

# General Anesthetic-Induced Channel Gating Enhancement of 5-Hydroxytryptamine Type 3 Receptors Depends on Receptor Subunit Composition

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## ABSTRACT

5-Hydroxytryptamine (serotonin) (5-HT) type 3 (5-HT<sub>3</sub>) receptors are members of an anesthetic-sensitive superfamily of Cys-loop ligand-gated ion channels that can be formed as homomeric 5-HT<sub>3A</sub> or heteromeric 5-HT<sub>3AB</sub> receptors. When the efficacious agonist 5-HT is used, the inhaled anesthetics halothane and chloroform (at clinically relevant concentrations) significantly reduce the agonist EC<sub>50</sub> for 5-HT<sub>3A</sub> receptors but not for 5-HT<sub>3AB</sub> receptors. In the present study, we used dopamine (DA), a highly inefficacious agonist for 5-HT<sub>3</sub> receptors, to determine whether the difference in sensitivity between 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors to the potentiating effects of halothane and chloroform is due to differential modulation of agonist affinity, channel gating, or both. Using the two-electrode voltage-clamp technique with 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors expressed in *Xenopus* oocytes, we found that chloro-

form and halothane enhanced currents evoked by receptor-saturating concentrations of DA for both receptor subtypes in a concentration-dependent manner but that the magnitude of enhancement was substantially greater for 5-HT<sub>3A</sub> receptors than for 5-HT<sub>3AB</sub> receptors. Isoflurane induced only a small enhancement of currents evoked by receptor-saturating concentrations of DA for 5-HT<sub>3A</sub> receptors and no enhancement for 5-HT<sub>3AB</sub> receptors. For both receptor subtypes, none of the three test anesthetics significantly altered the agonist EC<sub>50</sub> for DA, implying that these anesthetics do not affect agonist binding affinity. Our results show that chloroform, halothane, and (to a much lesser degree) isoflurane enhance channel gating for 5-HT<sub>3A</sub> receptors and that the incorporation of 5-HT<sub>3B</sub> subunits to produce heteromeric 5-HT<sub>3AB</sub> receptors markedly attenuates the ability of these anesthetics to enhance channel gating.

5-Hydroxytryptamine (serotonin) (5-HT) type 3 (5-HT<sub>3</sub>) receptors are members of an anesthetic-sensitive superfamily of Cys-loop ligand-gated ion channels that includes the nicotinic acetylcholine (nACh), GABA<sub>A</sub>, and glycine receptors. Members of this superfamily share important functional features, including channel gating upon agonist binding and desensitization with prolonged agonist exposure (Reeves and Lummis, 2002). Until fairly recently, only one subunit (5-HT<sub>3A</sub>) of the 5-HT<sub>3</sub> receptor had been cloned and characterized (Maricq et al., 1991), and homomeric 5-HT<sub>3A</sub> receptors

were the only subtype known. However, a second subunit (5-HT<sub>3B</sub>) has been identified and cloned, and although homomeric receptors cannot be formed with 5-HT<sub>3B</sub> subunits, they can be combined with 5-HT<sub>3A</sub> subunits to form heteromeric 5-HT<sub>3AB</sub> receptors (Davies et al., 1999). These heteromeric receptors possess functional properties that are distinct from those of homomeric 5-HT<sub>3A</sub> receptors, and their 5-HT-evoked responses are more similar to those observed in neuronal preparations (Davies et al., 1999; Dubin et al., 1999).

5-HT<sub>3</sub> receptors are thought to play important roles in regulating a variety of organ systems, in particular the cardiovascular and nervous systems. For example, there is evidence that 5-HT<sub>3</sub> receptors in the nucleus tractus solitarius are important modulators of the baroreflex (Comet et al., 2005), carotid chemoreflex (Sevoz et al., 1997), and the Bezold-Jarisch reflex (Pires et al., 1998). Presynaptic 5-HT<sub>3</sub> receptors also modulate GABA release (Koyama et al., 2000, Turner et al., 2004), an effect that may play a role in the mechanism of action of inhaled anesthetics. There is also

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**ABBREVIATIONS:** 5-HT, 5-hydroxytryptamine (serotonin); nACh, nicotinic acetylcholine; DA, dopamine; MAC, minimum alveolar concentration; P4S, piperidine-4-sulfonic acid.

substantial evidence that 5-HT<sub>3</sub> receptors are involved in nociception (Zeitz et al., 2002). In addition, 5-HT<sub>3</sub> receptors are important modulators of emesis, as evidenced by the efficacy of 5-HT<sub>3</sub> receptor antagonists in the treatment of postoperative nausea and vomiting, which has been strongly linked to the use of halogenated volatile anesthetics (Apfel et al., 2002). Thus, modulation of 5-HT<sub>3</sub> receptors may contribute to the mechanism of action as well as some of the undesirable side effects of volatile anesthetics.

At clinically relevant concentrations, physically small, halogenated volatile anesthetics such as halothane and chloroform significantly potentiate submaximal 5-HT<sub>3A</sub> receptor-mediated current responses evoked by 5-HT (Stevens et al., 2005b). However, heteromeric 5-HT<sub>3AB</sub> receptors have been found to be generally much less sensitive to such anesthetic-induced potentiation of current responses than homomeric 5-HT<sub>3A</sub> receptors (Stevens et al., 2005a). Studies using a range of 5-HT concentrations demonstrate that for 5-HT<sub>3A</sub> receptors, the presence of small, halogenated anesthetics significantly reduces the EC<sub>50</sub> for 5-HT without increasing the maximal response to 5-HT (Stevens et al., 2005b). However, this anesthetic-induced reduction in agonist EC<sub>50</sub> is greatly attenuated when 5-HT<sub>3B</sub> subunits are incorporated to produce heteromeric 5-HT<sub>3AB</sub> receptors (Stevens et al., 2005a). The present study sought to define the underlying mechanism accounting for the difference in anesthetic-induced sensitivity to agonist between the two 5-HT<sub>3</sub> receptor subtypes. We used dopamine (DA), a highly inefficacious agonist for 5-HT<sub>3</sub> receptors, to determine whether the difference in sensitivity of 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors to the potentiating effects of small, halogenated anesthetics is due to differential modulation of agonist affinity, channel gating, or both.

