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α Subunit Isoform Influences $GABA_A$ Receptor Modulation by Propofol

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Summary—We have investigated the role of the α subunit in the modulation of γ-aminobutyric acid type A ($GABA_A$) receptors by the general anesthetic propofol, using whole-cell patch clamp recordings made from distinct stable fibroblast cell lines which expressed only $\alpha_1\beta_3\gamma_2$ or $\alpha_6\beta_3\gamma_2$ $GABA_A$ receptors. At clinically relevant anesthetic concentrations, propofol potentiated submaximal GABA currents in $\alpha_1\beta_3\gamma_2$ receptors to a far greater degree than those in $\alpha_6\beta_3\gamma_2$ receptors. The α subunit influenced the efficacy of propofol for modulation, but not its potency. In contrast, direct gating of the ion channel by propofol, in the absence of GABA, was significantly larger in the α_6 than the α_1 containing receptors. The potentiation of submaximal GABA by trichloroethanol, and the potentiation and direct gating by methohexital was also studied, and showed the same relative trends as propofol. © 1997 Elsevier Science Ltd.

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$GABA_A$ (γ-aminobutyric acid type A) receptors are the major receptors for inhibitory neurotransmission in the mammalian brain. The $GABA_A$ receptor is a pentameric complex formed by different glycoprotein subunits (α_{1-6}, β_{1-4}, γ_{1-4}, δ) which combine to form a chloride channel (reviewed by Burt and Kamatchi, 1991; Olsen and Tobin, 1990; Rabow *et al.*, 1995). $GABA_A$ receptor subunit expression in the central nervous system is heterogeneous (Laurie *et al.*, 1992a,b; Wisden *et al.*, 1991). For example, the $GABA_A$ α_6 subunit isoform is localized solely in cerebellar granule cells, whereas α_1 is widely expressed throughout the brain (Lüddens *et al.*, 1990; Kato, 1990; Mertens *et al.*, 1993; Wisden *et al.*, 1992).

The $GABA_A$ receptor complex is modulated allosterically by a wide range of compounds which act at discrete but unknown sites on the receptor (Macdonald and Olsen, 1994). One major group of $GABA_A$ receptor modulators is the class of general anesthetics, many of which have been demonstrated to augment $GABA_A$ receptor chloride currents at clinically relevant concentrations (e.g. Zimmerman *et al.*, 1994), and are thought to elicit anesthesia by enhancing inhibitory synaptic transmission (Nicoll *et al.*, 1975; Gage and Robertson, 1985). General anesthetics known to potentiate the actions of GABA (γ-aminobutyric acid) include propofol [2,6-diisopropylphenol (PRO); Hales and Lambert (1991); Hara *et al.* (1994)], steroid anesthetics (Harrison and Simmonds, 1984; Harrison *et al.*, 1987), barbiturates (Study and Barker, 1981), chlormethiazole (Hales and Lambert, 1992), halogenated volatile anesthetics (Wakamori *et al.*, 1991; Jones *et al.*, 1992), and trichloroethanol (TCEt, the active metabolite of chloral hydrate; Lovinger *et al.*, 1993; Peoples and Weight, 1994).

Different combinations of $GABA_A$ receptor subunits show variable sensitivity to allosteric modulators (e.g. Horne *et al.*, 1993). The best documented role of specific subunits is for benzodiazepine modulation, where the presence of a γ subunit is required (Pritchett *et al.*, 1989), but benzodiazepine agonist sensitivity is also influenced critically by the type of α subunit isoform. Specifically, the exchange of a single amino acid confers benzodiazepine sensitivity on the normally benzodiazepine-insensitive α_6-containing $GABA_A$ receptor (Kleingoor *et al.*, 1993).

The role of $GABA_A$ receptor subunits in general anesthetic modulation is less clear. For example, modulation of $GABA_A$ receptors by PRO is qualitatively

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independent of the γ subunit (Jones *et al.*, 1995), and can be observed in heteromeric $\alpha\beta$ or even in homomeric β_1 receptors (Sanna *et al.*, 1995a,b). It was recently shown that the α subunit influences modulation by pentobarbital (Thompson *et al.*, 1996). This study was therefore designed to compare the effects of PRO on GABA-induced chloride currents in receptors containing two different α subunits against a common $\beta\gamma$ background. The α_6 subunit was chosen as it shares the least homology [along with α_4, see Wafford *et al.* (1996)] with the other α subunit isoforms (Tyndale *et al.*, 1995), and contributes to the differential pharmacology of benzodiazepines (Hadingham *et al.*, 1993). We hypothesized that if differences in PRO modulation were to exist among α subunit isoforms, they might be most apparent in comparisons with α_6-containing receptors. Portions of this work were previously presented in abstract form (O'Shea *et al.*, 1996).

MATERIALS AND METHODS

Tissue culture

An Ltk^- mouse fibroblast cell line from ATCC (Rockville, MD, U.S.A.) was stably transfected with dexamethasone-inducible $GABA_A$ receptor $\alpha_1\beta_3\gamma_2$ or $\alpha_6\beta_3\gamma_2$ cDNA, as previously described in detail (Hadingham *et al.*, 1992). The resultant cell lines were cultured in supplemented Eagle's minimum essential medium (Sigma, St. Louis, MO, U.S.A.) as previously described.

Electrophysiology. Electrophysiological recordings were performed at room temperature using the whole-cell patch clamp technique. The electrode solution contained (in mM): 147, *N*-methyl-D-glucamine hydrochloride; 5, CsCl; 5, K_2ATP; 5, 4-(2-hydroxyethyl-1-piperazine ethane sulfonic acid (HEPES); 1, $MgCl_2$; 0.1, $CaCl_2$; and 1.1, ethylene glycol bis(b-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), pH 7.2, osmolarity 315 mosmol. Pipette-to-bath resistance was 4–7 MΩ. During an experiment, cells were constantly perfused with extracellular solution containing (in mM) 145 NaCl, 3 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 5.5 D-glucose and 10 HEPES, pH 7.4, osmolarity 320–330 mosmol. Cells were voltage-clamped at −60 mV. Since the intracellular and extracellular solutions contained symmetrical chloride concentrations, the chloride reversal potential was *ca* 0 mV.

