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Molecular Properties Important for Inhaled Anesthetic Action on Human 5-HT_{3A} Receptors

Renna J. N. Stevens, BA, Dirk Rüsç, MD, Paul A. Davies, PhD, and Douglas E. Raines, MD
Department of Anesthesia and Critical Care, Massachusetts General Hospital, Boston, Massachusetts

Abstract

Although inhaled anesthetics have diverse effects on 5-hydroxytryptamine type 3 (5-HT_{3A}) receptors, the mechanism accounting for this diversity is not understood. Studies have shown that modulation of 5-HT_{3A} receptor currents by n-alcohols depends on molecular volume, suggesting that steric interactions between n-alcohols and their binding sites define their action on this receptor. Electrostatic interactions also play an important role in anesthetic action on other ligand-gated receptors. We aimed to determine the contribution of molecular volume and electrostatics in defining volatile anesthetic actions on 5-HT_{3A} receptors. Human 5-HT_{3A} receptors were expressed in, and recorded from, *Xenopus* oocytes using the two-electrode voltage-clamp technique. The effects of a range of volatile anesthetics, n-alcohols, and nonhalogenated alkanes on submaximal serotonin-evoked peak currents, and full serotonin concentration-response curves were defined. Volatile anesthetics and n-alcohols, but not alkanes, smaller than 0.120 nm³ enhanced submaximal serotonin-evoked peak currents whereas all larger agents reduced currents. Most compounds tested inhibited maximal serotonin-evoked peak currents to varying degrees. However, only agents smaller than 0.120 nm³ shifted the 5-HT_{3A} receptor's serotonin concentration-response curve to the left, whereas larger anesthetics shifted them to the right. Modulation of human 5-HT_{3A}-mediated currents by volatile anesthetics exhibits a dependence on molecular volume consistent with the n-alcohols, suggesting that both classes of agents may enhance 5-HT_{3A} receptor function via the same mechanism. Furthermore, the enhancing but not inhibiting effects of anesthetic compounds on 5-HT_{3A} receptor currents are modulated by electrostatic interactions.

5-hydroxytryptamine type 3 (5-HT₃) receptors are members of an anesthetic-sensitive superfamily of pentameric Cys-loop ligand-gated ion channels that also includes nicotinic acetylcholine (nACh), γ -aminobutyric acid type A (GABA_A), and glycine receptors (1). Mammalian 5-HT₃ receptors are found in a range of nervous tissues, including regions involved in pain and vomit reflex circuitry, such as the area postrema and nucleus tractus solitarius of the brainstem, consistent with a role for 5-HT₃ receptors in emesis. Although there have been fewer studies to define anesthetic action on 5-HT₃ receptors than on any

Address correspondence and reprint requests to: Douglas E. Raines, MD, Department of Anesthesia and Critical Care, Massachusetts General Hospital, 55 Fruit Street, Clinics Building 3, Boston, MA 02114. draines@partners.org.

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other member of this superfamily, inhaled anesthetics and n-alcohols have significant effects on 5-HT_{3A}-mediated currents even at subclinical concentrations.

One study demonstrated that inhaled anesthetics have diverse effects on 5-HT_{3A} receptors (2). For example, at 1 MAC, halothane substantially enhances currents evoked by a small concentration of agonist, whereas sevoflurane, xenon, and nitrous oxide (N₂O) reduce currents and isoflurane has relatively little effect. Studies of the 5-HT_{3A} receptor suggest that n-alcohol molecular volume is an important determinant of n-alcohol action (3–5). Analogous conclusions have been made in studies of the nACh receptor, where it has been proposed that steric interactions between alcohols and their protein binding sites critically modulate n-alcohol binding and action (6,7). In addition, it is thought that electrostatic interactions, such as hydrogen bonding or dipolar interactions, modulate anesthetic potentiation of other ligand-gated channels such as the GABA and nACh receptors (8,9).

The relative contributions of different biochemical and molecular characteristics in determining inhaled anesthetic modulation of 5-HT_{3A} receptors have not been defined. We hypothesized that both molecular volume and electrostatic interactions are important determinants of inhaled anesthetic action on 5-HT_{3A} receptors. To test this hypothesis, we studied the effects of 14 inhaled anesthetics and n-alcohols representing an array of molecular volumes and electrostatic properties on a range of serotonin-evoked currents.

