A Single Phenylalanine Residue in the Main Intracellular Loop of α1 γ-Aminobutyric Acid Type A and Glycine Receptors Influences Their Sensitivity to Propofol

Gustavo Moraga-Cid, Ph.D.,* Gonzalo E. Yevenes, Ph.D.,* Günther Schmalzing, M.D.,† Robert W. Peoples, Ph.D.,‡ Luis G. Aguayo, Ph.D.§

ABSTRACT

Background: The intravenous anesthetic propofol acts as a positive allosteric modulator of glycine (GlyRs) and γ-aminobutyric acid type A (GABA\textsubscript{A}R) receptors. Although the role of transmembrane residues is recognized, little is known about the involvement of other regions in the modulatory effects of propofol. Therefore, the influence of the large intracellular loop in propofol sensitivity of both receptors was explored.

Methods: The large intracellular loop of α\textsubscript{1} GlyRs and α\textsubscript{1}β\textsubscript{2} GABA\textsubscript{A}R was screened using alanine replacement. Sensitivity to propofol was studied using patch-clamp recording in HEK293 cells transiently transfected with wild type or mutant receptors.

Results: Alanine mutation of a conserved phenylalanine residue within the α\textsubscript{1} large intracellular loop significantly reduced propofol enhancement in both GlyRs (360 ± 30 vs. 75 ± 10%, mean ± SEM) and GABA\textsubscript{A}Rs (361 ± 49% vs. 80 ± 23%). Remarkably, propofol-hyposensitive mutant receptors retained their sensitivity to other allosteric modulators such as alcohols, etomidate, trichloroethanol, and isoflurane. At the single-channel level, the ability of propofol to increase open probability was significantly reduced in both α\textsubscript{1} GlyR (189 ± 36 vs. 22 ± 13%) and α\textsubscript{1}β\textsubscript{2} GABA\textsubscript{A}R (279 ± 29 vs. 29 ± 11%) mutant receptors.

Conclusion: In this study, it is demonstrated that the large intracellular loop of both GlyR and GABA\textsubscript{A}R has a conserved single phenylalanine residue (F380 and F385, respectively) that influences its sensitivity to propofol. Results suggest a new role of the large intracellular loop in the allosteric modulation of two members of the Cys-loop superfamily. Thus, these data provide new insights into the molecular framework behind the modulation of inhibitory ion channels by propofol.

What We Already Know about This Topic

• Propofol positively modulates receptors for the inhibitory transmitters γ-aminobutyric acid type A (GABA) and glycine, but the molecular mechanisms involved are unclear.

What This Article Tells Us That Is New

• A single homologous residue in the large M3-M4 intracellular loops of the α\textsubscript{1} subunits of GABA\textsubscript{A} and glycine receptors modulates the action of propofol but not of other general anesthetics.
critical for pore formation, and a large intracellular loop connecting TM3 and TM4. To date, molecular cloning has identified 5 subunits of the GlyR (α1–4 and β) and 19 subunits of the GABAα R, with the GlyR α, β, and GABAα R α, β, γ, combinations being predominant in the adult mammalian central nervous system.1,2

GABAα R and GlyR play important roles in the actions of general anesthetics, including propofol,5–9 which is widely used in intensive care units.10 Previous studies indicated that these receptors contain sites important for propofol action.6,11,12 For instance, residues in the TM domains in α1 and β2/3 subunits of the GABAα R were shown to be important for actions of anesthetics, including propofol.13–21 Experiments using a photoreactive analog of etomidate identified two residues (α1M236 in M1 and β2M286 in M3) as part of a binding pocket for this anesthetic.22 In addition, based on the capacity of propofol to protect a sulphydryl-specific reagent from reacting with a substituted cysteine, it was proposed that M286 in M3 served as an anesthetic binding site in β2.23 A more recent study showed that binding of the photoreactive analog of etomidate to this site was either directly or allosterically inhibited by other general anesthetics, suggesting complex intramolecular interactions.24 In addition to binding sites in TM2/TM3 of α/β subunits in GABAα R, a tyrosine in TM4 (Y444) was found to influence the action of propofol, but not etomidate, on the receptor.14

Studies in animal and molecular experimental models have shown that the sites of general anesthetics on GABAα R and GlyR are somewhat overlapping for different chemical structures. For example, transgenic mice carrying propofol-insensitive GABAα Rs (β3N265M) also showed resistance to etomidate and exhibited substantial reductions in the modulatory actions of the volatile anesthetic enfurane.19 Similarly, it was reported that residues S267 and A288 of α1 GlyR,25 which previously had been reported as critical for modulation by alcohols and enfurane,26 also affected propofol sensitivity.

