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Effects of isoflurane and hexafluorodiethyl ether on human recombinant $GABA_A$ receptors expressed in *Sf*9 cells

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Abstract

Effects of volatile anesthetics and a volatile convulsant on human recombinant γ-aminobutyric acid (GABA) type A receptor responses were studied using the whole cell configuration of the patch clamp technique. *Sf*9 cells were transfected with bacuroviruses carrying cDNAs of α1β2, α1β2γ2s, α3β2 and α3β2γ2s subunit combinations of the human $GABA_A$ receptor. Clinical concentrations of isoflurane (a volatile anesthetic) enhanced the GABA-induced current of the α1β2γ2s and α3β2γ2s $GABA_A$ subunit combinations. On the other hand, isoflurane suppressed the current of the α1β2 and α3β2 subunit combinations, indicating that the anesthetic effects depended upon the presence of γ2s subunit. A high concentration (2 mM) of isoflurane generated a surge current following the washout of GABA and the anesthetic. Hexafluorodiethyl ether (a volatile convulsant) decreased the GABA-response of the both α3β2γ2s and α3β2 constructs without generating a surge current. The results suggest that volatile agents affect the receptor–ionophore complex via direct interaction with proteins but not through a perturbation of the membrane lipid environment. A hypothetical sequential model for the anesthetic action is presented.

Keywords: Anesthetic, volatile; Convulsant, volatile; *Sf*9 cell; $GABA_A$ receptor, recombinant

1. Introduction

Volatile anesthetic agents have been used successfully in clinical practice for more than 150 years. It is, however, still controversial how these agents affect the central nervous system (CNS) to induce anesthesia in humans and animals. Recent studies have indicated that general anesthetics at clinical concentrations change the efficacy of excitatory and inhibitory neurotransmissions in the CNS. Suppression or enhancement of excitatory transmissions has been reported with glutamate receptors (Wakamori et al., 1991; Narimatsu et al., 1996), nicotinic acetylcholine receptors (Flood et al., 1997) and 5-hydroxytryptamine-3 receptors (Jenkins et al., 1996). However, enhancement of the inhibitory transmission mediated by γ-aminobutyric acid (GABA) is now thought to be a major mechanism of action of general anesthetics and to be involved in induction of general anesthesia. Experiments with stereoisomers of isoflurane further supported the significance of potentiation of $GABA_A$ system in general anesthesia (Hall et al., 1994; Harris et al., 1994; Moody et al., 1994). A similar volatile compound, hexafluorodiethyl ether which induces convulsions in humans and in animals, has also been reported to affect the $GABA_A$ response of dissociated neurons in a different manner, namely, marked suppression of the GABA-evoked current (Wakamori et al., 1991),

Molecular biology has revealed that a variety of different $GABA_A$ receptors exists in the CNS, probably as heteromeric pentamers composed of α, β, γ and/or δ subunits (McKernan and Whiting, 1996). Using a variety of expression systems, extensive studies have been carried out to elucidate which subunits or which amino acid residues in specific subunits are essential for the action of anesthetic compounds (Mihic et al., 1994a; Harris et al., 1995; Hill-Venning et al., 1997). In the *Xenopus* oocytes expression system, Mihic et al. (1997) showed that two specific amino acid residues in transmembrane domains 2 and 3 are critical for an allosteric modulation of the GABA-gated chloride currents by volatile anesthetics. In the present study, we used the *Sf*9 cell–bacurovirus system to express the human recombinant $GABA_A$ receptors

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and examined whether the effects of volatile anesthetic and convulsant compounds depended on the subunit composition of the $GABA_A$ receptor.

2. Materials and methods

2.1. Expression of $GABA_A$ receptors in Sf9 cells

*Sf*9 insect cells were grown in spinner flask cultures at 27°C in serum-free medium (Sf900-II-SFM, Life Technologies). *Sf*9 cells, at a density of 7.5×10^5 cells/Petri dish (35 mm i.d., Falcon), were infected with bacroviruses containing cDNAs of human $GABA_A$ receptor subunits. The combination of subunits was $\alpha 1-\beta 2-\gamma 2s$ or $\alpha 3-\beta 2-\gamma 2s$ at a multiplicity of infection of 3:1:2, or $\alpha 1-\beta 2$ or $\alpha 3-\beta 2$ at a multiplicity of infection of 3:1. The infected cells were incubated at 27°C for about 48 hours (Nabekura et al., 1998). *Sf*9 cells were purchased from Riken Cell Bank (Tsukuba, Ibaraki, Japan; Vaughn et al., 1977; Smith et al., 1985). The construction of the expression vector for the human $GABA_A$ receptor $\alpha 1$, $\alpha 3$, $\beta 2$, and $\gamma 2s$ has been described previously (Witt et al., 1996; Westh-Hansen et al., 1997). The amino acid sequence of the subunits were: $\alpha 1$ (see Schofield et al., 1989); $\alpha 3$ (see Hadingham et al., 1993a); $\beta 2$ (see Hadingham et al., 1993b) with a difference at amino acid residue 109 glycine instead of published valine; $\gamma 2s$ (see Pritchett et al., 1989; Mihic et al., 1994c) with two differences in amino acid residues, at position 81 threonine instead of published methionine and at position 142 threonine instead of published serine. Numbering is without signal sequence.

2.2. Electrophysiological experiments

At about 48 h after infection, *Sf*9 cells were constantly perfused with extracellular solution at a rate of 4 ml/min. The standard external solution was composed of (in mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10, HEPES 10. The pH was adjusted to 7.4 with Tris base. Patch pipette solution had a composition of (in mM): Cs_2SO_4 50, CsCl 78, $MgCl_2$ 6, EGTA 5, ATP 5, HEPES 10. The pH was adjusted to 7.2 with Tris base.