Electrophysiology-picospritzer. GABA was applied to the cell under study by brief pressure ejection (2–6 p.s.i., 10–100 msec) from low-resistance micropipettes filled with 20 μM GABA for cells containing $\alpha_1\beta_3\gamma_2$ receptors and 2 μM GABA for cells containing $\alpha_6\beta_3\gamma_2$ receptors. This produced transient inward currents which were standardized by varying the duration of the pressure pipette pulse. For both cell lines, a maximal response was elicited by a 1 sec pulse of GABA from the pressure pipette, which was then decreased to 25–50 msec to achieve transient chloride currents that were *ca* 20% of the maximum current obtainable by 20 or 2 μM (test response; 19.3 ± 0.89% and 19.2 ± 0.94% for the $\alpha_1\beta_3\gamma_2$ cells and $\alpha_6\beta_3\gamma_2$ cells, respectively). GABA was applied every 20 sec. Anesthetic agents were applied to the bath until a stable, maximal potentiation of the GABA response was achieved. Drugs were then washed out until the pre-drug current was regained. Using this method, anesthetic equilibrium and recovery typically took 5–10 min. Current responses were low-pass filtered at 5 kHz (−3 dB, Bessel filter 902; Frequency Devices, Inc, MA, U.S.A.), digitized (TL1-125 interface; Axon Instruments, Foster City, CA, U.S.A.), and stored for off-line analysis (AXOBASIC, Axon Instruments).

Electrophysiology-rapid solution changer. GABA and/or anesthetics were rapidly applied to the cell by local perfusion [as described in Koltchine *et al.* (1996)] using a motor-driven solution exchange device (Bio Logic Rapid Solution Changer RSC-100; Molecular Kinetics, Pullman, WA, U.S.A.). An approximate EC_{10} GABA test dose was used as the control value ($\alpha_1\beta_3\gamma_2$/PRO: 9.0 ± 2.2% of maximal current; $\alpha_1\beta_3\gamma_2$/methohexital (MTX): 8.4 ± 0.7%; $\alpha_6\beta_3\gamma_2$/PRO: 9.2 ± 0.2%; $\alpha_6\beta_3\gamma_2$/MTX: 10.8 ± 1.4%) for the potentiation experiments. The solution changer was controlled by protocols in the acquisition programs AXOBASIC or pCLAMP5 (Axon Instruments). Laminar flow was maintained by applying all solutions at identical flow rates via a multi-channel infusion pump (Stoelting, Wood Dale, IL, U.S.A.). Prior to recording, a blue dye (FD and C Blue No. 1 and FD and C Red No. 40, McCormick, Baltimore, MD, U.S.A.) was used to check the alignment of the solution streams from the rapid solution changer.

Data analysis-electrophysiology. Drug-induced potentiation of a GABA-induced current was defined as the percentage increase of the control GABA response (defined as the average of the pre-drug and post-drug GABA induced currents). Concentration–response data were fitted (KaleidaGraph; Reading, PA, U.S.A.) with the logistic equation: $I/I_{max} = 100*[drug]^N/([drug]^N + (EC_{50})^N)$, where I/I_{max} is the percentage of the maximum obtainable GABA response, EC_{50} is the concentration producing a half-maximal response, and N is the Hill coefficient. Pooled data are presented as mean ± SEM. Statistical significance was determined by Student's two-tailed, unpaired *t*-test.

[^{3}H]Muscimol binding. Binding was performed on homogenized membranes prepared from cells cultured as above. Cells were harvested in ice-cold binding buffer containing (in mM): 20, Tris; 2, ethylenediamine tetraacetic acid (EDTA); 150, KCl, pH 7.4, then centrifuged at 2700***g*** for 10 min at 4°C. The cell pellet was hypotonically lysed in deionized water and centrifuged at 48 000***g*** for 20 min at 4°C. Finally, the pellet was rinsed with binding buffer and stored at −80°C for up to 2 months. Prior to experiments, membranes were thawed on ice and resuspended in assay buffer containing (in mM): 124, NaCl; 1.3, $MgSO_4$; 25, HEPES; 2.9, KCl; 1.2, KH_2PO_4; D-glucose, 5.2, pH 7.4. The pellet was then homogenized with a Brinkman polytron (final protein concentration 0.3–0.5 mg/ml by Bradford assay, BSA

