Point Mutation in the First Transmembrane Region of the β2 Subunit of the γ-Aminobutyric Acid Type A Receptor Alters Desensitization Kinetics of γ-Aminobutyric Acid- and Anesthetic-induced Channel Gating*

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A conserved glycine residue in the first transmembrane (TM1) domain of the β2 subunit has been identified to be involved with desensitization induced by γ-aminobutyric acid (GABA) and anesthetics. Recombinant GABAA receptors expressed in SF9 cells were recorded using semi-agonist application. Upon direct activation by GABA or anesthetics, the main effect of the TM1 point mutation on the β2 subunit (G219F) was to slow the time constant (τ) of desensitization. At GABA concentrations eliciting maximum currents, the corresponding median τ values were 0.87 s (25–75% interval (0.76; 1.04 s)), 0.93 s (0.76; 1.23 s), and 1.36 s (1.17; 1.57 s) for α1β2γ2, α1(G223F)β2γ2, and α1β2(G219F)γ2, respectively. The τ value for the β2-mutant receptor was significantly longer than that for α1β2γ2 (p < 0.01) and α1(G223F)β2γ2 (p < 0.05). For pentobarbital-induced currents (500 μM), the corresponding median τ values were 1.36 s (0.81; 1.41 s), 1.47 s (1.31; 2.38 s), and 2.82 s (2.21; 5.56 s) for α1β2γ2, α1(G223F)β2γ2, and α1β2(G219F)γ2, respectively. The τ value for the β2-mutant receptor was significantly longer than that for α1β2γ2 (p < 0.01). The present findings suggest that this TM1 glycine residue is critical for the rate at which desensitization occurs and that both GABA and intravenous anesthetics implement an analogous pathway for generating desensitization.

Most volatile and intravenous anesthetics enhance the activity of γ-aminobutyric acid type A (GABAA) receptors and directly activate this ligand-gated chloride ion channel in the absence of its endogenous ligand, GABA (1, 2). Specifically, anesthetics are known to prolong GABA-induced Cl− channel opening (1, 2), and, depending on the type of anesthetic, this potentiates GABA-gated currents appears to alter deactivation and/or desensitization. For example, halothane, a volatile anesthetic, has been shown to slow the dissociation of GABA from its receptor, i.e. slows deactivation (3). Propofol, an intravenous anesthetic, slows both deactivation and the exit rate from desensitization (4), and neurosteroids, some of which have anesthetic qualities (5), also decrease the recovery rate from desensitization (6). From these studies, it is apparent that desensitization of GABAA receptors is altered in the presence of intravenous anesthetics.

Investigations using chimeras and site-directed mutagenesis have identified key amino acids in the second transmembrane domain (TM2) of both the α and β subunits, which are involved in the conformational state of desensitization (7, 8). Furthermore, for GABAA receptors, structural determinants of anesthetic action have been primarily located to both the TM2 and TM3 regions of GABAA receptors (9–12). These same regions have been described to be an integral part of the channel gating domain of the GABAA receptor (13, 14). These data suggest that the desensitization machinery appears to lie within the channel gating region and that this domain is also allosterically sensitive to anesthetics.

Another GABAA receptor domain to be allosterically sensitive to intravenous anesthetics is the TM1 region. As shown below in Fig. 1, the N-terminal of TM1 is highly conserved among GABAA receptor subunits, including the p1 subunit, which comprises the homomeric receptors that are insensitive to most anesthetics (1). However, the TM1 glycine residue, which is conserved across GABAA receptor subunits, is replaced by a phenylalanine in the p1 subunit (see Fig. 1). In the previous study by Carlson et al. (2000), the mutation of the TM1 glycine of the β2 subunit to the homologous residue, phenylalanine, in the p1 subunit, i.e. β2(G219F) was shown to affect receptor gating induced by both GABA and anesthetics (15). This finding was consistent with the suggestion that the TM1 region may work together with TM2 for channel gating (16). Because this TM1 glycine on the β2 subunit is perhaps linked with the channel gating region of TM2, the present study tests the hypothesis that glycine 219 on the β2 subunit affects the conformational events of desensitization induced by GABA and/or anesthetics. Kinetic analyses were performed on whole-cell patch clamp recordings from wild type GABAA receptors, α1β2γ2, and mutant GABAA receptors, α1(G223F)β2γ2 and α1β2(G219F)γ2, which were recombinantly expressed in SF9 cells. It was determined that a TM1 glycine on the β2 subunit is involved with desensitization of GABA-, pentobarbital-, and propofol-induced currents. These findings suggest
that the structural determinants for regulating desensitization resulting from direct activation of the $\text{GABA}_{A}$ receptor chloride ionophore are similar for $\text{GABA}_{A}$ and anesthetics.

