Anesthetic and Nonanesthetic Halogenated Volatile Compounds Have Dissimilar Activities on Nicotinic Acetylcholine Receptor Desensitization Kinetics

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Background: The Meyer-Overton rule predicts that an anesthetic's potency will correlate with its oil solubility. A group of halogenated volatile compounds that disobey this rule has been characterized. These compounds do not induce anesthesia in rats at partial pressures exceeding those predicted by the Meyer-Overton rule to be anesthetic. The observation that potentiation of GABA\(_A\) receptor responses by anesthetic and nonanesthetic halogenated volatile compounds correlates with their abilities to induce general anesthesia suggests that this receptor is involved in the mechanism of general anesthesia. However, the GABA\(_A\) receptor is only one member of a superfamily of structurally similar ligand-gated ion channels. This study compares the actions of both anesthetic and nonanesthetic halogenated volatile compounds on another member of this superfamily of receptors, the nicotinic acetylcholine receptor (nACChOR).

Methods: The actions of both anesthetic and nonanesthetic compounds on desensitization kinetics were characterized from the time-dependent binding of the fluorescent acetylcholine analogue, Dns-C\(_4\)Cho, to the nACChOR.

Results: At concentrations predicted by the Meyer-Overton rule to be equianesthetic, the anesthetics isoflurane and enflurane were significantly more effective than the nonanesthetics 1,2-dichlorobenzofuranocyclobutane and 2,3-dichloroacetofluorobutane in enhancing the fraction of receptors preexisting in the slow desensitized state and increasing the apparent rate of agonist-induced fast and slow desensitization.

Conclusions: The potencies with which anesthetic and nonanesthetic compounds enhance desensitization kinetics in the nACChOR parallel their in vivo anesthetic potencies. These results support the use of desensitization of the nACChOR as a mechanistic model for studies of general anesthesia and suggest that an insensitivity to nonanesthetic compounds may be a feature common to members of the superfamily of ligand-gated ion channels. (Key words: Anesthetics, volatile: enflurane; isoflurane. Nicotinic acetylcholine receptors: desensitization. Theories: anesthetic action.)

ALTHOUGH general anesthesia may be induced by a wide variety of structurally dissimilar compounds, the potencies with which general anesthetics act correlate remarkably well with their oil solubilities (the Meyer-Overton rule).1 This suggests that general anesthetics act by binding to hydrophobic components of neuronal membranes. One interpretation of the Meyer-Overton rule is that anesthetics alter critical interactions between neuronal proteins and their lipid environment. This theory has been supported by studies demonstrating that the potencies with which anesthetics disorder lipid bilayers correlate with their potencies as general anesthetics and that both can be reversed by applying pressure.2-5 On the other hand, the Meyer-Overton rule could reflect direct anesthetic binding to hydrophobic regions on proteins; general anesthetics inhibit the enzymatic activity of the (lipid-free) firefly luciferase protein in proportion to their oil solubilities and anesthetic potencies.6,7

The Meyer-Overton rule is not without exceptions. It has been demonstrated that several halogenated volatile compounds are unable to induce anesthesia in rats even at partial pressures that exceed the minimum alveolar concentration (MAC) values predicted by their oil solubilities.8 In addition, these compounds do not reduce the MAC of coadministered desflurane. Such compounds offer new pharmacologic tools for testing anesthetic theories.9 For example, they may be used to identify relevant anesthetic sites, because the pharmacology of anesthetic and nonanesthetic compounds on relevant sites are predicted to reflect their anesthetic potencies in vivo.10 In addition, these compounds may provide clues to defining the molecular features that

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bestow anesthetic properties to many hydrophobic compounds.

The nicotinic acetylcholine receptor (nAcChoR) is the best characterized member of the superfamily of ligand-gated ion channels. Members of this superfamily are sensitive targets of general anesthetics and frequently are used as protein models for the study of general anesthesia. Other members of this superfamily include the GABA_A, glycine, and glutamate receptors. However, because the nAcChoR can be purified in large quantities at high specific activity, it has become far better characterized than any other member. Consequently, detailed kinetic models have been developed and tested that provide a framework for studying the effects of anesthetics on nAcChoR conformational transitions. Pharmacologic studies indicate that general anesthetics stabilize the slow desensitized conformational state of the nAcChoR, an inactive state characterized by a high affinity for agonist. In addition, general anesthetics enhance the apparent rates at which low affinity receptors are converted by agonist to higher affinity states (agonist-induced fast and slow desensitization).