Although these previous studies have predicted that several residues might constitute a propofol binding pocket, the absence of high-resolution structures of drug-receptor complexes for eukaryotic receptors has hindered a complete understanding of the molecular mechanisms underlying propofol actions. Molecular analysis based on homology modeling approaches showed that the implicated TM domain residues form a water-filled cavity that might be able to accommodate structurally unrelated molecules.16,27 However, the structure and characteristics of these putative cavities remain unresolved.18,19,22–24 For example, in cysteine cross-linking studies, propofol weakly protected the M286 residue but was not able to protect the β2N265C residue from modification by p-chloromercuribenzenzene sulfonate, implying that this residue does not contribute significantly to the binding site.23 Moreover, the replacement of α1N265 or β2M286 with bulky hydrophobic residues promoted changes in channel gating and increased agonist potency, complicating the interpretation regarding the reduced propofol sensitivity.13,28

All these studies are in agreement with the idea that residues in the TM domains are important for propofol actions in both GABAα Rs and GlyRs. However, little is known about the contribution of other receptor regions. In this regard, a recent study has demonstrated that the large intracellular loop (LIL) of the α1 GlyR can influence the allosteric effects exerted by ethanol.29,30 which has been proposed to act at a site in the TM domains. Therefore, in the current study we investigated the influence of the LIL on the allosteric action of propofol in two members of the Cys-loop superfamily. Our results identified a single phenylalanine residue, conserved in the α1 subunit of both GABAα Rs and GlyRs, which affects their sensitivity to propofol. These results provide novel information about the relevance of the LIL in the allosteric modulation of the Cys-loop superfamily.

Materials and Methods

Complementary DNA Constructs. Mutations were inserted using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) in constructs encoding the human GlyR α1 subunit subcloned in the pCI vector (Promega, Madison, WI) and the rat α1 and β2, GABAα R subunits subcloned in the pRK5 vector (Clontech, Mountain View, CA). All mutations were confirmed by full sequencing. The GlyRs and GABAα R amino acids were numbered according to their position in the mature protein sequence.

Cell Culture and Transfection. HEK293 cells (CRL-1573; American Type Culture Collection, Manassas, VA) were cultured using standard methods. For the GlyR experiments, HEK293 cells were cotransfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) with the α1 GlyR plus the pGreenLantern plasmid (Invitrogen) conferring the green fluorescent protein (ratio 1:1; 2 μg DNA for each plasmid). Expression of green fluorescent protein was used as a marker of positively transfected HEK293 cells and recordings were made after 18–36 h. In some experiments in which GABAα R α1β2γ2 subunits were expressed, HEK293 cells were cotransfected using Lipofectamine 2000 (Invitrogen) with the α1 and β2 subunits subcloned in the pRK5 vector and the γ2 subunit subcloned in the vector internal ribosome entry site 2-enhanced green fluorescent protein (pIRE2-EGFP, Clontech) using a cotransfection ratio for α1β2γ2 of 1:2:5. To express the α1β2 subunit combination, cells were cotransfected with the α1 subunit subcloned in pRK5 and the β2 subunit subcloned in the pIRE2-EGFP, using the 1:2 ratio, respectively.

