**Inhibition of Glutamatergic Activation of Extracellular Signal–regulated Protein Kinases in Hippocampal Neurons by the Intravenous Anesthetic Propofol**

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*Background:* Intravenous anesthetics cause amnesia, but the underlying molecular mechanisms are poorly understood. Recent studies reveal a significant role of extracellular signal–regulated protein kinases (ERKs) in controlling synaptic plasticity and memory formation. As a major synapse-to-nucleus superhighway, NMDA receptor signals to inducible transcriptional events essential for NMDA receptor–dependent forms of synaptic plasticity and memory. This study investigated the role of the widely used intravenous anesthetic propofol in regulating NMDA receptor–dependent ERK phosphorylation.

**Methods:** The possible effect of propofol on NMDA receptor–mediated ERK phosphorylation was detected in cultured rat hippocampal neurons with Western blot analysis.

**Results:** The authors found that propofol at clinical relevant concentrations (1–10 μM) reduced NMDA receptor–mediated ERK phosphorylation. This reduction was independent of γ-aminobutyric acid transmission. The inhibition of the NMDA receptor seems to contribute to the effect of propofol on NMDA-stimulated ERK phosphorylation, because propofol reduced constitutive NMDA receptor NRI subunit phosphorylation and impaired NMDA receptor–mediated Ca2+ influx. Furthermore, by inhibiting the ERK pathway, propofol blocked NMDA receptor–dependent activation of two key transcription factors, Elk-1 and cyclic adenosine monophosphate response element–binding protein (CREB), and, as a result, attenuated Elk-1/CREB–dependent reporter gene (c-Fos) expression.

**Conclusions:** These results suggest that propofol possesses the ability to inhibit NMDA receptor activation of the ERK pathway and subsequent transcriptional activities in hippocampal neurons. These findings indicate a new avenue to explore a transcription-dependent mechanism that may underlie anesthetic interference with synaptic plasticity related to amnestic properties of intravenous anesthetics.

/MITOGEN-ACTIVATED protein kinases (MAPKs) refer to a large family of cytosolic and nuclear serine/threonine kinases that function in a variety of cellular activities in mammalian cells.1 2 The first subfamily of MAPKs was identified as extracellular signal–regulated protein kinases 1 and 2 (ERK1/2)3 and has drawn considerable attention because they are highly expressed in postmitotic neurons of the adult brain and are actively involved in many types of intracellular activities and synaptic plasticity.

ERK1/2 are activated via phosphorylation on Thr(202) and Tyr(204) by diverse stimuli. Once activated, ERK1/2 translocate from the cytosol to the nucleus to activate specific transcription factors, leading to inducible gene expression.2 γ-Glutamate is among neurotransmitters that readily activate ERK1/2 in neurons (for a review, see Wang et al.4). Activation of the ionotropic glutamate receptor, the N-methyl-D-aspartate receptor (NMDAR), markedly increased basal levels of phospho-ERK1/2 (pERK1/2) proteins5–9 through a Ca2+-dependent mechanism.7

An emerging role of ERK1/2 in controlling synaptic plasticity and memory formation in the adult brain has recently been established in several behavioral and cellular studies.10,11 ERK1/2 signaling seems to facilitate transcriptional events and, as a result, to regulate distribution and functions of synaptic proteins to control many forms of synaptic plasticity, including long-term potentiation (LTP) in the hippocampus12,13 a cellular model of learning and memory.14,15 In light of NMDAR–dependent forms of LTP in the hippocampal CA1 region, it has been shown that NMDAR-gated Ca2+ influx activates the ERK pathway to increase the transcription of a family of genes essential for the induction and/or maintenance of LTP.16–19

Propofol (2,6-diisopropylphenol) is a highly effective intravenous anesthetic and is now widely used for general anesthesia and for sedation with local anesthesia and in intensive care units.20,21 Like other intravenous anesthetics, propofol produces amnesia in addition to sedation, hypnosis, and general anesthesia. The amnestic effect of propofol is believed to be related to its known inhibition of LTP at Schaffer collateral–commissural pathway to CA1 pyramidal cell synapses in the hippocampus.22–24 However, cellular mechanisms underlying the propofol inhibition of hippocampal LTP are poorly understood.

