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Propofol Induces Changes in the Cytosolic Free Calcium Concentration and the Cytoskeletal Organization of Cultured Human Glial Cells and Primary Embryonic Rat Brain Cells

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Background: **The site of action of the intravenous anesthetic drug propofol is uncertain. Therefore, we examined the effects of propofol on the cytosolic free calcium levels of cultured primary embryonic rat brain cells (80–85% neurons), and on the organization of the cytoskeleton in these rat cells and in a model system of cultured human glial cells (astrocytes).**

Methods: **Propofol was added to the cells as the clinically available solution Diprivan. Cytosolic free calcium changes in neurons were studied using Fura2 and a single-cell microfluorometric method. Fluorescence microscopy was used to study the organization of actin filaments and tubulin in detergent-extracted cells.**

Results: **An increase in the cytosolic free calcium concentration of 116 ± 39 nM was seen shortly after the addition of 0.3 $\mu g \cdot ml^{-1}$ propofol, and a propofol concentration of 0.03 $\mu g \cdot ml^{-1}$ resulted in an increase in cytosolic free calcium concentration of the same magnitude, 119 ± 42 nM. Most of the calcium (60–75%) came from the extracellular environment, and the rest was from intracellular stores. When neurons were depleted of intracellular calcium by 1,2-bis-5-methyl-aminophenoxylethane-N,N′-tetra-acetoxymethylacetate (MAPT/AM), no changes were seen in the actin organization of the cytoskeleton. Actin organization was affected by all concentrations of propofol, 0.3–50 $\mu g \cdot ml^{-1}$ (1.7–280 μM), when exposure to the drug was achieved by a 30-min incubation. After the incubation, the exposed cells were more rounded and exhibited increased ruffling activity, both at the periphery and on the cellular surface, and ring-shaped actin structures were also seen. These effects were concentration dependent and reversible, and reached a maximum after 20 min of incubation. Propofol had no apparent effect on the organization of tubulin.**

Conclusions: **Propofol induced changes in the cytoskeletal organization of actin in cultured rat neurons and human glial cells. These changes must have been due to the increase in intracellular calcium seen shortly after the addition of propofol, since no effects on actin organization were seen when intracellular calcium was depleted. (Key words: Anesthetics, intravenous: propofol. Brain, human glial cells: cytoskeleton; cytosolic free calcium. Methods, culture: primary embryonic rat brain cell.)**

GENERAL anesthetic agents may exert their effects at several cellular and subcellular levels.[1] At very high MAC multiples some anesthetics (*e.g.*, halothane, enflurane, ether, and pentobarbital) hyperpolarize central neurons by increasing potassium conductance,[2] perhaps by an induced potentiation of resting cytosolic calcium ion concentrations.[3] Moreover, an increase in the cytosolic calcium level can activate actin-severing proteins, such as gelsolin, that are capable of inducing changes in the actin filament structure of the cytoskeleton.[4]

The cytoskeleton is a complex, three-dimensional network of protein filaments that extends throughout the cytoplasm.[5] This network allows the cell to adopt a variety of shapes, to control and coordinate movements and to actively move organelles within the cytoplasm. There are three main types of protein filaments in the cytoskeleton, and one of these, actin, is closely connected with the cell membrane, a fact that is of particular interest when considering the cellular mechanisms of lipid-soluble general anesthetics.[1,6] In 1968 it was hypothesized[7] that general anesthetics

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produce anesthesia by depolymerizing the microtubules of nerve cells. Microtubules are hollow tubes composed of the protein tubulin, hence it is also important to consider tubulin when discussing anesthetic action at the cellular level.

Propofol (2,6-diisopropylphenol) is a new intravenous anesthetic, chemically different from other drugs used to achieve general anesthesia.[8] As of yet, the site of action of propofol is unclear, although a recent report indicated that human brain sodium channels may be a molecular target of propofol.[9] In addition, propofol has been shown to dose-dependently inhibit the activity (firing rate) of neurons known to be inhibited by γ-aminobutyric acid–mimetic drugs (GABA).[10]

The aim of the current study was to further clarify the action of propofol. This was accomplished by studying the effects of the drug on changes in the cytosolic free calcium (Ca^{2+}) concentration in cultured rat neurons, and by investigating the effect of propofol on the organization of actin and tubulin in the rat neurons and in cultured human glial cells.

Materials and Methods

Cells and Cell Cultures

Human glial cells (PC 107, passages 7–15; gift from Professor Peter Collins, Department of Pathology, University of Gothenburg, Sweden) were cultured in modified Eagle's Minimum Essential Medium supplemented with 10% fetal calf serum, 2 mM glutamine, 50 $U \cdot ml^{-1}$ penicillin, and 50 $\mu g \cdot ml^{-1}$ streptomycin. The cells were grown for 3 or 4 days on sterile round cover glasses (12 mm in diameter) placed in the wells of a 24-well tissue culture plate at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. These cells were identified as astrocytes by adding antibodies against glial fibrillary acidic protein, a protein that specifically protein constituting the intermediate filaments of astrocytes.[11]