## Materials and Methods

**Xenopus Oocyte Expression.** *X. laevis* oocytes were harvested from adult female frogs (Xenopus One, Ann Arbor, MI) and isolated as described previously (Raines et al., 2003). The Massachusetts General Hospital Animal Care Committee approved all animal housing and surgical procedures.

cDNA encoding the 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> subunits (kindly provided by E. Kirkness, The Institute for Genomic Research, Rockville, MD) were transcribed into messenger RNA using the mMACHINE high yield capped RNA transcription kit (Ambion, Austin, TX). After treatment with collagenase IA for 1 h, stage V and VI oocytes were manually defolliculated and injected with 25 to 50 nl of RNA encoding the 5-HT<sub>3A</sub> subunit to express homomeric 5-HT<sub>3A</sub> receptors, or a mixture of RNA encoding the 5-HT<sub>3A</sub> and 5-HT<sub>3B</sub> subunits (in a ratio of 1:2:1 by volume for 5-HT<sub>3A</sub> subunit/5-HT<sub>3B</sub> subunit/water) to express heteromeric 5-HT<sub>3AB</sub> receptors. Oocytes were kept at 18°C in ND-96 incubation solution (containing 96 mM NaCl, 2 mM KCl, 10 mM HEPES, 1.8 mM CaCl<sub>2</sub>, 1.0 mM MgCl<sub>2</sub>, 5 units/ml penicillin, and 5 μg/ml streptomycin, pH adjusted to 7.5 with NaOH) for 3 to 7 days before electrophysiological experimentation.

**Electrophysiological Recordings.** Electrophysiological recordings were performed using the whole-oocyte two-electrode voltage-clamp technique at room temperature (22–24°C). Oocytes were held at a cross-membrane potential of –50 mV using a GeneClamp 500B amplifier (Molecular Devices, Inc., Sunnyvale, CA). Capillary glass electrodes filled with 3 M KCl and possessing open tip resistances less than 5 MΩ were used to impale oocytes. During experimentation, oocytes were continuously superfused at a rate of 5 ml/min with ND-96 buffer (containing 96 mM NaCl, 2 mM KCl, 10 mM HEPES, 1 mM CaCl<sub>2</sub>, 0.8 mM MgCl<sub>2</sub>, pH adjusted to 7.5 with NaOH) in a 0.04-ml recording chamber using a gas-tight closed syringe superfusion system, producing an estimated solution exchange time of 0.5 to

1 s. Control of buffer perfusion was accomplished using a six-channel valve controller (Warner Instruments, Hamden, CT) interfaced with a Digidata 1322A data acquisition system (Molecular Devices, Inc.) and driven by a Dell personal computer (Dell, Round Rock, TX). The perfusion apparatus was constructed with gas-tight glass syringes and Teflon tubing to minimize absorptive and evaporative loss of anesthetic drugs. In parallel experiments, gas chromatographic analysis of solutions entering the oocyte chamber indicated that such loss was less than 15%. Current responses were recorded using Clampex 9.0 software (Molecular Devices, Inc.) and filtered using a Bessel (eight-pole) low-pass filter with a –3-dB cut-off at 1.56 Hz (Clampfit 9.0; Molecular Devices, Inc.) before analysis.

**Preparation of Volatile Anesthetic and DA Solutions.** Volatile anesthetic solutions were prepared by adding an excess of anesthetic agent to a sealed glass bottle containing recording buffer solution and stirring with a Teflon-coated stir bar overnight. The resulting saturated anesthetic solution of known concentration was subsequently diluted with buffer solution using a gas-tight syringe to obtain the final desired anesthetic concentration. The anesthetizing concentrations of halogenated volatile anesthetics were defined as the aqueous concentrations corresponding to 1 MAC in humans, calculated using the aqueous/gas partition coefficient at 37° (Franks and Lieb, 1993).

Because DA oxidizes and precipitates in aqueous solution over time, buffer solutions were deoxygenated by stirring under vacuum for at least 30 min and subsequently put on ice before adding DA. DA-containing solutions were kept on ice in the dark until immediately before experimentation, at which time the solution was brought to room temperature using a water bath. Using these techniques, no visible DA precipitation was observed over the course of experimentation.

**Experiments to Establish 5-HT and DA Concentration-Response Relationships.** To establish 5-HT and DA concentration-response relationships, a control experiment was first performed by perfusing the oocyte for 15 s with buffer solution containing a concentration of 5-HT known to elicit maximal current (100 μM 5-HT for 5-HT<sub>3A</sub> receptors and 300 μM 5-HT for 5-HT<sub>3AB</sub> receptors). After a recovery period of 5 min, the test experiment was performed by perfusing the cell for 15 to 60 s with buffer solution containing either 5-HT or DA at the desired concentration. After another recovery period of 3 to 5 min, a second control experiment was performed using a concentration of 5-HT that elicits maximal current. Peak current responses were recorded, and the current response from the test experiment was normalized to the average of the two maximal 5-HT-evoked controls performed immediately before and after each test experiment.

