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The Actions of Sevoflurane and Desflurane on the γ-Aminobutyric Acid Receptor Type A

Effects of TM2 Mutations in the α and β Subunits

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Background: **Previous studies have shown that specific amino acid residues in the putative second transmembrane segment (TM2) of the γ-aminobutyric acid receptor type A ($GABA_A$) receptor play a critical role in the enhancement of $GABA_A$ receptor function by halothane, enflurane, and isoflurane. However, very little is known about the actions of sevoflurane and desflurane on recombinant $GABA_A$ receptors. The aim of this study was to examine the effects of sevoflurane and desflurane on potentiation of GABA-induced responses in the wild-type $GABA_A$ receptor and in receptors mutated in TM2 of the α1, α2, or β2 subunits.**

Methods: **$GABA_A$ receptor α1 or α2, β2 or β3, and γ2s subunit cDNAs were expressed for pharmacologic study by transfection of human embryonic kidney 293 cells and assayed using the whole cell voltage clamp technique. Concentration–response curves and EC_{50} values for agonist were determined in the wild-type α1β2γ2s and α2β3γ2s receptors, and in receptors harboring mutations in TM2, such as α1(S270W)β2γ2s, α1β2(N265W)γ2s, and α2(S270I)β3γ2s. The actions of clinically relevant concentration of volatile anesthetics (isoflurane, sevoflurane, and desflurane) on GABA activated Cl^- currents were compared in the wild-type and mutant $GABA_A$ receptors.**

Results: **Both sevoflurane and desflurane potentiated submaximal GABA currents in the wild-type $GABA_A$ α1β2γ2s receptor and α2β3γ2s receptor. Substitution of Ser270 in TM2 of the α subunit by a larger amino acid, tryptophan (W) or isoleucine (I), as in α1(S270W)β2γ2s and α2(S270I)β3γ2s, completely abolished the potentiation of GABA-induced currents by these anesthetic agents. In contrast, mutation of Asn265 in TM2 of the β subunit to tryptophan (W) did not prevent potentiation of GABA-induced responses. The actions of sevoflurane and desflurane in the wild-type receptor and in mutated receptors were qualitatively and quantitatively similar to those observed for isoflurane.**

Conclusions: **Positions Ser270 of the $GABA_A$ α1 and α2 subunits, but not Asn265 in the TM2 of the β2 subunit, are critical for regulation of the $GABA_A$ receptor by sevoflurane and desflurane, as well as isoflurane, consistent with the idea that these three volatile anesthetics share a common site of actions on the α subunit of the $GABA_A$ receptor.**

LIGAND-GATED ion channels have emerged as strong candidates to mediate the actions of volatile anesthetics.[1,2] In particular, γ-aminobutyric acid type A ($GABA_A$) receptors, the major inhibitory neurotransmitter receptors in the central nervous system, are regulated allosterically by a wide range of drugs, including halogenated volatile anesthetics, n-alcohols, propofol, barbiturates, benzodiazepines, and steroids.[3–7] The $GABA_A$ receptor is a heteromeric complex assembled from different glycoprotein subunits (α_{1-6}, β_{1-3}, γ_{1-4}, δ, ϵ, and π), which combine to form a chloride channel. $GABA_A$ receptors *in vivo* probably consist of pentameric complexes of α, β, and γ subunits with a stoichiometry of 2α: 2β: 1γ and have been proposed to contain four hydrophobic transmembrane segments (TM1–TM4).[8–10] The most prevalent receptor subtype in synapses of the adult mammalian central nervous system is α1β2γ2s, which accounts for approximately 40% of the total complement of $GABA_A$ receptors.[11] Previous studies have shown that specific point mutations at Ser270 or Ala291 in the $GABA_A$ receptor α2 subunit selectively abolish agonist potentiation by the inhaled anesthetic enflurane (fig. 1).[3] In addition, extensive mutagenesis at Ser270 in the α2 subunit suggests a critical role of this residue, not only for channel gating but also in anesthetic modulation of receptor function by halothane and isoflurane. Conversely, Asn265 in the β2 subunit seems not to be important for the actions of isoflurane,[7] but to be critical in the actions of certain intravenous agents.[12,13]

