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Differential modulatory actions of the volatile convulsant flurothyl and its anesthetic isomer at inhibitory ligand-gated ion channels

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Abstract

A challenge for theories of general anesthesia is the existence of compounds predicted to be anesthetics but which, instead, do not produce anesthesia and often elicit other behavioral effects such as convulsions. This study focused on flurothyl (bis[2,2,2-trifluoroethyl] ether), a potent volatile convulsant, and its anesthetic isomer, 'iso-flurothyl' (1,1,1,3,3,3-hexafluoro-2-methoxypropane). The effects of flurothyl and iso-flurothyl were studied using the whole-cell patch-clamp technique on agonist activated chloride currents in human GABA_A, glycine, and GABA_C ρ_1 receptors expressed in HEK 293 cells. GABA_A and glycine receptors are promising molecular targets for the actions of inhaled ether general anesthetics. Flurothyl acted as a non-competitive antagonist at GABA_A $\alpha_2\beta_1$ and $\alpha_2\beta_1\gamma_2$ receptors, but had no effect at glycine α_1 receptors. Flurothyl had biphasic actions on GABA responses at GABA_C ρ_1 receptors. In contrast, iso-flurothyl enhanced ('potentiated') submaximal agonist responses at GABA_A and glycine receptors, but had no effect on GABA responses at GABA_C ρ_1 receptors. Point mutations in GABA_A and glycine receptor subunits, which have been previously shown to abolish potentiation of agonist responses by the ether anesthetics enflurane and isoflurane, also ablated potentiation of agonist responses by iso-flurothyl. These same mutations in the GABA_A receptor had only modest effects on the inhibitory actions of flurothyl. GABA_A receptors with mutations conferring insensitivity to antagonism by picrotoxin were still inhibited by flurothyl, suggesting that picrotoxin and flurothyl antagonize GABA responses by distinct sites or mechanisms of action. In summary, antagonism of GABA_A receptors is likely to account for the convulsant effects of flurothyl, while the general anesthetic actions of iso-flurothyl, like those of other ether anesthetics, may be related to positive modulation of GABA_A and/or glycine receptors.

Keywords

GABA_A receptor; Glycine receptor; GABA_C ρ receptor; General anesthetics; Convulsants; Picrotoxin

1. Introduction

Although inhaled general anesthetics have been used in clinical practice for over 150 years, their molecular mechanisms of action remain controversial. The traditional dogma used to explain the mechanisms of action of volatile anesthetics has been that these compounds act 'non-specifically', perhaps by perturbing the properties of membrane lipids (reviewed by Franks and Lieb, 1994). However, research within the last several decades has demonstrated

numerous inconsistencies between experimental observations and non-specific theories of general anesthesia (Franks and Lieb, 1994; Koblin et al., 1994; Krasowski and Harrison, 1999). A major problem with the non-specific theories is that some chemical compounds predicted to be anesthetics based upon their lipophilicity do not in fact produce anesthesia (Koblin et al., 1994). These compounds, originally called 'nonanesthetics', are now more properly referred to as 'nonimmobilizers'. Most of the non-immobilizers also elicit convulsions in animals (Eger et al., 1999). One of the better known non-immobilizers is the fluorinated ether flurothyl (Indoklon; see Fig. 1), which is a potent volatile convulsant (Krantz et al., 1957a). Flurothyl was discovered inadvertently during the search for novel inhaled anesthetics (Krantz et al., 1957b). The convulsant properties of flurothyl at one time were used clinically as a chemical replacement for electroconvulsive therapy in patients with psychiatric disorders (Krantz et al., 1957a).

Intriguingly, a structural isomer of flurothyl, 'iso-flurothyl' (see Fig. 1), induces general anesthesia, and not convulsions, in mice and dogs (Koblin et al., 1981). These observations are difficult to reconcile with a nonspecific mechanism of anesthetic action. This study tests the hypothesis that interaction with specific receptor protein molecules in the central nervous system, in particular the inhibitory ligand-gated ion channels, may account for the contrasting behavioral actions of the two flurothyl isomers.

The ligand-gated ion channels have emerged as strong molecular candidates to mediate the effects of general anesthetics (Franks and Lieb, 1994; Harris et al., 1995; Krasowski and Harrison, 1999). Two especially promising molecular targets are γ -aminobutyric acid type A (GABA_A) and glycine receptors. These two 'inhibitory' neurotransmitter receptors are part of a ligand-gated ion channel gene superfamily that includes the nicotinic acetylcholine, serotonin₃, and GABA_C ρ receptors (Ortells and Lunt, 1995). The inhaled ether anesthetics, at clinically relevant concentrations, produce positive allosteric modulation of GABA_A and glycine receptors (Downie et al., 1996; Harrison et al., 1993; Jones et al., 1992; Mascia et al., 1996; Wakamori et al., 1991). In whole-cell patch-clamp experiments, the effects of general anesthetics are evident as an enhancement ('potentiation') of submaximal current responses to agonist (reviewed by Harris et al., 1995; Krasowski and Harrison, 1999).

The GABA_A receptor is a pentameric complex assembled from different glycoprotein subunits that combine to form a chloride channel (Barnard et al., 1998). GABA_A receptors *in vivo* predominantly consist of heteromeric complexes of α , β , and γ subunits, although receptors lacking the γ subunit ($\alpha\beta$ receptors) studied in heterologous expression systems retain many of the pharmacological characteristics of $\alpha\beta\gamma$ -containing receptors (Harrison et al., 1993; Pritchett et al., 1989). Strychnine-sensitive glycine receptors *in vivo* consist of both α homomers and $\alpha\beta$ heteromers, with a switch from homomeric to heteromeric receptors occurring during development (Betz, 1992; Kuhse et al., 1995). Glycine α subunits robustly form homomeric receptors in a variety of heterologous expression systems (Betz, 1992; Kuhse et al., 1995).

'GABA_C' receptors, formed by ρ_{1-3} subunits (Barnard et al., 1998; Cutting et al., 1991), are also members of the ligand-gated ion channel gene superfamily. GABA_C receptors do not represent a likely molecular target for general anesthetic actions; in fact, they are a useful tool for molecular studies precisely because of their atypical responses to general anesthetics (Mihic et al., 1997). In contrast to their actions at GABA_A and glycine receptors, ether anesthetics do *not* potentiate submaximal GABA responses at GABA_C ρ_1 receptors (Harrison et al., 1993; Mihic and Harris, 1996). The unusual anesthetic pharmacology of GABA_C ρ_1 receptors enabled Mihic et al. (1997) to identify amino acid residues within GABA_A and glycine receptor subunits as critical for potentiation of agonist responses by ether anesthetics (see below).

