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Actions of the General Anesthetic Propofol on Recombinant Human $GABA_A$ Receptors: Influence of Receptor Subunits[1]

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ABSTRACT

The intravenous general anesthetic 2,6-diisopropylphenol (propofol) potentiates $GABA_A$ receptor function, but the precise mechanisms and specificity of this action are still unclear. To study the influence of receptor subunit composition on the action of propofol, 18 different combinations of cloned cDNAs coding for human brain subunit isoforms of the $GABA_A$ receptor, as well as mRNAs from mouse brain, were expressed in *Xenopus* oocytes, and effects of this anesthetic were investigated by the voltage-clamp technique. We found that low concentrations (1–10 μM) of propofol dramatically potentiated GABA-evoked Cl^- currents in all $GABA_A$ receptor constructs tested. This action did not require specific subunits but was correlated inversely with the GABA sensitivity of each receptor construct. Larger concentrations (10–25 μM) of propofol produced direct activation of Cl^- currents, and this action was dependent on the expression of the β-subunit of the $GABA_A$ receptor and did not correlate with the GABA sensitivity of the receptors. These results suggest that propofol exerts a dual effect on $GABA_A$ receptors: a positive modulation of the GABA-mediated action on $GABA_A$ receptors that is not influenced by the receptor subunit composition, and a specific interaction with the β-subunit that directly activates the $GABA_A$ receptor-coupled Cl^- channel.

Propofol (2,6-diisopropylphenol) is a widely used injectable anesthetic agent that is structurally unrelated to other anesthetics. It has a wide range of clinical indications including induction and maintenance of anesthesia (Langley and Heel, 1988; Sebel and Lowdon, 1989). Propofol exhibits a rapid onset and a short duration of anesthesia, and the incidence of excitatory phenomena as well as nausea and emesis during recovery from anesthesia is generally low (Green and Jonsson, 1993; Gunawardene and White, 1988; McCollum *et al.*, 1987).

Although there is considerable information about the pharmacokinetic and pharmacodynamic properties of propofol (Cockshott, 1985; Dundee and Clarke, 1989; Langley and Heel, 1988), less is known about its neurochemical mechanism of action. A number of studies, however, have indicated that γ-aminobutyric $acid_A$ ($GABA_A$) receptors represent a sensitive target for the action of this intravenous anesthetic (Collins, 1988; Concas *et al.*, 1991; Hales and Lambert, 1991; Lin *et al.*, 1992) as well as other intravenous and volatile anesthetics (Franks and Lieb, 1994). In electrophysiological studies, propofol has been shown to enhance GABA-mediated inhibition in rat olfactory cortex slices, in the hippocampal dentate gyrus of urethane-anesthetized rats and in the rat substantia nigra (Albertson *et al.*, 1991; Collins, 1988; Peduto *et al.*, 1991). Moreover, propofol potentiated, in a reversible and concentration-dependent manner, whole cell currents elicited by GABA in bovine chromaffin cells and rat central neurons (Hales and Lambert, 1988, 1991). Subsequent biochemical studies carried out by Concas *et al.* (1990, 1991) demonstrated that propofol mimicked other general anesthetics such as pentobarbital and alphaxalone by enhancing the function of the $GABA_A$ receptor complex in different areas of the rat brain. In addition, the same authors observed that propofol, in the presence of either alphaxalone, pentobarbital or diazepam, induced an additive inhibition of ^{35}S-TBPS binding as well as an additive enhancement of ^{3}H-GABA binding and muscimol-stimulated $^{36}Cl^-$ uptake measured in brain membrane preparations, suggesting that the action of propofol may occur at a site different from that of other GABAergic modulators (Concas *et al.*, 1991). Supporting this hypothesis, Concas *et al.* (1992) used radiolabeled propofol to detect a specific ^{3}H-propofol recognition site in rat brain, suggesting that this anesthetic might bind directly to a site associated with ion channel proteins (Concas

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ABBREVIATION: GABA, γ-aminobutyric acid.

et al., 1992). However, whether the specific site of action of propofol on $GABA_A$ receptors is the receptor subunit proteins, other ion channel regulatory proteins, the protein-lipid interface or the lipid bilayer remains to be defined.

Recent molecular cloning and sequencing of multiple subunits of the $GABA_A$ receptor indicated that the $GABA_A$ receptor is a heterooligomeric ligand-gated chloride channel consisting of α-, β-, γ-, and δ-subunits (DeLorey and Olsen, 1992; Dunn *et al.*, 1994). The wide number of variants of each subunit allows for a great variety of different types of $GABA_A$ receptors that will likely differ in drug sensitivity as well as brain regional localization and developmental profile. Whereas anesthetic GABAergic modulators such as pentobarbital, 3α-OH-DHP and enflurane are able to potentiate $GABA_A$ receptors made of only α- and β-subunits (Lin *et al.*, 1993; Puia *et al.*, 1990; Sigel *et al.*, 1990), benzodiazepines require the coexpression of the γ_2-subunit for the potentiation of GABA function (Pritchett *et al.*, 1989). In addition, benzodiazepine pharmacology at $GABA_A$ receptors is also determined by the α-subunit isoform (α_{1-6}) (Hadingham *et al.*, 1993; Luddens *et al.*, 1990; Pritchett *et al.*, 1989).

The aim of this study was to investigate the role of different $GABA_A$ receptor subunits in the modulatory action of propofol on $GABA_A$ receptors. In addition, propofol also activates $GABA_A$ receptors in the absence of GABA (Hales and Lambert, 1988, 1991; Hara *et al.*, 1993), and we studied the influence of the subunit composition of the $GABA_A$ receptor on the direct effect of this anesthetic. To address these questions, we used the *Xenopus* oocyte expression system. A preliminary report of this work was published in abstract form (Sanna *et al.*, 1993).

