Korean J Physiol Pharmacol
Vol 5: 189-197, April, 2001

Effect of Propofol on Ion Channels in Acutely Dissociated Dorsal Raphe Neuron of Sprague-Dawley Rats

Bong-Jae Lee, Moo-Il Kwon, Min-Chul Shin[1], Youn-Jung Kim[1], Chang-Ju Kim[1], Soon Ae Kim[2], Ee-Hwa Kim[3], and Joo-Ho Chung[2]

Departments of Anesthesiology, [1]Physiology and [2]Pharmacology, College of Medicine, Kyung Hee University, Seoul 130-701; [3]Department of Meridianology, College of Oriental Medicine, Semyung University, Chungbuk 390-711, Korea

To investigate propofol's effects on ionic currents induced by γ-aminobutyric acid (GABA) and glycine as well as on those produced by the nicotinic acetylcholine- and glutamate-responsive channels, rat dorsal raphe neurons were acutely dissociated and the nystatin-perforated patch-clamp technique under voltage-clamp conditions was used to observe their responses to the administration of propofol. Propofol evoked ion currents in a dose-dependent manner, and propofol (10^{-4} M) was used to elicit ion currents through the activation of $GABA_A$, glycine, nicotinic acetylcholine and glutamate receptors. Propofol at a clinically relevant concentration (10^{-5} M) potentiated $GABA_A$-, glycine- and NMDA receptor-mediated currents. The potentiating action of propofol on $GABA_A$-, glycine- and NMDA receptor-mediated responses involved neither opioid receptors nor G-proteins. Apparently, propofol modulates inhibitory and excitatory neurotransmitter-activated ion channels either by acting directly on the receptors or by potentiating the effects of the neurotransmitters, and this modulation appears to be responsible for the majority of the anaesthetic and/or adverse effects.

Key Words: Propofol, GABA, Glycine, Glutamic acid, Patch-clamp technic

INTRODUCTION

Propofol (2,6-diisopropylphenol) is an intravenous anesthetic that is widely used in daily clinical practice on surgical patients. Structurally, it is similar to α-tocopherol and acetylsalicylic acid. General anesthetics range from chemically inert gases to complex steroidal agents. In view of this chemical diversity, it is surprising that many general anesthetics used clinically or as experimental agents share, at relevant concentrations, the effect of potentiation of γ-aminobutyric acid (GABA)-mediated activation of the $GABA_A$ receptor (Franks & Lieb, 1994; Belelli et al, 1996).

GABA is the major inhibitory neurotransmitter in the central nervous system (CNS). GABA is released from GABAergic neurons and binds to both $GABA_A$ and $GABA_B$ receptors. The $GABA_A$ receptor is a macromolecular protein that contains specific binding sites, at least for GABA, picrotoxin, barbiturates, benzodiazepines, and anesthetic steroids, and forms a chloride ion-selective channel (Macdonald & Olsen, 1994). Substantial experimental evidences indicate that $GABA_A$ receptors are major targets of propofol, being implicated in both the behavioral and the pharmacological actions of this agent (Tanelian et al, 1993; Franks & Lieb, 1994). The actions of propofol on $GABA_A$ receptors are complex: propofol induces the potentiation of GABA-mediated responses, directly activates the receptors in the absence of GABA and alters the pattern of receptor desensitization (Hales & Lambert, 1991; Hara et al, 1993; Sanna et al, 1995).

Electrophysiologic studies have implicated voltage-dependent Na^+ channels as another possible molecular site of action of propofol (Ratnakumari & Hemmings,

Corresponding to: Joo-Ho Chung, Department of Pharmacology, College of Medicine, Kyung Hee University, Seoul 130-701, Korea. (Tel) 82-2-961-0303, (Fax) 82-2-968-0569, (E-mail) jhchung@sbsmail.net

1997; Rehberg & Duch, 1999). Ratnakumari & Hemmings (1997) reported that inhibition of channel-mediated Na^+ influx, increase in intracellular [Na^+] and glutamate release occurred in synaptosomes at concentrations of propofol achieved clinically, and these results support the possibility of a role for neuronal voltage-dependent Na^+ channels as a presynaptic molecular target for general anesthetic effects. Additionally, there have been several reports about the relationship between propofol and Ca^{2+} currents (Wu et al, 1997; Todorovic & Lingle, 1998). Propofol is known to have direct dromotropic and chronotropic effects on the cardiac conduction system, and these changes have been attributed, at least in part, to the drug's direct dose-dependent suppression of the cardiac sodium, calcium and transient outward potassium currents (Wu et al, 1997). Antinociceptive effects of propofol have also been reported in humans (Anker-Moller et al, 1991), and studies have shown that positive modulation of the $GABA_A$ receptor in the spinal cord can cause antinociception *in vitro* (Edwards et al, 1990; Nadeson & Goodchild, 1997).

Although many suggestions have been made regarding the molecular action mechanism of propofol, little is known about the modulatory actions of propofol on inhibitory neurotransmitter-activated neuronal ion channels ($GABA_A$- and glycine-stimulated channels) and excitatory neurotransmitter-activated ion channels (nicotinic aetylcholine- and glutamate-stimulated channels). Dorsal raphe nuclei are major sites of origin of ascending pathways, which innervate the hippocampus, neocortex and striatum (Azmitia & Segal, 1978). In this study, modulatory action of propofol on these ion currents in acutely dissociated dorsal raphe neurons was investigated using the nystatin-perforated patch-clamp technique under voltage-clamp conditions.