One possible explanation for the dissimilar behavioral effects of flurothyl and iso-flurothyl is that these two isomers have differential effects on the function of inhibitory ligand-gated ion channels. Partial confirmation of this hypothesis came with the observation that flurothyl potently antagonizes the responses to GABA, but not to glycine, in dissociated neurons from the rat nucleus tractus solitarius. In contrast, the volatile ether anesthetic enflurane enhances both GABA_A - and glycine receptor-mediated responses in the nucleus tractus solitarius neurons (Wakamori et al., 1991).

To study the molecular details of the actions of flurothyl and iso-flurothyl at inhibitory ligand-gated ion channels, the modulatory actions of flurothyl and iso-flurothyl were examined on agonist responses at wild-type human GABA_A, glycine, and GABA_C ρ_1 receptors expressed in human embryonic kidney (HEK) 293 cells. These three receptors were studied to characterize the receptor selectivity of flurothyl and iso-flurothyl.

Further experiments explored putative sites of action of flurothyl and iso-flurothyl on GABA_A and glycine receptor subunits. Specific amino acid residues within putative transmembrane (TM) domains 2 and 3 of GABA_A and glycine receptors have been shown to be critical for positive modulation by the ether anesthetics enflurane and isoflurane. Specific point mutations of these amino acid residues abolish potentiation of agonist responses at GABA_A and glycine receptors by enflurane and isoflurane (Krasowski et al., 1998a; Mihic et al., 1997). The effect of these mutations on the ability of flurothyl and iso-flurothyl to modulate GABA_A and glycine receptor function was examined. Additional studies investigated whether flurothyl shares a mechanism of action with picrotoxin, a non-competitive antagonist at GABA_A receptors. The effects of flurothyl were measured in GABA_A receptors that contained mutations which render the receptor insensitive to antagonism by picrotoxin (Gurley et al., 1995). Lastly, the implications of the results for explaining the behavioral effects of flurothyl and iso-flurothyl are discussed. Portions of this work have previously appeared in abstract form (Harrison and Krasowski, 1998; Krasowski et al., 1998b).

2. Methods

2.1. Site-directed mutagenesis of receptor cDNAs

The GABA_A α_2 (Hadingham et al., 1993a), β_1 (Hadingham et al., 1993b), γ_2 s (Pritchett et al., 1989), GABA_C ρ_1 (Cutting et al., 1991), and glycine α_1 (Grenningloh et al., 1987) receptor subunit cDNAs used in this study are of human origin. Preparation of the S270I and A291W mutations of the GABA_A α_2 subunit, the S265I and M286W mutations of the GABA_A β_1 subunit, and the S267I and A288W mutations of the glycine α_1 subunit has been described previously (Krasowski et al., 1998a,c). The GABA_A α_2 (T261F/T267A) and β_1 (T256F/T262A) double mutations were created using the QuikChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA). Positive clones were confirmed by sequencing of both DNA strands of the complete receptor cDNA insert (Sequenase 2.0, United States Biochemical, Cleveland, OH, USA). The sequences of the sense mutagenic primers were (underlined nucleotides indicate alteration from the wildtype cDNA sequence):

5'-GTGTTTGGAGTCTTCACTGTCTAACAATGGCCACTCTAAGCATC-3' for the GABA_A α_2 (T261F/T267A) double mutation.

5'-GCACTAGGAATCTTTACGGTGCTTACAATGGCCACCATCAGCACC-3' for the GABA_A β_1 (T256F/T262A) double mutation.

2.2. Cell culture and transfection of receptor cDNAs

Wild-type or mutated receptor cDNAs were expressed via the pCIS2 vector, which contains a single copy of the strong promoter from cytomegalovirus and a polyadenylation sequence from

SV40. HEK 293 cells (American Type Culture Collection, Rockville, MD, USA) were maintained in culture on glass coverslips and transfected with the appropriate cDNAs by the calcium phosphate precipitation method to achieve transient expression (Harrison et al., 1993; Krasowski et al., 1998c).

2.3. Electrophysiological recordings

HEK 293 cells were used for electrophysiological experiments 2–5 days following cDNA transfection. Electrophysiological recordings were performed at room temperature using the whole-cell patch-clamp technique (Harrison et al., 1993; Krasowski et al., 1997). The extracellular medium contained (in mM): 145 NaCl, 3 KCl, 1.5 CaCl₂, 1 MgCl₂, 5.5 D-glucose, and 10 4-(2-hydroxyethyl)-1-piperazine) ethane sulfonic acid (HEPES), pH 7.4, osmolality 320–330 mosmol. The electrode solution contained (in Mm): 145 *N*-methyl-D-glucamine hydrochloride, 5 K₂ATP, 5 HEPES/KOH, 2 MgCl₂, 0.1 CaCl₂, and 1.1 ethylene glycol-bis (β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), pH 7.2, osmolality 315 mosmol. Pipette-to-bath resistance was 4–6 MV. Cells were voltage-clamped at -60 mV.

All drugs and agonists were rapidly (<50 msec exchange time) applied to the cell by local perfusion (Krasowski et al., 1997) using a motor-driven solution exchange device (Bio Logic Rapid Solution Changer RSC-100; Molecular Kinetics, Pullman, WA, USA). Laminar flow was maintained by applying all solutions at identical flow rates via a multi-channel infusion pump (Stoelting, Wood Dale, IL, USA). The solution changer was driven by protocols in the acquisition program pCLAMP5 (Axon Instruments, Foster City, CA, USA). Responses were low-pass-filtered at 5 kHz and digitized (TL-1-125 interface; Axon Instruments) using pCLAMP5 and stored for offline analysis.

2.4. Design of pharmacology experiments

Two experimental designs were used to assess drug-induced enhancement ('potentiation') or inhibition of agonist-induced responses at GABA_A, glycine, or GABA_C ρ₁ receptors. Agonist concentration–response curves for most of the wild-type and mutated receptors considered in this study have been described previously in detail (Krasowski et al., 1998a,c). Inhibition was assessed by co-application of the drug with an EC₇₀ concentration of agonist (i.e. the concentration of agonist that produces 70% of the maximal response to agonist). As in previous studies (Krasowski et al., 1998a,c), potentiation of agonist responses was assessed by coapplication of the drug with an EC₂₀ concentration of agonist (i.e. the concentration of agonist that produces 20% of the maximal response to agonist). Drug-induced potentiation of an agonist-induced current was expressed as the percentage increase of the control agonist response.