The primary embryonic rat brain cells were cultured according to a method described by Hansson and Rönnbäck.[12] The cells were obtained from 15–17-day-old rat embryos (Sprague-Dawley) as follows. A pregnant rat was anesthetized with ether, and the embryos were removed and placed on ice. The brain tissue was dissected and then homogenized by sieving through a nylon mesh (80 μm) into modified Eagle's minimum essential medium[12] supplemented with 20% fetal calf serum, 30 mM glucose, and 5 $\mu g \cdot ml^{-1}$ insulin (*p*H 7.3–7.4). Antibiotics were added to the medium as described above. The cells were cultured on poly-L-lysine–coated sterile round cover glasses (12 mm) placed in the wells of a 24-well tissue culture plate. After 24 h, the culture medium was changed to Eagle's minimum essential medium with 10% fetal calf serum, and thereafter changed every 2nd day. To suppress the growth of dividing cells, 10 μM cytosine-1-β-D arabinofuranoside was added to the culture medium on the 3rd day. The cells were grown for 8–10 days at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. These cell cultures contained 80–85% neurons. For quantification of changes in Ca^{2+} concentration only neurons were used since the method employed called for stimulation and microscopy of single cells. Neurons subjected to such quantification were obtained as described above, with the following exceptions: brain tissue originated from both newborn rats (E. Hansson, Department of Cell Biology, University Hospital, Linköping) and embryos, and the neurons were grown at the bottom of Petri dishes (35 × 10 mm, Flow, Edinburgh, Scotland) on round sterile cover glasses with a diameter of 25 mm. When performing fluorescence microscopy, both primary embryonic rat brain cells and human glial cells were used. All experiments were approved by the Ethics Committee for Animal Research of the University Hospital in Linköping, Sweden.

Propofol

Propofol, 2,6-diisopropylphenol, is a hydrophobic substituted phenol with a molecular weight of 178 and a *p*Ka in water of about 11. For clinical use propofol is dissolved in an oil-water emulsion composed of 10% soya bean oil, 1.2% egg phosphatide, 2.25% glycerol, and water (Diprivan, ICI Pharma, Gothenburg, Sweden). The emulsion is isotonic, has a physiologic *p*H, and is similar to the clinically available Intralipid (10%, Kabi Pharma AB, Stockholm, Sweden); we used both Diprivan and Intralipid (controls) in our experiments. Intralipid is ten times more concentrated than the Diprivan vehicle and, accordingly, the concentrations of Intralipid used were ten times those of Diprivan.

Determination of Cytosolic Free Calcium Concentrations

The concentration of Ca^{2+} was measured after incubating the rat neurons with the calcium indicator Fura2 acetoxymethyl ester.[13] This indicator is the membrane-permeant ester form which is hydrolyzed by cytosolic

esterases to the Fura2 free-acid form. The cells were incubated with 4 μM Fura2 acetoxymethyl ester in the culture medium for 45 min at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The cover glass was then placed in a specially designed chamber in which it constituted the bottom. To this chamber was added 0.5 ml of a physiologically balanced calcium-containing buffer[14]; in some cases this medium was modified by excluding $CaCl_2$ and adding 1 mM ethylene-bis-(oxyethylenenitrilo) tetraacetic acid (to study the intracellular mobilization of calcium) immediately prior to the exposure to Diprivan. Single cells, morphologically identified as neurons, were optically isolated by placing them individually in a circular diaphragm slightly larger than a cell. Dual excitation microfluorometry was then performed with an inverted microscope (Nikon, Tokyo, Japan). The excitation light alternated rapidly (four times per second) between λ_1 = 340 nm and λ_2 = 380 nm (SPEX fluorometer, Glen Creston, London, England). This set-up was equipped with a thermostatic chamber that allowed the cells to be maintained at 37°C during the entire experiment. The fluorescence was monitored through an interference filter (λ = 510 nm) by photon counting. The SPEX equipment was controlled by a microcomputer, which also handled the data acquisition. Data collection was synchronized with the movement of the chopping mirror, such that the fluorescence respectively emitted by the two alternating wavelengths was stored in separate files.

Consequently, during an experiment, the two distinct excitation wavelengths could be simultaneously recorded and stored. Using the formula proposed by Grynkiewicz and coworkers[13] the ratio of the fluorescence excited at the two wavelengths was calibrated to express the Ca^{2+} concentration. The calibration constants were determined in the same experimental set-up in separate experiments with Fura2 acid at approximately 10 nM Ca^{2+} (R_{min}) and at 5 mM Ca^{2+} (R_{max}). The Fura2 dissociation constant for Ca^{2+} (224 nM) was from the results of Grynkiewicz *et al.*[13] The Ca^{2+} level was measured after the addition of propofol in three concentrations: 0.03, 0.3 and 3 $\mu g \cdot ml^{-1}$.

Depletion of Intracellular Calcium

Primary embryonic rat brain cell cultures were first preincubated in a medium containing 1 mM Ca^{2+} and 75 μM 1,2-bis-5-methyl-amino-phenoxylethane-N,N'-tetra-acetoxymethylacetate (MAPT/AM) for 1 h and then stimulated with 3 $\mu g \cdot ml^{-1}$ propofol. MAPT/AM is a monofluorescent calcium chelator. Thereafter, the calcium signal was measured by the SPEX method and the organization of actin was studied by fluorescence microscopy on detergent extracted cells.