To establish DA concentration-response relationships in the presence of anesthetics, the cell was allowed to recover for 5 min after the control experiment using a concentration of 5-HT that elicits maximal current, and the test experiment was performed by first perfusing the oocyte with anesthetic solution alone for 30 s, and then immediately switching to a solution containing both anesthetic and DA for 75 s. A final control experiment was performed after a 3- to 5-min recovery period using a concentration of 5-HT that elicits maximal current. Peak current responses were recorded, and the current response from the test experiment was normalized to the average of the two maximal 5-HT-evoked controls performed immediately before and after each test experiment. All experiments using anesthetics had matched controls (without anesthetic) using the same oocytes, to account for cell-to-cell variability in current responses and agonist EC<sub>50</sub>.

**Experiments to Establish Anesthetic Concentration-Response Relationships.** To establish anesthetic concentration-response relationships in the presence of receptor-saturating concentrations of DA, a control experiment was first performed by perfusing the oocyte for 60 s with buffer solution containing a concentration of DA known to elicit maximal current (1 mM DA for 5-HT<sub>3A</sub> receptors and 2 mM DA for 5-HT<sub>3AB</sub> receptors). After a 3-min recovery period, the test experiment was performed by perfusing the cell with anes-

thetic solution alone for 30 s and then immediately switching to a solution containing both anesthetic and DA for 75 s. After another 3-min recovery period, a second control experiment was performed using a concentration of DA known to elicit maximal current. Peak current responses were recorded, and the current response from the test experiment was normalized to the average of the two maximal DA-evoked controls performed immediately before and after each test experiment.

**Statistical Analysis.** Normalized data were plotted as mean  $\pm$  standard deviation. 5-HT and DA concentration-response data were fitted to a Hill equation in the form  $I = I_{\max}/[1 + (EC_{50}/[\text{agonist}])^n]$ , where  $I$  is the peak current evoked by agonist,  $I_{\max}$  is the maximum current evoked by high agonist concentrations,  $EC_{50}$  is the concentration of agonist that elicits 50% of the maximal response, and  $n$  is the Hill coefficient. Data were analyzed using Igor Pro 4.01 software (Wavemetrics, Lake Oswego, OR). Statistical analyses were performed using Prism 4.0 software (GraphPad Software Inc., San Diego, CA).

**Drugs and Chemicals.** 5-HT, DA, ethyl 3-aminobenzoate methanesulfonate salt (tracaine), and collagenase IA were purchased from Sigma-Aldrich (St. Louis, MO). Isoflurane, halothane, and chloroform were purchased from Baxter (Deerfield, IL), Halocarbon Laboratories (River Edge, NJ), and Fisher Scientific (Fair Lawn, NJ), respectively. All other chemicals were reagent grade.

## Results

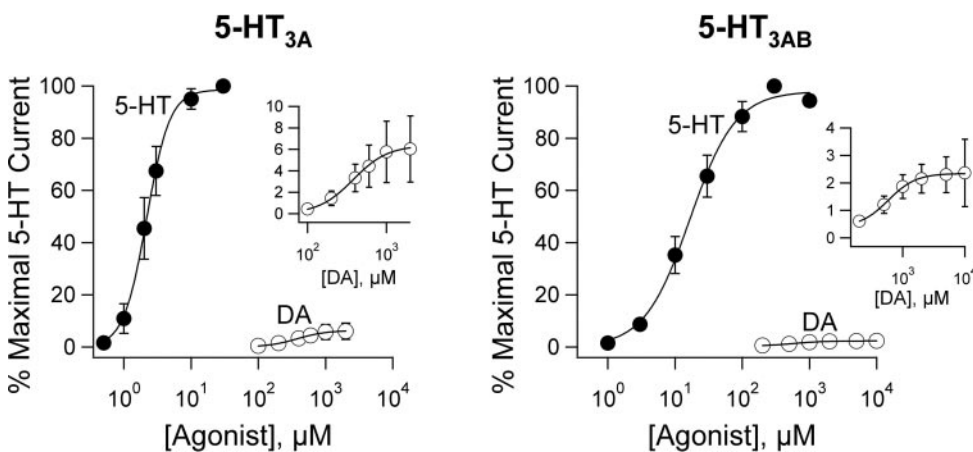
Figure 1 shows 5-HT and DA concentration-response relationships for currents mediated by 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors. For both receptor subtypes, 5-HT- and DA-evoked peak currents increased with agonist concentration before reaching a plateau (Fig. 1, insets show the DA concentration-response relationship for each receptor subtype with expanded scales to provide more detail). For 5-HT<sub>3A</sub> receptors, the  $EC_{50}$  for 5-HT was  $2.2 \pm 0.1 \mu\text{M}$  with a Hill coefficient of  $2.5 \pm 0.2$  and a maximal current at high concentrations ( $I_{\max}$ ) of  $99 \pm 1\%$ . DA was a relatively impotent and inefficacious agonist, because its  $EC_{50}$  was  $379 \pm 19 \mu\text{M}$  with a Hill coefficient of  $2.0 \pm 0.2$ , and  $I_{\max}$  was  $6.4 \pm 0.2\%$  of maximal 5-HT-evoked current response. For 5-HT<sub>3AB</sub> receptors, the  $EC_{50}$  for 5-HT was  $16.6 \pm 1.5 \mu\text{M}$  with a Hill coefficient of  $1.3 \pm 0.1$ , and  $I_{\max}$  of  $98 \pm 2\%$ . DA was also an impotent and inefficacious agonist for 5-HT<sub>3AB</sub> receptors, because its  $EC_{50}$  was  $583 \pm 42 \mu\text{M}$  with a Hill coefficient of  $1.9 \pm 0.2$ , and  $I_{\max}$  was  $2.35 \pm 0.03\%$  of maximal 5-HT-evoked current response.

