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Separate Domains for Desensitization of GABA ρ_1 and β_2 Subunits Expressed in *Xenopus* Oocytes

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Abstract. Desensitization of ligand-gated receptor channels is an intrinsic feedback mechanism and prevents the receptor/channels from becoming overly activated thereby maintaining biological function of the nervous system. Desensitization also plays an important role in neuronal plasticity. By taking advantage of biophysical and pharmacological diversities of GABA β_2 subunits from the brain and ρ_1 subunits from the retina, structural determinants that confer agonist-induced desensitization were identified. A synthetic chimeric receptor/channel was created from the β_2 and ρ_1 subunits for this investigation. The chimera was constructed from the extracellular N-domain of the β_2 subunit, extending from the amino terminus to the beginning region of the M1 transmembrane segment, and from the C-domain of the ρ_1 subunit extending from the M1 transmembrane segment to the carboxyl terminus. The C-domain region included the M1 to M4 transmembrane regions and the large intracellular loop between the M3 and M4 transmembrane segments. Homo-oligomeric GABA β_2, ρ_1, and β_2/ρ_1 chimeric receptor/channels were individually expressed in *Xenopus* oocytes, and the desensitization characteristics attributable to each type of subunit were compared. Results from the present study reveal that motifs in the amino-terminal and carboxyl-terminal domains of the β_2 subunit conferred the agonist-induced desensitization; chloroform modulation was linked to specific phases of the GABA-activated current decay.

Key words: Receptor/channel — Retinal ρ_1-subunit — Chimera — Voltage-clamp — Barbiturates

Introduction

GABA (γ-aminobutyric acid) is a major inhibitory neurotransmitter in the central nervous system and in the retina. The native GABA receptor/channel is composed of a pentameric protein with an integral GABA-gated Cl^- channel (Olsen & Tobin, 1990). In the central nervous system (CNS), the GABA receptor/channel is also a drug-targeting protein with distinct binding sites for allosteric modulators, including benzodiazepines, barbiturates, neurosteroids, bicuculline and picrotoxin (Olsen & Macdonald, 1994; Sieghart, 1989). Barbiturates and benzodiazepines potentiate the GABA-induced Cl^- currents while bicuculline and picrotoxin inhibit the currents (DeLorey & Olsen, 1992; Olsen et al., 1990). There are at least five distinct clases of subunits in GABA receptor/channels in the brain: α, β, γ, δ and ε. Sixteen different $GABA_A$ receptor subunits (6 α, 4 β, 3 γ, δ, ε and π) have been cloned from mammalian cells (Lolait et al., 1989; Schofield et al., 1987; Shivers et al., 1989; Ymer et al., 1989; Hedblom & Kirkness, 1997; Davies et al., 1997). Analysis of amino acid sequences reveals a high degree of identity (60 to 80%) within each subunit type. A 20 to 40% sequence identity exists between different subunit types (Olsen & Tobin, 1990; Shivers et al., 1989).

Recently, two novel subunits, GABA ρ_1 and GABA ρ_2, have been cloned from the retina (Cutting et al., 1991; Kusama et al., 1993; Shimada, Cutting & Uhl, 1992; Wang, Guggino & Cutting, 1994). Unlike receptors containing α, β, γ, or δ subunits, GABA receptor/channels with ρ_1 and ρ_2 subunits are insensitive to $GABA_A$ receptor modulators, including bicuculline, hexobarbital, and diazepam, but they are reversibly inhibited by halogenated fatty acids and $ZnCl_2$ (DelRaso, Huang & Lu, 1996; Wang, 1995). Homo-oligomeric receptors containing ρ_1 and ρ_2 receptor/channels are less sensitive to muscimol and are desensitized much more slowly than $GABA_A$ receptors are (Kusama et al., 1993; Wang et al., 1994).

Desensitization, the decay of current in the presence of ligand, is a biophysical characteristic of most ligand-gated receptor/channels, especially the nACh receptor/

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channel and GABA receptor/channel (Lohse, 1993). Desensitization of the GABA receptor/channel functions as a kind of negative-feedback loop that prevents the receptor/channel from becoming overly activated and serves to maintain physiological function of the nervous system. It has been reported that general anesthetics allosterically regulate some ligand-gated channels and modify the kinetics of ligand-gated receptor/channel desensitization (Hara, Kai, & Ikemoto, 1993; Orser et al., 1994; Raines, Rankin & Miller, 1995; Yang & Olsen, 1987). Although desensitization occurs only at moderately high concentrations of volatile general anesthetics, chloroform-induced desensitization of GABA receptor/channels is of clinical interest. It has been suggested that neuroexcitation observed during volatile general anesthesia may be due to GABA receptor/channel desensitization and disinhibition following the rapid application of a large bolus of drugs (Hara et al., 1993).

In addition to being subject to regulation by the ligand, desensitization is also determined by the intrinsic properties of the GABA receptor/channel itself. For example, the $GABA_A$ receptor/channel shows a high degree of desensitization, but, as noted above, the GABA receptor/channel containing ρ_1 and ρ_2 subunits, shows a very small degree of desensitization (Wang et al., 1994). As yet, the molecular basis of desensitization in GABA receptor/channel is not completely understood. In an attempt to elucidate the process of desensitization, we have taken advantage of the fact that receptors with ρ_1 subunits are desensitization resistant and that desensitization has fast and slow components in receptors with β_2 subunits. Our study model was a chimeric subunit constructed from two domains. One was the large extracellular domain of the β_2 subunit extending from the amino-terminus to the beginning of the M1 transmembrane segment; the other was the domain of the ρ_1 subunit containing the M1 to M4 transmembrane regions and the large intracellular loop between the M3 and M4 transmembrane segments. Pentobarbital and chloroform were used in this study of the domain-specific effect of agonist-induced GABA receptor/channel desensitization.

