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MULTIPLE ACTIONS OF PROPOFOL ON αβγ AND αβδ $GABA_A$ RECEPTORS

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Running title: Propofol modulation of recombinant $GABA_A$ receptors

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abbreviations:

CNS, central nervous system; DMEM, dulbecco's modified eagle medium; DMSO, dimethylsulfoxide; HEK, human embryonic kidney; propofol, 2, 6-di-isopropylphenol

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ABSTRACT

$GABA_A$ receptors are predominantly composed of αβγ and αβδ isoforms in the brain. It has been proposed that αβγ receptors mediate phasic inhibition while αβδ receptors mediate tonic inhibition. Propofol (2, 6-di-isopropylphenol), a widely used anesthetic drug, exerts its effect primarily by modulating $GABA_A$ receptors. However, the effects of propofol on the kinetic properties of αβγ and αβδ receptors are uncertain. We transfected human embryonic kidney (HEK293T) cells with cDNAs encoding rat α1, α6, β3, γ2L or δ subunits and performed whole cell patch clamp recordings to explore this issue. Propofol (3 μM) increased GABA concentration-response curve maximal currents similarly for both α1β3γ2L and α6β3γ2L receptors, but propofol increased those for α1β3δ and α6β3δ receptors differently, the increase being greater for α1β3δ than for α6β3δ receptors. Propofol (10 μM) produced similar alterations in α1β3γ2L and α6β3γ2L receptor currents when using a pre-application protocol; peak currents were not altered, desensitization was reduced, and deactivation was prolonged. Propofol enhanced peak currents for both α1β3δ and α6β3δ receptors, but the enhancement was greater for α1β3δ receptors. Desensitization of these two isoforms was not modified by propofol. Propofol did not alter the deactivation rate of α1β3δ receptor currents but did slow deactivation of α6β3δ receptor currents. The findings that propofol reduced desensitization and prolonged deactivation of γ2L subunit-containing receptors and enhanced peak currents or prolonged deactivation of δ subunit-containing receptors suggest that propofol enhancement of both phasic and tonic inhibition may contribute to its anesthetic effect in the brain.

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INTRODUCTION

γ-aminobutyric acid type A ($GABA_A$) receptors are ligand-gated pentameric chloride ion channels and mediate the majority of inhibition in the CNS. More than 16 different $GABA_A$ receptor subunit subtypes have been identified, including α1-α6, β1-β3, γ1-γ3, δ, ε, π and θ (Olsen and Macdonald, 2002). McKernan and Whiting (1996) suggested that $GABA_A$ receptors may exist *in vivo* predominantly as αβγ and αβδ isoforms. The αβγ isoforms are mainly localized in GABAergic synapses, but αβδ isoforms were found on extra or perisynaptic membranes (Nusser et al., 1998; Wei et al., 2003), suggesting that αβγ receptors may mediate phasic inhibition and αβδ receptors may be involved in tonic inhibition (Bai et al., 2001; Stell et al., 2003). Recombinant αβγ receptors expressed in mammalian cells exhibited rapid desensitization (Haas and Macdonald, 1999; Bianchi and Macdonald, 2001; Scheller and Forman, 2002). However, α1 or α4 subunit-containing αβδ $GABA_A$ receptors had relatively less desensitization (Brown et al., 2002; Wohlfarth et al., 2002; Feng et al., 2004), although α6 subunit-containing αβδ receptors were more desensitizing (Bianchi et al., 2002).

Several widely used general anesthetic drugs including propofol (2, 6-di-isopropylphenol) exert their effects in the CNS mainly by enhancing $GABA_A$ receptor currents (Olsen and Macdonald, 2002). Modulation of αβγ receptor current amplitudes by propofol has been substantially explored (Hill-Venning et al., 1997; Uchida et al., 1997; Lam and Reynolds, 1998; Pistis et al., 1999; Carlson et al., 2000; Davies et al., 2001; Krasowski et al., 2001; Williams and Akabas, 2002), but propofol effects on the kinetic properties of recombinant αβγ receptors are unclear. Although one study suggested that propofol slightly enhanced the function of α4β3δ receptors (Brown et al., 2002), its effects on current kinetics of other αβδ isoforms are unknown. Therefore, modulation of propofol on recombinant αβγ and αβδ receptors was examined to

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explore the potential effects of propofol on phasic and tonic GABAergic inhibition. $GABA_A$ receptor α1 subunit mRNA is ubiquitously expressed in the brain whereas α6 subunit mRNA is restrictively found in the cerebellum (Wisden et al., 1992). In addition, α6 subunits preferably coassemble with δ subunits, and the α6βδ receptor is one of the predominant δ subunit-containing $GABA_A$ receptor isoforms in the brain (Poltl et al., 2003). It is interesting to determine if propofol has different effects on α1 and α6 subunit-containing $GABA_A$ receptors. Therefore, modulation of recombinant α1βγ, α6βγ, α1βδ and α6βδ receptors by propofol was examined to explore the potential α subunit dependent effects of propofol.

In the present study, we demonstrated subunit-specific propofol activation of γ2L and δ subunit-containing $GABA_A$ receptors. Propofol evoked a greater maximal conductance change (ΔG) from γ2L than from δ subunit-containing receptors. Propofol similarly decreased the desensitization and prolonged the deactivation of α1β3γ2L and α6β3γ2L receptors without affecting the peak current amplitudes. Although propofol modulation of αβγ $GABA_A$ receptor currents was relatively insensitive to the α subunit subtype, α subtype-specific effects of propofol were observed for αβδ receptors. Propofol produced a greater enhancement of peak current amplitudes for α1β3δ than for α6β3δ receptors and prolonged the deactivation of α6β3δ receptor currents without altering deactivation of α1β3δ receptor currents.

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MATERIALS AND METHODS

Expression of recombinant $GABA_A$ receptors in human embryonic kidney (HEK) cells

Human embryonic kidney (HEK293T) cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen Corporation, Grand Island, NY) in an incubator at 37°C with 5 % CO_2 and 95 % air. The cells were seeded at a density of 400,000/dish in 60-mm culture dishes (Corning Incorporated, Corning, NY) and transfected the following day with the combinations of cDNAs encoding rat α1, α6, β3, γ2L and δ $GABA_A$ receptor subunits (2 μg of each subunit in different ternary combinations) along with 2 μg of pHOOK (Invitrogen, Carlsbad, CA) using a modified calcium phosphate precipitation method (Fisher and Macdonald, 1997). The cells were incubated at 37°C for 4 hrs with 3 % CO_2 and then shocked for 30 s with 15 % glycerol (Sigma Chemical Co., St. Louis, MO). The selection marker pHOOK encoded a cell surface antibody (sFv), which bound to the antigen (phOx) coated on the ferromagnetic beads (Invitrogen Corporation). The bead-bound transfected cells were separated from non-transfected cells using a magnetic stand (Greenfield et al., 1997). Electrophysiological recordings were obtained 24 hr later. Eighty two percent of cells that bound beads also expressed $GABA_A$ receptors (~ 80 % for α1β3γ2L receptors, ~ 79 % for α1β3δ receptors, 86 % for α6β3γ2L receptors and ~ 87 % for α6β3δ receptors).