In this study, I have tested the relevance of nAcChoR desensitization as a mechanistic model for general anesthesia by comparing the potencies with which anesthetic and nonanesthetic halogenated volatile compounds enhance nAcChoR desensitization kinetics. Because previous work has determined that anesthetic and nonanesthetic halogenated volatile compounds also differ in their abilities to potentiate GABA_A receptor currents, this study also tests the hypothesis that an insensitivity to nonanesthetic compounds is a general characteristic of members of the superfamily of ligand-gated ion channels.

Materials and Methods

*Torpedo nobiliana* was obtained from Biofish Associates (Georgetown, MA). Diisopropylfluorophosphate and (dansylaminoethyl) trimethylammonium perchlorate were purchased from Sigma Chemicals (St. Louis, MO). The fluorescent agonist, [1-(5-dimethylamino naphthalene)sulphonamido] n-hexanoic acid b-(N-trimethylammonium bromide) ethyl ester (Dans-C_6-Cho), was synthesized according to the procedure of Waksman et al. Isoflurane and enfurane were purchased from Anaquest (Murray Hill, NJ). The nonanesthetics 1,2-dichloroethanfluorocyclobutane and 2,3-dichloro-octafluorobutane were purchased from PCR (Gainesville, FL). Gas chromatography was performed on a Hewlett Packard 5890 gas chromatograph equipped with a J and W (Folsom, CA) DB-WAX 122-7033 column.

**Preparation and Characterization of nAcChoR Membranes**

Membrane fragments were prepared from the electric organs of *Torpedo nobiliana* as previously described and approved by the Massachusetts General Hospital Animal Care and Use Committee. Membranes fragments were stored in Torpedo physiologic saline (250 mm NaCl, 5 mm KCl, 3 mm CaCl, 2 mm MgCl_2, 5 mm NaH_2PO_4, and 0.02% NaNO_3, pH 7.0) at −80°C and used within 48 h of being thawed. The number of agonist binding sites was determined from the competition of (dansylaminoethyl) trimethylammonium perchlorate binding by acetylcholine as described by Neubig and Cohen. Acetylcholinesterase activity was inhibited by exposing membrane fragments to 0.5 mm diisopropylfluorophosphate for 30 min before mixing with Dans-C_6-Cho. Fluorescence intensity measurements were made with an SX.17 stopped-flow spectrofluorimeter (Applied Photophysics, Leatherhead, England) through a 560-nm high pass filter (Omega Optical, Brattleboro, VT).

