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# Potentiation of γ-Aminobutyric Acid Type A Receptor-Mediated Chloride Currents by Novel Halogenated Compounds Correlates with Their Abilities to Induce General Anesthesia

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## SUMMARY

The Meyer-Overton hypothesis, predicting that the potency of an anesthetic correlates with its affinity for lipid, is a cornerstone of modern anesthetic theory. Several halogenated compounds were recently found to deviate from this prediction, whereas others did not. We tested the abilities of enflurane and five of these compounds to potentiate γ-aminobutyric acid $(GABA)_A$ receptor responses in *Xenopus* oocytes expressing $\alpha1\beta2$ or $\alpha1\beta2\gamma2S$ $GABA_A$ receptors. Enflurane and the anesthetic 1-chloro-1,2,2-trifluorocyclobutane (F3) strongly potentiated chloride currents produced by 5 μM GABA with both $\alpha1\beta2$ and $\alpha1\beta2\gamma2S$ receptors. This potentiation decreased as the GABA concentration was raised. The transitional compound (less potent than predicted by its lipid solubility) 2-bromoheptafluoropropane produced modest enhancement, whereas three nonanesthetics (neither causing anesthesia *in vivo* nor decreasing the requirement for known anesthetics), 1,2-dichlorohexafluorocyclobutane, 2-chloroheptafluoropropane, and 2,3-chlorooctafluorobutane, did not affect $GABA_A$ receptor currents. Although all five compounds were predicted to be anesthetics by the Meyer-Overton hypothesis, only F3 behaved as an anesthetic *in vivo*, and only F3 markedly potentiated $GABA_A$ receptor responses in oocytes. These results strongly implicate the $GABA_A$ receptor in general anesthesia. Fluorescence polarization studies showed that anesthetics (enflurane and F3), but not nonanesthetics (1,2-dichlorohexafluorocyclobutane and 2,3-chlorooctafluorobutane), disordered membrane lipids. Thus, for the compounds studied, actions on both $GABA_A$ receptor function and lipid order distinguish between anesthetics and nonanesthetics.

Most theories describing the effects of general anesthetics predict that these compounds act on the cell membrane. The Meyer-Overton hypothesis (1) argues that lipid solubility, or affinity for a lipid-like phase, correlates with anesthetic potency. Although they were originally believed to act nonspecifically in the lipid portion of membranes, recent evidence suggests that anesthetics may inhibit neuronal activity (2) by modulating the function of specific membrane proteins. For example, Franks and Lieb (3, 4) found that anesthetics could inhibit the activity of the lipid-free enzyme luciferase and that inhalational general anesthetics have stereospecific effects on potassium-conducting ion channels.

Structurally diverse compounds such as inert gases, barbiturates, ethers, steroids, and halogenated carbons can all produce anesthesia (1). There are compelling behavioral, biochemical, and electrophysiological data suggesting that anesthesia may result from potentiation of chloride conductance through the $GABA_A$ receptor/chloride channel complex, the primary mediator of neuronal inhibition in the mammalian central nervous system. In rats and mice, the $GABA_A$ receptor analog THIP produces sedation, analgesia, and loss of righting reflex (5). Furthermore, muscimol and THIP (5) increase, whereas the $GABA_A$ receptor antagonist bicuculline (6) decreases, the duration of pentobarbital-induced loss of righting reflex. Our earlier work (7, 8) demonstrated that the structurally diverse anesthetics diethyl ether, enflurane, halothane, isoflurane, ketamine, pentobarbital, phenobarbital, 3α-hydroxy-5α-dihydroprogesterone, and propofol could all enhance GABAergic currents in *Xenopus* oocytes.

Thus, there appears to be a strong case for anesthesia resulting from the enhancement of GABAergic currents. The problem with that argument is that volatile anesthetics affect a number

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**ABBREVIATIONS:** GABA, γ-aminobutyric acid; DPH, 1,6-diphenyl-1,3,5-hexatriene; F3, 1-chloro-1,2,2-trifluorocyclobutane; F6, 1,2-dichlorohexafluorocyclobutane; F7-Br, 2-bromoheptafluoropropane; F7-Cl, 2-chloroheptafluoropropane; F8, 2,3-chlorooctafluorobutane; THIP, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; MAC, minimum alveolar concentration; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MBS, modified Barth's saline; GluR, glutamate receptor.

of other receptors (for a recent review, see Ref. 9) in a manner that could also explain anesthesia, such as by inhibiting receptors mediating excitatory transmission. For example, volatile anesthetics such as isoflurane and enflurane inhibit the function of *N*-methyl-D-aspartate, kainate, and AMPA receptors (10), whereas they enhance glycine receptor $\alpha$ subunit-activated currents (11) and 5-hydroxytryptamine type 3 receptor responses (12). The effects of volatile anesthetics may also depend on the particular receptor subunits studied. We recently discovered that enflurane inhibits kainate currents in *Xenopus* oocytes expressing GluR1 or GluR3 subunits; however, only GluR3, and not GluR1, receptors were sensitive to the anesthetic F3.[1] Our current knowledge does not allow us to discriminate among these potential sites of anesthetic action. The problem is compounded by the diverse effects of volatile anesthetics. They not only produce the anesthetic state but also have various effects on the central nervous system that, although important as side effects (e.g., nausea and depression of ventilation), are not relevant to anesthesia.