**EXPERIMENTAL PROCEDURES**

**Site-directed Mutagenesis and Generation of Recombinant Baculoviruses**—Point mutations were introduced into the cDNA of rat $\alpha_{1}$ and $\beta_{2} \text{GABA}_{A}$ receptor subunits with an in vitro mutagenesis system (Altered Sites II, Promega). The coding region of $\alpha_{1}$ (and $\beta_{2}$ subunit performed separately) was subcloned into pCruet. and both mutations, $\alpha_{1}(\text{G223F})$ and $\beta_{2}(\text{G219F})$, were incorporated using a mutagenic oligonucleotide. Following verification of mutagenesis by DNA sequencing, the point-mutated $\text{GABA}_{A}$ receptor subunits were subcloned into the baculovirus transfer vectors for generation of recombinant baculovirus according to BAC-BAC expression system (Invitrogen) or BaculoGold transfection kit (BD PharMingen). All procedures were performed according to the manufacturer’s suggestion and as previously described (15).

**Cell Culture and Baculovirus Infection**—Sf9 insect cells (Spodoptera frugiperda) were grown as a shaking culture (140 rpm) in serum-free solutions applied from a multibarreled perfusion pipette (18).

**Drug Applications**—Stock solutions of $\text{GABA}$ (Sigma) and pentobarbital sodium (GABAergic, in $\text{GABA}_{A}$ and $\beta_{2}$ subunit, on the other hand, significantly decreased the EC$_{50}$ of the receptor for anesthetics) and the current remaining after 5 s of application (end current). Rise time was estimated as the time needed for the current to increase from 10–90% of the peak value. The time constant for desensitization ($\tau_{\text{des}}$) was estimated from the current decay from the peak to the end of the 5-s application, whereas the time constant for deactivation ($\tau_{\text{act}}$) was estimated using the current decay from the termination of agonist application to baseline. Time constants were fitted by mono- and biexponential functions, using PulseFit (HEKA) software. The quality of the fit was evaluated by the root mean square value. In general, the fit was not significantly improved by using two exponentials.

**Statistics**—Current data (peak currents, end currents) were normally distributed. They were described using mean and standard error (S.E.) and compared with analysis of variance (ANOVA) and where relevant by a Tukey multiple comparison procedure. Time data (rise times, time constants) were often not normally distributed and therefore described using median, 25 and 75% quartiles. Comparisons were made using the Kruskal-Wallis one-way analysis of variance followed where relevant by Dunn’s multiple comparison procedure. Probabilities ($p$) < 0.05 were considered statistically significant.

**RESULTS**

**Comparison of Kinetics of Wild Type and TM1 Glycine-mutated $\text{GABA}_{A}$ Receptors at Maximum $\text{GABA}$ Currents**—The $\text{GABA}$ concentration-response relationships for the wild type ($\alpha_{1}\beta_{2}\gamma_{2}$) $\text{GABA}_{A}$ receptor and the mutated $\alpha_{1}(\text{G223F})\beta_{2}\gamma_{2}$ and $\alpha_{1}(\text{G219F})\beta_{2}\gamma_{2}$ receptors have been characterized in our previous study (15), and the vital data are summarized in Table I. Briefly, the $\alpha_{1}$ subunit did not significantly affect the concentration-response relationship for $\text{GABA}$-induced peak currents. The corresponding mutation in the $\beta_{2}$ subunit, on the other hand, significantly decreased the EC$_{50}$ of the receptor for $\text{GABA}$. The Hill coefficients determined for the three subunit combinations were not significantly different.