Incubation of Cell Cultures for Studies of the Cytoskeleton

Propofol or Intralipid at the various concentrations were mechanically mixed and stirred into modified Eagle's minimum essential medium of the same composition as the above-described culture media. For each cell culture some specimens were used as controls, which were incubated with fresh culture medium only and thereafter treated the same way as the test samples. Propofol concentrations of 3.3 and 50 $\mu g \cdot ml^{-1}$ were used in the experiments on human glial cells. In the experiments on the embryonic rat brain cell culture, we used 0.3, 3.3, 10, and 50 $\mu g \cdot ml^{-1}$ propofol; as indicated above, the corresponding concentrations of Intralipid were ten times higher. During anesthesia, the blood concentration levels of propofol in humans range from an immediate peak level of 10 $\mu g \cdot ml^{-1}$ after intravenous injection,[15] through levels of 3.3 $\mu g \cdot ml^{-1}$ during steady-state anesthesia[16] to 1.5–1.7 $\mu g \cdot ml^{-1}$ when patients awaken.[16] This means that the concentration of 50 $\mu g \cdot ml^{-1}$ used in our experiments is higher than the blood concentration level ordinarily seen in humans.

The cells were incubated with propofol or Intralipid for 30 min in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. At the same time the control cells were incubated with fresh culture media. To test the reversibility of the effect, some cells were incubated with 3.3 $\mu g \cdot ml^{-1}$ propofol as above, rinsed and thereafter allowed to "normalize" for 30 min in fresh culture medium. Furthermore, a time-response titration was performed at 5-min intervals on human glial cells incubated with 3.3 $\mu g \cdot ml^{-1}$ propofol for periods ranging from 5 to 45 min.

Fluorescence Microscopy

Human glial cells and embryonic rat brain cells were prepared for fluorescence microscopy as follows. After incubation with propofol or Intralipid, the cells were extracted for 10 min with 0.5% Triton X-100 in a microtubule-stabilizing buffer and then fixed for 15 min in 4% paraformaldehyde in Hanks balanced saline solution in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.[17] Actin was visualized by staining with tetramethylrhodamine isothiocyanate–conjugated phal-

loidin specific for filamentous actin.[18] The tubulin distribution was studied by adding monoclonal primary antibodies against alpha tubulin, followed by fluorescein isothiocyanate– or tetramethylrhodamine isothiocyanate–conjugated secondary antibodies. These preparatory procedures for fluorescence microscopy were performed according to Bell and coworkers[17] and are summarized in figure 1.

Quantification of Cytosolic Free Calcium

The peak area (arbitrary units) and ΔCa^{2+} (nanomolar) of each Ca^{2+} trace were calculated, and the mean and SD of the response seen after the addition of propofol or Intralipid in the calcium-free or calcium-containing medium were calculated from different experiments. Cells with a cytoskeletal organization differing from that of normal glial cells[19] were identified and their numbers are expressed as percentages of the total number of glial cells. At least 50 cells were identified for each concentration of propofol and Intralipid. Quantification was performed only on cells from experiments with normally looking control cells.[19] The embryonic rat brain cell cultures were maintained in culture until reaching confluence, and only cells that could be individually isolated were used for the quantitation.

Statistics

One-factor analysis of variance was used for calculation of statistical significance. The means and SD of the different concentrations were calculated and compared to controls by using Fisher's test. Differences between individual values were assessed by Fisher's test. Statistical significance was considered to be present at $P \leq 0.05$.

