

Differential Neurotoxic Effects of Propofol on Dissociated Cortical Cells and Organotypic Hippocampal Cultures

Isabelle Spahr-Schopfer, M.D.,* Lazlo Vutsits, M.D.,† Nicholas Toni, M.Sc.,‡ Pierre-Alain Buchs, Ph.D.,§ Lorena Parisi,|| Dominique Muller, M.D., Ph.D.#

Background: Propofol is a widely used anesthetic agent for adults and children. Although extensive clinical use has demonstrated its safety, neurologic dysfunctions have been described after the use of this agent. A recent study on a model of aggregating cell cultures reported that propofol might cause irreversible lesions of γ -aminobutyric acid-mediated (GABAergic) neurons when administered at a critical phase of brain development. We investigated this issue by comparing the effects of long-term propofol treatment on two models of brain cultures: dissociated neonatal cortical cell cultures and organotypic slice cultures.

Methods: Survival of GABAergic neurons in dissociated cultures of newborn rat cortex (postnatal age, 1 day) treated for 3 days with different concentrations of propofol was assessed using histologic and cytochemical methods. For hippocampal organotypic slice cultures (postnatal age, 1 and 7 days), cell survival was assessed by measuring functional and morphologic parameters: extracellular and intracellular electrophysiology, propidium staining of dying cells, and light and electron microscopy.

Results: In dissociated neonatal cell cultures, propofol induced dose-dependent lesions of GABAergic neurons and of glial cells. In contrast, no evidence for neurotoxic effects of

propofol were found after long-term treatment of organotypic slice cultures. Excitatory transmission was not affected by propofol, and inhibitory transmission was still functional. Histologic preparations showed no evidence for cell degeneration or death.

Conclusion: Although long-term applications of propofol to dissociated cortical cell cultures produced degeneration and death of GABAergic neurons and glial cells, no such lesions were found when using a model of postnatal organotypic slice cultures. This conclusion is based on both functional and morphologic tests. (Key words: Anesthesia; brain; rat; toxicity.)

PROPOFOL (2,6-diisopropyl phenol) is an alkyl phenol derivative dissolved in a lipid emulsion that has gained wide clinical use as an anesthetic and hypnotic agent since its introduction into clinical practice in 1986. Extensive clinical experience has demonstrated that propofol is a safe, intravenous anesthetic agent with relatively few side effects. Initial animal studies suggested that propofol is a pure hypnotic with little or no anticonvulsant or proconvulsant activity.¹ More recently, there have been several contradictory reports of both proconvulsant and anticonvulsant events associated with anesthesia in humans.²⁻⁵ Neurologic sequelae occurring after prolonged sedation with propofol,^{6,7} as well as convulsions after short anesthesia,⁸ have been described in children. Furthermore, a recent study conducted using a model of aggregating rat brain cell cultures provided evidence that propofol given at clinically relevant concentrations might cause irreversible lesions to γ -aminobutyric acid-mediated (GABAergic) neurons.⁹ Because GABA is a major inhibitory neurotransmitter, lesions of GABAergic neurons might explain some of the neurologic events described. Furthermore, although propofol is not recommended for use in children younger than 3 yr, it is used more and more extensively, including in neonatology.^{10,11} We therefore undertook the present study to evaluate the possibility that propofol might result in neurotoxic effects on the developing brain. For this purpose, we compared the effects of long-term propofol

* Consultant for Pediatric Anesthesia, Department of Anesthesiology, Pharmacology and Surgical Intensive Care.

† Research Resident, Department of Morphology.

‡ Student, Department of Anesthesiology, Pharmacology and Surgical Intensive Care.

§ Research Fellow, Department of Anesthesiology, Pharmacology and Surgical Intensive Care.

|| Technician, Department of Anesthesiology, Pharmacology and Surgical Intensive Care.

Professor of Neuropharmacology, Department of Anesthesiology, Pharmacology and Surgical Intensive Care.

Received from the Departments of Anesthesiology, Pharmacology and Surgical Intensive Care, and Morphology, University Medical Center, University Hospital of Geneva, Geneva, Switzerland. Submitted for publication November 30, 1998. Accepted for publication January 14, 2000. Supported by the Swiss National Research Fund, Bern, Switzerland.

Address reprint requests to Dr. Spahr-Schopfer: Department of Anesthesiology, University Hospital of Geneva, 1211 Geneva 14, Switzerland. Address electronic mail to: Isabelle.SpahrSchopfer@hcuge.ch

treatment on two distinct models of brain tissue. We used dissociated neonatal cell cultures that represent a model of immature, developing neurons close to the model in which toxicity was reported. In addition, we also tested organotypic slice cultures, which are prepared from postnatal animals and represent a more complex, three-dimensional preparation exhibiting many of the features of developing brain tissue.

Materials and Methods

Primary Culture of Newborn Rat Cerebral Cortex

After obtaining approval from the Animal Care Committee of the University Medical Center, primary cultures of newborn Sprague-Dawley rat cerebral cortex were prepared as described previously.¹² Briefly, after removal of the meningeal tissue, the cerebral cortex was mechanically dissociated in Hank's calcium- and magnesium-free medium and centrifuged for 10 min at 1,000 rpm. The pellet was resuspended in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and plated on polylysine-coated coverslips in petri dishes with a seeding density of 3.5×10^6 cells/petri dish. Under these conditions, cultures became confluent at the sixth day, and at this time the serum-containing medium was replaced by serum-free medium (Dulbecco's modified Eagle's medium [Gibco, Basel, Switzerland]: 15 mg/ml insulin, 20 mg/ml transferrin, 20 nM progesterone, 100 mM putrescine, and 30 nM sodium selenite) in the presence of cytosine-arabinoside (10^{-7} M) and Brain Derived Neurotrophic factor (Regeneron Pharmaceuticals, Tarrytown, NY). Under these conditions, neurons remained viable up to more than 2 weeks in culture.