To estimate possible differences in the kinetics of the different $\text{GABA}$ receptors, the lowest concentrations of $\text{GABA}$ giving rise to maximum peak responses (saturating concentrations) were investigated (i.e. 2 mM for the wild type and the $\alpha_{1}(\text{G223F})\beta_{2}\gamma_{2}$ receptors, and 0.2 mM for the $\alpha_{1}(\text{G219F})\beta_{2}\gamma_{2}$ receptor (Fig. 2). The rate of current onset was described using the 10–90% rise time. As shown in Fig. 3A, the rise time did not significantly differ between the wild type and the $\alpha_{1}(\text{G223F})$ and $\beta_{2}(\text{G219F})$ mutant receptors. Increasing the $\text{GABA}$ concentration from 0.2 to 2 mM for the $\beta_{2}$-mutant receptor did not further decrease the rise time. None of the 10–90% rise times for saturating $\text{GABA}$ concentrations were significantly different from the 10–90% exchange time for extracellular solution (median 112 ± 12 s, 25–75% interval 79; 141 ms) determined in nine cells expressing the $\alpha_{1}\beta_{2}\gamma_{2}$ receptor. The time constant for solution exchange was 52 ms (37; 67 ms).

**Differences in the extent of desensitization were estimated from the proportion of the peak current remaining at the end of the 5-s application. No significant differences in the extent of current fade between the wild type and the mutant receptors**

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**Table I**

| Subunit combination | $\text{EC}_{50}$/µM | Hill coefficient |
|---------------------|---------------------|------------------|
| $\alpha_{1}\beta_{2}\gamma_{2}$ | 1.19 (0.98–1.40) | 1.40 |
| $\alpha_{1}(\text{G223F})\beta_{2}\gamma_{2}$ | 1.27 (0.91–1.62) | 2.01 |
| $\alpha_{1}(\text{G219F})\beta_{2}\gamma_{2}$ | 5.4 (4.3–6.5) | 3.9 (3.7–6.1) |

*p < 0.001, significant difference between the $\text{EC}_{50}$ of the $\beta_{2}$-mutant receptor compared with the wild type and the $\alpha_{1}$-mutant receptors. These data are from Ref. 15.*

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**FIG. 1. Sequence alignment of the pre-TM1/TM1 amino acids of $\text{GABA}_{A}$ receptor subunits. The glycine residue (G, in boldface) is conserved in all of the subunits except the $\rho_{1}$ (as indicated by the symbol #), which has a phenylalanine (P, in boldface and underlined). There are five amino acids that are conserved in all the subunits listed, including $\rho_{1}$ (asterisks) and seven amino acids that are conserved within subunit families (dots). The sources for all sequences were GenBank™ andCutting et al. (25).**
were detected using the saturating GABA concentrations mentioned above (Fig. 3B).

The desensitization kinetics were described using the time course of current fade, which for all three receptor types was described by one exponential component (Figs. 2 and 3C). Although the corresponding time constants ($\tau_{\text{desens}}$) for the wild type GABA$_A$ receptor and the $\alpha 1$(G223F) mutated receptor were similar, the $\tau_{\text{desens}}$ for the $\beta 2$(G219F) mutated receptor was significantly longer than the $\tau_{\text{desens}}$ of the wild type ($p < 0.01$) and the $\alpha 1$-mutant ($p < 0.05$) receptors. Increasing the GABA concentration to 2 mM on the $\beta 2$-mutant decreased $\tau_{\text{desens}}$ ($p < 0.05$) and the end current ($p < 0.05$) significantly.