Representative current traces from experiments using receptor-saturating concentrations of DA (1 mM for the 5-HT<sub>3A</sub>

receptor and 2 mM for the 5-HT<sub>3AB</sub> receptor) in the absence and presence of chloroform, halothane, and isoflurane at 2 MAC are shown in Fig. 2. The second control trace after washout of anesthetic is shown for each experiment to demonstrate the reversibility of anesthetic-induced effects. For this set of experiments using 5-HT<sub>3A</sub> receptors, chloroform and halothane both elicited dramatic increases in maximal DA-evoked currents (1083 and 495%, respectively), whereas isoflurane elicited a significantly smaller (55%) increase in such currents. Compared with the dramatic current enhancements observed for 5-HT<sub>3A</sub> receptors, chloroform and halothane elicited only modest increases in maximal DA-evoked currents for 5-HT<sub>3AB</sub> receptors (144 and 91%, respectively, for this set of experiments), and isoflurane elicited a mere 11% increase in such currents. Chloroform and halothane also seemed to enhance the rate of DA-induced desensitization (particularly for 5-HT<sub>3A</sub> receptors), although this effect was not studied in further detail.

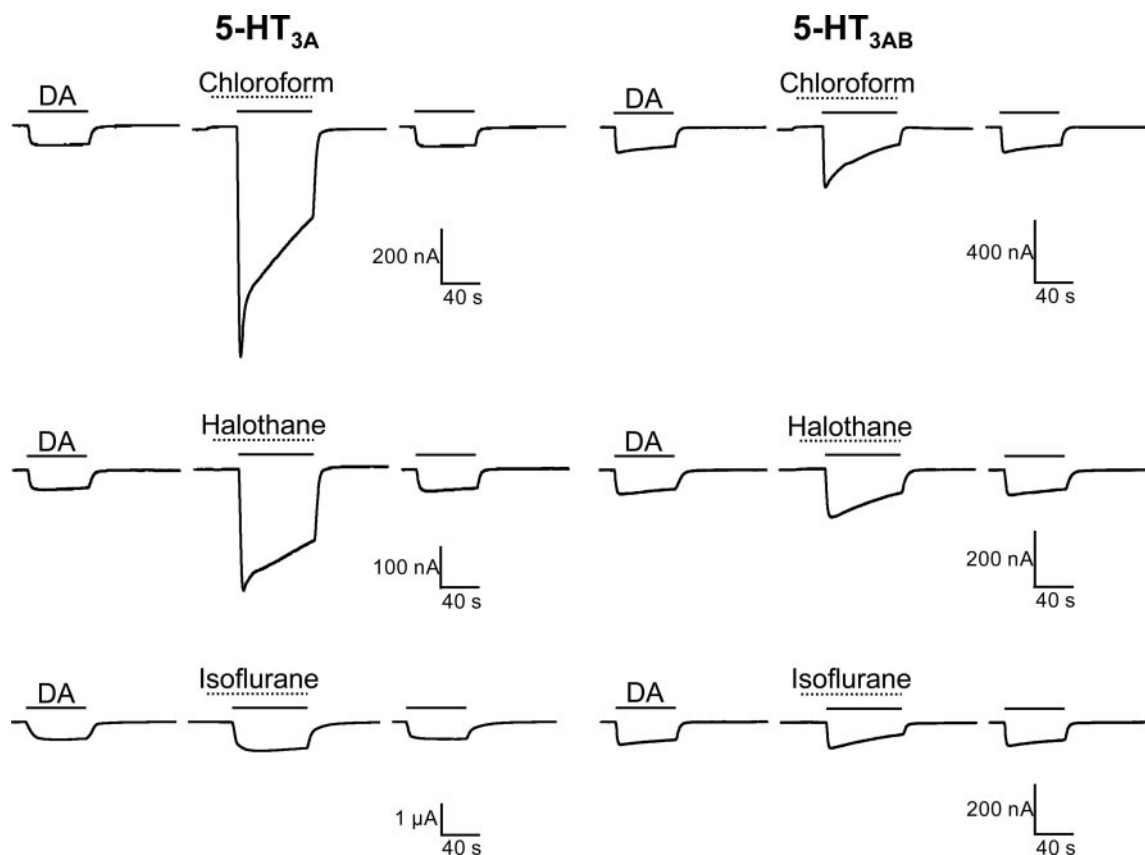
The potentiation of maximal DA-evoked currents by chloroform, halothane, and isoflurane over a range of clinically relevant concentrations (0.25–2 MAC) is shown in Fig. 3. For both receptor subtypes, anesthetic-induced potentiation of DA-evoked current responses increased in an anesthetic concentration-dependent manner. For 5-HT<sub>3A</sub> receptors, 2 MAC chloroform yielded the greatest current potentiation ( $745 \pm 97\%$ ), followed by halothane ( $507 \pm 128\%$ ) and isoflurane ( $60 \pm 14\%$ ). The same was true for 5-HT<sub>3AB</sub> receptors, although the magnitude of the current potentiation for all three anesthetics was substantially less than that observed for 5-HT<sub>3A</sub> receptors (at 2 MAC, chloroform, halothane, and isoflurane elicited increases of  $107 \pm 5$ ,  $87 \pm 5$ , and  $8 \pm 4\%$ , respectively). Using a paired two-tailed  $t$  test, all anesthetic-induced increases in DA-evoked currents were found to be statistically significant ( $p < 0.05$ ) for 5-HT<sub>3A</sub> receptors, with the single exception of isoflurane at 0.25 MAC. For 5-HT<sub>3AB</sub> receptors, all anesthetic-induced increases in DA-evoked currents were found to be statistically significant ( $p < 0.05$ ), except for halothane at 0.25 MAC and isoflurane at 0.25 MAC, 0.5 MAC, and 2 MAC.