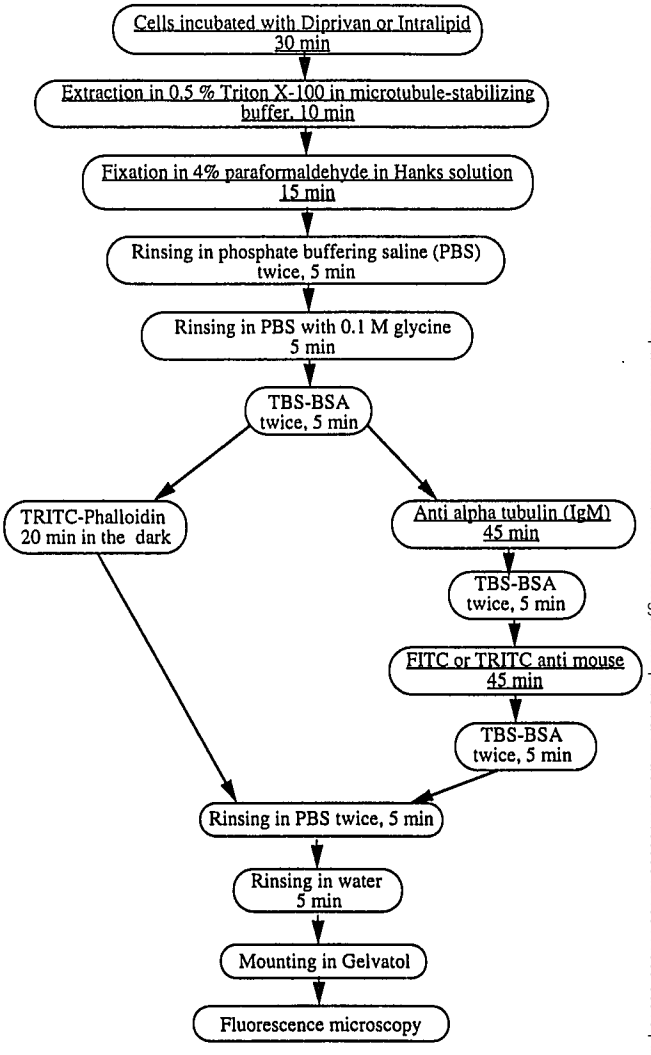

Fig. 1. Preparatory procedure for fluorescence microscopy. Underlined text = work carried out at 37°C in a humidified atmosphere of 5% CO_2 and 95% air; plain text = work done in ambient air at room temperature; TBS-BSA = Tris-HCl–buffered saline with 1% bovine serum albumin.

Results

Effects of Propofol on Cytosolic Free Calcium Concentrations in Primary Embryonic Rat Brain Cells

A significant increase in Ca^{2+} was seen immediately after the addition of 3 $\mu g \cdot ml^{-1}$ propofol, whereas only a slight increase was seen after addition of Intralipid. The increase in Ca^{2+} concentration minus the background concentration (denoted ΔCa^{2+}) was 244 ± 83 nM after treatment with 3 $\mu g \cdot ml^{-1}$ propofol and 18 ± 4 nM after Intralipid treatment. For propofol, similar ΔCa^{2+} responses after treatment with 0.03 and 0.3 $\mu g \cdot ml^{-1}$ of 119 ± 42 nM and 116 ± 39 nM were seen, respectively. The ΔCa^{2+} response after 3 $\mu g \cdot ml^{-1}$ propofol was approximately twice that seen after the addition of 0.3 $\mu g \cdot ml^{-1}$ of the drug (244 ± 83 and 116 ± 39 nM, respectively). These results are illustrated in figure 2. The increase in peak area (arbitrary units) under the calcium ion curve was 1089 ± 503 (mean ± SD) after propofol treatment and 91 ± 59 after Intralipid ($P < 0.0001$). The duration of the change in calcium ion levels was 128 ± 39 s for propofol and 30 ± 5 s for Intralipid ($P < 0.0001$); the calcium con-

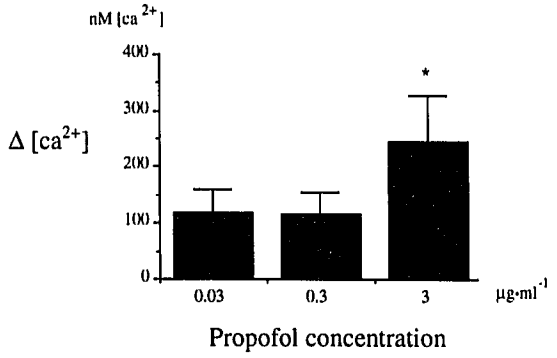

Fig. 2. The increase in cytosolic free calcium concentration (ΔCa^{2+}) in neurons at various propofol concentrations. ΔCa^{2+} is the increase in peak Ca^{2+} concentration minus the background concentration (the level in an unstimulated cell). Values are means with SDs. A significantly greater response was elicited by 3 $\mu g \cdot ml^{-1}$ propofol than by the other two (lower) concentrations (Fisher's test for differences between individual values; $^*P < 0.05$).

centrations returned to normal after these short changes. Approximately 75% of the cells stimulated with propofol showed some response, whereas less than 50% of the cells stimulated with Intralipid responded. An increase in Ca^{2+} concentration was also seen in cells studied in the calcium-depleted buffer. The propofol-induced peak area (arbitrary units) was 446 ± 180, which represents approximately 40% of the area seen in cells studied in the calcium-containing buffer. The duration of the peak area was 106 ± 34 s in cells studied in the calcium-depleted buffer, not significantly different from the duration of peak areas obtained for cells studied in a calcium-containing buffer. The changes in Ca^{2+} concentration after propofol and Intralipid treatment are shown in figures 3 and 4.

Effects of Propofol on Primary Embryonic Rat Brain Cells Preincubated with MAPT/AM

Preincubation of the embryonic rat brain cells with MAPT/AM, a monofluorescent calcium chelator, reduced the calcium signal very dramatically. Only a small, slow calcium signal of long duration could be observed in these cells after propofol addition. The organization of actin, as revealed by fluorescence microscopy, was unchanged. Only 8 of 306 cells exposed to 3 $\mu g \cdot ml^{-1}$ propofol exhibited ruffling and only 10 showed signs of ring formation (features defined below). Of 250 untreated cells that served as controls, 9 displayed ruffling, and ring formation was seen in 12. These results are not statistically significant.