**FIG. 2.** Examples of current traces (gray) showing GABA-induced currents at saturating concentrations in the $\alpha 1\beta 2\gamma 2$, $\alpha 1$(G223F)$\beta 2\gamma 2$, and $\alpha 1\beta 2$(G219F)$\gamma 2$ receptor combinations. The corresponding fits of the desensitization and deactivation phases are shown as black curves. The time constants for desensitization ($\tau_{\text{desens}}$) are: $\alpha 1\beta 2\gamma 2$, 807 ms; $\alpha 1$(G223F)$\beta 2\gamma 2$, 873 ms; $\alpha 1\beta 2$(G219F)$\gamma 2$, 1.34 s. The time constants for deactivation ($\tau_{\text{deact}}$) are: $\alpha 1\beta 2\gamma 2$, 452 ms; $\alpha 1$(G223F)$\beta 2\gamma 2$, 728 ms; $\alpha 1\beta 2$(G219F)$\gamma 2$, 717 ms. Please refer to Fig. 3 for a summary of the data set of $\tau$ values with saturating GABA concentrations.

**FIG. 3.** Comparison of GABA kinetics at maximum GABA currents in the $\alpha 1\beta 2\gamma 2$ (2 mM GABA), $\alpha 1$(G223F)$\beta 2\gamma 2$ (2 mM GABA), and $\alpha 1\beta 2$(G219F)$\gamma 2$ (0.2 mM and 2 mM GABA) GABA$_A$ receptor combinations. A, the 10–90% rise time. Each column represents the median ± 25–75% interval of 6–26 Sf9 cells. B, the proportion of peak current remaining after 5 s of GABA application. The end current of the $\alpha 1\beta 2$(G219F)$\gamma 2$ receptor was significantly smaller at 2 mM GABA than at 0.2 mM. Each column represents the means ± S.E. of 6–22 Sf9 cells (*, $p < 0.05$). C, time constants ($\tau_{\text{desens}}$) of desensitization. The median ($n = 6–22$ cells) $\tau$ for the $\beta 2$-mutant at 0.2 mM GABA was significantly slower than at 2 mM GABA and for the wild type and $\alpha 1$ mutant receptors (*, $p < 0.05$; **, $p < 0.01$). D, time constants of deactivation ($\tau_{\text{deact}}$) shown as median ± 25–75% interval of 7–22 cells. At 2 mM GABA $\tau_{\text{deact}}$ was significantly longer for the $\alpha 1\beta 2$(G219F)$\gamma 2$ than for the $\alpha 1$(G223F)$\beta 2\gamma 2$ receptor combinations (**, $p < 0.01$).
Deactivation time course of the GABA-elicited currents were also adequately described by one exponential component (Fig. 3D), with similar time constants (τ_deact) for the wild type and mutant receptors at saturating concentrations. When 2 mM GABA concentration was applied to the β2 mutant receptor, the τ_deact increased, and it was significantly longer than that for the α1(G223F) mutant receptor (p < 0.01).

**Table II**

| Subunit combination | GABA (EC_{20}) | GABA (EC_{20}) + propofol with pretreatment |
|---------------------|----------------|---------------------------------------------|
| α1β2γ2             | 190 ± 31*      | 285 ± 58*                                   |
| α1(G223F)β2γ2       | 115 ± 3        | 166 ± 12                                    |
| α1β2(G219F)γ2       | 120 ± 6        | 155 ± 14                                    |

* (p < 0.05).

**Mutation of TM1 Glycine on α1 and β2 Subunit Diminishes Propofol-induced Enhancement of GABA Currents**—The modulating effect of propofol on currents induced by GABA at EC_{20} in the wild type and mutant receptors is summarized in Table II, which shows the effect of the highest propofol concentration tested for each receptor combination without inducing direct activation: 50 μM for the α1β2γ2 and α1(G223F)β2γ2 and 5 μM for the α1β2(G219F)γ2 combinations. In all three receptor combinations, pretreatment with propofol was necessary to significantly enhance peak currents induced by GABA (wild type: p < 0.01, α-mutant and β-mutant: p < 0.001). The modulating effect of propofol was significantly smaller (p < 0.05) for the α1(G223F)β2γ2 combination as compared with the α1β2γ2 combination at both 10 and 50 μM propofol (Table II and Fig. 4). The α1β2(G219F)γ2 receptor showed significantly smaller modulation of GABA-induced currents when comparing 1 and 5 μM propofol for the β2 mutant with 10 and 50 μM propofol for the wild type (p < 0.05). There was no significant difference in the modulating effect of propofol between the α1(G223F)β2γ2 and the α1β2(G219F)γ2 receptors (Table II). Neither the rise times nor the end currents remaining after 5-s application were significantly altered by propofol in any of the receptor combinations (data not shown). Due to the slow time course of current decay during GABA application at EC_{20} the corresponding time constants could not be calculated.