To test the effect of propofol on neuronal survival, cultures were exposed to propofol (Disoprivan; Astra-Zeneca, London, United Kingdom) at concentrations of 0 (control), 10, 20, 50, and 100 $\mu\text{g/ml}$ for 72 h at the time when serum-free medium was administered, *i.e.*, from the sixth day *in vitro*. Electrophysiologic experiments conducted regarding synaptic activity at the surface of the slice culture confirmed that these concentrations of propofol were active. After 3 days of propofol exposure, cultures were fixed and processed for immunocytochemistry. To expose the two types of cultures (dissociated and organotypic) to the same conditions, a subsequent set of experiments were conducted using 10% fetal calf serum. Cells were exposed to the same concentrations of propofol for 72 h.

Immunocytochemistry

Cultures were fixed in 2% paraformaldehyde–2% glutaraldehyde diluted in 0.1 M phosphate buffer for 60 min at 4°C, then washed several times in phosphate-buffered saline solution. The monoclonal antibody anti-GABA 3D5 (a gift from P. Streit, Brain Research Institute, Zurich, Switzerland) was used to reveal GABAergic neurons. This antibody was diluted 1:2,000 in phosphate-buffered saline/0.5% bovine serum albumin/0.3% Triton X-100 solution. Cultures were incubated with the primary antibody at room temperature for 2 h or at 4°C overnight. Bound antibodies were revealed with rhodamine-conjugated sheep antimouse immunoglobulin G (dilution 1:40; Roche Diagnostic, Rothkreuz, Confederatio Helvetica [Switzerland]) secondary antibodies (diluted in phosphate-buffered saline/0.5% BSA solution).

Cultures were examined by either a fluorescence microscope (Axiophot; Zeiss, Oberlochen, Germany) or a confocal microscope (Bio-Rad MRC-600 laser scanning confocal imaging system; Hertfordshire, United Kingdom). Neurons were counted based on their morphology and GABA staining with the help of a square graticule placed into the ocular of the microscope. On each coverslip, 35 samples were randomly taken and pooled together. In each experimental group, at least three coverslips were examined.

Organotypic Hippocampal Slice Cultures

Organotypic hippocampal slice cultures were prepared from 7-day-old Sprague-Dawley rats as described previously.¹³ In some experiments, cultures were prepared from newborn animals (day 1 after birth). Briefly, animals were decapitated, the brain was excised, and the hippocampi were dissected under sterile conditions using procedures approved by the Animal Care Committee. Transverse hippocampal slices (six slices per hippocampus) were cut at 400 μm using a McIlwain tissue chopper (McIlwain, Surrey, UK). The slices were then placed on a porous Teflon membrane (millicell CM; Millipore Corp., Bedford, MA) and maintained at the interface between the culture medium and an atmosphere equilibrated with 5% CO_2 in air at 33°C. The culture media used in these experiments consisted of 50% minimal essential medium, 25% Hank's balanced salt solution, and 25% heat-inactivated horse serum, supplemented with Tris, bicarbonate, penicillin-streptomycin, and glucose. The culture media were changed twice a week.

The hippocampal slices were cultured for 6 days in the culture medium to allow the macrophages to remove the

cells that had been injured by the explantation.¹⁴ The slices were subsequently randomized into five groups: control, or propofol 100 $\mu\text{g}/\text{ml}$ for 3, 5, 7, or 10 days. The culture medium was changed three times a week. Propofol concentration was deliberately chosen at a value 10 times higher than peak clinical concentrations.¹⁵ To compare the two types of cultures under the same conditions as the dissociated cultures, a set of experiments was conducted using newborn rats (post-natal day 1). Organotypic slices were cultured with 10% serum only and exposed to 0 (control) and 100 $\mu\text{g}/\text{ml}$ propofol for 7 days.

Electrophysiologic Recordings

Slice cultures were placed in an interface recording chamber and continuously perfused with artificial cerebral fluid of the following composition: 140 mM NaCl, 3.5 mM KCl, 1.23 mM NaH_2PO_4 , 25.0 mM NaHCO_3 , 2.4 mM CaCl_2 , 1.5 mM MgSO_4 , and 10.0 mM dextrose, saturated with 95% O_2 –5% CO_2 , 35°C. Orthodromic and antidromic responses were evoked by stimulation pulses (200 μs ; 0.05 Hz) applied through a bipolar electrode made of twisted nichrome wires and positioned in the CA3 area. Evoked synaptic responses were recorded in the pyramidal layer of CA1 using a glass pipette filled with perfusion medium. Slices were equilibrated for 15–30 min before recordings with the artificial cerebral fluid to wash out the propofol. Responses were digitized and analyzed on-line using a homemade data acquisition system and stored for statistical analysis.

Recordings of membrane potential and current were conducted using whole-cell patch approaches and voltage-clamp techniques. Patch pipettes (3–8 M Ω) were filled with a medium containing 140 mM potassium gluconate, 8 mM NaCl, 20 mM Hepes, 10 mM EGTA, 2 mM MgCl_2 , 1 mM CaCl_2 , 2 mM Na-ATP, 2 mM Mg-GTP, pH 7.25. Responses were recorded using an Axoclamp 2B (Axon Instruments, Foster City, CA) amplifier (series resistance compensation set to 70–80%, access resistance between 10 and 20 M Ω).

Propidium Iodide Fluorescence Microscopy

Cell lysis can be identified by staining the nucleus of dying cells with propidium iodide (PI; Sigma, St. Louis, MO).¹⁶ PI was dissolved in phosphate buffer to a final concentration of 4.6 $\mu\text{g}/\text{ml}$. The cultured slices were incubated for 2 h at 33°C. They were fixed with paraformaldehyde 4% and mounted on glass. As positive control, we used slices treated with 300 μM HgCl for 2 h. PI signal was viewed with a fluorescence microscope,

and slices were photographed at low magnification under identical conditions of light intensity and exposure time. Because the number of labeled cells was low in control and propofol-treated cultures, quantification was conducted by counting the number of labeled cells per slice.