Sevoflurane and desflurane are volatile anesthetics that were developed relatively recently, and are now widely used in clinical anesthesia. Previous papers have reported the effects of these two volatile anesthetics on synaptic transmission in the central nervous system. Although sevoflurane has been reported to potentiate GABA-induced currents in cultured neurons[14,15] and to prolong synaptic currents mediated by $GABA_A$ receptors in hippocampal pyramidal neurons and interneurons,[16] little is known about the actions of sevoflurane or desflurane on recombinant $GABA_A$ receptors. To begin to investigate the actions of these anesthetics on $GABA_A$ receptors at the molecular level, we have examined the effects of sevoflurane and desflurane on the wild-type α1β2γ2s and α2β3γ2s receptors and on receptors harboring mutations in TM2 such as α1(S270W)β2γ2s, α1β2(N265W)γ2s and α2(S270I)β3γ2s. The aim of this study was to compare the effects of these volatile anesthetics with those of isoflurane on $GABA_A$ receptor function and to examine the effects of TM2 mutations.

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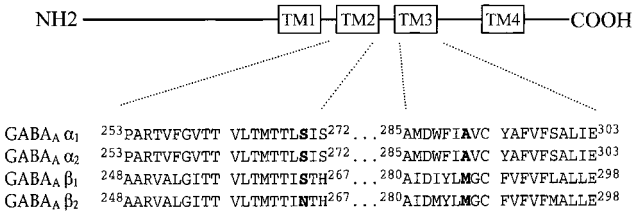

Fig. 1. Putative γ-aminobutyric acid type A (GABA$_A$) receptor subunit topology. GABA$_A$ receptor subunits have four hydrophobic transmembrane regions (TM1–TM4), a large hydrophilic ligand-binding domain in the extracellular N-terminus,[32] an intracellular loop between TM3 and TM4, and a short extracellular C-terminus (*top*). Amino acid sequence alignment of TM2 and TM3 from human α1, α2, β1, and β2 subunits (*bottom*). Residue positions in *bold type* within TM2 and TM3 of these receptor subunits are critical for potentiation of agonist responses by n-alcohols and volatile anesthetics.[3]

Materials and Methods

Site-directed Mutagenesis

Mutagenesis of the GABA$_A$ receptor subunit cDNA was performed with the unique site elimination method as described previously.[17] In brief, the unique site elimination method uses a two-primer system in which one primer is a mutagenic primer (Operon Technologies, Alameda, CA) and the other is a selection primer; a unique S*sp* I site in the expression vector pCIS2 was mutated to an alternative restriction site (*Mlu* I). These primers were 5'-phosphorylated using polynucleotide kinase, and then used to create mutations using the unique site elimination kit (Amersham Pharmacia Biotech, Piscataway, NJ). S*sp* I digestion was used to select in favor of desired mutants and against template DNA; positive clones of transformed *Escherichia coli* were screened for the appearance of the desired mutations by digestion with H*pa* II. The sequences of all cDNA inserts were confirmed by double-stranded DNA sequencing.

Cell Culture and Transfection of Receptor cDNA

Human embryonic kidney (HEK) 293 cells have been very useful for the transient expression and electrophysiologic analysis of GABA$_A$ receptors.[18,19] The GABA$_A$ receptor subunit cDNAs were obtained as follows: human α1, human α2, rat β2, rat β3, and human γ2 from the laboratory of the late Dr. Dolan Pritchett. The wild-type or mutant GABA$_A$ receptor cDNAs were expressed *via* the vector pCIS2, which contains one copy of the strong promoter from cytomegalovirus and a polyadenylation sequence from simian virus 40. These constructs were used to transfect HEK 293 cells (American Type Culture Collection, Rockville, MD). HEK 293 cells were maintained in culture on glass coverslips; cells were passaged weekly by trypsin treatment up to 20 times before being discarded and replaced with early passage cells. Cells was transfected using the $CaPO_4$ precipitation technique.[20] Three to five micrograms of each cDNA was added to 65 μl of distilled water, 8 μl of 2.5 M $CaCl_2$ and 75 μl of 50 mM N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid for each coverslip. The precipitation reaction was allowed to proceed for approximately 60 min. The cDNA was in contact with the HEK cells for 24 h in an atmosphere containing 3% CO_2 (37°C) before being removed and replaced with fresh culture medium in an atmosphere of 5% CO_2 (37°C).