Materials and Methods

Materials. Adult *Xenopus laevis* female frogs were purchased from Xenopus I (Ann Arbor, MI). Plasmid purification kits were obtained from Qiagen, Inc. (Chatsworth, CA). Propofol (2,6-diisopropylphenol) was purchased from Aldrich Chemical Company Inc. (Milwaukee, WI) and subsequently purified by distillation. GABA (γ-aminobutyric acid) and bicuculline methiodide were from Research Biochemicals Inc. (Natick, MA). Diazepam and flumazenil (Ro15–1788; ethyl-8-fluoro-5,6,-dihydro-5-methyl-6-oxo-4H-imidazo(1,5a)(1,4)benzodiazepine-3-carboxylate) were kindly provided by Hoffman-La Roche (Basel, Switzerland). All other reagents used were of analytical grade and were obtained from Sigma Chemical Company (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). After distillation, propofol was stored in small aliquots at −20°C under argon and dissolved daily using dimethyl sulfoxide (DMSO, final concentration in Modified Barth's saline was 0.01–0.1%). Determination of propofol concentrations in the recording chamber was carried out as described by Lin *et al.* (1993).

Preparation of cDNAs. cDNAs for the human α_1, α_2, α_5, β_1, γ_{2S}, γ_{2L}, and the rat β_2 $GABA_A$ receptor subunits were subcloned into the pCDM8 expression vector (Invitrogen, San Diego, CA) (Hadingham *et al.*, 1993). cDNAs were purified by CsCl gradient centrifugation or the Qiagen plasmid kit. The cDNAs were then resuspended in diethyl pyrocarbonate-treated water, aliquoted, and stored at −20°C until used for injection.

Isolation of mouse cortical messenger RNA. Poly(A)+ mRNA was isolated from adult (6–8 weeks) male C57BL/6 mouse brains with the Fast Track mRNA isolation kit (Invitrogen, San Diego, CA), then purified by phenol/chloroform extraction and subsequently precipitated with 0.15 volumes of 2 M sodium acetate and 2 volumes of absolute ethanol and stored at −80°C. The concentration of extracted poly(A)+ mRNA was determined spectrophotometrically (Dildy-Mayfield and Harris, 1992).

Isolation of *Xenopus* oocytes and microinjection of cDNAs and poly(A)+ mRNA. Stage V and VI oocytes were isolated from a section of ovary and exposed to collagenase Type IA (Sigma) as described elsewhere (Dildy-Mayfield, 1992). A mixture of $\alpha\beta$ or $\alpha\beta\gamma$ cDNAs (1.5 ng/30 nl) was injected into the oocyte nucleus using a 10-μl glass micropipette (10–15 μm tip diameter). Poly(A)+ mRNA was reconstituted in diethyl pyrocarbonate-treated water, and each oocyte was injected with 100 to 150 ng of mRNA/50 nl using a sterile glass pipette (tip diameter of 20–25 μm). The injected oocytes were incubated in Modified Barth's saline (MBS) (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 10 mM HEPES, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.91 mM $CaCl_2$, pH 7.5) supplemented with 2 mM sodium pyruvate, penicillin (10,000 U/l), streptomycin (10 U/l), gentamycin (50 U/l) and 0.5 mM theophylline, at 16–19°C until used. Oocytes were usually kept up to 4 days, during which they were transferred to fresh incubation medium once a day.

Electrophysiological recording. Electrophysiological recording began approximately 24 hr after cDNA injection or 2 to 3 days after mRNA injection and was carried out as described by Sanna *et al.* (1994). In brief, oocytes were placed in a rectangular recording chamber (100 μl volume) and continuously perfused with MBS solution at a flow rate of 2 ml/min at room temperature. Oocytes were impaled in the animal pole with two microelectrodes (1–5 MΩ) filled with filtered 3 M KCl and voltage-clamped at −70 mV using an Axoclamp IIA amplifier (Axon Instruments, Inc., Burlingame, CA). Resting membrane potentials usually ranged between −30 and −60 mV. The drugs were perfused for 20 sec unless otherwise noted (7–10 sec were required to reach equilibrium in the recording chamber). Five-minute intervals were allowed between applications of low concentrations of GABA alone and at least 10 min when GABA was applied at higher concentrations or with other drugs.

Statistical analysis. Data were expressed as percentage of control current responses (nA) obtained with GABA alone. A GABA control response was obtained before and after each drug application to take into account possible shifts in the control currents. Each oocyte represents a single "*n*," and oocytes from at least two different frogs were used for each experiment. One-way analysis of variance (ANOVA), followed by Fisher's LSD *post hoc* test, was used for determination of statistical significance. Linear regression (GraphPad software) was used for the correlational analysis.

Results

Direct actions of propofol on the function of $GABA_A$ receptors. In agreement with other results (Hales and Lambert, 1988, 1991; Hara *et al.*, 1993), bath perfusion of propofol (1–25 μM) induced inward currents in oocytes expressing $GABA_A$ receptors. These currents are $GABA_A$ receptor-mediated Cl^- currents because they were almost completely abolished by voltage-clamping the oocytes at −20 mV, *i.e.*, near the reversal potential for Cl^- in oocytes (Kusano *et al.*, 1982), and were not observed in uninjected oocytes (results not shown). In addition, intracellular injection of the calcium chelator EGTA (~0.2 mM final concentration) failed to affect propofol-induced currents, suggesting that they were not Ca^{++}-activated Cl^- currents (results not shown).