Recently, several volatile compounds with anesthetic potencies that deviate from those predicted by the Meyer-Overton hypothesis were described (13). Some do not produce anesthesia (nonanesthetics), whereas others (transitional compounds) are far less potent than would be predicted from their lipophilicity. These compounds offer a stringent test of the relevance of putative sites of anesthetic action. A relevant site should be affected by anesthetics in proportion to their *in vivo* potencies and should not be affected by nonanesthetics. Transitional compounds should have weaker effects commensurate with their lower potencies.

There are other approaches that one could take to test putative sites of anesthetic action. For example, the anesthetic potencies of alkanols are known to increase with carbon backbone length, but not indefinitely. Alcohols longer than 12 carbon atoms are without anesthetic efficacy, i.e., an anesthetic cutoff occurs (14). However, whether the cutoff for alcohol potentiation of GABAergic currents occurs at the same point as loss of anesthetic efficacy has not been determined. Nakahiro *et al.* (15), studying the series from ethanol to *n*-octanol, found a correlation between potentiation of GABAergic currents and alcohol carbon backbone length, but those authors did not test alcohols on both sides of the anesthetic cutoff at $C_{12}$.

In the present study, we tested one anesthetic, one transitional, and three nonanesthetic compounds for their effects on $GABA_A$ receptors expressed in *Xenopus* oocytes. The dependence of enflurane potentiation of GABAergic currents on GABA concentration and receptor subunit composition was also examined. Our present goal was to determine whether the ability to potentiate GABAergic currents could consistently distinguish anesthetics from nonanesthetics. Such a finding would provide a new line of evidence strengthening the hypothesis that the $GABA_A$ receptor/chloride channel complex is a major site of anesthetic action. We also tested some of these compounds for their abilities to disorder egg yolk lecithin vesicles and mouse brain membranes. Some of this work has previously been presented in abstract form (16).

## Experimental Procedures

**Materials.** Penicillin, streptomycin, gentamicin, and egg yolk lecithin were obtained from Sigma Chemical Co. (St. Louis, MO). GABA was purchased from Research Biochemicals (Natick, MA). Enflurane was bought from Anaquest Co. (Madison, WI), whereas F3, F6, F8, F7-Cl, and F7-Br were obtained from PCR Inc. (Gainesville, FL). DPH was purchased from Molecular Probes (Junction City, OR). All other reagents used were of reagent grade.

**Oocyte preparation and cDNA nuclear injection.** Oocytes were obtained from *Xenopus laevis* frogs kept in aquarium tanks at 19–21°, on a 12-hr light/dark cycle. Frogs were fed chopped beef hearts and mealworms thrice weekly and had surgery at most once per month. They were anesthetized by immersion in ice-cold water for 30–60 min. A small incision was then made in the abdominal wall and a piece of ovary was removed. Stage V and VI oocytes were isolated as described previously (7). The oocytes were placed in isolation medium (108 mM NaCl, 1 mM EDTA, 2 mM KCl, 10 mM HEPES, pH 7.5), and theca and epithelial layers were removed with forceps. The follicular layer was removed by a 10-min immersion in 0.5 mg/ml collagenase (type 1A; Sigma) dissolved in collagenase buffer (83 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES). The oocytes were then placed in MBS [88 mM NaCl, 1 mM KCl, 10 mM HEPES, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 0.91 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, adjusted to pH 7.5]. A 30-nl mixture of $GABA_A$ receptor subunit cDNAs (1.5 ng/30 nl) was injected into the animal pole of oocytes by the "blind" method of Colman (17), using a digital microdispenser (Drummond Scientific, Broomall, PA) loaded with a micropipette (10–15-$\mu$m tip). The methods used in the cloning of the human $GABA_A$ receptor subunits were described earlier (18). After injection, the eggs were singly placed in Corning cell wells (Corning Glass Works, Corning, NY) containing incubation medium (MBS supplemented with 2 mM sodium pyruvate, 0.5 mM theophylline, 10,000 units/liter penicillin, 10 mg/liter streptomycin, and 50 mg/liter gentamicin) that had been sterilized by passage through a 0.2-$\mu$m filter (Nalge Company, Rochester, NY). The oocytes were maintained at room temperature (21°) and usually expressed $GABA_A$ receptors the day after injection. Oocytes were used in electrophysiological recordings for up to 3–4 days after cDNA injection.

**Electrophysiological recording.** Oocytes were placed in a rectangular chamber (approximately 100-$\mu$l volume) during recording. They were perfused (2 ml/min) with MBS with or without drugs, using a roller pump (Cole-Parmer Instrument Co., Chicago, IL), through 18-gauge polyethylene tubing (Clay Adams Co., Parsippany, NJ) that connected the drug vials to the recording chamber. Oocytes rested in a small depression in the recording chamber and were oriented with the animal pole facing upwards. They were impaled with two glass electrodes (0.5–10 M$\Omega$) filled with 3 M KCl and were voltage clamped at −70 mV, using a Warner Instruments (Hamden, CT) model OC-725A oocyte clamp. A strip-chart recorder (Cole-Parmer Instrument Co.) continuously plotted the clamping currents.