Electrophysiologic Recordings

The coverslips were transferred, between 48 and 72 h after removal of the cDNA, to a large recording chamber and perfused continuously with extracellular solutions (145 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, 6 mM D-glucose, 10 mM HEPES/NaOH adjusted to pH 7.4). Whole cell patch clamp recordings from HEK 293 cells were made using the Axopatch 200 amplifier (Axon Instruments, Foster City, CA) as described previously.[7] The resistance of the patch pipette was 4–6 MΩ when filled with internal solutions (145 mM *N*-methyl-D-glucamine HCl, 5 mM dipotassium ATP, 1.1 mM EGTA, 2 mM $MgCl_2$, 5 mM HEPES/KOH, 0.1 mM $CaCl_2$ adjusted to pH 7.2). Cells were voltage clamped at −60 mV. Because the chloride concentrations in the intracellular and extracellular solutions were almost identical, the calculated chloride equilibrium potential was approximately 0 mV. In addition to the continuous bath perfusion with extracellular medium, solutions including GABA and/or volatile anesthetics were applied to the cell by local perfusion using a motor-driven solution-exchange device (Rapid Solution Changer RSC-100; Molecular Kinetics, Pullman, WA). This system exchanges solutions around the cells within approximately 20–50 ms.[21] Laminar flow out of the rapid solution changer head was achieved by driving all solutions at identical flow rates (1–1.5 ml/min) *via* a multichannel infusion pump (Stoelting, Wood Dale, IL). The solution changer was driven by protocols in the acquisition program of pCLAMP version 5 (Axon Instruments). For GABA concentration–response studies, cells were typically superfused with extracellular saline before switching into one of seven GABA concentrations for 3 s, and returning to saline for at least 15 s before any subsequent GABA application. GABA responses typically exhibited a small degree of desensitization during agonist applications, such that the amplitude of the responses declined by 10–15% from the peak current. However, recovery from desensitization was typically rapid after agonist removal, and GABA current amplitudes were stable in response to repeated applications. Currents were low-pass filtered at 2 kHz and digitized with the TL-1–125 interface (Axon Instruments) using pCLAMP 5 and stored for off-line analysis. All experiments were performed at room temperature (21°–24°C).

Drugs and Preparation of Volatile Anesthetic Solutions

Stock solutions of GABA and anesthetic compounds were diluted into the extracellular solutions daily shortly before use. All volatile anesthetic solutions were prepared by injection of liquid anesthetic with a gas-tight syringe (Hamilton, Reno, NV) into intravenous drip bags containing defined volumes of extracellular solutions (100 ml) and used for up to 4 h.[21] The clinically relevant "minimum alveolar concentration (MAC) equivalent" aqueous concentrations defined for this study and most often applied to cells were isoflurane 370 μM, sevoflurane 360 μM, and desflurane 280 μM. Losses of volatile anesthetics in this perfusion system has been measured using gas chromatography and represents only 5–10% of the total applied drug concentration.[19] The sources of the anesthetics used were as follows: Ayerst Laboratories (Philadelphia, PA) for halothane, Abbott Laboratories (North Chicago, IL) for isoflurane and sevoflurane, Ohmeda (Liberty Corner, NJ) for desflurane.

Data Analysis and Statistics

Control GABA concentration–response data were expressed as a fraction of the maximal response to GABA in each cell, allowing normalized data from different cells to be combined. Pooled data were fitted using a weighted sum of least-squares method (KaleidaGraph version 3.5, Synergy Software, Reading, PA) to a Hill equation:

$$I/I_{max(GABA)} = [drug]^{nH}/([drug]^{nH} + (EC_{50})^{nH});$$

where *I*/*I*max is the whole cell current amplitude expressed as a percentage of the current maximal peak current, [drug] is the GABA concentration, EC_{50} is the GABA concentration eliciting a current equal to half amplitude of *I*max, and nH is the Hill coefficient. Anesthetic-induced potentiation of a submaximal GABA response was then calculated as the percentage increase above the control (EC_{20}) response to GABA in the presence of the anesthetics. Anesthetics were always preapplied for 3 s before coapplication with agonist to ensure that the anesthetic had reached equilibrium with the receptors. All data are presented as mean ± SEM with *n* number of cells tested. Statistical significance was determined using one-way ANOVA to assess differences between groups.