Whole cell recordings

Whole cell macroscopic currents were recorded using patch clamp technique at room temperature. The recording electrodes were pulled from the thin-wall borosilicate glass tubing (i.d. = 1.12 mm, o.d. = 1.5 mm) (World Precision Instruments Inc., Sarasota, FL) on a P-2000 Quartz Micropipette Puller (Sutter Instrument Company, Novato, CA). The electrodes were fire polished on an MF-830 Micro Forge (Narishige, Tokyo, Japan), and the resistances of the

electrodes were 0.9 to 1.6 MΩ when filled with an internal solution (see following for ionic composition).

Currents were recorded with an Axopatch 200A patch clamp amplifier (Axon Instruments, Foster City, CA) and Digidata 1200 series interface (Axon Instruments). Series resistance was not compensated since we previously reported that desensitization rate and extent were not affected by the current size we usually obtained from these recombinant $GABA_A$ receptors (Bianchi and Macdonald, 2002), suggesting that series resistance errors did not significantly affect our interpretations.

Chemicals, solutions and drug application

All chemicals were purchased from Sigma Chemical Co. The external bath solution was composed of (in mM) 142 NaCl, 1 $CaCl_2$, 6 $MgCl_2$, 8 KCl, 10 glucose and 10 HEPES (pH 7.4, 328 - 330 mOsm). The internal micropipette solution consisted of (in mM) 153 KCl, 1 $MgCl_2$, 10 HEPES, 2 MgATP and 5 EGTA (pH 7.3, 301 - 309 mOsm). This combination of the external and internal solutions produced an E_{Cl} near 0 mV and an E_K at –75 mV.

GABA was dissolved in water and propofol was dissolved in dimethylsulfoxide (DMSO) to make 1 M stock solutions. The working solutions were prepared by diluting the stock solution with external solution on the day of the experiment. The maximal final concentration of DMSO in working solutions was 0.3 %. Drugs were applied by gravity using an ultra-fast delivery device consisting of multi-barrel tubes connected to a Perfusion Fast-Step system (Warner Instruments Inc., Hamden, CT). The 10 - 90 % open electrode tip rise time of solution exchange was approximately 0.4 msec. Consecutive drug applications were separated by an interval of at least 45 sec to minimize accumulation of desensitization. The duration of GABA or propofol application was 4 sec.

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Data analysis

Whole cell currents were analyzed offline using Clamp fit 8.1 (Axon Instruments). Peak currents were measured manually from the baseline to the transient peak. Potentiation of GABA current by propofol (% of GABA current) was determined by dividing the peak current of co-application of GABA and propofol by the peak current evoked by GABA alone and multiplying by 100. Normalized concentration-response data were fitted using a logistic equation with a variable slope: $I = I_{max}/(1 + 10^{(LogEC_{50}\text{-}Logdrug)*Hill\ slope})$. I was the peak current evoked by a given concentration of GABA or GABA and propofol co-application. I_{max} was the maximal peak current. EC_{50} was defined as the GABA concentration at which a 50 % of maximal response was evoked. Peak conductance change (ΔG) was calculated by dividing the peak current by the holding potential. The extent of desensitization (%) was calculated by dividing the amount of current loss after 4 sec drug application by peak current and multiplying by 100. The deactivation current phase was analyzed by fitting using the standard exponential Levenberg-Marquardt methods, and the exponential components were expressed in the form of $\Sigma\ a_n\tau_n$, where *a* was the relative amplitude, τ was the time constant and *n* (=1 or 2) was the number of exponential components. A weighted τ was used to compare the rates of deactivation: $a_1*\tau_1/(a_1 + a_2) + a_2*\tau_2/(a_1 + a_2)$, where a_1 and a_2 were the relative amplitudes of the fast and slow exponential components (at time zero), and τ_1 and τ_2 were the corresponding time constant. Data were reported as mean ± SEM. Paired Student's *t* test was used to compare the changes prior to and after propofol treatment. Unpaired Student's *t* test was utilized to compare the alterations between different treatment groups. The difference was considered to be statistically significant if *p* was less than 0.05.

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RESULTS

GABA sensitivity of γ2L and δ subunit-containing $GABA_A$ receptors assembled with either an α1 or α6 subunit

We first examined the GABA sensitivity of the four isoforms to be studied using standard concentration-response experiments. Whole cell currents were recorded from recombinant α1β3γ2L, α6β3γ2L, α1β3δ and α6β3δ $GABA_A$ receptors (Figure 1A, B, C, D). Cells were voltage clamped at –20 mV for cells transfected with γ2L subunit-containing receptors, and due to the smaller amplitudes of αβδ currents, cells were voltage clamped at –50 mV for cells transfected with δ subunit-containing receptors (Wohlfarth et al., 2002; Feng et al., 2004). $GABA_A$ receptor channel activation is not voltage-dependent at negative membrane potentials (-10 to –75 mV), although some reports show different degrees of nonlinearity (rectification) at positive potentials (Bianchi et al., 2002). In a recent report on pentobarbital modulation of α1β3δ and α1β3γ2L receptors (Feng et al., 2004), membrane potential was held at both -20 and -50 mV for each isoform and pentobarbital-evoked effects were consistent for both receptor isoforms at both holding potentials. In addition, membrane potential was clamped from -10 to -75 mV to study neurosteroid modulation of these receptor isoforms, and no voltage-dependent effects were observed (Wohlfarth et al., 2002). As GABA concentrations were increased, the GABA-evoked whole cell peak conductance change (ΔG) increased (Figure 1E). The maximal ΔG for the α1β3γ2L isoform was 270.3 ± 47.6 nS (n = 6), which was not significantly different from that for α6β3γ2L receptors (206.9 ± 65.6 nS, n = 7), but which was significantly greater than that for α6β3δ (81.5 ± 25.3 nS, n = 6) ($p<0.01$) and α1β3δ (22.1 ± 11.1 nS, n = 5) ($p<0.01$) receptors. The maximal ΔG evoked by GABA from α6β3γ2L receptors was not significantly different from that evoked from α6β3δ receptors but was significantly greater than that from

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α1β3δ receptors ($p<0.05$). The maximal ΔG evoked by GABA from α6β3δ receptors was not significantly different than that from α1β3δ receptors.