Drugs were applied for 20 sec, by which time the peak current response was obtained. Anesthetics or other compounds were always coapplied with GABA, i.e., there was no preincubation of oocytes with anesthetics or other compounds in the absence of GABA. The solutions of volatile compounds were freshly prepared immediately before use. Saturated solutions of the anesthetics refer to the solutions in the vials; the concentrations in the bath would be lower. A 5-min washout period was allowed between drug applications when low GABA concentrations (<10 $\mu$M) were used, increasing to 15 min for higher GABA concentrations. Data were obtained from oocytes isolated from at least two different frogs.

**Fluorescence polarization.** Measurements of membrane order were performed at 28° using an HH-1 spectrofluorimeter (BHL Associates, Burlingame, CA), exactly as described by Buck *et al.* (19) and Harris and Groh (20). Egg yolk lecithin (360 nmol/sample) in chloroform was dried under argon with 10 $\mu$g of DPH. The film of egg yolk lecithin/DPH was dispersed in phosphate-buffered saline (20) and sonicated for 30 sec to produce lipid vesicles. ICR mouse (Harlan Sprague Dawley, Indianapolis, IN) brain microsacs (19) were used at a protein concentration of 50 $\mu$g/ml. A decrease in fluorescence polarization reflects a decrease in bulk membrane order.

[1] J. E. Dildy-Mayfield, E. I. Eger II, and R. A. Harris, unpublished observations.

**Quantitation of volatile compound bath concentrations in the oocyte recording chamber.** Samples (5 ml) of oocyte bath perfusate (MBS plus anesthetic) were collected in glass syringes sealed with three-way stopcocks. Control samples included syringes containing saturated solutions or known concentrations of compounds that had not passed through the oocyte bath tubing. The syringes were sealed and were analyzed the next day. An aliquot of each sample was transferred to a calibrated 20-ml glass syringe capped with a three-way stopcock. By weighing the 20-ml syringe first empty and then with the aliquot, the precise volume of the aliquot was determined. Room air was added to the 20-ml mark. The syringes were then placed in a 37° water bath for 2 hr. Every 30 min the syringes were taken out of the bath and shaken vigorously to facilitate equilibration. After 2 hr, the gas phase was analyzed by gas chromatography.

## Results

Some of the properties of the novel compounds used in this study are shown in Table 1. The oil/gas and saline/gas partition coefficients, vapor pressures, and predicted and actual MACs were obtained from the report by Koblin *et al.* (13). MAC refers to the lung concentration of an inhaled anesthetic at 1-atm pressure that is required to eliminate movement in response to a noxious stimulus. It is an anesthetic or population $EC_{50}$.

We tested the recently identified anesthetic F3 for its effects on $GABA_A$ receptors. F3 (2 mM; 2.5 × MAC, correcting for temperature according to eq. 4 in Ref. 21) potentiated GABA-mediated currents in *Xenopus* oocytes expressing either $\alpha1\beta2$ or $\alpha1\beta2\gamma2S$ receptors (Fig. 1A). Using 5 μM GABA, significant potentiation was seen with both receptor types, with greater potentiation being found for receptors containing the $\gamma2S$ subunit (Fig. 1B). F3 potentiation with both $\alpha1\beta2$ and $\alpha1\beta2\gamma2S$ receptors displayed a bell-shaped concentration-response curve, similar to those seen with halothane (7, 22). Maximal potentiation was seen at an F3 concentration of 2–3 mM. F3 (2 mM) shifted the GABA concentration-response curves to the left for both receptors (Fig. 2A). Replotting these data as percentage potentiation versus GABA concentration showed that enhancement of GABAergic currents decreased at higher GABA concentrations (Fig. 2B). F3 potentiation was greater for $\alpha1\beta2\gamma2S$ than for $\alpha1\beta2$ receptors at lower GABA concentrations, whereas the reverse was seen at higher GABA concentrations.