**Stopped-flow Fluorescence Spectroscopy of nAcChoR Membranes**

Membrane fragments containing nAcChoRs (0.8 μM in agonist binding sites in Torpedo physiologic saline) and the fluorescent agonist Dans-C_6-Cho (4.0 μM in Torpedo physiologic saline) were introduced into two separate scaled 10-ml glass containers attached directly to the gas-tight mixing syringes of the spectrofluorimeter through a Teflon valve. A gas inlet and outlet line passed into each container. Using an agent-specific or Copper Kettle vaporizer with nitrogen as the carrier gas, the desired anesthetic or nonanesthetic compound was introduced into the containers through the inlet lines. Volatilized anesthetic and nonanesthetic compounds were intermittently bubbled through the nAcChoR and Dans-C_6-Cho solutions for 45 min at flow rates of at least 0.5 ml/min to reach equilibrium at room temperature. Solutions were aerated to facilitate equilibration between the gas and aqueous phases by rapidly drawing the solutions in and out of the stopped-flow spectrofluorimeter's mixing syringes every few minutes. Equilibration using this technique was confirmed in parallel experiments by measuring the partial pres.
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sure of volatile agents in aliquots of the aqueous solution and comparing them to solutions of known concentrations using gas chromatography. Furthermore, evidence of equilibration was provided by the observation that the actions of anesthetics and nonanesthetics on nAcChoR desensitization kinetics plateaued well within the timeframe allowed for equilibration. Anesthetic and nonanesthetic concentrations in the headspace gas were verified by gas chromatography. The temperature of the Copper Kettle vaporizer remained at 23 ± 1.5°C during the equilibration period. The receptor membrane and Dns-C$_6$-Cho solutions were drawn into the two mixing syringes. The contents of the syringes were rapidly mixed, yielding final concentrations of 0.4 μM agonist binding sites and 2.0 μM Dns-C$_6$-Cho. Fluorescence was excited at 290 nm using a 150-W xenon arc lamp and monochromator. The time-dependent change in fluorescence intensity above 560 nm was measured through a high pass filter. Fluorescence intensity was recorded (2,000 points typically over 100 s) on a logarithmic time base. For each experiment, three to six individual shots were signal averaged to improve the signal:noise ratio. Signal-averaged fluorescent traces were transferred to a Macintosh computer and fit to the sum of exponentials using a nonlinear least-squares algorithm.

The fraction of nAcChoR preexisting in the slow desensitized state and the apparent rates of fast and slow desensitization were determined from fluorescence traces as previously described in detail in this journal. Briefly, mixing nAcChoR membranes with Dns-C$_6$-Cho results in a time-dependent increase in fluorescence intensity as nAcChoR binding Dns-C$_6$-Cho. At concentrations of 2.0 μM Dns-C$_6$-Cho and 0.4 μM receptor binding sites, there is an initial rapid fluorescent component corresponding to the rapid, diffusion-limited binding of agonist to preexisting slow desensitized receptors. At this Dns-C$_6$-Cho concentration, this rapid component occurs on the time scale of 10–20 ms (fig. 1A). Because all receptors are converted to the slow desensitized state at equilibrium under the experimental conditions described above, the fraction of all receptors preexisting in the slow desensitized receptors is equal to the amplitude of this fast component divided by the total change in fluorescence intensity observed between mixing and the final equilibrium that is reached 1–2 min later. Dns-C$_6$-Cho also converts preexisting resting state receptors to the high-affinity state (fig. 1B). This process is largely biphasic, corresponding to agonist-induced fast desensitization (100 ms-to-second scale) and slow desensitization (seconds-to-minute scale). The apparent rates of fast and slow desensitization are derived from the rates of these components. The temperature of the stopped-flow spectrofluorimeter was maintained at 20 ± 0.3°C with a thermostatted water jacket. Because there is some variation between Torpedo preparations, the results presented here are from a single preparation. Another preparation yielded similar results. For convenience, the total increase in fluorescence intensity that occurs between mixing the fluorescent agonist with receptor membranes and the final equilibrium has been normalized to 1.0 in all figures. Anesthetic concentrations are expressed as aqueous concentrations and/or multiples of the EC$_{50}$ predicted by the Meyer-Overton rule. The latter permitted the actions of anesthetic and nonanesthetic compounds to be compared at concentrations that are predicted by the Meyer-Overton rule to be equianesthetic. The physical and pharmacologic properties of the anesthetic and nonanesthetic compounds used in this study are given in table 1.

The choice of nonanesthetic compounds used in this study was governed by several factors. First, the vapor pressures and partition coefficients of the compounds tested allowed their delivery at concentrations predicted by the Meyer-Overton rule to be many times their MAC. Second, their vapor pressures are similar to those of clinical anesthetics, permitting the delivery of a wide range of partial pressures via a Copper Kettle vaporizer. Finally, they do not contain bromine, an efficient quencher of fluorescence. Both 1,2-dichlorohexafluorocyclobutane and 2,3-dichlorooctafluorobutane are very hydrophobic (their oil/saline partition coefficients are 3,660 and 13,000, respectively). Therefore, adding nonanesthetics as aqueous solutions can lead to a significant depletion of the aqueous concentration and result in a serious underestimation of their activities. This was avoided by equilibrating receptor (and agonist solutions) with known partial pressures of compounds in a large volume of carrier gas. Because the buffer:gas partition coefficients are very low and the volume of carrier gas is high, depletion is negligible. At equilibrium, the partial pressure of the compound in both the aqueous and gas phases are equal. The reported aqueous concentrations are calculated from the buffer:gas partition coefficients at room temperature (23 ± 1°C).