As reported previously, α6 subunit-containing $GABA_A$ receptors had a lower GABA EC_{50} than α1 subunit-containing receptors (Saxena and Macdonald, 1996; Fisher et al., 1997). The EC_{50} for α6β3γ2L receptors (0.49 ± 0.14 μM) was smaller than that for α1β3γ2L receptors (6.17 ± 2.33 μM) ($p<0.05$). The EC_{50} for α6β3δ receptors (0.28 ± 0.05 μM) was also smaller than that for α1β3δ receptors (5.24 ± 0.43 μM) ($p<0.001$). No significant differences in EC_{50}s between α6β3γ2L and α6β3δ receptors or between α1β3γ2L and α1β3δ receptors were observed (Figure 1E). The mean Hill coefficient for α1β3γ2L receptors was 1.6 ± 0.2, and that for α6β3γ2L receptors was 1.5 ± 0.1. The mean Hill coefficients for α1β3δ and α6β3δ receptors were 1.0 ± 0.1 and 1.3 ± 0.1, respectively.

Differences in direct activation of γ2L or δ subunit-containing $GABA_A$ receptors by propofol

Propofol has been reported to activate directly $GABA_A$ receptors (Orser et al., 1994; Lam and Reynolds, 1998; Pistis et al., 1999; Davies et al., 2001; Krasowski et al., 2001; Brown et al., 2002; Dong and Xu, 2002). Propofol directly activated both γ2L and δ subunit-containing $GABA_A$ receptors (Figure 2A, B, C, D). However, similar to the results obtained with GABA, propofol evoked a greater maximal ΔG from γ2L than from δ subunit-containing receptors whether an α1 or α6 subtype was present (Figure 2E).

For both γ2L and δ subunit-containing $GABA_A$ receptors, propofol-evoked direct current showed no or little desensitization at propofol concentrations up to 300 μM. However at very high concentrations (>1 mM), propofol currents were rapidly activating, showed extensive desensitization, and a "rebound" current appeared upon washout of propofol (Figure 2A, B, C, D). The increased desensitization and appearance of "rebound" current might have resulted from

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propofol blocking open $GABA_A$ receptor channels at a low affinity site (Adodra and Hales, 1995; Davies et al., 2001). It appeared that the rate of desensitization was faster and the extent of desensitization was larger for α6 than for α1 subunit-containing receptors. The mechanisms underlying this phenomenon remain unknown. One possibility may be that the affinity of propofol to the channel binding site is greater for α6 than for α1 subunit-containing receptors so that more complete block is observed in α6 subunit-containing receptors. The multiphasic nature of the propofol concentration-response curve may also be partly explained by open channel block. However, the basis for the rapid change in current activation rate at high concentrations was unclear and was not further investigated.

Propofol enhanced peak currents evoked by a high concentration of GABA from α1β3δ more than from α1β3γ2L, α6β3γ2L and α6β3δ $GABA_A$ receptors

Performing concentration-response curves in the presence of a modulator such as propofol can provide an initial assessment of possible mechanisms of action by evaluating changes in EC_{50} and maximal current amplitudes. Propofol (3 μM) was co-applied with increasing GABA concentrations (from 0.01 to 1000 μM). Propofol slightly enhanced currents at high GABA concentrations, and thus shifted the GABA concentration-response curve upward similarly (~115 % of maximal current) for α1β3γ2L and α6β3γ2L receptors (Figure 3A, B, C). The GABA EC_{50} was not significantly altered by propofol for these receptor isoforms.

Co-application of propofol with high concentrations of GABA evoked a greater current enhancement for α1β3δ receptors than for α6β3δ receptors (139.1 ± 4.9 % versus 106.4 ± 2.6 %) ($p<0.001$) (Figure 3D, E, F). The maximal enhancement for α1β3δ receptors was also significantly greater than that for α1β3γ2L ($p<0.01$) and α6β3γ2L ($p<0.05$) receptors. The EC_{50} for α1β3δ receptors for GABA co-applied with propofol (7.05 ± 0.65 μM) was greater than that

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for GABA alone (5.24 ± 0.43 μM) (p = 0.05). The EC_{50} for α6β3δ receptors was unchanged by propofol (Figure 3F). The actual amount of enhancement of $GABA_A$ receptor current by propofol is somewhat difficult to interpret since the enhancement of α1β3γ2L and α6β3γ2L receptor currents is likely increased by the direct activation of propofol on these receptors. Nonetheless these concentration-response curves give the GABA concentration dependence in the presence of or absence of propofol and therefore are functionally useful.

Modulation by propofol of peak currents, desensitization and deactivation evoked by a saturating concentration of GABA was similar for α1β3γ2L and α6β3γ2L $GABA_A$ receptors

We were interested in exploring the modulation by propofol of peak $GABA_A$ receptor currents and kinetic properties evoked by a saturating GABA concentration. Long pulses of 1 mM GABA provide information about multiple phases of desensitization, as well as the deactivation following washout of GABA. To resolve better the fast phase of desensitization, the cells were lifted from recording dish to improve solution exchange rate. Propofol (10 μM) was pre-applied followed by co-application of propofol (10 μM) and a saturating GABA concentration (1 mM), allowing controlled duration of pre-equilibration as well as resolution of the direct effect of propofol. Propofol did not potentiate the peak GABA-evoked current of either α1β3γ2L (98.3 ± 1.4 %, n = 6) or α6β3γ2L (97.4 ± 4.7 %, n = 6) receptors (Figure 4A, B, C).

Mean desensitization of α1β3γ2L receptor current induced by GABA alone was 54.5 ± 5.0 %, which was significantly smaller than that of α6β3γ2L receptor current (81.9 ± 3.1 %) ($p<0.001$). Propofol reduced the extent of desensitization for both α1β3γ2L and α6β3γ2L receptor currents. Mean desensitization was significantly reduced to 44.0 ± 5.6 % ($p<0.01$) for α1β3γ2L receptors, and to 72.1 ± 3.9 % ($p<0.01$) for α6β3γ2L receptors (Figure 4A, B, D).

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These results were consistent with prior studies of propofol on neuronal $GABA_A$ receptors (Bai et al., 1999; Dong and Xu, 2002).

Propofol also prolonged deactivation of both α1β3γ2L and α6β3γ2L receptor currents. The mean weighted deactivation rate for α1β3γ2L receptor currents was significantly increased from 420.6 ± 63.2 ms to 534.6 ± 76.1 ms by propofol ($p<0.01$), and that of α6β3γ2L receptor currents was significantly increased from 346.3 ± 32.8 ms to 533.2 ± 68.6 ms ($p<0.05$) (Figure 4A, B, E).

Modulation by propofol of peak currents and deactivation with a saturating concentration of GABA was different for α1β3δ and α6β3δ $GABA_A$ receptors

The pre-application and lifted cell techniques were also used to explore propofol modulation of peak currents and kinetic properties of α1β3δ and α6β3δ receptor currents. The mean peak current enhancement of α1β3δ receptors by propofol (205.8 ± 33.1 %, n = 7) was significantly greater than that of α6β3δ receptors (107.6 ± 5.0 %, n = 7) ($p<0.05$) (Figure 5A, B, C).