It is known that potentiation of $GABA_A$ receptor-mediated currents by both enflurane (7, 8) and halothane (7) decreases with increasing GABA concentration. Thus, comparison of anesthetic sensitivity of $GABA_A$ receptors with different subunit compositions requires evaluation of a range of GABA concentrations. Enflurane, like F3, also significantly shifted GABA concentration-response curves to the left (Fig. 3A); this was greater for $\alpha1\beta2$ receptors (3.5-fold) than for $\alpha1\beta2\gamma2S$ receptors (1.8-fold). $GABA_A$ receptors displayed differential sensitivity to 1.5 mM enflurane (approximately 2 × MAC), at various GABA concentrations, depending on whether the $\gamma2S$ subunit was coexpressed with the $\alpha1$ and $\beta2$ subunits (Fig. 3B). As seen using F3 (Fig. 2B), the $\alpha1\beta2\gamma2S$ receptors displayed greater enhancement than did the $\alpha1\beta2$ receptors at lower GABA concentrations (<20 μM), whereas the reverse occurred at higher GABA concentrations. We found that the GABA $EC_{50}$ for $\alpha1\beta1$ receptors was lower than that for $\alpha1\beta1\gamma2S$ receptors (8). Because the data in Figs. 2B and 3B were expressed in terms of absolute GABA concentrations and because enflurane and F3 potentiation strongly depends on GABA concentration, part of the difference between the $\alpha1\beta2$ and $\alpha1\beta2\gamma2S$ receptors could be due to the different GABA $EC_{50}$ values for the two receptors. The data in Figs. 2B and 3B were regraphed with the percentage of maximal GABA effect on the abscissa. If, for example, the application of 10 μM GABA produced a current of 250 nA, whereas 300 μM GABA (with which maximal currents were seen) produced 1000 nA, then the abscissa value used was 250/1000 × 100 = 25 (an $EC_{25}$ effect). Therefore, 10 μM GABA might be the $EC_{50}$ for $\alpha1\beta2$ receptors but only the $EC_{20}$ for $\alpha1\beta2\gamma2S$ receptors. Reanalysis in this manner eliminated most of the differences in anesthetic sensitivity between the $\alpha1\beta2$ and $\alpha1\beta2\gamma2S$ receptors (Figs. 2C and 3C).

Despite having a predicted MAC of 17.8 μM at 21°, F6 had no effect on $GABA_A$ receptor-mediated chloride currents, using $\alpha1\beta1\gamma2S$ receptors expressed in *Xenopus* oocytes [$F(2,11) = 0.78$, $p > 0.77$] (Fig. 4). F8, with a predicted MAC of 8.8 μM at 21°, also failed to enhance GABAergic currents [$F(2,11) = 3.57$, $p > 0.07$]. It is conceivable that these compounds might increase both the GABA desensitization rate and the probability of channel opening in such a manner that the two effects cancel each other out, resulting in no net enhancement of GABAergic currents. We tested this possibility by using a very low concentration of GABA (250 nM, producing 0.2% of the maximal current), which did not show diminution of the GABAergic current over a 2-min application period (i.e., no desensitization). F6 used under these conditions, at the same concentrations as used in Fig. 4, still had no effect.

Neither F8 or another nonanesthetic (F7-Cl), tested at saturating concentrations, potentiated the effect of GABA (Fig. 5). F8 was tested on both $\alpha1\beta2$ and $\alpha1\beta2\gamma2S$ receptors, whereas F7-Cl was tested only on $\alpha1\beta2\gamma2S$ receptors. The transitional

TABLE 1

**Properties of compounds used in this study**

The predicted MAC values were obtained by dividing 1.82 by the oil/gas partition coefficient (13). The Meyer-Overton hypothesis predicts that the product of the MAC and the oil/gas partition coefficient should be a constant (typically these range from 1 to 3). The actual rat MAC values, oil/gas and saline/gas partition coefficients (at 37°), and vapor pressures (at 22–24°) were obtained from Ref. 13.

| Compound | Actual MAC | Predicted MAC | Partition coefficient | | | Vapor pressure |
|---|---|---|---|---|---|---|
| | | | Oil/gas | Oil/saline | Saline/gas | |
| | *atm* | *atm* | | | | *atm* |
| Enflurane | 0.022 | 0.0193 | 103.4 | 124 | 0.78 | 0.27 |
| F3 | 0.014 | 0.0073 | 248 | 159 | 1.58 | 0.12 |
| F6 | Nonanesthetic | 0.042 | 43.5 | 3,655 | 0.0119 | 0.24 |
| F8 | Nonanesthetic | 0.13 | 14.3 | 13,158 | 0.0019 | 0.3 |
| F7-C1 | Nonanesthetic | 0.88 | 2.1 | 1,750 | 0.0012 | 2.7 |
| F7-Br | 3.7 (transitional) | 0.27 | 6.7 | 2,393 | 0.0028 | 1.4 |