Methods

Adult female *Xenopus laevis* frogs (Xenopus One, Ann Arbor, MI) underwent surgery for oocyte removal as previously described (10). All procedures were approved by the Massachusetts General Hospital Animal Care Committee.

cDNAs encoding the 5-HT_{3A} subunit were generously provided by E. Kirkness (TIGR, Rockville, MD) and transcribed into messenger RNA using the mMESSAGE mMACHINE High Yield Capped RNA Transcription Kit (Ambion, Inc., Austin, TX). Defolliculated stage V and VI oocytes were injected with 25–50 nL of cRNA and incubated at 18°C in ND-96 solution (containing in mM: NaCl 96, KCl 2, HEPES 10, CaCl₂ 1.8, MgCl₂ 1.0, 5 U/mL penicillin and 5 µg/mL streptomycin, pH adjusted to 7.5 with NaOH).

Cells were recorded at room temperature between 18 h and 8 days postinjection using the whole oocyte two-electrode voltage clamp technique. Oocytes were voltage-clamped at –50 mV using a GeneClamp 500B amplifier (Axon Instruments, Union City, CA). Glass pipettes (A-M Systems Inc., Carlsborg, WA) were filled with 3M KCl (resistance <5 MΩ). During each experiment, oocytes were constantly superfused at a rate of 5 mL/min with buffer (containing in mM: NaCl 96, KCl 2, HEPES 10, CaCl₂ 1, MgCl₂ 0.8, pH adjusted to 7.5 with NaOH) in a 0.04 mL recording chamber using a closed syringe superfusion system. Current responses were recorded using Clampex V.8 software (Axon Instruments), filtered at 1 kHz, and sampled at 33.3 Hz on a personal computer for analysis. Data were analyzed using IgorPro V.3 software (Wavemetrics, Inc., Lake Oswego, OR).

A control peak current response was obtained by superfusing the oocyte for 60 s with buffer containing 1 µM serotonin, an agonist concentration that evokes approximately 10% of the

maximal peak current evoked by large serotonin concentrations (commonly referred to as an EC₁₀ concentration). After a 3-min recovery period, the effect of an anesthetic or n-alcohol was determined by superfusing the oocyte with buffer containing the desired agent for 30 s, which was then rapidly switched to buffer containing the agent plus 1 μ M serotonin for 60 s. After another 3-min recovery period, the control peak current response was measured again to ensure reversibility. For analysis, the control current was taken as the average of the two control measurements. The molecular volumes of all agents were determined using MacSpartan Pro V1.01 (Wavefunction Inc., Irvine, CA) on an Apple MacIntosh G4 computer. Geometry optimization was performed using *ab initio* molecular orbital calculations (Hartree-Fock, 3–21G basis set).

The oocyte was first superfused for 15 s with buffer containing 100 μ M serotonin, a concentration that evokes the maximal peak current response. After a 5-min recovery period, the control peak current response was obtained by superfusing the oocyte with buffer containing serotonin (0.5–30 μ M; 15–60 s exposure time, depending on concentration). After another recovery period (3–5 min, depending on the serotonin concentration), the maximal peak current response was again determined. After a 5-min recovery period, the effect of an anesthetic or n-alcohol was determined by preapplying the oocyte with buffer containing the desired agent for 30 s and then rapidly switching to buffer containing the agent and serotonin. After another 3–5 min recovery period, the maximal current response was measured again to ensure reversibility.

Serotonin (5-Hydroxytryptamine), tricaine methanesulfate (aminobenzoic acid ethyl ester), collagenase IA, butane, cyclopropane, and hexanol were purchased from Sigma Chemicals (St. Louis, MO). Propanol, pentanol, heptanol, and octanol were purchased from Aldrich Chemical (Milwaukee, WI), and butanol was from Fluka Chemika (Buchs, Switzerland). Both desflurane and isoflurane were from Baxter Healthcare (Deerfield, IL). Sevoflurane was purchased from Abbott Laboratories (Chicago, IL), halothane was from Ayerst Laboratories (New York, NY), and enflurane was purchased from Anaquest, Inc. (Liberty Corner, NJ). The anesthetizing concentrations of volatile anesthetics were defined as the aqueous concentrations corresponding to 1 MAC calculated using the aqueous:gas partition coefficient at 37°. MAC for all volatile anesthetics except chloroform was for humans (11–15). MAC for chloroform was taken as 0.5% atm, the average value reported by 2 groups studying mice (11,16). The MAC for cyclopropane was for humans (15). For butane, MAC was approximated as the EC₅₀ value reported by Firestone et al. (15). The anesthetizing concentrations of n-alcohols were defined as the aqueous concentrations that cause a loss of righting reflex in tadpoles (17).