Materials and Methods

Construction of the Chimeric GABA Receptor/Channel

The GABA receptor/channel chimera was constructed from cDNA that encode genes for the rat brain GABA β_2 subunit and the human retinal GABA ρ_1 subunit. The sequences of these fragments were based on published amino acid sequences of GABA receptor channels (Cutting et al., 1991; Schofield et al., 1987; Ymer et al., 1989). The particular cDNA fragments that were chosen encoded that portion of the GABA β_2 subunit from the amino terminus to the beginning of the M1 transmembrane segment (defined as the N-domain) and that portion of the GABA ρ_1 subunit from the M1 transmembrane segment to the carboxyl terminus (defined as the C-domain). The final fragment, thus, was composed of two intracellular loops (between M1-M2 and M3-M4) and four transmembrane segments. Amplification of these cDNA fragments was done by a polymerase chain reaction (PCR) using cDNAs encoding β_2 and ρ_1 subunits as templates. The amplified fragments were then inserted into PCR_3 plasmid following the protocol of the Eukaryotic TA Cloning Kit (Invitrogen). Vectors containing PCR-amplified fragments were confirmed by restriction enzyme digestion (*Esp I*) and identification on agarose gel.

The unidirectional fragment encoding the GABA ρ_1 C-domain was cleaved by restriction enzyme digestion using *Esp I, Kpn I,* and *Not I* (NEBiolabs) and purified from low-melting gel by β-agarase digestion. The cDNA for encoding the chimeric receptor/channel subunit was constructed by inserting the unidirectional cDNA fragment encoding the ρ_1 subunit C-domain into the linearized PCR_3-β_2 plasmid at a location downstream to the β_2 N-domain fragment. The connecting junction of the two parts of the chimera was at position T_{233}, the first transmembrane region of the β_2 subunit, and position M_{291} in the ρ_1 subunit. The junction was included the *Esp I* restriction enzyme site. The correct orientation and possible frame shift of the constructed chimeric subunit were examined by restriction enzyme digestion and then confirmed by DNA sequencing using Sequenase V2.0 (Amersham).

Synthesis of cRNA in vitro

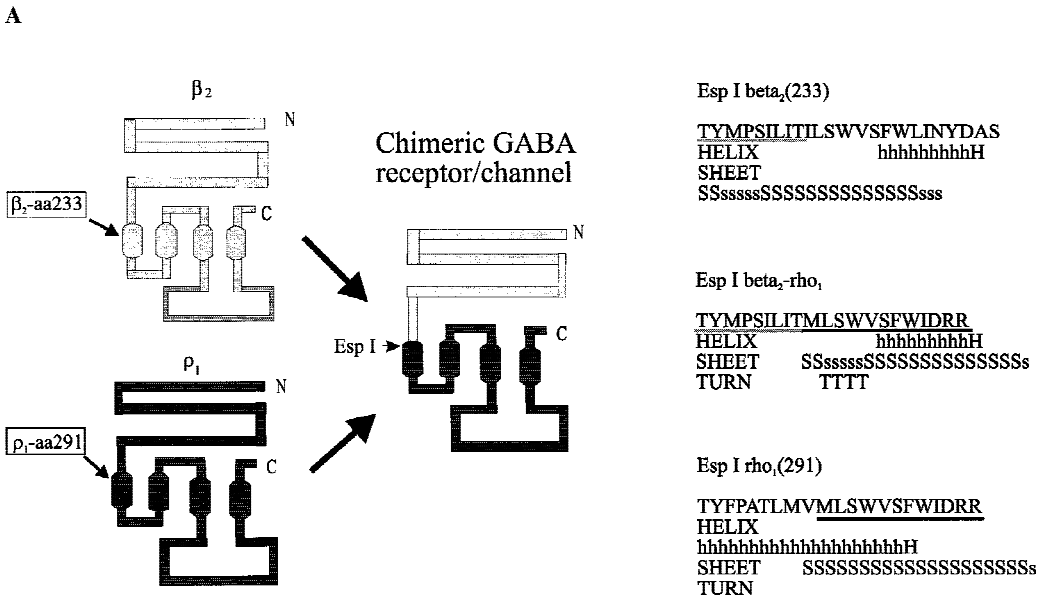
The cDNAs used to synthesize cRNA were linearized by the restriction enzyme *Xba I* (NEBiolabs). Transcription reactions were carried out using T_7 RNA polymerase and 10 μg linearized DNA in the presence of diguanosine triphosphate, an analogue of catabolite gene activator protein (CAP). Synthesis was in vitro at 37°C for 2 hr. The DNA template was removed by digestion with three units of RNase-free DNase I and ethanol precipitation. The synthesized cRNA was dissolved in diethyl-pyrocarbonate (DEPC)-treated water (1 μg/μl). Quality and quantity of transcribed cRNAs were monitored by gel electrophoresis and measured with a RNA calculator (Pharmacia).

Expression of GABA Receptor/Channels in *Xenopus* Oocytes

Adult female *Xenopus laevis* frogs (*Xenopus* I) were anesthetized by immersion in 0.15% tricaine methanesulfonate (Ayerst) for 20 min. Ovarian lobes were excised, and oocytes were defollicated by digestion with 3 mg/ml Type IA collagenase (Sigma, St. Louis, MO) in OR-2 solution (in mM): 100 NaCl, 2 KCl, 1 $MgCl_2$, 5 HEPES-TRIS, pH 7.5 for 3 hr. Stage V and VI oocytes were selected and stored at 18°C in MBS solution (in mM): 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.3 $Ca(NO_3)_2$, 0.4 $CaCl_2$, 0.8 $MgSO_4$, 1.5 HCl, penicillin, 100 units/ml, streptomycin, 100 mg/ml, pH 7.6. After a 24-hr recovery period, each oocyte was injected with 50 ng cRNA using a positive displacement microinjector (Drummond Scientific). Injected oocytes were used for voltage-clamp experiments after 72 hr incubation at 18°C in MBS.