Morphologic Analyses

For electron microscopic analyses, hippocampal organotypic cultures were processed as previously described.¹⁷ Briefly, cultures were fixed with a solution of 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Three rinses in 0.1 M phosphate buffer were followed by postfixation in 1% OsO_4 in 0.1 M phosphate buffer for 1 h at 20°C. After three rinses in phosphate buffer, the cultures were dehydrated through an ascending series of ethanol concentrations (25%, 50%, 75%, 95%, and 100%). Ethanol was then replaced by propylene oxide, and the slices were infiltrated through graded propylene oxide:epoxy embedding medium mixtures (1:1, 1:3, 2 h each) and left overnight in epoxy embedding medium. The slices were flat embedded in epoxy embedding medium between polyester foils for 48 h at 60°C, then re-embedded in epoxy embedding medium for two additional days. Blocks were then trimmed to include the CA1 region. Ultrathin sections of approximately 60–80 nm were stained with uranyl acetate and lead citrate and examined with a Philips CM10 electron microscope (Eindhoven, The Netherlands) at 80 kV. For histologic analyses, 2-mm-thick sections were stained with a solution of methylene blue and azur II in borax and observed with light microscope.

Statistics

Kruskal-Wallis and Tukey-Kramer multiple comparisons tests were used for analysis of PI staining. Unpaired *t* test was used for electrophysiologic data. $P < 0.05$ was considered significant.

Results

Effects of Propofol on Dissociated Cultures

Propofol was recently reported to produce neurotoxic effects on inhibitory GABAergic interneurons in a model of aggregating cell cultures. As illustrated in figure 1, we also observed such a toxic effect of propofol when added in the medium in dissociated cortical cell cultures. These dissociated cell cultures were prepared from new-

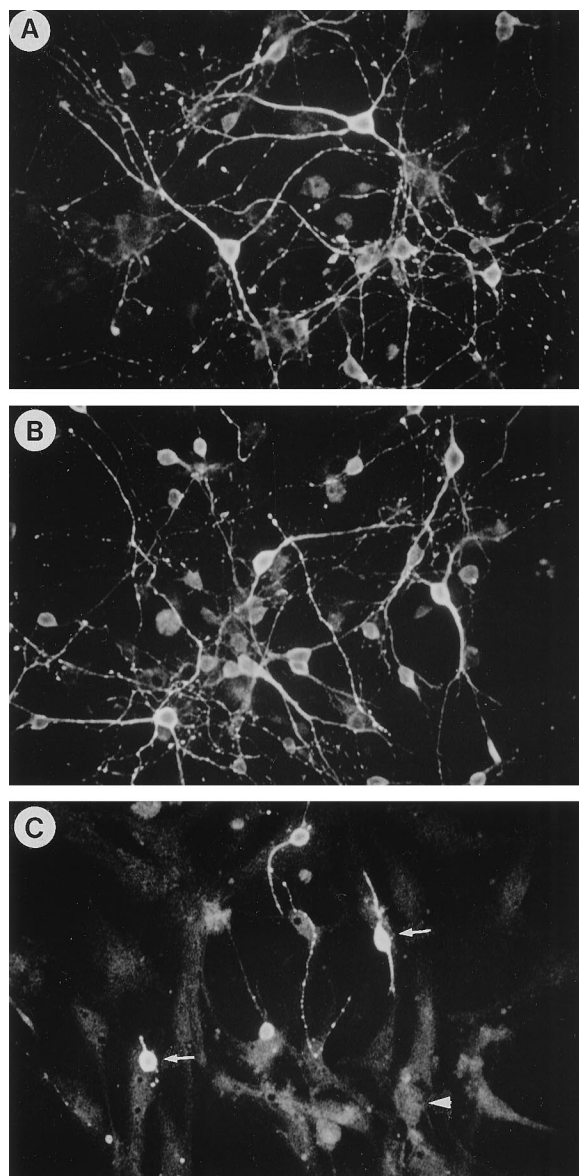


Fig. 1. Neurotoxic effect of long-term application of propofol on dissociated cortical cell cultures. GABAergic neurons are stained with an anti-GABA antibody. (A) Illustration of a control culture maintained 3 days under serum-free conditions showing viable neurons with a well-developed arborization. (B) Cultures treated for 3 days with 10 $\mu\text{g/ml}$ propofol show no detectable effect on neuronal survival and arborization. (C) Illustration of a culture treated for 3 days with 100 $\mu\text{g/ml}$ propofol. GABAergic cells (arrows) are pycnotic, indicating cell death. Glial cells (arrow head) are also swollen, indicating a poor viability. (A–C) Magnification 330 \times .

born tissue and were shown by previous work to mainly contain glial cells and GABAergic interneurons.¹⁸ Application of propofol at concentrations between 5 and 100 $\mu\text{g/ml}$ for 3 days, with or without the presence of serum in the culture media, resulted in a dose-dependent accumulation of pycnotic cells. Although concentrations of 5 and 10 $\mu\text{g/ml}$ had no effect on neuronal survival and morphology, at doses greater than 20 $\mu\text{g/ml}$, most cells in the culture became pycnotic, with very poorly developed arborizations (fig. 1). Cells surviving after propofol treatment were counted, and results show a dramatic decrease in the number of cells as a function of propofol concentration. Results of cell counts are expressed as percentage of the initial number before treatment (control) and are as follows (\pm SD): propofol 10 $\mu\text{g/ml}$, 98.4% (\pm 34.3); 20 $\mu\text{g/ml}$, 69% (\pm 28.7); 50 $\mu\text{g/ml}$, 46.2% (\pm 22.98); and 100 $\mu\text{g/ml}$, 18.4% (\pm 5.64). In addition, a progressive degeneration of glial cells was also apparent as the propofol concentration was increased. Note that glial cells were also faintly stained by the GABA antibody.¹⁹ As illustrated in figure 1C, glial cells exposed to 100 $\mu\text{g/ml}$ propofol became swollen thereby indicating a poor state of preservation. These results were reproduced in three separate experiments for cultures containing either 10% serum or no serum and were not observed if only the intralipid vehicle was applied to the cultures.

To test further the possibility of neurotoxic effects of propofol on the developing central nervous system, we then used a model of organotypic slice cultures. These cultures are prepared from postnatal animals, they retain a well-preserved morphologic and physiologic organization, and they share many similarities with the developmental events observed *in situ*. To evaluate the possibility of neurotoxic effects produced by propofol on this model, we used both functional and morphologic tests.

