Modulation of Human 5-HT_{3AB} Receptors by Volatile Anesthetics and n-Alcohols

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Abstract

Functional 5-hydroxytryptamine type 3 (5-HT_3) receptors can be formed by 5-HT_{3A} subunits alone or in combination with the 5-HT_{3B} subunit, but only the 5-HT_{3A} receptor has been previously studied with respect to the modulation by volatile anesthetics and n-alcohols. Using two-point voltage-clamp we show for the first time the modulation of heteromeric human (h)5-HT_{3AB} receptors, expressed in *Xenopus* oocytes, by a series of n-alcohols and halogenated volatile anesthetics. At twice their anesthetic concentration, compounds having a molecular volume of less than 110 $Å^3$ enhanced sub-maximal 5-HT-evoked current. Compounds larger than 110 $Å^3$ inhibited sub-maximal 5-HT-evoked current. In experiments examining 5-HT concentration-response relationships, chloroform and butanol caused a slight decrease in the 5-HT EC_{50}. Sevoflurane and octanol inhibited 5-HT-evoked current at all 5-HT concentrations tested but had no effect upon the 5-HT EC_{50}. Compared to previous data on homomeric h5-HT_{3A} receptors, the presence of the h5-HT_{3B} subunit reduces the enhancement of h5-HT_3 receptors by smaller halogenated volatile anesthetics and n-alcohols. In summary, these results suggest that heteromeric h5-HT_{3AB} receptors are modulated by halogenated volatile anesthetics at clinically relevant concentrations, in addition to n-alcohols, suggesting that these receptors maybe be another physiological target for these compounds. The modulation is dependent upon the molecular volume of the compound further supporting the concept of an anesthetic binding pocket of limited volume common on other Cys-loop ligand-gated ion channels. Incorporation of the 5-HT_{3B} subunit alters either the anesthetic binding site or the allosteric interactions between anesthetic binding and channel opening.

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Introduction

5-hydroxytryptamine (serotonin) type 3 (5-HT_3) receptors belong to a superfamily of Cys-loop ligand-gated ion channels (LGIC) that includes the nicotinic acetylcholine (nACh), γ-aminobutyric acid type A ($GABA_A$), glycine and zinc-activated channel (ZAC) receptors (Davies et al., 2003). The structure of the 5-HT_3 receptor is presumed to be similar to all receptors in the Cys-loop superfamily of ligand-gated ion channels, namely, oligomers consisting of five subunits arranged to form a non-selective cation channel (Reeves and Lummis, 2002). The human genome contains five genes encoding for different 5-HT_3 subunits (5-HT_{3A-E}) (Niesler et al., 2003). Presently, only two of these subunits, 5-HT_{3A} and 5-HT_{3B}, have been shown to be involved with the formation of functional receptors. Unlike the 5-HT_{3A} subunit, the 5-HT_{3B} subunit alone is unable to form homomeric receptors, but it can combine with 5-HT_{3A} subunits to form heteromeric 5-HT_{3AB} receptors that differ from homomeric 5-HT_{3A} receptors in pharmacological and biophysical properties (Davies et al., 1999; Dubin et al., 1999). Although the exact subunit composition of receptors *in vivo* is not fully known, there does appear to be a region specific expression of the different subunits (Morales et al., 2001; Morales and Wang, 2002; Niesler et al., 2003). Expression of the 5-HT_{3B} subunit may even be species dependent as Northern blot analysis and reverse transcription-polymerase chain reaction (RT-PCR) revealed message for 5-HT_{3B} subunits in human and monkey brain (Davies et al., 1999; Dubin et al., 1999) whereas, *in situ* hybridization and RT-PCR techniques failed to find 5-HT_{3B} subunits in rat brain (Morales and Wang, 2002). However, interneurons expressing the 5-HT_{3B} subunit have been identified in the rat hippocampus with the use of a polyclonal antibody (Monk et al., 2001).