Whole-Cell Current Recording

Two-microelectrode voltage-clamp experiments were performed in a chamber continuously perfused with normal Ringers solution containing (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, 1.2 $CaCl_2$, HEPES-NaOH, pH 7.4 and at a rate of 30 ml/min at room temperature (22°C). Microelectrodes with resistances of 1.5 to 2.2 MΩ were made by a horizontal puller (PD-5, Narishige) and filled with 3M KCl. GABA-induced currents were recorded with an Axonclamp 2A amplifier (Axon Instruments, Foster City, CA) using the two-microelectrode voltage-clamp



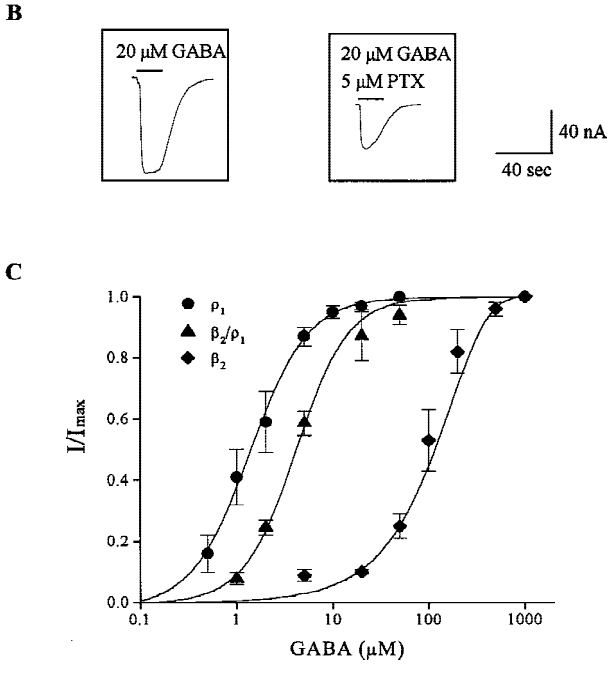


Fig. 1. Functional expression of β_2/ρ_1 chimeric receptor/channels in *Xenopus* oocytes. (*A*) Diagrammatic representation of synthetic β_2/ρ_1 chimeric GABA subunit. The scheme in the figure represents the chimera that ultimately was used in this study. The design of the chimera was constructed under the guidance of computer analysis with the DNAsis program. With this design, the secondary structures of the protein were conserved. (*B*) Whole-cell currents were activated in oocytes injected with 50 ng of cRNA encoding β_2/ρ_1 chimeric subunits. The GABA-activated current was partially blocked by 5 μM picrotoxin (PTX). Horizontal bars above each trace represent the period during which the agonist was applied. (*C*) Dose-response relationship of GABA-activated currents in oocytes expressing homo-oligomeric receptor/channels of β_2, β_2/ρ_1, or ρ_1 subunits. Fractional current (I/I_{max}) activated by GABA was plotted as a function of GABA concentrations. I_{max} represents the current induced by initial application of 500 μM GABA and was a reference for normalization. I represents the current activated by different concentrations of GABA. GABA was removed from the bath solution by perfusion with normal Ringers solution for at least 5 min after each application. Data points were fitted by the Hill equation, $I/I_{max} = [G]^n/[EC_{50}{}^n + [G]^n]$, where n represents the Hill coefficient and [G] represents GABA concentration. All data points were collected from three to ten independent experiments and plotted as the mean ± SE of the mean.

mode. The bath was connected through an Ag-AgCl-Agar 3M KCl bridge to the voltage-recording amplifier. Membrane currents were measured in Ringers solution with a holding potential of −80 mV. Currents were filtered through a four-pole bessel filter at 500 Hz. Data acquisition was performed by a 486 IBM computer using pCLAMP software (Axon Instruments), and graphics were generated using *Origin* software (Microcal) with a pCLAMP module.

Results

Construction of the Chimera

The structure of a chimeric subunit composed of the N-domain from the GABA β_2-subunit and the C-domain of the GABA ρ_1 subunit is shown in Fig. 1*A*. The first subunit that we attempted to construct with the junction of the GABA β_2 N-domain and GABA ρ_1 C-domain was at T_{216} (β_2 subunit) and T_{273} (ρ_1 subunit). A lack of response to GABA indicated that this chimeric subunit failed to functionally express with α_1 and γ_2 subunits in cRNA-injected oocytes (*data not shown*). An analysis of the protein secondary structure of the junction in the chimera and of corresponding sites in β_2 and ρ_1 subunits indicated that the protein secondary structure at the junction site was completely altered. A second construction of a chimera using $\beta_2(233)$ and $\rho_1(291)$ as the junction site was done with the aid of computer analysis. In this

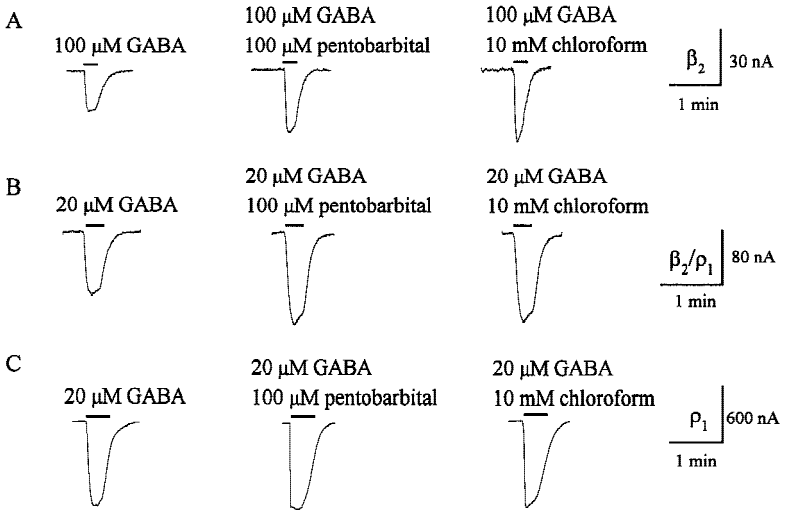


Fig. 2. Effects of pentobarbital and chloroform on homo-oligomeric ρ_1, β_2, or β_2/ρ_1 chimeric receptor/channels expressed in *Xenopus* oocytes. Representative whole-cell currents were elicited after application of 100 μM GABA, 100 μM GABA plus 100 μM pentobarbital, or 100 μM GABA plus 10 mM chloroform to oocytes expressing the β_2 subunit (*A*), or after application of 20 μM GABA, 20 μM GABA plus 100 μM pentobarbital, or 20 μM GABA plus 10 mM chloroform to oocytes expressing the β_2/ρ_1 chimeric subunit (*B*), or ρ_1 subunit (*C*). All oocytes were injected with the same amount of respective cRNAs (50 ng). Inward currents were recorded in normal Ringers solution at a holding potential of −80 mV. Agonists were applied in each experiment for 20 sec to allow the current to reach the peak response. Horizontal bars above each trace represent the application period of agonists. Downward deflections indicate inward currents. Note the differences in scale for different subunits. Data obtained from 4–6 independent experiments were very consistent.

chimera, the protein's secondary structure was largely conserved in the junction site of the β_2 and ρ_1 subunits (Fig. 1*A*).