All currents (obtained in the absence or presence of anesthetic or n-alcohol) were normalized to the peak current evoked by 100 μ M serotonin. Normalized data were plotted as mean $\pm$ SD. The serotonin concentration-response curves were fitted with a Hill equation in the form:

$$I = I_{\max} / [1 + (EC_{50} / [\text{serotonin}])^n]$$

where I is the peak current evoked by serotonin, $I_{\max}$ is the maximum current evoked by large serotonin concentrations, EC_{50} is the concentration of serotonin that elicits 50% of the maximal response, and n is the Hill coefficient.

For observations of anesthetic and n-alcohol modulation of submaximal (EC_{10}) serotonin-evoked currents, control responses were compared to test ones using a paired Student's t -test and statistical significance was set at $P < 0.05$.

Results

Figure 1 shows representative current traces demonstrating that at 2 times their anesthetizing concentrations, 2 relatively small agents (chloroform and butanol) significantly enhanced currents evoked by $1 \mu\text{M}$ serotonin (EC_{10}) whereas 2 larger ones (sevoflurane and octanol) significantly reduced currents ($P < 0.05$). The effects of a range of chloroform, sevoflurane, butanol, and octanol concentrations on currents evoked by $1 \mu\text{M}$ serotonin are shown in Figure 2. Over the concentration range studied (0.5–2 times their anesthetizing concentrations), chloroform and butanol produced a concentration-dependent increase in sub-maximal agonist-evoked current whereas sevoflurane and octanol produced a concentration-dependent inhibition of current.

We next surveyed the effects of volatile anesthetics and n-alcohols (ranging in size from 0.075 nm^3 to 0.160 nm^3) at 2 times their anesthetizing concentrations on submaximal ($1 \mu\text{M}$, EC_{10}) agonist-evoked currents (Fig. 3). Among the volatile anesthetics, chloroform (1.7 mM), desflurane (1.16 mM), halothane (0.43 mM), enflurane (1.17 mM), and isoflurane (0.55 mM) enhanced $1 \mu\text{M}$ serotonin-evoked currents to $315.2\% \pm 50.7\%$, $226.7\% \pm 25.6\%$, $192.2\% \pm 32.4\%$, $185.3\% \pm 25.9\%$, and $132.7\% \pm 29\%$ of control, respectively. However, sevoflurane (0.66 mM) inhibited $1 \mu\text{M}$ serotonin-evoked currents to $35.2\% \pm 11.2\%$ of control. Among the n-alcohols, propanol (146 mM), butanol (21.6 mM), and pentanol (5.8 mM) enhanced control currents to $368.9\% \pm 34.9\%$, $289.9\% \pm 60.6\%$, and $258\% \pm 30.3\%$ of control, respectively. Hexanol (1.14 mM) had virtually no effect on the control serotonin-evoked current ($96.7\% \pm 21.9\%$), whereas heptanol (0.46 mM) and octanol (0.11 mM) reduced serotonin-evoked currents to $36.3\% \pm 9.5\%$ and $29.1\% \pm 6.7\%$ of control respectively. A plot of enhancement of $1 \mu\text{M}$ serotonin-evoked currents by volatile anesthetics and n-alcohols versus agent molecular volume suggests an inverse relationship, as volatile anesthetics and n-alcohols of intermediate size tended to produce less enhancement of control responses as their molecular volumes increased (Fig. 3).

Concentration-response curves for serotonin were generated in the absence or presence of three representative volatile anesthetics (chloroform, isoflurane, and sevoflurane) and 4 representative n-alcohols (butanol, pentanol, hexanol, and octanol) at 2 times their anesthetizing concentrations. Each pair of agonist concentration-response curves (control and test) was obtained using the same set of oocytes to minimize the potentially confounding effects of cell-to-cell variability and, thus, improve sensitivity to changes in serotonin EC_{50} . Chloroform (1.7 mM) shifted the agonist concentration-response curve leftward and reduced the 5-HT_{3A} receptor's EC_{50} for serotonin by 54%, whereas sevoflurane (0.66 mM) increased the agonist EC_{50} by 21% (Fig. 4, Table 1). Both

anesthetics inhibited maximal currents evoked by large concentrations of serotonin, although inhibition by sevoflurane was more than that by chloroform (55% versus 9%, respectively). At 2 times its anesthetizing concentration, isoflurane (0.56 mM), which has a molecular volume intermediate between chloroform and sevoflurane, produced little or no change in either the EC₅₀ for serotonin or the maximum serotonin-evoked current response. However, at 4 times its anesthetizing concentration, isoflurane reduced the EC₅₀ for serotonin by 30% and reduced the maximum serotonin current response by 47% (Table 1).