Oocytes expressing $alpha_1$ $beta_1$ $gamma_{2S}$ receptors were much more sensitive to direct effects of propofol than oocytes expressing $alpha_1$ $beta_1$ receptors (fig. 1). Expression of receptors containing the γ_{2S} subunit (fig. 1, B and C) produced receptors that gave appreciable Cl^- currents with propofol concentrations of 5 to 25 μM (~37% of the control 5 μM GABA response with 25 μM propofol), but $alpha_1$ $beta_1$ receptors gave much smaller responses (less than 5% of the

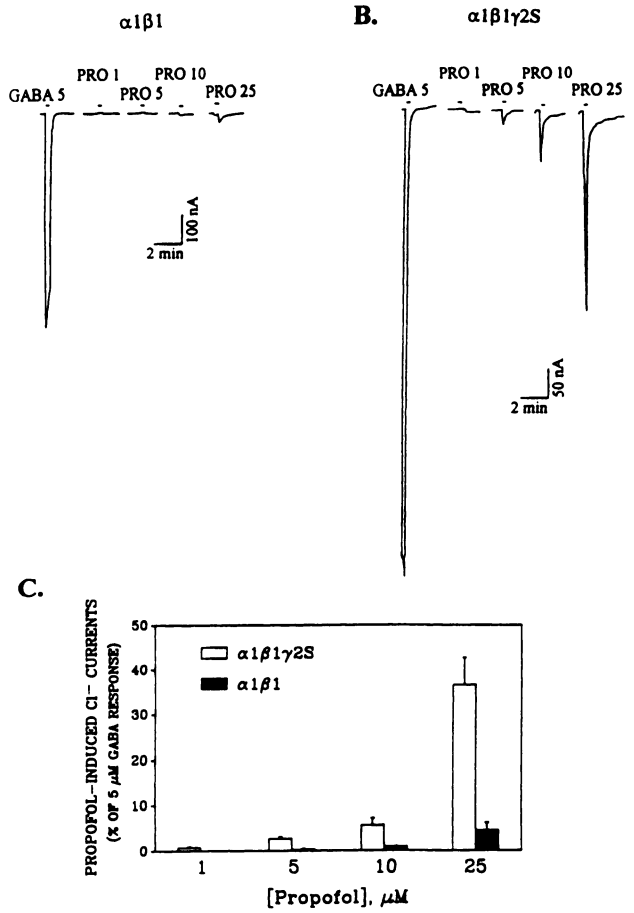

Fig. 1. Direct activation of $GABA_A$ receptor-mediated Cl^- currents by propofol. Tracings obtained from single oocytes expressing *alpha*$_1$ *beta*$_1$ (A) or *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ (B) receptors showing Cl^- currents produced by the perfusion of propofol (1–25 μM) in the absence of GABA, compared with the response to 5 μM GABA. Horizontal bars above each response indicate the drug application. Drugs were perfused for 20 sec. In C, values represent the currents induced by propofol and are expressed as mean percent of the control response with 5 μM GABA ± S.E.M. of 8 to 12 different oocytes.

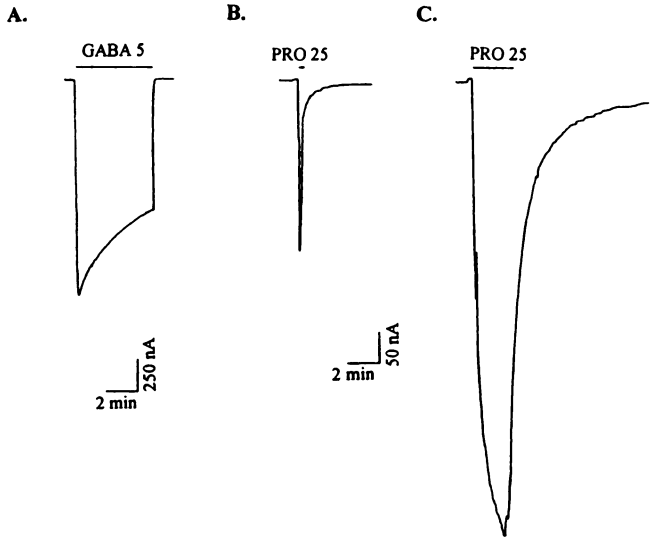

Fig. 2. $GABA_A$ receptor-mediated Cl^- currents induced by GABA or propofol show differential desensitization patterns. Tracings obtained from single oocytes expressing *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ receptors showing Cl^- currents induced by a 5-min perfusion of 5 μM GABA (A), a 20-sec (B) or a 2.5-min (C) perfusion of 25 μM propofol in the absence of GABA. Similar results were obtained in five other oocytes.

control 5 μM GABA response with 25 μM propofol) (fig. 1, A and C).

Activation of Cl^- currents by propofol showed a pattern that was different from the activation of these currents by GABA. As illustrated by the tracings in figure 2, prolonged (5 min) perfusion of 5 μM GABA evoked Cl^- currents that showed rapid desensitization (about 60% of the initial current amplitude was left by 5 min of GABA perfusion) (fig. 2A), but the amplitude of the current evoked by 25 μM propofol increased as a function of the length of drug perfusion and showed little desensitization, as indicated by comparing the currents produced by a 20-sec (fig. 2B) *versus* a 2.5-min (fig. 2C) propofol application. Thus, the results in figure 1 (obtained with a 20-sec application of propofol) underestimate the effects of propofol.

These propofol-induced currents, measured in oocytes expressing *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ receptors, were due to a direct activation of $GABA_A$ receptors because they were inhibited by both the $GABA_A$ receptor antagonist bicuculline (10 μM) and the $GABA_A$ receptor/channel blocker picrotoxin (25 μM); however, these currents were not altered by the benzodiazepine receptor antagonist Ro 15–1788 (1 μM) (fig. 3). In addition, propofol-evoked Cl^- currents were potentiated (~75%) by 300 nM diazepam (fig. 3, A and C). As expected, diazepam failed to increase propofol-mediated currents in oocytes expressing *alpha*$_1$ *beta*$_1$ receptors (result not shown).