METHODS

Preparation of the dorsal raphe neuron

Dorsal raphe neurons were freshly dissociated using a technique described previously (Kim et al, 1997; Han et al, 1999). In brief, 10- to 15-day-old Sprague-Dawley rats of both sexes were decapitated under anesthesia induced by Zoletil 50 (10 mg/kg i.m; Vibac, Carros, France). The brains were removed and transverse slices (400 μm thickness) were made with a microslicer (DTK-1000, DSK, Tokyo, Japan). The slices were preincubated in an incubation solution that had been well-saturated with 95% O_2 and 5% CO_2 at room temperature for 30 min. Then the slices were treated with pronase (protease XIV, 1 mg/6 ml of the oxygenated incubation solution) for 40~80 min at 32°C and subsequently with thermolysin (protease X, 1 mg/6 ml) for 10~20 min, also at same temperature. Following the enzyme treatment, the slices were immersed in enzyme-free incubation solution for 1 hour.

The dorsal raphe region was identified in slices in 60 mm culture dishes coated with silicone under a binocular microscope (SZ-ST, Olympus, Tokyo, Japan), and micropunched out from the slices with an electrolytically polished injection needle. The micropunched dorsal raphe regions were mechanically dissociated in a different dish with fire-polished fine glass Pasteur pipettes in 35 mm plastic culture dishes (3801, Falcon, Becton Dickinson, USA) filled with the standard solution. The dissociation procedure was performed under an inverted phase-contrast microscope (CK-2, Olympus, Tokyo, Japan). Most of the dissociated neurons adhered to the bottom of the dish within 20 min.

Solutions

The ionic composition of the incubation solution was (in mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, glucose 10 and $NaHCO_3$ 24. The pH was adjusted to 7.4 by continuous bubbling with 95% O_2 and 5% CO_2. The composition of the standard external solution was (in mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, and *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulphonic acid (HEPES) 10. The pH was adjusted to 7.4 with tris-hydroxymethylaminomethane (Tris-base). The composition of the internal pipette solution for nystatin-perforated recording was (in mM): KCl 150 and HEPES 10. The pH was adjusted to 7.2 by adding Tris-base. A stock solution containing 10 mg/ml nystatin was prepared and added to the patch pipette solution to reach a final concentration of 200 μg/ml.

Drugs

Propofol (2,6-diisopropylphenol), D-2-amino-5- phosphonopentanoic acid (D-AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and kainate were obtained from TOCRIS (Ballwin, MO, USA) and strychnine

from RBI (Natick, MA, USA). Pronase, thermolysin, nystatin, bicuculline, tubocurarine, GABA, glycine, nicotine, glutamate, *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), naltrexone, *N*-ethylmaleimide (NEM) and most of the other drugs used in this experiment were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Drugs were added to the standard solution to reach the final concentrations indicated in the text and were applied using a rapid application system termed the "Y-tube method" as described previously (Kim et al, 1997; Han et al, 1999). Using this technique, the standard solution surrounding a neuron could be exchanged within 10~20 ms.

Electrical measurements

Electrical recordings were performed in the nystatin-perforated patch recording mode under voltage-clamp conditions. Patch pipettes were prepared from glass capillaries with an outer diameter of 1.5 mm using a two-stage puller (PB-7, Narishige, Tokyo, Japan). The resistance between the recording electrode filled with the internal pipette solution and the reference electrode was 6~8 MΩ. After the formation of a stable perforated patch, the series resistance ranged from 16 to 25 MΩ.

Electrical stimulation, current recordings and filtration of currents (at 2.9 kHz) were made with an EPC-7 patch-clamp amplifier (List-Electronic, Darmstadt/Eberstat, Germany). The current and voltage were monitored using a pen recorder (Recti-Horiz-8K, NEC San-ei, Tokyo, Japan). All experiments were performed at room temperature (22~24°C).

Statistical analysis

Data are presented as mean±S.E.M., and Student's *t*-test was performed for statistical analysis using SPSS (version 7.5) and p-values less than 0.05 were considered to represent statistical significance.

RESULTS

Ion currents activated by propofol ($I_{propofol}$)

Experiments were carried out in the nystatin-perforated patch-clamp mode at a holding potential (V_H) of −50 mV. Propofol was applied every 2 min and the magnitude of the ion current elicited by propofol at a concentration of 10^{-4} M was used as the reference value. The inward current was recorded at various concentrations of propofol. At a concentration of 3×10^{-6} M propofol did not evoke any ion current (n=8). The magnitude of relative ion current evoked by 10^{-5} M propofol was 0.05±0.00 (n=8) times the reference value, and the relative ion currents magnitudes of 0.19±0.01 (n=7), 1.60±0.04 (n=9) and 1.82±0.08 (n=9) times the reference value were elicited by propofol at concentration of 3×10^{-5} M, 3×10^{-4} M and 10^{-3} M respectively. Fig. 1 shows the magnitude of the propofol-activated ion current plotted as a function of propofol concentration. Maximal current was produced by 10^{-3} M propofol and calculated EC_{50} was 8×10^{-5} M.

