg/ml. A fixed volume (25 μl) of mitochondrial suspension was diluted in 1 ml bovine serum albumin–free and EDTA-free sucrose buffer at 25°C. Ca2+-induced swelling of mitochondria was monitored as the decrease in light scattering at 540 nm for 5 min with a Perkin-Elmer Lambda 5 spectrophotometer, according to Halestrap et al.30 Propofol (30 or 100 μM final concentration) dissolved in dimethyl sulfoxide was added to the mitochondrial suspension 1 min before CaCl2 (100–1,000 μM) exposure. Controls were pricucbated with an equal concentration of dimethylsulfoxide (0.1%, final concentration).

**Transmission Electron Microscopy**

The effects of propofol on the mitochondrial ultrastructural changes of CA1 pyramidal cells evoked by OGD were analyzed in organotypic hippocampal slices by transmission electron microscopy. Slices were gently removed from the culture dishes with a brush, fixed in Karnowsky solution for 2 h at room temperature, and then postfixed in 1% osmium tetroxide (in 0.1 M phosphate buffer, pH 7.4) for 1 h at 4°C. Hippocampal slices were then dehydrated in graded acetone, passed through propylene oxide, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and alkaline bismuth and viewed under a Jeol 1010 (Tokyo, Japan) electron microscope at 80 kV.

**Permanent MCAO in Rats**

Adult male Sprague-Dawley rats weighing 250–280 g were used. All animals were fasted overnight before the experiment but had free access to water. Animals were initially anesthetized in a container with 5% isoflurane until the righting reflex became unapparent; then the anesthetic state was maintained with isoflurane (1–2%) delivered in an air–oxygen mixture (60 and 40%, respectively) through a facemask. Efficacy of ventilation and oxygenation was assessed by measuring carbon dioxide and oxygen tension in tail artery blood samples. Blood pressure was monitored noninvasively. Body temperature was monitored with a rectal probe and maintained at 37° ± 0.5°C with a thermostatically controlled heating pad. Permanent MCAO was induced using a relatively noninvasive technique with an intraluminal filament according to Zea Longa et al.34 Silicon-coated nylon filaments (external diameter, 0.28 mm) were prepared. The external and internal right carotid arteries were dissected under an operating microscope, and a silk suture was tied loosely around the external carotid stump. The silicone-coated nylon filament was then inserted through the external into the internal carotid artery up to the circle of Willis to occlude the right middle cerebral artery. The silk suture was finally tightened around the intraluminal filament to prevent bleeding and dislodgement. Recovery of the righting reflex occurred approximately 5 min after isoflurane administration was terminated, and the animals were returned to their cages. Vehicle-treated animals received an equivalent concentration of 10% Intralipid immediately after MCAO; treated animals received 100 mg/kg Diprivan intraperitoneally immediately after and at 15, 30, and 90 min after MCAO.

Twenty-four hours after ischemia, rats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal) and then decapitated. Brains were quickly removed and inspected to confirm the position of the intraluminal filament and the absence of subarachnoid hemorrhage. Brains were then coronally sectioned with a tissue chopper at 2-mm intervals, incubated for 30 min in a 2% solution of 2,3,5-triphenyl-2H-tetrazolium chloride at 37°C for vital staining, and fixed by immersion in 4% paraformaldehyde in phosphate buffer (pH 7.4). 2,3,5-Triphenyl-2H-tetrazolium chloride–stained sections were computer scanned, and the infarct areas were outlined and measured using an image analysis system (Image Pro Plus 3.0; Silver Spring, MD). Infarct volumes were calculated as previously described in detail35 and, to correct for swelling of the ischemic hemisphere, the following formula was applied, in which brain swelling corresponds to the difference in brain volume between the two hemispheres: Corrected Infarct Volume = Left Hemisphere Volume – (Right Hemisphere Volume – Measured Infarct Volume).36