2.5. Data analysis and curve fitting

Concentration–response curves for agonist activation or for drug-induced potentiation of submaximal agonist responses were fitted (KaleidaGraph; Reading, PA, USA) with the equation: $I = [L]^{nH} / ([L]^{nH} + [EC_{50}]^{nH})$, where [L]=concentration of ligand, *I*=normalized response, [EC₅₀]=concentration of ligand producing a half-maximal response, and *nH*=Hill coefficient. Data from inhibition concentration–response curves were fitted with the equation: $I/I_{max} = 100 - \{ [A]^{nH} / ([A]^{nH} + [IC_{50}]^{nH}) \}$, where [A]=concentration of antagonist, *I/I*_{max}=percent remaining control response, IC₅₀=concentration of antagonist producing half of the maximal degree of inhibition, and *nH*=Hill coefficient. Pooled data are presented throughout as mean ± standard error of the mean. Statistical significance was determined by one way analysis of variance with Dunnet's post-hoc test.

2.6. Preparation of flurothyl and iso-flurothyl solutions and determination of compound loss by gas chromatography analysis

Flurothyl and iso-flurothyl solution preparation and gas chromatography measurements were adapted from methods described previously (Jones et al., 1992). Flurothyl and iso-flurothyl solutions were prepared by injection with Hamilton Gastight syringes (Reno, NV, USA) of pure liquid flurothyl or iso-flurothyl into evacuated intravenous drip bags containing defined volumes of extracellular solution. A series of experiments using gas chromatography (the methodological details are described in Krasowski and Harrison, 2000) was undertaken to estimate the amount of flurothyl and isoflurothyl lost prior to cellular perfusion. The control experiments demonstrated that losses of flurothyl and iso-flurothyl from the aqueous solutions were small (.5% total losses) and would minimally influence the results of the pharmacology experiments.

2.7. Conversion of flurothyl and iso-flurothyl concentrations expressed in units of partial pressure to aqueous equivalents

In vivo potencies for inhaled compounds are often expressed in units of partial pressure in the gaseous phase. The most common measure of *in vivo* volatile anesthetic potency is minimum alveolar concentration (MAC), the partial pressure of volatile anesthetic in the lungs required to produce immobility in response to a noxious stimulus in 50% of trials (Eger et al., 1965; Quasha et al., 1980). Potencies of inhaled convulsants are often defined as the concentration that elicits convulsions in 50% of animals (Koblin et al., 1981).

Anesthetic MAC values or convulsant EC₅₀ values in partial pressures (at body temperature, considered here to be 37°C) were converted into aqueous equivalent concentrations with the appropriate equations (Franks and Lieb, 1993) and saline/gas partition coefficients (Koblin et al., 1981). Temperature corrections (Franks and Lieb, 1996) were then applied to obtain aqueous equivalents at 20°C (room temperature). The aqueous equivalent concentration reflects the concentration of inhaled compound in the aqueous phase that is in equilibrium with a particular partial pressure in the gas phase. It is believed that the lungs and brain are in equilibrium with respect to anesthetic concentration following induction of volatile anesthesia (Eger et al., 1965; Quasha et al., 1980). In contrast, a caveat to bear in mind is that the partial pressure of flurothyl in the lungs may not always be in equilibrium with the concentration of flurothyl in the brain, especially at high concentrations of inspired flurothyl (Koblin et al., 1981).

The MAC value for iso-flurothyl is 4.6% atm of partial pressure in dogs, while the EC₅₀ for loss of righting reflex in mice by iso-flurothyl is 2.65% atm (Koblin et al., 1981). Applying the conversions described above, the aqueous equivalents at 20°C for iso-flurothyl are 250 µM (MAC in dogs) and 110 µM (loss of righting reflex in mice). The EC₅₀ for flurothyl production of convulsions has been determined to be 0.45% atm in chicks (Cohen et al., 1975) and 0.122% atm in mice (Koblin et al., 1981). These values correspond to aqueous equivalents at 20°C of 100 µM (chicks) and 28 µM (mice). Concentrations of saturated aqueous solutions of flurothyl and iso-flurothyl at room temperature are 7.7 and 5.8 mM, respectively (Krantz et al., 1957b; Eger et al., 1999).

2.8. Sources of drugs

Drugs were obtained from the following sources: GABA, glycine, dimethyl sulfoxide (Sigma, St. Louis, MO, USA), flurothyl (PCR Inc., Gainesville, FL, USA), and picrotoxin (Research Biochemicals International, Natick, MA, USA). Iso-flurothyl was a generous gift from Dr Ross Terrell (Inhalon Pharmaceuticals, Bethlehem, PA, USA). Picrotoxin was first prepared as a stock solution in dimethyl sulfoxide before being dissolved in the extracellular medium. The maximum final concentration of dimethyl sulfoxide was 0.1% (v/v), which was determined

during carrier control experiments to have no effects on GABA-induced currents on the GABA_A receptor constructs utilized for the experiments involving picrotoxin.

3. Results

3.1. Contrasting modulation of wild-type GABA_A, glycine, and GABAC $\rho 1$ receptors by flurothyl and iso-flurothyl

Flurothyl has been characterized previously as an antagonist at neuronal GABA_A receptors, yet exerts no actions at neuronal glycine receptors (Wakamori et al., 1991). To determine whether similar observations also apply to recombinant GABA_A and glycine receptors, the effects of flurothyl were first assessed on responses to EC₇₀ concentrations of agonist at GABA_A $\alpha 2\beta 1$ and glycine $\alpha 1$ receptors. Co-application of flurothyl produced a concentration-dependent decrease of responses to 10 μ M GABA at the GABA_A $\alpha 2\beta 1$ receptor (Fig. 2A). The effects of flurothyl were readily reversible, even at high concentrations. In contrast, co-application of flurothyl had no effect on responses to 100 μ M glycine at the glycine $\alpha 1$ receptor, even at flurothyl concentrations approaching those of an aqueous saturated solution (Fig. 2B).

The general anesthetic iso-flurothyl would be expected to enhance submaximal agonist responses at GABA_A and glycine receptors, similar to the actions of clinically important ether anesthetics such as isoflurane. Indeed, iso-flurothyl potentiated responses to EC₂₀ concentrations of agonist at both GABA_A $\alpha 2\beta 1$ and glycine $\alpha 1$ receptors (Fig. 3). At either receptor, the effects of iso-flurothyl were rapidly reversible. Iso-flurothyl also activated these receptors in the absence of applied agonist (Fig. 3; see pre-application of 2 mM iso-flurothyl in particular). This 'direct activation' of GABA_A $\alpha 2\beta 1$ and glycine $\alpha 1$ receptors by iso-flurothyl was not an artifact of potentiation of responses to residual GABA or glycine in the bath, since direct activation by iso-flurothyl was observed even when no GABA or glycine was present anywhere in the perfusion system. The maximal degree of direct activation by iso-flurothyl at GABA_A $\alpha 2\beta 1$ and glycine $\alpha 1$ receptors was approximately 10% of the maximal responses to agonist, consistent with previous reports of direct activation by ether anesthetics at GABA_A and glycine receptors (Downie et al., 1996; Krasowski et al., 1998a; Wakamori et al., 1991; Yang et al., 1992).