Although equivalent concentrations of GABA with respect to response amplitude (EC_{20}) were chosen for the modulation experiments, the corresponding deactivation time constants differed between the receptor combinations. The deactivation time constants of the unmodulated responses were (median and 25–75% interval in milliseconds): α1β2γ2, 268 (219; 385); α1(G223F)β2γ2, 247 (207; 385); α1β2(G219F)γ2, 471 (358; 551). The deactivation time constant of the β2-mutant receptor was significantly longer (p < 0.01) than those of the wild type and the α1-mutant receptors, whereas the latter two were similar. Due to these absolute differences, the effects of the anesthetics on the deactivation time constant was estimated by calculating the ratio of τ_deact of the modulated and unmodulated responses in each cell. The propofol-modulated τ_deact relative to unmodulated were (median and 25–75% interval in percent of unmodulated): α1β2γ2, 151 (121; 157); α1(G223F)β2γ2, 108 (101; 118); α1β2(G219F)γ2, 118 (105; 129). The relative τ_deact on the wild type receptor was significantly greater than 100% (p < 0.01). The effect of propofol was to slow the τ_deact on the wild type receptor. In addition, pentobarbital-modulated τ_deact relative to unmodulated were assessed: α1β2γ2, 116 (95; 134); α1(G223F)β2γ2, 102 (98; 108); α1β2(G219F)γ2, 86 (84; 100). For pentobarbital, none of the relative τ_deact differed significantly from 100%. Pentobarbital did not alter the τ_deact as compared with unmodulated τ_deact.

**Comparison of Anesthetic Kinetics of Wild Type and TM1 Glycine-mutated α1(G223F)β2γ2 Receptors in the Absence of GABA**—As illustrated in Fig. 5A, the peak response levels of 50 μM pentobarbital on the α1β2(G219F)γ2 receptor and 500 μM pentobarbital on the α1β2γ2 and α1(G223F)β2γ2 receptors were not...
significantly different, whereas the peak response of 500 μM pentobarbital on the α1β2γ2 receptor was significantly larger than any of these (p < 0.001 in all cases). The rise-time for the wild type and the α1-mutant receptors at 500 μM pentobarbital and for the β2-mutant receptor at 50 and 500 μM pentobarbital did not significantly differ from each other (Fig. 8A).

To compare the desensitization kinetics, the fading of direct
pentobarbital-induced currents was compared (Fig. 8B). Upon application of 500 μM pentobarbital to the β2-mutant receptor, a significantly larger \((p < 0.01)\) end current remained than with 500 μM pentobarbital for the wild type and the α1-mutant receptors. For all combinations, the time course of current fade was described by one exponential component. The \(\tau_{\text{desens}}\) value for 500 μM pentobarbital in the β2-mutant receptor was significantly larger \((p < 0.01)\) than 50 μM in the same receptor and 500 μM pentobarbital in the wild type receptor (Fig. 8C).

The deactivation time course of the pentobarbital-elicited currents were also adequately described by one exponential component (Fig. 8D), with no significant differences between \(\tau_{\text{desact}}\) Values for the wild type and mutant receptors at the same concentrations.

**DISCUSSION**

**β2(G219F)-mutant Receptors and GABA-induced Currents—**

The present study examined the effect of a point mutation in the TM1 region of the α1 and β2 GABA<sub>A</sub> receptor subunits on the kinetics of GABA-mediated Cl⁻ currents. As indicated previously (15), the apparent affinity for GABA was increased in the α1β2(G219F)γ2 receptors.