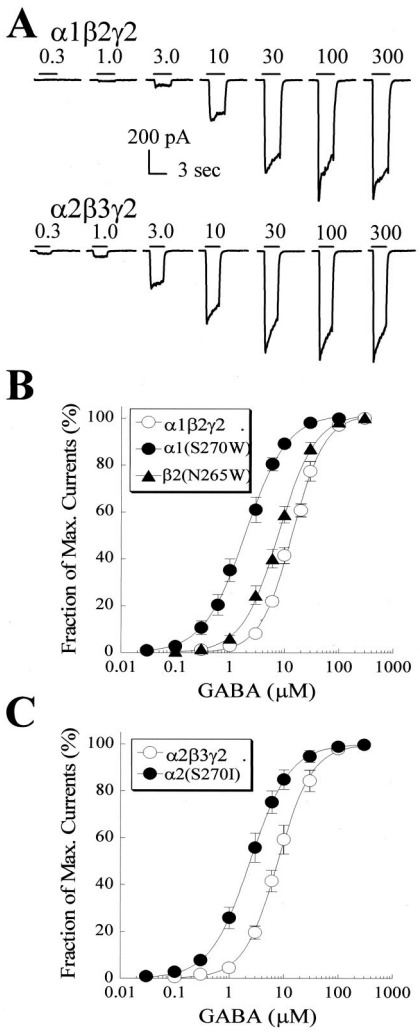

Fig. 2. (*A*) γ-Aminobutyric acid (GABA)-induced Cl^- currents recorded from a human embryonic kidney 293 cell expressing the wild-type $GABA_A$ *α1β2γ2s* receptor (*top*) or *α2β3γ2s* receptor (*bottom*). Cells were voltage clamped at −60 mV and GABA was applied with the rapid solution changer. *Bars* over *current traces* show the duration of rapid GABA application with the concentration (μM) of applied GABA. (*B*) Concentration–response curves for the wild-type $GABA_A$ *α1β2γ2s* receptor (*open circles*) and mutant receptors, *α1*(S270W) *β2γ2s* (*filled circles*) and *β2*(N265W) *β2γ2s* (*filled triangles*). GABA currents were expressed as a fraction of the maximal GABA response and these normalized data were fitted by a Hill equation. *Data points* are shown as the means of at least six cells and *error bars* indicate SEM. In some cases, the error bars are smaller than the symbols. (*C*) Concentration–response curves were also constructed in the wild-type $GABA_A$ *α2β3γ2s* receptor (*open circles*) and receptor mutated at *α2*(S270I) (*filled circles*). Data are constructed from at least eight dose–response curves.

Results

Concentration–Response Curves for the Wild-type $GABA_A$ α1β2γ2s Receptor, α2β3γ2s Receptor and Receptors Mutated in the TM2 Segment

The wild-type $GABA_A$ $\alpha1\beta2\gamma2$s receptor, $\alpha2\beta3\gamma2$s receptor and receptors harboring mutations in TM2 segment such as $\alpha1$(S270W)$\beta2\gamma2$s, $\alpha1\beta2$(N265W)$\gamma2$s and $\alpha2$(S270I)$\beta3\gamma2$s were transiently expressed in HEK 293 cells. Large inward chloride currents induced by GABA (0.01 μM to 1 mM) were recorded in the absence of the anesthetics, at a holding potential of −60 mV. Representative recordings of GABA-induced Cl^- currents are presented for the wild-type $\alpha1\beta2\gamma2$s receptor (fig. 2A, top) and for the wild-type $\alpha2\beta3\gamma2$s receptor (fig. 2A, bottom). To examine the effects of mutation in TM2 of the

Table 1. A Summary of the Characteristics of the Wild-type $\alpha1\beta2\gamma2$s Receptor, $\alpha2\beta3\gamma2$s Receptor, and Receptors Mutated at TM2 Segment

GABA Receptor	EC_{50} (μM)	Hill	*I*max (pA)	N Cells
$\alpha1\beta2\gamma2$s (WT)	14.4 ± 1.2	1.5 ± 0.2	543.0 ± 184.1	15
$\alpha1$(S270W)$\beta2\gamma2$s	1.8 ± 0.3*	1.3 ± 0.2	489.2 ± 109.0	10
$\alpha1\beta2$(N265W)$\gamma2$s	9.1 ± 1.1†	1.4 ± 0.2	467.9 ± 171.8	13
$\alpha2\beta3\gamma2$s (WT)	7.9 ± 0.8	1.4 ± 0.1	667.2 ± 113.0	18
$\alpha2$(S270I)$\beta3\gamma2$s	2.4 ± 0.4*	1.2 ± 0.1	343.0 ± 104.1	16

EC_{50} concentrations, Hill coefficient, and maximal current (*I*max) are given for each receptor as mean ± SEM for N number of cells tested, using the methods described. Statistical significance was determined using the Student *t* test.