Functional Expression of Homo-oligomer GABA Subunits

A recording of the whole-cell GABA-activated current in *Xenopus* oocytes injected with the $\beta_{2(233)}/\rho_{1(291)}$ chimera cRNA is shown in Fig. 1*B*. This figure also illustrates that the GABA-activated inward currents were partially blocked by 5 μM picrotoxin, a physical blocker of the Cl^- ion conductive pore. The dose-response curve to GABA for the receptor with the β_2/ρ_1 chimeric subunit was shifted to the left compared to that for the receptor with β_2 subunits and to the right compared to that for the receptor with ρ_1 subunits (Fig. 1*C*). The EC_{50} values of GABA activated currents were 1.8 μM for the receptor with ρ_1 subunits, 4.1 μM for the receptor with the β_2/ρ_1 chimeric subunits, and 103 μM for the receptor with the β_2 subunits.

Potentiation Effect of Pentobarbital and Chloroform on the GABA Receptor/Channel

GABA-activated currents obtained from oocytes injected with either cRNA encoding the GABA β_2 subunit or cRNA encoding the β_2/ρ_1 chimera were potentiated by 100 μM pentobarbital and by 10 mM chloroform, a general anesthetic drug with an EC_{50} of 1.8 mM for nAch receptors (Fig. 2*A* and *B*, respectively). However, the presence of 100 μm pentobarbital or 10 mM chloroform did not enhance GABA-activated currents in oocytes expressing the GABA ρ_1 subunit (Fig. 2*C*). The percent of enhancement of currents induced by coapplication of GABA and chloroform was 84.0 ± 16.0% in oocytes injected with the cRNA for β_2 subunits, 58.3 ± 6.8% in oocytes with the β_2/ρ_1 chimera cRNA and 0.01 ± 0.002% in oocytes with the cRNA for ρ_1 subunits ($n = 3$ to 7). The potentiation effect of chloroform on both the GABA receptor/channels with β_2 subunits and those with chimeric subunits was significantly different from the effect on the receptor/channels with ρ_1 subunits (all data presented as mean + SE, $n = 7$, Student *t*-test, $P < 0.001$).

The enhancement of GABA-induced current by pentobarbital was 41.7 ± 6.9% in oocytes with cRNA encoding for the GABA β_2 subunits and 65.6 ± 13.6% for the oocytes injected with cRNA for the chimeric subunits. This difference was not significant ($P > 0.05$). As was observed with the coapplication of chloroform, there was no effect of pentobarbital on oocytes carrying cRNA for encoding of ρ_1 subunits. The effect of pentobarbital in oocytes carrying cRNA for the ρ_1 subunits was significantly different from that observed in cells with cRNA for either β_2 or β_2/ρ_1 subunits ($P < 0.001$). The observed resemblance in behavior conferred by the β_2/ρ_1 chimeric subunit and the β_2 subunit in drug-induced potentiation indicates that the effects of pentobarbital and chloroform on GABA-mediated current may be determined by the N-domain of the β_2 subunit.

Agonist- and Drug-Induced Desensitization of the GABA Receptor/Channel

Properties of desensitization of homo-oligomeric receptor/channels formed by GABA ρ_1, β_2, or β_2/ρ_1 chimeric