Mean desensitization of α1β3δ receptor currents for GABA alone was 8.9 ± 3.5 %, which was significantly smaller than that for α6β3δ receptor currents (49.3 ± 5.2 %) ($p<0.001$) (Figure 5A, B, D), consistent with previous reports (Bianchi et al., 2002). Propofol did not alter the extent of desensitization for either isoform.

The deactivation rate of α1β3δ receptor currents following application of GABA alone was 103.9 ± 16.0 ms, which was significantly faster than that for α6β3δ receptor currents (315.0 ± 23.8 ms) ($p<0.001$) (Figure 5A, B, E). Although propofol did not alter deactivation of α1β3δ receptor currents, it increased the deactivation rate to 411.5 ± 44.3 ms for α6β3δ receptor currents ($p<0.05$).

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DISCUSSION

The enhancement of maximal peak currents by propofol was greater for α1β3δ receptors than for α1β3γ2L, α6β3γ2L and α6β3δ receptors

For all recombinant $GABA_A$ receptor isoforms examined in the present study, propofol directly activated the receptors with multiphasic concentration-response curves. The mechanisms underlying these properties remain unknown. One possibility is that there are multiple receptor binding sites with different affinities for propofol. Propofol in the presence of high GABA concentrations produced a substantial upward shift of the GABA concentration-response curve for α1β3δ more than for α1β3γ2L, α6β3γ2L and α6β3δ receptors. Thus propofol was similar to pentobarbital and neurosteroids in exerting greater potentiation of this receptor isoform (Wohlfarth et al., 2002; Feng et al., 2004). Co-application of 3 μM propofol with high concentrations of GABA produced a small enhancement of α1β3γ2L and α6β3γ2L receptor currents. A part of the enhancement might be contributed by direct activation by propofol of $GABA_A$ receptors since propofol at this concentration evoked direct currents from α1β3γ2L and α6β3γ2L receptors. Consistent with this interpretation, when propofol was pre-applied, and thus the direct activation current could be taken into account, α1β3γ2L and α6β3γ2L receptor peak currents were not increased by propofol. Thus, propofol modulation was similar to those of pentobarbital and neurosteroids (Wohlfarth et al., 2002; Feng et al., 2004), which did not potentiate maximal GABA-evoked αβγ currents. In contrast, propofol enhanced both α1β3δ and α6β3δ receptor peak currents. δ subunit-containing $GABA_A$ receptors have been reported to be modulated by a variety of structurally different compounds (Lees and Edwards, 1998; Thompson et al., 2002; Wohlfarth et al., 2002; Wallner et al., 2003; Feng et al., 2004). The consistent observation with δ subunit-containing receptors is that, unlike most αβγ isoforms, the maximal

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currents evoked by GABA can be increased by allosteric modulators. These observations are reminiscent of the increased efficacy of positive modulators on αβγ receptor currents activated by partial agonists. Also, there is direct evidence that GABA is not a "full" agonist at αβδ isoforms since the synthetic GABA analog THIP activated larger currents than GABA (Adkins et al., 2002; Brown et al., 2002). These data support the idea that GABA may be a partial agonist for δ subunit-containing $GABA_A$ receptors (Bianchi and Macdonald, 2003). Interestingly, propofol was reported to slightly enhance the saturating GABA-evoked peak currents of $GABA_A$ receptors on native hippocampal neurons (Bai et al., 1999), but the contribution of δ subunit-containing receptors on these neurons was not known (Wisden et al., 1992).

Subunit-dependent modulation of recombinant $GABA_A$ receptor kinetic properties by propofol

Propofol significantly decreased the extent of desensitization and prolonged the deactivation of α1β3γ2L and α6β3γ2L receptors in a similar manner, suggesting that the kinetic modifications induced by propofol are predominantly dependent on the γ2L rather than the α subunit in these receptors. This finding is consistent with a report on modulation of $GABA_A$ receptor kinetic properties by propofol in native hippocampal neurons (Bai et al., 1999), since the predominant $GABA_A$ receptor isoform is αβγ2L in hippocampal pyramidal cells (Wisden et al., 1992). Similar alterations in kinetic properties produced by propofol were also reported for $GABA_A$ receptors in native spinal cord neurons (Dong and Xu, 2002), implying that αβγ2L receptors may also be predominantly present on these neurons. Although propofol decreased desensitization of γ2L subunit-containing receptors, we observed prolonged current deactivation. If the decreased macroscopic desensitization reflected reduced stability of desensitized states, faster deactivation would be predicted based on the proposal that prolongation of deactivation is

"coupled" with increased desensitization (Jones and Westbrook, 1995; Haas and Macdonald, 1999). A similar pattern of modulation was observed for pentobarbital modulation of α1β3γ2L receptor currents (Feng et al., 2004). We and others have recently suggested that increasing gating efficacy can secondarily decrease macroscopic desensitization as well as prolong deactivation (Bianchi and Macdonald, 2001; Scheller and Forman, 2002). Consistent with this mechanism, increased gating efficacy (frequency) was reported for propofol modulation of single $GABA_A$ receptor channels from neurons (Orser et al., 1994). Simulation studies also suggested that propofol-evoked prolongation of GABA current deactivation and reduced desensitization might be achieved by propofol stabilization of the ligand-bound pre-open state (Bai et al., 1999).

Propofol did not significantly affect desensitization but differentially modified the deactivation of α1β3δ and α6β3δ receptor currents, providing an additional example of independent modulation of desensitization and deactivation. Pentobarbital and neurosteroids have been reported to increase desensitization and prolong deactivation of α1β3δ receptor currents (Wohlfarth et al., 2002; Feng et al., 2004). However, propofol did not significantly alter the desensitization and deactivation of α1β3δ receptors in the present study, which is unexpected since any kinetic parameter that could increase maximal open probability of a non-desensitizing receptor should also prolong deactivation. We do not have an explanation for this observation. It is possible that we could not resolve changes in deactivation at the whole cell level. The possible mechanism underlying the different effect of propofol and pentobarbital or neurosteroids on α1β3δ receptor desensitization might be that these general anesthetics differentially modulated the rate constants of α1β3δ receptor desensitized state. Consistent with this possibility, these drugs had different effects on channel open states. Pentobarbital was reported to increase mean open duration of recombinant receptor single channel currents

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including α1β3δ receptors (Feng et al., 2004), while in contrast, propofol has been reported to increase channel open frequency (Orser et al., 1994). Propofol did not significantly modify desensitization of α6β3δ receptor currents but significantly prolonged deactivation. The mechanisms for this propofol effect remains unclear. One parsimonious explanation may be that propofol slowed the agonist unbinding, which has been reported for another anesthetic drug halothane (Li and Pearce, 2000). These data suggest that modulation by propofol of $GABA_A$ receptor kinetic properties is subunit-dependent.