**Neurobehavioral Evaluation after MCAO in Rats**

After MCAO, the occurrence of a turning behavior ipsilateral to the ischemic side upon tail stimulation was searched as an index of ischemia in the territory of the middle cerebral artery. To assess the neuroprotective effects of propofol, a neurobehavioral examination according to Garcia et al.37 was performed immediately before and 24 h after MCAO. Each rat was assigned a score in six different tests (maximal global score = 18, minimal global score = 3). This testing assesses the following: spontaneous activity—no movement (0 points) to normal behavior (3 points); motor symmetry in all limbs—no movement at the left side (0 points) to normal behavior (3 points); symmetry in the outstretching of forelimbs—no movement (0 points) to normal motor symmetry (3 points); climbing—falls trying to climb (1 point) to normal climbing (3 points); body proprioception—no response at the left side (1 point) to symmetrical response (3 points); and response to vibrissae touch—no response on the left side (1 point) to symmetrical response (3 points). Neurobehavioral testing was performed by a single observer blinded to group assignment. To avoid the influence of daily fluctuations...
Fig. 1. Propofol attenuates CA1 injury induced by 30 min of oxygen-glucose deprivation (OGD) in rat organotypic hippocampal slices. Cultured slices were exposed to OGD, incubated with propidium iodide (5 μg/ml) for 24 h, and then photographed under fluorescence optics. (A) Hippocampal slice observed 24 h after 30 min of OGD displaying intense and selective propidium iodide labeling in the CA1 region. (B) CA1 injury was prevented by the presence of 30 μM propofol in the incubation medium during OGD and the subsequent 24-h recovery period. (C) OGD was applied for 30 min, and 24 h later, the extent of cell death was evaluated by measuring the propidium iodide fluorescence in the CA1 area. Background fluorescence was determined in control sister slices not exposed to OGD and was subtracted from all experimental values. Data are expressed as percentage of maximal propidium iodide fluorescence observed in untreated slices 24 h after OGD. Cell death in CA1 was significantly attenuated by the addition of 10–100 μM propofol to the incubating medium during OGD and the subsequent 24-h recovery period. Each point represents the mean ± SEM of at least five experiments. *P < 0.05 and **P < 0.01 versus untreated ischemic slices (analysis of variance followed by Tukey w test).

Results

Neuroprotective Effects of Propofol against OGD in Rat Organotypic Hippocampal Slices

Hippocampal slices cultured for 14 days in vitro retained an organotypic organization in which the pyramidal and granule cell layers could be clearly defined when observed under phase-contrast microscopy or after toluidine blue staining.29 Control slices incubated with PI displayed low fluorescence levels, but when cultures were subjected to OGD for 30 min, PI staining increased dramatically in the hippocampal CA1 area 24 h later (fig. 1A). Selective vulnerability of CA1 pyramidal cell death is a hallmark of severe global ischemia in patients successfully resuscitated from cardiorespiratory arrest38 or in experimental animal models.39,40 Quantitative analysis revealed that OGD induced an increase in PI fluorescence in the CA1 region that was approximately 65% of the maximal fluorescence intensity obtained by exposing the slices to 10 mM glutamate (see Pellegrini-Giampietro et al.28). The addition of 10, 30, and 100 μM propofol (but not of the vehicle dimethylsulfoxide) to the incubation medium during OGD and the subsequent 24-h recovery period significantly reduced CA1 injury by 35 ± 4, 40 ± 5, and 43 ± 5%, respectively (figs. 1B and C). Exposure of slices to propofol alone for 24 h did not induce any increase in PI fluorescence (not shown).

Effects of Propofol on Mitochondrial Swelling

To investigate the possible mechanism of action of propofol, we then examined its effects on brain mitochondria.

Propofol Reduces Ca2+-induced Brain Mitochondrial Swelling. The exposure of rat brain isolated mitochondria to CaCl2 (100–1,000 μM) induced a dose-dependent increase in mitochondrial swelling, as detected by a decrease in light transmission (fig. 2A). The addition of 100 μM propofol prevented the Ca2+-induced decrease in light transmission (figs. 2B and C); a similar effect was observed with 30 μM propofol (not shown). The effect was significant at 200–300 μM CaCl2 and, notably, persisted for at least 5 min after the stimulus.