Electrical recordings were carried out using the conventional whole cell mode of the patch clamp technique. Patch pipettes were made of glass capillaries with an outer

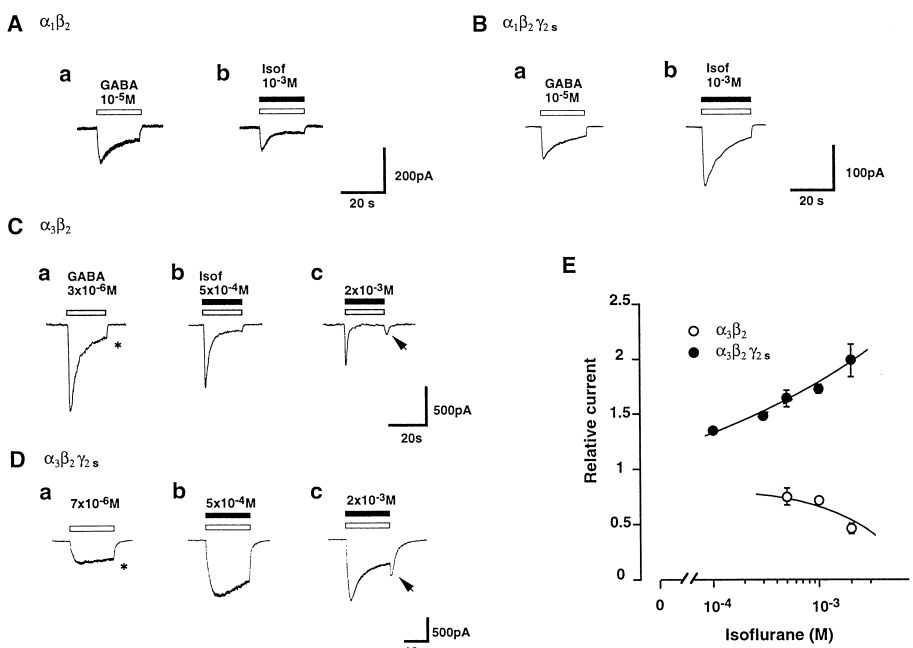


Fig. 1. Differential effects of isoflurane (Isof) on the GABA-induced current of $GABA_A$ receptors with or without $\gamma 2s$ subunit. (A) (a) In an *Sf*9 cell expressing $\alpha 1\beta 2$ subunit combination, GABA (10 μM) evoked an inward current at −40 mV. (b) The current was reduced by simultaneously applied isoflurane (1 mM). The decay rate of the current in the presence of the agonist and the anesthetic became greater. (B) (a) and (b) In receptor complexes composed of $\alpha 1\beta 2\gamma 2s$ subunits, isoflurane increased the current. (C) (a) GABA (3 μM) induced an inward current in an *Sf*9 cell transfected with $\alpha 3\beta 2$ subunit combination (holding potential −40 mV). The asterisk indicates the rapid deactivation phase of the current following washout of GABA. (b) The current was decreased by 500 μM isoflurane. (c) A higher concentration (2 mM) further decreased the current, making the decay in the continuous presence of GABA faster. A surge current was generated following the washout of both GABA and isoflurane (shown by an arrow). (D) (a) GABA (7 μM) evoked a current in an *Sf*9 cell expressing $\alpha 3\beta 2\gamma 2s$ subunit combination (holding potential −40 mV). (b) Isoflurane (500 μM) augmented the GABA response. (c) A higher concentration of the anesthetic accelerated the decay of the current and generated a surge current (arrow, note the slow decay of the surge current). (E) The concentration–effect curve of isoflurane, where the peak currents are plotted against the anesthetic concentration. Isoflurane increased the GABA response of $\alpha 3\beta 2\gamma 2s$ subtype dose-dependently (closed circle), whereas the agent suppressed the current of $\alpha 3\beta 2$ construct dose-dependently (open circle). Solid lines were drawn by eye. Each data point and vertical bar indicate the mean ± S.E.M. of five to eight experiments.

diameter of 1.5 mm using a vertical puller (BP-7, Narishige, Japan). The cells were voltage-clamped with a voltage-clamp amplifier (Axopatch 1D, Axon Instruments, USA). All signals were filtered with a low pass filter at a cut-off frequency of 1 kHz, monitored on a syncroscope (CS-8010, Kenwood, Japan) and a pen recorder (WR-3300, Graphtec, Japan), then digitized at a rate of 44 kHz. The data were stored on magnetic tapes (RD-120 TE, Teac, Japan) for later analysis. Rapid application of drugs was achieved by the Y-tube method as described previously (Murase et al., 1990). In the present study, each drug was applied at an interval of more than 3 min.

At the beginning of experiments transfecting $\alpha 1$ or $\alpha 3$, $\beta 2$ and $\gamma 2s$ subunits, we compared GABA responses in the presence and absence of $ZnCl_2$ (10 μM) in order to confirm the co-expression of the $\gamma 2s$ subunit in the particular cell under study, since it has been reported that Zn^{2+} ions reduce the GABA-induced current of receptors complexes devoid of γ subunits (Draguhn et al., 1990; Smart et al., 1991). In some experiments, GABA and volatile agents were thereafter applied without $ZnCl_2$, and in others they were applied in the continuous presence of $ZnCl_2$ (10 μM). Similar results were obtained in the both conditions. All data in the present report are those in the presence of 10 μM $ZnCl_2$.

The drugs used were GABA, isoflurane, sevoflurane, enflurane, methoxyflurane and hexafluorodiethyl ether. All volatile agents were resolved in the external solution just before use (Wakamori et al., 1991). All experiments were carried out at room temperature of approximately 22°C.