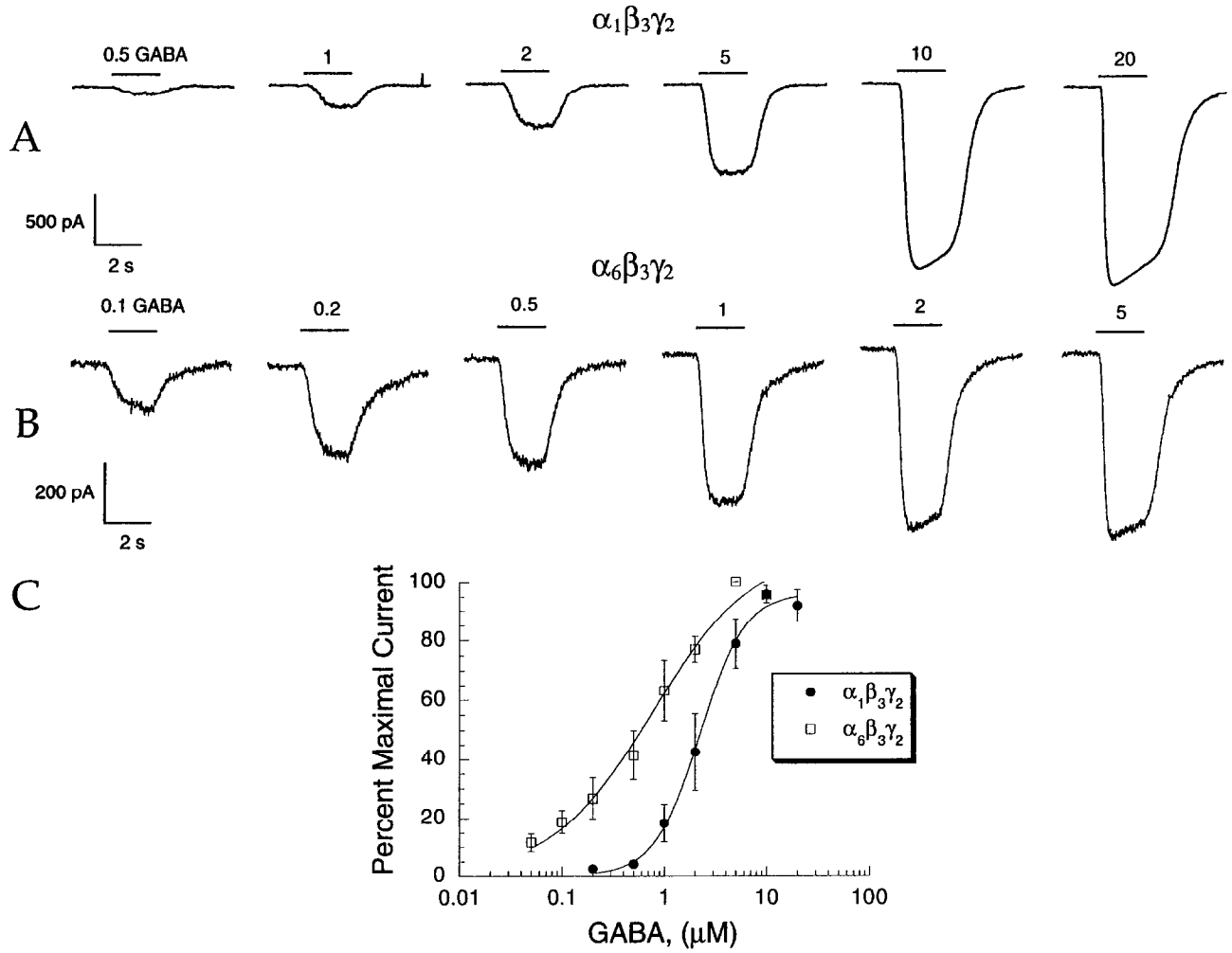

Fig. 1. GABA has higher apparent affinity for $\alpha_6\beta_3\gamma_2$ than $\alpha_1\beta_3\gamma_2$. GABA was applied with the rapid solution changer. (A and B) GABA responses from a cell expressing $GABA_A\alpha_1\beta_3\gamma_2$ (A) or $\alpha_6\beta_3\gamma_2$ (B) receptors. Bars over current traces indicate the duration of rapid GABA application, with the concentration of applied GABA in μM. (C) Concentration–response curves for $GABA_A$ $\alpha_1\beta_3\gamma_2$ or $\alpha_6\beta_3\gamma_2$ receptors. Data points are shown as the normalized means of multiple experiments ($5 \leqslant n \leqslant 7$), error bars indicate SEM.

standard). Membrane homogenates, along with eight concentrations of [^{3}H]muscimol (saturation curve) or GABA plus a fixed concentration of [^{3}H]muscimol (inhibition curve), were incubated in triplicate. After equilibration at room temperature, the assay mixtures were vacuum filtered through GF/B filter paper (Whatman, Clifton, NJ, U.S.A.) using a cell harvester (Brandel model MB-48, Gaithersburg, MD, U.S.A.), and quickly washed 10 times with 0.25 ml assay buffer. Specific binding was determined by subtracting the radioactive counts in the presence of 10 mM GABA from the radioactive counts in buffer alone. Similar results were obtained with a centrifugation assay using binding buffer (data not shown).

Data analysis-binding. Data points are taken from specific binding in individual experiments, and represent the mean of triplicate assays ± SEM. Saturation binding data were fit (KaleidaGraph; Reading, PA, U.S.A.) with the equation: $B = (B_{max} * [L])/([L] + K_d)$, where B is the specific binding, $[L]$ is [muscimol], and K_d is the dissociation constant for muscimol. Inhibition data were fit with the equation:

$$B = B_{max} - [\text{drug}]/(IC_{50} + [\text{drug}]) * (B_{max} - B_{min}),$$

where B_{min} is the lowest specific binding, [drug] is the final concentration of GABA, IC_{50} is the concentration producing a half-maximal displacement of [^{3}H]muscimol. The K_i value for GABA was calculated from: $K_i = IC_{50}/1 + ([L]/K_d)$.