We further investigated the receptor subunit influence on the direct action of this anesthetic by expressing different $GABA_A$ receptor subunit combinations. To compare propofol actions on subunit combinations that vary in receptor density (*e.g.*, degree of expression) as well as GABA sensitivity, effects of propofol were normalized relative to actions of a submaximal or maximal concentration of GABA. Table 1 illustrates the currents induced by 25 μM propofol, expressed as a percentage of the response to 5 μM GABA or a maximally effective GABA concentration, in 18 different $GABA_A$ receptor constructs. From these data it is clear that homomeric *beta*$_1$ receptors were the most sensitive to activation by propofol; "dilution" of the β_1-subunit with either α or γ_2 markedly reduced this action of propofol. In fact, the Cl^- currents evoked by 25 μM propofol were about 10-fold greater than those evoked by 5 μM GABA and about 140% of those induced by a maximal concentration (10 mM) of GABA (table 1 and fig. 4C). In addition, we tried to express monomeric *beta*$_2$ receptors but failed to observe an appreciable GABA response, even with very high GABA concentrations. However, in a few oocytes we could measure very small inward currents (in the range of 5–10 nA) with 10 mM GABA; in these few cells, perfusion of 25 μM propofol induced currents of similar amplitude to those induced by maximal concentration of GABA (results not shown). The importance of the β-subunit is also emphasized by the observation that the only subunit combination not affected by propofol (but sensitive to GABA) was one lacking β_1 (the $\alpha_1\gamma_{2S}$) (fig. 4, table 1). There were no consistent differences between the three α-subunits tested or the two β-subunits (table 1). When the actions of propofol are expressed as a percentage of the re-

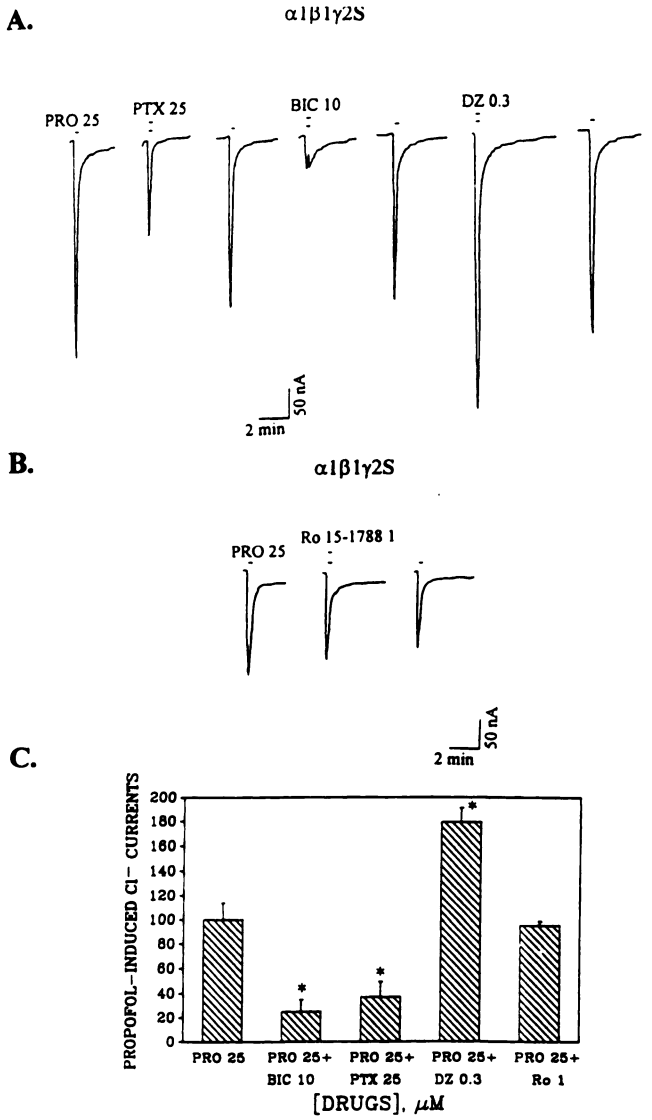

Fig. 3. $GABA_A$ receptor-mediated Cl^- currents evoked by propofol are altered by positive and negative modulators of GABAergic function. Tracings in A and B were obtained from single oocytes expressing *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ receptors and show the effects of 25 μM picrotoxin (PTX), 10 μM bicuculline (BIC), 0.3 μM diazepam (DZ) and 1 μM Ro 15–1788 on the Cl^- currents elicited by 25 μM propofol in the absence of GABA. Values in C are expressed as a percentage of the control propofol response ± S.E.M. of 8 to 12 different oocytes. * $P < .01$ *vs.* control response (ANOVA and Fisher's LSD *post hoc* test).

sponse to 5 μM GABA, addition of a γ_2-subunit to an *alpha beta* receptor increases the effect of propofol by 2- to 9-fold. However, most of this increase is because a γ_2-subunit reduces the sensitivity of the receptor to GABA (discussed below), and the γ_2-subunit has no consistent effect if the data are expressed in terms of the maximal response to GABA (tables 1 and 2).

Propofol enhancement of GABA action. Propofol (1–25 μM) potentiated Cl^- currents evoked by 5 μM GABA in oocytes expressing *alpha*$_1$ *beta*$_1$ or *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ $GABA_A$ receptors. This effect was concentration-dependent and reversible when propofol was washed out from the perfusion bath (fig. 5, A and B). When 5 μM GABA was used to activate the receptor, propofol potentiation of GABA responses was greater when measured in oocytes expressing

TABLE 1

Propofol Directly Activates Cl^- Currents in *Xenopus* Oocytes Expressing Different Subunits of the $GABA_A$ Receptor

Receptor Composition	Current Induced by 25 μM Propofol (% 5 μM GABA response)	% of Maximal GABA Response
$\alpha_1\beta_1$ (12)	4.6 ± 1.6	2.2 ± 0.8
$\alpha_1\beta_1\gamma_{2S}$ (14)	36.6 ± 6.1	7.1 ± 1.2
$\alpha_1\beta_1\gamma_{2L}$ (9)	29.4 ± 8.5	1.0 ± 0.3
$\alpha_1\beta_2$ (5)	7.2 ± 2.0	2.0 ± 0.6
$\alpha_1\beta_2\gamma_{2S}$ (4)	31.4 ± 15.7	1.9 ± 0.9
$\alpha_2\beta_1$ (8)	27.9 ± 3.9	7.4 ± 1.0
$\alpha_2\beta_1\gamma_{2S}$ (14)	46.4 ± 9.7	2.9 ± 0.6
$\alpha_2\beta_1\gamma_{2L}$ (4)	87.0 ± 22.6	6.5 ± 1.7
$\alpha_2\beta_2$ (9)	59.1 ± 6.9	19.5 ± 2.3
$\alpha_2\beta_2\gamma_{2S}$ (13)	352 ± 54	4.9 ± 0.7
$\alpha_2\beta_2\gamma_{2L}$ (7)	560 ± 127	14.0 ± 3.2
$\alpha_5\beta_1$ (7)	8.0 ± 2.7	2.6 ± 0.9
$\alpha_5\beta_1\gamma_{2S}$ (19)	29.1 ± 7.0	2.5 ± 0.6
$\alpha_5\beta_1\gamma_{2L}$ (9)	29.3 ± 2.7	2.3 ± 0.2
$\alpha_1\gamma_{2S}$ (7)	N.D.	N.D.
β_1 (13)	955 ± 138	134 ± 19
$\beta_1\gamma_{2S}$ (5)	150 ± 13	25 ± 2
$\beta_1\gamma_{2L}$ (7)	118 ± 12	8.3 ± 0.8