The MAC of halogenated volatile agents are predicted by the Meyer-Overton rule to be 1.82 atm/
(oil:gas partition coefficient) at 37°C. The predicted EC50 at 20°C (EC50pred) was calculated by converting the predicted MAC (at 37°C) to an aqueous EC50 concentration (at 37°C) and then temperature-correcting to 20°C according to equation 4 in reference 28.

**Estimation of the Buffer-Gas Partition Coefficients for Anesthetic and Nonanesthetic Compounds**

Buffer-gas partition coefficients at room temperature (23 ± 1°C) were estimated generally as described by Ionescu.29 Approximately 10 ml of the compound in air at about 20% of its saturated vapor pressure and 10 ml of buffer was drawn into a weighed and calibrated gas-tight syringe (with stop-cock) and allowed to equilibrate for 2 h at room temperature. To facilitate equilibration, the mixture was vortexed for 60 s every 15 min. The concentration in the gas phase was analyzed using gas chromatography. The air was removed carefully to avoid trapping bubbles within the syringe, and approximately 10 ml of fresh room air was added. After equilibration (another 2 h with vortexing for 60 s every 15 min), the syringe and contents were weighed, and the exact volume of buffer and air was calculated. The concentration in the gas phase was analyzed using gas chromatography. The buffer-gas partition coefficient (λ2) was estimated as:

\[ \lambda = \frac{V_a/V_s}{C_2/(C_1 - C_2)} \]

**Statistical Analysis**

All data points are the average of at least three separate experiments. The error bars on each point indicate the standard deviation between experiments. The significance of anesthetic and nonanesthetic-induced alterations in kinetic parameters was determined with a Student’s two-tailed t-test. A P < 0.05 was considered to be statistically significant.

**Table 1. Physical and Pharmacologic Properties of Anesthetic and Nonanesthetic Compounds**

<table>
<thead>
<tr>
<th>Halogenated Volatile Compound</th>
<th>Predicted MAC* (atm)</th>
<th>MAC† (atm)</th>
<th>Oil-Gas Partition Coefficient‡</th>
<th>Vapor Pressure§ (atm)</th>
<th>Buffer-Gas Partition Coefficient¶</th>
<th>Predicted EC50** (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enflurane</td>
<td>0.109</td>
<td>0.020</td>
<td>96.5</td>
<td>0.28</td>
<td>1.1 ± 0.12</td>
<td>0.48</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>0.020</td>
<td>0.015</td>
<td>90.8</td>
<td>0.38</td>
<td>0.81 ± 0.045</td>
<td>0.35</td>
</tr>
<tr>
<td>1,2-Dichlorohexafluorocyclobutane</td>
<td>0.042</td>
<td>Not anesthetic</td>
<td>43.5</td>
<td>0.24</td>
<td>0.024 ± 0.001</td>
<td>0.016</td>
</tr>
<tr>
<td>2,3-Dichloroocafuoroobutane</td>
<td>0.073</td>
<td>Not anesthetic</td>
<td>25</td>
<td>0.30</td>
<td>0.0037 ± 0.00043</td>
<td>0.0045</td>
</tr>
</tbody>
</table>

* In rats at 37°C. Defined as 1.82 atm/oil:gas partition coefficient.
† Determined in rats.
‡ At 37°C.
§ At 22-25°C.
¶ At 23 ± 1°C (room temperature).
** Aqueous concentration at the predicted MAC for rats (temperature corrected to 20°C using Equation 4 in reference 28).