The effects of butanol and octanol on serotonin concentration-response curves paralleled those of chloroform and sevoflurane, respectively (compare Fig. 4A with 4C, and Fig. 4B with 4D). Butanol (22 mM) reduced the serotonin EC₅₀ by 50%, whereas octanol (0.11 mM) increased the EC₅₀ by 29% (Table 1). Both n-alcohols inhibited maximal currents evoked by high concentrations of serotonin, but inhibition by octanol was more than that by butanol (66% versus 23%, respectively). Although pentanol and hexanol differ by only a single methylene group, they produced very different effects on the 5-HT_{3A} receptor's agonist concentration-response curve. Pentanol (5.8 mM) reduced the EC₅₀ for serotonin by 34% and inhibited the currents evoked by a maximum serotonin concentration by 25%, whereas hexanol (1.14 mM) increased the receptor's agonist EC₅₀ by 26% and inhibited the currents elicited by a maximal agonist concentration by 34%.

We also tested the effects of two nonhalogenated alkane anesthetics on submaximal agonist-induced currents and on full agonist concentration-response curves. Unlike the physically smaller alcohols and volatile inhaled anesthetics, butane and cyclopropane failed to enhance control currents elicited by 1 μ M serotonin at 1/2 $\times$ and 1 $\times$ their anesthetizing concentrations. In fact, both inhibited currents at these concentrations (Figs. 3 and 5). As its concentration increased, cyclopropane continued to inhibit the control response in a concentration-dependent manner, whereas butane mildly enhanced the 1 μ M serotonin current at concentrations larger than 0.3 mM (approximately 2 times its anesthetizing concentration; Fig. 5).

Full agonist concentration-response curves in the absence and presence of cyclopropane and butane are shown in Figure 6A. Cyclopropane (1.62 mM, 2 times its anesthetizing concentration) produced a small rightward-shift in the concentration-response curve (serotonin EC₅₀ increased from 1.66 ± 0.1 μ M to 1.94 ± 0.1 μ M), but did not alter maximal agonist-evoked responses. Conversely, butane produced no change in the serotonin concentration-response curve at 0.16 mM (1 $\times$ its anesthetizing concentration) and only a modest change even at 0.64 mM (reducing the EC₅₀ from 2.17 ± 0.04 μ M to 1.71 ± 0.03 μ M). This concentration of butane did, however, inhibit the maximal agonist response by 26% (Figs. 6B and 6C, respectively).

Discussion

Our studies demonstrate that the actions of volatile anesthetics on agonist-evoked 5-HT_{3A} receptor currents vary with their molecular volumes, a pattern of behavior that closely parallels those of n-alcohols. At 2 times their anesthetizing concentrations, smaller volatile anesthetics and n-alcohols enhanced submaximal serotonin-evoked currents. The magnitude

of this enhancement tended to decrease with increasing molecular volume, and no volatile anesthetic or n-alcohol larger than 0.120 nm^3 produced current enhancement. Indeed, all agents larger than 0.124 nm^3 (sevoflurane, heptanol, and octanol) reduced submaximal serotonin-evoked currents. In general, the n-alcohols produced somewhat more enhancement than the volatile anesthetics, perhaps reflecting the different *in vivo* end-points used to define their anesthetic potencies (loss of righting reflex for n-alcohols versus MAC for volatile anesthetics). All of the alcohols and volatile anesthetics possess inhibitory activity, as they all inhibited maximal agonist-evoked currents, although larger agents produced relatively more inhibition than smaller ones. Despite their inhibitory actions, smaller halogenated volatile anesthetics and n-alcohols enhanced submaximal serotonin-evoked currents because this receptor inhibition is counterbalanced at small agonist concentrations by a simultaneous leftward shift in the serotonin concentration-response curve. In contrast, larger volatile anesthetics and n-alcohols decreased submaximal serotonin-evoked currents because they inhibited currents without producing a leftward shift in the serotonin concentration-response curve. In fact, larger volatile anesthetics and n-alcohols produced a modest rightward shift in serotonin concentration-response curves. The striking similarity between the actions of volatile anesthetics and n-alcohols of similar molecular volumes on serotonin-evoked currents suggests that these two classes of agents may act at the same receptor sites and that steric interactions critically modulate anesthetic and n-alcohol binding.