2.3. Statistical analysis

Experimental values were presented as mean ± S.E.M. Student's t test was applied when appropriate. For the relationship between GABA concentration and the peak current amplitude, continuous lines were fitted according to the following equation with a least square fitting routine after normalizing the amplitudes of the responses:

$$I = I_{max} C^n / (K_D^n + C^n) \quad (1)$$

where I is the normalized value of the current, I_{max} the maximal response, C the GABA concentration, K_D the concentration corresponding the half-maximal response, and n the Hill coefficient.

3. Results

3.1. Differential effects of volatile anesthetics on the GABA receptors with or without $\gamma 2s$ subunit

GABA did not evoke any current in those *Sf*9 cells which were transfected with cDNAs of $\alpha 1$ (six experiments), $\alpha 3$ (six experiments), or $\beta 2$ (20 experiments) subunit alone, indicating that the homomeric $\alpha 1$, $\alpha 3$, or $\beta 2$ receptor did not assemble functional GABA receptor–chloride ionophore complexes (data not shown). In heteromeric $GABA_A$ receptors, GABA induced a chloride current and the effects of methoxyflurane, enflurane, isoflurane and sevoflurane on the current were investigated. All the volatile anesthetics tested (1 mM) increased the GABA-induced current of the $\alpha 1\beta 2\gamma 2s$ and $\alpha 3\beta 2\gamma 2s$ subtypes, but reduced the current of the $\alpha 1\beta 2$ and $\alpha 3\beta 2$ subunit constructs (partly shown in Ikemoto et al., 1998). Fig. 1A and B show experiments with $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2s$ constructs, respectively. GABA (10 μM) induced a desensitizing inward current with both receptors at −40 mV (Fig. 1Aa and Ba). In the former combination, isoflurane

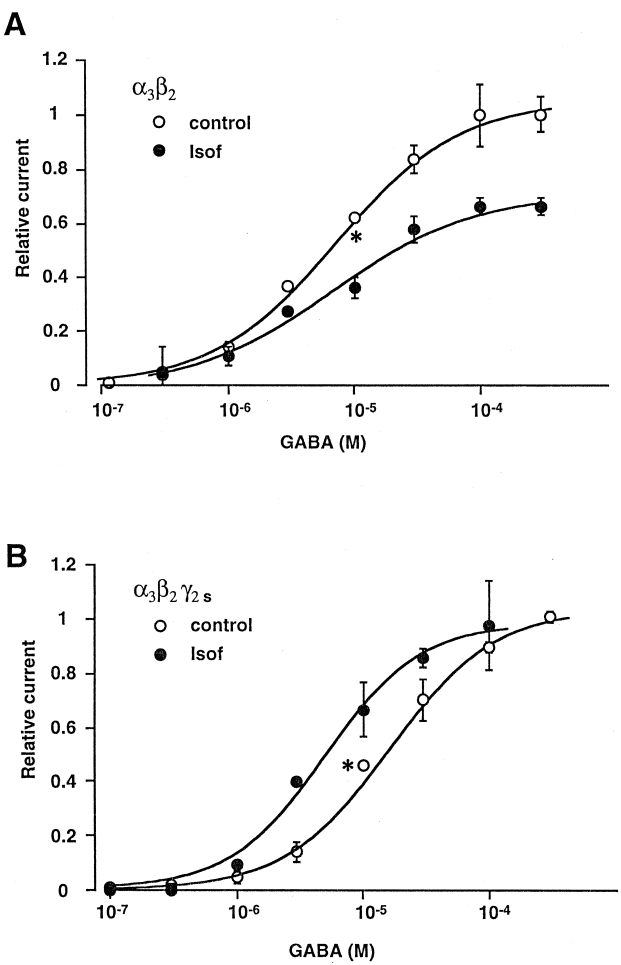


Fig. 2. Effects of isoflurane on the concentration–response curve of the both subtypes of the recombinant $GABA_A$ receptors. (A) In $\alpha 3\beta 2$ $GABA_A$ receptor subunit combination, isoflurane (Isof, 1 mM) shifted the curve downward, reducing the peak response to about 60% of control and shifting the K_D from 6.7 to 7.7 μM and the Hill coefficient from 0.92 to 0.80. (B) In $\alpha 3\beta 2\gamma 2$ $GABA_A$ receptor subunit combination, isoflurane shifted the curve to the left, changing the K_D from 12.8 to 3.99 μM and the Hill coefficient from 1.08 to 1.12. The holding potential was −40 mV. The peak current was normalized to that evoked by 10 μM GABA (shown by an asterisk) and plotted against the GABA concentration. Curve fitting was made according to Eq. (1) in the text. Each data point and vertical bar indicate the mean ± S.E.M. of five to eight experiments.

(1 mM) decreased the current with a concomitant acceleration of the decay in the presence of both GABA and isoflurane (Fig. 1Ab). Whereas, the anesthetic increased the GABA-induced current of $\alpha 1\beta 2\gamma 2s$ construct (Fig. 1Bb).