Effects of antagonists on the propofol-activated ion current

The effects of bicuculline (a $GABA_A$ receptor antagonist), tubocurarine (a nicotinic acetylcholine receptor antagonist), strychnine (a glycine receptor anta-

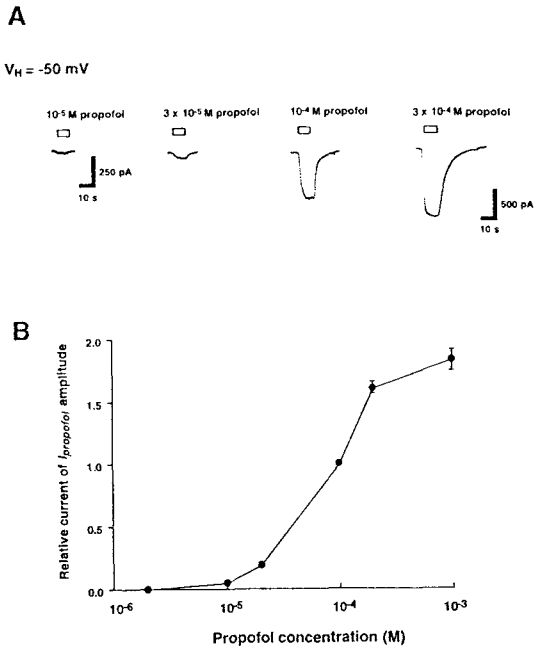

Fig. 1. Ion current elicited by propofol. Nystatin-perforated patch-clamp under voltage clamp condition (V_H= −50 mV) was performed on acutely dissociated dorsal raphe neurons. Propofol was applied every 2 min and the resultant ion currents ($I_{propofol}$) were measured.

gonist), D-AP5 (an NMDA sensitive glutamate receptor antagonist) and CNQX (AMPA/kainate- sensitive glutamate receptor antagonist) were examined.

Bicuculline (10^{-5} M) reduced the magnitude of the ion current evoked by propofol at concentration of 10^{-4} M to about 65.17±1.63% (n=6, $p<0.05$) of the reference value. The magnitude of the ion current evoked by 10^{-4} M propofol was decreased to about 56.40±2.67% of the reference value by 10^{-5} M tubocurarine (n=6, $p<0.05$), about 45.47±2.75% (n=6, $p<0.05$) by 10^{-5} M strychnine, about 9.86± 2.08% by 10^{-4} M D-AP5 (n=6, $p<0.05$) and about 11.78±2.39% by 10^{-4} M CNQX (n=6, $p<0.05$)(Fig. 2). In this experiment, it could be seen that the ion current induced by propofol was suppressed by several types of ion channel antagonists, suggesting that propofol at high concentrations (10^{-4} M in this experiment) directly activate $GABA_A$ receptors, nicotinic acetylcholine reeptor, glycine receptors and both the NMDA-sensitive and the non-NMDA-sensitive subtypes of glutamate receptors.

Modulation by propofol of neurotransmitter-activated ion currents

Because 10^{-5} M propofol produces little direct activation of most of the receptors tested, this particular concentration was used. Propofol at concentration of 10^{-5} M was chosen to test the potentiation of neurotransmitter-activated ion currents by the drug.

To investigate the modulatory action of propofol on the GABA-induced current, the magnitude of ion current elicited by 10^{-5} M GABA was used as the reference value, and 10^{-5} M propofol was applied simultaneously with GABA. Propofol potentiated the GABA-elicited current, to about 160.30±8.84% (n=6, $p<0.05$) of the reference value. Using similar methods, propofol was observed to enhance the current evoked by 10^{-5} M glycine to about 157.20±

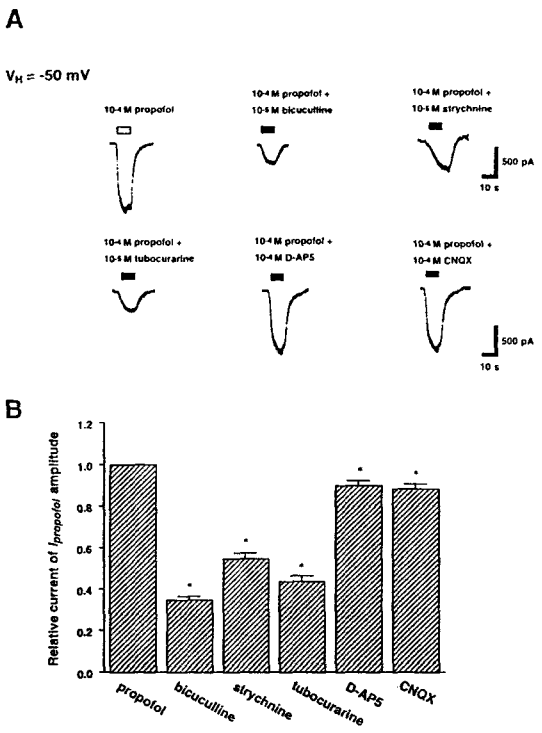

Fig. 2. Effects of different types of ion channel antagonists (bicuculline: $GABA_A$ receptor antagonist, strychnine: glycine receptor antagonist, tubocurarine: nicotinic acetylcholine receptor antagonist, D-AP5: NMDA-sensitive glutamate receptor antagonist, CNQX: non-NMDA-sensitive glutamate receptor antagonist) on propofol-elicited ion current. The ion current induced by propofol was suppressed by these antagonists. *means $p<0.05$ compared to control.