Physiologic Analyses in Organotypic Cultures

As a first test, we measured the size of the excitatory field potential evoked by supramaximal stimulation in slices treated up to 10 days with concentrations of propofol 10–20 times larger than those used in clinical situations. The results are illustrated in figure 2 and show that no statistically significant difference could be detected between control and treated cultures, thereby indicating that excitatory transmission was not affected by propofol.

Because these experiments could not exclude that a subpopulation of GABAergic interneurons could have

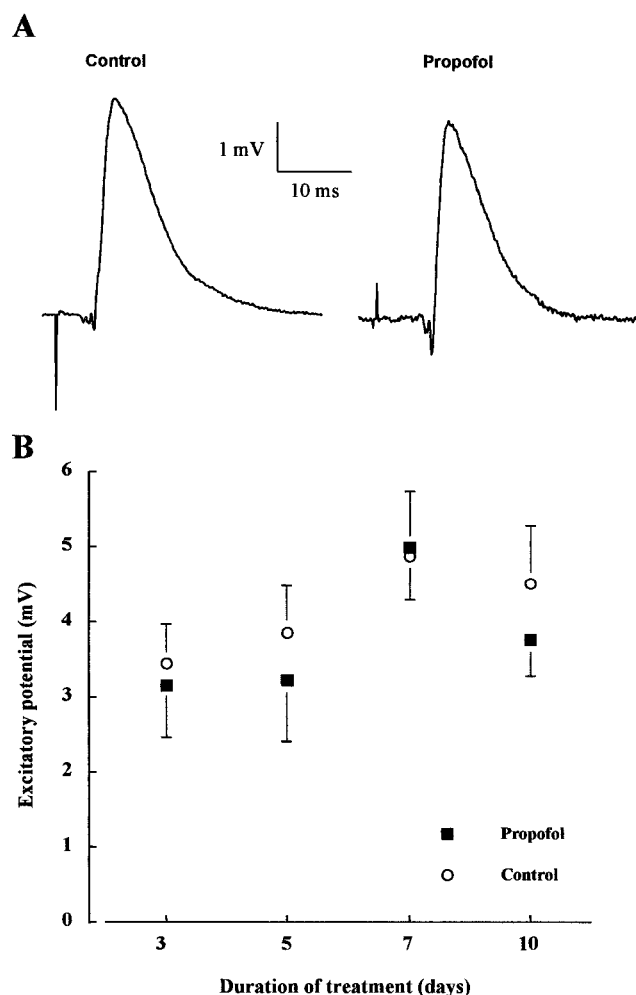


Fig. 2. Long-term treatment with propofol does not affect excitatory synaptic responses in a model of organotypic hippocampal slice cultures. (A) Illustration of excitatory postsynaptic potentials evoked by supramaximal stimulation and recorded in the pyramidal layer of CA1 in control and propofol-treated cultures. (B) Graph representing the size of excitatory field potentials (in millivolts) evoked by supramaximal stimulation in slice cultures treated for 3, 5, 7, and 10 days with 100 μ g/ml propofol. Values are mean \pm SD, $n = 7$. There is no statistically significant difference between the groups.

been affected, we then measured whether GABA_A-mediated inhibition was still functional after 1-week treatment with propofol. To test this, we analyzed the effects of bicuculline, a GABA_A-receptor antagonist, on field potentials elicited in control and propofol-treated slices. Under control conditions, blockade of inhibition quickly results in the generation of epileptiform discharges. As illustrated in figure 3, we found, in both cases, that blockade of GABA_A receptors by bicuculline triggered epileptiform activity and prolonged in a similar way the

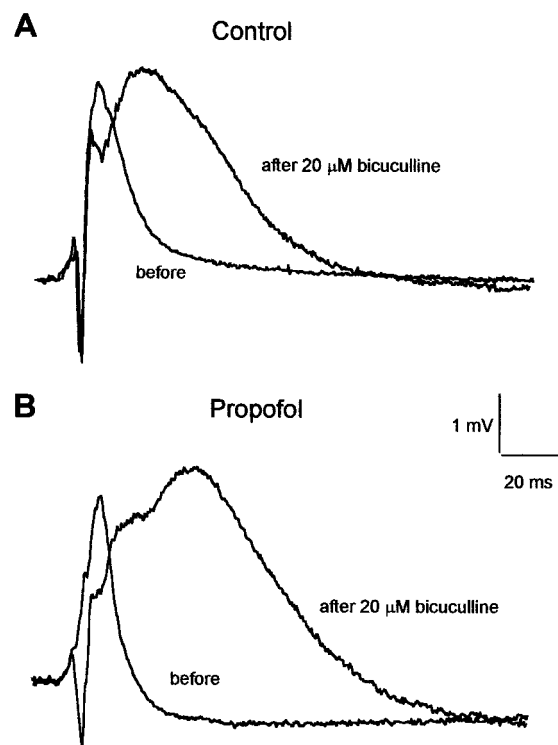


Fig. 3. Preserved inhibitory activity in long-term propofol treated slice cultures. The existence of a functional inhibitory network is tested by comparing excitatory field potentials recorded in the stratum pyramidale of the CA1 region before and after application of 20 μ M bicuculline, a GABA_A receptor antagonist. (A) Representative responses recorded in control cultures. (B) Representative responses recorded in a slice culture treated for 7 days with 100 μ g/ml propofol. Similar results were obtained in five other experiments ($n = 6$).

excitatory postsynaptic potential recorded in the CA1 pyramidal layer.