In this study, we therefore examined the effect of propofol on the NMDAR-dependent ERK1/2 phosphorylation in cultured rat hippocampal neurons. We found that propofol at clinical relevant concentrations (1–10 μM) for producing clinical amnesia inhibited the NMDAR-mediated activation of the ERK pathway as well.
as the ERK-sensitive activation of transcription factors and gene expression. These findings create a novel avenue to investigate transcription-dependent mechanisms for the amnesic property of propofol.

Materials and Methods

Primary Hippocampal Neuronal Cultures

Standardized procedures preparing primary neuronal cultures from embryonic day 17/18 (E17/E18) rats (Charles River, New York, NY) were used in this study. Time-mated pregnant rats were killed by carbon dioxide asphyxiation in accordance with institutional guidelines for the care and use of animals. Fetuses were removed by cesarean section and transferred to a sterile Petri dish with cold phosphate-buffered saline (PBS). Fetal hippocampi were dissected from surrounding brain tissue, and pooled tissue from each litter was mechanically dissociated and plated on a poly-D-lysine-coated 24-well plate at 200,000–250,000 cells/well. Cells were cultured in a plating DMEM/F12 medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum, 10 g/l glucose, 10 mg/l gentamicin, and 10 mg/l penicillin–streptomycin. After 24 h, the plating medium was replaced by a fresh mixture of a maintenance medium composed by 30% DMEM–F12 medium and 70% neurobasal. Approximately half of the medium was replaced once a week, and 5 μM of 1-β-d-arabinofuranosylcytosine was added on day 4. Cultures were maintained for 9–11 days and contained virtually pure neurons. All procedures performed were approved by the Institutional Animal Care and Use Committee (Kansas City, Missouri) and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Western Blot Analysis

Cell lysates from cultures were sonicated, protein concentrations were determined, and the equal amount of protein (20 μg in 20 μl/lane) was loaded on NuPAGE Novex 4–12% gels (Invitrogen) for separation of proteins. Proteins were transferred to polyvinylidene fluoride membrane (Immobilon-P, 0.45 mm; Millipore, Bedford, MA) and blocked in blocking buffer (5% nonfat dry milk in PBS and 0.1% Tween 20) for 1 h. The blots were incubated in a primary rabbit polyclonal antibody against phospho-ERK1/2 (Thr202/Tyr204) (Cell Signaling, Beverly, MA; 1:1,000), ERK1/2 (Cell Signaling; 1:1,000), c-Fos (Oncogene Research Products, San Diego, CA; 1:1,000), phospho-ERK1/2 (Cell Signaling; 1:1,000), Elk-1 (Santa Cruz Biotechnology; 1:100), c-Fos (Oncogene Research Products, San Diego, CA; 1:1,000), phospho-ERK1/2 (Cell Signaling; 1:1,000), Elk-1 (Santa Cruz Biotechnology; 1:100), c-Fos (Oncogene Research Products, San Diego, CA; 1:1,000), phospho-ERK1/2 (Cell Signaling; 1:1,000), Elk-1 (Santa Cruz Biotechnology; 1:100), and phosphpo-NR1-Ser896 (Upstate; 1:1,000), or β-actin (Santa Cruz Biotechnology; 1:1,000) overnight at 4°C. This was followed by 1 h of incubation in goat anti-rabbit horseradish peroxidase–linked secondary antibodies (Jackson Immunoresearch Laboratory, West Grove, PA) at 1:5,000. Immunoblots were developed with the enhanced chemiluminescence reagents (ECL; Amersham Pharmacia Biotech, Piscataway, NJ, and captured into Kodak Image Station 2000R (Rochester, NY). Kaleidoscope-preserved standards (Bio-Rad, Hercules, CA) were used for protein size determination. The density of immunoblots was measured using the Kodak 1D Image Analysis software and were expressed as percentage of control values.

Intracellular Ca²⁺ Concentration Measurements

Intracellular Ca²⁺ concentration ([Ca²⁺]) measurements were performed according to our previous procedures. Briefly, the culture was loaded with HEPES-buffered balanced salt solution aerated with 95% O₂–5% CO₂, pH 7.4, and contained 3 μM fura-2 AM (Sigma, St. Louis, MO). The fluorescence of fura-2 was sequentially excited at 340 and 380 nm. Emitted lights were collected from the sample through a cooled, intensified charge-coupled device video camera (IC-110; Photon Technology International Inc., Lawrenceville, NJ). The fluorescent signal was measured at a single neuronal cell. Baseline was recorded for 3–5 min before bath application of drugs. [Ca²⁺], was calculated from ratios of the intensities of emitted fluorescence at two excitation wavelengths (F340/F380) with Northern Eclipse Image software (Empix Imaging, Inc., Mississauga, Ontario, Canada). When needed, fluorescence ratios (340/380) were converted to an absolute [Ca²⁺] using the equation of [Ca²⁺] = K_d(F_min/F_max)(R – R_min)/(R_max – R), where K_d is the dissociation constant for fura-2, F is the emitted fluorescence from 380 nm excitation, and R is the fluorescence ratio.