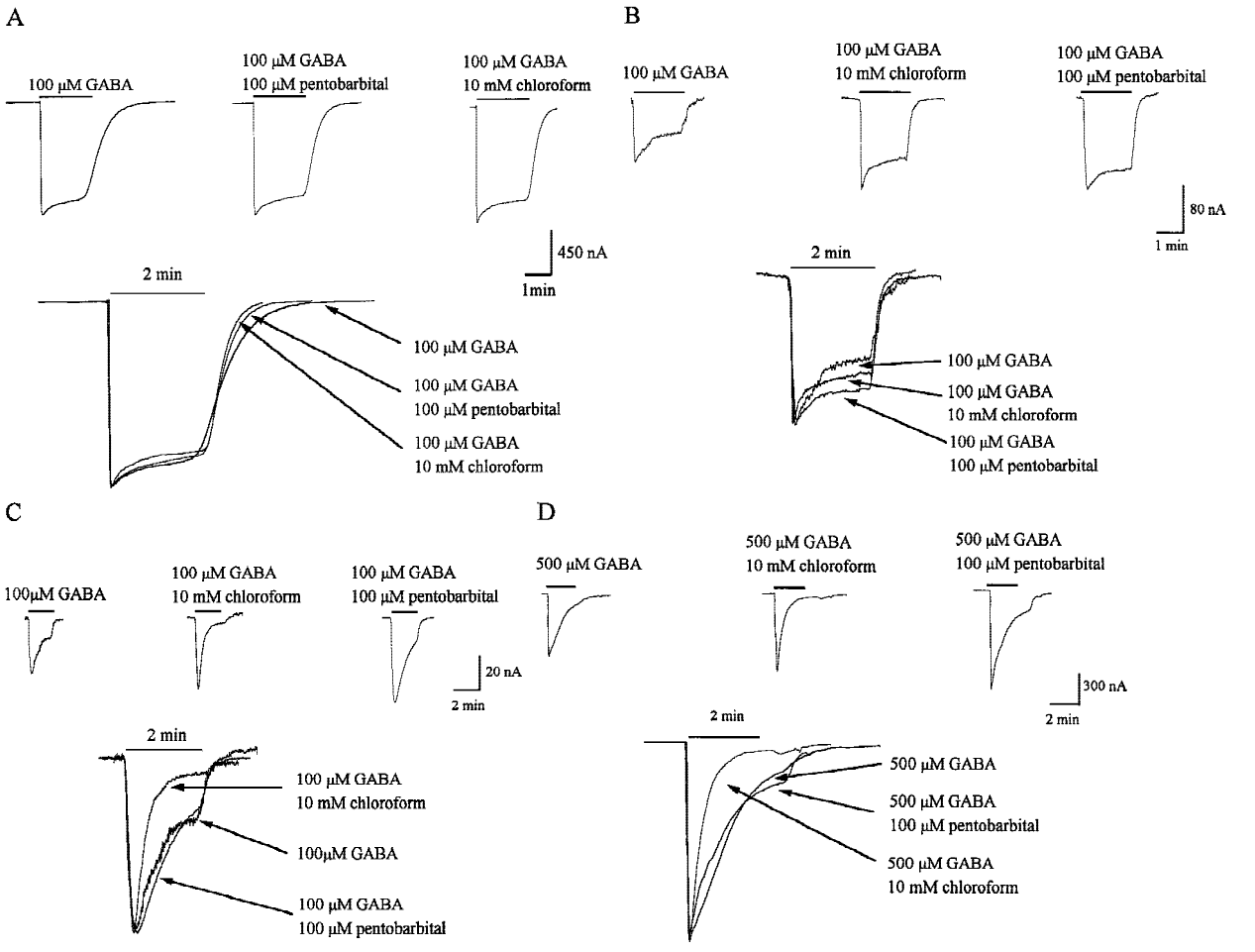


Fig. 3. Desensitization of homo-oligomeric GABA receptor/channels with β_2, ρ_1, or β_2/ρ_1 chimeric subunits and a hetero-oligomeric receptor/channel of α_1, β_2, γ_2 combination. Comparison of GABA, pentobarbital, and chloroform effects on the desensitization of GABA receptor/channels having ρ_1 subunits (*A*), β_2/ρ_1 chimeric subunits (*B*), β_2 subunits (*C*), or α_1, β_2, γ_2 combination (*D*). Currents were induced by fast perfusion of 100 μM GABA (receptors with β_2, ρ_1, or β_2/ρ_1 chimeric subunits) or 500 μM GABA (receptors with α_1, β_2, γ_2 subunits). The perfusion speed was approximately 400 μl/sec; the bath solution was totally replaced in 250 msec. Horizontal bars represent continuous perfusion of agonists (for two min). Currents were recorded at a membrane potential of −80 mV and were very consistent in 6–8 individual experiments. Current traces were superimposed by a scale-matching technique and are presented in the lower panel of each group.

subunits were compared to those of heterooligomeric receptor/channels composed of α_1, β_2, γ_2 subunits coexpressed in *Xenopus* oocytes. It has been shown that desensitization of the GABA receptor/channel is affected by several factors, including membrane potentials, agonist dosage, and chemical modulations. The use of a fixed dose of GABA and a constant membrane potential facilitated our examination of the process of desensitization in the presence of the various subunits of the GABA receptor/channel. In accordance with the dose-response curves shown in Fig. 1*C*, GABA was applied to oocytes expressing either the ρ_1 or β_2/ρ_1 chimeric subunit (20 μM GABA) or the β_2 subunit (100 μM GABA) at a holding potential of −80 mV.

Relative to each other, the decline of the GABA-activated current in the oocytes was very slow in the presence of homo-oligomeric GABA receptor/channels with ρ_1 subunits (Fig. 3*A*), intermediate in cells with receptors modified with the β_2/ρ_1 chimera (Fig. 3*B*), and rapid in the presence of receptor/channels with β_2 subunits (Fig. 3*C*). The hetero-oligomeric receptor channel (α_1, β_2, γ_2) also demonstrated a rapid decline phase (Fig. 3*D*). Decay of GABA-activated currents in cells with homo-oligomeric β_2-subunit receptor/channels and with hetero-oligomeric receptor/channels were biphasic, having a fast and a slow component. The decline phase of the GABA-activated current in the presence of the GABA receptor/channels with the chimeric β_2/ρ_1 subunits was attenuated compared to that in cells with GABA receptor/channels with β_2 or α_1, β_2, γ_2 subunits. To better demonstrate these observations, the activation curves for these four types of receptor/channels were

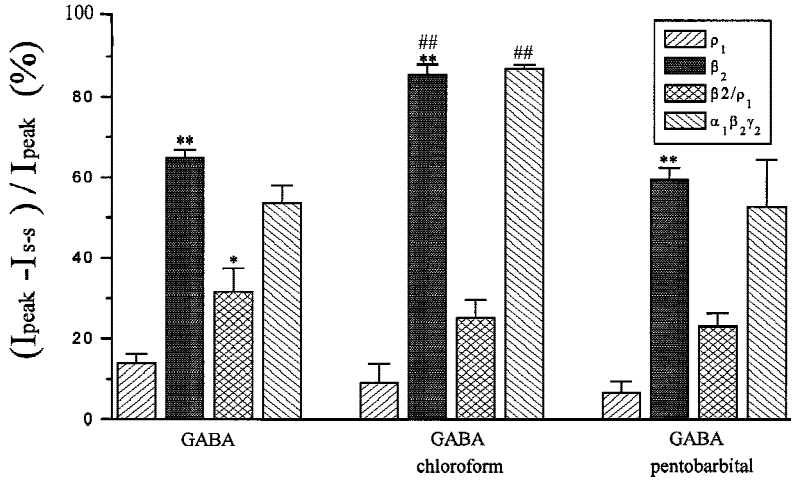


Fig. 4. Analysis of the effects of GABA, chloroform, and pentobarbital on desensitization of GABA ρ_1, β_2, β_2/ρ_1, or α_1, β_2, γ_2 receptor/channels. The degree of desensitization was calculated by $(I_{peak} - I_{s\text{-}s})/I_{peak}$, where I_{peak} represents the GABA-activated current measured at the peak time, and $I_{s\text{-}s}$ represents the steady-state current measured at the end of the two-minute application of agonists. All data were collected from three to eight independent experiments. Columns and error bars represent means with standard error of the mean. *Indicates significant differences at an α level of 0.05; **indicates significant differences at an α level of 0.001. ##indicates a significant difference at the α level of 0.001 between the application of GABA and the coapplication of GABA and 10 mM chloroform. GABA (100 μM) was applied to ρ_1, β_2, or β_2/ρ_1 chimeric subunits, and 500 μM GABA was used for coexpressed α_1, β_2, γ_2 subunits.

scaled to match their amplitudes and superimposed (lower panels of Fig. 3*A* to *D*).