Using ultra-fast agonist application to GABA<sub>A</sub> receptors in outside-out membrane patches, it is possible to achieve 10–90% rise times of ~1 ms at saturating GABA concentrations \((e.g. \alpha1β1γ2 receptors) (20)\). In the present experiments the maximum activation rate and peak current were limited by the speed of agonist application. This limit comes into effect at the GABA concentrations giving rise to maximum peak currents where the rise times reach the lower limit set by the extracellular solution exchange rate (Fig. 3). Accordingly, an increase of the GABA concentration from 0.2 to 2 mM for the β-mutant did not further decrease the rise time.

As measured by the end-current remaining during a saturating GABA application, the degree of desensitization was not different between the wild type and the two mutant receptors. Part of the current fade may be due to the Cl⁻ gradient across the cell membrane (21), but because of the similar extent of current fade for the three receptor combinations tested, it is likely that the contribution of Cl⁻ shift is of similar magnitude. The time constant for desensitization, however, was significantly longer for 200 μM GABA in the β2-mutant receptor. This slower desensitization could be significantly accelerated by raising the GABA concentration at the β2(G219F) combination to 2 mM \((as used for the other combinations), whereby a time constant comparable to the other combinations was achieved. At the same time, the amount of desensitization increased significantly.

Ultra-fast agonist application to GABA<sub>A</sub> receptors in outside-out membrane patches often reveals a desensitization time course with two exponential components \((e.g. a fast component of \(\tau < 10\) ms and a slower component of \(\tau ~ 150\) ms for \(\alpha1β3γ2 \) receptors) (22), although some investigations have found monoeXponential desensitization \((e.g. \tau ~ 500\) ms, \(\alpha1β1γ2 \) receptors) (20). A fast component of desensitization would not be resolved in the present experiments due to the limited rate of extracellular solution exchange. Even though our experiments do not reveal the true magnitude and time constants of desensitization, the differences observed between the receptor combinations reflect actual differences in desensitization kinetics. Although the approximately 10-fold decrease of EC₅₀ and the concentration required to achieve the minimum rise time and maximum peak current could be explained
by a selective increase in agonist binding rate for the β2-
mutant receptor, the changes of the magnitude and time con-
stant of desensitization imply that the TM1 glycine on the
β2 subunit is part of the desensitization machinery of GABAA
receptors.

How a decrease in functional EC_{50} for GABA is associated
with an increase in the τ value for desensitization is difficult to
resolve. However, it should be noted that the ρ GABAA recep-
tors have a lower EC_{50} for GABA than α1β2γ2 receptors, and ρ
receptors do not desensitize or desensitize at an extremely slow
rate (23, 24). Moreover, this TM1 glycine residue is conserved
across all GABAA receptor subunits, except the ρ subunit,
which has a phenylalanine (25). A working theory on channel
gating described by Akabas and Karlin (16), hypothesizes that
the N-terminal region of the TM1 domain works in tandem
with the TM2 domain to elicit the conformational events of
gating (i.e. activation, desensitization, and deactivation).
Because glycine residues allow for conformational flexibility
(26), subunits containing this residue may transfer the ago-
nist binding energy more readily to the conformational state
of desensitization than the bulkier hydrophobic residue, phe-
nylalanine. Thus, upon channel activation, the β2 mutant
receptors have increased channel currents due to the slowing
of desensitization.

Other studies using chimeras and site-directed mutagenesis
have shown that the TM1 region of the β2 and γ2 subunits
harbor important residues for fast desensitization (22, 27).
Interestingly, two TM1 residues on the γ2 subunit, directly
adjacent to the conserved TM1 glycine residue that is mutated
in the present study, have been shown to be important for fast
desensitization (22). Fast desensitization in the γ2-containing
GABAA receptors was not eliminated by mutating these γ2
TM1 residues alone (22), but other structural determinants in
the extracellular N-terminal are most likely required, indicat-
ing that there are multiple determinants on the γ2 subunit and
perhaps other subunits for fast desensitization. The data with
our α1β2(G219F)γ2 receptor support the claim that the N-
terminal end of TM1 domain of the β subunit is involved with
desensitization.