Effects of Propofol on the Cytoskeleton of Human Glial Cells

The organization of the cytoskeleton was affected by incubation with propofol for 30 min. The observed changes were primarily seen in the actin filament organization, and tubulin distribution was apparently unaffected. Control cells were flat, had well-defined, distinct edges and a distribution of actin similar to that noted in human glial cells by other investigators.[19] The propofol-treated cells (at all concentrations) appeared more rounded and showed an increased number of changes in actin filament distribution as compared to control cells. The observed morphologic phenomena comprised actin-containing surface extensions, such as peripheral ruffles (membrane foldings), microspikes,

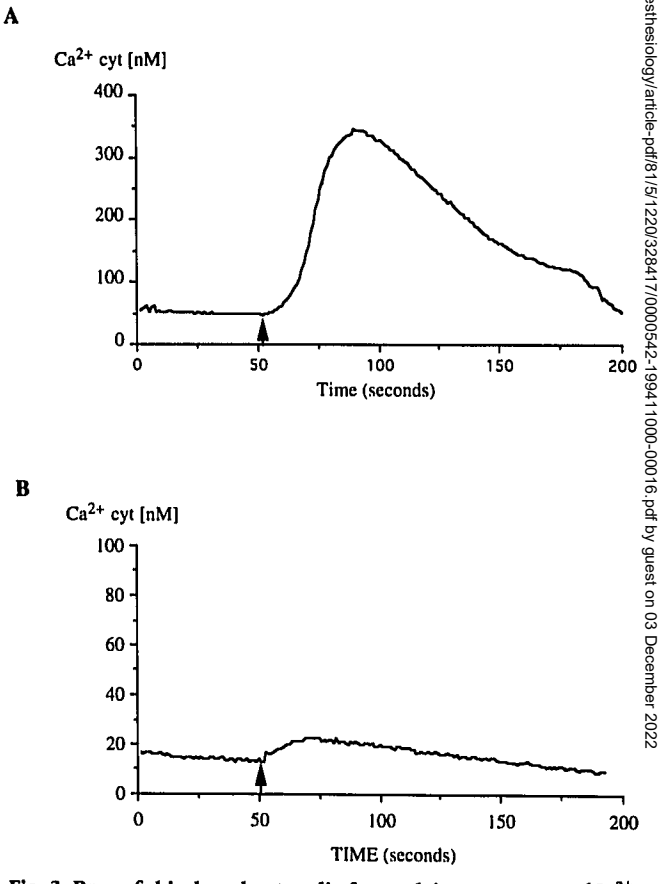

Fig. 3. Propofol-induced cytosolic free calcium responses (Ca^{2+} cyt) in single neurons. (*A*) Trace from a neuron stimulated with 3 $\mu g \cdot ml^{-1}$ propofol in a calcium-containing medium. (*B*) Trace from a neuron stimulated with 3 $\mu g \cdot ml^{-1}$ propofol in a calcium-depleted medium. The traces shown are representative of at least 16 separate experiments. Arrow = time of propofol addition.

and flat membrane protrusions (lamellipodia). When a cell moves some of these ruffles sweep backward over its surface; this has been quantitated and is referred to as "ruffling activity." Circular arrangements of actin were also observed in an increased number of cells treated with propofol as compared to control cells. These morphologic structures were also quantitated and are referred to as "ring structures." (fig. 5)

The proportion of cells exhibiting ruffling activity increased from 18 ± 8% (mean ± SD) in the control cells to 52 ± 11% and 58 ± 11% in cells treated with 3.3 $\mu g \cdot ml^{-1}$ and 50 $\mu g \cdot ml^{-1}$ propofol, respectively ($P < 0.001$). The percentage of cells showing ring structures increased from 4 ± 2% in the control cultures to 11 ± 5% ($P < 0.001$) and 9 ± 5% ($P < 0.02$), respectively, in cells exposed to 3.3 and 50 $\mu g \cdot ml^{-1}$ propofol.

The time-response titration showed that both the formation of ring structures and ruffling activity increased with time and reached maxima after 20–25 min (fig. 6). Although the number of propofol-induced ring structures successively declined to the same level as in the control cells, the increase in ruffling activity (as compared to controls) was still seen 45 min after addition of propofol (fig. 6).

Effects of Propofol on the Cytoskeleton of Primary Embryonic Rat Brain Cells

Similar to the human glial cells, the propofol-treated neurons were more rounded and showed approximately the same change in the distribution of actin filament, as described above. The proportion of neurons with ruffling activity increased with increasing propofol concentrations, and this effect was reversible (fig. 7). The highest concentration of Intralipid (500 $\mu g \cdot ml^{-1}$) also induced an increase in ruffling activity in the rat cells (fig. 7). After incubation with propofol, an increased number of cells with actin ring structures was seen; this effect was reversible and increased with increasing concentrations to 10 $\mu g \cdot ml^{-1}$ propofol. However, percentage-wise, fewer cells exhibited actin rings than showed changes in ruffling activity (fig. 8). As in the human glial cells, tubulin distribution in the rat neurons was not affected by propofol. Incubation with Intralipid caused no changes in the number of ring structures or tubulin organization.