The effects of pentobarbital and chloroform on desensitization of GABA-induced currents were analyzed by calculating the decay ratio of currents using the equation of:

$$decay\ ratio = (I_{peak} - I_{s\text{-}s})/I_{peak}$$

where I_{peak} represents the current amplitude at the peak time and $I_{s\text{-}s}$ is the relative steady-state current measured two minutes after activation of the current. The current generated by oocytes expressing ρ_1 subunits in GABA receptor/channels was not affected by the presence of either 10 mM chloroform or 100 μM pentobarbital as indicated by decay ratios of 14.0 ± 2.24% ($n = 6$) for application of GABA alone (control), 9.2 ± 4.64% ($n = 3$) for coapplication of GABA and chloroform, and 8.8 ± 2.77% ($n = 3$) for coapplication of GABA and pentobarbital (Fig. 4). These ratios were not significantly different ($P > 0.05$).

In oocytes expressing the β_2 subunit in the GABA receptor/channel, on the other hand, 10 mM chloroform markedly accelerated the decay phase of the current (Fig. 3*C*). Figure 4 shows that, in the presence of β_2 subunits, the decay ratio of the current induced by 100 μM GABA alone (control) was 65.0 ± 1.94% ($n = 7$) and increased to 85.7 ± 2.67% ($n = 3$) after coapplication of the chloroform. This increase was significant ($P < 0.001$). In contrast, the addition of 100 μM pentobarbital did not produce a significant increase in the decay ratio (59.7 ± 2.91%; $n = 3$, $P > 0.05$) even though, as seen in Fig. 3*C*, it had a much stronger potentiation effect on the receptor/channel than did chloroform. The GABA-activated current in oocytes expressing the β_2/ρ_1 chimeric subunit was potentiated by both 10 mM chloroform and 100 μM pentobarbital (Fig. 3*B*); however, as shown in Fig. 4, no significant change in decay ratios was observed ($P > 0.05$). Decay ratios of this current for GABA alone, pentobarbital coapplied with GABA, and chloroform coapplied with GABA were 31.6 ± 5.70% ($n = 7$), 25.2 ± 4.56% ($n = 8$), and 23.3 ± 2.96% ($n = 3$), respectively (Fig. 4).

Desensitization was also observed in oocytes coexpressing receptor/channels containing α_1, β_2, γ_2 subunits. The decay ratio of the current was 53.8 ± 4.19% ($n = 6$) for the application of 500 μM GABA alone (Fig. 4). Coapplication of GABA and 100 μM pentobarbital did not alter the decay ratio (52.1 ± 7.3%; $n = 4$), but coapplication of GABA with 10 mM chloroform significantly increased the decay ratio to 87.3 ± 0.95% ($P < 0.001$). The results for agonist- and drug-induced desensitization are summarized in the Table.

Kinetics of GABA Receptor/Channel Desensitization

A scale-matching method was also used to compare the current traces recorded from oocytes expressing the homo-oligomeric β_2, ρ_1, and β_2/ρ_1 chimeric receptor/channels in the absence of and in the presence of 10 mM chloroform (Fig. 5*A*). Current traces from cells with β_2 subunits and cells with β_2/ρ_1 chimeric subunits were enlarged and superimposed upon the trace from oocytes with ρ_1 subunits. In the presence of GABA alone, the decay of the initial portion of the fast phase of the GABA-activated current was nearly the same in the presence of β_2 subunits or β_2/ρ_1 chimeric subunits. However, the later phase of the fast decay was substantially accelerated in the presence of β_2 subunits as compared to that in the β_2/ρ_1 chimera (Fig. 5*A*, left panel). In a com-

Table. GABA receptor/channel desensitization

Subunit	Decay rate (%)			Potentiation		Phase acceleration		Time constant (s)	
	GABA	GABA + Pento	GABA + Chlor	GABA + Pento	GABA + Chlor	GABA	GABA + Chlor	GABA	GABA + Chlor
β_2	65 ± 1.9	60 ± 2.9	86 ± 2.7*	⊕⊕	⊕	Fast Slow	Initial accel. Late accel.	τ_f: 6 ± 0.8 τ_s: 73 ± 15.4	τ_f: 3 ± 1.7 τ_s: 20 ± 0.3
β_2/ρ_1	32 ± 5.7	25 ± 4.6	23 ± 3.0	⊕	⊕	Initial like β_2 Slower than β_2	Initial like β_2 Late like ρ_1	τ_f: 25 ± 5.5 τ_s: 61 ± 20.0	τ_f: 8 ± 1.9 No change
ρ_1	14 ± 2.2	9 ± 2.8	9 ± 4.6	⊖	⊖	Slow Slow	Unaffected Unaffected	 τ_s: 66 ± 2.8	 No change
$\alpha_1\beta_2\gamma_2$	54 ± 4.2	52 ± 7.3	87 ± 1.0*	⊕	⊕	Fast Slow			

Symbols: ⊕ = positive response; ⊖ = no response; * = significant difference ($P < 0.05$).

parable fashion, with the coapplication of chloroform, the initial portion of the fast decay was accelerated in the presence of both β_2 subunits and β_2/ρ_1 chimeric subunits. In the later portion of the fast decay phase, however, the acceleration was extremely rapid in the presence of β_2 subunits compared to acceleration in the presence of β_2/ρ_1 subunits (Fig. 5*A*, right panel), indicating that chloroform was more effective in desensitizing receptors with β_2 subunits than those with the chimeric subunits.

For the purposes of quantification, the decay portions of the GABA-activated currents shown in Fig. 5*A* were analyzed and plotted (Fig. 5*B*). The decay phases were usually best fitted with a two-exponential function for the homo-oligomeric β_2/ρ_1-subunit and β_2-subunit receptor/channels and with a single-exponential function for the homo-oligomeric ρ_1-subunit receptor/channels. With the addition of chloroform, the time constant for the initial rapid component of the fast decay (τ_f) was reduced from 5.7 ± 0.8 sec (GABA only) to 2.9 ± 1.7 sec in the presence of the β_2 subunits and from 25.4 ± 5.5 sec (GABA only) to 8.4 ± 1.9 sec in the presence of the β_2/ρ_1 chimeric subunits. The reduction in the time constant was significant in both instances ($P < 0.05$). Time constants for the later slow component (τ_s) of the fast current decay where also accelerated when the receptor/channels had β_2 subunits, changing from 72.5 ± 15.4 sec (GABA only) to 20.3 ± 0.7 sec. In the presence of receptor/channels with β_2/ρ_1 chimeric subunits, however, τ_s was not significantly affected by the coapplication of GABA and chloroform ($P > 0.05$). The data for the time constants is also summarized in the Table.