Electrophysiology. Whole cell recordings were performed as previously described.29,30 A holding potential of ~60 mV was used. Patch electrodes were filled with (in mM): 140 CsCl, 10 2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid, 10 HEPES (pH 7.4), 4 MgCl2, 2 adenosine-5′-triphosphate and 0.5 guanosine-5′-triphosphate. The external solution contained (in mM): 150 NaCl, 5 KCl, 2.0
CaCl₂, 1.0 MgCl₂, 10 HEPES (pH 7.4), and 10 glucose. The amplitude of the γ-aminobutyric acid (GABA) or glycine current was assayed using a brief (1–2 s) pulse of GABA or glycine every 60 s. The modulation of the GABA or glycine current by propofol (2,6 diisopropylphenol; Sigma–Aldrich, St Louis, MO) was assayed using a pulse of glycine (EC₁₀) or GABA (EC₁₀) coapplied with propofol for each receptor studied, without any preapplication. The EC₁₀ and EC₅₀ values were obtained from concentration-response curves for GABA (1–1000 μM) and glycine (1–100 μM), and the response was normalized to saturating concentrations of the agonist (100%). In all the experiments, a brief pulse of 1 mM GABA or glycine was performed at the end of the recording period to verify that the concentration used corresponded to the actual EC₁₀ in each cell. Cells that displayed responses < EC₅ or > EC₁₅ were discarded. The methodology for single-channel recordings in the outside-out configuration has been previously published.³⁰,³¹ Briefly, patch pipettes were coated with R6101 elastomer (Dow-Corning, Midland, MI) and had tip resistances of 7–15 megohms after fire polishing. Cells were voltage clamped at −60 mV for GlyRs and −100 mV for GABAᵦRs and the data were filtered (5 kHz low-pass 8-pole Bessel) and acquired at 50 kHz using pClamp software (Molecular Devices, Sunnyvale, CA). Etomidate, ethanol, butanol, and trichloroethanol were purchased from Sigma–Aldrich. Isoflurane was purchased from Baxter (Baxter International Inc., Deerfield, IL). Agonist and allosteric drug solutions were applied to cells using a stepper motor-driven rapid solution exchanger (Warner Instrument Corp., Hamden, CT). Cells were maintained in extracellular medium containing (in mM): 150 NaCl, 5 KCl, 2 CaCl₂, 10 HEPES, 10 glucose;

![Diagram](image_url)

**Fig. 1.** Deletion of the segment between residues E326 and A384 in the large intracellular loop (LIL) of the α₁ glycine receptor (GlyRs) reduced its sensitivity to propofol. (A) Schematic representation of the GlyR subunit topology. The entire sequence of the LIL for human α₁ GlyRs is shown. Three functional mutants were generated for α₁ GlyR by deletion of different regions of the LIL. The arrows indicate the deleted segments and truncated sequences are illustrated by the dashed line. The numbers indicate the positions in the mature polypeptide. (B) Glycine-activated (EC₁₀) current in wild type GlyRs was enhanced by propofol (PRO, 30 μM). In contrast, the sensitivity to propofol was significantly reduced in the Δ326–384 and Δ355–384 truncated GlyRs, whereas the sensitivity of the Δ326–355 mutant did not change. (C) The graph shows that the sensitivity to propofol was significantly reduced when the segment Δ355–384 was deleted (one-way ANOVA with Bonferroni correction post test). The bars represent the mean ± SEM. *** Significance of P < 0.001.
pH 7.4. The intracellular recording solution contained (in mM): 140 CsCl, 2 MgATP, 10 2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid and 10 HEPES; pH 7.2.

Data Analysis. Whole-cell data analysis was performed using OriginPro 7.0 (OriginLab, Northampton, MA). Nonlinear regression analysis was used to fit concentration-response curves for glycine or GABA responses. Determination of significant differences between control and drug treatment groups were performed using one-way ANOVA or paired Student t tests followed by the Bonferroni correction post hoc test. Concentration-response curves were generated by fitting the data to the Hill equation: 

\[ I = \frac{I_{\text{max}}}{1 + \frac{[A]}{EC_{50}}} \]

where \( I \) is the current, \( I_{\text{max}} \) is the maximum current, \([A]\) is the agonist concentration, and \( n \) is the Hill coefficient. All results are expressed as mean ± SEM; values of \( P < 0.05 \) were considered statistically significant. Data from single-channel recordings were idealized using the segmentation K-means algorithm in the QUB software suite (The Research Foundation State University of New York, Buffalo, NY). Dwell time histograms were fitted with three or four exponential components using Clampfit software (Molecular Devices), and mean open times were obtained from the proportionally weighted averages of the individual components. Values for open probability were calculated from idealized records. All-points amplitude histograms were generated and fitted with Gaussian functions using ClampFit.