3.2. Concentration-dependent effects of isoflurane on $GABA_A$ receptor of $\alpha 3\beta 2\gamma 2s$ and $\alpha 3\beta 2$ constructs

The differential effects of isoflurane were studied in more detail on the recombinant $GABA_A$ receptors of $\alpha 3\beta 2\gamma 2s$ and $\alpha 3\beta 2$ subunit combinations. An *Sf*9 cell transfected with $\alpha 3$ and $\beta 2$ subunits was voltage-clamped at -40 mV and an inward current was evoked by 3 μM of GABA, resulting in 30% of the maximal chloride ion current (Fig. 1C, see also the control concentration–response curve in Fig. 2A). Simultaneously applied isoflurane (500 μM) markedly decreased the current, with further reduction by a higher concentration (2 mM) of the anesthetic. The decay rate of the current in the continuous presence of GABA was increased dose-dependently. Following washout of both GABA and the anesthetic, a surge current was recorded as shown by the arrow in Fig. 1Cc. In $\alpha 3\beta 2\gamma 2s$ GABA receptor complexes, the current was evoked by 7 μM of GABA, which also led an approximately 30% activation of the maximal chloride current (see Fig. 2B). The current was increased by 500 μM of isoflurane. When 2 mM of isoflurane was applied, the decay of the current in the presence of GABA became faster, as was observed with the $\alpha 3\beta 2$ subunit combination. A large surge current followed the washout and decayed slowly (arrow in Fig. 1Dc) compared with the deactivation of the GABA-induced current (asterisk in Fig. 1Da). The dose–effect relationship of isoflurane on the both receptor subunit combinations is shown in Fig. 1E. The inhibition of the GABA-gated current in $\alpha 3\beta 2$ subunit combination as well as the enhancement observed in $\alpha 3\beta 2\gamma 2s$ subunit construct were dose-dependent.

Fig. 2 illustrates the effects of isoflurane on the concentration–response curve of GABA of the both subtypes. In $\alpha 3\beta 2$ combination, 1 mM isoflurane shifted the curve downward, reducing the maximum response to about 60% of the control. The dissociation constant (K_D) was slightly changed from 6.7 to 7.7 and the Hill coefficient (n) from 0.92 to 0.80 (Fig. 2A). In the receptor with $\gamma 2s$ subunit (namely, $\alpha 3\beta 2\gamma 2s$ composition; Fig. 2B), the anesthetic

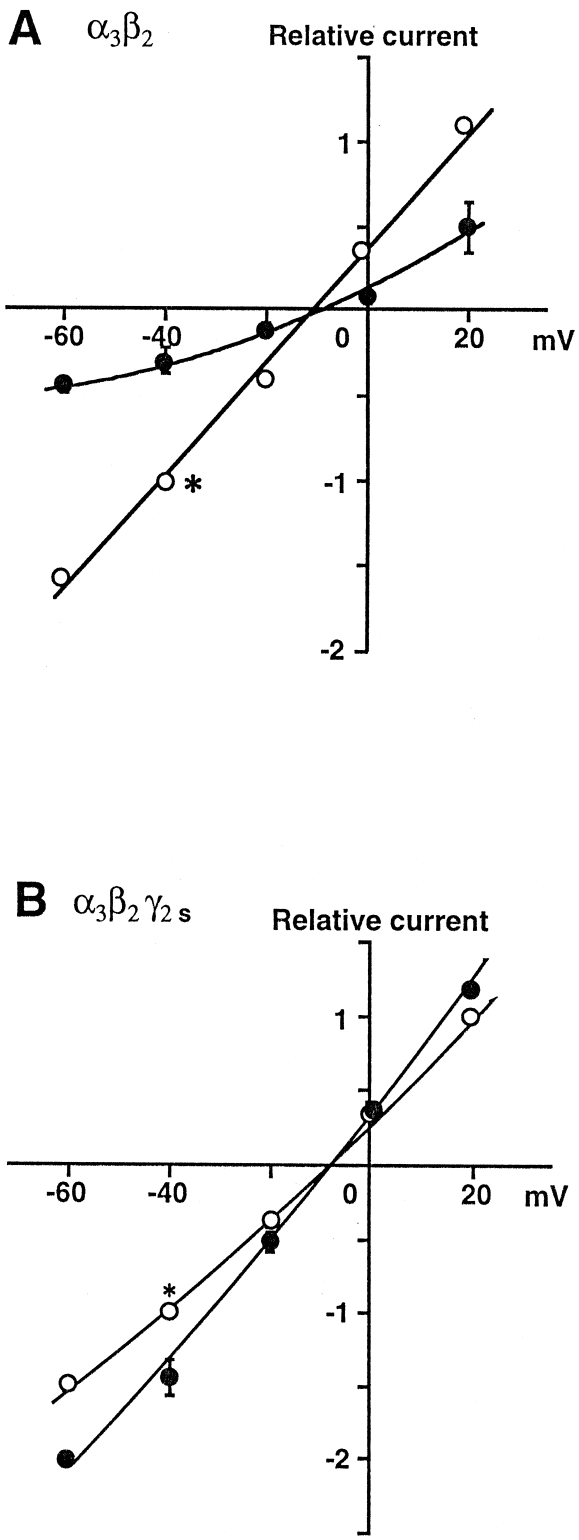


Fig. 3. Current–voltage relationship of the GABA response in the presence and the absence of isoflurane. (A) In $\alpha 3\beta 2$ $GABA_A$ receptor subunit combination, the response evoked by 3 μM GABA reversed its polarity at -11.0 mV (control, open circle), which is close to the equilibrium potential for chloride ions in the present condition (-13.5 mV). Isoflurane (1 mM) suppressed the current at both sides of the reversal potential (closed circle) without changing the reversal potential value (-8.8 mV). (B) In $\alpha 3\beta 2\gamma 2s$ $GABA_A$ receptor subunit combination, the current induced by 7 μM GABA reversed its polarity at -10.7 mV (control, open circle). Isoflurane (1 mM) increased the current without changing the reversal potential (closed circle; -10.5 mV). Currents are normalized to that evoked at -40 mV (asterisk). Each data point and vertical bar represent the mean ± S.E.M. of four to six experiments.