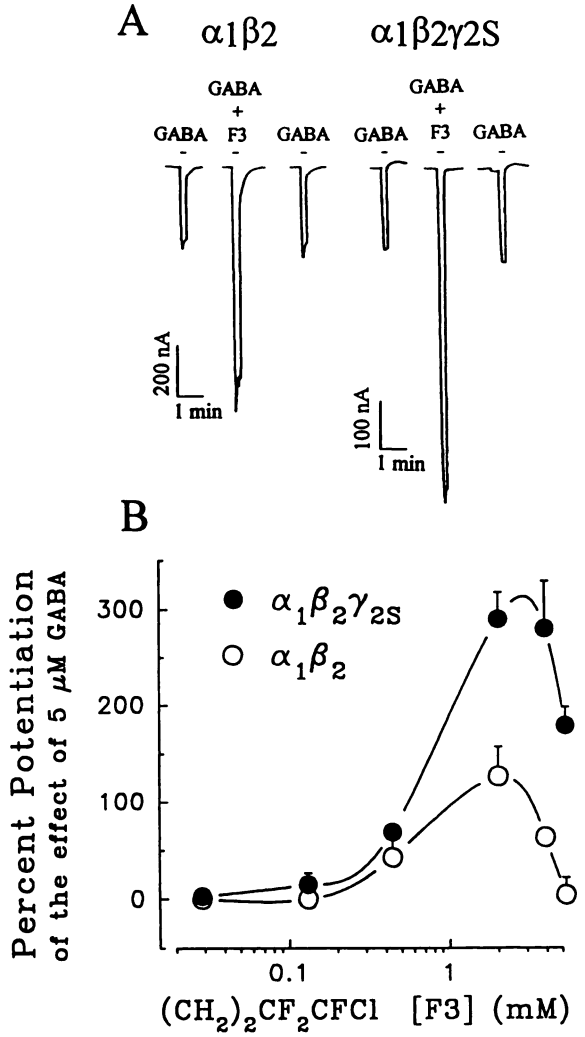


**Fig. 1.** A, Representative tracings of inward currents induced by 5 μM GABA in the presence or absence of 2 mM F3, with α1β2 (*left*) or α1β2γ2S (*right*) receptors. B, Anesthetic F3 potentiation of the effects of 5 μM GABA in *Xenopus* oocytes expressing either α1β2 or α1β2γ2S receptors. GABA was bath-applied with various concentrations of F3 for 20 sec. The bath concentrations of F3 are shown on the *abscissa*. Data represent the percentage enhancement of the effect produced by 5 μM GABA alone. Values are presented as mean + standard error for four to six oocytes.

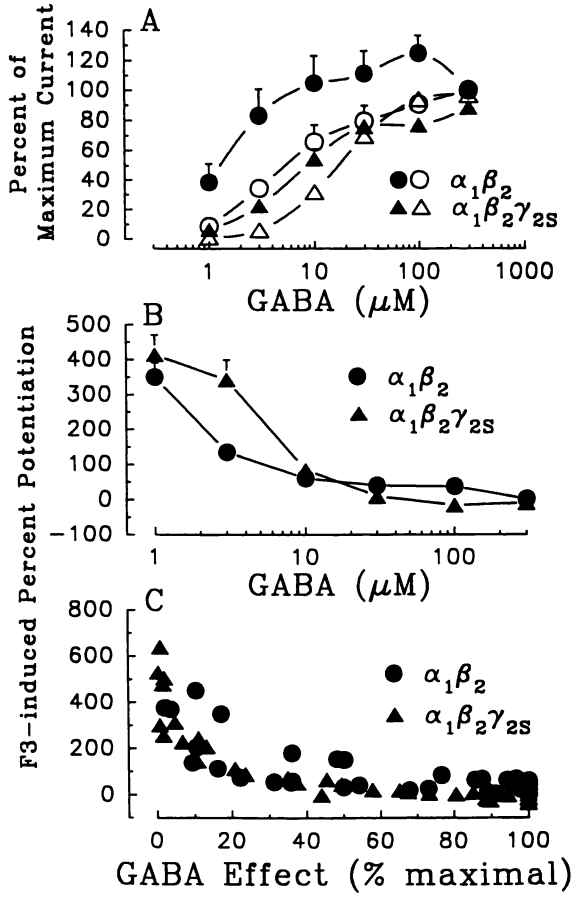


**Fig. 2.** A, F3 (2.0 mM bath concentration) shifts the GABA concentration-response curve to the left with receptors composed of α1β2 (*circles*) or α1β2γ2S (*triangles*) subunits. *Open symbols*, effect of GABA alone; *solid symbols*, effect of GABA plus F3. For α1β2 receptors, the GABA $EC_{50}$ was 6.2 μM in the absence of F3 and 1.5 μM in the presence of F3. For α1β2γ2S receptors, the $EC_{50}$ values were 17 μM and 6.5 μM, respectively. GABA was applied with F3 for 20 sec; the maximal current observed was used for analysis. Data represent the percentage enhancement of the effect produced by GABA alone. Values are presented as mean + standard error for five to seven oocytes. B, The potentiation of GABAergic currents produced by F3 diminishes as the GABA concentration is increased. F3, like enflurane, had a greater effect on the γ2S-containing receptors at low GABA concentrations and a greater effect on the α1β2 receptors at higher GABA concentrations. Note that significant potentiation can still be seen at higher GABA concentrations (>20 μM) with the α1β2 but not the α1β2γ2S receptors. Data (from A) represent the percentage enhancement seen at each GABA concentration. C, The difference in F3 potentiation of GABAergic currents with α1β2 and α1β2γ2S receptors is due to the same fixed concentrations of GABA being used for both receptors, despite their having different GABA $EC_{50}$ values. *Abscissa*, effect of GABA as a percentage of the maximal effect (see text for more details). Each *point* on the graph represents an individual measurement.

compound F7-Br, which is less potent *in vivo* than predicted by the Meyer-Overton hypothesis, produced a modest enhancement of the effect of GABA, at a saturating concentration (Fig. 5), consistent with its limited potency (MAC = 3.7 atm) (13). Neither enflurane, F3, F6, F8, F7-Cl, nor F7-Br produced any currents in the absence of exogenously applied GABA (data not shown).