* $P < 0.001$, † $P < 0.01$ compared to the corresponding wild-type receptor.

GABA = γ-aminobutyric acid, TM = transmembrane, WT = wild-type.

α or β subunit on the apparent affinity for the agonist, full concentration–response curves for GABA were determined in all receptors. Currents elicited by at least seven different concentrations of GABA were expressed as a fraction of the maximal GABA response, and the normalized data were fitted by a Hill equation. Figures 2B and C display GABA concentration–response curves for the wild-type receptor and for receptors mutated at TM2 of either α or β subunit. Mutation of Ser270 to a large amino acid such as tryptophan or isoleucine produced a leftward shift in the curves, consistent with an increased apparent affinity for GABA. The GABA EC_{50}, Hill coefficient and maximal current amplitude data for all receptors tested are summarized in table 1. There was no significant difference in Hill coefficient or in the amplitude of peak GABA currents between the wild-type receptor and mutant receptors.

Effects of Point Mutation on Potentiation of GABA Responses by Sevoflurane and Desflurane

To determine whether the mutated amino acid residues influenced the ability of volatile anesthetics to potentiate $GABA_A$ receptor function, the effects of desflurane, sevoflurane, and isoflurane on the amplitude of GABA responses were examined. Figure 3A shows representative recordings from experiments in which we measured the potentiation of submaximal (EC_{20} concentration) GABA-induced currents by sevoflurane and desflurane in cells expressing the wild-type $\alpha1\beta2\gamma2$s receptor. Clinically relevant concentrations of isoflurane (1 MAC ≅ 370 μM), sevoflurane (1 MAC ≅ 360 μM), and desflurane (1 MAC ≅ 280 μM) significantly enhanced the amplitude of GABA responses by 99.0 ± 13.1, 59.3 ± 11.2, and 55.7 ± 12.6% above control, respectively (fig. 3B). These data are consistent with our previous observations that clinical concentration equivalents of isoflurane potentiated EC_{20} GABA currents by approximately 100%.[7] When Ser270 of the $\alpha1$ subunit was substituted by a large amino acid tryptophan (W), the mutant receptor $\alpha1$(S270W)$\beta2\gamma2$s showed no significant potentiation by isoflurane, sevoflurane, and desflurane (8.2 ± 7.1, 7.5 ± 5.5, 3.6 ± 9.8% above control, fig. 3B, bottom),