Values represent the mean ± S.E.M. Numbers in parentheses represent the number of oocytes tested. N.D., no detectable current induced with propofol. Values for percent of maximal response were calculated from the data for 5 μM GABA from this table and table 2.

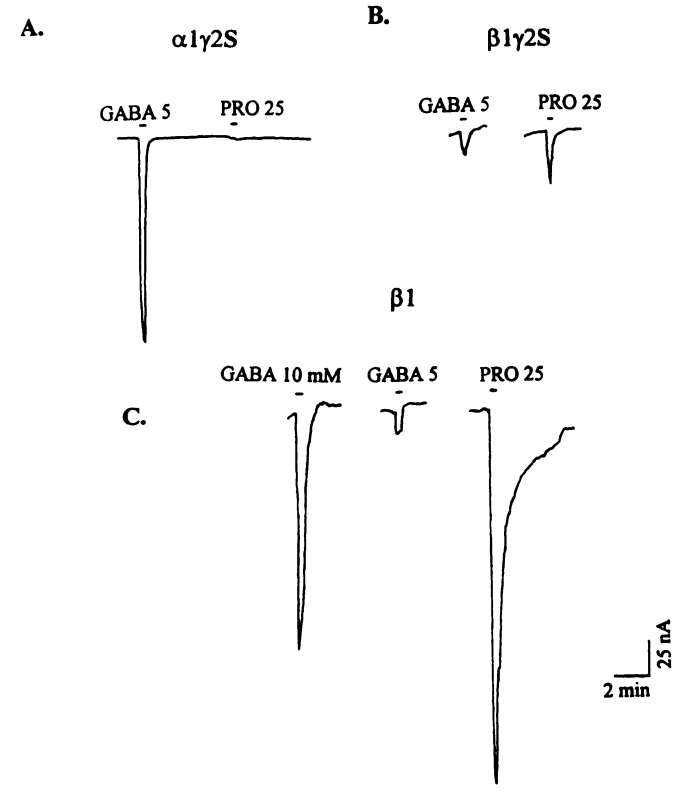

Fig. 4. The sensitivity of $GABA_A$ receptors to the direct activation by propofol depends on the expression of the β-subunit. The tracings show the Cl^- currents induced by GABA (5 μM in A and B, or 5 μM and 10 mM in C) or propofol (25 μM) measured in single oocytes expressing *alpha*$_1$ *gamma*$_{2S}$ (A), *beta*$_1$ *gamma*$_{2S}$ (B), or *beta*$_1$ (C) receptors. These are representative of similar results obtained in 5 to 13 different oocytes.

alpha$_1$ *beta*$_1$ *gamma*$_{2S}$ receptors compared with those receptors without the a γ_2-subunit. With *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ receptors, the highest concentration (25 μM) of propofol

TABLE 2

Potentiation by Propofol of GABA-evoked Cl^- Currents in *Xenopus* Oocytes Expressing Different Subunits of the $GABA_A$ Receptor

Receptor Composition	Propofol, 5 μM (% potentiation)	Propofol, -fold increase by γ_2	GABA, 5 μM (% maximum GABA)
$\alpha_1\beta_1$ (12)	195 ± 9		48.3 ± 7.4
$\alpha_1\beta_1\gamma_{2S}$ (14)	338 ± 64	1.7	19.4 ± 6.1
$\alpha_1\beta_1\gamma_{2L}$ (9)	318 ± 41	1.6	3.3 ± 1.1
$\alpha_1\beta_2$ (5)	211 ± 57		27.8 ± 5.2
$\alpha_1\beta_2\gamma_{2S}$ (4)	382 ± 83	1.8	6.2 ± 1.7
$\alpha_2\beta_1$ (8)	239 ± 58		26.5 ± 3.3
$\alpha_2\beta_1\gamma_{2S}$ (14)	342 ± 58	1.4	6.3 ± 2.6
$\alpha_2\beta_1\gamma_{2L}$ (4)	333 ± 132	1.4	7.5 ± 3.6
$\alpha_2\beta_2$ (9)	246 ± 33		33.0 ± 4.4
$\alpha_2\beta_2\gamma_{2S}$ (13)	585 ± 74	2.4	1.4 ± 0.8
$\alpha_2\beta_2\gamma_{2L}$ (7)	606 ± 104	2.5	2.5 ± 1.1
$\alpha_5\beta_1$ (7)	137 ± 41		33.2 ± 3.9
$\alpha_5\beta_1\gamma_{2S}$ (19)	307 ± 26	2.2	8.6 ± 2.3
$\alpha_5\beta_1\gamma_{2L}$ (9)	339 ± 29	2.5	7.8 ± 2.8
$\alpha_1\gamma_{2S}$ (7)	521 ± 128		6.9 ± 1.9
β_1 (13)	482 ± 72		14.0 ± 1.5
$\beta_1\gamma_{2S}$ (5)	194 ± 18	0.4	16.7 ± 7.3
$\beta_1\gamma_{2L}$ (7)	342 ± 8	0.7	7.0 ± 1.0

Values represent the mean ± S.E.M. Numbers in parentheses represent the number of oocytes tested. Values for percent of maximal response were calculated from the data for 5 μM GABA from this table and table 1.