Implications for propofol actions on γ2L or δ subunit-containing receptor currents: multiple anesthetic mechanisms

General anesthetics exert their effect in the brain largely by modulating $GABA_A$ receptor synaptic currents (Olsen and Macdonald, 2002). However, substantial recent evidence suggests that nonsynaptic or tonic forms of inhibition can have profound effects on neuronal excitability. Pentobarbital and several other general anesthetics have been reported to potentiate the currents of δ subunit-containing $GABA_A$ receptors (Lees and Edwards, 1998; Brown et al., 2002; Wohlfarth et al., 2002; Feng et al., 2004). These and the present studies suggest that δ subunit-containing receptors are an important target for general anesthetics. δ subunit-containing $GABA_A$ receptors are involved in tonic inhibition (Stell et al., 2003; Wei et al., 2003), suggesting that general anesthetics may partly exert their effect by enhancing tonic inhibition. This may be one explanation for the findings that the tonic inhibition was enhanced by propofol in hippocampal neurons (Bai et al., 2001; Bieda and MacIver, 2004). Also, propofol as well as pentobarbital (Feng et al., 2004) decreased the desensitization and prolonged the deactivation of γ2L subunit-containing receptors, which may mediate the phasic inhibition. This modification of kinetic properties by propofol has been demonstrated to prolong the synaptic currents (Bai et al.,

1999). Therefore, some general anesthetics such as propofol may also exert their effect by enhancing phasic inhibition. In addition, it was reported that a clinically relevant concentration of propofol is 0.4 μM (Dong and Xu, 2002). Interestingly, propofol at this concentration evoked direct currents from γ2L subunit-containing receptors. Thus, besides modulation of $GABA_A$ receptor currents, propofol may directly activate $GABA_A$ receptors to contribute its anesthetic effects in the brain.

The α1βγ2L receptor isoform is ubiquitously distributed in the brain and may be one of the major targets for propofol anesthetic effects. In contrast, α6βγ2L and α6βδ isoforms are restricted to the cerebellum (Wisden et al., 1992), a structure that may be involved in propofol anesthetic side effects such as ataxia. In the present study we report that propofol substantially enhanced α1β3δ receptor peak currents. However, a previous study suggested α1βδ receptors may be a minor isoform of δ subunit-containing $GABA_A$ receptors in the brain (Poltl et al., 2003). Therefore, it is likely that α1β3δ receptors have limited contribution to propofol effects in the brain. More importantly, the present findings that propofol similarly modulated the deactivation and/or desensitization of α1β3γ2L, α6β3γ2L and α6β3δ receptors suggest that propofol may exert similar effect on GABAergic inhibition in different regions of the brain. These data also suggest that enhancement of tonic inhibition and phasic inhibition may be equally important for propofol anesthetic effects as well as side effects. Further experiments are needed to confirm these speculations *in vivo*.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. GABA concentration-response patterns of αβγ2L and αβδ $GABA_A$ receptors containing either an α1 or α6 subunit subtype.

A-D, Representative whole cell current traces evoked by different concentrations of GABA from recombinant α1β3γ2L, α6β3γ2L, α1β3δ and α6β3δ $GABA_A$ receptors are presented. E, The GABA concentration-response curve was expressed as mean peak conductance changes (ΔG) vs a series of GABA concentrations for α1β3γ2L (n = 6, squares), α6β3γ2L (n = 7, triangles), α1β3δ (n = 5, circles) and α6β3δ (n = 6, diamonds) receptors. The solid line above each current trace represents the duration (4 sec) of GABA application. The error bars represent SEMs.

Figure 2. Propofol evoked greater direct response from γ2L than δ subunit-containing $GABA_A$ receptors.

A-D, Representative whole cell current traces evoked by different concentrations of propofol from recombinant α1β3γ2L, α6β3γ2L, α1β3δ and α6β3δ $GABA_A$ receptors are presented. E, The propofol concentration-response curve was expressed as mean peak conductance changes (ΔG) vs a series of propofol concentrations for α1β3γ2L (n = 6, squares), α6β3γ2L (n = 6, triangles), α1β3δ (n = 7, circles) and α6β3δ (n = 5, diamonds) receptors. The solid line above each current trace represents the duration (4 sec) of propofol application. The error bars represent SEMs.

Figure 3. Propofol produced greater enhancement of α1β3δ receptor than α1β3γ2L, α6β3γ2L and α6β3δ receptor currents at high GABA concentrations.

A, B, Examples of whole cell current traces evoked by GABA alone as well as co-application of GABA and propofol (3 μM) from α1β3γ2L and α6β3γ2L receptors are presented.

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C, The concentration-response curves for GABA alone (open symbols) and co-application of GABA with 3 μM propofol (solid symbols) were plotted for α1β3γ2L (squares) and α6β3γ2L (triangles) receptors. D, E, Examples of whole cell current traces evoked by GABA alone as well as co-application of GABA and propofol (3 μM) from α1β3δ and α6β3δ receptors are presented. F, The concentration-response curves for GABA alone (open symbols) and co-application of GABA with 3 μM propofol (solid symbols) were plotted for α1β3δ (circles) and α6β3δ (diamonds) receptors. The solid line above each current trace represents the duration (4 sec) of GABA application or GABA and propofol co-application. N = 5-7 cells for each GABA or GABA+propofol concentration-response curve. The error bars represent SEMs.

Figure 4. Propofol modulated the peak current, desensitization and deactivation of α1β3γ2L and α6β3γ2L receptors similarly.

A, B, Representative whole cell current traces evoked by GABA (1 mM) alone as well as co-application of GABA (1 mM) and propofol (10 μM) with propofol pre-applied from α1β3γ2L and α6β3γ2L receptors are presented. The GABA control current (grey trace) was normalized to the current evoked by co-application of GABA and propofol to demonstrate the changes in desensitization and deactivation. C, Propofol did not potentiate the mean GABA peak currents of α1β3γ2L (n = 6) and α6β3γ2L (n = 6) receptors. The grey dashed line indicates 100 %. D, Propofol treatment significantly decreased the mean desensitization of α1β3γ2L and α6β3γ2L receptors. E, Propofol treatment significantly increased the mean time constant of deactivation of α1β3γ2L and α6β3γ2L receptors. The solid line above each representative current trace denotes the duration of GABA application, and the black dashed line denotes that of propofol application. The error bars denote the SEMs.

* Significantly different from corresponding GABA control at $p<0.05$; ** $p<0.01$

Significantly different from GABA+propofol of α1β3γ2L isoform at p<0.01

+++ Significantly different from GABA control of α1β3γ2L isoform at p<0.001

Figure 5. Propofol differentially modulated the peak current and deactivation of α1β3δ and α6β3δ receptors.