Fig. 4 summarizes the modulatory actions of flurothyl and iso-flurothyl at GABA_A $\alpha 2\beta 1$, $\alpha 2\beta 1\gamma 2s$, glycine $\alpha 1$, and GABAC $\rho 1$ receptors. Flurothyl inhibited responses to EC₇₀ concentrations of GABA at GABA_A $\alpha 2\beta 1$ and $\alpha 2\beta 1\gamma 2s$ receptors with IC₅₀ values of 152 ± 31 and 83 ± 12 μ M, respectively (Fig. 4A; Table 1). These IC₅₀ values are close to the EC₅₀ values for flurothyl induction of convulsions in chicks (EC₅₀=100 μ M) and mice (EC₅₀=28 μ M; see Methods). Flurothyl, at all concentrations studied, did not alter responses to 100 μ M glycine at the glycine $\alpha 1$ receptor (Fig. 4A). Curiously, flurothyl produced a biphasic action on GABA responses at GABAC $\rho 1$ receptors. At 100 and 200 μ M, flurothyl slightly, yet significantly, enhanced responses to 7 μ M GABA. At concentrations of 500 μ M and above, flurothyl inhibited GABA responses at the GABAC $\rho 1$ receptor, with approximately 40% inhibition produced by the co-application of 5 mM flurothyl with 7 μ M GABA (Fig. 4A).

As seen in Fig. 4B, iso-flurothyl potentiated responses to EC₂₀ concentrations of agonist at GABA_A $\alpha 2\beta 1$, $\alpha 2\beta 1\gamma 2s$, and glycine $\alpha 1$ receptors in a concentration-dependent manner. Summary data from the curve fits for the potentiation of agonist responses by iso-flurothyl in Fig. 4B are ($n=5-10$): GABA_A $\alpha 2\beta 1$ (EC₅₀= 133 ± 12 μ M iso-flurothyl, Hill slope= 2.4 ± 0.4 , $E_{\max}$ = $145 \pm 4.0\%$ potentiation), GABA_A $\alpha 2\beta 1\gamma 2s$ (EC₅₀= 211 ± 34 μ M, Hill slope= 1.7 ± 0.3 , $E_{\max}$ = $158 \pm 6.6\%$), and glycine $\alpha 1$ (EC₅₀= 808 ± 126 μ M, Hill slope= 1.3 ± 0.2 , $E_{\max}$ = $122 \pm 7.9\%$ potentiation). The potentiation of agonist responses by iso-flurothyl had significantly higher potencies at the GABA_A $\alpha 2\beta 1$ and $\alpha 2\beta 1\gamma 2s$ receptors, as compared to the glycine $\alpha 1$ receptor. The EC₅₀ values for potentiation of GABA responses at GABA_A $\alpha 2\beta 1$ or $\alpha 2\beta 1\gamma 2s$ receptors by

iso-flurothyl are both close to the anesthetic potencies for loss of righting reflex in mice (110 μM) and MAC (250 μM) in dogs (see Methods). Iso-flurothyl had no effect on submaximal responses to GABA at the GABA_C ρ_1 receptor at all concentrations tested (Fig. 4B).

3.2. Effect of co-application of flurothyl and isoflurothyl on agonist concentration–response relationships at GABA_A $\alpha_2\beta_1$ and glycine α_1 receptors

The experiments described above only assessed the effects of flurothyl and iso-flurothyl on fixed concentrations of agonist at GABA_A and glycine receptors. To study how the modulatory effects of flurothyl and isoflurothyl vary with agonist concentration, agonist concentration–responses curves at GABA_A $\alpha_2\beta_1$ and glycine α_1 receptors were determined in the presence and absence of flurothyl and iso-flurothyl.

Flurothyl produced a concentration-dependent reduction of the maximal response (E_{max}) to GABA at GABA_A $\alpha_2\beta_1$ receptors with only a small (less than twofold) increase in the EC_{50} for GABA (Fig. 5A). Summary data from the curve fits in Fig. 5A are ($n=5-9$): GABA alone ($\text{EC}_{50}=8.7\pm0.4\text{ }\mu\text{M}$, Hill slope= 1.9 ± 0.2), GABA plus 0.1 mM flurothyl ($\text{EC}_{50}=12.4\pm0.7\text{ }\mu\text{M}$, Hill slope= 1.3 ± 0.1 , $E_{\text{max}}=0.93\pm0.01$ of the maximal response to GABA alone), GABA plus 1 mM flurothyl ($\text{EC}_{50}=14.3\pm1.0\text{ }\mu\text{M}$, Hill slope= 1.3 ± 0.1 , $E_{\text{max}}=0.66\pm0.01$), and GABA plus 5 mM flurothyl ($\text{EC}_{50}=12.1\pm0.7\text{ }\mu\text{M}$, Hill slope= 1.2 ± 0.1 , $E_{\text{max}}=0.57\pm0.01$). The EC_{50} , Hill slope, and E_{max} values differed significantly for the concentration–response curves for GABA in the presence of 0.1, 1, or 5 mM flurothyl, as compared to the concentration–response relationship for GABA alone ($p<0.05$ for each). The effects of flurothyl at GABA_A $\alpha_2\beta_1$ receptors are consistent with noncompetitive antagonism, with inhibition by flurothyl observed at all GABA concentrations, including a reduction of the E_{max} value for GABA.

In contrast to flurothyl, 1 mM iso-flurothyl shifted the concentration–response curve for GABA at GABA_A $\alpha_2\beta_1$ receptors to the left in a parallel manner (Fig. 5B). EC_{50} , Hill slope, and E_{max} values determined from the curve fits in Fig. 5B are ($n=5-10$): GABA alone ($\text{EC}_{50}=8.7\pm0.4\text{ }\mu\text{M}$, Hill slope= 1.9 ± 0.2) and GABA plus 1 mM iso-flurothyl ($\text{EC}_{50}=0.85\pm0.07\text{ }\mu\text{M}$, Hill slope= 1.7 ± 0.2 , $E_{\text{max}}=1.0\pm0.01$). Iso-flurothyl did not significantly alter the Hill slope or E_{max} for GABA but did produce a significant decrease in the EC_{50} value for GABA ($p<0.005$).