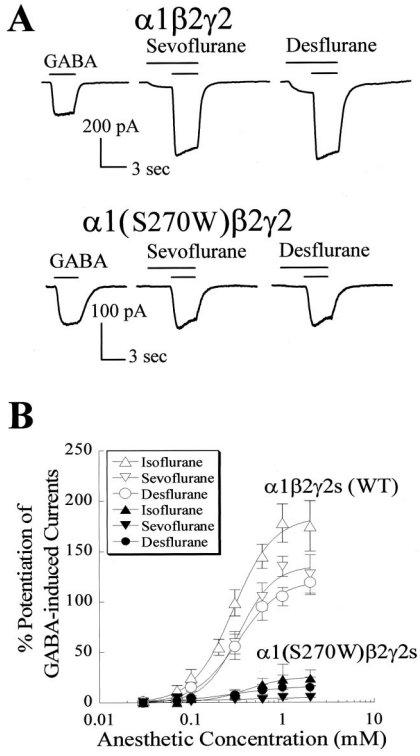

Fig. 3. Submaximal γ-aminobutyric acid (GABA) currents in the wild-type $GABA_A$ $\alpha1\beta2\gamma2$s receptor are strongly enhanced by coapplication of clinically relevant concentrations of sevoflurane and desflurane, but not in $\alpha1$(S270W)$\beta2\gamma2$s. (*A*) Representative examples of enhancement of submaximal GABA responses by sevoflurane and desflurane. Sample tracings were obtained from human embryonic kidney cells expressing the wild-type $\alpha1\beta2\gamma2$s receptor (*top*) and mutant receptor, $\alpha1$(S270W)$\beta2\gamma2$s (*bottom*). The anesthetics were preapplied for 3 s before coapplication with submaximal concentration (EC_{20}) of GABA (5 μM for wild-type and 0.5 μM for mutant receptor). Note that preapplied volatile anesthetics directly activated small GABA currents in the absence of GABA in the wild-type receptors. (*B*) Concentration–response relationships for potentiation of GABA by three volatile anesthetics. Although all volatile anesthetics significantly potentiated EC_{20} GABA responses at all concentrations of more than 0.3 mM in the wild-type receptor ($P < 0.05$ for each concentration; six to eight experiments for each data point), no significant potentiation of an EC_{20} GABA concentration was observed in $\alpha1$(S270)$\beta2\gamma2$s. From the curve fit, the EC_{50} value for potentiation of $\alpha1\beta2\gamma2$s receptor by isoflurane, sevoflurane, and desflurane was 0.28, 0.33, and 0.32 mM, respectively.

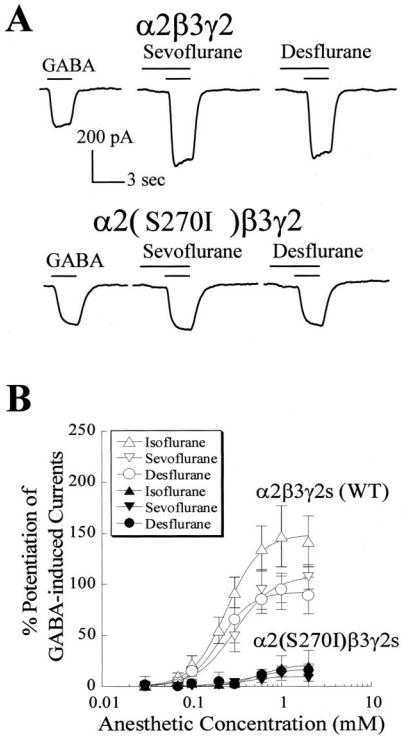

Fig. 4. Submaximal γ-aminobutyric acid (GABA) currents in the wild-type α2β3γ2s receptor are also strongly enhanced by sevoflurane and desflurane, but not in α2(S270I)β3γ2s. (*A*) Representative examples of enhancement of submaximal GABA responses by sevoflurane and desflurane. Sample tracings were obtained from human embryonic kidney cells expressing the wild-type α2β3γ2s receptor (*top*) and mutant receptor, α2(S270I)β3γ2s (*bottom*). The anesthetics were preapplied for 3 s before coapplication with submaximal concentration (EC_{20}) of GABA (3 μM for the wild-type and 0.7 μM for mutant receptor). In contrast to the wild-type α2β3γ2s receptor, submaximal GABA currents in α2(S270I)β2γ2s mutant receptor were not enhanced by coapplication of volatile anesthetics (up to 2 mM). (*B*) Concentration–response relationships for potentiation of GABA-induced currents by three volatile anesthetics were summarized. Similarly, the EC_{50} value for potentiation of α2β3γ2s receptor by isoflurane, sevoflurane, and desflurane was 0.28, 0.29, and 0.20 mM, respectively.