To analyze more quantitatively whether the ratio between excitation and inhibition could have been modified by propofol treatment, we used intracellular whole-cell patch techniques and measured the current to voltage relation of the compound synaptic response elicited by stimulation of Schaffer collaterals. Under these conditions, fiber stimulation triggered an excitatory postsynaptic potential-inhibitory postsynaptic potential sequence characterized by an inward current followed by an outward current at potentials below the reversal potential for GABA_A-mediated inhibition (fig. 4). To be able to compare the size of these currents between different cells, the stimulation intensity was adjusted in all experiments so as to evoke an inward excitatory current of -0.5 nA at -65 mV. We then measured the size of the excitatory and GABA_A-mediated currents at 10

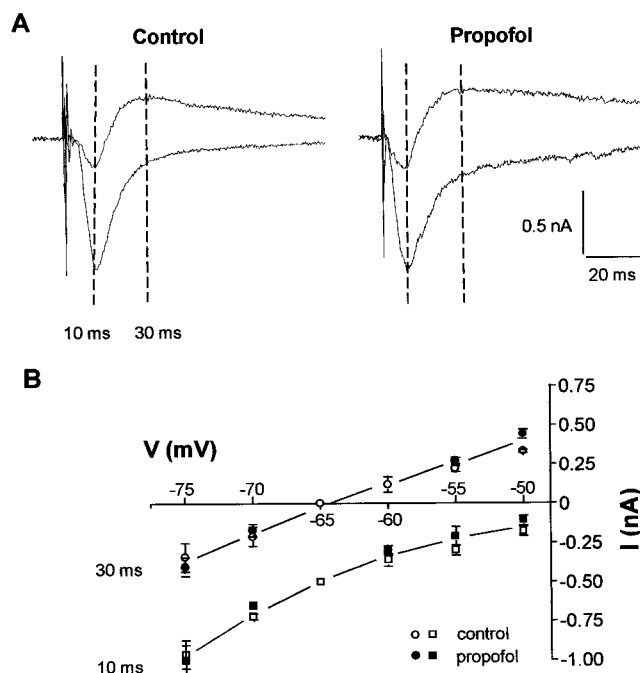


Fig. 4. Quantitative analysis of excitatory and inhibitory synaptic currents recorded using whole-cell clamp techniques in control and propofol-treated slice cultures. (*A, left*) Illustration of the compound synaptic currents recorded under control conditions with the cell clamped at -55 mV and -70 mV. The biphasic nature of the response at -55 mV indicates that it is composed of an inward excitatory component (best measured 10 ms after stimulation) and an outward inhibitory component (that is maximal 30 ms after stimulation). (*A, right*) Traces are similar responses recorded under the same conditions but after 1-week treatment with propofol. (*B*) Current to voltage relationship representing the size of the excitatory (squares; measures made at 10 ms) and inhibitory (circles; measures made at 30 ms) components of the synaptic responses recorded at different membrane potentials under control condition (open symbols) and after 7 days treatment with $100 \mu\text{g/ml}$ propofol (filled symbols). Data are mean \pm SD of the values measured in three different CA1 neurons. The current to voltage curves are superposable, indicating no difference in the ratio between excitation and inhibition after propofol treatment.

and 30 ms, respectively, after the stimulation and compared these values at various levels of resting membrane potentials. As illustrated on the *i-v* curve of figure 4B, the size of the currents measured at 10 and 30 ms after stimulation were found to be very similar under control conditions to those recorded in slice cultures treated for 7 days with propofol.

Morphologic Analyses

To detect the possibility that propofol produced neurotoxic effects in these cultures, we first measured cell death using PI, a fluorescent marker that stains the nu-

cleus of permeabilized, dying cells. Under control conditions, hippocampal organotypic slice cultures prepared from neonatal rats show very scarce PI staining: only a few fluorescent cells could be detected (fig. 5A). However, when the slice was exposed to a neurotoxic agent such as HgCl₂, the level of fluorescence markedly increased, and most cells in the pyramidal layer became stained (fig. 5B). Slice cultures were then treated for 3, 5, 7, and 10 days with propofol, were fixed, and the number of labeled cells were counted (fig. 5C). As shown in figure 5D, the mean number of cells stained with PI was not significantly different from control after propofol treatment.

To confirm these results, we also analyzed semithin sections stained with toluidine blue. These histologic preparations are of interest to analyze more globally the state of preservation of the tissue and the possibility to observe cell swelling or gliotic processes. As illustrated in figure 6, no evidence for cell degeneration, cell death, cell swelling, or scar formation could be detected even 10 days after treatment with propofol.

Finally, to confirm the existence of a functional GABAergic inhibitory network in propofol-treated cultures, we also conducted morphologic analyses at the electron microscopic level. Well-preserved inhibitory interneurons, characterized by a markedly segmented nucleus, were routinely found in cultures treated for 7 days with propofol. In addition, a dense network of inhibitory symmetrical synapses were clearly observed on the soma and proximal dendrites of pyramidal neurons (fig. 6C). Although quantitative comparisons were difficult with this approach, these experiments provided evidence for the existence of a well-preserved inhibitory network in propofol-treated slice cultures.

Effects of Propofol on Slice Cultures of Different Ages

Because one important difference between dissociated cell cultures and organotypic cultures was the age and developmental stage of the tissue at the time of explantation, we also analyzed the effects of propofol treatment (for 7 days) on organotypic cultures prepared from newborn rats (postnatal day 1).

To assess the possible neurotoxic effects of propofol on these young cultures, we measured the amplitude of the excitatory and inhibitory responses evoked by supra-maximal stimulation in control cultures and in cultures treated for 7 days with $100 \mu\text{g/ml}$ propofol. As illustrated in figure 7C (top graph), the excitatory postsynaptic potentials recorded in control and propofol-treated cul-