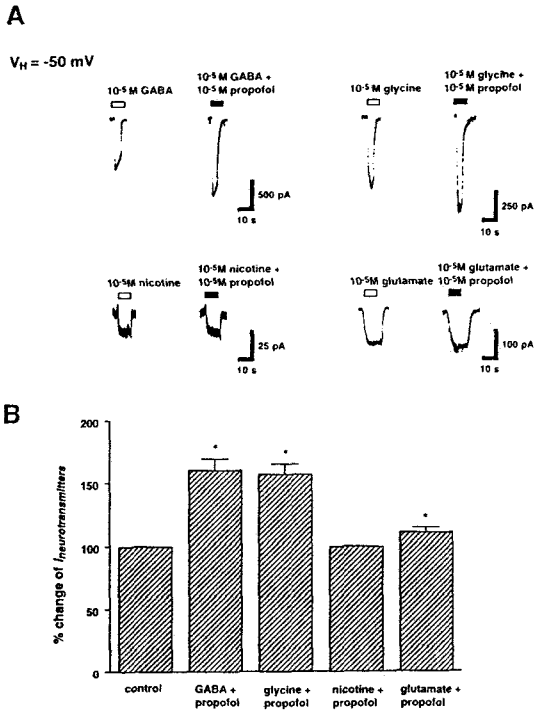

Fig. 3. Modulation by propofol of neurotransmitter-induced ion currents. Propofol potentiated GABA- and glycine- induced chloride currents, while it exerted no effect on nicotinic acetylcholine receptor-mediated current. Propofol also enhanced the glutamate-elicited response, albeit only slightly. *means $p<0.05$ compared to control.

8.10% of the reference value (n=6, $p<0.05$). However, propofol exerted no effect on the nicotinic acetylcholine receptor-mediated current elicited by 10^{-5} M nicotine (n=7, $p>0.05$). The current activated by 10^{-5} M glutamate was potentiated by propofol to about 110.59±3.40% (n=8, $p<0.05$) of the reference value (Fig. 3).

Modulation by propofol of glutamate receptors subtypes

In the previous section, it was shown that propofol potentiated glutamate-induced current. In this experiment, the involvement of the subtypes of glutamate receptors in propofol-mediated potentiation of glutamate-induced response was investigated. Application of 10^{-4} M NMDA, 10^{-5} M AMPA and 10^{-5} M kainate on dorsal raphe neurons itself elicited ion currents. Concurrent application of 10^{-5} M propofol did not alter either AMPA- or kainate-induced ion currents, but potentiated NMDA-elicited current to about 115.67±10.00% (n=8, $p<0.05$) of the reference value (Fig. 4).

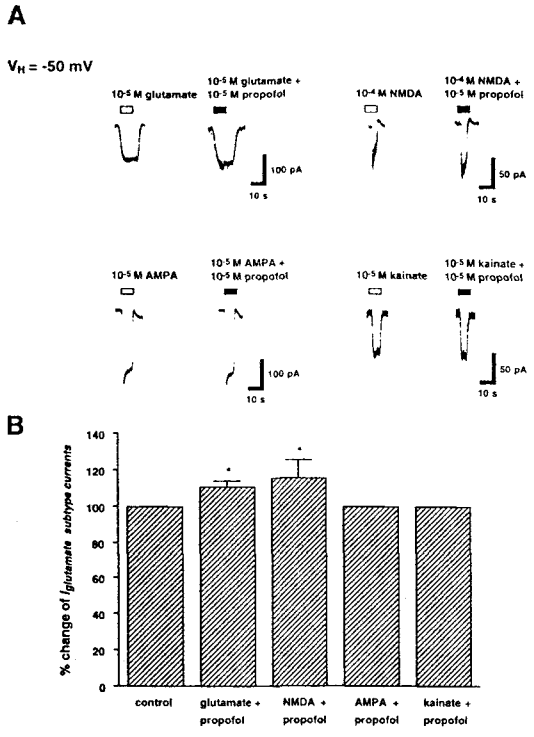

Fig. 4. Propofol potentiated NMDA-induced current but not AMPA- and kainate-induced currents. *means $p<0.05$ compared to control.

Effects of naltrexone on propofol-mediated potentiation of GABA-, glycine- and NMDA-induced currents

To evaluate the involvement of opioid receptors in propofol-mediated potentiation of GABA-, glycine- and NMDA-induced currents in dorsal raphe neurons, naltrexone, an opioid antagonist and a stable naloxone analogue, was applied at a concentration of 10^{-5} M concurrently with 10^{-5} M propofol. Propofol enhanced the GABA-elicited current to about 160.30± 8.84% of the reference value, and naltrexone applied with propofol brought this figure to 159.35±8.84%. This result showed that naltrexone exerts no effect on propofol-mediated potentiation of GABA-elicited response (n=6, $p>0.05$). Propofol enhanced the glycine-induced current to about 157.20±8.10% of the reference value, and naltrexone administrated with propofol brought this figure to about 157.30±9.10%, showing that naltrexone exerts no effect on propofol-