Iso-flurothyl (2 mM) also shifted the concentration–response curve for glycine at the glycine α_1 receptor to the left in a parallel fashion (Fig. 5C). Flurothyl (2 mM), on the other hand, had no effect on the concentration–response relationship for glycine at the glycine α_1 receptor (Fig. 5C). EC_{50} , Hill slope, and E_{max} values determined from the curve fits in Fig. 5C are ($n=5-11$): glycine alone ($\text{EC}_{50}=46.3\pm2.5\text{ }\mu\text{M}$, Hill slope= 1.7 ± 0.1), glycine plus 2 mM flurothyl ($\text{EC}_{50}=44.2\pm2.2\text{ }\mu\text{M}$, Hill slope= 1.6 ± 0.1 , $E_{\text{max}}=0.99\pm0.01$), and glycine plus 2 mM iso-flurothyl ($\text{EC}_{50}=7.6\pm0.6\text{ }\mu\text{M}$, Hill slope= 1.4 ± 0.2 , $E_{\text{max}}=1.01\pm0.02$). Flurothyl did not significantly alter the EC_{50} , Hill slope, and E_{max} values for glycine at the glycine α_1 receptor, while iso-flurothyl produced a significant decrease of the EC_{50} value for glycine ($p<0.005$). The parallel left-shifts of the agonist concentration–response curves at GABA_A $\alpha_2\beta_1$ and glycine α_1 receptors produced by iso-flurothyl are similar to the effects of other ether anesthetics on agonist concentration–response curves at neuronal GABA_A and glycine receptors (Downie et al., 1996; Wakamori et al., 1991).

3.3. Effect of mutations within TM2 and TM3 of GABA_A and glycine receptor subunits on the modulatory actions of flurothyl and iso-flurothyl

Previous work has shown that two amino acid residue positions within TM2 and TM3 of GABA_A and glycine receptor subunits are critical for the positive modulatory actions of volatile ether anesthetics (Fig. 6). Site-directed mutations at these amino acid positions can completely abolish the ability of volatile ether anesthetics to potentiate submaximal responses to agonist

at GABA_A and glycine receptors (Koltchine et al., 1999; Krasowski et al., 1998a; Mihic et al., 1997). It was hypothesized that mutations at these positions might also affect modulation by flurothyl and/or iso-flurothyl at GABA_A and glycine receptors.

Fig. 7 depicts the ability of iso-flurothyl to potentiate responses to EC₂₀ concentrations of agonist at GABA_A α_2 (S270I) β_1 , α_2 (A291W) β_1 , $\alpha_2\beta_1$ (S265I), $\alpha_2\beta_1$ (M286W), glycine α_1 (S267I), and α_1 (A288W) receptors. Iso-flurothyl failed to potentiate responses to agonist at GABA_A α_2 (S270I) β_1 , α_2 (A291W) β_1 , and $\alpha_2\beta_1$ (M286W) receptors, as well as at the glycine α_1 (A288W) receptor, but enhanced the magnitude of currents at GABA_A $\alpha_2\beta_1$ (S265I) and glycine α_1 (S267I) receptors. This pattern of activity is similar to that described for the ether anesthetics diethyl ether, enflurane, isoflurane, methoxyflurane, and sevoflurane (Krasowski et al., 1998a; Mihic et al., 1997; Krasowski and Harrison, 2000).

Flurothyl inhibition of GABA responses was assessed at the GABA_A α_2 (S270I) β_1 , α_2 (A291W) β_1 , $\alpha_2\beta_1$ (S265I), and $\alpha_2\beta_1$ (M286W) receptors. Flurothyl inhibited responses to EC₇₀ concentrations of GABA at all these receptors, although in some cases the concentration–response relationships differed from that at the wild-type GABA_A $\alpha_2\beta_1$ receptor (Fig. 8A,B; Table 1). The most obvious difference was in the maximal amount of inhibition produced by flurothyl at the GABA_A α_2 (S270I) β_1 and α_2 (A291W) β_1 receptors (Fig. 8A; Table 1). The maximal percent inhibition by flurothyl (the ‘relative efficacy’) at these receptors was each less than half of that at the GABA_A $\alpha_2\beta_1$ receptor (Fig. 8A; Table 1). The GABA_A receptors with mutations in the β_1 subunit, GABA_A $\alpha_2\beta_1$ (S265I) and $\alpha_2\beta_1$ (M286W), did not influence the relative efficacy of inhibition by flurothyl, although the IC₅₀ value for flurothyl at the GABA_A $\alpha_2\beta_1$ (M286W) receptor was over threefold greater than at the wild-type GABA_A $\alpha_2\beta_1$ receptor (Fig. 8B; Table 1).

3.4. GABA responses at picrotoxin-insensitive GABA_A receptors are inhibited by flurothyl

The non-competitive antagonism by flurothyl at GABA_A receptors raised the possibility that flurothyl might share a mechanism of action with picrotoxin, another compound known to inhibit GABA_A-mediated responses in a non-competitive manner. Picrotoxin appears to bind to the GABA_A receptor at or near the ion channel pore, which is proposed to be lined predominantly by amino acid residues from TM2 (Xu and Akabas, 1996; Xu et al., 1995). A double point mutation within TM2 of either the GABA_A α_1 or β_2 subunit has been described which completely abolishes antagonism of GABA responses by picrotoxin at GABA_A $\alpha_1\beta_2\gamma_{2s}$ receptors (Gurley et al., 1995). The analogous double point mutations to those described by Gurley et al. (1995) were constructed in the GABA_A α_2 (T261F/T267A) and GABA_A β_1 (T256F/T262A) subunits (see Fig. 6). As a control, the actions of picrotoxin were assessed at wild-type GABA_A $\alpha_2\beta_1$ and GABA_A $\alpha_2\beta_1\gamma_{2s}$ receptors. Picrotoxin inhibited responses to an EC₇₀ concentration of GABA (15 μ M) at GABA_A $\alpha_2\beta_1$ receptors with an IC₅₀ of 4.5 ± 1.3 μ M, a Hill slope of 1.0 ± 0.2 , and a maximal percent inhibition of $72.6 \pm 5.2\%$ ($n=5-10$). Picrotoxin inhibited responses to an EC₇₀ concentration of GABA (25 μ M) at GABA_A $\alpha_2\beta_1\gamma_{2s}$ receptors with an IC₅₀ of 1.7 ± 0.2 μ M, a Hill slope of 1.3 ± 0.2 , and a maximal percent inhibition of $94.8 \pm 2.9\%$ ($n=5-6$).