Cell Viability Assay

Cell viability was measured using a double fluorescein diacetate–propidium iodide staining procedure. Fluorescein diacetate is membrane permeable and freely enters intact cells, in which it is hydrolyzed by cytosolic esterase and converted to membrane-impermeable fluorescein with a green fluorescence, exhibited only by live cells. Propidium iodide is nonpermeable to live cells, but penetrates the membranes of dying or dead cells, showing red fluorescence. Cells were rinsed twice with 1× PBS and incubated at 37°C for 5 min with 1× PBS (0.5 ml/well) containing 10 μg/ml fluorescein diacetate (Sigma) and 5 μg/ml propidium iodide (Sigma). Cultures were washed once with PBS and examined under fluorescent light microscopy. The total numbers of viable cells stained by green fluorescein and dead cells stained by red propidium iodide were determined by counting.
cells in five random fields. Positive control was produced by treating cultures with kainic acid (500–1,000 μM, 24 h).

**Drugs and Drug Treatments**

Propofol was purchased from Sigma. N-Methyl-d-aspartate (NMDA), (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate (MK801), bicuculline, (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl)phosphinic acid (CGP55845), and U0126 were purchased from Tocris Cookson Inc. (Ballwin, MO). All drugs were freshly made on the day of experiment. Drugs were dissolved in 1/100 PBS with or without an aid of dimethyl sulfoxide (DMSO). Propofol was dissolved in DMSO. The final concentration of DMSO was 0.1%, at which DMSO itself had no effect on basal (fig. 1A) and NMDA-stimulated ERK1/2 phosphorylation. Cultures were washed with PBS and preincubated at 37°C for 60 min in the humidified atmosphere of 5% CO₂ in HEPES-buffered balanced salt solution (HBS) (154 mM NaCl, 5.6 mM KCl, 2 mM CaCl₂, 2 mM MgSO₄, 5.5 mM glucose, and 20 mM HEPES-KOH or HEPES-NaOH, pH 7.4). For NMDA treatments, MgSO₄ was omitted from, and 1 μM of glycine was added to, the HBS. A Na⁺-free HBS was made by iso-osmotically substituting N-methyl-D-glucamine for NaCl, and adjusting the pH to 7.2 with HCl. To have parameters comparable, none of the HBSs contained sodium bicarbonate. Cells were treated by adding drug to the HBS. At the end of drug treatment, the cells were quickly washed with ice-cold PBS (pH 7.4, Ca²⁺ free) and placed immediately on ice. The cell monolayer was rapidly scraped in ice-cold lysis buffer.

**Statistics**

The results are presented as mean ± SEM. The data were evaluated using one- or two-way analysis of variance, as appropriate, followed by a Bonferroni (Dunn) comparison of groups using least squares-adjusted means. The EC₅₀ value is the effective concentration at which 50% of the maximum effect is reached, which was estimated by using nonlinear least-squares regression techniques (GraphPad Prism version 4.0 for Windows; GraphPad Software, San Diego, CA). Probability levels of less than 0.05 were considered statistically significant.

**Results**

**Selectivity of pERK1/2 and ERK1/2 Antibodies**

Control experiments were first conducted to evaluate the selectivity of phospho-specific antibodies raised against pERK1/2 and antibodies against ERK1/2. Omission of the primary antibodies against pERK1/2 or ERK1/2 in immunoblot with protein extract from cultured rat hippocampal neurons provided no visible
immunoreactivity (data not shown). With the primary antibodies in immunoblot, we found a single band in a molecular weight predicted for the size of pERK1 and pERK2 (fig. 1A). Similar results were observed for ERK1 and ERK2 (fig. 1A). These results demonstrated the selectivity of pERK1/2 and ERK1/2 antibodies. In addition, the effect of DMSO on basal pERK1/2 and ERK1/2 levels was examined. We found no significant change in either pERK1/2 or ERK1/2 protein levels after incubation of DMSO (2–5 min) at 0.1%, a final concentration in cultures when it was used to dissolve propofol (fig. 1A).