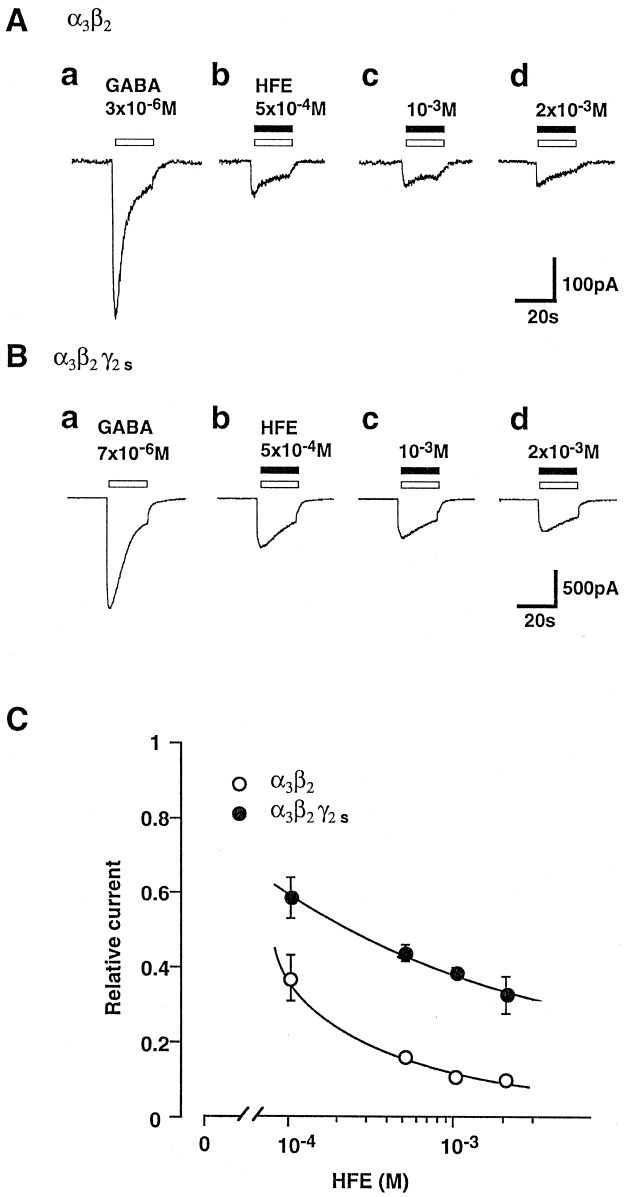


Fig. 4. Effects of a volatile convulsant, hexafluorodiethyl ether (HFE), on the both types of the $GABA_A$ receptor. (A) (a) An *Sf*9 cell expressing $\alpha 3\beta 2$ subtype was voltage clamped at −40 mV and 3 μM of GABA evoked a chloride current. (b) Simultaneous application of hexafluorodiethyl ether greatly reduced the peak current. (c and d) Higher concentrations of the convulsant further suppressed the peak current. The decay rate of the current in the presence of GABA was not increased. (d) Simultaneous washout of GABA and hexafluorodiethyl ether (2 mM) did not generate a surge current. (B) (a) GABA (7 μM) induced a chloride current with a cell expressing $\alpha 3\beta 2\gamma 2s$ construct at −40 mV. (b, c and d) Hexafluorodiethyl ether suppressed the current dose-dependently and no surge current was observed. (C) The dose–inhibition curve of hexafluorodiethyl ether on $\alpha 3\beta 2\gamma 2s$ and $\alpha 3\beta 2$ $GABA_A$ receptor combinations. The reduction was dose-dependent and was greater with $\alpha 3\beta 2$ (open circle) than with $\alpha 3\beta 2\gamma 2s$ combination (closed circle). Each data point and vertical bar represent the mean ± S.E.M. of five to seven experiments.

shifted the curve to the left, reducing the K_D from 12.8 to 3.99 μM with an insignificant change in the Hill coefficient from 1.08 to 1.12.

The current–voltage relationships are depicted in Fig. 3. The reversal potential for the $\alpha 3\beta 2$ combination was −11.0 mV, and that for the $\alpha 3\beta 2\gamma 2s$ composition −10.7 mV. These values are close to the equilibrium potential for Cl ions (−13.5 mV) calculated with the Nernst equation in