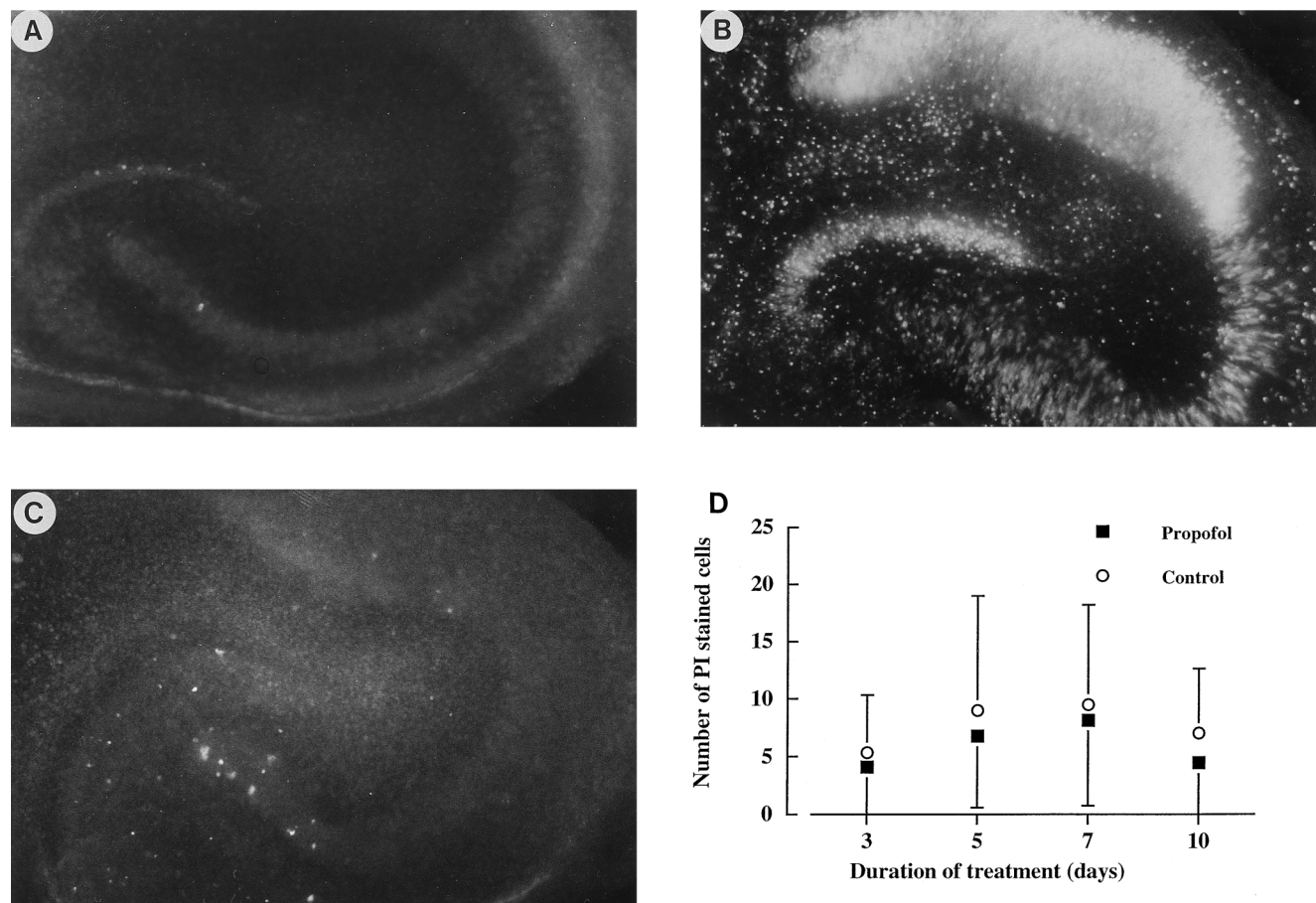


Fig. 5. Absence of cell death revealed by propidium iodide staining in propofol-treated slice cultures. (A) Illustration of propidium iodide staining in a healthy control hippocampal slice culture. Only a few labeled cells are present. (B) Propidium iodide staining obtained after treatment with the neurotoxic agent HgCl_2 . (C) Propidium iodide staining of a slice culture treated for 7 days with 100 $\mu\text{g/ml}$ propofol. (D) Quantitative analysis of the number of propidium iodide-stained cells counted at different times in control (open circles) and propofol-treated (black squares) slice cultures. Data are mean \pm SD ($n = 6-9$).

tures were not different, thus confirming the results obtained in slice cultures prepared from older animals. To analyze GABA_A -receptor-mediated responses, slice cultures were incubated in the presence of NBQX (5 μM), an antagonist of AMPA receptors, and CPP (10 μM), an antagonist of NMDA receptors. Under these conditions, a smaller synaptic response can still be evoked that is sensitive to bicuculline, a GABA_A -receptor antagonist (fig. 7B). We then compared the size of these GABA_A -mediated synaptic responses evoked by supramaximal stimulation in control cultures and propofol-treated cultures. As illustrated in figure 7C (bottom graph), no difference could be detected between the two conditions.

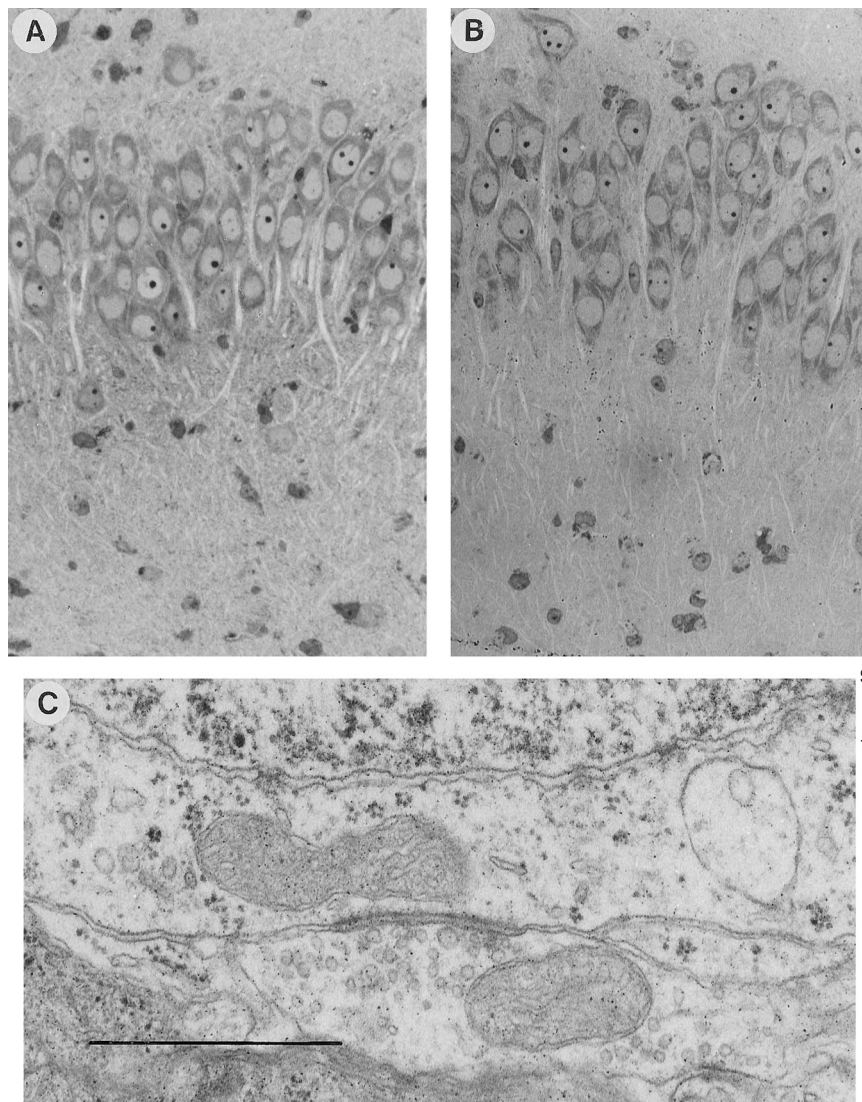
Similarly, propidium iodide staining revealed no difference between control cultures and slices treated for 7