Figure 4 shows DA concentration-response relationships for 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors in the absence and presence of 2 MAC chloroform, halothane, and isoflurane, normalized to maximal 5-HT-evoked current (by 100  $\mu\text{M}$  5-HT for 5-HT<sub>3A</sub> receptors and 300  $\mu\text{M}$  5-HT for 5-HT<sub>3AB</sub> receptors). Tables 1 and 2 summarize the  $EC_{50}$  and  $I_{\max}$  data

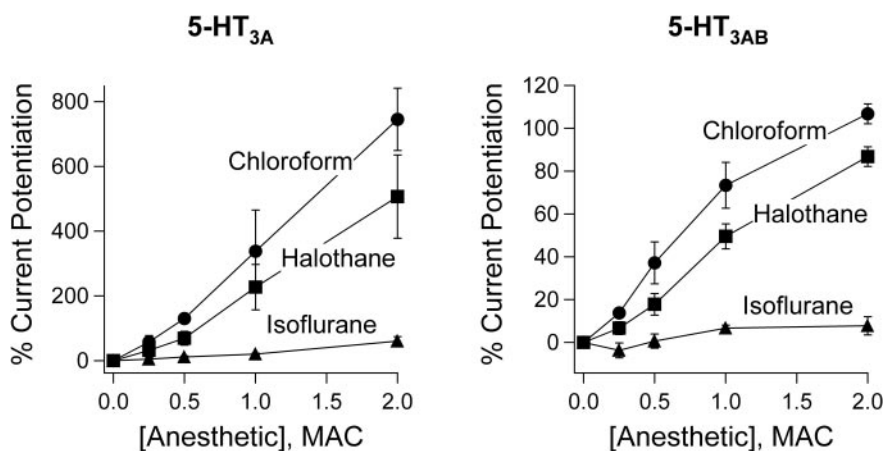


**Fig. 1.** 5-HT and DA concentration-response relationships for 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors, normalized to maximal 5-HT-evoked current responses. Note the low efficacy and potency of DA relative to 5-HT. Insets show DA concentration-response relationships with expanded scales to provide more detail. Data are expressed as mean  $\pm$  standard deviation ( $n = 20$ –48 for 5-HT<sub>3A</sub> receptors and 4–18 for 5-HT<sub>3AB</sub> receptors).





**Fig. 2.** Representative current traces illustrating the effects of 2 MAC chloroform, halothane, and isoflurane on current responses elicited by receptor-saturating concentrations of DA on 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors. The solid lines above the traces represent perfusion of the oocyte with DA, and the dotted lines represent perfusion with anesthetic. DA concentration was 1 mM for 5-HT<sub>3A</sub> receptors and 2 mM for 5-HT<sub>3AB</sub> receptors.



**Fig. 3.** Anesthetic-induced potentiation of current responses elicited by receptor-saturating concentrations of DA for 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors over a range of clinically relevant anesthetic concentrations. Note the different scales on the vertical axes for 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors. DA concentration was 1 mM for 5-HT<sub>3A</sub> receptors and 2 mM for 5-HT<sub>3AB</sub> receptors. Data are expressed as mean  $\pm$  standard deviation ( $n = 3$ ).

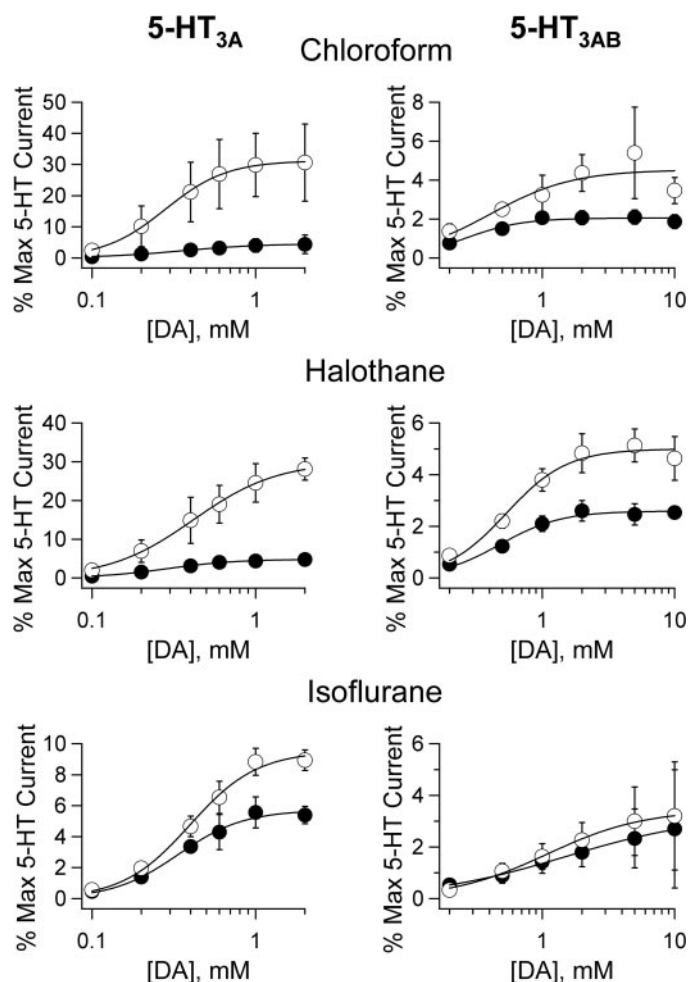
obtained from these concentration-response relationships for 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors, respectively. Chloroform and halothane had profound effects on the DA concentration-response relationship for 5-HT<sub>3A</sub> receptors, increasing the maximum DA-evoked current response ( $I_{max}$ ) by 578 and 525%, respectively. However, isoflurane only increased  $I_{max}$  by 67%.