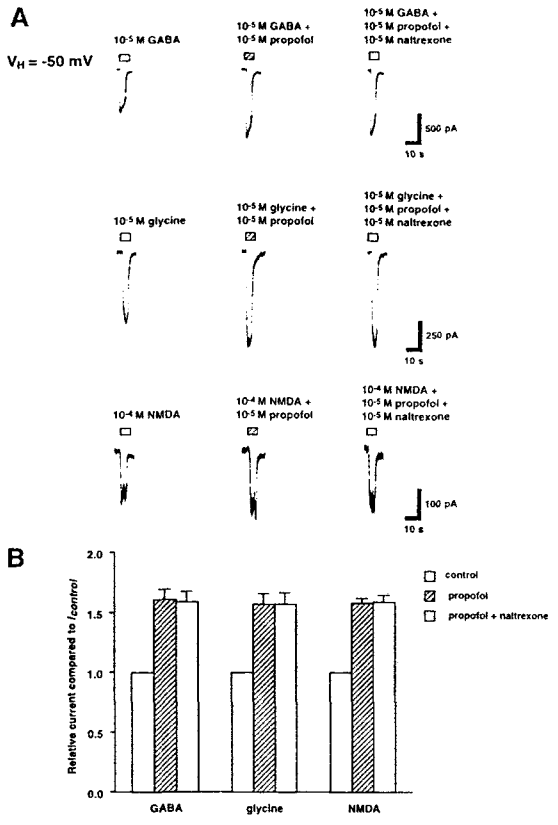

Fig. 5. Naltrexone's effects on propofol-mediated potentiation of GABA-, glycine- and NMDA-evoked responses. Naltrexone, an opioid antagonist and a stable naloxone analogue, did not alter propofol-mediated potentiation of GABA-, glycine- and NMDA-induced currents.

mediated potentiation of glycine-elicited response (n=6, $p>0.05$). Propofol augmented the NMDA-elicited current to about $158.02\pm4.05\%$ of the reference value and naltrexone applied with propofol brough this figure to about $158.72\pm5.78\%$ (n=6, $p>0.05$) (Fig. 5).

Effects of N-ethylmaleimide (NEM) on propofol-mediated potentiation of GABA-, glycine- and NMDA-induced currents

In order to elucidate the involvement of GTP-binding proteins (G-proteins) in propofol-mediated potentiation of GABA-, glycine- and NMDA- induced currents, we examined the effects of NEM on the potentiation of GABA-, glycine and NMDA- evoked responses.

It is known that NEM at a concentration of 50 μM inhibits the agonist-activated currents mediated by pertussis toxin (PTX)-sensitive G-proteins (Ueda et al, 1990). The advantage of using NEM is that it allows to examine PTX-sensitive G-protein-mediated action before and after its inhibition within the same recording. Propofol potentiated the GABA-induced current to $144.96\pm9.04\%$ of the reference value, and after perfusion with the standard solution containing NEM at a concentration of 5×10^{-5} M for 2 min, the potentiation by propofol of the GABA-elicited current brought the figure to $147.20\pm7.76\%$ (n=6, $p>0.05$). Propofol was observed to potentiate the glycine-induced current to $143.00\pm2.62\%$ of the reference figure. After perfusion with NEM at a concentration of 5×10^{-5} M for 2 min, the potentiation by propofol of glycine brought this figure to $150.00\pm4.23\%$ (n=6, $p>0.05$). Propofol increased the NMDA-elicited current to about $128.45\pm2.52\%$ of the reference value, and after perfusion with NEM at a concentration of 5×10^{-5} M for 2 min, this figure was brought to $128.62\pm3.93\%$ (n=6, $p>0.05$). These results showed that the potentiating action of propofol on GABA-, glycine- and NMDA-induced currents does not involve G-proteins (Fig. 6).

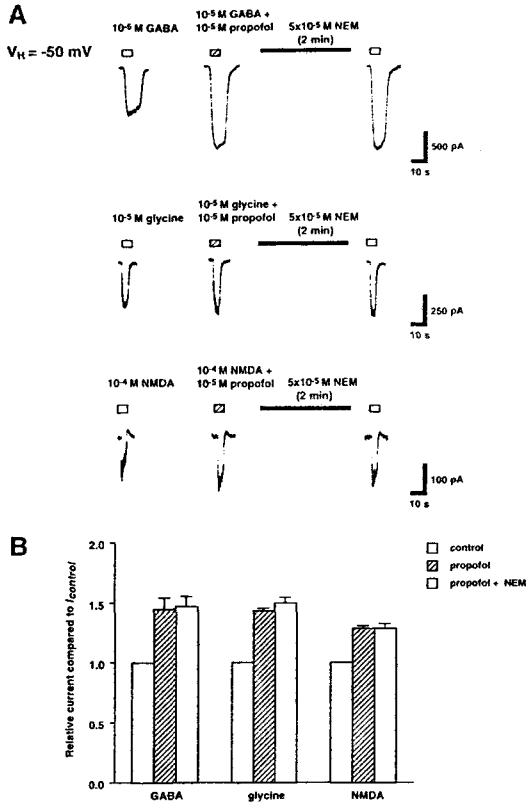

Fig. 6. Effects of *N*-ethylmaleimide on propofol-mediated potentiation of GABA-, glycine- and NMDA-mediated responses. The potentiating actions of propofol on GABA-, glycine- and NMDA-induced currents were not altered by pretreatment with NEM, a sulfhydryl alkylating agent which selectively inhibits PTX-sensitive G-proteins.