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Fig. 2. Increase in fluorescence intensity recorded after mixing membranes containing the nAcChoR with Dns-C₆-Cho. Solutions were preequilibrated with either isoflurane (A) or 1,2-dichlorohexafluorocyclobutane (B) at the indicated concentrations. The fraction of receptors preexisting in the slow desensitized state equals the normalized amplitude of the fast (1st) fluorescent component. The EC₅₀ predicted by the Meyer-Overton rule (EC₅₀pred M-O) is 0.35 mM for isoflurane and 16 μM for 1,2-dichlorohexafluorocyclobutane (correcting for temperature using equation 4 in reference 28). Final concentrations were 0.1 μM agonist binding sites and 2.0 μM Dns-C₆-Cho.

Results

Actions of Anesthetic and Nonanesthetic Compounds on the Fraction of Receptors Preexisting in the Slow Desensitized Conformational State

In the absence of anesthetic or nonanesthetic compounds, the fraction of all receptors preexisting in the slow desensitized state was 0.26 ± 0.028. Equilibration with either isoflurane or enflurane resulted in a concentration-dependent increase in the fraction of preexisting slow desensitized nAcChoRs (fig. 2A). This increase was statistically significant at concentrations as low as 0.91 mM enflurane (1.9 × EC₅₀pred M-O) and 1.0 mM isoflurane (2.9 × EC₅₀pred M-O). At the highest concentrations studied, isoflurane (2.5 mM) and enflurane (3.6 mM) increased the fraction of all receptors preexisting in the slow desensitized state to 0.59 ± 0.013 and 0.70 ± 0.056, respectively.

The nonanesthetic 1,2-dichlorohexafluorocyclobutane was less effective in shifting the receptor equilibrium toward the desensitized state than was either anesthetic relative to its oil solubility; a statistically significant increase in the fraction of preexisting slow desensitized receptors was observed only after equilibration with 120 μM (7.5 × EC₅₀pred M-O; fig. 2B). At saturation (240 μM; 15 × EC₅₀pred M-O), 1,2-dichlorohexafluorocyclobutane increased the fraction of preexisting slow desensitized receptors to 0.45 ± 0.024 (fig. 3). The nonanesthetic 2,3-dichloroacetofluorobutane also was less effective than either anesthetic in shifting the receptor equilibrium (fig. 3). A statistically significant increase was observed only after equilibration with a concentration of 46 μM (10 × EC₅₀pred M-O).

Actions of Anesthetic and Nonanesthetic Compounds on the Apparent Rates of Fast and Slow Desensitization

In the absence of anesthetic or nonanesthetic compounds, the apparent rates of fast and slow desensitization were 0.27 ± 0.042 s⁻¹ and 0.043 ± 0.005 s⁻¹, respectively. Enflurane and isoflurane increased

Fig. 3. The effect of anesthetic and nonanesthetic compounds on the fraction of nAcChoRs preexisting in the slow desensitized conformational state. The EC₅₀ predicted by the Meyer-Overton rule (EC₅₀pred M-O) is given in table 1. Final concentrations were 0.4 μM agonist binding sites and 2.0 μM Dns-C₆-Cho.
the apparent rates of fast and slow desensitization in a concentration-dependent manner (fig. 4A). The increases in the apparent rates of fast and slow desensitization became statistically significant at concentrations of 0.45 and 0.50 mM for enfurane and isoflurane, respectively. This corresponds to 0.93 $\times$ EC$_{50}$pred M-O and 1.4 $\times$ EC$_{50}$pred M-O for enfurane and isoflurane, respectively. At high concentrations, enfurane and isoflurane shifted a large fraction of the nAChORs to the slow desensitized state before agonist-induced desensitization. Under these conditions, the fluorescent components corresponding to fast and slow desensitization of the nAChOR are relatively small and difficult to resolve. Therefore, the apparent rates of fast and slow desensitization were determined only at anesthetic concentrations that shifted no more than 60% of the nAChORs to the desensitized state before agonist-induced desensitization. At the highest anesthetic concentrations for which the apparent rates of desensitization could be resolved, enfurane and isoflurane increased the apparent rate of fast desensitization by 10-and 13-fold, respectively, and the apparent rate of slow desensitization by 6- and 4-fold, respectively.