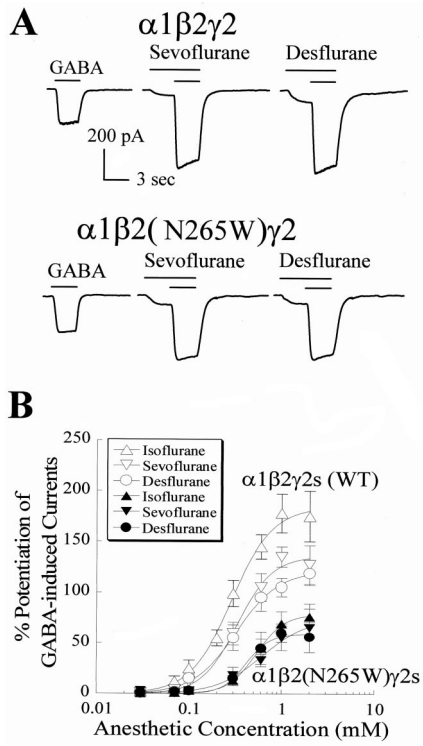

Fig. 5. Mutation within TM2 of γ-aminobutyric acid type A ($GABA_A$) β2 subunit, α1β2(N265W)γ2s, did not abolish positive modulation by the volatile anesthetics. (*A*) Representative examples of enhancement of submaximal GABA (5 μM for wild-type and 2.5 μM for mutant receptor) responses of the wild-type receptor by sevoflurane and desflurane. (*B*) Concentration–response relationships for potentiation of GABA-induced currents by three volatile anesthetics. Although all anesthetics significantly potentiated GABA-induced currents in the α1β2(N265W)γ2s receptor ($P < 0.05$ *versus* control at 0.3 mM or higher), the degree of potentiation was smaller than those of the wild-type receptor. From the curve fit, the EC_{50} value for potentiation of α1β2(N265W)γ2s receptor by isoflurane, sevoflurane, and desflurane was 0.24, 0.30, and 0.17 mM, respectively. Data are constructed from at least eight dose–response curves.

suggesting that α1(S270) is one of the key residues for the modulation of $GABA_A$ receptors by these volatile anesthetics. In fact, no significant potentiation was observed in the α1(S270W)β2γ2s receptor for any of the three volatile agents, up to 2 mM anesthetic.

To confirm the importance of Ser270 of the α subunit, we also studied the effects of this mutation in another subunit combination. The α2β3γ2s receptor subtype was used because this is the second most abundant subunit combination in mammalian brain.[11] As shown in figure 2C and table 1, the α2β3γ2s GABA receptor also expressed well in HEK cells. As shown in figure 4A, the three volatile anesthetics significantly enhanced GABA-induced currents in the α2β3γ2s wild-type receptor (89.2 ± 15.1, 49.3 ± 15.5, and 65.3 ± 22.1% of control, $n \geq 8$, $P < 0.001$ for each anesthetic). The degree of potentiation was similar to that observed in α1β2γ2s receptor (fig. 3). When Ser270 of the α2 subunit was substituted by the large amino acid isoleucine (I), the mutant receptor α2(S270I)β3γ2s was insensitive to potentiation of GABA responses by sevoflurane and desflurane (7.6 ± 8.8, 3.2 ± 7.6, and 5.5 ± 4.3% above control, respectively, fig. 4B).

As shown in figure 1, Asn265 in the β2 subunit is homologous with position Ser270 in the α1 subunit. In fact, this position is an important amino acid residue for intravenous anesthetic modulation of the receptor.[12,13,22] We therefore examined the effects of TM2 mutation in the β2 subunit using α1β2(N265W)γ2s. In contrast to α1(S270W)β2γ2s, isoflurane, sevoflurane, and desflurane potentiated GABA-induced currents strongly in the α1β2(N265W)γ2s receptor (fig. 5), although the degree of potentiation produced by these anesthetics was somewhat smaller than that measured for the wild-type α1β2γ2s receptor.

Discussion

All $GABA_A$ receptors tested in this study were successfully expressed in HEK 293 cells and the apparent affinity for GABA could be measured easily. We believe from our data that amino acid substitutions in the TM2 segment of the α1, α2 or β2 subunits of the $GABA_A$ receptor did not compromise ion channel function significantly, consistent with the notion that these point mutations did not produce large structural changes in the region of the channel pore, or in the GABA binding sites of the extracellular N-terminus.

Regulation of the Wild-type $GABA_A$ $\alpha1\beta2\gamma2s$ Receptor and Mutated Receptors by Sevoflurane and Desflurane

As illustrated in figure 3A, clinically relevant concentrations of isoflurane, sevoflurane, and desflurane significantly increased the amplitude of submaximal GABA-induced currents in the wild-type $GABA_A$ $\alpha1\beta2\gamma2s$ receptors. These data are consistent with previous observations that volatile anesthetics potentiated $GABA_A$ receptor-mediated chloride currents in recombinant $GABA_A$ $\alpha2\beta1$ receptor,[4,23] $GABA_A$ $\alpha1\beta2\gamma2s$ receptor,[7,24] $GABA_A$ receptors of cultured neurons,[25,26] and in brain slices.[27,28] These data are consistent with the hypothesis that neuronal inhibition mediated by $GABA_A$ receptors plays an important role in the production of anesthesia by these agents.