Results

A Conserved Phenylalanine Residue is Important for the Sensitivity of GlyRs and GABA\(_{\alpha}R\) to Propofol

We tested the propofol sensitivity in a series of truncated GlyRs in which a large extension of the LIL was deleted. Three truncated forms of the GlyR, referred to in this study as Δ326–384, Δ326–355, and Δ355–384, were constructed and examined (fig. 1A). When the major portion of the LIL was deleted in Δ326–384, the potentiation of the glycine-activated current by propofol (30 μM) was significantly reduced from 363 ± 33% to 70 ± 12% (fig. 1, B and C). In contrast, the sensitivity of the Δ326–355 mutant (358 ± 48%) was similar to wild type (tested at EC\(_{50}\)). Sensitivity of the Δ355–384 mutant to propofol was attenuated to 71 ± 13% (fig. 1C), which is consistent with the view that propofol effects on GlyRs are influenced by residues located in the C-terminal region. In order to identify critical amino acids involved in propofol modulation within the G326-Q384 sequence, a sequential series of substitutions of the wild type amino acids with alanine and concentration-response curves for potentiation of the glycine current by propofol (1–100 μM) were constructed. These analyses showed that with the exception of the mutant 376MRKLF\(_{380} \rightarrow 376AAAAA_{380}\) (376–380A), all the other mutants retained their normal sensitivity to propofol (fig. 2A). For example, the sensitivity to 30 μM propofol was reduced to 65 ± 14% in the 376–380A mutant (open circles, n = 18) compared with 360 ± 30% in wild type (closed circles, n = 22). Additional single alanine substitutions showed that only the replacement of the phenylalanine residue (F380A) was able to affect the propofol modulation of GlyRs (75 ± 10%, fig. 2, B and C).

Given the functional and structural homology of the GABA\(_{\alpha}R\)s with the GlyRs,\(^1\) we hypothesized that the action of propofol on the GABA\(_{\alpha}R\) could similarly be influenced by residues located within the LIL. Because residues of both \( \alpha \) and \( \beta \) subunits have been implicated in the sensitivity of GABA\(_{\alpha}R\)s to propofol,\(^1\) we deleted a homologous intracellular loop of both \( \alpha \) and \( \beta \) subunits (fig. 3A). The \( \gamma \) subunit, however, does not appear to be required for the potentiation of GABA-evoked currents by propofol.\(^32\) To further confirm these results, we tested the sensitivity of GABA\(_{\alpha}R\) \( \alpha_1\beta_2\gamma_2 \) and \( \alpha_1\beta_2 \) combinations and did not find any significant differences (345% potentiation for \( \alpha_1\beta_2\gamma_2 \) and 340% potentiation for \( \alpha_1\beta_2 \)). Based on these data, we used GABA\(_{\alpha}R\)s composed of \( \alpha_1\beta_2 \).
subunits in our subsequent experiments. Deletion of the sequence between positions R354 and S388 in the subunit (1) significantly reduced the potentiation of GABAAR by propofol (7418%, n = 14) (fig. 3, B and C). However, when the homologous deletion in the subunit was examined, the sensitivity to propofol was unaltered (361 ± 49%, n = 8) (fig. 3C). Therefore, we carried out alanine scanning of the C-terminal region of the LIL of the subunit. We found that propofol produced equivalent modulation of all mutants, with the exception of 384TFNSV388 (384 –388A), which was potentiated by only 98123% (fig. 4A, open circles). Consequently, we next determined which amino acids in this region were involved. Similar to the GlyR, there is a conserved phenylalanine residue (F385) in the homologous position in the subunit, which when mutated caused a significant reduction in propofol potentiation (80 ± 23%) (fig. 4, B and C). In contrast, mutations at flanking residues had no effect on the potentiation by propofol (fig. 4C).