Fast desensitization has been shown to correlate with pro-
longed deactivation, probably due to reopening of channels
after leaving the long-lived desensitized states (28). Desensiti-
zation and deactivation can, however, be uncoupled by muta-
tion of the above-mentioned two amino acids in the TM1 region
of the GABAA receptor γ2 subunit, which selectively acceler-
ated deactivation without altering desensitization (22). Al-
though our agonist application rate does not allow us to resolve
fast desensitization, the resulting states still become populated
during agonist application and would be expected to influence
the time course of deactivation. Indeed, an increase in GABA

![Figure 7](http://www.jbc.org/)
concentration from EC$_{20}$ to a saturating GABA concentration prolonged deactivation for all three receptor types in the present investigation. The $\beta 2$G219F mutation gave rise to a significant prolongation of $\tau_{\text{deact}}$ at EC$_{20}$ concentrations compared with the wild type and $\alpha$-mutant receptors, but this difference vanished at the saturating GABA concentrations. Further increase in the GABA concentration from 200 $\mu$M to 2 mM with the $\beta$-mutant receptor resulted in both increased amount of desensitization and prolongation of the deactivation (Fig. 3). Thus, the $\alpha 1$G223F and $\beta 2$G219F mutations did not have any prominent effect on desensitization-deactivation coupling.

**TM1 Glycine and Anesthetic-modulated GABA Currents**—Because propofol can induce direct activation of GABA$_A$ receptors, modulation experiments with propofol were conducted with concentrations that elicited only enhancement and were not confounded with direct activation effects. For the $\alpha 1$- and $\beta 2$-mutant receptors, propofol-induced enhancement of GABA currents was diminished. A similar finding for the $\beta 2$-mutant receptor has been shown for pentobarbital-modulated GABA currents (15). Upon analyzing the decay of the propofol-modulated currents, it was assessed that there was no change in the magnitude of desensitization for both the $\alpha 1$- and $\beta 2$-mutant receptors as compared with wild type receptors. The same results were also assessed for pentobarbital-modulated GABA currents (results not shown). Desensitization time constants could not be determined for the modulation experiments due to the slow decay of current elicited by GABA (EC$_{20}$). Other studies have shown that the mechanism by which anesthetics modulate GABA currents is achieved by slowing the desensitization and deactivation rates (4, 6). For the wild type receptor we observed that deactivation was slowed significantly by propofol, whereas for the $\alpha 1$- and $\beta 2$-mutant receptors, the effect on $\tau_{\text{deact}}$ was insignificant. The reduced effect of propofol on deactivation in the two mutant receptors thus parallels the reduced enhancement of peak current. A reduced dissociation rate of GABA from the receptor has been suggested as one reason for the slowing of deactivation (4), which may also contribute to the increase in $\tau_{\text{deact}}$ and peak current by propofol in the wild type receptor in this study (although other mechanisms are possible). For pentobarbital-modulated currents, the $\tau_{\text{deact}}$ was not altered in any receptor combination tested, suggesting that other mechanisms that perhaps include desensitization are
Role of GABA$_A$ Receptor TM1 in Desensitization

more important for pentobarbital enhancement of GABA$_A$ currents.

A decrease in anesthetic-modulation observed with the TM1 mutant receptors is consistent with two other studies which showed that the sensitivity to GABA was enhanced with point mutations at the TM2 9’ leucine (β2L259) and the 15’ serine (β1S265 and α2S270) (29, 30). In addition, positive allosteric potentiation was reduced (29, 30). A decrease in GABA-induced desensitization with the TM2 9’ point mutation was demonstrated, as well (29). Because in the present study the TM1 β2(G219F) point mutation significantly decreased the EC$_{50}$ for GABA, the conformational changes needed to allosterically potentiate GABA on these receptors are most likely at or near its intrinsic maximum and thus cannot be modulated any further. However, it is important to note that the TM2 9’ and 15’ point mutations created spontaneously active channels and that all positive allosteric modulators, including benzodiazepines, were affected. The TM1 point mutation in the present study did not create spontaneously active channels, and in our previous study, benzodiazepine potentiation of GABA$_A$ currents in the β2(G219F) receptor combination was shown to be unchanged (15).