Propofol Reduces OGD-induced Mitochondrial Swelling in CA1 Pyramidal Cells. To determine whether these findings on isolated brain mitochondria are relevant to ischemia, we used transmission electron microscopy to examine the effects of propofol on the OGD-induced mitochondrial damage of CA1 pyramidal cells in hippocampal slices. Observation of control hippocampal slices under the electron microscope (fig. 3) revealed the appearance of healthy, triangular-shaped CA1 pyramidal cells, displaying a large nucleus, dispersed chromatin, a well-defined nucleolus, and a cytoplasm rich in organelles and lipofuscin (not shown; see
Mitochondria were elongated or round and had numerous transversae cristae with a regular pattern and an electron-dense intramitochondrial matrix. After OGD, CA1 pyramidal cell mitochondria showed substantial ultrastructural alterations, such as swelling (i.e., enlarged size with an electron-lucent matrix), disruption of cristae, and fragmentation of internal membranes. Treatment with 30 or 100 µM propofol exerted a protective effect on CA1 pyramidal cell mitochondria: The frequency and the extent of the ultrastructural abnormalities were markedly reduced. In particular, the treatment substantially decreased the ischemia-induced mitochondrial swelling.

**Propofol Reduces the Infarct Size after Permanent MCAO in Rats**

Rats not displaying a clear-cut turning behavior after MCAO and rats with subarachnoid hemorrhage at postmortem examination were excluded from the study. Physiologic parameters did not differ significantly among rat groups (table 1). Permanent MCAO in vehicle-treated rats led to the formation of a large infarct in the ipsilateral hemisphere that 24 h later affected a significant portion of the frontal and parietal cortex, the caudate-putamen, and the diencephalon (fig. 4A). Administration of 100 mg/kg intraperitoneal propofol (but not of the Intralipid vehicle) immediately after the occlusion reduced the extent of the infarct (fig. 4B); the effect was particularly evident and reached statistical significance in the most anterior portions of the brain (figs. 4B and C). The volume of the infarct produced by MCAO was 426 ± 24 mm³ (mean ± SEM); propofol significantly reduced the extension by approximately 30 ± 4%. A quantitatively similar degree of protection was obtained when the drug was administered 15 and 30 but not 90 min after MCAO (fig. 4D). Finally, a comparable degree of neuroprotection was observed when the infarct volume was corrected for swelling of the ischemic hemisphere (data not shown).

**Propofol Improves Spontaneous Activity after Permanent MCAO in Rats**

As reported, MCAO induced a significant reduction in the global neurobehavioral score, when observed 24 h after the occlusion (table 2). The global neurobehavioral global score was not modified by treatment with propofol. However, when individual tasks were compared, propofol administered within 30 min after MCAO significantly preserved spontaneous activity in ischemic rats.

**Discussion**

Our results show that propofol at clinically relevant concentrations is neuroprotective in models of cerebral ischemia in vitro and in vivo. In vitro, propofol...
attenuated CA1 pyramidal cell death in rat organotypic hippocampal slices exposed to OGD in a concentration-dependent fashion, prevented the Ca^{2+}-induced increase in membrane permeability in isolated rat brain mitochondria, and reduced the ultrastructural mitochondrial changes evoked by OGD in CA1 pyramidal cells. In vivo, rats subjected to permanent MCAO and treated with propofol displayed, 24 h later, smaller infarcts and improved spontaneous activity as compared with vehicle-treated rats, even when propofol administration was delayed up to 30 min after vascular occlusion.

Propofol (10–100 μM) was able to attenuate CA1 pyramidal cell injury in organotypic hippocampal slices exposed to OGD. Previous reports had shown that propofol reduces neurotransmission damage induced by anoxia but not by OGD in acute hippocampal slices. These controversial results may be ascribed to the fact that organotypic cultures are quite different from acute hippocampal slices. These controversial results may be ascribed to the fact that organotypic cultures are quite different from acute hippocampal slices.