Dependence of anesthetic or n-alcohol action on molecular volume has also been noted in the other members of this receptor superfamily. In the nACh receptor, the predominant effect of short chain n-alcohols is to shift the agonist concentration-response curve for ion flux leftward, whereas the predominant effect of long chain alcohols is to inhibit maximal agonist-evoked currents (6,18). Competition studies to examine potential interactions between ethanol and octanol indicate that short-chain and long-chain n-alcohols do not compete for a single site on the nACh receptor, implying that the enhancing and inhibiting actions of n-alcohols are mediated by different sites on this receptor (6). Partial agonist studies suggest that ethanol reduces agonist EC_{50} primarily by stabilizing the open channel state, a kinetic mechanism that has also been proposed to account for ethanol's agonist EC_{50} reducing action on 5-HT₃ receptors (19,20). In the GABA_A receptor, n-alcohol potency for enhancing submaximal agonist-evoked currents increases from methanol through decanol, as predicted by the increasing n-alcohol hydrophobicity. However, potency reaches a plateau beyond decanol and disappears at dodecanol (21). Such loss of activity of larger, more hydrophobic members of a homologous series is termed "cutoff," and it is considered to be evidence of discrete binding sites of limited size. In the α_1 glycine receptor, enhancement of submaximal agonist-evoked current by n-alcohols also exhibits a cutoff that may be altered by changing the size of amino acid residues in transmembrane domains that are thought to line the n-alcohol binding cavity (22).

The apparent size of the cavity which modulates anesthetic and n-alcohol enhancement of agonist action on the 5-HT_{3A} receptor is $\sim 0.120 \text{ nm}^3$. This is significantly smaller than the apparent cavity size that modulates anesthetic and n-alcohol enhancement of agonist action on GABA_A and glycine receptors, which has been estimated to be $\sim 0.25\text{--}0.37 \text{ nm}^3$.

However, the 5-HT_{3A} receptor cavity appears to be similar in size to that of the nACh receptor, as alcohols larger than butanol inhibit, rather than enhance, submaximal agonist-evoked currents in the nACh receptor. The similarity in cavity size between the 5-HT_{3A} and nicotinic acetylcholine receptors versus the GABA_A and glycine receptors is not entirely surprising, as the two groups of receptors are functionally distinct (cation-selective versus anion-selective, respectively).

Despite their smaller size, the nonhalogenated alkane anesthetics butane and cyclopropane did not enhance submaximal serotonin 5-HT_{3A}-mediated currents at clinically relevant concentrations but rather inhibited them. These results parallel those observed in other members of the ligand-gated ion channel superfamily. For example, in contrast to n-alcohols and volatile anesthetics, nonhalogenated alkane anesthetics fail to enhance submaximal agonist evoked currents mediated by GABA_A and Torpedo nACh receptors at clinically relevant concentrations (8). Similarly, whereas isoflurane and n-alcohols reduce the apparent agonist dissociation constant of the nACh receptor, the nonhalogenated alkanes do not (8,23,24).

We have previously proposed that nonhalogenated alkanes, such as butane and cyclopropane, are particularly ineffective enhancers of agonist action in the GABA_A and nACh receptors because they are unable to engage in electrostatic interactions that can enhance binding affinity to amino acid residues that line anesthetic binding sites on proteins (8). The same interactions appear to be important for the enhancing actions of inhaled anesthetics on 5-HT_{3A} receptor currents and could explain why neither xenon nor N₂O (whose abilities to engage in electrostatic interactions are relatively weak) enhance 5-HT_{3A} receptor currents evoked by small concentrations of agonist (2). However, butane and cyclopropane (as well as xenon and N₂O) retain the ability to inhibit 5-HT_{3A} receptor-mediated currents, suggesting that electrostatic interactions do not modulate the inhibitory potencies of anesthetics and n-alcohols on 5-HT_{3A} receptor currents.

If these inhaled anesthetics and n-alcohols alter the agonist EC₅₀ of the 5-HT_{3A} receptor by modulating the equilibrium between closed (preopen) and open channel states, then our data imply that smaller and larger agents stabilize and destabilize, respectively, the open channel state relative to the closed state. Within the context of typical kinetic models describing agonist binding to Cys-loop receptors and channel gating, destabilization of the open channel state relative to the closed state will also reduce the current evoked by large concentrations of an effective agonist. This may explain, at least in part, why larger anesthetics produce greater inhibition of maximal serotonin-evoked currents than smaller ones.