Thus, these data allow us to conclude that a conserved phenylalanine residue, in both the GlyR and GABAAR subunits, is critically important for the allosteric modulation exerted by propofol.

Replacement of a Phenylalanine Residue Within the LIL Selectively Reduced the Sensitivity to Propofol but Not to Other Allosteric Modulators

Suggested putative sites of propofol action in the TM domains in both GlyRs and GABAARs were often associated with the effects of other structurally unrelated molecules. These observations suggest that these sites might be related to more than one allosteric modulator. To address this issue, we tested the sensitivity of the propofol-resistant mutants that we identified to alcohols, neurosteroids and other intravenous or volatile anesthetics. Contrary to the idea that there is a common molecular site for pharmacological modulators,16–19,26,33,34 our electrophysiological data show that the sensitivity of the mutant receptors to modulation by propofol.
other allosteric regulators was unaffected (fig. 5A, B). For instance, the volatile anesthetic isoflurane potentiated both the GlyR WT (183 ± 19%, n = 8) and the F380A mutant (186 ± 18%, n = 8) to a similar degree (fig. 5A). Notably, GABA-evoked currents in the GABA\textsubscript{A}R \(\alpha_1\), \(\beta_2\) subunits between T384 and V388. The F385A mutant was less sensitive to propofol than the wild type (fig. 6B). In agreement with the results obtained in whole cell recordings, the application of propofol to membrane patches containing an F380A mutant channel did not increase channel activity (22 ± 13% above control, n = 5, fig. 6B). Similar to GlyRs, the analysis of the GABA\textsubscript{A}R \(\alpha_1\) F385A\(\beta_2\) mutant receptor showed that conductance and open probability were not changed (table 1). However, the GABA\textsubscript{A}R \(\alpha_1\) \(\beta_2\) wild type was strongly enhanced by propofol (fig. 6D–F), whereas the GABA\textsubscript{A}R \(\alpha_1\) F385A\(\beta_2\) mutant did not show any significant potentiation (fig. 6E). Thus, these results demonstrate that mutations in intracellular sites did not cause noticeable effects in either GlyR or GABA\textsubscript{A}R channel function, but specifically altered the sensitivity to propofol of these receptors.

**Discussion**

In the current study, we provide evidence supporting a new role of the LIL for propofol actions in two members of the Cys-loop superfamily. The data show that mutation of a phenylalanine residue, which is conserved in both GABA\textsubscript{A}R and GlyRs, significantly reduced sensitivity to propofol. Single-channel recordings showed that kinetic parameters of wild type and mutant receptors were very similar, suggesting that the reduction in propofol sensitivity was not caused by changes in ion channel properties.

Previous studies showed that GABA\textsubscript{A}R and GlyRs carrying mutations that affected the sensitivity to allosteric modulators displayed altered gating properties.13,28,35,36 For instance, mutant GABA\textsubscript{A}R and GlyRs with reduced propofol, etomidate, and general anesthetic sensitivity showed significant changes in agonist potency and channel gating.13,28,35,36 In addition, the mutant (S267Q in GlyRs)37 that showed reduced sensitivity to ethanol and general anesthetics also displayed reduced channel gating activity.36 Thus, one can argue that the impaired effects of several allosteric modulators in these mutant receptors were caused by changes in gating mechanisms.13,28,35–37 In contrast, we found that mutation of F380 in \(\alpha_1\) GlyRs and F385 in \(\alpha_1\),
GABA subunits strongly reduced the allosteric modulation exerted by propofol without noticeable changes in the channel properties. At the single-channel level, we found that low concentrations of glycine or GABA elicited single-channel currents with conductances and mean open times similar to those previously published. In agreement with previous studies, we found that propofol increased the open probability, without changes in mean open time, of GlyR and GABAARs. The absence of an effect of propofol on open time suggests that it does not affect the channel closing rate. Thus, the increased open probability observed in this study is either attributable to an increase in burst duration, which is in agreement with a study in GABAARs, or to an increase in opening frequency. Further studies will be required to distinguish between these possibilities.