Enflurane, F3, F6, and F8 were tested for their abilities to disorder egg yolk lecithin vesicles (Fig. 6A) and mouse brain microsac membranes (Fig. 6B). Membrane disordering was observed with enflurane and F3 but not with the nonanesthetics F6 and F8. In contrast, F6 produced ordering in the egg yolk lecithin vesicles but only at very high concentrations (4–10 times greater than the predicted MAC). Although irrelevant for mechanisms of anesthesia, this finding does demonstrate F6 incorporation into the egg yolk lecithin vesicles.

## Discussion

General anesthesia can be produced by a large number of structurally diverse compounds that have in common the ability to potentiate responses at the $GABA_A$ receptor. Barbiturates (23–26), diethyl ether (27), steroid anesthetics (28–31), and propofol (7, 32, 33) all potentiate the effects of GABA in electrophysiological and chloride uptake assays. In addition, diethyl ether, halothane, enflurane, and methoxyflurane enhance $^{36}Cl^-$ uptake into cerebral cortical brain vesicles in a concentration-dependent, picrotoxin-reversible manner (27,

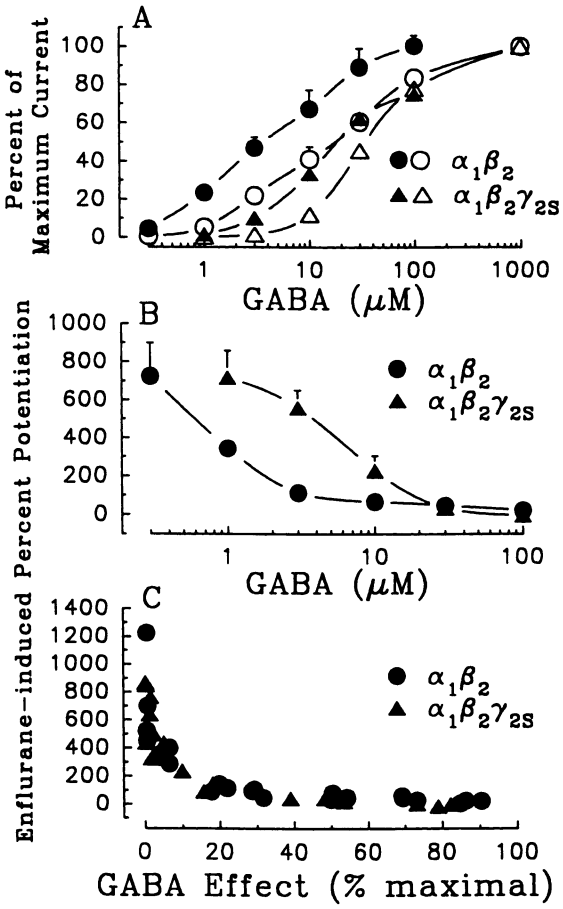


**Fig. 3.** A, Enflurane (1.5 mM bath concentration) shifts the GABA concentration-response curve to the left in receptors composed of $\alpha1\beta2$ (*circles*) or $\alpha1\beta2\gamma2S$ (*triangles*) subunits. *Open symbols*, effect of GABA alone; *solid symbols*, effect of GABA plus enflurane. For $\alpha1\beta2$ receptors, the GABA $EC_{50}$ was 17.6 μM in the absence of enflurane and 5.0 μM in the presence of enflurane. For $\alpha1\beta2\gamma2S$ receptors, the $EC_{50}$ values were 36.6 μM and 20.3 μM, respectively. GABA was applied with enflurane for 20 sec; the maximal current observed was used for analysis. Data represent the percentage enhancement of the effect produced by GABA alone. Values are presented as mean + standard error for three or four oocytes. B, The potentiation of GABAergic currents produced by enflurane diminishes as the GABA concentration is increased. Enflurane appeared to have a greater effect on the $\gamma2S$-containing receptors at low GABA concentrations and a greater effect on the $\alpha1\beta2$ receptors at higher GABA concentrations. The data from A were replotted as GABA concentration versus percentage potentiation. C, The difference in enflurane potentiation of GABAergic currents with $\alpha1\beta2$ and $\alpha1\beta2\gamma2S$ receptors is due to the same fixed concentrations of GABA being used for both receptors, despite their having different GABA $EC_{50}$ values. *Abscissa*, effect of GABA as a percentage of the maximal effect (see text for more detail). Each *point* on the graph represents an individual measurement.