days with propofol (fig. 7A). Numbers of PI-stained cells were $5.4 (\pm 3)$ for controls and $6.2 (\pm 2.4)$ for propofol (data are mean \pm SD; $n = 8$).

Discussion

This study was undertaken to investigate whether propofol, a commonly used agent in pediatric anesthesia, can result in neurotoxic effects on the developing brain. This possibility was raised by the observation that propofol produces toxic effects on a model of aggregating cell cultures and confirmed here using a model of dissociated cortical cell cultures. In a model of embryonic cell culture, Honegger and Matthieu⁹ showed that application of propofol for 8 h produced dose-dependent lesions with loss of GABAergic neurons at a con-

Fig. 6. Absence of morphologic alterations produced by long-term treatment of slice cultures with 100 $\mu\text{g/ml}$ propofol. (A) Toluidine blue-stained semithin section of the CA1 region of a control hippocampal slice culture. (B) Same as in (A) but after 10 days of treatment with 100 $\mu\text{g/ml}$ propofol. (C) Typical symmetric inhibitory synapse on a CA1 pyramidal cell body of a propofol-treated culture (bar: 10 μm).



centration of 10 $\mu\text{g/ml}$. In our model, we found that treatment of dissociated cultures for 3 days with propofol at concentrations higher than 20 $\mu\text{g/ml}$ was accompanied by the degeneration and death of most GABAergic neurons. However, this effect was not selective of GABAergic interneurons, because we found that the viability of glial cells was also affected. These observations thus confirmed and strengthened the conclusion that propofol produces neurotoxic effects on dissociated cell culture models.

Surprisingly, however, this conclusion is in marked contrast with the effects produced by propofol on a more complex *in vitro* model of brain tissue. Organotypic slice cultures were prepared from newborn and

7-day-old animals; they contain all the cellular types present *in situ* and they retain a well-preserved three-dimensional architecture. From a functional point of view, these cultures also exhibit properties that are very similar to those reported in the developing hippocampus.^{17,20} They therefore represent an interesting model of postnatal developing central nervous system.

Using this model, we found that long-term treatment of slice cultures with propofol at concentrations 10–20 times higher than those used under clinical conditions did not result in neurotoxic effects. This conclusion is based on both functional and morphologic tests, showing that there is no major alteration of excitatory and inhibitory transmission, that the ratio between excita-

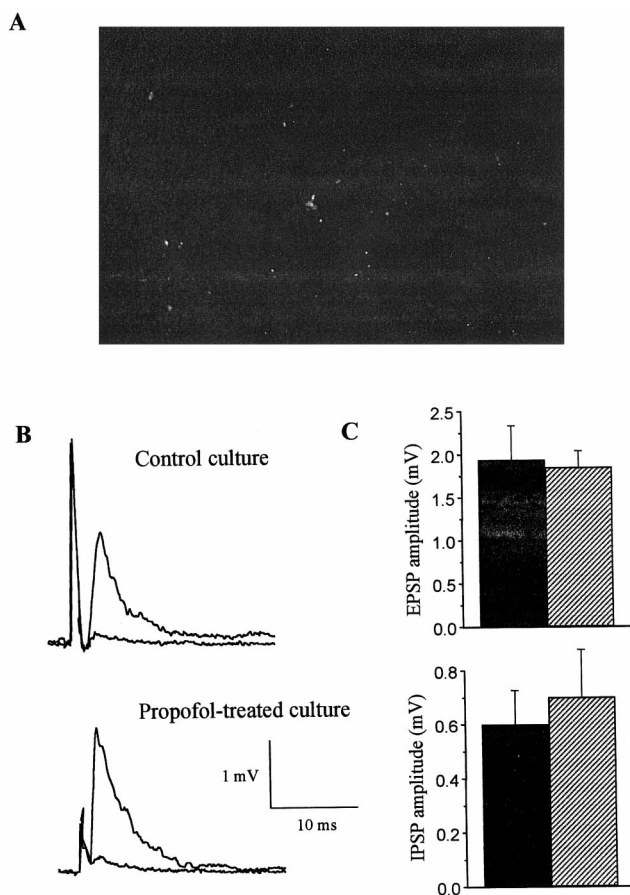


Fig. 7. Absence of neurotoxic effects of propofol (100 $\mu\text{g/ml}$) on organotypic slice cultures prepared from neonate rats (postnatal age, 1 day). (A) Illustration of propidium iodide staining in a slice culture prepared from a neonate rat on postnatal day 1, exposed for 7 days to propofol in a medium containing 10% horse serum as for cortical cell cultures. Only a few labeled cells can be detected. (B) Illustration of GABA_A-mediated inhibitory postsynaptic potentials recorded in the presence of 5 μM NBQX and 10 μM CPP in a slice culture prepared from a rat on postnatal day 1 in control conditions and after propofol treatment. Traces (averages of three) are illustrated before and after application of 10 μM bicuculline, a GABA_A-receptor antagonist. (C) Summary of amplitude measurements of excitatory (top) and inhibitory (bottom) responses evoked by supramaximal stimulation in control and propofol-treated slice cultures. Data are mean \pm SD of the values obtained in five different cultures. No statistically significant differences were detected.