Drugs used. Stock solutions of GABA, bicuculline methiodide, TCEt (Sigma), PRO (Aldrich, Milwaukee, WI, U.S.A), MTX sodium (Brevital®, Eli Lilly, Indianapolis, IN, U.S.A.), and midazolam hydrochloride (Versed®, intravenous/intramuscular solution preparation; Roche Pharmaceuticals, Manati, PR, U.S.A.) were diluted into extracellular solution daily before use. PRO was first dissolved into dimethyl sulfoxide (DMSO; Sigma) to form a stock solution of 10 mM PRO which

Table 1. A summary of electrophysiological data using the rapid solution changer (values shown are means ± SEM with number of experiments in parentheses)

Cell line	EC_{50} (μM)	Hill coefficient	Maximum (pA)
$\alpha_1\beta_3\gamma_2$	2.2 ± 0.2 (6)	1.9 ± 0.2 (6)	2690 ± 400 (18)
$\alpha_6\beta_3\gamma_2$	0.8 ± 0.1 (7)	0.9 ± 0.2 (7)	1186 ± 448 (8)

Table 2. A summary of radioligand binding data using [3H]muscimol (values represent the pooled means ± SEM from curve fits of individual experiments with number of experiments in parentheses)

Cell line	Muscimol K_d (nM)	GABA IC_{50} (nM)	GABA K_i (nM)
$\alpha_1\beta_3\gamma_2$	90 ± 23 (5)	370 ± 78 (3)	337 ± 71 (3)
$\alpha_6\beta_3\gamma_2$	28 ± 5 (5)	45 ± 14 (3)	34 ± 10 (3)

was then dissolved into the extracellular solution to form the final PRO solutions (maximum final concentration of DMSO was 0.05% for a 50 μM PRO solution). Carrier controls were performed with 0.05% DMSO in extracellular medium. No significant effects of this DMSO solution were observed on GABA-induced currents in cells expressing either $\alpha_1\beta_3\gamma_2$ or $\alpha_6\beta_3\gamma_2$ receptors. [^{3}H]muscimol (sp. act. 14.9 and 19.1 Ci/mmol) was obtained from NEN (Wilmington, DE, U.S.A.).

RESULTS

Receptor characteristics

We initially characterized the electrophysiological responses to GABA of the $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ combinations. Application of GABA to either cell line by the rapid solution changer elicited concentration-dependent inward currents [Fig. 1(A, B)]. Analysis of the concentration–response data [Fig. 1(C) and Table 1] shows that the $\alpha_6\beta_3\gamma_2$ receptor has a higher apparent affinity for GABA (EC_{50} 0.8 ± 0.1 μM) and a lower Hill coefficient ($N = 0.9 \pm 0.2$) than the $\alpha_1\beta_3\gamma_2$ line (EC_{50} 2.2 ± 0.2 μM, $N = 1.9 \pm 0.2$, respectively; $p < 0.05$ for both comparisons). These results are in accord with other investigations that compared GABA responses in α_1 and α_6-containing $GABA_A$ receptor combinations (Ducic *et al.*, 1995; Thompson *et al.*, 1996). Also, the $\alpha_1\beta_3\gamma_2$ line produced larger maximal GABA currents than the $\alpha_6\beta_3\gamma_2$ line (2690 ± 400 pA vs 1186 ± 448 pA, $p < 0.05$). Midazolam (1 μM) potentiated GABA currents in cells containing the α_1 subunit, but not in cells containing the α_6 subunit, and GABA currents in both receptor subtypes were blocked by 20 μM bicuculline (data not shown).

As shown in Fig. 2(A) and Table 2, the $\alpha_6\beta_3\gamma_2$ receptor shows a higher affinity for [^{3}H]muscimol than $\alpha_1\beta_3\gamma_2$ ($\alpha_6\beta_3\gamma_2$ K_d 28 ± 5 nM versus $\alpha_1\beta_3\gamma_2$ K_d 90 ± 23, $p < 0.05$). A Scatchard plot of the data (Fig. 2(B)) illustrates that binding at both receptors was appropriately fit by a one-site model. In displacement assays (Fig. 2(C)), GABA binding affinity to α_6 was also higher than at α_1-containing receptors, as shown by its lower IC_{50} (Table 2: $\alpha_6\beta_3\gamma_2$: 45 ± 14 nM vs $\alpha_1\beta_3\gamma_2$: 370 ± 78, $p < 0.05$), and lower K_i value ($\alpha_6\beta_3\gamma_2$: 34 ± 10 versus $\alpha_1\beta_3\gamma_2$: 337 ± 71, $p < 0.05$).

Propofol potentiation of GABA responses

GABA potentiation by PRO was measured electrophysiologically using two separate methods: the pico-