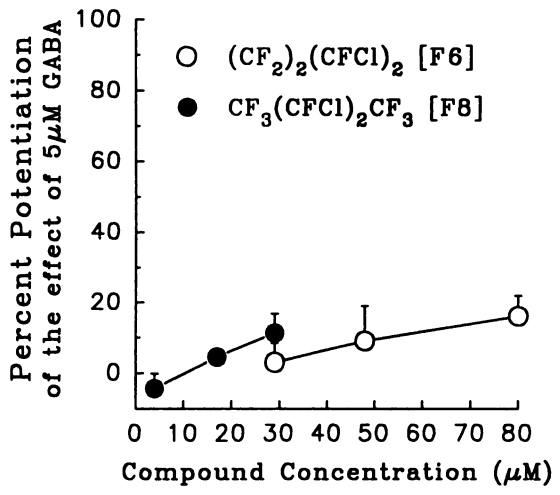


**Fig. 4.** Failure of the nonanesthetics F6 and F8 to potentiate the effect of 5 μM GABA in *Xenopus* oocytes expressing $\alpha1\beta1\gamma2S$ $GABA_A$ receptors. The predicted MACs of F6 and F8 at 21° are 17.8 μM and 8.8 μM, respectively. GABA was bath-applied with F6 or F8 for 20 sec. Data represent the percentage enhancement of the effect produced by 5 μM GABA alone. Values are presented as mean + standard error for four oocytes.

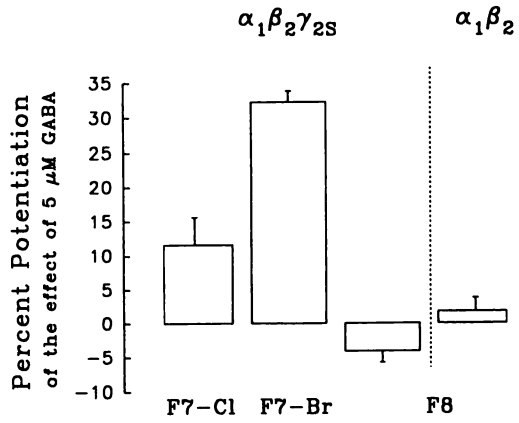


**Fig. 5.** Lack of effect of the nonanesthetics F8 and F7-Cl, at saturating concentrations, on $GABA_A$ receptor-mediated currents. The oocyte bath concentrations of these compounds were not determined. F8 was tested with $\alpha1\beta2$ and $\alpha1\beta2\gamma2S$ receptors, whereas F7-Cl was tested only with $\alpha1\beta2\gamma2S$ receptors. The transitional compound F7-Br, at a saturating concentration, produced modest enhancement of the effect of 5 μM GABA. GABA was bath-applied with the compounds for 20 sec. Data represent the percentage enhancement of the effect produced by 5 μM GABA alone. Values are presented as mean + standard error for three to 10 oocytes.

34). Halothane potentiates GABA-mediated neuronal depression in electrophysiological studies (35–38) by prolonging the decay time constant of $GABA_A$ receptor-mediated currents (39, 40), including spontaneous inhibitory postsynaptic currents (41, 42).

Our results provide a new line of evidence implicating the $GABA_A$ receptor/chloride channel complex as a major mediator of anesthetic action *in vivo*. We previously showed, using the *Xenopus* oocyte expression system, that enflurane, halothane, isoflurane, diethyl ether, ketamine, 3α-hydroxy-5α-dihydroprogesterone, pentobarbital, phenobarbital, and propofol could all enhance the effects of GABA (7, 8). This effect of these structurally diverse anesthetics suggests a connection between anesthesia *in vivo* and the $GABA_A$ receptor system. Our current results take these findings a step further, by showing that only halogenated compounds that can produce anesthesia *in vivo* can potentiate GABAergic currents *in vitro*. Clear enhancement of the effect of GABA was produced by the anesthetic F3 and was not seen with the nonanesthetics F6, F8, and F7-Cl. Furthermore, the transitional compound F7-Br, which is less potent than predicted by the Meyer-Overton hypothesis, produced only modest potentiation, in agreement with its limited anesthetic effect.

It is important to consider the usefulness of the *Xenopus* oocyte expression system in the study of anesthetic actions on mammalian $GABA_A$ receptors. Do these receptors mimic those

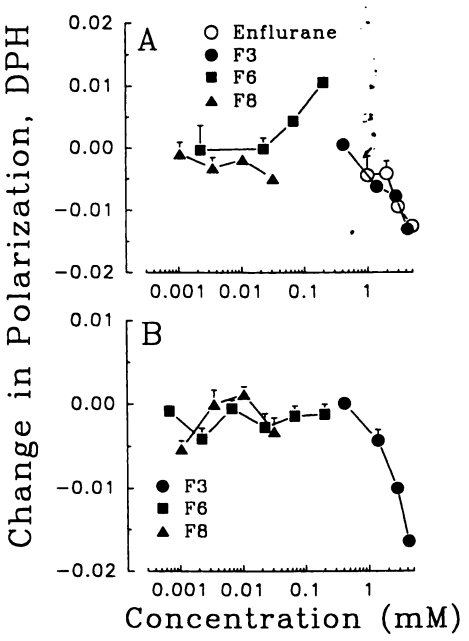


**Fig. 6.** Effects of anesthetics/nonanesthetics on membrane order, measured with the probe DPH. Negative numbers indicate decreased order, compared with readings obtained with DPH alone. A, Only anesthetics were found to decrease order; enflurane and F3, but not the nonanesthetics F6 and F8, decreased order in egg yolk lecithin vesicles. At higher concentrations, F6 had an ordering effect. Data are represented as mean ± standard error for three or four separate determinations. B, F3, but not F6 and F8, decreased membrane order in mouse brain microsacs. Data are represented as mean ± standard error for three to six separate determinations.

expressed in mammalian cells? Modulators of $GABA_A$ receptor function, such as barbiturates, benzodiazepines, zinc, and steroid anesthetics, all appear to act in oocytes as they do in other systems (7, 43, 44). Evidence also suggests that the compounds we studied in oocytes act similarly in rat spinal cord preparations. Kendig *et al.* (45) showed that F3, but not F6, potentiated dorsal root potentials, which are indicators of $GABA_A$ receptor function.