**Effects of Propofol on Basal ERK1/2 Phosphorylation**

We then tested whether propofol had any effect on basal levels of pERK1/2 and ERK1/2 in cultured rat hippocampal neurons. Propofol was added to cultures and incubated for 5 min at a series of concentrations (0.1, 1, 3, and 10 μM). These concentrations were selected based on the clinical relevant blood concentrations (approximately 3.5–3.7 μM) for producing 50% loss of memory29,30 as compared with the clinical relevant blood concentrations (approximately 5–28 μM) for maintaining general anesthesia.31 We found that propofol at all concentrations surveyed did not alter basal levels of either pERK1/2 or ERK1/2 (figs. 1B–D). Similar results as to changes in pERK1/2 and ERK1/2 proteins were obtained when propofol was incubated at the same range of concentrations for 1 or 15 min (data not shown). Propofol at all concentrations did not alter basal levels of β-actin (fig. 1B). In addition, there was no significant difference in cell viability between control and propofol-treated cultures as detected by the double fluorescein diacetate–propidium iodide staining.

**Propofol Inhibits NMDA-stimulated ERK1/2 Phosphorylation**

Activation of NMDARs was able to increase ERK1/2 phosphorylation in cultured striatal neurons.5–7 Similarly, NMDA incubation (100 μM) for different durations (1–40 min) caused a rapid and transient increase in ERK1/2 phosphorylation in cultured hippocampal neurons (fig. 2A). A reliable increase in pERK1/2 levels was seen 1 min after propofol incubation. At 40 min, no significant change in pERK1/2 levels was induced by NMDA. Throughout the time course tested, NMDA did not alter total levels of ERK1/2 proteins. Therefore, the elevated level of pERK1/2 was due to an increased phosphorylation process of ERK1/2 in response to NMDA stimulation. In the presence of MK801, a noncompetitive open-channel blocker of NMDAR channels, NMDA no longer induced a significant change in pERK1/2 levels (fig. 2A). Therefore, the stimulatory effect of NMDA on ERK1/2 phosphorylation relies on effective activation of NMDAR channels.

To determine whether propofol affects the NMDAR-dependent ERK1/2 phosphorylation, the ability of NMDA to elevate ERK1/2 phosphorylation was tested in the presence of propofol. As shown in figure 2B, in the presence of 0.1 μM of propofol, NMDA still induced a significant increase in basal levels of pERK1/2, and this increase was not statistically different from that observed after NMDA treatment alone. In cultures pretreated with 1 μM of propofol, however, NMDA did not induce a significant increase in pERK1/2 levels. At 10 μM, propofol completely prevented NMDA from inducing an increase in pERK1/2. The EC50 values for propofol to inhibit NMDA-stimulated ERK1 and ERK2 phosphorylation were 1.1 and 1.0 μM, respectively. In all drug treatments, ERK1/2 remained with no change. These data showed a concentration-dependent inhibition of NMDA-regulated ERK1/2 phosphorylation.

**The Inhibitory Effect of Propofol Is Independent of GABA Transmission**

Propofol is a known allosteric potentiator and agonist of γ-aminobutyric acid type A (GABA_A) receptors.32,33 In the hippocampal CA1 region, propofol potentiated GABA_A receptor-mediated inhibitory postsynaptic potentials.34–36 To evaluate the possible contribution of GABAergic transmission to the propofol action, the propofol effect on NMDA-stimulated ERK1/2 phosphorylation was tested in the presence of the GABA_A receptor antagonist bicuculline or the GABA_B receptor antagonist CGP55845. We found that propofol (10 μM) blocked the NMDA-stimulated ERK1/2 phosphorylation in cultures pretreated with 20 μM bicuculline (fig. 3A). Similarly, propofol (10 μM) displayed the ability to block the NMDA-stimulated ERK1/2 phosphorylation in the presence of the GABA_A receptor antagonist CGP55845 (1 μM; fig. 3B). Neither bicuculline nor CGP55845 had any effect on basal pERK1/2 and ERK1/2 levels (figs. 3A and B). Both antagonists did not seem to alter the ERK1/2 phosphorylation induced by NMDA (figs. 3A and B). Furthermore, direct activation of the GABA receptor with its ligand GABA (5 or 50 μM; 5 min) had no detectable effect on the NMDA-stimulated ERK1/2 phosphorylation (data not shown). These results indicate that activation of GABAergic transmission after propofol administration, if there is any, plays an insignificant role in the propofol-sensitive inhibition of NMDA actions.