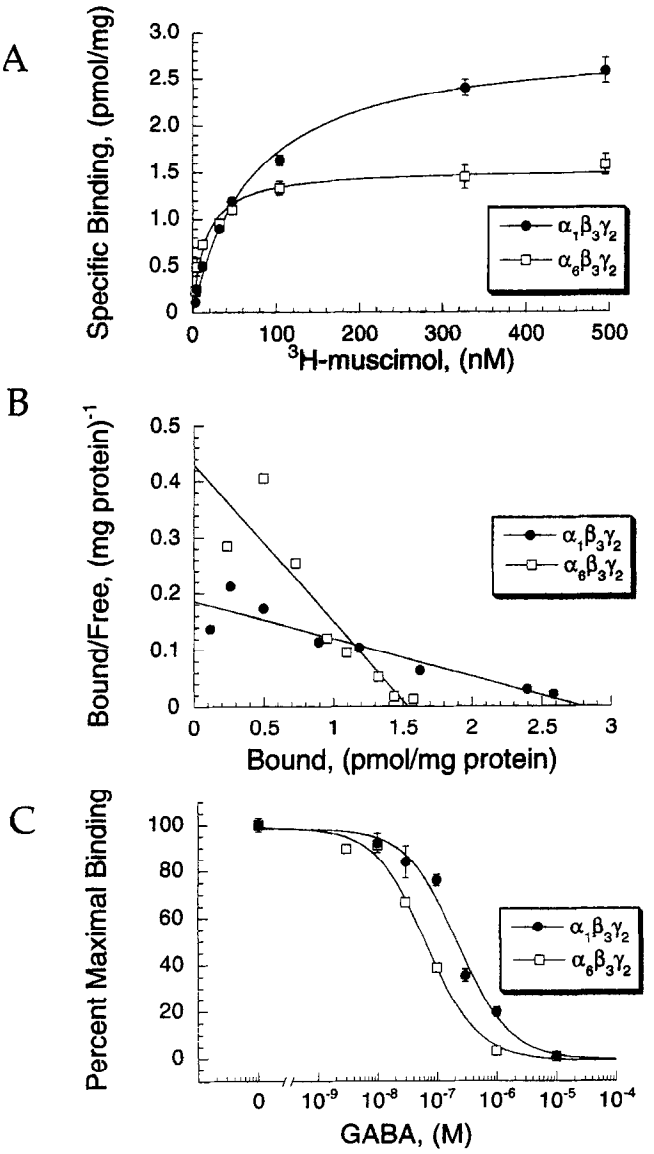

Fig. 2. Binding affinity is higher for muscimol and GABA at $\alpha_6\beta_3\gamma_2$ than $\alpha_1\beta_3\gamma_2$. (A) Saturation binding isotherms for $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ receptors obtained by using increasing concentrations of [^{3}H]muscimol. Data points show the means ± SEM from a single experiment, with each concentration performed in triplicate. This experiment was performed five times, with similar results. (B) Shows a Scatchard plot from the same experiment from (A). Data points are the means of triplicate measurements. (C) An inhibition curve showing displacement of 10 nM [^{3}H]muscimol with increasing concentrations of GABA. This figure represents the means ± SEM from a single experiment, with each point performed in triplicate. This experiment was performed three times with similar results.

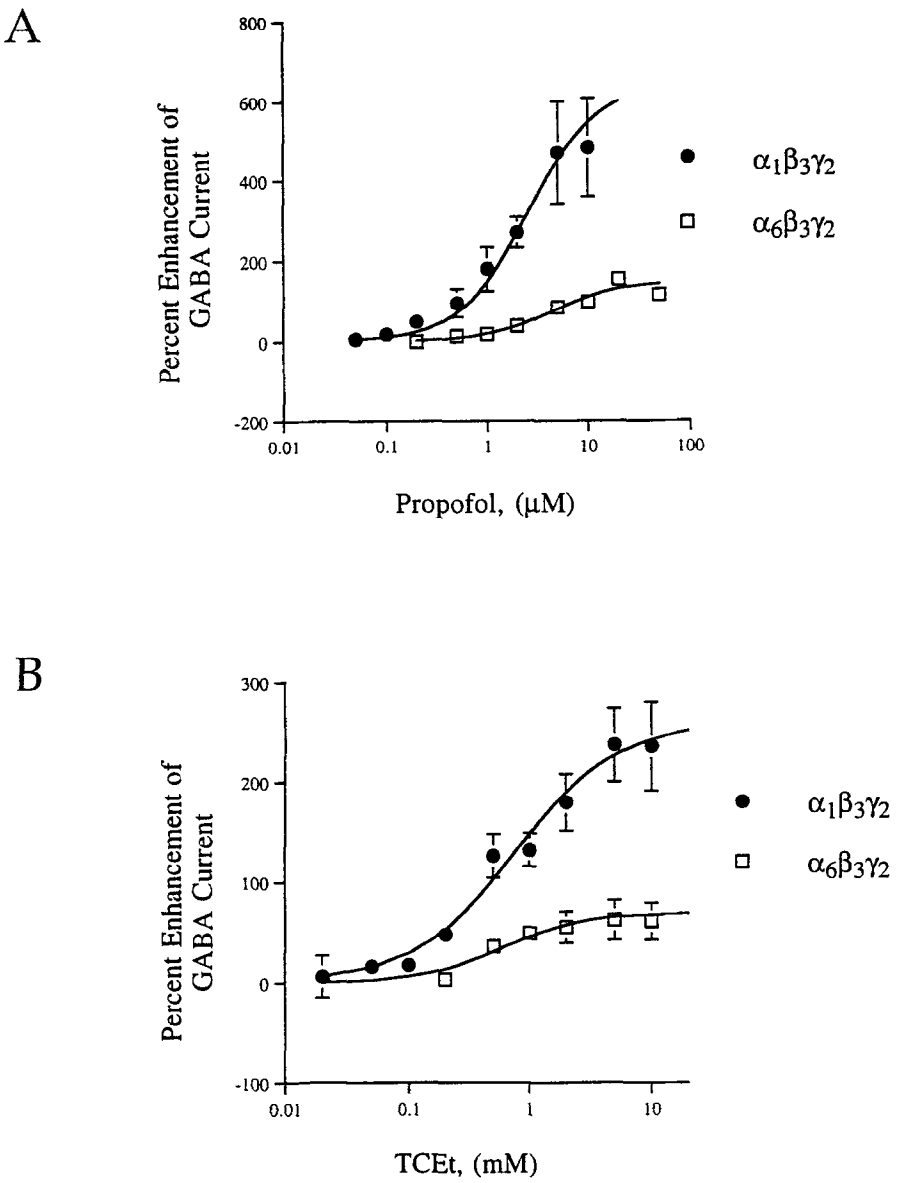

Fig. 3. α subunit affects the efficacy, but not potency of PRO. Submaximal GABA was applied by picospritzer as detailed in Materials and Methods; anesthetics were applied to the bath. (A) PRO (0.05–50 μM) potentiates GABA-induced currents in both $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ $GABA_A$ receptors. Data points are means ± SEM from pooled experiments ($4 \leqslant n \leqslant 13$). (B) TCEt (0.02–10 mM) potentiates GABA-induced currents in both receptor combinations ($5 \leqslant n \leqslant 15$; pooled data are shown).

spritzer and rapid solution changer. The picospritzer was used for the initial set of experiments (Fig. 3, Table 3), since this method allowed for a wide range of modulator concentrations to be tested rapidly. However, once the appropriate PRO concentration range was established, later experiments with the rapid solution changer (Fig. 4) provided the means to apply known concentrations of GABA.