Fig. 3. Ultrastructural evidence that propofol reduces CA1 pyramidal cell mitochondrial swelling after oxygen-glucose deprivation in organotypic hippocampal slices. (A and D) Control CA1 pyramidal cell mitochondria, displaying a healthy and regularly shaped morphologic appearance and an electron-dense intramitochondrial matrix. (B and E) Thirty minutes of oxygen-glucose deprivation induced mitochondrial swelling and dramatic ultrastructural alterations, including disruption of cristae and fragmentation of the internal membranes. (C and F) Treatment of organotypic hippocampal slices with 100 μM propofol markedly prevented the mitochondrial alterations induced by oxygen-glucose deprivation. Bar: 500 nm (A–C) and 200 nm (D–F).

Anesthesiology, V 104, No 1, Jan 2006
Downloaded From: http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931078/ on 11/28/2018

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.
notypic hippocampal slice model. It should be noted that in their study, OGD injury was more severe, because the duration of exposure was more prolonged (1 hr vs. 30 min) and neuronal death was not selective to the CA1 region, as occurs in vivo. Our model, therefore, may be more amenable to pharmacologic intervention with drugs as propofol that are neuroprotective in models of transient forebrain ischemia in vivo.16,50,51

There are several mechanisms that may explain the neuroprotective effects of propofol, including its capability of modulating glutamate neurotransmission and its antioxidant properties. However, the results of studies on these mechanisms have not been consistent. Propofol restores glutamate uptake in ascorbate-depleted astrocytes exposed to peroxyl radicals,5 reduces potassium-evoked glutamate release in rat cerebrocortical slices,52 and significantly counteracts OGD-induced increase of extracellular glutamate concentrations in neuronal cultures, presumably by restoring glutamate uptake.3 However, in freshly prepared rat hippocampal slices, propofol impairs the recovery of CA1 population spikes from N-methyl-D-aspartate–induced damage.5,46 Moreover, propofol directly inhibits N-methyl-D-aspartate receptors only at high concentrations (> 160 μM),19 and its neuroprotective effect in rats subjected to global ischemia does not seem to be due to a reduction in extracellular glutamate concentrations.50 Propofol has repeatedly been demonstrated to have antioxidant properties,14,42 although in an in vivo study, neuroprotection with propofol could not be ascribed to a modification of low-molecular-weight antioxidants.12

Postischemic energy failure and activation of apoptosis in neurons are regulated at multiple levels, and an increase in mitochondrial membrane permeability is now accepted to play a major role.21 Propofol has been shown to ameliorate reperfusion injury in the rat heart by inhibiting the opening of the MPTP.26,27 but no studies have yet been conducted in the brain. We therefore examined the effects of propofol on brain mitochondria under two conditions that simulate cerebral ischemia: an overload of CaCl25,54 in isolated brain mitochondria and OGD injury in hippocampal slices. Propofol prevented mitochondrial swelling in both circumstances at 30 and 100 μM, concentrations that have been reported to directly inhibit the MPTP in the heart27 and are generally retained to correspond to the concentrations that, because of its high lipid–water coefficient partition, can be reached in the brain during anesthesia.17,11,43,55 These findings suggest that an effect of propofol on mitochondria might also be important in the ischemic setting in vivo, by directly inhibiting the MPTP or, indirectly, by modulating the production/concentration of other cytotoxic mediators, such as free radicals, glutamate, or calcium that can alter the integrity of mitochondria or trigger the opening of the MPTP.21,27