A, B, Representative whole cell current traces evoked by GABA (1 mM) alone as well as co-application of GABA (1 mM) and propofol (10 μM) with propofol pre-applied from α1β3δ and α6β3δ receptors are presented. C, Propofol produced significantly greater mean enhancement from α1β3δ (n = 7) than from α6β3δ (n = 7) receptors. The grey dashed line indicates 100 %. D, Propofol treatment did not significantly affect the mean desensitization of α1β3δ and α6β3δ receptors. E, Propofol treatment significantly increased the mean time constant of deactivation of α6β3δ receptors but did not alter that of α1β3δ receptors. The solid line above each representative current trace denotes the duration of GABA application, and the black dashed line denotes that of propofol application. The error bars denote the SEMs.

* Significantly different from corresponding α6β3δ GABA control or α6β3δ isoform at p<0.05

Significantly different from GABA+propofol of α1β3δ isoform at p<0.001

+++ Significantly different from GABA control of α1β3δ isoform at p<0.001

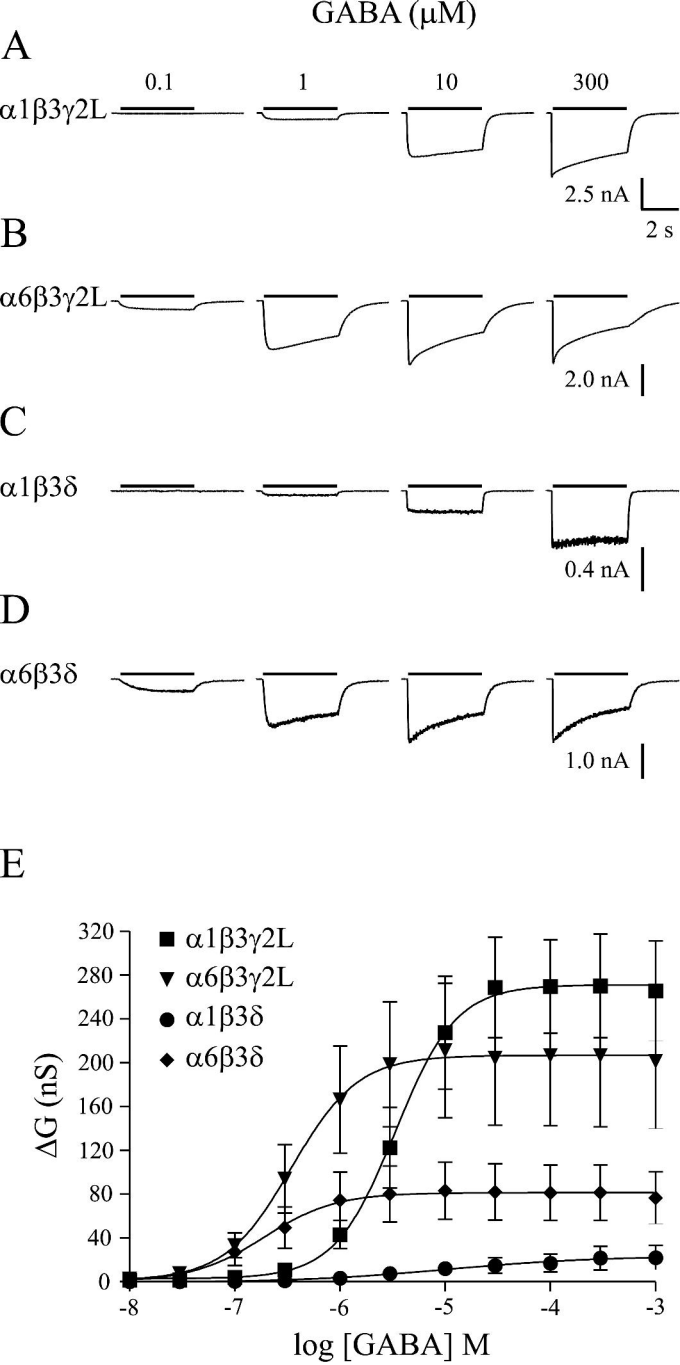
GABA (μM)
A
0.1
1
10
300
α1β3γ2L
2.5 nA
2 s
B
α6β3γ2L
2.0 nA
C
α1β3δ
0.4 nA
D
α6β3δ
1.0 nA
E
α1β3γ2L
α6β3γ2L
α1β3δ
α6β3δ
ΔG (nS)
320
280
240
200
160
120
80
40
0
-8
-7
-6
-5
-4
-3
log [GABA] M

Fig. 1

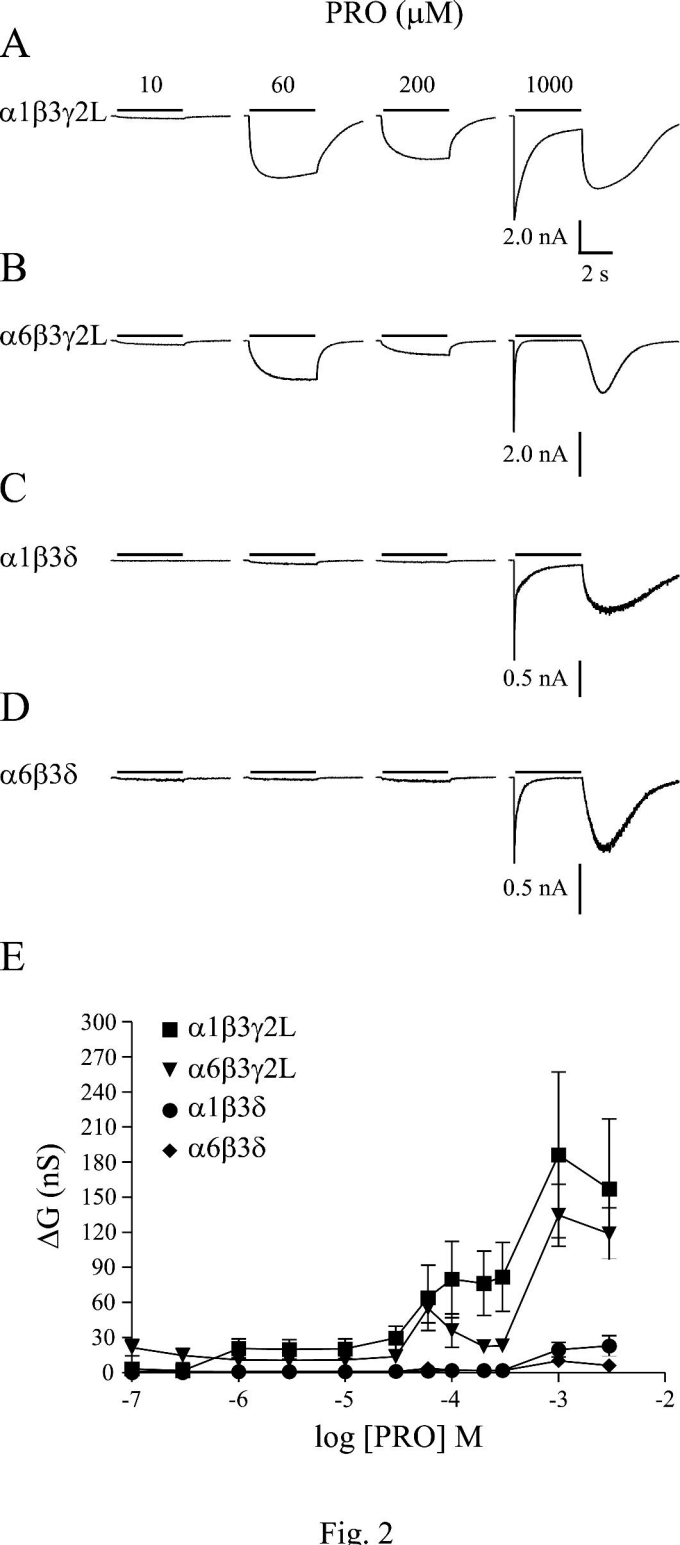
PRO (μM)
A
10
60
200
1000
α1β3γ2L
2.0 nA
2 s
B
α6β3γ2L
2.0 nA
C
α1β3δ
0.5 nA
D
α6β3δ
0.5 nA
E
α1β3γ2L
α6β3γ2L
α1β3δ
α6β3δ
ΔG (nS)
300
270
240
210
180
150
120
90
60
30
0
-7
-6
-5
-4
-3
-2
log [PRO] M