In contrast to the effects observed for 5-HT<sub>3A</sub> receptors, 2 MAC chloroform and halothane had relatively minor effects on the DA concentration-response relationship for 5-HT<sub>3AB</sub> receptors, increasing  $I_{max}$  by 125 and 92%, respectively. 2 MAC isoflurane had no effect on  $I_{max}$  for 5-HT<sub>3AB</sub> receptors ( $3.3 \pm 0.2\%$  of maximal 5-HT-evoked response in the absence

of isoflurane versus  $3.4 \pm 0.1\%$  of maximal 5-HT-evoked response in the presence of isoflurane). None of the three test anesthetics altered the  $EC_{50}$  of the DA concentration-response relationship to a significant degree for either receptor subtype.

## Discussion

As depicted in Scheme 1, in the simplest sequential model that defines agonist-evoked activation of 5-HT<sub>3</sub> receptors, agonist (A) first binds to closed, resting state receptors ( $R_c$ ), and then agonist-bound closed receptors ( $AR_c$ ) isomerize to an open channel state ( $AR_o$ ). Within the context of this



**Fig. 4.** DA concentration-response relationships for 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors in the absence and presence of 2 MAC chloroform, halothane, and isoflurane, normalized to maximal 5-HT-evoked current responses. Closed circles represent controls (i.e., current responses evoked by DA alone), and open circles represent current responses evoked by DA in the presence of anesthetic. The controls were matched (i.e., the same oocytes were used for experiments with and without anesthetic). Note the different scales on the vertical axes for 5-HT<sub>3A</sub> and 5-HT<sub>3AB</sub> receptors. Data are expressed as mean  $\pm$  standard deviation ( $n = 3-6$ ).

scheme, volatile anesthetics may increase the 5-HT<sub>3</sub> receptor's sensitivity to agonist by modulating either 1) the agonist binding step (i.e., increasing agonist affinity) or 2) the channel gating step (i.e., increasing agonist efficacy). When using a highly efficacious agonist such as 5-HT, an anesthetic-induced increase in either parameter will reduce the agonist EC<sub>50</sub> for current activation, without increasing the current response evoked by high agonist concentrations (Colquhoun, 1998). Thus, a highly efficacious agonist cannot be used to determine the kinetic step upon which an anesthetic acts. However, when using a highly inefficacious agonist such as DA, effects on each step become readily distinguishable, because enhancement of channel gating produces an increase in current responses evoked by high agonist concentrations, whereas an increase in agonist affinity does not.

The results of this study indicate that at clinically relevant concentrations, chloroform, halothane, and isoflurane increase the agonist sensitivity of homomeric 5-HT<sub>3A</sub> receptors by enhancing channel gating. Our previous work with 5-HT<sub>3A</sub> receptors using a concentration of 5-HT that elicits

**TABLE 1**

EC<sub>50</sub> and  $I_{max}$  data derived from DA concentration-response relationships for 5-HT<sub>3A</sub> receptors in the absence and presence of anesthetics

	EC <sub>50</sub>	$I_{max}$
	mM	% max 5-HT
Chloroform control	0.35 $\pm$ 0.02	4.6 $\pm$ 0.1
Chloroform 2 MAC	0.28 $\pm$ 0.01	31.2 $\pm$ 0.4
Halothane control	0.29 $\pm$ 0.01	4.8 $\pm$ 0.1
Halothane 2 MAC	0.42 $\pm$ 0.02	30.0 $\pm$ 0.8
Isoflurane control	0.34 $\pm$ 0.02	5.7 $\pm$ 0.3
Isoflurane 2 MAC	0.40 $\pm$ 0.03	9.5 $\pm$ 0.5

**TABLE 2**

EC<sub>50</sub> and  $I_{max}$  data derived from DA concentration-response relationships for 5-HT<sub>3AB</sub> receptors in the absence and presence of anesthetics

	EC <sub>50</sub>	$I_{max}$
	mM	% max 5-HT
Chloroform control	0.27 $\pm$ 0.04	2.0 $\pm$ 0.1
Chloroform 2 MAC	0.4 $\pm$ 0.2	4.5 $\pm$ 0.8
Halothane control	0.48 $\pm$ 0.05	2.6 $\pm$ 0.1
Halothane 2 MAC	0.53 $\pm$ 0.06	5.0 $\pm$ 0.2
Isoflurane control	1.5 $\pm$ 0.3	3.3 $\pm$ 0.2
Isoflurane 2 MAC	1.1 $\pm$ 0.1	3.4 $\pm$ 0.1



**Scheme 1.** Simplest sequential model defining agonist-evoked activation of 5-HT<sub>3</sub> receptors.

10% of the maximal obtainable current (EC<sub>10</sub>) showed that at 2 MAC, chloroform induced the greatest amount of current enhancement (215%), followed by halothane (92%) and isoflurane (33%) (Stevens et al., 2005b). Consistent with those results, the present study using receptor-saturating concentrations of DA as agonist found that at 2 MAC, chloroform induced the greatest amount of channel gating enhancement, followed by halothane and then isoflurane.