Propofol has been repeatedly demonstrated to be neuroprotective in transient models of global50,56 and focal ischemia induced by both MCAO8,10,57 or endothelin-1 intrastratial injection in awake rats.11,12 Despite that acute ischemic stroke in humans is frequently thrombembolic in nature and rarely undergoes spontaneous reperfusion,58 the effects of propofol in permanent MCAO have not been yet investigated. A report by Tsai et al.15 failed to demonstrate a protective action of propofol after permanent MCAO, but in this study, it was combined with bilateral carotid occlusion. We observed that the overall mean infarction size was significantly reduced in rats receiving propofol within 30 min after MCAO and that the protection was particularly evident in the most anterior coronal sections (slices 1–3), in areas that include the frontoparietal cortex. Propofol also preserved spontaneous activity after MCAO but did
not affect other neurologic tasks or the mean global neurobehavioral score. Permanent MCAO in rats produces an ischemic core in the caudate-putamen and a penumbral area in the frontoparietal cortex, where a network of surface collateral connections exists and allows a certain degree of reperfusion. Spontaneous activity, as evaluated according to Garcia et al., is an index of rat normal exploring behavior rather than of motor function and thus requires the integrity of the frontal cortex.

In our permanent MCAO model, propofol induced neuroprotection to a somewhat lesser degree when compared with that reported in models of transient focal ischemia. This could be attributed to the model itself, which in our case is more severe and does not include reperfusion of the right middle cerebral artery, or to the fact that we administered propofol in a single administration and not by continuous infusion. The total amount of propofol we administered, however, is in accord with the study of Arcadi et al. showing that propofol at a single dose of 50 and 100 but not 25 mg/kg intraperitoneally attenuates CA1 injury after global ischemia in gerbils. In our study, we limited the period of evaluation of neuroprotection, in terms of both anatom-
ical lesion and neurologic outcome, to the first 24 h after MCAO. Therefore, we cannot rule out a transient protective effect of propofol in our model, as reported for other anesthetics.59 However, in a model of incomplete cerebral ischemia and reperfusion, propofol offered long-term neuroprotection.60 Moreover, early evaluation of the neuroprotective effects of propofol seems to be predictive of prolonged functional improvement in rats exposed to mild focal ischemia.12

There are a number of issues in this study that still must be clarified. (1) Although we have demonstrated that propofol directly reduces Ca2+ -induced swelling in isolated mitochondria, it is possible that propofol may act upstream of mitochondria in our in vitro and in vivo models of ischemia, perhaps by interacting with other cytotoxic events (i.e., glutamate receptor activation, impairment of glutamate uptake, free radical production) that may or may not involve changes in intracellular free Ca2+ concentrations and mitochondrial membrane permeability. (2) Because we used a recovery interval of 24 h in hippocampal slices exposed to OGD and in rats after MCAO, we cannot exclude a transient neuroprotective effect for propofol. (3) The concentrations of propofol that we used have been reported to be in the anesthetic range by some authors43,44,45 but are considered to be high by others.

Despite these limitations, our study shows that propofol is neuroprotective in organotypic hippocampal slices exposed to OGD in vitro, possibly by preventing the ischemic increase in brain mitochondrial membrane permeability. In a severe model of focal cerebral ischemia in vivo, propofol reduces the size of the infarct and preserved spontaneous activity, even when drug administration is delayed up to 30 min after the occlusion of the middle cerebral artery.

The authors thank Prof. Lucia Formigli, B.Sc. (Dipartimento di Anatomia, Istotologia e Medicina Legale, Università di Firenze, Florence, Italy), for help with the transmission electron microscopy.