Discussion

The major findings of this study were the increase in cytosolic calcium ion concentration and the changes

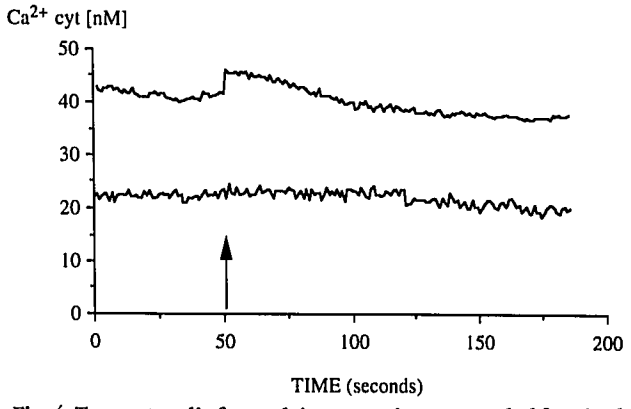

Fig. 4. Two cytosolic free calcium transients recorded for single neurons stimulated with 30 $\mu g \cdot ml^{-1}$ Intralipid. The top trace represents a cell in calcium-containing medium and the lower trace a cell in calcium-depleted medium. Each of the traces is representative of at least 10 separate experiments. Arrow = time of Intralipid addition.

seen in the organization of actin in the presence of propofol. The changes in actin filament organization caused by propofol were concentration dependent and reversible, and they were observed in both human glial cells and primary embryonic rat brain cells. The only difference between the drug-treated cells and the controls was exposure to propofol, hence the observed effects in this study must logically have been caused by this drug.

An increase in cytosolic calcium can be due to an influx from the extracellular environment *via* calcium ion channels or to a release from intracellular stores in, for example, mitochondria and endoplasmic reticulum. Eriksson[20] found that propofol inhibited calcium-induced permeabilization of rat liver mitochondria in a concentration-dependent manner. The major effect of propofol in the cited study was an inhibition of the Ca^{2+} efflux from the mitochondria. The ability of other anesthetic agents (*e.g.*, halothane and diethylether) to increase resting intracellular ionized calcium levels, when studied at supraclinical concentrations is correlated to their membrane-fluidizing actions,[21] and this could indicate that the calcium increase is of extracellular origin. However, we found both an increased inflow of extracellular calcium and a release from intracellular stores after stimulation of neurons with propofol, findings that appear contradictory to those of Eriksson.[20] An increase in Ca^{2+} has been found to hyperpolarize vertebrate neurons by increasing potassium conductance.[2] This makes neurons less responsive to action potentials, and could explain the