Within the central and peripheral nervous systems, activation of presynaptic 5-HT_3 receptors is associated with a modulation in release of neuropeptides and neurotransmitters such as ACh,

GABA, dopamine, glutamate, and vasoactive intestinal peptide (Férézou et al., 2002; Koyama et al., 2000; Wang et al., 1998), hence, any modulation 5-HT_3-mediated release by anesthetics will have potentially significant physiological effects. For example, 5-HT_3 receptors are involved with autonomic reflexes including emesis (Hornby, 2001), blood pressure and heart rate (Comet et al., 2004; Veelken et al., 1993). In fact, 5-HT_3 receptor antagonists are used to prevent and treat post-operative nausea and vomiting (PONV) which has a strong association with the use of halogenated volatile anesthetics (Apfel et al., 2002).

5-HT_3 receptors, along with nACh, GABA and glycine receptors, are anesthetic-sensitive. Previous studies have shown that volatile anesthetics and n-alcohols modulate currents mediated by rodent 5-HT_{3A} (Jenkins et al., 1996; Machu and Harris, 1994; Zhou and Lovinger, 1996) and human 5-HT_{3A} receptors (Stevens et al., 2005; Suzuki et al., 2002). The molecular volume of the anesthetic determines the modulation of sub-maximal 5-HT-evoked currents with currents being enhanced by smaller (<120 $Å^3$) compounds (Stevens et al., 2005). Because the subunit composition of LGIC can influence the modulation by anesthetics, the aim of this study was to examine the modulation of heteromeric h5-HT_{3AB} receptors by anesthetic compounds and determine the influence the 5-HT_{3B} subunit has on their modulation.

This is the first report on the effects of halogenated volatile anesthetics and n-alcohols on heteromeric human 5-HT_{3AB} receptors. Here, we show that a variety of volatile anesthetics and n-alcohols do indeed modulate human 5-HT_{3AB} receptor-mediated currents, and that the modulation characteristics differ compared to those of 5-HT_{3A} receptors.

Materials and Methods:

Xenopus Oocyte Preparation and Receptor Expression Oocytes from HCG-injected adult female *Xenopus laevis* frogs (Xenopus One, Ann Arbor, MI) were surgically removed under anesthesia with 0.2% tricaine and hypothermia. cDNA encoding the human 5-HT_{3A} and 5-HT_{3B} subunits were generously supplied by E. Kirkness (TIGR, Rockville, MD) and transcribed into mRNA 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 encoding for h5-HT_{3A} subunit or a cRNA mixture encoding for both the h5-HT_{3A} and h5-HT_{3B} subunits (in the ratio of 1:2:1 by volume for 5-HT_{3A} subunit: 5-HT_{3B} subunit: water). Oocytes were incubated at 18°C for 18 hours to 8 days in filter-sterilized ND-96 solution (in mM): NaCl 96, KCl 2, HEPES 10, $CaCl_2$ 1.8, $MgCl_2$ 1.0, penicillin 5 units/ml, streptomycin 5 mcg/ml, pH adjusted to 7.5 with NaOH prior to performing electrophysiological experiments.

Electrophysiological Recording Currents from oocytes expressing 5-HT_3 receptors were recorded using the two-electrode voltage-clamp technique. Oocytes were placed in a 40 μl recording chamber, impaled with two capillary glass electrodes (A-M Systems, Carlsborg, WA) filled with 3M KCl (resistance <5 MΩ) and were voltage-clamped at –50 mV using a GeneClamp 500B amplifier (Axon Instruments, Union City, CA). During electrophysiological recording, oocytes were constantly superfused at a rate of 5 ml/min with buffer solution (in mM) NaCl 96, KCl 2, HEPES 10, $CaCl_2$ 1.0, $MgCl_2$ 0.8, pH adjusted to 7.5 with NaOH, using a closed syringe superfusion system when volatile anesthetics were present. Currents were filtered at 1 KHz, sampled at 33.3 Hz and recorded using Clampex v.9 software (Axon Instruments).

Protocols and Data Analysis The effects of several volatile and alcohol anesthetics on agonist-mediated currents were surveyed using 2 μM 5-HT, an agonist concentration that evokes

approximately 10% of the maximal peak current for 5-HT_{3AB} receptors (EC_{10} concentration). The EC_{10} concentration is commonly measured when examining the modulation of Cys-loop ligand-gated ion channels as it allows any enhancements to be measured. Oocytes were preincubated with anesthetic for 30 seconds prior to co-application of anesthetic plus 5-HT for 60 seconds. Each experiment was preceded and followed by a control application of 5-HT, both to normalize data and to ensure the reversibility of any drug-induced modulation of currents.