tion and inhibition is preserved, that there is no evidence for cell death or changes in the state of preservation of neuronal cells, and that a well-preserved inhibitory network of interneurons and inhibitory synapses can still be observed in treated cultures. In view of the different tests conducted, it seems very unlikely that a selective loss of a population of GABAergic interneurons could have occurred undetected. To compare more precisely

the two types of cultures, we realized experiments with identical age of explantation, duration of culture before treatment, and proportion of serum in the culture media, and we found the same results. We therefore conclude that propofol, although it has overt toxic effects on embryonic or young dissociated cell cultures, does not affect postnatal developing central nervous system in organotypic cultures. However, it should be kept in mind that the toxicity was observed on dissociated cortical and not hippocampal neurons. The reasons for the discrepancy observed between these models remain unclear. It may be that propofol interferes with physiological parameters that are different in embryonic or very young compared with postnatal neurons. For example, there is evidence that GABA_A-mediated currents are more likely to be depolarizing in embryonic neurons, whereas they are hyperpolarizing in more mature cells.²¹ However, we found no signs of propofol toxicity in organotypic slice cultures prepared from newborn animals. Alternatively, it is also possible that dissociated cell cultures, because of a loss of integrity of cellular contacts between neuronal and glial cells, represent a more sensitive and less physiologic model for toxicologic analyses than slice cultures. This possibility is not unlikely considering that slice cultures can survive *in vitro* for a much longer period of time than dissociated cells.

From a clinical point of view, although the absence of neurotoxic effects of long-term propofol treatment on organotypic slice cultures is rather encouraging, the possibility confirmed here that this compound affects survival of embryonic or immature neurons or glial cells should not be underscored.

References

1. Sebel P, Lowdon JD: Propofol: A new intravenous anesthetic (letter). *ANESTHESIOLOGY* 1989; 71:260-77
2. Hodkinson BP, Frith RW, Mee EW: Propofol and the electroencephalogram. *Lancet* 1987; 2:1518
3. Makela JP, Iivanainen M, Pieninkeroinen IP, Waltimo O, Lahdesmäki M: Seizures associated with propofol anesthesia. *Epilepsia* 1993; 34:832-5
4. Wood PR, Browne GPR, Pugh S: Propofol infusion for the treatment of status epilepticus. *Lancet* 1988; 1:480-1
5. Samra SK, Sneyd JR, Ross DA, Henry TR: Effects of propofol sedation on seizures and intracranially recorded epileptiform activity in patients with partial epilepsy. *ANESTHESIOLOGY* 1995; 82:843-51
6. Lanigan C, Sury M, Bingham R, Howard R, Mackersie A: Neurological sequelae in children after prolonged propofol infusion. *Anaesthesia* 1992; 47:810-1
7. Trotter C, Serpell MG: Neurological sequelae in children after prolonged propofol infusion. *Anaesthesia* 1992; 47:340-2

NEUROTOXIC EFFECTS OF PROPOFOL

8. Finley GA, MacManus B, Sampson SE, Fernandez CV, Retallick R: Delayed seizures following sedation with propofol. *Can J Anaesth* 1993; 40:863-5
9. Honegger P, Matthieu JM: Selective toxicity of the general anesthetic propofol for GABAergic neurons in rat brain cell cultures. *J Neurosci Res* 1996; 45:631-6
10. Westrin P: The induction dose of propofol in infants 1-6 months of age and in children 10-16 years of age. *ANESTHESIOLOGY* 1991; 74: 455-8
11. Murat I, Billard V, Vernois J, Zaouter M, Marsol P, Souron R, Farinotti R: Pharmacokinetics of propofol after a single dose in children aged 1-3 years with minor burns. *ANESTHESIOLOGY* 1996; 84:526-32
12. Kiss JK, Wang C, Olive S, Rougon G, Lang J, Baetens D, Harry D, Pralong WF: Activity-dependent mobilization of the adhesion molecule polysialic NCAM to the cell surface of neurons and endocrine cells. *EMBO J* 1994; 13:5284-92
13. Stoppini L, Buchs PA, Muller D: A simple method for organotypic slice cultures of nervous tissues. *J Neurosci Methods* 1991; 37:173-82
14. Okada M, Sakagushi T, Kawasaki K: Correlation between anti-ubiquitin immunoreactivity and specific neuronal death in N-methyl-D-aspartate-treated rat hippocampal organotypic cultures. *Neurosci Res* 1995; 22:359-66
15. Kay NH, Sear JW, Uppington J, Cockshott ID, Douglas EJ: Disposition of propofol in patients undergoing surgery: A comparison in men and women. *Br J Anaesth* 1986; 58:1075-9
16. Macklis JD, Madison RD: Progressive incorporation of propidium iodide in cultured mouse neurons correlate with declining electrophysiological status: A fluorescence scale of membrane integrity. *J Neurosci Methods* 1990; 31:43-6
17. Buchs PA, Stoppini L, Muller D: Structural modifications associated with synaptic development in area CA1 of rat hippocampal organotypic cultures. *Dev Brain Res* 1993; 71:81-91
18. Dichter MA: Physiological identification of GABA as the inhibitory transmitter for mammalian cortical neurons in cell culture. *Brain Res* 1980; 190:111-21
19. Dutton GR: Astrocytes amino acids: Evidence for release and possible interactions with neurons, Astrocytes, Pharmacology and Function. Edited by Murphy S. San Diego, Harcourt Brace Jovanovich Academic Press Inc., 1993, pp 183-220
20. Gahwiler BH, Capogna M, McKinney RA, Thompson SM: Organotypic slice cultures: A technique has come to age. *Trends Neurosci* 1997; 20:471-7
21. Ben-Ari Y, Khazipov R, Leinikugel X: GABA_A, NMDA and AMPA receptors: A developmentally regulated "menage à trois." *Trends Neurosci* 1997; 20:523-9