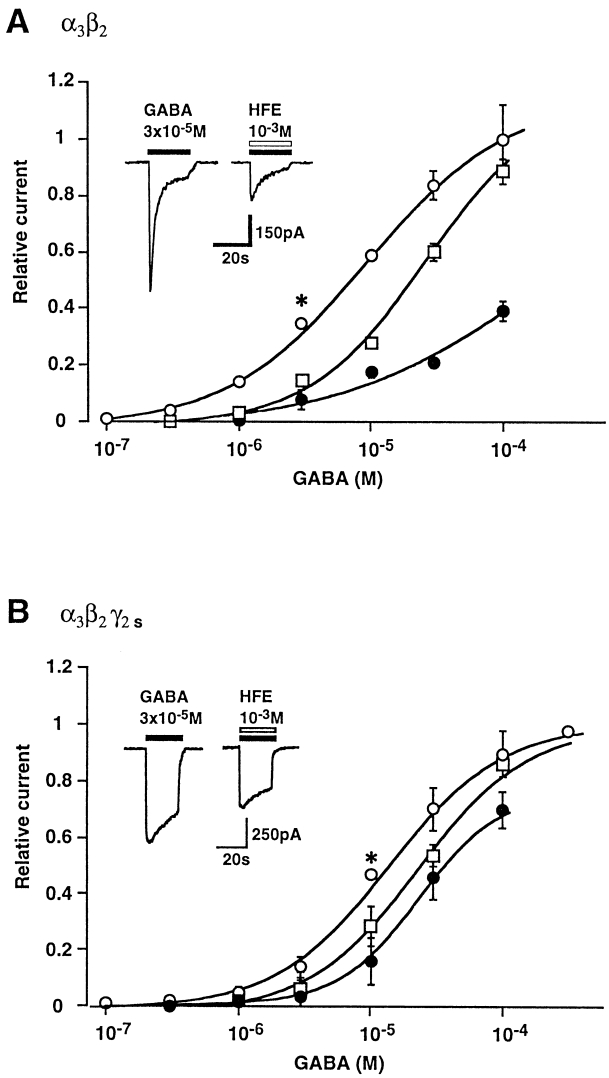
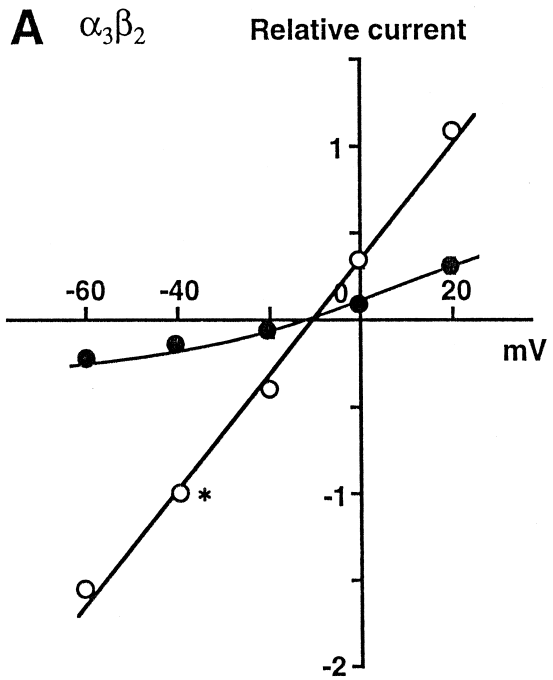
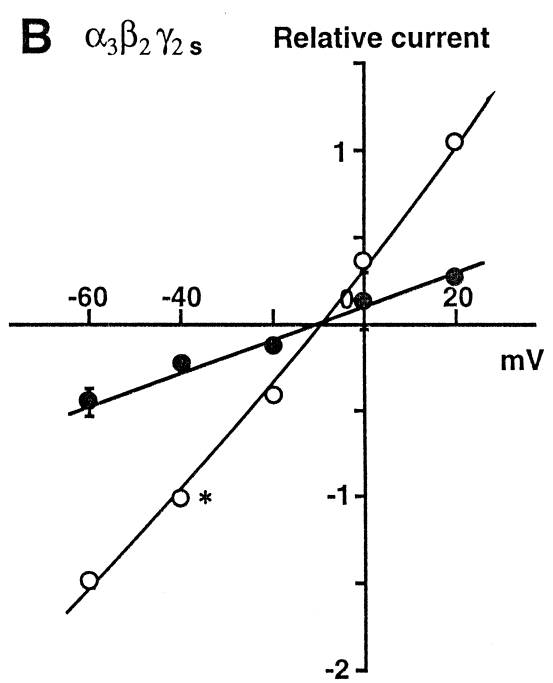


Fig. 5. Effects of hexafluorodiethyl ether on the concentration–response curve for GABA of $\alpha 3\beta 2$ and $\alpha 3\beta 2\gamma 2s$ $GABA_A$ receptor subunit combinations. (A) A low concentration of hexafluorodiethyl ether (100 μM, open squares) shifted the curve of the $\alpha 3\beta 2$ combination to the right, changing K_D from 6.7 to 26 μM and the Hill coefficient from 0.92 to 1.0. A high concentration (1 mM, closed circles) seems to shift the curve downward (fitting was not made). Inset: Hexafluorodiethyl ether (1 mM) markedly reduced the current evoked by 30 μM GABA. All data points are normalized to the current evoked by 3 μM GABA (asterisk). (B) With the $\alpha 3\beta 2\gamma 2s$ subtype, 100 μM (open squares) and 1 mM (closed circles) of hexafluorodiethyl ether shifted the curve to the right, with K_D being changed from 13 to 26 and 42 μM by 100 μM and 1 mM of the convulsant, respectively. The Hill coefficient of 1.1 was not altered. Inset: Hexafluorodiethyl ether (1 mM) reduced the current evoked by 30 μM GABA. The peak currents are normalized to that evoked by 10 μM GABA (asterisk). Each data point and vertical bar represent the mean ± S.E.M. of five to nine experiments.

the present condition. The suppression in $\alpha 3\beta 2$ combination (Fig. 3A) and the augmentation in $\alpha 3\beta 2\gamma 2s$ construct (Fig. 3B) were voltage-independent.





3.3. Effects of hexafluorodiethyl ether on the both subtypes

A volatile convulsant, hexafluorodiethyl ether, dose-dependently suppressed the GABA-induced current of the both subunit combinations (Fig. 4A and B). The peak current was decreased but the decay phase in the presence of GABA was not accelerated. Fig. 4C depicts the concentration–effect curve of hexafluorodiethyl ether. Greater suppression was observed with the $\alpha 3\beta 2$ combination.

Effects of hexafluorodiethyl ether on the concentration–response curve of GABA are illustrated in Fig. 5. In the $\alpha 3\beta 2$ combination, a low concentration of the convulsant (100 μM) shifted the curve to the right, changing the K_D from 6.7 to 26 μM and the Hill coefficient from 0.92 to 1.0. A higher concentration of the agent (1 mM) seems to shift the curve downward. The curve obtained using the $\alpha 3\beta 2\gamma 2s$ receptor construct was shifted to the right by the both concentration of the convulsant (Fig. 5B). Hexafluorodiethyl ether did not change the reversal potential for the GABA response and the depressant effect was voltage-independent for the both receptor subunit constructs (Fig. 6A and B).