In an earlier study (8), we found that enflurane produced greater potentiation of the $EC_{50}$ of GABA with $\alpha1\beta1$ receptors than with $\alpha1\beta1\gamma2S$ receptors. The GABA $EC_{50}$ was 10 $\mu$M with the former and 30 $\mu$M with the latter. This agrees with the present results, because enflurane and F3 had greater effects on the GABA $EC_{50}$ with $\alpha1\beta2$ receptors than with $\alpha1\beta2\gamma2S$ receptors (Figs. 2A and 3A). It appears that at higher effective GABA concentrations greater potentiation of GABAergic currents can be seen with $\alpha1\beta2$ receptors. The 'crossover' of the concentration-response curves in Figs. 2B and 3B illustrates this point. This is not clearly evident in Fig. 3C because of the large numbers (1300%) on the ordinate. We have also noticed this 'crossing over' phenomenon using anesthetic concentrations of ethanol and butanol (46). High concentrations of alcohols also share with volatile and steroid anesthetics and barbiturates the ability to potentiate currents with $GABA_A$ receptors lacking a $\gamma2$ subunit (8, 46–48).

The replacement of hydrogen atoms in F3 with fluorine, giving F6, converts a potent anesthetic that is capable of enhancing $GABA_A$ receptor-mediated currents into a nonanesthetic that is not. The replacement of bromine in F7-Br with chlorine, giving F7-Cl, converts a transitional compound into a nonanesthetic. It seems that relatively minor changes in structure can profoundly affect anesthetic efficacy both *in vivo* and in the enhancement of GABAergic currents. What might explain these findings? The Meyer-Overton hypothesis, as presently formulated, would seem to fail. Note that the predicted MAC of the nonanesthetic F6 falls between those of enflurane and the transitional compound F7-Br. The oil/gas partition coefficients listed in Table 1 thus cannot account for the difference in anesthetic efficacy between the anesthetics and nonanesthetics, supporting our contention that the Meyer-Overton hypothesis is, by itself, insufficient to predict whether a compound will be an anesthetic. However, if one examines the saline/gas partition coefficients of these compounds (Table 1), a pattern appears. F3 and enflurane have much higher saline/gas partition coefficients than do F6 and F8. Furthermore, halothane has a saline/gas partition coefficient of 0.75 and isoflurane one of 0.55 (49). It may be that the hydrophilicity of these compounds also determines whether they produce anesthesia and enhance GABAergic currents. However, the transitional compound F7-Br has a saline/gas partition coefficient lower than that of F6, suggesting that the saline/gas partition coefficient dependence of anesthesia may vary among different chemical series. One possible explanation for this observation is that the saline/gas partition coefficient partially but incompletely reflects the physical properties critical for interaction with the $GABA_A$ receptor and for production of anesthesia. Additional experiments using other compounds are necessary to resolve this issue.

We tested several of these novel compounds for their abilities to decrease order in egg yolk lecithin vesicles and mouse brain microsac membranes. Only the anesthetics enflurane and F3 were able to disorder these membranes, whereas the nonanesthetics F6 and F8 either were without effect or actually increased order (at higher concentrations). However, the fluidizing effects of F3 and enflurane were minor at pharmacologically relevant concentrations. For example, 0.8 mM F3 (~1 × MAC) produced minimal disordering (Fig. 6); however, the same concentration of F3 markedly enhanced GABA-mediated currents (Fig. 2). Furthermore, the magnitudes of the changes in membrane order are so small that they can be mimicked by changes in temperature as small as 1°, decreasing their physiological relevance (9). It is also worth noting that membrane disordering appears to inhibit, rather than enhance, GABAergic currents and is unlikely to be responsible for the actions of anesthetics on $GABA_A$ receptors (19).

We conclude that anesthetics consistently differ from nonanesthetics in their abilities to potentiate $GABA_A$ receptor-mediated chloride currents in *Xenopus* oocytes. The anesthetics enflurane and F3 produced strong enhancement, whereas the transitional compound F7-Br produced only modest enhancement of the effect of GABA. The nonanesthetics F6, F8, and F7-Cl had no effects on GABAergic currents. Although the Meyer-Overton hypothesis predicts that all of these compounds should act as complete anesthetics *in vivo*, only F3 and enflurane did so, and F7-Br was less potent *in vivo* than predicted. These findings correlate with the abilities of these compounds to enhance $GABA_A$ receptor-mediated currents, and they strengthen the hypothesis that general anesthesia may be mediated by an action on the $GABA_A$ receptor/chloride channel complex.

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