Discussion

Characteristics of GABA B_2/P_1 Chimeric Receptor/Channel

In the present study, possible agonist-induced effects by separate domains of GABA β_2 subunits were investigated using a synthetic receptor/channel containing chimeric subunits constructed from the amino-terminal structure of a β_2 subunit and the portion of a ρ_1 subunit extending from the M1 transmembrane region to the carboxyl terminal. cRNA encoding for the chimeric subunits was injected into *Xenopus laveis* oocytes. We chose to express the GABA β_2, ρ_1, and chimeric β_2/ρ_1 homo-oligomeric receptor/channels in *Xenopus* oocytes because homo-oligomeric receptor/channels formed by these subunit types presented much higher levels of expressed GABA-activated currents compared to other GABA receptor subunits, such as α and γ (*data not shown*). Taking advantage of biophysical and pharmacological diversities in β_2 and ρ_1 subunits, we have identified determinant domain that confer subunit-selective modulation of GABA receptor/channel function by anesthetic drugs. The GABA receptor/channel is a target protein of anesthetic drugs, such as pentobarbital, and other volatile general anesthetics, such as chloroform (Amin & Weiss, 1993; Hara et al., 1993; Orser et al., 1994; Twyman, Rogers, & Macdonald, 1989*a*,*b*; Young & Sigman, 1983). The possible modulation sites in the receptor/channel are just began to be understood (Bellelli et al., 1997; Mihic et al., 1997; Moody et al., 1997).

Data presented in this study extend our understanding of the structural mechanism of ligand-gated receptor/channel desensitization.

Among the three homo-oligomers, we compared the properties of channel desensitization by examining the decay of GABA-activated currents and by analysis of the decay ratio. We compared potentiation effects, phase acceleration in the early stages of current activation, and time constant characteristics of the phases. The effects of potentiation by a hetero-oligomer, the α_1, β_2, γ_2 receptor/channel, were also evaluated. It was demonstrated in the study of channel desensitization that the decay of GABA-activated current was rapid and biphasic in oocytes expressing the hetero-oligomeric receptor/channel and in oocytes expressing the homo-oligomeric receptor/channel with β_2 subunits. Decay of the GABA-

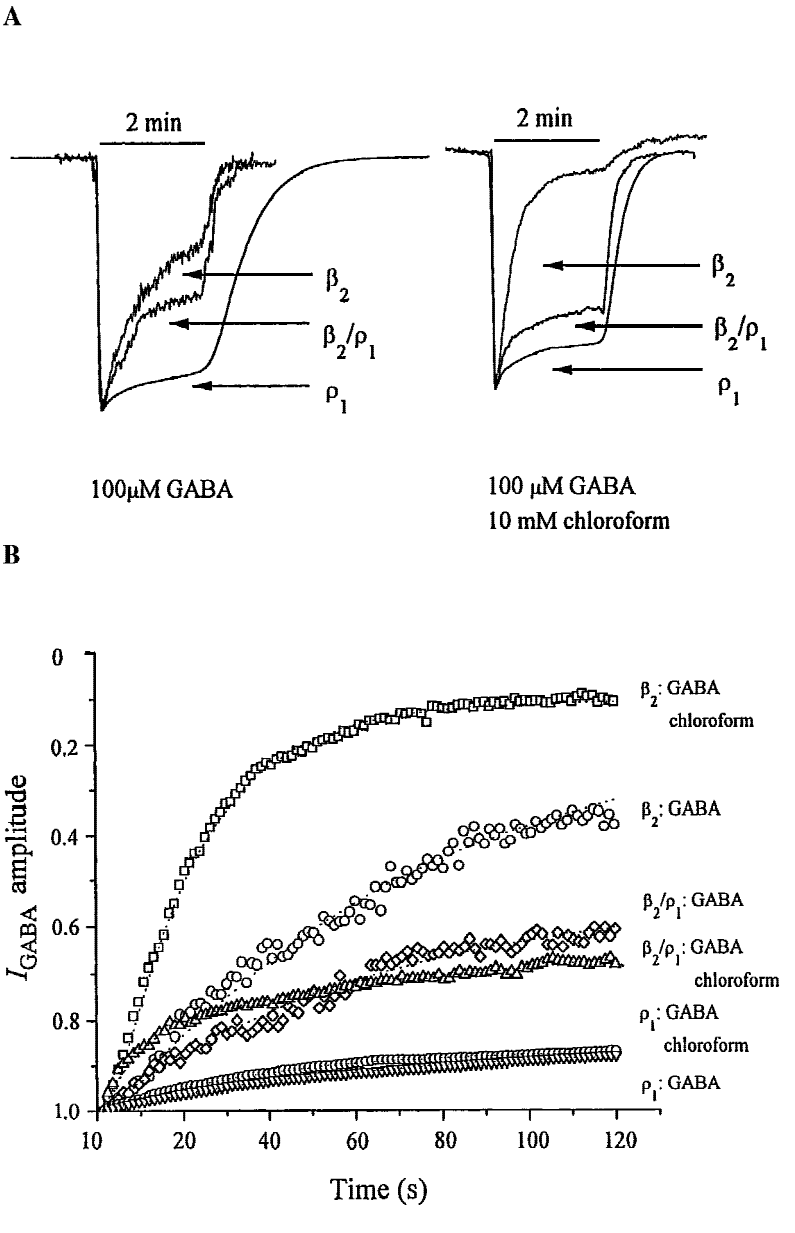


Fig. 5. Comparison of desensitization of GABA ρ_1, β_2, β_2/ρ_1, or α_1, β_2, γ_2 chimeric subunits induced by GABA or GABA plus 10 mM chloroform. (*A*) Superimposed current traces showing activation by GABA in the absence (plots on left) and in the presence of chloroform (plots on right). Current traces with smaller amplitudes were enlarged for the purpose of matching the scale. Horizontal bar represents the period of the application of agonists. (*B*) Decay curves for GABA-induced currents plotted from the recordings shown in panel (*A*). Decay curves from expressed β_2 or β_2/ρ_1 chimeric subunits were fitted with a two-exponential function; decay curves from the ρ_1 subunits were fitted with a single-exponent function. All current decays were normalized to the peak current induced in oocytes expressing each subunit type. Time constant (τ) is indicated as τ_f for the fast phase component or τ_s for the slow phase component.