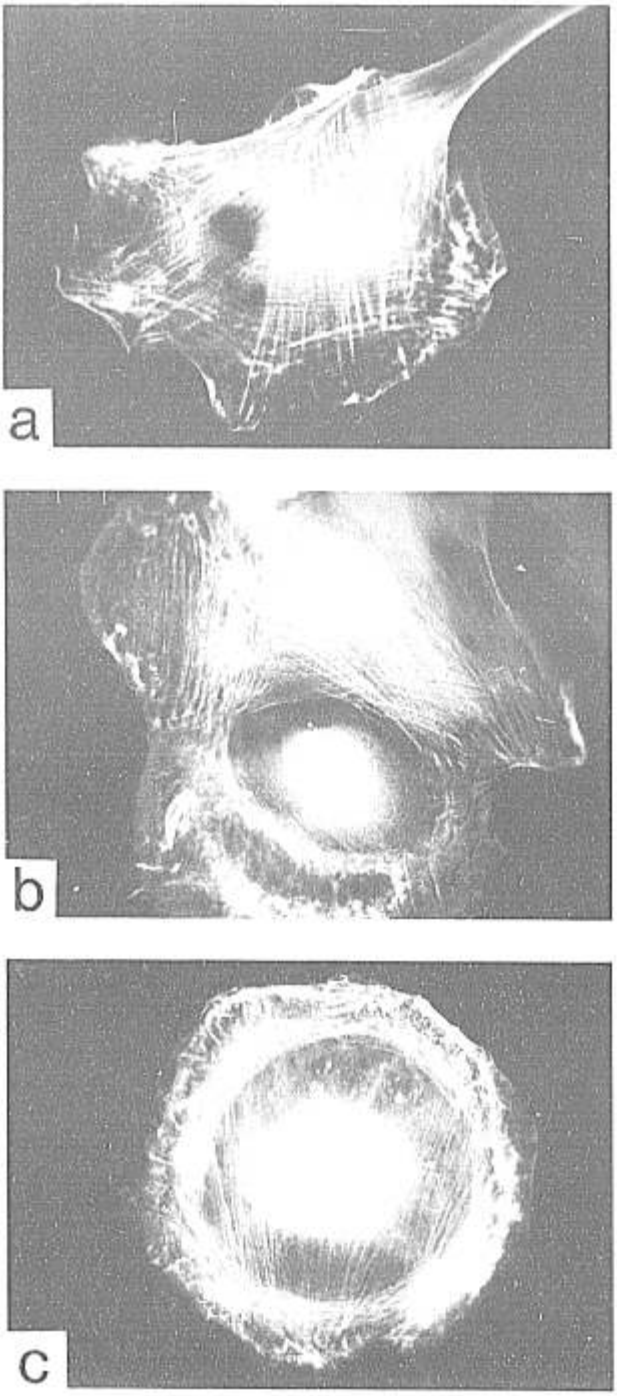

Fig. 5. Fluorescence micrographs of cultured human glial cells. The organization of actin is shown after labeling of the filaments with tetramethylrhodamine isothiocyanate–conjugated phalloidin. (*A*) Control cell; (*B* and *C*) cells incubated with 3.3 $\mu g \cdot ml^{-1}$ propofol for 30 min. An actin ring structure is seen in *B*; *C* shows a typical round cell with peripheral ruffling activity.

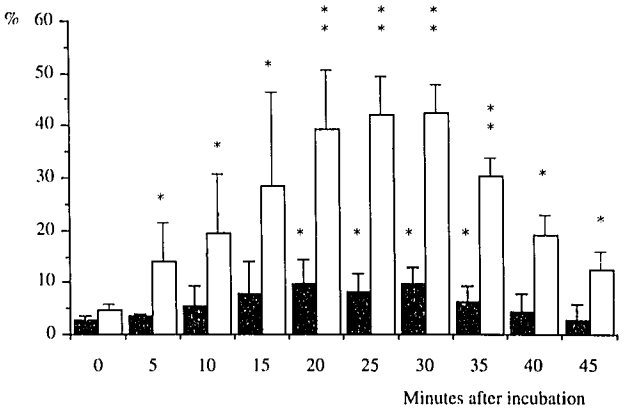

Fig. 6. Means and SDs of ruffling activity (open squares) and formation of ring structures (filled squares) with time in human glial cells exposed to 3.3 $\mu g \cdot ml^{-1}$ propofol for various periods of time. Significant differences were obtained by using Fisher's test for differences between individual values. *$P < 0.05$; **$P < 0.01$.

biochemical event behind the lower firing rate seen in neurons treated with propofol.[10]

Increases in Ca^{2+} are known to activate gelsolin, a protein that initiates severing of actin filaments and thereby changes the actin filament network.[4] It has been suggested that the inositol phospholipids (phospho-

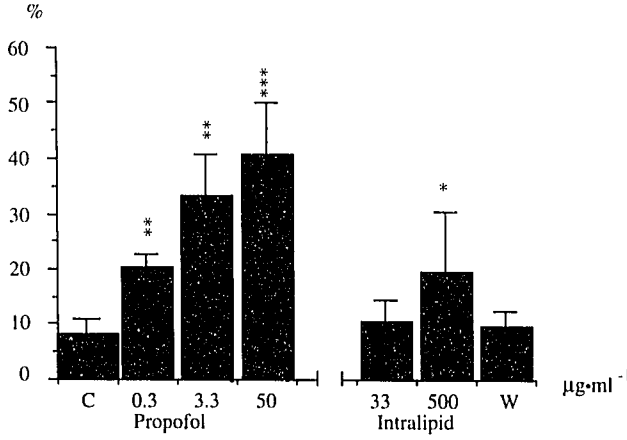

Fig. 7. Means and SDs of percentages of primary embryonic rat brain cells exhibiting ruffling activity. Results are shown for controls (C), for cells in various concentrations of propofol in lipid (Diprivan) and Intralipid, and for propofol-exposed cells after the drug was washed out (W). Cells were incubated in test medium for 30 min. *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$ for individual values compared with control. The response to 3.3 $\mu g \cdot ml^{-1}$ propofol was statistically greater than the response to 0.3 $\mu g \cdot ml^{-1}$ ($P = 0.01$), and the response to 50 $\mu g \cdot ml^{-1}$ was greater than to 3.3 $\mu g \cdot ml^{-1}$ ($P = 0.05$). Fisher's test for differences between individual values was used.

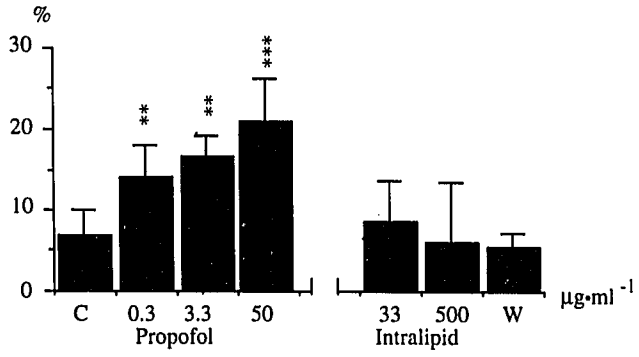

Fig. 8. Means and SDs of percentages of primary embryonic rat brain cells with actin ring structures. Results are shown for control cells (C), for cells in various concentrations of propofol in lipid (Diprivan) and Intralipid, and for propofol-exposed cells after the drug was washed out (W). Cells were incubated in test medium for 30 min. **$P < 0.01$ and ***$P < 0.001$ for individual values compared with control. There was no statistically significant difference between the responses at 0.3 and 3.3 $\mu g \cdot ml^{-1}$ propofol ($P = 0.07$). The response to 50 $\mu g \cdot ml^{-1}$ propofol was significantly greater than the responses to the other two (lower) concentrations ($P = 0.01$). Fisher's test for differences between individual values was used.