DISCUSSION

Currents were observed to be directly evoked by propofol in a dose-dependent manner in acutely dissociated rat dorsal raphe neurons at sufficiently high concentrations (Fig. 1). The systemic blood concentration of propofol required to produce unconsciousness in laboratory animals was reported to be in the range of $1\sim4$ μg/ml ($5.6\sim22.4\times10^{-6}$ M) (Adam et al, 1980). The arterial plasma concentration needed to induce loss of consciousness in humans was reported to be around $3\sim4$ μg/ml ($16.8\sim22.4\times10^{-6}$ M) (Vuyk et al, 1992). The inward current evoked by propofol was suppressed by bicuculline, strychnine and tubocurarine, which suggests that propofol at high concentrations (10^{-4} M in this study) directly activates $GABA_A$, glycine and nicotinic acetylcholine receptors. The current evoked by propofol was also suppressed by D-AP5 and CNQX, which suggests that high concentrations of propofol directly activates glutamate receptors of both the NMDA-sensitive and the non-NMDA-sensitive subtypes (as well)(Fig. 2).

It has been reported that propofol-induced currents are suppressed by bicuculline and strychnine, and that

the direct activation of $GABA_A$ receptors occurs *via* propofol's interaction with the β-subunit (Hara et al, 1993; Sanna et al, 1995). The present study showed that both inhibitory (GABA and glycine) and excitatory (nicotinic acetylcholoine and glutamate) neurotransmitter receptors are activated by high concentration (10^{-4} M in this study) of propofol. $GABA_A$ receptors are members of a ligand-gated ion channel superfamily which also includes glycine, serotonin-3, GABA ρ ($GABA_C$) and nicotinic acetylcholine receptors. $GABA_A$ receptors share significant amino acid sequence homology with these receptors (Ortells & Lunt, 1995; Rothlin et al, 1999). Glycine receptor function is modulated positively by clinical concentrations of volatile anesthetics (Harrison et al, 1993; Downie et al, 1996). Some general anesthetics have potent effects on neuronal but not muscular nicotinic acetylcholine receptors (Flood et al, 1997; Violet et al, 1997). This observation could explain the suppression of propofol-induced ion currents by antagonists of different types of ion channels.

The concentration of 10^{-4} M for propofol is higher than that used clinically. Propofol-mediated activation of excitatory neurotransmitter receptors - nicotinic acetylcholine, NMDA-sensitive and non-NMDA-sensitive subtypes of glutamate receptors - at such a high concentration could explain the unintended actions of propofol. Some excitatory side effects of propofol have been observed during induction of anaesthesia with various doses ranging from 1.5 to 3.0 mg/kg body weight. Manifestations of excitatory effects have been known to occur during maintenance of anesthesia, of which spontaneous movement was reported to be the most common form, and this may be explained by the fact that high concentrations of propofol activate excitatory neurotransmitter receptors (Bansinath et al, 1995).

The major mechanism of action of propofol is potentiation of the effect of inhibitory neurotransmitters (Tanelian et al, 1993; Franks & Lieb, 1994; Downie et al, 1996; Pistis et al, 1997; Bai et al, 1999). Many reports have shown that $GABA_A$ and glycine receptors are positively modulated by propofol at clinically relevant concentrations. The results of the present study also show that clinically relevant concentrations of propofol (10^{-5} M in this study) potentiate GABA and glycine receptor-mediated currents profoundly. As for excitatory neurotransmitter receptors, however, propofol was shown to slightly potentiate NMDA-sensitive glutamate receptors-mediated responses, while nicotinic acetylcholine receptors were unaffected (Fig. 3).

Nadeson & Goodchild (1997) showed that propofol causes antinociception in rats *via* its actions on the $GABA_A$ and *delta* opioid receptors; these mechanisms suggest the possibility of propofol potentiation of opioid analgesia. However, in this experiment, naltrexone did not alter the propofol-mediated potentiation of GABA- and glycine-induced responses, suggesting that the propofol-mediated modulation does not involve opioid receptors (Fig. 5).

Neurotransmitters acting through G-protein-coupled receptors modify the electrical excitability of neurons. Activation of these receptors can affect the voltage dependence, speed of gating and probability of opening of various ion channels, thus altering the computational state and output of a neuron. The G-proteins are heterotrimeric molecules with α-, β- and γ-subunits. The α-subunit can be classified into one of three families, depending on whether it is a target for pertussis toxin, cholera toxin or neither. In neurons, the most widespread modulatory signaling pathway is characterized by its sensitivity to PTX, which indicates that the relevant receptors are coupled to G-proteins of the Gi family, such as Gi or Go (Hille, 1994). NEM was used in this study to block PTX-sensitive G-protein action in acutely dissociated dorsal raphe neurons. NEM is a sulfhydryl alkylating agent that can selectively inhibit PTX-sensitive G-protein-mediated effects in central (Han et al, 1999; Tang & Lovinger, 2000), peripheral (Shapiro et al, 1994) and invertebrate neurons (Fryer, 1992). In this study, the potentiation of GABA- and glycine- induced currents by propofol was not altered by NEM pretreatment lasting 2 min. These data suggest that the potentiating action of propofol on GABA- and glycine-evoked currents in dorsal raphe neurons does not involve G-proteins (Fig. 6).