**Propofol Inhibits NMDAR NR1 Phosphorylation and Ca^{2+} Influx**

N-Methyl-D-aspartate receptor NR1 subunits undergo a tight phosphorylation at distinct sites, serine 897 and 896, of their intracellular C-terminus under normal conditions.57 Such serine phosphorylation represents a major posttranslational modification of this subunit and is required for normal NMDAR function as assayed in several experimental manipulations.38–41 To determine
whether propofol affects NR1 phosphorylation at these serine sites, effects of propofol exposure on basal levels of NR1 subunits with phosphorylated serine 897 (pNR1-Ser897) or serine 896 (pNR1-Ser896) were tested with a phospho-site–specific antibody. We found that propofol at 1–50 μM reduced basal levels of pNR1-Ser897 (figs. 4A and B). Similar results were obtained for pNR1-Ser896 (figs. 4A and B). However, propofol did not change basal levels of NR1 proteins. Propofol at 0.1 μM or 0.1% DMSO had no significant effect on pNR1-Ser897 or pNR1-Ser896 (data not shown). To determine whether the propofol effect was mediated through a GABA receptor–dependent mechanism, the effect of propofol on NR1 phosphorylation was tested in the presence of bicuculline. We found that in cultures pretreated with bicuculline (20 μM), propofol (10 μM) reduced basal levels of pNR1-Ser897 and pNR1-Ser896 to an extent comparable to that produced by propofol in cultures without treatment with bicuculline (fig. 4C). Therefore, the propofol effect was independent of GABA receptors. To determine whether propofol affects NMDAR-mediated Ca²⁺ influx, we tested effects of propofol on NMDA-induced Ca²⁺ increase. A typical elevation of [Ca²⁺]ᵢ was induced after addition of NMDA (50–100 μM) into the culture as detected by somatic fura-2 ratio fluorescent measurements (fig. 4D), which was blocked by MK801 (0.1 μM) or removing extracellular Ca²⁺ ions (data not shown), confirming an NMDAR-mediated Ca²⁺ increase. In the presence of propofol (10 μM), NMDA (100 μM) was still able to induce a significant increase of [Ca²⁺]ᵢ as compared with basal levels (fig. 4D). However, the amplitude of Ca²⁺ increases was significantly smaller than that
observed in the absence of propofol (fig. 4D). The effect of propofol at the two lower concentrations (1 and 3 μM) on Ca^{2+} responses to NMDA (50–100 μM, 20 s) was also detected. We found that propofol at 5 μM significantly reduced the amplitude of Ca^{2+} responses (127.4 ± 10.3 nM after NMDA vs. 107.2 ± 6.8 nM after propofol + NMDA, n = 14; P < 0.05). Propofol at 1 μM slightly reduced the amplitude of Ca^{2+} responses (129.6 ± 9.5 nM after NMDA vs. 118.4 ± 10.3 nM after propofol + NMDA, n = 13), which did not reach a statistically significant level (P > 0.05). Propofol itself had no effect on basal levels of intracellular Ca^{2+}. These results indicate that propofol is capable of inhibiting NR1 phosphorylation and reducing NMDAR-mediated Ca^{2+} signals in cultured hippocampal neurons.

**Propofol Inhibits NMDAR/ERK-dependent Transcriptional Activities**

One of particularly noticeable roles that active ERK plays is to facilitate gene expression via phosphorylating nuclear transcription factors. We then tested Elk-1 and CREB, two specific factors downstream to the ERK pathway, for their responses to NMDA stimulation in the presence of propofol. In addition, an immediate early gene, c-Fos, was tested as a reporter of inducible gene expression downstream to Elk-1 and CREB to determine a final output of gene expression. We found that the NMDA-stimulated phosphorylation of ERK1/2 (fig. 5A), Elk-1 (fig. 5B), and CREB (fig. 5C) and c-Fos expression (fig. 5D) were blocked by a MAPK kinase–selective inhibitor, U0126 (5 μM), confirming the role of the ERK pathway in mediating NMDA-stimulated Elk-1 and CREB phosphorylation and c-Fos expression. Interestingly, like U0126, propofol (10 μM) substantially reduced Elk-1 (fig. 5B) and CREB expression (fig. 5C) and c-Fos expression (fig. 5B) induced by NMDA stimulation. No significant differences were found in total ERK1/2, Elk-1, CREB, and β-actin proteins after any drug treatments (fig. 5). These results suggest that propofol can reduce the NMDA-stimulated phosphorylation of Elk-1 and CREB.
and c-Fos expression in parallel with the reduction of NMDA-stimulated ERK1/2 phosphorylation.