Our results suggest that the mutation of the conserved phenylalanine residue generates propofol-hyposensitive GABA and GlyRs through a mechanism that does not involve changes in ion channel gating. Therefore, our results are consistent with the hypothesis that the impaired propofol sensitivity is caused by an alteration in allosteric mechanisms rather than impaired ion channel function.

Regarding the subunits involved in propofol effects on GABAAR, previous reports have suggested that mutation of residues in the subunit were sufficient to abolish the sensitivity to propofol of αβ heteropentamers. In contrast, our results showed that mutation of the F385 residue in the α1 subunit strongly reduced the sensitivity of GABAAR α1β2 subunits to propofol. In agreement with our finding, it was demonstrated that GABAAR containing the α6 subunit were fourfold less sensitive to propofol than those with α1, independent of the presence of β and γ subunits. Interestingly, sequence alignment of the LIL showed that although the α1 subunit has the phenylalanine residue, the α6 subunit has an isoleucine residue in the homologous position. Thus, our results are consistent with the hypothesis that the presence or absence of this phenylalanine residue within the LIL influences propofol sensitivity.

Several studies have suggested that residues in TM regions of GABAARs and GlyRs can form a water-filled cavity capable of binding propofol. In addition, these residues also influence the receptor sensitivity to several structurally unrelated molecules. However, studies addressing the specificity of the residues involved in anesthetic actions have yielded conflicting results. Although a single residue (Y444W) within the GABAAR β2 subunit was shown to be important for the action of propofol, a recent report showed that this mutation also reduced potentiation by menthol, suggesting a lack of specificity. Furthermore, it was found that residues in TM2 and TM3 of GlyRs and the homologous residues in the GABAAR reduced the sensitivity to propofol of αβ heteropentamers.

![Fig. 5. Propofol-hyposensitive receptors retain normal sensitivity to other positive allosteric modulators.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931109/)
to alcohols and volatile anesthetics without changes in the sensitivity to propofol. However, it was recently shown that the same mutations affected the sensitivity to propofol. Our electrophysiological results, in contrast with both of these studies, demonstrated that both GlyRs and GABA<sub>R</sub> mutants conserved their sensitivity to other allosteric modulators. For instance, the sensitivity to etomidate, previously suggested to share a binding site with propofol in GABA<sub>R</sub>s, was not altered in the GABA<sub>R</sub> mutant. Our findings suggest that the intracellular phenylalanine residue is a determinant of propofol sensitivity in both GlyRs and GABA<sub>R</sub>s but does not appear to affect the sensitivity to any other allosteric modulator.

Currently, high-resolution molecular features for the intracellular region connecting TM3 and TM4 in eukaryotic Cys-loop ion channels are not available and only initial structural assessments can be obtained from homology modeling. Previous studies in the 5-hydroxytryptamine<sub>3</sub> receptor have suggested that this region might be structured as an α-helix (termed membrane-associated stretch). In agreement with this finding, a previous model of α<sub>1</sub> GlyRs generated using the Torpedo nicotinic acetylcholine receptor as a template predicted an α-helical structure in this region. Even though our results can only suggest that the phenylalanine residue forms a binding site, we speculate that propofol is accommodated by antiparallel helices and stabilized by hydrophobic interactions with two phenylalanine residues nearby. This agrees with data obtained in crystallized proteins in complex with propofol that showed that binding pockets are formed by the arrangement of two helices, and that the putative cavity was lined by basic amino acids and hydrophobic residues, which provide the necessary environment to accommodate the anesthetic molecule.