Similarly, our previous work with 5-HT<sub>3AB</sub> receptors using a submaximal (EC<sub>10</sub>) concentration of 5-HT showed that at 2 MAC, chloroform (121%) induced more current enhancement than halothane (43%), whereas isoflurane induced no appreciable current enhancement (Stevens et al., 2005a). Consistent with those results, the present study using receptor-saturating concentrations of DA as agonist found that chloroform induced more channel gating enhancement than halothane, whereas isoflurane did not enhance gating. For both receptor subtypes, none of the three test anesthetics significantly altered the agonist EC<sub>50</sub> for DA, indicating that these anesthetics do not affect agonist binding affinity to 5-HT<sub>3</sub> receptors.

The present work also reveals that compared with homomeric 5-HT<sub>3A</sub> receptors, heteromeric 5-HT<sub>3AB</sub> receptors have markedly attenuated sensitivity to anesthetic-induced channel gating enhancement. This explains previous results demonstrating that anesthetics produce relatively little reduction in the 5-HT<sub>3AB</sub> receptor's EC<sub>50</sub> for 5-HT in comparison with that seen with the 5-HT<sub>3A</sub> receptor (Stevens et al., 2005a). If these results are considered within the context of the classic Monod-Wyman-Changeux model for allosteric proteins (Monod et al., 1965), then our results imply that chloroform

and halothane bind with higher affinity to 5-HT<sub>3A</sub> receptors in the open state versus the closed state, thus preferentially stabilizing the open state relative to the closed state. Incorporation of 5-HT<sub>3B</sub> subunits reduces anesthetic-induced gating enhancement because it reduces anesthetic binding affinity and/or conformational state selectivity. Similarly isoflurane produces little or no gating enhancement in either receptor subtype at clinically relevant concentrations because it possesses lower binding affinity and/or state selectivity than either chloroform or halothane.

Why do some anesthetics stabilize the open state whereas others do not? Our group's previous studies with both 5-HT<sub>3A</sub> (Stevens et al., 2005b) and 5-HT<sub>3AB</sub> receptors (Stevens et al., 2005a) using an EC<sub>10</sub> concentration of 5-HT as agonist showed that there is a strong correlation between an anesthetic's ability to potentiate currents and its molecular volume. Specifically, only physically small volatile anesthetics (molecular volumes <120 Å<sup>3</sup>) potentiate 5-HT-evoked currents, whereas larger ones either had no effect or actually inhibited currents. This suggests that anesthetic binding to (and stabilizing of) the open state is sterically limited such that only inhaled anesthetics smaller than isoflurane (i.e., chloroform and halothane) significantly enhance channel gating efficacy.

The technique of using partial (i.e., inefficacious) agonists to distinguish between anesthetic effects on agonist binding versus channel gating is well established. Wu et al. (1994) used suberyldicholine, a partial agonist of the nACh receptor, to show that ethanol enhances channel gating (i.e., stabilizes the open state) of the nACh receptor, although a small increase in agonist affinity was also observed in that study. Suberyldicholine has also been used to show that isoflurane increases the apparent agonist affinity of the nACh receptor, by slowing the dissociation of agonist from its binding site (Raines and Zachariah, 2000).

Similarly, there is evidence that halothane slows agonist unbinding from the GABA<sub>A</sub> receptor, prolonging receptor activation (Li and Pearce, 2000). On the other hand, Harrison and colleagues used the combination of a partial agonist for the GABA<sub>A</sub> receptor, piperidine-4-sulfonic acid (P4S), and a mutant GABA<sub>A</sub> receptor with a gating defect, to demonstrate that isoflurane potentiates maximal P4S-evoked currents and thus acts, at least in part, by enhancing channel gating (Topf et al., 2003). In a separate report, the same research group also used a similar approach with wild-type GABA<sub>A</sub> receptors and P4S to show that propofol enhances channel gating of the GABA<sub>A</sub> receptor (O'Shea et al., 2000).

Lovinger and colleagues used DA to show that ethanol and trichloroethanol enhance channel gating in 5-HT<sub>3</sub> receptors expressed in NCB-20 neuroblastoma cells (Lovinger et al., 2000). However, because NCB-20 cells express mRNA for both 5-HT<sub>3A</sub> and 5-HT<sub>3B</sub> subunits (Hanna et al., 2000), it is difficult to ascertain whether the agonist-evoked currents observed in that study were mediated by 5-HT<sub>3A</sub> receptors, 5-HT<sub>3AB</sub> receptors, or a combination of both. Our study using a heterologous expression system allowed us to define anesthetic actions on each receptor subtype separately, and our results demonstrate that the effects are clearly distinct.

In conclusion, the physically small, halogenated anesthetics chloroform and halothane decrease the agonist EC<sub>50</sub> for 5-HT of homomeric 5-HT<sub>3A</sub> receptors by enhancing channel gating (i.e., stabilizing the open state relative to the closed

state of the receptor) and without increasing agonist affinity. The incorporation of 5-HT<sub>3B</sub> subunits to produce heteromeric 5-HT<sub>3AB</sub> receptors markedly attenuates the ability of these anesthetics to enhance channel gating, illustrating the subunit dependence of these anesthetic effects. Isoflurane elicits only a minor gating enhancement effect for 5-HT<sub>3A</sub> receptors, which is abolished upon incorporation of 5-HT<sub>3B</sub> subunits to form 5-HT<sub>3AB</sub> receptors.