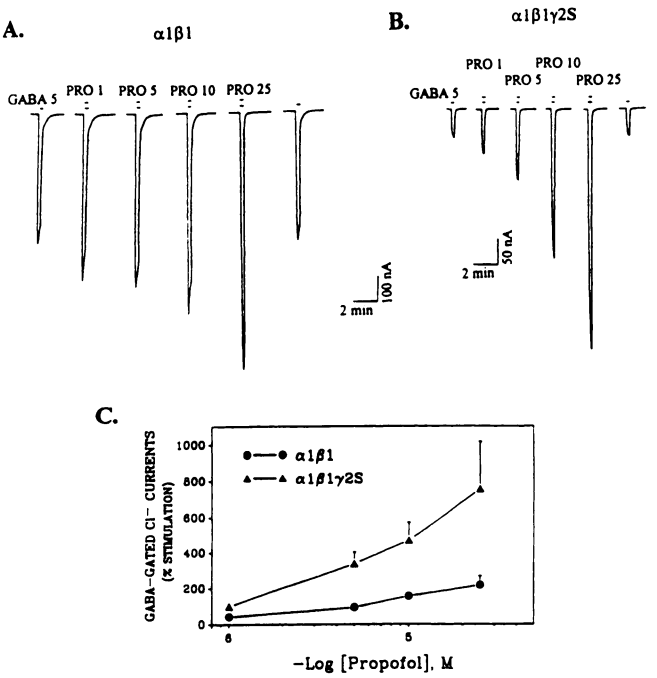

Fig. 5. Effect of propofol on GABA-evoked Cl^- currents in oocytes expressing $GABA_A$ receptors with and without the γ_{2S}-subunit. Tracings were obtained from single oocytes expressing *alpha*$_1$ *beta*$_1$ (A) or *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ (B) $GABA_A$ receptors, and illustrate the potentiation of 5 μM GABA response by 1 to 25 μM propofol. Values in C represent the mean percent potentiation ± S.E.M. of 12 to 14 different oocytes.

tested enhanced 5 μM GABA-mediated Cl^- currents by about 750%, but the responses of GABA on *alpha*$_1$ *beta*$_1$ receptors were potentiated by only about 200% (fig. 5). There was no consistent effect of varying the α-subunit (α_1 *vs.* α_2 *vs.* α_5) or β (β_1 *vs.* β_2). Because prolonged application of propofol enhanced the direct action of this drug, we asked if preapplication would also enhance propofol potentiation of GABA action. We found that preapplication of propofol (1 μM) for 3.5 min resulted in 80 ± 7% potentiation of GABA, whereas co-application of GABA and propofol for 20 sec produced 19 ± 2% potentiation (n = 3) (fig. 6). Thus, preapplication increased the action of propofol by about 4-fold; this action of propofol was readily reversed with washing (fig. 6). The data in fig. 5C underestimate the effects of propofol because they were obtained with a 20-sec application of the drug.

Propofol (5 μM) potentiation of GABA-evoked Cl^- currents in oocytes expressing $GABA_A$ receptors with or without the γ_{2S}-subunit was dependent on the concentration of GABA being greater at lower concentrations of the agonist and was reduced when the concentration of GABA was increased (fig. 7A). The response evoked by a very large concentration of GABA (1 mM) was inhibited by 5 μM propofol (fig. 8).

One important point when comparing the action of drugs on different $GABA_A$ receptors is that the sensitivity to GABA depends on the subunit composition of the receptors, and this can influence the magnitude of potentiation produced by anesthetics (Mihic *et al.*, 1994a,b). In agreement with others (Lin *et al.*, 1993), we found that *alpha*$_1$ *beta*$_1$ receptors display a lower EC_{50} value (~5 μM) as compared with *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ receptors (~20 μM) (fig. 7B). Thus, the fixed concentration (5 μM) of GABA used to test propofol's effects (see fig. 5) induced a response that was about EC_{48} and EC_{19} (see also table 2) for *alpha*$_1$ *beta*$_1$ and *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ receptors, respectively. To determine if the difference in sensitivity to GABA of the receptors with and without the γ_{2S}-subunit might account for the difference in the propofol's action, we replotted the data in fig. 7A as percent of propofol potentiation *versus* percent of maximal GABA response (produced by 1 mM GABA). We found that the potentiation

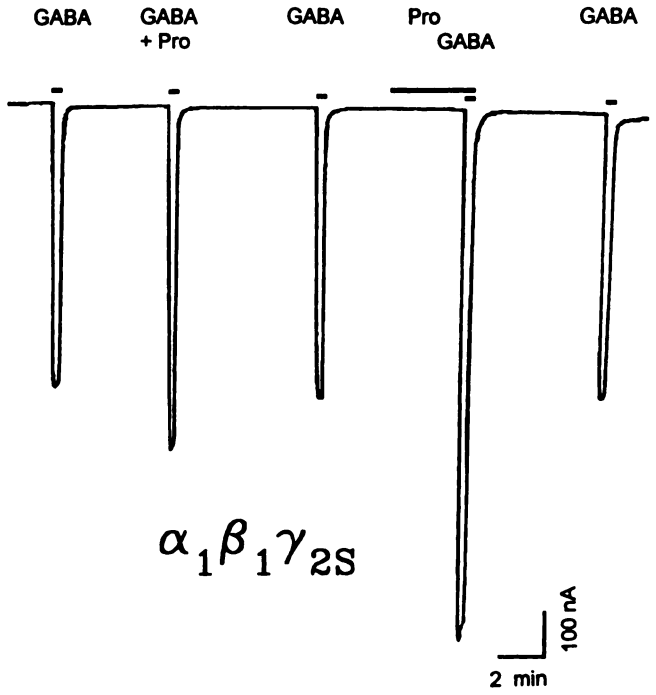

Fig. 6. Prolonged application of propofol enhances potentiation of GABA action. Propofol (1 μM) was applied for 20 sec to give the second response or 3.5 min to give the fourth response. GABA (1 μM) was applied for 20 sec in all cases. This experiment was repeated three times with similar results; mean values are given in the text.