Fig. 2

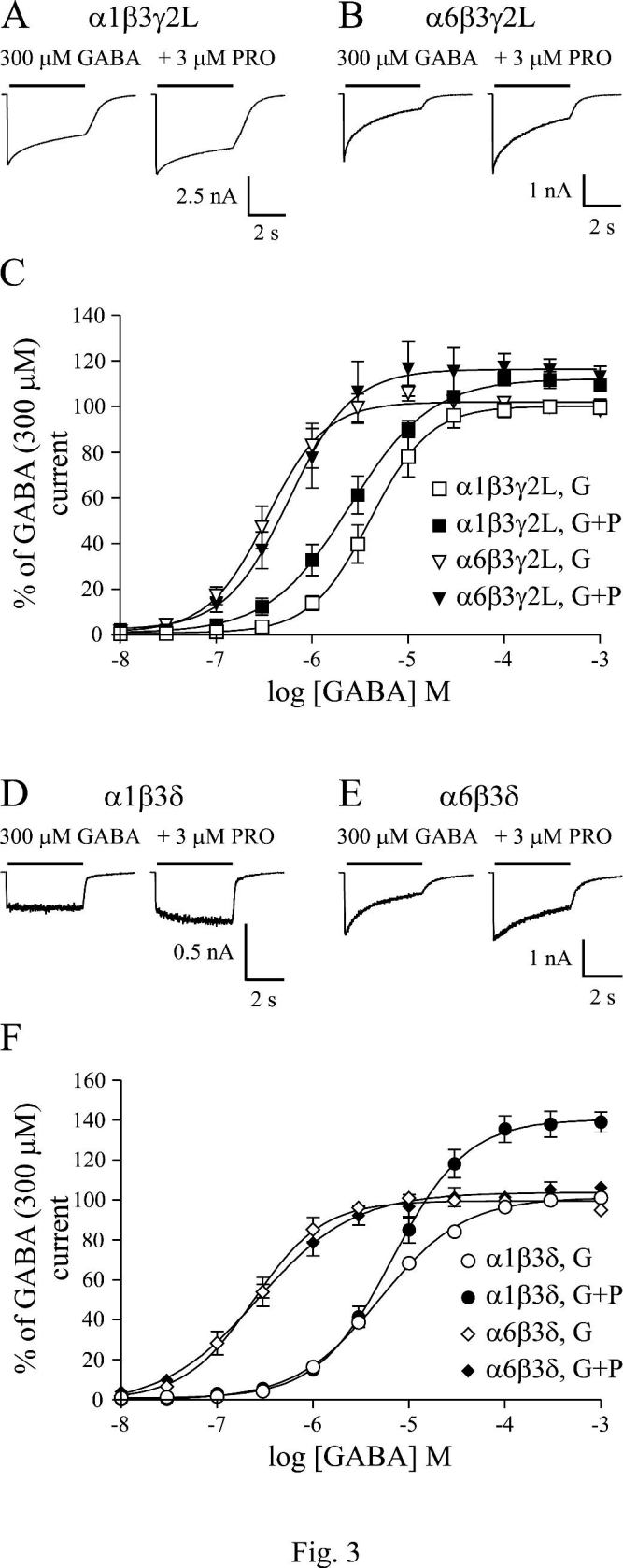
A
α1β3γ2L
300 μM GABA
+ 3 μM PRO
2.5 nA
2 s
B
α6β3γ2L
300 μM GABA
+ 3 μM PRO
1 nA
2 s
C
% of GABA (300 μM) current
log [GABA] M
α1β3γ2L, G
α1β3γ2L, G+P
α6β3γ2L, G
α6β3γ2L, G+P
D
α1β3δ
300 μM GABA
+ 3 μM PRO
0.5 nA
2 s
E
α6β3δ
300 μM GABA
+ 3 μM PRO
1 nA
2 s
F
% of GABA (300 μM) current
log [GABA] M
α1β3δ, G
α1β3δ, G+P
α6β3δ, G
α6β3δ, G+P