## References

- Apfel CC, Kranke P, Katz MH, Goepfert C, Papenfuss T, Rauch S, Heineck R, Greim CA, and Roewer N (2002) Volatile anaesthetics may be the main cause of early but not delayed postoperative vomiting: a randomized controlled trial of factorial design. *Br J Anaesth* **88**:659–668.
- Colquhoun D (1998) Binding, gating, affinity and efficacy: the interpretation of structure-activity relationships for agonists and of the effects of mutating receptors. *Br J Pharmacol* **125**:924–947.
- Comet MA, Laguzzi R, Hamon M, and Sevoz-Couche C (2005) Functional interaction between nucleus tractus solitarius NK<sub>1</sub> and 5-HT<sub>3</sub> receptors in the inhibition of baroreflex in rats. *Cardiovasc Res* **65**:930–939.
- Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG, and Kirkness EF (1999) The 5-HT<sub>3B</sub> subunit is a major determinant of serotonin-receptor function. *Nature (Lond)* **397**:359–363.
- Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC, Wilson SJ, Galindo JE, Glass CA, Luo L, et al. (1999) The pharmacological and functional characteristics of the serotonin 5-HT<sub>3A</sub> receptor are specifically modified by a 5-HT<sub>3B</sub> receptor subunit. *J Biol Chem* **274**:30799–30810.
- Franks NP and Lieb WR (1993) Selective actions of volatile general anaesthetics at molecular and cellular levels. *Br J Anaesth* **71**:65–76.
- Hanna MC, Davies PA, Hales TG, and Kirkness EF (2000) Evidence for expression of heteromeric serotonin 5-HT<sub>3</sub> receptors in rodents. *J Neurochem* **75**:240–247.
- Koyama S, Matsumoto N, Kubo C, and Akaike N (2000) Presynaptic 5-HT<sub>3</sub> receptor-mediated modulation of synaptic GABA release in the mechanically dissociated rat amygdala neurons. *J Physiol (Lond)* **529**:373–383.
- Li X and Pearce RA (2000) Effects of halothane on GABA<sub>A</sub> receptor kinetics: evidence for slowed agonist unbinding. *J Neurosci* **20**:899–907.
- Lovinger DM, Sung KW, and Zhou Q (2000) Ethanol and trichloroethanol alter gating of 5-HT<sub>3</sub> receptor-channels in NCB-20 neuroblastoma cells. *Neuropharmacology* **39**:561–570.
- Marić AV, Peterson AS, Brake AJ, Myers RM, and Julius D (1991) Primary structure and functional expression of the 5HT<sub>3</sub> receptor, a serotonin-gated ion channel. *Science (Wash DC)* **254**:432–437.
- Monod J, Wyman J, and Changeux J (1965) On the nature of allosteric transitions: a plausible model. *J Mol Biol* **12**:88–118.
- O'Shea SM, Wong LC, and Harrison NL (2000) Propofol increases agonist efficacy at the GABA<sub>A</sub> receptor. *Brain Res* **852**:344–348.
- Pires JG, Silva SR, Ramage AG, and Futuro-Neto HA (1998) Evidence that 5-HT<sub>3</sub> receptors in the nucleus tractus solitarius and other brainstem areas modulate the vagal bradycardia evoked by activation of the von Bezold-Jarisch reflex in the anesthetized rat. *Brain Res* **791**:229–234.
- Raines DE, Claycomb RJ, and Forman SA (2003) Modulation of GABA<sub>A</sub> receptor function by nonhalogenated alkane anesthetics: the effects on agonist enhancement, direct activation and inhibition. *Anesth Analg* **96**:112–118.
- Raines DE and Zachariah VT (2000) Isoflurane increases the apparent agonist affinity of the nicotinic acetylcholine receptor by reducing the microscopic agonist dissociation constant. *Anesthesiology* **92**:775–785.
- Reeves DC and Lummis SCR (2002) The molecular basis of the structure and function of the 5-HT<sub>3</sub> receptor: a model ligand-gated ion channel. *Mol Membr Biol* **19**:11–26.
- Sevoz C, Callera JC, Machado BH, Hamon M, and Laguzzi R (1997) Role of serotonin<sub>3</sub> receptors in the nucleus tractus solitarius on the carotid chemoreflex. *Am J Physiol* **272**:H1250–H1259.
- Stevens R, Rüschi D, Solt K, Raines DE, and Davies PA (2005a) Modulation of human 5-HT<sub>3AB</sub> receptors by volatile anesthetics and n-alcohols. *J Pharmacol Exp Ther* **314**:338–345.
- Stevens RJ, Rüschi D, Davies PA, and Raines DE (2005b) Molecular properties important for inhaled anesthetic action on human 5-HT<sub>3A</sub> receptors. *Anesth Analg* **100**:1696–1703.
- Topf N, Jenkins A, Baron N, and Harrison NL (2003) Effects of isoflurane on gamma-aminobutyric acid type A receptors activated by full and partial agonists. *Anesthesiology* **98**:306–311.
- Turner TJ, Mokler DJ, and Luebke JI (2004) Calcium influx through presynaptic 5-HT<sub>3</sub> receptors facilitates GABA release in the hippocampus: in vitro slice and synaptosome studies. *Neuroscience* **129**:703–718.
- Wu G, Tonner PH, and Miller KW (1994) Ethanol stabilizes the open channel state of the Torpedo nicotinic acetylcholine receptor. *Mol Pharmacol* **45**:102–108.
- Zeitze KP, Guy N, Malmberg AB, Dirajlal S, Martin WJ, Sun L, Bonhaus DW, Stucky CL, Julius D, and Basbaum AI (2002) The 5-HT<sub>3</sub> subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. *J Neurosci* **22**:1010–1019.

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