4. Discussion

4.1. Isoflurane increases the GABA response of $\alpha 3\beta 2\gamma 2s$ subunit combination

The *Sf*9 cell–bacurovirus expression system has been used successfully in electrophysiological studies of $GABA_A$ receptor–ionophore complex (Birnir et al., 1995, 1997; Nabekura et al., 1998). Homomeric isomers of $\alpha 1$, $\alpha 3$ or $\beta 2$ subunit did not assemble functional $GABA_A$ receptor–ionophore complex. In heteromeric $\alpha 3\beta 2$ and $\alpha 3\beta 2\gamma 2s$ $GABA_A$ receptor subunit combinations, GABA evoked a current which reversed its polarity near the equilibrium potential for chloride ions (Figs. 3 and 6), indicating that chloride ions were the charge carrier, as were reported with endogenous $GABA_A$ receptor–ionophore complex. Birnir et al. (1995, 1997) used the extracellular solution of pH 6.2 (which, they described, is physiological for the insect cells). In the present study, the

Fig. 6. Effects of hexafluorodiethyl ether on the current–voltage relationship of the GABA-induced current. (A) In $\alpha 3\beta 2$ $GABA_A$ receptor subunit combination, 30 μM GABA induced a current which reversed its polarity at -10.7 mV (control; open circles). Hexafluorodiethyl ether reduced the current voltage-independently without changing the reversal potential (closed circles; -9.2 mV). (B) With $\alpha 3\beta 2\gamma 2s$ subtype, 10 μM GABA evoked a current which reversed its polarity at -11.0 mV (open circles). Hexafluorodiethyl ether suppressed the current also voltage-independently (closed circles). The reversal potential was not altered either (-11.0 mV). Peak currents are normalized to that evoked at -40 mV in the control condition (asterisk). Each point and vertical bar indicated the mean $\pm$ S.E.M. of four to five experiments.

amplitude of the GABA response may be larger than in the physiological condition, since it was reported that higher extracellular proton concentration decreased the GABA response of the rat dorsal root ganglion cells (Zhai et al., 1998).

It has been reported that volatile anesthetics increased the GABA-induced chloride current at clinical concentrations in dissociated neurons of mammalian CNS (Nakahiro et al., 1989; Wakamori et al., 1991). Using recombinant $GABA_A$ receptors of a variety of subunit compositions expressed in various host cells, investigators have described similar augmentation of the current by general anesthetics. Harrison et al. (1993) showed that isoflurane increased the current of human recombinant $GABA_A$ receptors composed of $\alpha1\beta1$, $\alpha2\beta1$, $\alpha1\beta1\gamma2s$ or $\alpha2\beta1\gamma2s$ subunits, which were expressed in human embryonic kidney (HEK) 293 cells. Enflurane was reported to increase the GABA response of the $\alpha1\beta1$ and $\alpha1\beta1\gamma2s$ subunit constructs expressed in *Xenopus* oocytes (Lin et al., 1993). Alcohols also enhanced the GABA response of both $\alpha1\beta1$ and $\alpha1\beta1\gamma2s$ subunit constructs expressed in *Xenopus* oocytes (Mihic et al., 1994b). In the present study using *Sf*9 cell–bacurovirus system, isoflurane at clinical concentrations increased the current of $\alpha1\beta2\gamma2s$ and $\alpha3\beta2\gamma2s$ subunit combinations. The concentration–response curve of GABA was shifted to the left, indicating that the affinity of the agonist to the receptor was increased (Fig. 2B), as has been shown with native GABA receptors of central neurons (Wakamori et al., 1991). The enhancement was voltage-independent, reflecting that the anesthetic action was not affected by the electric field across the *Sf*9 cell membrane (Fig. 3). Higher concentration of isoflurane facilitated the current decay in the continuous presence of GABA. A surge current was generated following simultaneous removal of GABA and isoflurane (Fig. 1). The surge current waned very slowly (arrow in Fig. 1Dc) compared with the deactivation of the GABA-evoked current (asterisk in Fig. 1Da; see also Ikemoto et al., 1998). These findings are similar to those found in dissociated neurons.

4.2. Suppression by isoflurane of the GABA response of the $\alpha3\beta2$ subunit combination

Clinical concentrations of isoflurane dose-dependently suppressed the GABA-gated current of the $\alpha3\beta2$ subunit combination (Fig. 1). The concentration–response curve for GABA was shifted downward, suggesting a non-competitive inhibition of the receptor–ionophore complex (Fig. 2A). These findings suggest that the $\gamma2s$ subunit determines whether volatile anesthetics enhance or suppress the $GABA_A$ response in the *Sf*9 cell–bacurovirus expression system. Namely, the actions of volatile anesthetics may be affected by the expression system. The host cell-dependence of the function of a recombinant protein was pointed out by Wang et al. (1998), who described that the sodium channels expressed in HEK 293 cell membrane differed in the inactivation kinetics and the potency of local anesthetics from those expressed in the *Xenopus* oocyte membrane. Lewis et al. (1997) examined the channel properties of a class of rat recombinant nicotinic acetylcholine receptor ($\alpha3$ and $\beta4$ subunits composition) using two host cell types, mouse fibroblast L929 cells and *Xenopus* oocytes, and showed that the single channel conductance and the burst duration differed in the two cell types. Since the $GABA_A$ receptor belongs to the same receptor superfamily as the nicotinic acetylcholine receptor, similar dependence on the host cell type may have taken place. In other words, the insect cells may yield difference in assembly of the subunits, stoichiometry of construction and/or posttranslational modification of the subunits, resulting in functional diversity of the proteins. It remains possible that other types of subunits, such as $\alpha2$, $\beta1$, etc., are involved in the differential effect, although no data are available on such subunits with the present expression system.