**Discussion**

This study investigated a hypothesis that propofol inhibits the NMDAR-mediated activation of the ERK1/2 pathway in cultured rat hippocampal CA1 neurons. We found that propofol at the clinical relevant concentrations blocked the NMDAR-mediated activation of ERK1/2. Propofol also attenuated the NMDA-stimulated phosphorylation of transcription factors, CREB and Elk-1, two downstream targets to the ERK1/2 pathway, and a reporter gene expression (c-Fos). In the presence of the GABAA or GABA B receptor antagonist, propofol preserved its potency in inhibiting the NMDAR-mediated ERK1/2 phosphorylation, indicating a GABA-independent mechanism mediating the effect of propofol. Because propofol inhibited serine phosphorylation of NMDAR NR1 subunits and NMDAR-mediated Ca\(^{2+}\)/H\(^{+}\) influx, the inhibition of NMDAR-mediated Ca\(^{2+}\)/H\(^{+}\) influx may partially account for the inhibition of NMDAR-stimulated ERK1/2 phosphorylation. The results obtained here suggest that propofol possesses the ability to produce the potent inhibition of NMDAR-mediated ERK activation and ERK-mediated gene expression.

Propofol has been well documented as an allosteric potentiator and agonist of GABA\(_{A}\) receptors.\(^{32,33}\) By enhancing GABAergic transmission, propofol produces its anesthetic effect. Because propofol potentiated GABA\(_{A}\) receptor-mediated inhibitory postsynaptic potentials in the hippocampal CA1 region,\(^{34–36}\) it is possible that this agent may inhibit the NMDA-stimulated ERK1/2 phosphorylation through a GABAergic mechanism. However, results from this study seem to disagree with this notion. Pharmacologic blockade of GABAA or GABA B receptors did not affect the propofol inhibition of NMDA-stimulated ERK1/2 phosphorylation. Direct stimulation of GABA receptors with GABA did not inhibit the NMDAR-mediated ERK1/2 phosphorylation.

**N-Methyl-D-aspartate receptors regulate ERK1/2 phosphorylation via a Ca\(^{2+}\)-sensitive mechanism.** The NMDAR-mediated Ca\(^{2+}\) influx activates the Ca\(^{2+}\)-sensitive protein kinases, such as phosphatidylinositol 3-kinase and Ca\(^{2+}/\)calmodulin-dependent protein kinases, to increase ERK1/2 phosphorylation.\(^{7}\) In this study, propofol at 3 and 10 \(\mu M\) was found to decrease NMDA-stimulated Ca\(^{2+}\) influx. Therefore, at these two concentrations, propofol may inhibit the ERK1/2 phosphorylation through a Ca\(^{2+}\)-dependent mechanism. In contrast, propofol at 1 \(\mu M\) did not significantly affect NMDA-stimulated Ca\(^{2+}\) influx, whereas it was effective to inhibit the ERK1/2 phosphorylation. Therefore, at this low concentration, propofol may reduce the ERK1/2 phos-
phorylation through an unclear and likely Ca\(^{2+}\)-independent mechanism. In support of this, NMDARs have been found to increase ERK1/2 phosphorylation via a prime synaptic protein, PSD-95, in a Ca\(^{2+}\)-independent manner. Nevertheless, at all concentrations surveyed (1, 3, and 10 \(\mu\)M), propofol showed the ability to inhibit NMDAR phosphorylation at the two NR1 serine sites (serine 897 and 896). This may cause inhibition of NMDAR function in activating either the Ca\(^{2+}\)-dependent or Ca\(^{2+}\)-independent signaling pathway, leading to reduction of NMDA-stimulated ERK1/2 responses. Phosphorylation of NR1 at these serine sites has been thought to be a major posttranslational modification of this subunit, and the state of NR1 serine phosphorylation is positively correlated to enhanced NMDAR function in several experimental manipulations. In our previous work conducted in cultured striatal neurons, we found that propofol at 1–10 \(\mu\)M can cause a rapid decrease in NR1 phosphorylation at the two serine sites. In this study, we observed the same inhibitory effect of propofol on NR1 serine phosphorylation in hippocampal neurons. Therefore, the propofol action in suppressing NR1 phosphorylation is not cell-type specific.