When the $GABA_A$ receptor α1(S270W) $\beta2\gamma2s$ was tested for modulation by sevoflurane and desflurane, there was a strong effect of the larger side chains such as tryptophan, which completely abolished the anesthetic potentiation by sevoflurane and desflurane (fig. 3A, bottom). These data confirm previous findings that mutation of α2(S270) in $\alpha2\beta1$ can ablate potentiation by the inhaled anesthetic enflurane,[3] isoflurane,[4,23] and halothane,[4] suggesting that S270 of the α subunit is a key residue for potentiation of $GABA_A$ receptors by sevoflurane and desflurane, and for other volatile anesthetics. In contrast, GABA-induced currents in a receptor mutated in the TM2 segment of the β subunit, $\alpha1\beta2$(N265W)$\gamma2s$, retained sensitivity to the anesthetics (fig. 5). The contrasting effects of α1(S270) and β2(N265) subunit mutations in the current study suggest that it is more likely that N265 governs critical allosteric transitions, rather than directly binding volatile anesthetics.

Regulation of the Wild-type $GABA_A$ $\alpha2\beta3\gamma2s$ Receptor and $\alpha2$(S270I)$\beta3\gamma2s$ Receptor by Sevoflurane and Desflurane

The importance of Ser270 of the α subunit was confirmed not only in the $\alpha1\beta2\gamma2s$ receptor, but also in the $\alpha2\beta3\gamma2s$ receptor. The mutations analyzed in the current study all result from the replacement of a smaller amino acid by a larger amino acid (*e.g.*, serine to isoleucine, serine to tryptophan, asparagine to tryptophan). If residues within TM2 of the GABA α subunit do indeed form part of a binding pocket, then substitution by larger amino acid residues at these positions should hinder volatile anesthetic binding, and thereby ablate positive modulation of receptor function. We therefore hypothesize that the serine residue in TM2 of α1 and α2 subunits forms part of a binding site not only for halothane and isoflurane, but also for sevoflurane and desflurane. The molecular volumes for desflurane and sevoflurane are very similar to that of isoflurane, so that the isoflurane-like pattern of activity of these drugs in the wild-type and mutant receptors studied here is not unexpected. Further investigation will be needed verify whether general anesthetics such as sevoflurane and desflurane do indeed bind directly to amino acids in the TM2 segment of the α subunit. A high-resolution structure of the $GABA_A$ receptor must first be resolved.

The Role of β Subunit in Volatile Anesthetic Modulation of the $GABA_A$ Receptor

Site-directed mutagenesis studies have demonstrated the importance of the residue N265 in the β subunit for potentiation of GABA responses by etomidate and the anticonvulsant loreclezole[22,29]. In fact, recent work has demonstrated that mutation of Asn289 within the $GABA_A$ β3 subunit (homologous with Asn265 in the β2 subunit) to methionine abolishes direct activation and GABA-induced potentiation by etomidate *in vitro*,[12] but the involvement of the β subunit in regulation by volatile anesthetic is still unclear at this time.[7] In addition, a knock-in mouse harboring the N289 M mutation in the β3 subunit shows a drastically reduced behavioral response to both propofol and etomidate,[13] using both the loss of righting reflex and withdrawal reflex assays. The evidence for involvement of the $GABA_A$ receptor in these behavioral actions of etomidate and propofol therefore appears incontrovertible, and this strengthens the case in favor of similar knock-in mouse experiments with the volatile anesthetics studied here.

In conclusion, the data presented here confirm previous reports that Ser270 of the α1 subunit is important in the actions of inhaled agents at the $GABA_A$ receptor, and extends these observations to sevoflurane and desflurane. In addition, we provide new information that the importance of Ser270 of the α subunit is conserved in the wild-type $GABA_A$ $\alpha2\beta3\gamma2s$ receptor. Several lines of evidence have converged to infer the existence of a common site of action for halothane and isoflurane within the α subunit.[4,7,24,30,31] The present data are consistent with the existence of a cavity that may be occupied by isoflurane, sevoflurane, or desflurane because the same mutations block potentiation by all three agents.

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