With the α1(G223F)/β2γ2 receptors, the EC$_{50}$ for GABA was not altered, yet propofol-induced potentiation was significantly less than in the wild type receptors, whereas pentobarbital-induced potentiation was not altered by the α1 point mutation (15). Perhaps, the TM1 glycine residue on the α1 subunit may be a component of the binding pocket for propofol. This suggestion is supported by the finding that propofol-induced enhancement of GABA$_A$ agonist binding was also reduced in this same mutant receptor complex (15). These findings indicate that the same conserved glycine residue on the α1 and β2 subunit may contribute in different ways to the conformational events elicited by different anesthetics.

The β2 TM1 Glycine and the Kinetics of Anesthetic-induced Direct Activation of GABA$_A$ Receptors—Although the activation of GABA-gated currents at high concentrations were effectively limited by the application system, the observed rise times and desensitization time constants for anesthetic-gated currents were considerably larger than for GABA-gated currents. Although this does not exclude the existence of unresolved fast components, it allowed us to resolve some differences on a slower time scale, which reflect actual kinetic differences between the receptor combinations. For pentobarbital-induced direct activation of the GABA$_A$ chloride channel, the α1β2(G219F)/γ2 receptors demonstrated a biphasic concentration-response curve. The first phase was shifted leftward relative to the concentration-response curves of the α1β2γ2 and α1(G223F)/β2γ2 receptors, and the peak current, rise time, end current, and time constants for desensitization and deactivation of 50 μM pentobarbital for the β2-mutant receptor were similar to the same parameters of 500 μM pentobarbital for the α1β2γ2 and α1-mutant receptors. Thus the first phase of the concentration-response curve of the β2-mutant receptor could be explained by an increased association rate of pentobarbital. The effect of 500 μM pentobarbital on the β2-mutant receptor was significantly different from that on the other two receptor combinations when comparing peak current, decay, and τ$_{desens}$.

Specifically, the amount of decay induced by 500 μM pentobarbital in the β2-mutant receptor was significantly smaller and developed significantly slower than in the α1β2γ2 receptor.

The biphasic nature of the pentobarbital concentration-response curve of the α1β2(G219F)/γ2 receptor was further emphasized by the concentration-dependent kinetics. Although a normal concentration-dependent decrease of the rise time was observed, the pattern of current decay was atypical. At 500 μM pentobarbital, the decay was less extensive and developed with a longer time constant than at 50 or 1500 μM pentobarbital. At the same time, the deactivation time constant tended to become longer with 500 μM than with 50 μM pentobarbital. It should be noted that for agonists the extent and rate of desensitization normally increase with increasing concentration. The present findings suggest that a second (or additional) binding site with lower affinity for pentobarbital was exposed in the presence of the β2(G219F) point mutation and elicited a different pattern of desensitization and that may appear to become uncoupled from deactivation.

For propofol, similar to GABA and pentobarbital, α1β2(G219F)/γ2 conferred a receptor that was activated by lower agonist concentrations. Desensitization was more extensive (but with a rate that tended to be slower) and deactivation was faster at a higher concentration as compared with a lower one. Although a larger fraction of receptors were in one of the desensitized states after application of the higher propofol concentration, we cannot determine whether a smaller fraction of these were in states corresponding to fast desensitization and therefore expected to slow deactivation. Thus the intactness of desensitization-deactivation coupling cannot be determined.

As seen with the GABA-induced currents, the point mutation (G⇒F), in the TM1 domain on the β2 subunit, alters the conformational changes involved in desensitization upon direct activation by an agonist, in this case, anesthetics. However, it should be kept in mind that the complete cascade of events for desensitization may not be identical between GABA- and anesthetic-induced direct activation (21).

Concluding Remarks—The molecular basis of desensitization in GABA$_A$ receptors is not well understood, but there is increasing evidence, including the present study, in support of the claim that the TM1 domain is involved in mediating conformational changes that lead to desensitization (22, 27, 31). To this end, the present findings suggest that GABA and anesthetics appear to implement similar conformational events, which elicit desensitization. Because it appears that the allosteric regulation of GABA$_A$ receptors by anesthetics is related to, in part, the regulation of desensitization, identifying structural determinants involved with desensitization will be essential to further elucidate the mechanism of anesthetic action.

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