Significant potentiation of submaximal GABA-induced chloride currents by PRO was first observed at

Table 3. A comparison of electrophysiological data from $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ using bath-applied PRO or TCEt, picospritzer-applied GABA (values are pooled means ± SEM, $4 \leqslant n \leqslant 15$)

Anesthetic	Cell line	EC_{50}	Hill coefficient	Maximum (% enhancement)
PRO	$\alpha_1\beta_3\gamma_2$	2.6 ± 0.5 μM	1.3 ± 0.2	564 ± 53
	$\alpha_6\beta_3\gamma_2$	4.6 ± 1.9 μM	1.3 ± 0.3	136 ± 17
TCEt	$\alpha_1\beta_3\gamma_2$	0.8 ± 0.2 mM	1.0 ± 0.6	258 ± 21
	$\alpha_6\beta_3\gamma_2$	0.6 ± 0.2 mM	1.3 ± 0.3	60 ± 3

0.2 μM and 0.5 μM PRO for the $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ $GABA_A$ receptors, respectively (Fig. 3(A), $p < 0.05$ for each). Table 3 shows that the magnitude of the maximal potentiation of GABA-induced currents by PRO in these picospritzer experiments was significantly greater in cells expressing the α_1 isoform relative to the α_6 isoform (maximum: 4.1-fold greater efficacy; $p < 0.05$). Statistically significant differences between the efficacy for PRO modulation of α_1 and α_6 receptors were observed at all concentrations $\geqslant 0.2$ μM PRO.

The rapid solution changer was then used to investigate $GABA_A$ receptor modulation in greater detail. As shown in Fig. 4, and Table 4, the efficacy of PRO potentiation applied at 10 μM was higher in α_1 than α_6-containing receptors (1024 $\pm$ 203% vs 120 $\pm$ 39%; percent enhancement of an EC_{10} GABA test concentration by $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$-containing receptors, respectively; $p < 0.05$).