In this study, the action of propofol on glutamate-elicited responses in dorsal raphe neurons was shown to differ from those described in reports. In cultured murine hippocampal neurons, it has been shown using the patch clamp technique that propofol inhibits the NMDA-sensitive subtype of glutamate receptors, probably through allosteric modulation of channel gating rather than through blocking of the open channel (Orser et al, 1995). In *Xenopus* oocytes propofol at clinical concentrations was shown to mildly suppress NMDA receptor channel activity (Yamakura et al, 1995). The experiments on rat dorsal

raphe neurons presented in this study showed that propofol potentiated the overall glutamate receptor response by about 10.59%; propofol potentiated NMDA receptor response by about 15.67%, while it exerted no effect on AMPA- and kainate-induced responses (Fig. 4). From these results, it can be suggested that propofol-mediated potentiation of glutamate-induced response is due to the modification of NMDA receptors, and that the propofol-mediated potentiation of NMDA-evoked currents dose not involve opioid receptors (Fig. 5) or G-proteins (Fig. 6). There are many controversies regarding propofol-induced neurotoxicity. Some reports have suggested that propofol prevents certain neuronal injuries (Hans et al, 1994; Lee & Cheun, 1999). Contradictory results have also been reported (Honegger & Matthieu, 1996; Zhu et al, 1997). Zhu et al (1997) investigated the effects of thiopental and propofol on NMDA- and AMPA-induced neuronal damage; while thiopental was shown to improve the recovery of population spikes after the administration of NMDA and AMPA, propofol worsened the recovery of population spikes following NMDA-induced damage and did not significantly alleviate AMPA-induced neuronal damage, and consequently it was concluded that propofol aggravates NMDA-induced neuronal damage. The results of the present study suggest the possibility that the potentiation of currents by propofol, which are elicited by NMDA-sensitive subtype of glutamate receptors in dorsal raphe neurons is one of mechanisms of propofol neurotoxicity.

In this study, the modulatory action of propofol on ion channels activated by inhibitory and excitatory neurotransmitters was examined, and it appears that this action is mainly responsible for the anesthetic action and/or adverse effects of propofol.

REFERENCES

Adam HK, Glen JB, Hoyle PA. Pharmacokinetics in laboratory animals of ICI 35 868, a new i.v. anaesthetic agent. *Br J Anaesth* 52: 743–746, 1980

Anker-Moller E, Spangsberg N, Arendt-Nielsen L, Schutz P, Kristensen MS, Bjerring P. Subhypnotic dose of thiopentone and propofol cause analgesia to experimentally induced acute pain. *Br J Anaesth* 66: 185–188, 1991

Azmitia EC, Segal M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *J Comp Neurol* 179: 641–667, 1978

Bai D, Pennefather PS, MacDonald JF, Orser BA. The general anesthetic propofol slows deactivation and desensitization of $GABA_A$ receptors. *J Neurosci* 19: 10635–10646, 1999

Bansinath M, Shukla VK, Turndorf H. Propofol modulates the effects of chemoconvulsants acting at GABA-ergic, glycinergic, and glutamate receptor subtypes. *Anesthesiology* 83: 809–815, 1995

Belelli D, Callachan H, Hill Venning C, Peters JA, Lambert JJ. Interaction of positive allosteric modulators with human and *Drosophila* recombinant GABA receptors expressed in *Xenopus laevis* oocytes. *Br J Pharmacol* 118: 563–576, 1996

Downie DL, Hall AC, Lieb WR, Franks NP. Effects of inhalational general anaesthetic on native glycine receptors in rat medullary neurones and recombinant glycine receptors in *Xenopus* oocytes. *Br J Pharmacol* 118: 493–502, 1996

Edwards M, Serrao JM, Gent JP, Goodchild CS. On the mechanism by which midazolam causes spinally mediated analgesia. *Anesthesiology* 73: 273–277, 1990

Flood P, Ramirez-Latorre J, Role L. $\alpha 4\beta 2$ neuronal nicotinic acetylcholine receptors in the central nervous system are inhibited by isoflurane and propofol, but α 7-type nicotinic acetylcholine receptors are unaffected. *Anesthesiology* 86: 859–865, 1997

Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. *Nature* 367: 607–614, 1994

Fryer MW. An *N*-ethylmaleimide-sensitive G-protein modulates aplysia Ca^{2+} channels. *Neurosci Lett* 146: 84–86, 1992

Hales TG, Lambert JJ. The actions of propofol on inhibitory amino acid receptors of bovine adrenomedullary chromaffin cells and rodent central neurones. *Br J Pharmacol* 104: 619–628, 1991

Han SH, Cho YW, Kim CJ, Min BI, Rhee JS, Akaike N. μ-opioid agonist-induced activation of G-protein-coupled inwardly rectifying potassium current in rat periaqueductal gray neurons. *Neuroscience* 90: 209–219, 1999

Hans P, Bonhomme V, Collette J, Albert A, Moonen G. Propofol protects cultured rat hippocampal neurons against N-methyl-D-aspartate receptor-mediated glutamate toxicity. *J Neurosurg Anesthesiol* 6: 249–253, 1994

Hara M, Kai Y, Ikemoto Y. Propofol activates $GABA_A$ receptor-chloride ionophore complex in dissociated hippocampal pyramidal neurons of the rat. *Anesthesiology* 79: 781–788, 1993