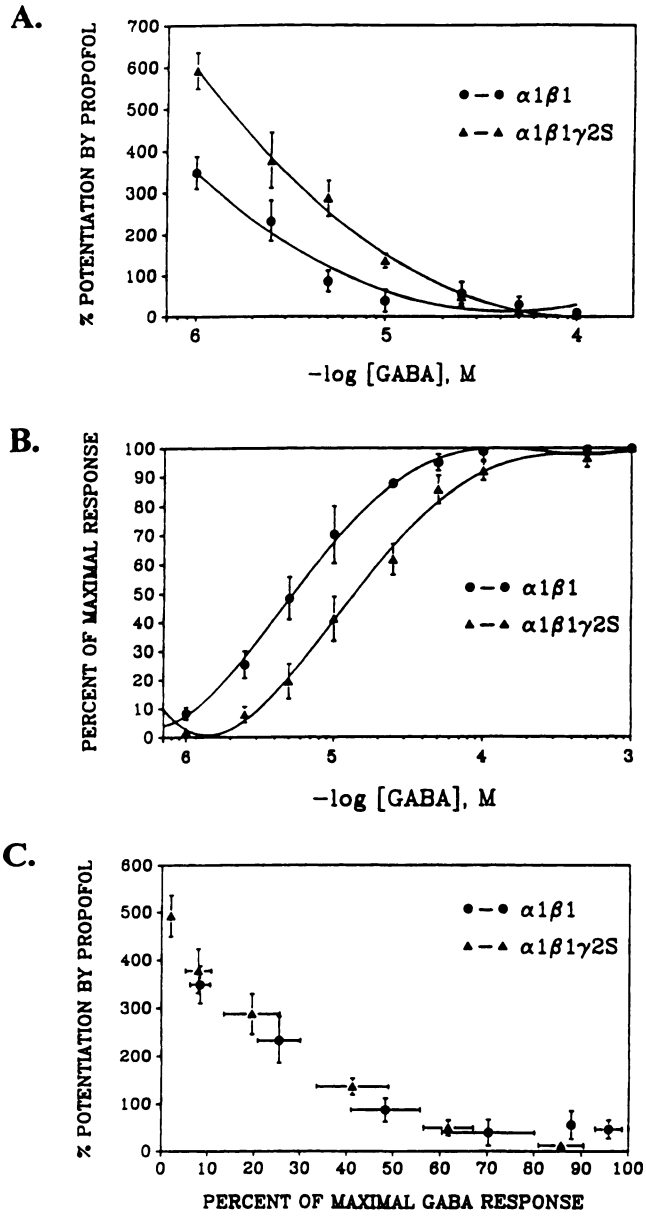

Fig. 7. Propofol potentiation of GABA-evoked Cl^- currents in *alpha*$_1$ *beta*$_1$ or *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ $GABA_A$ receptors depends upon the concentration of GABA. Points in A represent the percent potentiation of GABA responses by 5 μM propofol *versus* the absolute concentration of GABA (1–100 μM). B illustrates the concentration-response curves for GABA (1–1000 μM). In C, the values in the ordinate of the graph (A) were replotted as percent of propofol potentiation versus percent of maximal GABA response (1 mM). Values were obtained from seven to eight different oocytes.

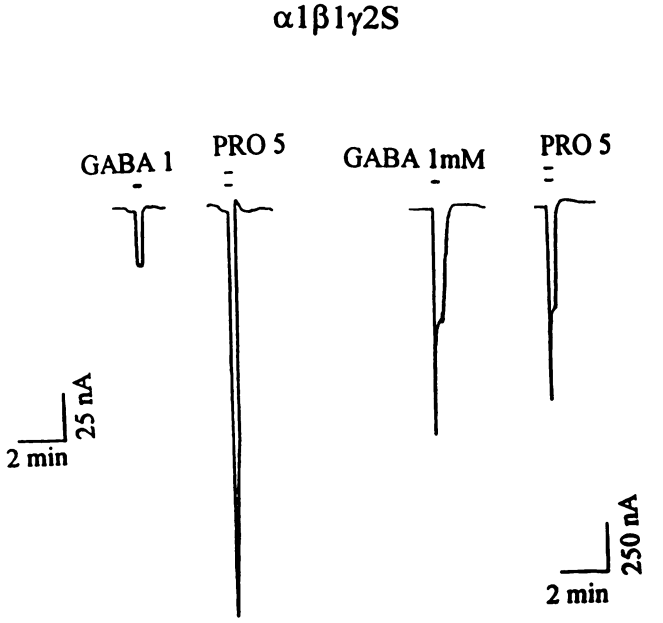

Fig. 8. Propofol inhibits the maximal GABA response. The tracings were obtained from a single oocyte expressing *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ $GABA_A$ receptors and show the effect of 5 μM propofol on Cl^- currents induced by 1 μM and 1 mM GABA, respectively. Similar results were obtained from 14 other oocytes.

induced by 5 μM propofol was similar in *alpha*$_1$ *beta*$_1$ and *alpha*$_1$ *beta*$_1$ *gamma*$_{2S}$ receptors, as indicated by the superimposed points in fig. 7C.

Another possible concern is that the direct effect of propofol contributes significantly to the potentiation of GABA action for some of the subunit combinations. However, 5 μM propofol produced little direct activation of Cl^- currents, and this direct effect did not contribute appreciably to the potentiation of GABA responses (tables 1 and 2). At this concentration, propofol was able to significantly enhance GABA responses in all receptors tested, even in *alpha*$_1$ *gamma*$_{2S}$ receptors that were found completely insensitive to the direct activation of this anesthetic (see table 1 and fig. 4A).