Direct activation of $GABA_A$ receptors

As Fig. 4 illustrates, some of the single cell responses

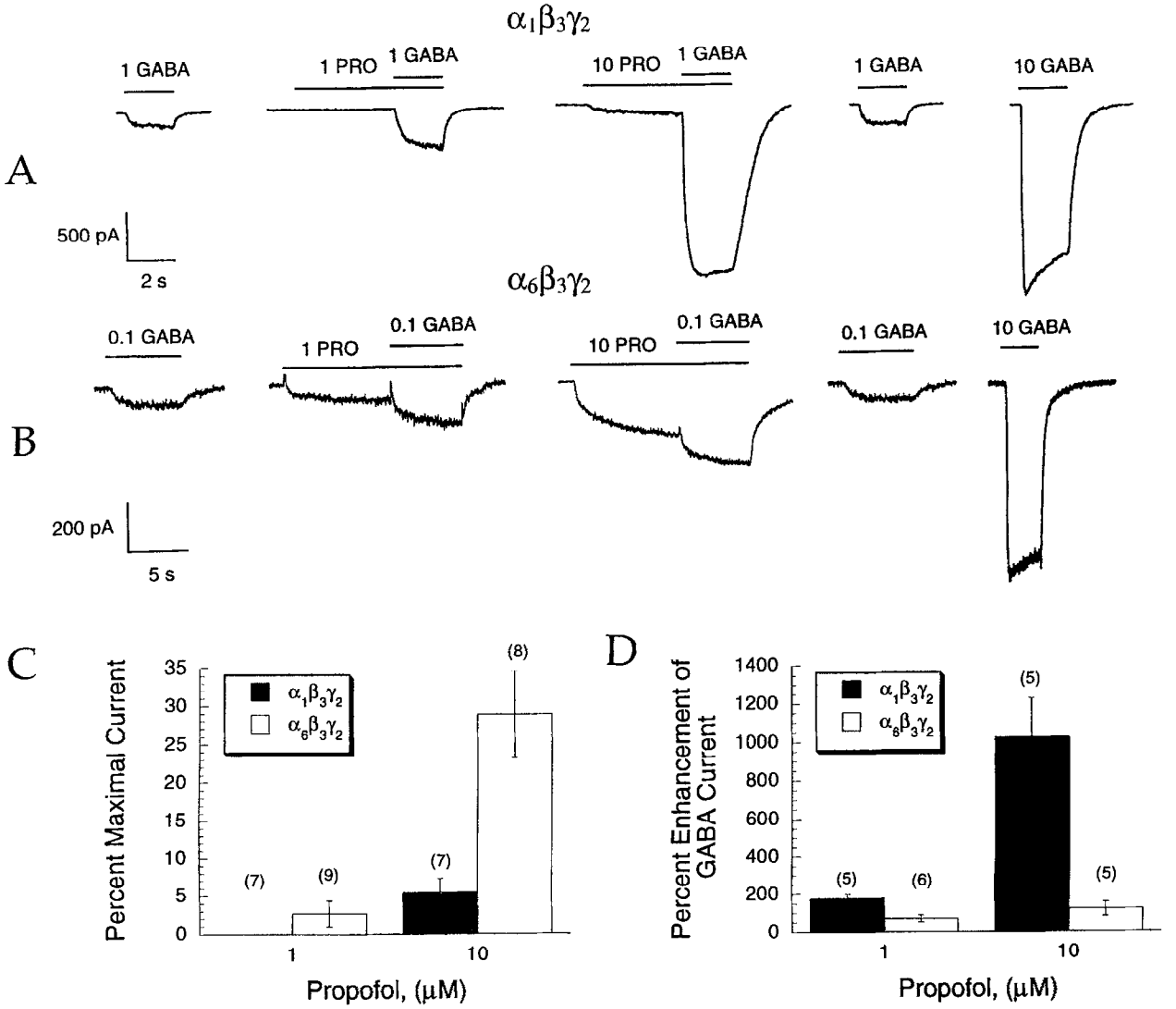

Fig. 4. PRO causes greater direct gating in α_6 than α_1, but greater potentiation in α_1 than α-containing receptors. GABA and anesthetics were applied with the rapid solution changer. (A and B) PRO enhances EC_{10} GABA responses in either (A) $\alpha_1\beta_3\gamma_2$ or (B) $\alpha_6\beta_3\gamma_2$ $GABA_A$ receptors. The first trace shows a pre-anesthetic control response to GABA; the last trace shows the response to a maximal concentration of GABA. Bars over current traces indicate GABA and PRO applications, respectively, with the concentration of each drug applied given in μM. In both receptor combinations, preapplication of PRO often elicited a direct inward current in the absence of GABA. (C) PRO directly elicits an inward current in the absence of applied GABA, displayed here as a percentage of the maximal GABA response. Bars show the percentage of direct gating $\pm$ SEM from pooled experiments, with the number of experiments given in parentheses. This direct current is significantly greater at 10 μM PRO for the $\alpha_6\beta_3\gamma_2$ receptors ($p < 0.05$). (D) Enhancement of an EC_{10} GABA by 1 and 10 μM PRO is significantly greater in the $\alpha_1\beta_3\gamma_2$-containing receptors. The ordinate shows the percentage enhancement of the GABA dose ($\pm$ SEM) in the presence of PRO compared to the GABA dose alone. (Number of experiments shown in parentheses.)

Table 4. A comparison of $\alpha_1\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$ using the rapid solution changer to apply GABA and PRO or MTX (values represent the means ± SEM pooled from multiple experiments with number of experiments in parentheses)

Cell line	Concentration (μM)	PRO (% Enhancement)	MTX (% Enhancement)
$\alpha_1\beta_3\gamma_2$	1	178 ± 21 (5)	125 ± 16 (6)
	10	1024 ± 203 (5)	816 ± 63 (6)
$\alpha_6\beta_3\gamma_2$	1	70 ± 18 (6)	124 ± 43 (7)
	10	120 ± 39 (5)	309 ± 86 (6)

to PRO show an inward current during pre-equilibration of the anesthetic, prior to co-application with GABA. This direct channel gating by 10 μM PRO was much more pronounced in $\alpha_6\beta_3\gamma_2$ than $\alpha_1\beta_3\gamma_2$-containing receptors (Fig. 4(C): 24.0 ± 6.4% versus 5.3 ± 1.9% of maximal current, respectively; $p < 0.05$).

Effects of other anesthetics

To determine whether these findings applied to other GABA modulators, the barbiturate MTX was also studied (Fig. 5 and Table 4). As with PRO, the efficacy of MTX enhancement of an EC_{10} GABA test concentration was higher in α_1 than α_6-containing receptors (Table 4:

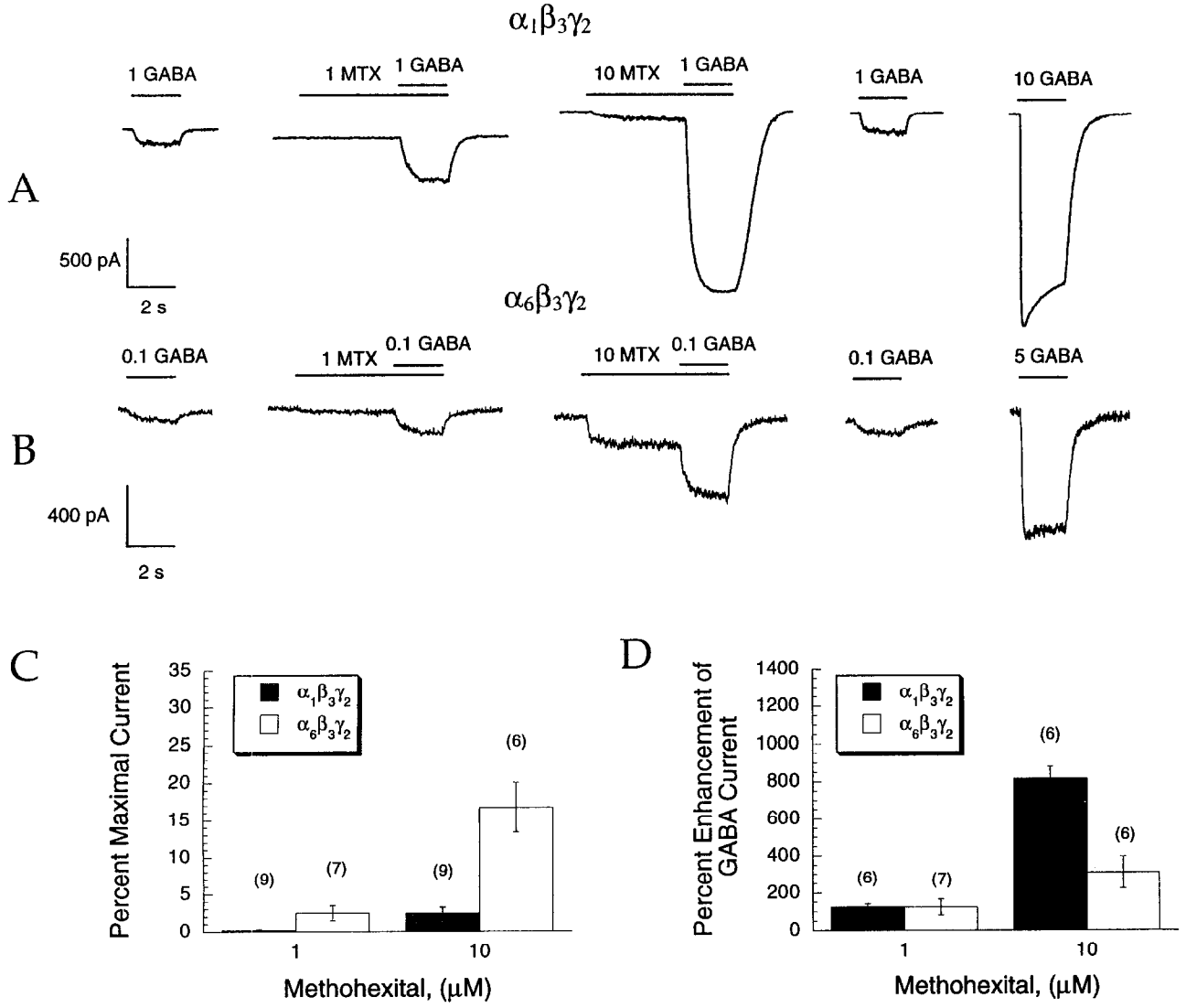

Fig. 5. MTX causes greater direct gating in α_6 than α_1, but greater potentiation in α_1 than α-containing receptors. Experimental design and figure representations are analogous to Fig. 4. (A and B) Enhancement of submaximal GABA responses by MTX in cells expressing either (A) $\alpha_1\beta_3\gamma_2$ or (B) $\alpha_6\beta_3\gamma_2$ $GABA_A$ receptors. As with PRO, preapplication of MTX often elicited an inward current directly. (C) MTX directly activates an inward current in the absence of applied GABA, shown as a percentage of the maximal GABA response. This direct current is significantly greater at both 1 and 10 μM MTX for the $\alpha_6\beta_3\gamma_2$ receptors ($p < 0.05$). (D) Enhancement of an EC_{10} GABA current response by 1 and 10 μM MTX is significantly greater in the $\alpha_1\beta_3\gamma_2$ at 10 μM.

816 ± 63% vs 309 ± 86%, respectively; $p < 0.05$). Figure 5 shows that, in agreement with previously published data studying pentobarbital modulation of $GABA_A$ receptors expressed in *Xenopus* oocytes (Thompson *et al.*, 1996), 10 μM MTX also elicits significantly larger direct current in $\alpha_6\beta_3\gamma_2$ than $\alpha_1\beta_3\gamma_2$ containing receptors (16.7 ± 3.3% versus 2.4 ± 0.8% of maximal current, respectively; $p < 0.05$).

Trichloroethanol was also studied, using picospritzer-applied GABA (Fig. 3(B), Table 3). This drug also potentiated submaximal GABA currents, although it was less efficacious than PRO (2.2-fold less efficacious at both receptor subtypes). In a similar fashion to PRO, the efficacy of TCEt potentiation was significantly greater in $\alpha_1\beta_3\gamma_2$ than $\alpha_6\beta_3\gamma_2$-containing receptors ($p < 0.05$).

DISCUSSION

The concentrations of PRO, TCEt, and MTX that caused enhancement of GABA-induced currents in this study correlate with clinically relevant anesthetic concentration ranges determined *in vivo*. PRO induces general anesthesia in rats and dogs with an estimated EC_{50} of 0.4 μM free PRO (Franks and Lieb, 1994), and TCEt anesthetizes canines and humans at concentrations in the range of 0.2–2 mM (Breimer, 1977; Garrett and Lambert, 1973). Threshold anesthetic concentrations for MTX in humans are estimated to range from 12 to 37 μM (Lauven *et al.*, 1987).

The results of this study underline the importance of subunit composition in the modulatory effects of PRO. As summarized in Tables 3 and 4, replacing an α_6 with an α_1 subunit in $GABA_A$ receptors with an identical $\beta_3\gamma_2$ subunit background markedly increased the modulatory action of PRO. Although the larger maximal current (as shown in Table 1) and higher maximal binding [B_{max}; Fig. 2(A and B)] suggest that more α_1-containing receptors are being expressed, normalization of the enhancement against parallel GABA test concentrations demonstrates that the differential enhancement cannot be explained by differences in receptor expression levels.

Our data with MTX contrast slightly with other investigations of the role of the α subunit in modulation by barbiturates. Our finding that direct activation by MTX is greater in α_6 than α_1-containing receptors is in agreement with two studies assessing pentobarbital modulation in $GABA_A$ receptors expressed in *Xenopus* oocytes (Wafford *et al.*, 1996; Thompson *et al.*, 1996). However, in contrast to our findings, those two studies found that pentobarbital potentiated submaximal GABA currents to a slightly greater degree in α_6 than α_1-containing receptors. These differences may be accounted for by the use of different expression systems, barbiturates, and $\beta\gamma$ backgrounds between those studies and ours. Use of the rapid solution changer allows for rapid equilibration of the applied GABA concentration, a condition which may be more difficult to achieve in whole oocytes. Nevertheless, from these studies, as well as our own, it is clear that the α subunit plays a significant role in both PRO and barbiturate modulation.

Inspection of Table 3 reveals a difference in the maximal potentiation (efficacy), but not the EC_{50}, for GABA potentiation by PRO between $\alpha_1\beta_3\gamma_2$ or $\alpha_6\beta_3\gamma_2$ $GABA_A$ receptors. Thus, differences between the structures of the α_1 and α_6 subunit isoforms may be important in the extent of allosteric modulation by PRO, but perhaps not direct binding *per se*, of the drug to the $GABA_A$ receptor. Previous work has already shown that modulation by PRO does not require the γ subunit (Jones *et al.*, 1995), and in fact, is even seen in β_1 homomers expressed in *Xenopus* oocytes (Sanna *et al.*, 1995b). Our finding that the α subunit isoform type does not affect the apparent affinity of PRO is consistent with the primary determinants of PRO binding being located on the β subunit, or on components of the α subunit which are highly conserved.

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