For analysis, the average of these two measurements was used as the control. A 3-minute recovery period was allowed after each application of agonist (with or without anesthetic). Experiments were repeated in at least three oocytes.

The molecular volumes of all anesthetic agents were determined using Mac Spartan 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 correlation coefficient relating anesthetic and n-alcohol action with molecular volume was calculated using SPSS 9.0 (SPSS, Inc., Chicago, IL).

For experiments examining the effects of halogenated volatile anesthetics and n-alcohols on agonist concentration-response relationships, the oocyte was first exposed for 15 seconds to 300μM 5-HT, a concentration that yields maximal current. Following a 5-minute recovery period, a control current was measured at a specific 5-HT concentration (15-75 seconds exposure time, depending on concentration). Following another recovery period (3-5 minutes, depending on 5-HT concentration), the maximal current response was again determined using 300μM 5-HT. After a further 5-minute recovery period, the oocyte was preincubated with anesthetic for 30 seconds prior to the coapplication of anesthetic and 5-HT. Finally, after another 3-5 minute

recovery period, the maximal current response was again measured using 300 μM 5-HT to ensure reversibility.

For analysis, all currents were normalized to the average maximal current elicited by 300 μM 5-HT immediately preceding and following each measurement. Normalized data were plotted as mean ± standard deviation. The 5-HT concentration-response curves were fitted to the following Hill equation:

$$I = I_{max} / [1 + (EC_{50}/[5\text{-HT}])^{n}] \quad (1)$$

where I is the peak current at a certain concentration of 5-HT, I_{max} is the maximum 5-HT-evoked current, EC_{50} is the concentration of 5-HT that elicits 50% of the maximal response, and n is the Hill coefficient. Serotonin EC_{50} values were calculated to evaluate shifts in the 5-HT concentration-response relationship curves in the presence of volatile anesthetics and alcohols.

Data were analyzed *post-hoc* using Graphpad Prism v.4 software (Graphpad Software, Inc., San Diego, CA). Statistical analysis was performed by using a Student's t-Test with statistical significance set at $p < 0.05$.

Drugs and Chemicals 5-hydroxytryptamine (serotonin), aminobenzoic acid ethyl ester (tricaine), collagenase IA, and hexanol were purchased from Sigma Chemicals Co. (St. Louis, MO). Pentanol and octanol were purchased from Aldrich Chemical Co. (Milwaukee, WI), and butanol was purchased from Fluka Chemika (Buchs, Switzerland). Isoflurane was purchased from Baxter Healthcare Corp. (Deerfield, IL). Sevoflurane was purchased from Abbott Laboratories (Chicago, IL). Saturated solutions of volatile anesthetics were prepared by adding an excess of agent to a sealed bottle of recording solution, and stirred overnight. These saturated solutions of known concentration were then diluted using gas-tight syringes to yield the final desired anesthetic concentration for experimentation. The anesthetizing concentrations of

halogenated volatile anesthetics were defined as the aqueous concentrations corresponding to 1 minimal alveolar concentration (MAC) calculated using the aqueous:gas partition coefficient at 37ºC. Except for chloroform, MAC for all volatile anesthetics was in humans (Eger et al., 2003; Strum and Eger, 1987; Wadhwa et al., 2003). MAC for chloroform was taken as 0.5% atm, the average value of two studies in mice (Deady et al., 1981; Miller et al., 1973). The anesthetizing concentrations of n-alcohols were defined as the aqueous concentrations that cause loss of righting reflex in tadpoles (Alifimoff et al., 1989).

Results

Application of 5-HT to oocytes injected with mRNA encoding for h5-HT_{3A} and h5-HT_{3B} subunits caused a concentration dependent activation of inward current that desensitized in the continued presence of high concentration of 5-HT. Examining the 5-HT concentration-response of h5-HT_{3A} and h5-HT_{3AB} receptors expressed in oocytes demonstrated a difference between the two receptors. The measured EC_{50}'s were 1.8 ± 0.05 μM and 20 ± 3 μM for h5-HT_{3A} and h5-HT_{3AB} receptors respectively (Figure 1A). In addition, heteromeric h5-HT_{3AB} receptors had a lower Hill coefficient (1.2 ± 0.2) compared to homomeric h5-HT_{3A} receptors (2.9 ± 0.4).