Responses to EC₇₀ concentrations of GABA at GABA_A α_2 (T261F/T267A) β_1 (T256F/T262A) receptors were completely insensitive to antagonism by picrotoxin. The effects of picrotoxin on responses to 40 μ M GABA at this receptor were ($n=5$): 1 μ M picrotoxin ($-0.5 \pm 3.8\%$ inhibition), 10 μ M picrotoxin ($2.2 \pm 5.0\%$ inhibition), and 100 μ M picrotoxin ($2.6 \pm 1.0\%$ inhibition). Similarly, picrotoxin did not inhibit responses to 75 μ M GABA at GABA_A α_2 (T261F/T267A) β_1 (T256F/T262A) γ_{2s} receptors ($n=5$): 1 μ M picrotoxin ($0.9 \pm 2.8\%$ inhibition), 10 μ M picrotoxin ($-0.3 \pm 3.3\%$ inhibition), and 100 μ M picrotoxin ($0.8 \pm 2.0\%$ inhibition). In contrast to the lack of effect by picrotoxin, flurothyl antagonized responses to GABA at GABA_A α_2 (T261F/T267A) β_1 (T256F/T262A) and α_2 (T261F/T267A) β_1 (T256F/T262A) γ_{2s}

receptors. The concentration–response curves for flurothyl inhibition of GABA responses at these two mutated GABA_A receptors overlapped that for wild-type GABA_A $\alpha_2\beta_1$ and $\alpha_2\beta_1\gamma_{2s}$ receptors (Fig. 8C; Table 1).

4. Discussion

4.1. Contrasting modulatory effects of flurothyl and iso-flurothyl on the inhibitory ligand-gated ion channels

Flurothyl and iso-flurothyl, despite being structural isomers of one another, demonstrated strikingly different actions on the inhibitory ligand-gated ion channels studied. Iso-flurothyl had actions at GABA_A, glycine, and GABA_C ρ_1 receptors which were similar to other ether anesthetics such as isoflurane and sevoflurane (see Table 2). The ether anesthetics characterized to date have been shown to be positive allosteric modulators of GABA_A and glycine receptors (Downie et al., 1996; Harrison et al., 1993; Mascia et al., 1996; Wakamori et al., 1991), while none potentiates submaximal GABA responses at the GABA_C ρ_1 receptor (Harrison et al., 1993; Mihic and Harris, 1996).

Flurothyl, on the other hand, has a very different pattern of activity from iso-flurothyl and the ether anesthetics (see Table 2). Flurothyl non-competitively antagonized the effects of GABA at GABA_A receptors, yet had no actions on glycine receptors. The effects demonstrated in this study for recombinant GABA_A and glycine receptors parallel previous observations in brainstem neurons of the rat (Wakamori et al., 1991). In further contrast to the volatile ether anesthetics, flurothyl exerted a biphasic action on GABA-mediated responses at the GABA_C ρ_1 receptor (Table 2).

4.2. Sites of action for flurothyl and iso-flurothyl on GABA_A and glycine receptors

This study also demonstrated that site-directed mutations within TM2 and TM3 of GABA_A and glycine receptor subunits, previously observed to abolish potentiation of agonist responses by the ether anesthetics enflurane and isoflurane, also abolished potentiation of agonist responses by iso-flurothyl. Although it is not yet clear whether these mutations disrupt the actual binding sites for volatile ether anesthetics on GABA_A and glycine receptors (see Koltchine et al., 1999; Krasowski and Harrison, 2000), iso-flurothyl clearly depends on similar receptor requirements to other ether anesthetics.

In contrast, these site-directed mutations within GABA_A receptor subunits had less dramatic effects on inhibition of GABA responses by flurothyl. None of the mutations abolished the inhibitory effects of flurothyl, although mutations in the GABA_A α_2 subunit reduced the maximal magnitude of inhibition by flurothyl.

The inhibition of GABA responses by flurothyl at GABA_A $\alpha_2\beta_1$ and $\alpha_2\beta_1\gamma_{2s}$ receptors was unaffected by double point mutations within the α_2 and β_1 subunits that completely abolished the inhibitory actions of picrotoxin. Flurothyl also differs from picrotoxin in that flurothyl had no effects on glycine responses at glycine α_1 receptors, whereas picrotoxin has potent antagonistic effects on homomeric glycine receptors (see Table 2; Pribilla et al., 1992). Much like the steroids pregnenolone sulfate and dehydroepiandrosterone sulfate, which inhibit both picrotoxin-sensitive and picrotoxin-insensitive GABA_A receptors (Shen et al., 1999), flurothyl would appear to utilize a mechanism of action distinct from that for picrotoxin to antagonize GABA responses. This contrasts with some other GABA_A receptor antagonists. For example, the inhibitory actions of certain substituted butyrolactones at GABA_A receptors are abolished by point mutations that also eliminate antagonism of GABA responses by picrotoxin (Williams et al., 1997).

Where might flurothyl interact on the GABA_A receptor molecule? One possibility is that the binding site responsible for inhibition by flurothyl is the same as that responsible for the 'blocking effects' caused by high concentrations of the ether anesthetics at GABA_A receptors. The blocking action of volatile anesthetics on GABA-induced currents can be inferred from the 'rebound' currents that follow washout of GABA and anesthetic during experiments to examine anesthetic-induced potentiation of GABA responses. Rebound currents are believed to reflect the relief of low-affinity block of GABA-induced current by the volatile anesthetic (Banks and Pearce, 1999; Jones et al., 1992; Wu et al., 1996). The blocking effect of high concentrations of the volatile anesthetic isoflurane is clearly revealed in mutated GABA_A receptors that are insensitive to potentiation of GABA responses by isoflurane (Koltchine et al., 1999).

One might speculate that flurothyl and volatile ether anesthetics interact with an inhibitory site on the GABA_A receptor that is distinct from the site that ether anesthetics interact with to potentiate GABA responses (see also Banks and Pearce, 1999). The affinity of flurothyl for this site would presumably be higher than that for the ether anesthetics. Interestingly, inhibitory effects of volatile ethers at glycine receptors have not been reported in the literature (see Downie et al., 1996; Wakamori et al., 1991), and flurothyl itself fails to inhibit agonist responses at glycine receptors. Presumably, flurothyl and the ether anesthetics do not inhibit glycine receptor-mediated responses because the glycine receptor simply lacks this inhibitory binding inhibitory site.

4.3. Possible chemical basis for the contrasting effects of flurothyl and iso-flurothyl

What chemical features might account for the differences between flurothyl and iso-flurothyl with respect to their interactions with the ligand-gated ion channels and for their contrasting behavioral effects? From the large number of low-molecular-weight halogenated ethers that have been synthesized and screened for anesthetic activity, one important factor appears to be the pattern of halogenation (Koblin et al., 1999). Extensive or symmetric halogenation of ethers tends to decrease anesthetic potency and increase convulsive activity, whereas asymmetric halogenation favors anesthetic activity. The halogenated ether anesthetics of greatest clinical importance during the past century (e.g. desflurane, enflurane, isoflurane, methoxyflurane, and sevoflurane) are all asymmetric (e.g. methyl ethyl or methyl propyl) ethers with irregular patterns of halogenation (Eger et al., 1999; Koblin et al., 1999). Iso-flurothyl fits this pattern and, in fact, differs from sevoflurane by one fluorine atom. On the other hand, flurothyl is a diethyl ether with a symmetric pattern of fluorination.