In summary, our results demonstrate that modulation of 5-HT_{3A} receptors by volatile anesthetics varies with their molecular volumes. This volume dependence closely parallels that of n-alcohols, suggesting that volatile anesthetics and n-alcohols bind to common receptor sites and that this binding is modulated by steric interactions. Furthermore, our results suggest that electrostatic interactions between anesthetics and their binding sites are critical for the 5-HT_{3A}-enhancing, but not inhibiting, actions of anesthetics on the 5-HT_{3A} receptor.

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References

1. Reeves DC, Lummis SC. The molecular basis of the structure and function of the 5-HT₃ receptor: a model ligand-gated ion channel (review). *Mol Membr Biol.* 2002; 19:11–26. [PubMed: 11989819]
2. Suzuki T, Koyama H, Sugimoto M, et al. The diverse actions of volatile and gaseous anesthetics on human-cloned 5-hydroxytryptamine₃ receptors expressed in *Xenopus* oocytes. *Anesthesiology.* 2002; 96:699–704. [PubMed: 11873047]
3. Jenkins A, Franks NP, Lieb WR. Actions of general anaesthetics on 5-HT₃ receptors in N1E-115 neuroblastoma cells. *Br J Pharmacol.* 1996; 117:1507–15. [PubMed: 8730747]
4. Machu TK, Harris RA. Alcohols and anesthetics enhance the function of 5-hydroxytryptamine₃ receptors expressed in *Xenopus laevis* oocytes. *J Pharmacol Exp Ther.* 1994; 271:898–905. [PubMed: 7965811]
5. Zhou Q, Lovinger DM. Pharmacologic characteristics of potentiation of 5-HT₃ receptors by alcohols and diethyl ether in NCB-20 neuroblastoma cells. *J Pharmacol Exp Ther.* 1996; 278:732–40. [PubMed: 8768725]
6. Wood SC, Forman SA, Miller KW. Short chain and long chain alkanols have different sites of action on nicotinic acetylcholine receptor channels from Torpedo. *Mol Pharmacol.* 1991; 39:332–8. [PubMed: 1706469]
7. Wood SC, Hill WA, Miller KW. Cycloalkanemethanols discriminate between volume- and length-dependent loss of activity of alkanols at the Torpedo nicotinic acetylcholine receptor. *Mol Pharmacol.* 1993; 44:1219–26. [PubMed: 8264559]
8. Raines DE, Claycomb RJ, Scheller M, Forman SA. Nonhalogenated alkane anesthetics fail to potentiate agonist actions on two ligand-gated ion channels. *Anesthesiology.* 2001; 95:470–7. [PubMed: 11506122]
9. Eckenhooff RG, Johansson JS. Molecular interactions between inhaled anesthetics and proteins. *Pharmacol Rev.* 1997; 49:343–67. [PubMed: 9443162]
10. Raines DE, Claycomb RJ, Forman SA. Modulation of GABA(A) receptor function by nonhalogenated alkane anesthetics: the effects on agonist enhancement, direct activation, and inhibition. *Anesth Analg.* 2003; 96:112–8. [PubMed: 12505935]
11. Firestone LL, Sauter JF, Braswell LM, Miller KW. Actions of general anesthetics on acetylcholine receptor-rich membranes from Torpedo californica. *Anesthesiology.* 1986; 64:694–702. [PubMed: 3717633]
12. Strum DP, Eger EI II. Partition coefficients for sevoflurane in human blood, saline, and olive oil. *Anesth Analg.* 1987; 66:654–6. [PubMed: 3605675]
13. Eger EI II, Laster MJ, Gregory GA, et al. Women appear to have the same minimum alveolar concentration as men: a retrospective study. *Anesthesiology.* 2003; 99:1059–61. [PubMed: 14576539]
14. Wadhwa A, Durrani J, Sengupta P, et al. Women have the same desflurane minimum alveolar concentration as men: a prospective study. *Anesthesiology.* 2003; 99:1062–5. [PubMed: 14576540]
15. Firestone, LL.; Miller, JC.; Miller, KW. Table of physical and pharmacological properties of anesthetics, *Molecular and Cellular Mechanisms of Anesthetics.* Roth, SH.; Miller, KW., editors. New York: Plenum Press; 1986. p. 455-70.
16. Deady JE, Koblin DD, Eger EI II, et al. Anesthetic potencies and the unitary theory of narcosis. *Anesth Analg.* 1981; 60:380–4. [PubMed: 7195159]
17. Alifimoff JK, Firestone LL, Miller KW. Anaesthetic potencies of primary alkanols: implications for the molecular dimensions of the anaesthetic site. *Br J Pharmacol.* 1989; 96:9–16. [PubMed: 2784337]
18. Zuo Y, Aistrup GL, Marszalec W, et al. Dual action of n-alcohols on neuronal nicotinic acetylcholine receptors. *Mol Pharmacol.* 2001; 60:700–11. [PubMed: 11562431]