As previously observed (Davies *et al*., 1999; Dubin *et al*., 1999; Stewart *et al*., 2001), incorporation of h5-HT_{3B} subunits into heteromeric h5-HT_3 receptors resulted in 5-HT-evoked currents that were distinct from those mediated by homomeric h-5HT_{3A} receptors (Figure 1B). Serotonin-evoked currents from heteromeric h5-HT_{3AB} receptors decayed faster relative to that observed with homomeric h5-HT_{3A} receptors. The decay of current was examined by calculating the percentage of the maximal 5-HT-evoked current amplitude remaining after a 15 s application of 5-HT (Figure 1C). No difference in maximal 5-HT-evoked current amplitude was observed between h5-HT_{3A} receptors (100 μM) and h5-HT_{3AB} receptors (300 μM). However, a significant difference in current decay at the end of a 15s application of maximal 5-HT was observed between h5-HT_{3A} receptors (43.6 ± 21.6 %, n = 14) and h5-HT_{3AB} receptors (93.5 ± 2.6 %, n = 11, $p < 0.0001$).

Initial screening of volatile anesthetic and n-alcohol effects on heteromeric h5-HT_{3AB} receptors was performed using a concentration of 5-HT (2 μM), which elicits approximately 10% of maximal current (EC_{10}). The volatile anesthetics and n-alcohols were all used at equipotent concentrations, specifically, at twice their anesthetizing concentrations (see Methods).

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Anesthetic modulation of agonist-elicited currents varied with anesthetic molecular volume, a plot of the modulation by n-alcohols and halogenated volatile anesthetics of 5-HT (2 μM) evoked currents versus agent molecular volume shows a negative correlation, r_s = -0.962 (Figure 2). The physically smaller (molecular volumes <110Å^3) volatile anesthetics chloroform (1.7 mM) and halothane (0.43 mM) along with the n-alcohol, butanol (21.6 mM), enhanced 2 μM 5-HT-elicited currents by 121 ± 50.7%, 42.6 ± 4.5% and 48.6 ± 23.2% respectively. However, the larger (molecular volumes >110Å^3) volatile anesthetic sevoflurane (0.66 mM) and n-alcohols hexanol (1.14 mM) and octanol (0.11 mM) inhibited currents by -49.6 ± 9%, -37.8 ± 14.7% and -65.7 ± 8.1% respectively. Amplitudes of 2 μM 5-HT-evoked currents were minimally affected by 5.8 mM pentanol (13.9 ± 21%) and 0.55 mM isoflurane (-10.3 ± 9.5%), an alcohol and anesthetic that are intermediate in molecular volume.

Anesthetic modulation of EC_{10} 5-HT-evoked currents mediated by heteromeric h5-HT_{3AB} receptors was compared with that mediated by homomeric h5-HT_{3A} receptors (Stevens et al. 2005). Chloroform, halothane, butanol and pentanol were significantly less effective at enhancing EC_{10} 5-HT-evoked current amplitudes meditated by h5-HT_{3AB} receptors compared to h5-HT_{3A}-receptors (Figure 3). Isoflurane caused a small enhancement of h5-HT_{3A}-mediated currents, but slightly inhibited currents mediated by h5-HT_{3AB} receptors. Although hexanol caused a significantly greater inhibition of currents recorded from h5-HT_{3AB} compared to h5-HT_{3A} receptors, inhibition by sevoflurane and octanol appeared identical for both receptors.

Agonist concentration-response relationships were constructed in the absence and presence of halogenated volatile anesthetics (chloroform, isoflurane and sevoflurane) and n-alcohols (butanol and octanol), to resolve anesthetic actions on agonist EC_{50} and maximal agonist-elicited currents in oocytes expressing h5-HT_{3AB} receptors. Each anesthetic and alcohol was applied at

one and two times their anesthetizing concentrations. None of the halogenated volatile anesthetics tested had any significant effect on 5-HT EC_{50} values. Chloroform (0.85 and 1.7 mM) had no effect on maximally 5-HT-evoked currents and only slightly decreased 5-HT EC_{50} (Figure 4A). At 2 MAC, chloroform (1.7 mM) decreased the EC_{50} by only 22.5% from 16 ± 0.9 μM to 12.4 ± 1.1 μM with no change in the Hill slope. Two MAC isoflurane (0.55 mM) increased the 5-HT EC_{50} by only 23.2% in addition to an inhibition of peak current amplitude (Figure 4B). Sevoflurane caused a concentration-dependent decrease in maximal 5-HT-evoked current, 2 MAC sevoflurane (0.66 mM) decreased peak current by 73% while causing only a small (5.6%) increase in EC_{50} (Figure 4C). All compounds tested had no effect on the Hill coefficient.