Eger et al. (1999) has attempted to classify the volatile non-immobilizers based on physicochemical properties, and has suggested that the convulsant non-immobilizers fall into two general classes. For most of the non-immobilizers, the convulsant potency correlates strongly with the oil/gas partition coefficient; for the remainder, including flurothyl, this correlation does not hold (Eger et al., 1999). It is noteworthy that the convulsants in the first category, such as the halogenated cyclobutanes, do not appear to alter the function of any of the ligand-gated ion channels, including GABA_A, glycine, and neuronal nicotinic acetylcholine receptors (Cardoso et al., 1999; Mascia et al., 1996; Mihic et al., 1994). By contrast, flurothyl and one other convulsant ether, CF₂Cl-O-CH₂CF₃ (M.D. Krasowski and E.I. Eger, II, unpublished observations), both antagonize GABA responses at GABA_A receptors. This suggests that the volatile nonimmobilizers characterized to date produce convulsions either by antagonism of GABA_A receptors (e.g. flurothyl, CF₂Cl-O-CH₂CF₃) or by interacting with other as yet undefined target molecule(s) (e.g. the halogenated cyclobutanes).

4.4. Relationship to the in vivo actions of flurothyl and iso-flurothyl

The data in this study are consistent with a role for GABA_A and/or glycine receptors in the general anesthetic actions of iso-flurothyl. By contrast, the convulsive properties of flurothyl may be explained by inhibition of GABA_A receptor-mediated synaptic transmission. The convulsions produced by flurothyl in mammals (Krantz et al., 1957a,b) resemble those elicited by picrotoxin (Browne, 1875) but not by the glycine receptor antagonist strychnine (Dusser de Barenne, 1933), consistent with the lack of effect of flurothyl at recombinant or neuronal glycine receptors.

An interesting phenomenon observed with the convulsions produced by flurothyl in mice is a bell shape to the concentration–response curve for flurothyl-induced convulsions (Koblin et al., 1981). In particular, a maximal number of convulsions by flurothyl in mice are elicited at 0.24% atm partial pressure of inhaled flurothyl (approximately 55 μ M aqueous equivalent concentration in plasma; see Methods). Beyond 0.6% atm (=138 μ M), flurothyl produced far fewer convulsions and these occurred only at the very onset of application of flurothyl. This led Koblin et al. (1981) to suggest that flurothyl may actually possess both anesthetic and convulsant properties, with the anesthetic effect produced by flurothyl being of much lower potency than the convulsant effect. In support of this hypothesis, 0.3% to 0.4% atm of flurothyl (=69 to 92 μ M) increased the minimum alveolar concentration (MAC; see Methods) for isoflurane while 3–4% atm (=688 to 918 μ M) of flurothyl decreased the MAC for isoflurane. In other words, low concentrations of flurothyl functionally antagonized the anesthetic action of isoflurane, while higher flurothyl concentrations actually augmented the anesthetic effect of isoflurane. Koblin et al. (1981) determined that flurothyl produced loss of righting reflex in mice at 1.2% atm (=275 μ M), an anesthetic effect which appears to be unrelated to its convulsant action. Flurothyl also appears to have an MAC value at some concentration greater than 4% atm (=918 μ M).

What might account for the mixed anesthetic and convulsant properties of flurothyl? The results described in this study and by Wakamori et al. (1991) support a role for GABA_A receptors in the convulsive effects of flurothyl, but not for any anesthetic actions of flurothyl. In recombinant or neuronal GABA_A receptors, the concentration–response curves for inhibition of GABA responses by flurothyl appear to follow a simple sigmoidal relationship, with no reduction of the inhibition produced by flurothyl at concentrations of flurothyl approaching that of an aqueous saturated solution. An alternative hypothesis to explain the anesthetic actions is that flurothyl, at high concentrations, alters the function of other molecular targets in the nervous system, although there have been few studies that have explored this issue. Wakamori et al. (1991) detected only small effects of flurothyl on responses to *N*-methyl-D-aspartate, quisqualate, and kainate in brainstem neurons and only at flurothyl concentrations greater than 1 mM. It would be interesting to know whether flurothyl has effects on other receptors postulated to play a role in general anesthesia, such as the neuronal nicotinic acetylcholine receptors.

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Fig. 1.
Chemical structures of flurothyl and iso-flurothyl.

Fig. 2.

Flurothyl antagonizes responses to GABA at human GABA_A $\alpha_2\beta_1$ receptors, but has no effect on responses to glycine at human glycine α_1 receptors. (A) Flurothyl inhibits responses to an EC₇₀ concentration (10 mM) of GABA at the GABA_A $\alpha_2\beta_1$ receptor. (B) In contrast, flurothyl, even at concentrations approaching that of an aqueous saturated solution, has no effect on the responses to an EC₇₀ concentration (100 μ M) of glycine at the glycine α_1 receptor. Flurothyl was pre-applied to the cells before co-application of agonist and flurothyl. Traces shown are individual recordings from HEK 293 cells transfected with cDNAs encoding the indicated receptor subunit combinations.

Fig. 3.

Iso-flurothyl enhances responses to submaximal concentrations of agonist at both GABA_A $\alpha_2\beta_1$ and glycine α_1 receptors. (A) Iso-flurothyl potentiates responses to an EC₂₀ concentration (2 μ M) of GABA at the GABA_A $\alpha_2\beta_1$ receptor. (B) Iso-flurothyl potentiates responses to an EC₂₀ concentration (20 μ M) of glycine at the glycine α_1 receptor. Iso-flurothyl also produces some direct activation of GABA_A $\alpha_2\beta_1$ and glycine α_1 receptors, which is manifest during the pre-application of iso-flurothyl prior to co-application of agonist and iso-flurothyl. Traces shown are individual recordings from HEK 293 cells transfected with cDNAs encoding the indicated receptor subunit combinations.