Fig. 5. Effects of propofol (Pro) and U0126 (U) on the \(N\)-methyl-\(D\)-aspartate (NMDA)-stimulated phosphorylation of extracellular signal–regulated protein kinases 1 and 2 (ERK1/2; \(A\)), Elk-1 (\(B\)), and cyclic adenosine monophosphate response element–binding protein (CREB; \(C\)), and c-Fos expression (\(D\)) in cultured rat hippocampal neurons. Note that both propofol and U0126 blocked the NMDA-induced phosphorylation of ERK1/2, Elk-1, and CREB, without changing cellular levels of the three proteins. Propofol (10 \(\mu\)M) or U0126 (5 \(\mu\)M) was incubated 30 min before and during treatment with NMDA for 15 min (pERK1/2, pElk-1, and pCREB) or 30 min (c-Fos). Representative immunoblots are shown at left of the quantified data (mean ± SEM, \(n = 6\)). * \(P < 0.05\) versus basal levels. + \(P < 0.05\) versus NMDA alone.
Ca²⁺ influx in the degree of inhibition of NMDA-stimulated Ca²⁺ responses, in contrast to a statistically significant reduction of the Ca²⁺ influx observed at 3 μM of propofol between this study and previous studies. This slight difference in the degree of inhibition of NMDA-stimulated Ca²⁺ influx observed at 3 μM of propofol between this study and that conducted by Grasshoff and Gillessen may reflect differences in cultures (cortical cultures in their studies vs. hippocampal cultures in this study), application of NMDA (300 μM for 180 s in their studies vs. 50–100 μM for 20 s in this study), and other culturing conditions.

Long-term potentiation is an activity-dependent event reflecting enhancement of synaptic efficacy. In the hippocampal CA1 region, LTP can last for hours and requires de novo transcription and protein synthesis. Like LTP, various forms of learning behaviors, especially at the late phase of learning, are messenger RNA– and protein synthesis–dependent. In exploring signaling mechanisms mediating inducible gene expression needed for LTP, increasing evidence indicates that the ERK pathway plays a pivotal role. The ERK pathway is a major synapse-to-nucleus superhighway mediating new gene expression in response to a variety of cellular stimuli. Recent reports show that the induction, maintenance, or both of NMDAR-dependent LTP require activation of this pathway. For example, NMDAR-dependent LTP in CA1 area was associated with an increase in active ERK1/2. The inhibitors selective for the ERK pathway blocked NMDAR-dependent LTP. Like the inhibitors for the ERK pathway, propofol blocked NMDAR-dependent LTP in the hippocampal CA1 region. Likely through its inhibition of NMDAR-dependent LTP, propofol induces amnesia, and its suppression of memory function can persist for several hours after propofol administration.

In this study, we demonstrated that propofol inhibited NMDAR-dependent activation of the ERK pathway in hippocampal CA1 neurons. Although the current study conducted no electrophysiologic and behavioral experiments to define the role of the inhibited ERK phosphorylation by propofol in regulating NMDAR-dependent LTP and memory behaviors, our findings here seem to add a piece of evidence for a possible transcription-dependent mechanism responsible for the propofol inhibition of NMDAR-dependent LTP and for the amnesic effect of propofol.

The ERK pathway transmits glutamate receptor signals to Elk-1 and CREB for the facilitation of gene expression. The Elk-1- and CREB–facilitated gene expression is implicated in the development and maintenance of LTP and learning and memory. It is then intriguing to investigate whether propofol that inhibits the NMDAR/ERK pathway could inhibit NMDAR/ERK-dependent Elk-1 and CREB phosphorylation. We found that propofol at concentrations that blocked NMDAR-dependent activation of ERK1/2 attenuated increases in Elk-1 and CREB phosphorylation and c-Fos expression (fig. 6). Therefore, propofol possesses the ability to suppress Elk-1– and CREB-sensitive gene expression likely through the inhibition of NMDAR-dependent activation of the ERK pathway. This suppression of Elk-1– and CREB-sensitive gene expression may contribute to the inhibition of transcriptionally dependent LTP.

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Anesthesiology, V 105, No 6, Dec 2006

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