Harrison NL, Kugler JL, Jones MV, Greenblatt EP, Pritchett DB. Positive modulation of human γ-aminobutyric acid type A and glycine receptors by the inhalation anesthetic isoflurane. *Mol Pharmacol* 44: 628–632,

1993

Hille B. Modulation of ion-channel function by Gprotein-coupled receptors. *Trends Neurosci* 17: 531−536, 1994

Honegger P, Matthieu JM. Selective toxicity of the general anesthetic propofol for GABAergic neurons in rat brain cell cultures. *J Neurosci Res* 45: 631−636, 1996

Kim CJ, Rhee JS, Akaike N. Modulation of high-voltage activated Ca^{2+} channels in the rat periaqueductal gray neurons by μ-type opioid agonist. *J Neurophysiol* 77: 1418−1424, 1997

Lee SR, Cheun JK. Propofol administration reduces hippocampal neuronal damage induced by kainic acid in rats. *Neurol Res* 21: 225−228, 1999

Macdonald RL, Olsen RW. $GABA_A$ receptor channels. *Annu Rev Neurosci* 17: 569−602, 1994

Nadeson R, Goodchild CS. Antinociceptive properties of propofol: Involvement of spinal cord γ-amino $acid_A$ receptors. *J Pharmacol Exp Ther* 283: 81−186, 1997

Orser BA, Bertlik M, Wang LY, MacDonald JF. Inhibition by propofol (2,6 di-isopropylphenol) of the *N*-methyl-D-aspartate subtype of glutamate receptor in cultured hippocampal neurones. *Br J Pharmacol* 116: 1761−1768, 1995

Ortells MO, Lunt GG. Evolutionary history of the ligand-gated ion-channel superfamily of receptors. *Trends Neurosci* 18: 121−127, 1995

Pistis M, Belelli D, Peters JA, Lambert JJ. The interaction of general anaesthetics with recombinant $GABA_A$ and glycine receptors expressed in *Xenopus laevis* oocytes: a comparative study. *Br J Pharmacol* 122: 1707−1719, 1997

Ratnakumari L, Hemmings HC Jr. Effects of propofol on sodium channel-dependent sodium influx and glutamate release in rat cerebrocortical synaptosomes. *Anesthesiology* 86: 428−439, 1997

Rehberg B, Duch DS. Suppression of central nervous system sodium channels by propofol. *Anesthesiology* 91: 512−520, 1999

Rothlin CV, Katz E, Verbitsky M, Elgoyhen AB. The α9 nicotinic acetylcholine receptor shares pharmacological properties with type A γ-aminobutyric acid, glycine, and type 3 serotonin receptors. *Mol Pharmacol* 55: 248−254, 1999

Sanna E, Garau F, Harris RA. Novel properties of homomeric 1 γ-aminobutyric acid type A receptors: actions of the anesthetics propofol and pentobarbital. *Mol Pharmacol* 47: 213−227, 1995

Shapiro MS, Wollmuth LP, Hille B. Modulation of Ca^{2+} channels by PTX-sensitive G-proteins is blocked by *N*-ethylmaleimide in rat sympathetic neurons. *J Neurosci* 14: 7109−7116, 1994

Tanelian DL, Kosek P, Mody I, Maclver MB. The role of the $GABA_A$ receptor/chloride channel complex in anesthesia. *Anesthesiology* 78: 757−776, 1993

Tang KC, Lovinger DM. Role of pertussis toxin-sensitive G-proteins in synaptic transmission and plasticity at corticostriatal synapses. *J Neurophysiol* 83: 60−69, 2000

Todorovic SM, Lingle CJ. Pharmacological properties of T-type Ca^{2+} current in adult rat sensory neurons: Effects of anticonvulsant and anesthetic agents. *J Neurophysiol* 79: 240−252, 1998

Ueda H, Misawa H, Katada T, Ui M, Takagi H, Satoh M. Functional reconstruction of purified Gi and Go with μ-opioid receptors in guinea-pig striatal membranes pretreated with micromolar concentrations of *N*-ethylmaleimide. *J Neurochem* 54: 841−848, 1990

Violet JM, Downie DL, Nakisa RC, Lieb WR, Franks NP. Differential sensitivities of mammalian neuronal and musle nicotinic acetylcholine receptors to general anesthetics. *Anesthesiology* 86: 866−874, 1997

Vuyk J, Engbers FH, Lemmens HJ, Burm AG, Vletter AA, Glandines MP, Bovill JG. Pharmacodynamics of propofol in female patients. *Anesthesiology* 77: 3−9, 1992

Wu MH, Su MJ, Sun SM. Comparative direct electrophysiological effects of propofol on the conduction system and ionic channels of rabbit hearts. *Br J Pharmacol* 121: 617−624, 1997

Yamakura T, Sakimura K, Shimoji K, Mishina M. Effects of propofol on various AMPA-, kainate and NMDA-selective glutamate receptor channels expressed in *Xenopus* oocytes. *Neurosci Lett* 188: 187−190, 1995

Zhu H, Cottrell JE, Kass IS. The effect of thiopental and propofol on NMDA- and AMPA-mediated glutamate excitotoxicity. *Anesthesiology* 87: 944−951, 1997