Fig. 4. Concentration–response data for the modulatory effects of flurothyl and iso-flurothyl at $GABA_A \alpha_2\beta_1$, $\alpha_2\beta_1\gamma_{2s}$, glycine α_1 , and $GABA_C \rho_1$ receptors. (A) The effects of flurothyl on responses to EC_{70} concentrations of agonist at $GABA_A \alpha_2\beta_1$, $\alpha_2\beta_1\gamma_{2s}$, glycine α_1 , and $GABA_C \rho_1$ receptors. The ordinate indicates percent current remaining, compared to the control response to agonist (where 100% indicates no effect), when flurothyl is co-applied with agonist. See Table 1 for summary information on the curve fits. (B) The effects of iso-flurothyl on responses to EC_{20} concentrations of agonist at $GABA_A \alpha_2\beta_1$, $\alpha_2\beta_1\gamma_{2s}$, glycine α_1 , and $GABA_C \rho_1$ receptors. The ordinate indicates percent enhancement of a control response to

agonist when iso-flurothyl is co-applied with agonist (where 0% indicates no effect). See Results for summary information on the curve fits.

Fig. 5.

Concentration–response relationships for agonist at GABA_A $\alpha_2\beta_1$ and glycine α_1 receptors in the absence or presence of flurothyl and iso-flurothyl. (A) GABA concentration–response curves at the GABA_A $\alpha_2\beta_1$ receptor in the absence or presence of increasing concentrations of flurothyl. (B) GABA concentration–response curves at the GABA_A $\alpha_2\beta_1$ receptor in the absence or presence of 1 mM iso-flurothyl. (C) Glycine concentration–response curves at the glycine α_1 receptor in the absence or presence of 2 mM flurothyl or 2 mM iso-flurothyl. See Results for summary information on the curve fits in (A), (B), and (C).

	TM2	TM3
Gly α_1	250 PARVGLGITT VLTMTT QSSG 269...282 AIDIW MAVC LLFVFSALLE300	
GABA _A α_2	253 PARTVFGV TT VLTMT TL SIS272...285 AMDWFI AVC YAFVFSALIE303	
GABA _A β_1	248 AARVALG ITT VLTMT TI STH267...280 AIDIY LMGC FVFVFLALLE298	
GABA _C ρ_1	280 PARVPLGITT VLTMT TI ITG300...315 AVDIY LMVS FVFVFLSVLE340	

Fig. 6.

Partial amino acid sequence alignment of TM2 and TM3 from human glycine α_1 , GABA_A α_2 , β_1 , and GABA_C ρ_1 receptor subunits. Residue positions in **bold type** are important for the current study. The GABA_A α_2 (T261), α_2 (T267), β_1 (T256), and β_1 (T262) positions have been shown to be important for the antagonism by picrotoxin of GABA responses at GABA_A receptors (Gurley et al., 1995). Site-directed mutations at the glycine α_1 (S267), α_1 (A288), GABA_A α_2 (S270), α_2 (A291), β_1 (S265) and β_1 (M286) amino acid residue positions can reduce or abolish potentiation of submaximal agonist responses by ether anesthetics at GABA_A and glycine receptors (Koltchine et al., 1999; Krasowski et al., 1998a; Mihic et al., 1997).

Fig. 7.

Summary of the effects of mutations in TM2 or TM3 of GABA_A α₂ and β₁ or glycine α₁ receptor subunits on potentiation of agonist responses by iso-flurothyl. (A) Iso-flurothyl (1 mM) potentiates responses to EC₂₀ concentrations of GABA at GABA_A α₂β₁ and α₂β₁(S265I) receptors, yet fails to alter GABA responses at GABA_A α₂(S270I)β₁, α₂(A291W)β₁ and α₂β₁(M286W) receptors. The ordinate indicates percent enhancement of a control response to GABA when iso-flurothyl is co-applied with GABA. (B) Iso-flurothyl (2 mM) potentiates responses to EC₂₀ concentrations of glycine at glycine α₁ and α₁(S267I) receptors but does not affect glycine responses at glycine α₁(A288W) receptors. The asterisk indicates that the potentiation of agonist responses at the mutated receptor differed significantly from that at the

corresponding wild-type receptor with a significance level of $p < 0.01$ ($n = 5-13$ for all experiments).

Fig. 8.

The effects of mutations in GABA_A α_2 or β_1 subunits on flurothyl inhibition of GABA responses at GABA_A $\alpha_2\beta_1$ receptors. (A) The GABA_A α_2 (S270I) β_1 and α_2 (A291W) β_1 receptors are still inhibited by flurothyl, although the maximal inhibition by flurothyl at these receptors is reduced. (B) The GABA_A $\alpha_2\beta_1$ (S265I) and $\alpha_2\beta_1$ (M286W) receptors are inhibited by flurothyl. (C) The GABA_A α_2 (T261F/T267A) β_1 (T256F/T262A) receptor, which is insensitive to antagonism by picrotoxin, has a concentration–response curve for inhibition by flurothyl which overlaps that for the wild-type GABA_A $\alpha_2\beta_1$ receptor. See Table 1 for summary information on the curve fits in (A), (B), and (C) ($n=5-13$ for all experiments).

Summary data for concentration-response relationships for GABA activation and flurothyl inhibition at wild-type and mutated GABA_A receptor combinations

Table 1

GABA _A receptor combination	GABA EC ₅₀	Hill slope	n	Flurothyl inhibition IC ₅₀ (μM)	Hill slope	Maximal percent inhibition	n
α ₂ β ₁	8.7±0.4	1.9±0.2	9	152±31	1.3±0.2	83.8±4.7	5-8
α ₂ β ₁ γ _{2s}	19.4±1.6	1.4±0.1	6	83±12	2.4±0.7	53.3±2.7	6
α ₂ (S270D)β ₁	14.6±0.1	2.4±0.1	6	210±44	1.8±0.5	33.4±2.1	5-9
α ₂ (A288W)β ₁	2.4±0.1	1.7±0.2	5	386±74	1.0±0.1	40.9±2.5	5-10
α ₂ β ₁ (S265I)	37.5±8.5	1.2±0.2	7	70±6	1.6±0.2	76.7±1.9	6
α ₂ β ₁ (M286W)	8.7±0.4	0.8±0.2	7	533±130	1.0±0.2	76.5±11.4	5-12
α ₂ (T261F/T267A) + β ₁ (T256F/T262A)	21.8±2.1	1.4±0.2	6	89±4	2.0±0.2	75.0±1.2	5
α ₂ (T261F/T267A) + β ₁ (T256F/T262A) + γ _{2s}	48.4±6.7	1.0±0.1	7	107±35	1.9±0.3	57.6±2.4	6

Table 2

Comparison of the modulatory actions of flurothyl, picrotoxin, iso-flurothyl, and ether anesthetics on submaximal responses to agonist at inhibitory ligand-gated ion channels

Compound	GABA _A receptors	Glycine receptors	GABAC ρ_1 receptors
Flurothyl	Inhibition	No effect	Biphasic (see text)
Picrotoxin	Inhibition	Inhibition	Inhibition
Iso-flurothyl	Potentiation	Potentiation	No effect
Sevoflurane and other ether anesthetics	Potentiation	Potentiation	No effect