activated current was slow and greatly attenuated in oocytes with ρ_1-subunit receptor/channels. These results are in agreement with results from previous studies (Frosch, Lipton & Dichter, 1992; Moss et al., 1992; Oh & Dichter, 1992). In the oocytes expressing the chimera, the decay was intermediate, showing evidence of biphasic features; the amplitude of the current was attenuated compared to that in oocytes expressing the homo-oligomeric β_2-subunit receptor/channels and in the hetero-oligomeric receptor/channels.

A comparison of the decay ratios and potentiations, which are shown in the Table, demonstrates that the decay of GABA-activated currents induced by high doses of GABA was enhanced by chloroform in a pharmacological dosage range in oocytes expressing the GABA β_2 homo-oligomeric receptor/channels and GABA α_1, β_2, γ_2 hetero-oligomeric receptor/channels. Potentiation by pentobarbital was observed in the β_2 homo-oligomeric receptor/channels and in the hetero-oligomeric receptors. These data are consistent with other results showing that some volatile general anesthetics can potentiate and desensitize the GABA receptor/channel at μM to mM concentrations (Hara et al., 1993; Orser et al., 1994; Raines et al., 1995). The decay ratios for coapplication of pentobarbital and of chloroform in the oocytes expressing the ρ_1 subunits were not significantly different from the decay ratio for GABA alone and, for this subunit type, there was no potentiation by either pentobarbital or chloroform. The effects on decay ratio and potentiation in the presence of the β_2/ρ_1 subunits reflected the chimeric

nature of the subunits. As was observed in the presence of the ρ_1 subunits, there was no significant increase in the decay ratio with the coapplication of either chloroform or pentobarbital in oocytes expressing the chimeric subunits. However, as was observed in the presence of the β_2 subunits, potentiation of the GABA-activated current did occur with the coapplication of either chloroform or pentobarbital. Thus, the oocytes expressing the β_2/ρ_1 subunits showed characteristics that reflected the origin of each domain.

As shown in Figs. 2 and 3*A–C*, both pentobarbital and chloroform enhanced the GABA-activated current in oocytes expressing the β_2 or the β_2/ρ_2 subunits but not in oocytes expressing only the ρ_1 subunits. Recalling that the chimera was composed of the N-domain from the β_2 subunit and the C-domain of the ρ_1 subunit and noting that the β_2 N-domain was common only to the receptors with β_2 or chimeric subunits, it can be deduced from our results that the modulation site of pentobarbital and chloroform is most likely located in the amino-terminal region (N-domain) of the GABA β_2 subunit.

As the decay ratio and potentiation studies demonstrated, the kinetic properties observed in oocytes expressing the chimeric subunits reflected the origin of the N- and C-domains. Recall that the results of the channel desensitization study showed that the GABA-activated current in oocytes expressing β_2 subunits and the heterooligomeric receptors, could be described as biphasic. As shown in the Table and Fig. 5, in the presence of the chimeric β_2/ρ_1 subunits, the fast phase was accelerated, as was seen in the presence of the β_2 subunits. The slow phase was slower than that observed with β_2 subunits but faster than that in the presence of the ρ_1 subunits.

With the coapplication of chloroform in the presence of β_2 subunits, a decrease was observed in the time constants for both the initial (τ_f) and late (τ_s) portions of the fast phase. When the receptors were chimeric, coapplication of chloroform resulted in a decrease in the time constant for the initial portion of the fast phase, that is, the phase was accelerated, but the time constant for the later portion of the fast phase of the current was not affected by the addition of chloroform. Chloroform also had no effect when the receptors were composed of ρ_1-subunits. It appears, then, that the effect of chloroform that was observed in the presence of the β_2-subunit receptors was dependent on both the N- and the C-domain. These data suggest that the N-domain of the β_2 subunits contributed to the initial portion of the fast phase. Because there was an effect due to chloroform in the late portion of the fast phase in the oocytes expressing the β_2 subunits, but not in the oocytes expressing the ρ_1 or β_2/ρ_1 subunits, the data also suggest that the C-domain of the β_2 subunits determined the strong acceleration observed in the late portion of the fast phase of the GABA-activated current. These results provide direct evidence, for the first time, that volatile general anesthetics can modulate GABA receptor/channels and regulate GABA-mediated current through the amino-terminal region (N-domain) and/or the M1 transmembrane to carboxy-terminal region (C-domain) of the receptor/channel.

Two tenable mechanisms for the action of volatile anesthetics on the membrane-bound GABA receptor/channels are: direct binding to the protein or anesthetic-induced disruption of protein-lipid interactions. Chloroform is highly lipid soluble, but, as our data showed, the ρ_1 subunit was not affected by the use of chloroform at the same dosage levels as was used with the β_2 and β_2/ρ_1 subunits. Hence, the physical nature of the interaction between drugs and GABA receptor/channels is not via the lipid membrane partition. Recently, it has been found that volatile anesthetics, including chloroform, can alter macromolecular conformations from lipid-free α-helixes to β-sheets essentially by their solvent effects, thus, destroying the solvation water shell surrounding the macromolecule (Chiou et al., 1992). In view of the Chiou et al. findings, it is reasonable to hypothesize that the modulation of chloroform on the GABA β_2 receptor or the chimeric β_2/ρ_1 receptor is not through an interaction with the bilayer-lipid membrane, affecting transmembrane regions embedded in the membrane, but by induction of the transition of the secondary structure in the amino-terminal and carboxyl-terminal regions of the GABA receptor/channel, resulting in conformational changes (Birnir et al., 1997).

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