In contrast, the nonanesthetics 1,2-dichlorohexafluorocyclobutane and 2,3-dichlorooctafluorobutane had little effect on the apparent rates of fast and slow desensitization (fig. 4B). Although there was a trend toward higher apparent rates after equilibration with increasing concentrations of these compounds, this increase was so small that, even when nAChORs were equilibrated with nonanesthetic compounds at their maximally attainable concentrations (saturation), the apparent rates increased by no more than 0.7-fold and fall within the normal range previously reported for nAChORs (figs. 5 and 6).$^{20,26}$

Dns-C$_6$-Cho was used at a concentration of 2.0 μM in this study because: (1) it provides a reasonable signal-to-noise ratio; (2) at equilibrium, it converts essentially all of the nAChORs to the slow desensitized state without significant depletion of the free aqueous concentration of Dns-C$_6$-Cho; (3) diffusion-controlled binding of Dns-C$_6$-Cho to preexisting slow desensitized receptors (first fluorescent component) can be followed easily with a stopped-flow spectrofluorimeter having a 1

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Fig. 4. Increase in fluorescence intensity recorded after mixing membranes containing the nAChOR with Dns-C$_6$-Cho. Solutions were preequilibrated with either isoflurane (A) or 1,2-dichlorohexafluorocyclobutane (B) at the indicated concentrations. On the time scale shown, the increase in fluorescence intensity reflects agonist-induced fast and slow desensitization. Final concentrations were 0.4 μM agonist binding sites and 2.0 μM Dns-C$_6$-Cho. The EC$_{50}$ predicted by the Meyer-Overtor rule (EC$_{50}$pred M-O) is 0.35 mM for isoflurane and 16 μM for 1,2-dichlorohexafluorocyclobutane (correcting for temperature using equation 4 in reference 28).

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Fig. 5. The effect of anesthetic and nonanesthetic compounds on the apparent rate of fast desensitization. Rates are plotted on a logarithmic axis. Final concentrations were 0.4 μM agonist binding sites and 2.0 μM Dns-C$_6$-Cho.
ms mixing time; and (4) the kinetic processes of fast and slow desensitization can be resolved on a single experimental fluorescence trace. However, experiments using 0.20 and 20 μM Dns-C₆-Chol demonstrated that nonanesthetics were less effective than anesthetics in enhancing agonist binding to nAChRs (fig. 7). Thus, the lack of efficacy exhibited by nonanesthetics relative to anesthetics is not unique to the particular concentration of agonist chosen for these studies but occurs over a wide range of agonist concentrations.

Discussion

In agreement with previous reports, general anesthetics increase the fraction of nAChR preexisting in the slow desensitized state before agonist-induced desensitization. The concentration of isoflurane required to shift half of all receptors to the slow desensitized state is approximately 2.0 mm, a value similar to that previously reported from this laboratory in which membranes were mixed with aqueous solutions of anesthetics. Although isoflurane is a more potent general anesthetic than enflurane in vivo, it is somewhat less potent than enflurane in increasing the fraction of receptors in the slow desensitized state. In addition, the concentration of anesthetic required to significantly increase the fraction of slow desensitized receptors exceeds that required to induce general anesthesia. These findings may be considered limitations of this mechanistic model of anesthetic action.