Table 2. Neurobehavioral Score after Permanent MCAO

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Vehicle T0</th>
<th>Propofol T15</th>
<th>Propofol T30</th>
<th>Propofol T90</th>
<th>Propofol T90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous activity</td>
<td>3 (3, 3)</td>
<td>2 (1, 3)*</td>
<td>3 (2, 3)</td>
<td>3 (2, 3)</td>
<td>3 (3, 1)</td>
<td>2 (1, 3)*</td>
</tr>
<tr>
<td>Symmetry</td>
<td>3 (3, 3)</td>
<td>2 (1, 2)*</td>
<td>1/2 (1, 2)*</td>
<td>2 (1, 2)*</td>
<td>1/2 (1, 2)*</td>
<td>1/2 (1, 2)*</td>
</tr>
<tr>
<td>Outstretching</td>
<td>3 (3, 3)</td>
<td>2 (1, 2)*</td>
<td>2 (1, 2)*</td>
<td>2 (1, 2)*</td>
<td>2 (1, 2)*</td>
<td>2 (1, 2)*</td>
</tr>
<tr>
<td>Climbing</td>
<td>3 (3, 3)</td>
<td>2 (1, 3)*</td>
<td>2 (1, 3)*</td>
<td>2 (1, 3)*</td>
<td>2 (1, 3)*</td>
<td>2 (1, 3)*</td>
</tr>
<tr>
<td>Proprioception</td>
<td>3 (3, 3)</td>
<td>2 (1, 2)*</td>
<td>2 (1, 2)*</td>
<td>2 (1, 2)*</td>
<td>2 (1, 2)*</td>
<td>2 (1, 2)*</td>
</tr>
<tr>
<td>Vibrisse touch</td>
<td>3 (3, 3)</td>
<td>2 (1, 2)*</td>
<td>1/2 (1, 3)*</td>
<td>1 (1, 2)*</td>
<td>2 (1, 3)*</td>
<td>2 (1, 2)*</td>
</tr>
<tr>
<td>Global score</td>
<td>18 (18, 18)</td>
<td>10 (7, 12)*</td>
<td>11 (8, 13)*</td>
<td>11 (10, 13)*</td>
<td>11 (10, 12)*</td>
<td>10 (7, 13)*</td>
</tr>
</tbody>
</table>

Neurobehavioral evaluation was performed by means of six different tasks according to the scale originally described by Garcia et al.77 and was assessed on awake animals immediately before induction of anesthesia for right middle cerebral artery occlusion (MCAO; sham) and 24 h after MCAO (ischemia). The six tasks evaluated spontaneous activity, symmetry in the movement of four limbs, forepaw outstretching, climbing, body proprioception, and response to vibrissee touch. In vehicle-treated rats, the mean global ability to perform the tasks was significantly impaired after MCAO. The treatment with propofol within 30 min after MCAO did not significantly improve the global score but preserved spontaneous activity. Data are expressed as median (minimum, maximum) of at least eight animals per group.

* P < 0.05 vs. sham (Kruskal-Wallis test followed by Dunn post hoc test).

References

18. Yamakura T, Sakimura K, Shimoji K, Mishina M. Effects of propofol on

Anesthesiology. V 104, No 1, Jan 2006

ADEMBRI ET AL.

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.
various AMPA, kainate- and NMDA-selective glutamate receptor channels expressed in Xenopus oocytes. Neurosci Lett 1995; 188:187–90
30. Halestrap AP: Calcium-dependent opening of a nonspecific pore in the mitochondrial inner membrane is inhibited at pH values below 7: Implications for the protective effect of low pH against chemical and hypoxic cell damage. Biochem J 1991; 278 (pt. 3):715–9
32. Di Lisa F, Menabo R, Canton M, Barile M, Bernardi P. Opening of the mitochondrial permeability transition pore causes depletion of mitochondrial and cytosolic Na+ and is a causative event in the death of myocytes in postischemic reperfusion of the heart. J Biol Chem 2001; 276:2751–5
39. Kirino T: Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 1982; 239:57–69
49. Abdel-Hamid KM, Tymianski M: Mechanisms and effects of intracellular calcium buffering on neuronal survival in organotypic hippocampal cultures exposed to anoxia/aglycemia or to excitotoxins. J Neurosci 1997; 17:3538–53
50. Yan V, Nakayama R, Ushijima K: Intracerebroventricular propofol is neuroprotective against transient global ischemia in rats: Extracellular glutamate level is not a major determinant. Brain Res 2000; 883:69–76
54. Kristian T: Metabolic stages, mitochondria and calcium in hypoxic/ischemic brain damage: Cell Calcium 2004; 36:221–33