19. Wu G, Tonner PH, Miller KW. Ethanol stabilizes the open channel state of the Torpedo nicotinic acetylcholine receptor. *Mol Pharmacol*. 1994; 45:102–8. [PubMed: 8302268]
20. Lovinger DM, Sung KW, Zhou Q. Ethanol and trichloroethanol alter gating of 5-HT3 receptor-channels in NCB-20 neuroblastoma cells. *Neuropharmacology*. 2000; 39:561–70. [PubMed: 10728877]
21. Nakahiro M, Arakawa O, Nishimura T, Narahashi T. Potentiation of GABA-induced Cl[−] current by a series of n-alcohols disappears at a cutoff point of a longer-chain n-alcohol in rat dorsal root ganglion neurons. *Neurosci Lett*. 1996; 205:127–30. [PubMed: 8907333]
22. Wick MJ, Mihic SJ, Ueno S, et al. Mutations of gamma-aminobutyric acid and glycine receptors change alcohol cutoff: evidence for an alcohol receptor? *Proc Natl Acad Sci U S A*. 1998; 95:6504–9. [PubMed: 9600996]
23. Raines DE, Zachariah VT. Isoflurane increases the apparent agonist affinity of the nicotinic acetylcholine receptor. *Anesthesiology*. 1999; 90:135–46. [PubMed: 9915322]
24. Raines DE, Zachariah VT. The alkyl chain dependence of the effect of normal alcohols on agonist-induced nicotinic acetylcholine receptor desensitization kinetics. *Anesthesiology*. 1999; 91:222–30. [PubMed: 10422948]

Figure 1.

Representative traces showing the effects of chloroform (A), sevoflurane (B), butanol (C), and octanol (D) at 2× their anesthetizing concentrations on current responses evoked by serotonin (1 μM, ~EC₁₀). Chloroform (1.7 mM) substantially enhanced the control current response whereas sevoflurane (0.66 mM) inhibited it. Correspondingly, butanol (21.6 mM) enhanced and octanol (0.11 mM) inhibited the serotonin-evoked response to similar degrees.

Figure 2.

Enhancement or inhibition of current responses evoked by serotonin (1 μ M, $\sim$ EC₁₀) by chloroform and sevoflurane (A) and butanol and octanol (B) plotted against anesthetic concentration. Enhancement of current responses by chloroform and butanol and inhibition by sevoflurane and octanol were concentration dependent. Modulation of serotonin-evoked currents by the n-alcohols closely resembles that of the volatile anesthetics. Data were collected using 3–6 oocytes for each concentration and expressed as mean $\pm$ SD.

Figure 3.

Enhancement or inhibition of current responses evoked by serotonin (1 μ M, $\sim$ EC10) by various n-alcohols and anesthetics (all at 2 $\times$ their anesthetizing concentrations) plotted against agent molecular volume. With the exception of cyclopropane and butane, compounds smaller than hexanol (0.124 nm³) enhanced the serotonin-evoked current response whereas larger compounds inhibited serotonin-evoked currents. Data were collected using 3–6 oocytes for each agent and expressed as mean $\pm$ SD.

Figure 4.

Normalized serotonin concentration-response curves in the absence (closed circles) and presence of chloroform (A), sevoflurane (B), butanol, (C), and octanol (D) all at 2 $\times$ anesthetic concentration. Chloroform (1.7 mM) reduced the serotonin EC_{50} from $2.32 \pm 0.2 \mu\text{M}$ to $1.07 \pm 0.11 \mu\text{M}$ and the response to maximal concentrations of serotonin by 9%. Sevoflurane (0.66 mM) increased the serotonin EC_{50} from $2.94 \pm 0.06 \mu\text{M}$ to $3.74 \pm 0.26 \mu\text{M}$ and reduced the maximum serotonin response by 55%. In analogy with chloroform and sevoflurane, butanol (21.6 mM) reduced the EC_{50} for serotonin from $1.71 \pm 0.07 \mu\text{M}$ to $0.85 \pm 0.11 \mu\text{M}$ and inhibited the maximal serotonin response by 23%, and octanol (0.11 mM) increased the serotonin EC_{50} from $1.95 \pm 0.02 \mu\text{M}$ to $2.52 \pm 0.04 \mu\text{M}$ and reduced the maximal serotonin response by 66%. Data were collected from at least 3 oocytes for each concentration-response experiment, normalized to 100 μM serotonin and expressed as mean $\pm$ SD.