The nonanesthetic 1,2-dichlorohexafluorocyclobutane increased the fraction of receptors preexisting in the slow desensitized state, although only at concentrations of 120 μM (7.5 × EC₅₀pred M-O) or greater. Even a saturated solution of 1,2-dichlorohexafluorocyclobutane (which is 15 × the EC₅₀ predicted by the Meyer-Overton rule) shifted fewer than half of all receptors to the desensitized state. In general, the multiple of EC₅₀pred M-O required to induce a similar increase in the fraction of nAChR preexisting in the slow desensitized state was three- to fourfold greater for 1,2-dichlorohexafluorocyclobutane than for enflurane or isoflurane. Similarly, 2,3-dichlorooctfluorobutane was relatively less potent in increasing the fraction of slow desensitized nAChRs than predicted by the Meyer-Overton rule.
In addition to increasing the fraction of preexisting slow desensitized receptors, general anesthetics increase the apparent rates of agonist-induced fast and slow desensitization. In contrast to their effect on the fraction of preexisting slow desensitized receptors, anesthetics increase the apparent rates of agonist-induced fast and slow desensitization at clinically relevant anesthetic concentrations. The relationship between the potency with which anesthetics induce anesthesia and enhance the apparent rates of agonist-induced fast and slow nAChOR desensitization is quite good when one considers a group of anesthetics representing a wide range of potencies; the anesthetic potencies of methanol, butanol, cyclopentanemethanol, chloroform, and isoflurane span a range of nearly 2,000-fold, but the potencies with which equianesthetic concentrations enhance the apparent rates of fast and slow desensitization differ by no more than 50%. This study extends this observation to nonanesthetic compounds because compounds that are unable to induce anesthesia have little effect on the rates of agonist-induced fast and slow desensitization. Statistically significant increases in the rates of agonist-induced fast and slow desensitization can be detected only at concentrations that approach 10 times that predicted to be anesthetic. In the case of 1,2-dichloroethanehexylcyclobutanate, this is a saturated aqueous solution at 20°C. In contrast, the in vivo anesthetic activities of these compounds have been characterized at partial pressures that are only about twice their predicted MAC. Higher partial pressures were lethal. In an attempt to detect “partial” anesthetic effects, Koblin et al. measured the MAC of desflurane in the presence of nonanesthetic agents. Partial anesthetics are predicted to lower the MAC of coadministered desflurane. The nonanesthetic compounds 1,2-dichloroethanehexylcyclobutanate and 2,3-dichlorooctylfluorobutane failed to lower desflurane’s MAC. However, an important assumption of this approach is that anesthetic effects are additive. This assumption may not be valid for halogenated volatile compounds such as these, which produce excitatory behavior. Thus, the ability of near saturated aqueous solutions of nonanesthetics to slightly enhance agonist-induced fast and slow desensitization may not represent a limitation of this model.

The data in this study do not identify the specific kinetic steps leading to agonist-induced desensitization perturbed by halogenated volatile anesthetics. One possibility is that anesthetics increase the affinity of agonist for the nAChOR. The observation that enfurane’s effect on Dns-Cα-

Cho binding kinetics is relatively modest at a high agonist concentration (20 μM) is consistent with such a mechanism. Further evidence that volatile halogenated anesthetics increase agonist affinity has been provided by electrophysiologic studies using nAcChoRs expressed by the clonal BC3H-1 cell line. A previous study comparing the abilities of anesthetics and nonanesthetics to enhance GABA-induced chloride currents in GABA target receptors reported that anesthetics significantly potentiated GABA currents, and nonanesthetics did not. This was interpreted as evidence that the GABA receptor was not only a relevant anesthetic target but perhaps the neuronal target responsible for general anesthesia. When considered with the current study, it appears that an insensitivity to nonanesthetic halogenated volatile compounds may be a more general characteristic of members of the superfamily of ligand-gated ion channels. The ability of both the nAChOR and the GABA receptor to distinguish between anesthetic and nonanesthetic halogenated volatile compounds suggests that the anesthetic binding site in these two membrane protein systems may be similar. The considerable degree of amino acid sequence homology between the hydrophobic membrane spanning regions of these two receptors is generally consistent with a site of action in this region. However, studies using other members of this superfamily will be necessary to confirm this hypothesis. In summary, at concentrations predicted to be equianesthetic by the Meyer-Overton rule, nonanesthetic halogenated volatile compounds are significantly less effective than anesthetic compounds at shifting the preexisting nAChOR conformational equilibrium toward the slow desensitized state and enhancing the apparent rates of agonist-induced fast and slow desensitization. Thus, the activities with which halogenated volatile compounds alter nAChOR desensitization kinetics generally parallel their abilities to induce general anesthesia and potentiate GABA currents. These results support the use of the nAChOR as a model for studying anesthetic actions and are consistent with homologous anesthetic sites of action in nAChOR and GABA receptor membranes.

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