Fig. 3

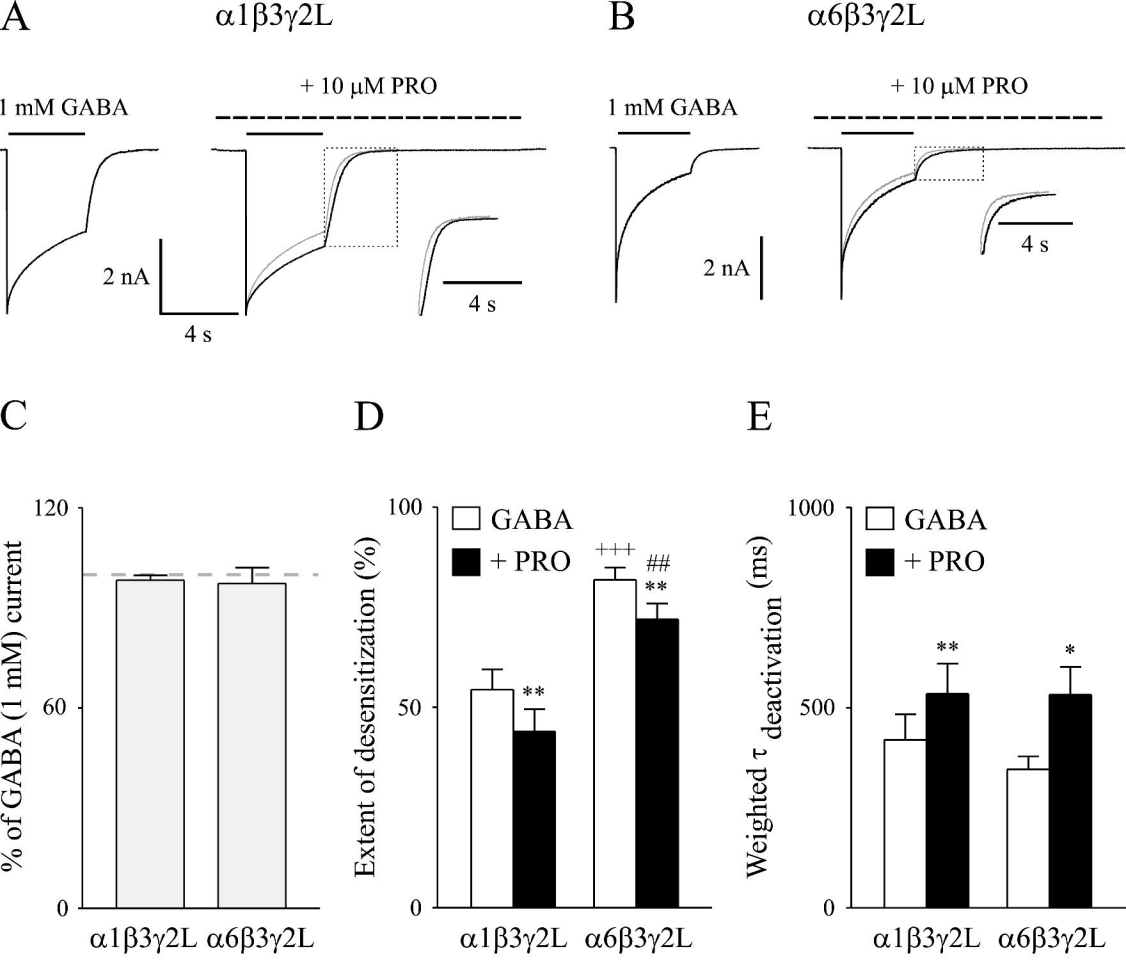
A
α1β3γ2L
1 mM GABA
+ 10 μM PRO
2 nA
4 s
4 s
B
α6β3γ2L
1 mM GABA
+ 10 μM PRO
2 nA
4 s
C
% of GABA (1 mM) current
120
60
0
α1β3γ2L
α6β3γ2L
D
Extent of desensitization (%)
100
50
0
GABA
+ PRO
+++
##
**
**
α1β3γ2L
α6β3γ2L
E
Weighted τ deactivation (ms)
1000
500
0
GABA
+ PRO
**
*
α1β3γ2L
α6β3γ2L

Fig. 4

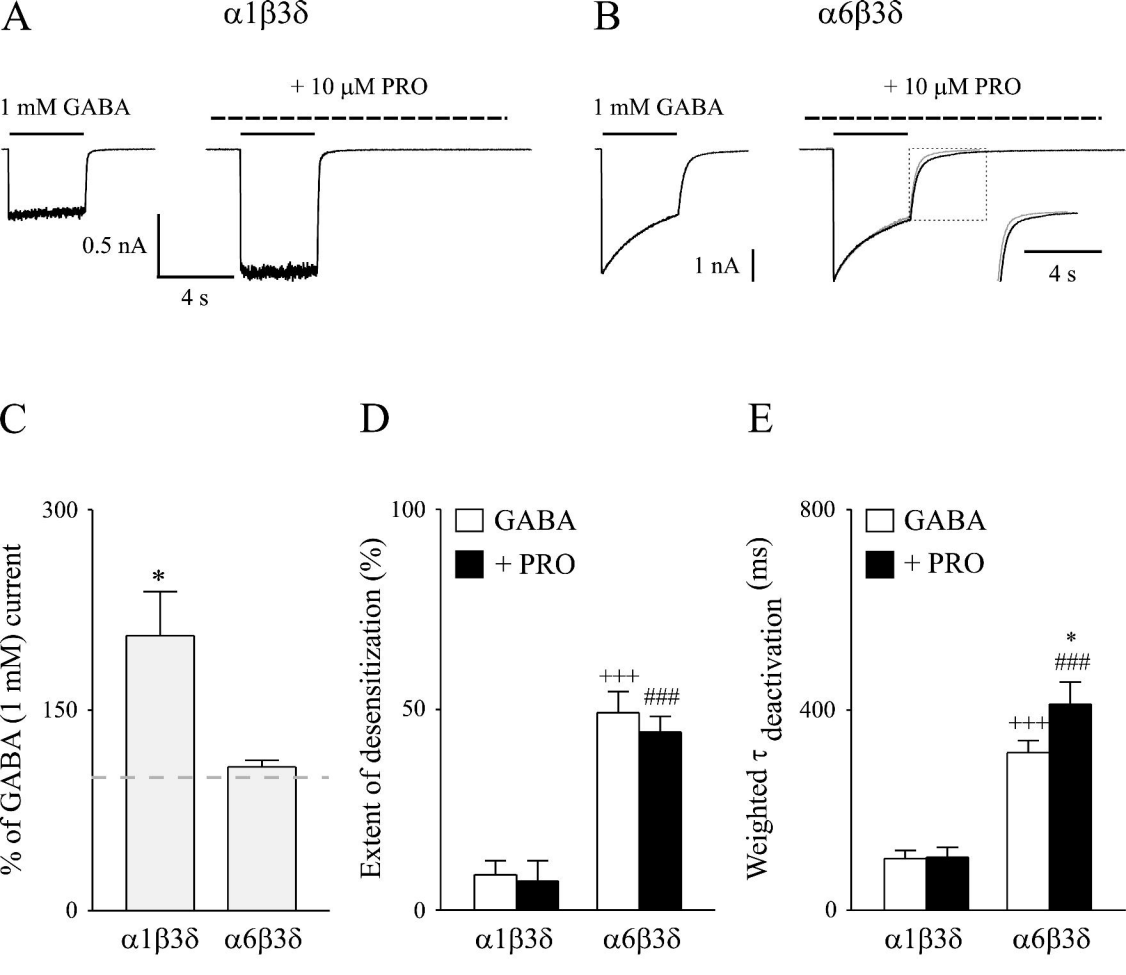
A
α1β3δ
1 mM GABA
+ 10 μM PRO
0.5 nA
4 s
B
α6β3δ
1 mM GABA
+ 10 μM PRO
1 nA
4 s
C
% of GABA (1 mM) current
300
150
0
*
α1β3δ
α6β3δ
D
Extent of desensitization (%)
100
50
0
GABA
+ PRO
+++
###
α1β3δ
α6β3δ
E
Weighted τ deactivation (ms)
800
400
0
GABA
+ PRO
+++
*
###
α1β3δ
α6β3δ

Fig. 5