In conclusion, we have identified a conserved phenylalanine residue localized in the LIL, which influences the propofol effect on single-channel function in wild type and mutated glycine receptor (GlyRs) and γ-aminobutyric acid type A receptor (GABA<sub>A</sub>R). (A) Single-channel activity recorded in wild type and F380A GlyRs (calibration bar: 5 pA, 10 ms). (B) Percentage of the open probability (nPo) potentiation by propofol in the wild type and F380A GlyRs. (C) Main conductance of the wild type and F380A GlyRs. (D) Single-channel activity recorded in wild type and F385A GABA<sub>A</sub>R (calibration bar: 2 pA, 10 ms). (E) Percentage of nPo potentiation in the wild type and F385A GABA<sub>A</sub>R by propofol. (F) Main conductance of the wild type and F385A GABA<sub>A</sub>R receptor. The bars represent the mean ± SEM. *** Significance of P < 0.001.
fol sensitivity of GlyRs and GABA_ARs. These results provide the first evidence indicating that the LIL plays a role in anesthetic effects on inhibitory Cys-loop ion channels. Thus, these data provide new insights into the molecular mechanism of modulation of inhibitory ion channels by propofol, and will contribute to the understanding of the complex molecular framework underlying the modulation of central nervous system activity by general anesthetics.

The authors thank Lauren Aguayo, B.S. (Technician, Department of Physiology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile), for technical assistance and help with the GlyR constructions.

References
1. Lynch JW: Molecular structure and function of the glycine receptor chloride channel. Physiol Rev 2004; 84:1051–95
9. Sebel LE, Richardson JE, Singh SP, Bell SV, Jenkins A: Additive effects of sevoflurane and propofol on gamma-aminobutyric acid receptor function. Anesthesiology 2006; 104:1176–83
14. Richardson JE, Garcia PS, O’Toole KK, Derry JM, Bell SV, Jenkins A: A conserved tyrosine in the β2 subunit M4 segment is a determinant of γ-aminobutyric acid type A receptor sensitivity to propofol. Anesthesiology 2007; 10:412–8
17. Krasowski MD, Koltchine VV, Ricc CE, Ye Q, Finn SE, Harrison NL: Propofol and other intravenous anaesthetics have sites of action on the gamma-aminobutyric acid type A receptor distinct from that for isoflurane. Mol Pharmacol 1998; 53:530–8

Table 1. Kinetics Parameters of Wild Type and Mutant GlyRs and GABA_ARs in Absence or Presence of Propofol

<table>
<thead>
<tr>
<th>GlyRs</th>
<th>Glycine 1 μM</th>
<th>Glycine 1 μM + Propofol 1 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>MOT (ms)</td>
<td>MST (ms)</td>
</tr>
<tr>
<td>Wild type</td>
<td>1.15 ± 0.03</td>
<td>25.2 ± 4</td>
</tr>
<tr>
<td>F380A</td>
<td>1.35 ± 0.05</td>
<td>26.1 ± 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GABA_ARs</th>
<th>GABA 1 μM</th>
<th>GABA 1 μM + Propofol 1 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condition</td>
<td>MOT (ms)</td>
<td>MST (ms)</td>
</tr>
<tr>
<td>Wild type</td>
<td>1.03 ± 0.03</td>
<td>18.3 ± 0.04</td>
</tr>
<tr>
<td>F385A</td>
<td>1.18 ± 0.04</td>
<td>17.7 ± 0.03</td>
</tr>
</tbody>
</table>

GABA = γ-aminobutyric acid; GABA_ARs = γ-aminobutyric acid receptor type A; GlyRs = glycine receptors; MOT = mean open time; MST = mean shut time; nPo = open probability; γ = conductance.
34. Krasowski MD, Nishikawa K, Nikolaeva N, Lin A, Harrison NL: Methionine 286 in transmembrane domain 3 of the GABA(A) receptor beta subunit controls a binding cavity for propofol and other alkylphenol general anesthetics. Neuropharmacology 2001; 41:952–64
35. Lobo IA, Mascia MP, Trudell JR, Harris RA: Channel gating of the glycine receptor changes accessibility to residues implicated in receptor potentiation by alcohols and anesthetics. J Biol Chem 2004; 279:33919–27
40. O’Shea SM, Williams CA, Jenkins A: Inverse effects on gating and modulation caused by a mutation in the M2–M3 Linker of the GABA(A) receptor gamma subunit. Mol Pharmacol 2009; 76:641–51