4.3. Hexafluorodiethyl ether suppresses the GABA-induced current in $\alpha3\beta2$ and $\alpha3\beta2\gamma2s$ subunit combinations

Hexafluorodiethyl ether decreased the GABA responses of the both receptor subtypes (Fig. 4). A low concentration of the convulsant (0.1 mM) shifted the GABA concentration–response curve of $\alpha3\beta2$ subunit combination to the right, although a higher concentration shifted the curve downward (Fig. 5A). With $\alpha3\beta2\gamma2s$ subunit construct, a rightward shift of the concentration–response curve is apparent (Fig. 5B). These findings suggest a competitive inhibition of the current by the convulsant. The suppression was voltage-independent, reflecting no apparent effects of membrane electrical field on the binding of the convulsant (Fig. 6). These results are consistent with the actions of the agent on the native GABA receptors of rat central neurons (Wakamori et al., 1991).

4.4. Differential effects of the volatile agents on $\alpha3\beta2\gamma2s$ and $\alpha3\beta2$ subunit combinations

In bacurovirus–*Sf*9 cell expression system, recombinant human $GABA_A$ receptors of $\alpha3\beta2\gamma2s$ subunit composition may possess three classes of binding sites for general anesthetics, with high and low affinities (Fig. 7). Binding of the anesthetics to a high affinity site may allosterically decrease the apparent K_D value of GABA, resulting in the leftward shift of the GABA concentration–response curve. Binding to a low affinity site may physically block the channel resulting in facilitation of the decay of the current in the presence of GABA (GB^* in Fig. 7; as suggested for muscle type nicotinic acetylcholine receptor by Dilger et al., 1995). Binding to a second low affinity site may allosterically induce a new non-conducting conformation ($GR^*\#$ in Fig. 7). Cessation of the open channel blockade following instantaneous washout of both GABA and the anesthetic unmasks the non-desensitized portion of the

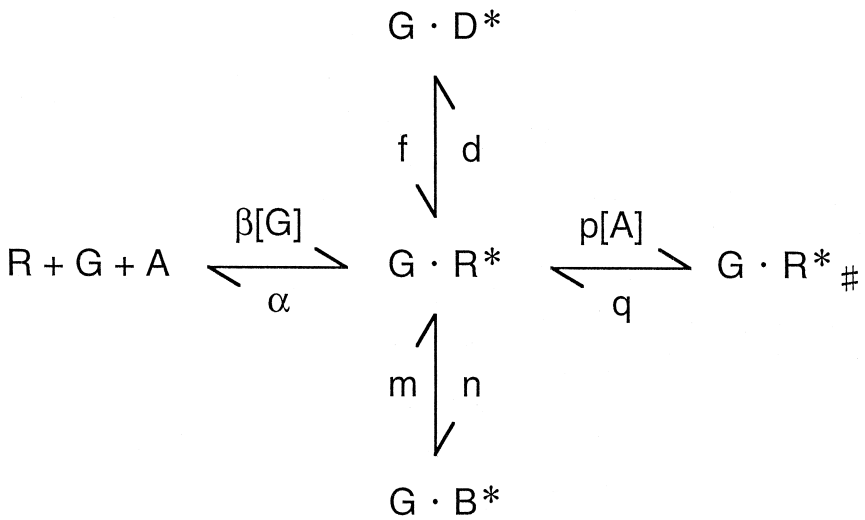


Fig. 7. A hypothetical sequential model for anesthetic action on the α3β2γ2s subtype expressed in *Sf*9 cells. R denotes the receptor in the activatable state; G, GABA; A, anesthetic; GR*, conducting state bound with GABA and with anesthetic at the high affinity site; GD*, desensitized state bound with anesthetic at the high affinity site; GB*, blocked state by binding of anesthetic at a low affinity site; GR*#, a non-conducting state bound with anesthetic at the both low affinity sites. α, β, d, f, n, m, p and q are rate constants. [G] is the GABA concentration and [A] the concentration of anesthetic. Following washout of both GABA and anesthetic, the blocked state GB* enters a conducting state GR* (and/or GR without anesthetic at the high affinity site), rendering a rapid onset of the surge current, whose peak depends on the degree of desensitization. The conducting state(s) will be provided from GR*# and decline slowly according to the rate constant q, since α is much larger and f is much smaller than q. Some intermediate states are omitted for simplicity (see Ikemoto et al., 1988).

current, resulting in rapid appearance of the surge current, which declines slowly according to the rate constant, q, since the recovery from desensitization is slow (f is small) and the deactivation rate constant (α) is large. Hexafluorodiethyl ether may have distinct single class of binding site which reduces the affinity of GABA for the receptor to suppress the GABA-evoked current.

The α3β2 subtype may have only the low affinity binding sites for the anesthetics, lacking the high affinity site to enhance the current, due to absence of the γ subunit. Channel blocking action may become evident at rather lower concentrations of isoflurane, which results in facilitation of the decay phase in the presence of GABA, and a higher concentration generates the surge current reflecting the formation of the GR*# state. The affinity of hexafluorodiethyl ether for its binding site may be greater in the absence of the γ subunit.

In conclusion, highly lipid-soluble volatile agents affected two subtypes of human recombinant $GABA_A$ receptors differentially at clinical concentrations, indicating that these agents interact directly with the receptor–ionophore proteins but not through a perturbation of the lipid environment of the cell membrane.

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