One question that arises is whether the direct effects and the potentiating action of propofol on $GABA_A$ receptor function are mediated by the same mechanism and whether either is related to the GABA sensitivity of the subunit combinations. To address these questions, we asked whether these actions covaried. The direct action of propofol (expressed relative to the maximal GABA response) was not correlated with the potentiating action of propofol or with GABA sensitivity (expressed as the action of 5 μM GABA relative to the maximal GABA effect). The potentiating action of propofol was correlated with GABA sensitivity (r = .71, $P < .001$). Thus, GABA sensitivity of the different receptors could account for about 50% of the variance in propofol potentiation, suggesting that other factors must also influence this action of propofol.

Effect of propofol on $GABA_A$ receptors expressed from mouse cortical mRNA. GABA responses were also measured in oocytes injected with mouse cortical mRNA. After determining the maximal current response by 10 mM GABA, the concentration of GABA that induced a response representing 20 ± 3% of the maximal response was assessed for each individual oocyte and subsequently used to test the effects of propofol. GABA responses were potentiated in a concentration-dependent manner by concentrations of propofol ranging from 1 to 25 μM (table 3). When compared with human recombinant $GABA_A$ receptors, those expressed from mouse cortical mRNA were generally less sensitive to the action of propofol.

Discussion

The molecular mechanism by which many general anesthetic agents exert their effects in the mammalian nervous

TABLE 3
Effect of Propofol on GABA-evoked Cl^- Currents in *Xenopus* Oocytes Expressing Mouse Cortical mRNA

Propofol (μM)	% Potentiation of EC_{20} GABA Response
1	30 ± 3
10	151 ± 16
25	228 ± 28

system is presently unknown, and the classic problem of "lipids or proteins as the target for anesthetics" is still an unsolved question. In recent years, however, it has become increasingly clear the important role played by $GABA_A$ receptors in mediating some of the effects induced by general anesthetics as well as depressant drugs such as benzodiazepines, barbiturates, steroids and ethanol (Franks and Lieb, 1994; Tanelian *et al.*, 1993). All these compounds have been shown to potentiate the action of GABA on $GABA_A$ receptors.

More recently, propofol, an intravenous agent chemically unrelated to other anesthetics, has been characterized as a potent and effective modulator of the GABAergic function in brain preparations and cultured cells (Albertson *et al.*, 1991; Concas *et al.*, 1990, 1991; Hales and Lambert, 1991). Propofol has two actions on the $GABA_A$ receptor: a direct activation of the receptor and potentiation of the actions of GABA (Hales and Lambert, 1991; Peduto *et al.*, 1991; Hara *et al.*, 1993). Comparing these two actions with 18 different combinations of receptor subunits provides new information about the subunit requirements for these actions. The direct action of propofol required a β-subunit, suggesting that this subunit contains a binding site for this drug (see also Sanna *et al.*, 1995). Addition of α-subunits to the β_1-subunit markedly reduced the ability of propofol to activate the receptor but enhanced the action of GABA. It is possible that the propofol site on the β_1-subunit is occluded by α-subunits or that the affinity of the propofol site is markedly reduced in the presence of α-subunits. In contrast to the subunit specificity for the direct actions of propofol, potentiation of GABA actions by propofol did not require a β-subunit and was observed with all subunit combinations tested. In addition, correlation analysis showed no relationship between the direct and potentiating actions of propofol. Thus, it is necessary to postulate two sites of action for propofol, one on the β-subunit that produces the direct action and a second that cannot be placed on any single subunit and is responsible for potentiation of GABA action. These two sites also differ in their sensitivity to propofol as the potentiating effect is clear with 1 to 5 μM propofol, but the direct action requires 10 to 25 μM. This distinction is especially explicit with heteromeric *alpha beta gamma*$_2$ receptors (as occur in neurons), where 5 μM propofol produced 300 to 600% potentiation of GABA but only weak direct effects. The potentiating action of propofol varied about 5-fold between the different subunits, with most of the variation due to enhanced action of propofol in the presence of a γ_2-subunit. However, these differences mainly reflect the GABA sensitivities of the receptors. The γ_2-subunits reduce the GABA sensitivity of the receptor making the test concentration of GABA (5 μM) further from a maximal concentration, thereby increasing the percent potentiation (Mihic *et al.*, 1994a,b).

The lack of subunit dependence for the potentiating action of propofol is different from the actions of benzodiazepines that require a γ-subunit but is similar to other anesthetic agents including pentobarbital, butanol and enflurane (Dunn *et al.*, 1994; Lin *et al.*, 1993; Mihic *et al.*, 1994a,b). The direct action of propofol is unusual in requiring a β-subunit. The only other drug reported to act on this subunit is the anticonvulsant loreclezole, which acts on receptors containing $\beta_{2/3}$-subunits, but not those containing β_1 (Wafford *et al.*, 1994; Wingrove *et al.*, 1994). However, our recent studies indicate that the direct actions of pentobarbital, but not those of alphaxalone, require a β-subunit (Sanna *et al.*, 1995).

It is of interest to consider the possible role of these actions of propofol in its clinical use as an anesthetic. The plasma concentration of propofol for anesthesia is estimated at 8 μM, but extensive protein binding leads to calculation of a free concentration of 0.4 μM (Franks and Lieb, 1994). These values were measured at 37°C, whereas our experiments were carried out at room temperature, but a range of 0.5 to 5 μM would seem to be reasonable pharmacologically. Heteromeric receptors and those expressed from brain mRNA are likely to be more relevant to neuronal receptors than homomeric receptors, and these receptors show potentiation of GABA action by propofol concentrations in the range of 1 to 5 μM but display little or no direct activation by these concentrations. Thus, potentiation of GABA responses, but not direct activation of the receptors, is likely important for the anesthetic actions of propofol. This is similar to most findings with volatile anesthetics (Tanelian *et al.*, 1993; Mihic *et al.*, 1994b).

In summary, direct activation of $GABA_A$ receptors by propofol is likely due to a site on the β-subunit, but the potentiation of GABA action occurs at a separate site that cannot be placed on any one subunit. It may be on a conserved region that exists on several different subunits or on another protein that regulates the function of the receptor complex.

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