Figure 5.

Enhancement or inhibition of current responses evoked by serotonin ($1 \mu\text{M}$, $\sim\text{EC}_{10}$) by cyclopropane and butane plotted against anesthetic concentration. Inhibition by cyclopropane was concentration-dependent over the range studied ($0.5\text{--}8$ times its anesthetizing concentration, $410 \mu\text{M}\text{--}6.6 \text{ mM}$) with a maximum level of inhibition of $84.8\% \pm 3\%$ at 6.6 mM . Butane, conversely, produced a biphasic effect. At $0.5\times$ and $1\times$ its anesthetizing concentration ($80 \mu\text{M}$ and $160 \mu\text{M}$, respectively) butane reduced control agonist-induced responses, with a maximum inhibition of $28\% \pm 5.5\%$ at $160 \mu\text{M}$. However, at $2\times$ its anesthetizing concentration and larger, butane modestly enhanced control responses, with a maximum enhancement of $42.9\% \pm 21.9\%$ at $4\times$ its anesthetizing concentration, $640 \mu\text{M}$.

Figure 6.

Normalized serotonin concentration-response curves in the absence (closed circles) and presence of 2 $\times$ the anesthetizing concentration of cyclopropane (A), and butane (160 μM , B; 640 μM , C). Cyclopropane increased the 5-HT EC₅₀ from $1.66 \pm 0.1 \mu\text{M}$ to $1.94 \pm 0.1 \mu\text{M}$ but had no effect on the maximal agonist-evoked response. At 160 μM , butane produced no change in the serotonin concentration-response curve. Even at 640 μM , 4 $\times$ its anesthetizing concentration, butane slightly shifted the serotonin concentration-response curve to the left but did not appreciably alter the serotonin EC₅₀ ($2.17 \pm 0.04 \mu\text{M}$ and $1.71 \pm 0.03 \mu\text{M}$ in the absence and presence of butane, respectively). This large concentration of butane did, however, inhibit the maximal serotonin-evoked response by 26%. Data were collected from at least 3 oocytes for each concentration-response experiment, normalized to 100 μM serotonin and expressed as mean $\pm$ SD

Table 1

Serotonin EC₅₀s (μ M) and I_{max}s Derived from Serotonin Concentration-Response Curves Obtained in the Absence (Control) or Presence of Anesthetics and n-Alcohols

	Control	+Anesthetic or n-alcohol	EC ₅₀ Cntrl/EC ₅₀ test
Chloroform			
EC ₅₀	2.32 $\pm$ 0.20	1.07 $\pm$ 0.11	2.2
I _{max}	101 $\pm$ 4.92	91.4 $\pm$ 4.05	
Sevoflurane			
EC ₅₀	2.94 $\pm$ 0.06	3.74 $\pm$ 0.26	0.79
I _{max}	100 $\pm$ 1.06	44.6 $\pm$ 1.35	
Isoflurane			
EC ₅₀ (2 $\times$ MAC)	2.09 $\pm$ 0.15	2.09 $\pm$.04	1
I _{max}	96.9 $\pm$ 0.5	89.5 $\pm$ 1.06	
Isoflurane			
EC ₅₀ (4 $\times$ MAC)	2.45 $\pm$ 0.08	1.72 $\pm$.08	1.4
I _{max}	96.5 $\pm$ 1.83	50.7 $\pm$ 1.3	
Butanol			
EC ₅₀	1.71 $\pm$.07	0.85 $\pm$ 0.11	2
I _{max}	97.6 $\pm$ 2.02	75.6 $\pm$ 4.2	
Octanol			
EC ₅₀	1.95 $\pm$ 0.02	2.52 $\pm$ 0.04	0.77
I _{max}	93.5 $\pm$ 0.49	31.9 $\pm$ 0.25	
Pentanol			
EC ₅₀	1.97 $\pm$ 0.07	1.30 $\pm$ 0.09	1.5
I _{max}	99.7 $\pm$ 1.88	75.0 $\pm$ 2.43	
Hexanol			
EC ₅₀	2.16 $\pm$ 0.12	2.72 $\pm$ 0.13	0.79
I _{max}	97.9 $\pm$ 3.11	64.3 $\pm$ 2.31	

MAC = minimum alveolar concentration; EC₅₀ = serotonin concentration that produces a half-maximal current response; I_{max} = maximal current evoked by high serotonin concentrations (expressed as a percent of control).