inositides) in the cell membrane affect the cellular state of actin[22] and they may therefore be involved in the effects of propofol on actin organization. Phospholipid metabolism leads to the formation of two messenger substances, inositol trisphosphate and diacylglycerol. The function of inositol trisphosphate is to release calcium from intracellular stores. Diacylglycerol phosphorylates a number of proteins by activating protein kinases and it can also stimulate both the formation of actin nucleation sites at plasma membranes and changes in the organization of the cytoskeleton.[23,24]

In any case, it is possible that propofol-induced changes in the cytoskeletal distribution of actin can be explained by other hypotheses as well. In 1899 Meyer[25] suggested that the molecular mechanisms of anesthesia are based on the solubility of anesthetics in lipids. Modern studies[26] have confirmed this "Meyer-Overton" theory of anesthesia by concluding that the critical site of anesthetic action has hydrophobic solubility properties such as those occurring in specific regions of lipids and proteins in neuronal membranes. A molecule of a lipid-soluble anesthetic such as propofol can, when bound within the plasma membrane, interfere with the normal function and structure of that organelle. This could, in turn, induce the type of intracellular changes described in the current study, and also affect the normal function of ion channels in the plasma membrane. Support for this theory of action of propofol comes from a study showing that clinically relevant concentrations of propofol depressed two major sodium-channel functions.[9] The effect of this depression was a reduction in the fractional open-time and interaction with the steady-state activation. The current effects seen after incubation with 500 $\mu g \cdot ml^{-1}$ Intralipid could have been mediated by interactions between the egg phosphatide in Intralipid and phosphatides in the cell membrane, leading to effects similar to those seen after the addition of propofol.

The cytoskeleton provides the cell with a framework for the attachment and movement of cytoplasmic components, and it is connected with the cell membrane and is involved in changes of shape and motility.[5] Changes in the cytoskeleton induce changes in the membranes, and thereby in the entire cell, and such cytoskeletal reorganization can be transmitted from one cell to another, across cell membranes. The described phenomena are usually seen in active cells that are migrating or changing shape. We were unable to demonstrate changes in the tubulin structure in our cells. Our findings do not support the hypothesis that anesthetics depolymerize microtubules,[7] a hypothesis that has been questioned by other investigators as well.[27,28]

Morphologic changes seen in human glial cells after stimulation with platelet-derived growth factor (PDGF)[19] are similar to the cytoskeletal rearrangements observed in the current study. PDGF has also been shown to induce the formation of actin ring structures in human fibroblasts.[29] Since similar morphologic changes are elicited by PDGF and propofol, similar mechanisms of action could be at hand. In our study, propofol-treated cells differed significantly from control cells in regard to the number of actin ring structures visible. In previous studies on glial cells grown in serum-free media before the addition of PDGF, ring structures were not seen in the control cells.[19] We have noted (unpublished observation) that after the addition of PDGF to human fibroblasts grown without serum, ring structures occur within minutes and subsequently disappear after about 1 h. All cells used in the current experiments were grown in the presence (10–20%) of fetal calf serum which contains about 1 $ng \cdot ml^{-1}$ of PDGF. One possible explanation for the few actin rings seen in the control cells is that the medium surrounding both propofol-exposed and control cells was renewed immediately before performing the tests.

The propofol concentration of 3 $\mu g \cdot ml^{-1}$ used in our experiments is similar to the blood concentration levels

measured during clinical anesthesia of humans. A tenfold higher propofol concentration of 33.8 (SE 4.57) $\mu g \cdot ml^{-1}$ is needed to induce anesthesia in rats, based on the finding of burst suppression on the electroencephalogram‖. Propofol concentrations prevailing in the cerebrospinal fluid during anesthesia are unknown, although, the volume of distribution of propofol is very high, 20–40 l,[30] and pharmacokinetic modeling indicates that equilibration across the blood brain barrier is rapid and completed within 2–3 min. Therefore, the concentrations found in the blood are similar to those in cerebrospinal fluid, and we chose the former for use in the current study.

Sleep is usually produced within 1 min when using propofol for clinical anesthesia.[31] The changes in cytosolic calcium ion concentration we observed in cultured cells occurred immediately after the addition of propofol. The morphologic effects on the cytoskeleton were found to be at a maximum after 20–25 min of incubation and we do not interpret them as changes that would occur during anesthesia, but rather as a consequence of the increase in cytosolic calcium that is seen in cultured cells stimulated with propofol.

In conclusion, propofol in concentrations exceeding or equivalent to clinically relevant concentrations induces changes in the morphology of cultured brain cells. These changes are a consequence of the increase in Ca^{2+} concentration seen after stimulation with propofol. Evidence of this is that no changes in actin organization were observed when intracellular calcium was depleted.

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