The actions of the n-alcohols butanol (11 and 22 mM) and octanol (0.11 and 0.22 mM) on 5-HT concentration response relationships resembled those described above for the volatile anesthetics chloroform and sevoflurane respectively. Butanol caused a reduction in the 5-HT EC_{50}, reducing the control EC_{50} from 15 ± 0.7 μM to 13.1 ± 3.7 μM and 9.5 ± 0.8 μM ($p < 0.05$) for 11 and 22 mM butanol respectively. Butanol (11 mM) showed negligible inhibition (2.5%) at high 5-HT concentrations but at 22 mM, butanol caused an inhibition (16%) of maximal 5-HT-evoked current amplitude (Figure 5A). The n-alcohol of larger molecular volume, octanol, inhibited currents evoked by most 5-HT concentrations but did not effect the 5-HT EC_{50}. At 0.22 mM, octanol inhibited the maximal 5-HT-evoked current by 65%, yet EC_{50} remained unchanged, 13.4 ± 1.7 μM and 13.3 ± 0.4 μM for the 5-HT EC_{50}'s in the absence and presence of octanol (Figure 5B).

Figure 6 summarizes the data on EC_{50}'s and I_{max} from our 5-HT concentration-response relationship experiments and compares them to what we have previously reported for the 5-HT_{3A}

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receptor (Stevens et al., 2005). Both chloroform and butanol caused a smaller leftward shift in 5-HT EC_{50} with less inhibition of maximal evoked currents mediated by h5-HT_{3AB} receptors compared to h5-HT_{3A} receptors. The ability of octanol and sevoflurane to right shift the 5-HT EC_{50} was greatly reduced by the incorporation of the 5-HT_{3B} subunit. Sevoflurane was slightly better at blocking maximal-evoked current in h5-HT_{3AB} receptors. While isoflurane had no effects upon the 5-HT concentration-response relationship of h5-HT_{3A} receptors it inhibited 5-HT_{3AB}-mediated current evoked by high concentrations of 5-HT. No volatile anesthetic or n-alcohol tested affected the Hill coefficients of any 5-HT concentration-response relationship.

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Discussion

This study demonstrates that heteromeric h5-HT_{3AB} receptors are modulated by halogenated volatile anesthetics and n-alcohols. Similar to what we have previously reported for homomeric h5-HT_{3A} receptors (Stevens et al., 2005), molecular volume of n-alcohols and halogenated volatile anesthetics determines their ability to modulate h5-HT_{3AB}-mediated current amplitude at sub-maximal concentrations of 5-HT. There exists a negative correlation between molecular volume of compound and their ability to modulate sub-maximal current amplitude, such that all agents smaller than 110 $Å^3$ (chloroform, halothane, butanol and pentanol) enhance sub-maximal 5-HT-evoked current amplitude and compounds larger than 110 $Å^3$ (isoflurane, sevoflurane, hexanol and octanol) inhibit sub-maximal 5-HT-evoked current amplitude.

We propose that allosteric interactions modulate anesthetic action on 5-HT_3 receptors and suggest that volatile anesthetics and n-alcohols enhance sub-maximal 5-HT responses by binding to a small binding site that physically limits the binding of volatile anesthetics or n-alcohol having molecular volumes > 110 $Å^3$. The modulation by both halogenated volatile anesthetics and n-alcohols of similar molecular volumes suggests common site(s) of action. An additional larger binding site is also proposed. This site allows for the binding of compounds having molecular volumes in excess of 110 $Å^3$ and mediates the inhibitory actions of the compounds. In contrast to the enhancing properties of the volatile anesthetics and n-alcohols, incorporation of the 5-HT_{3B} subunit does not appear to affect the inhibitory properties of the compounds. Similar n-alcohol enhancing and inhibitory sites have been shown for the nicotinic receptor. The enhancing site being similar in volume to the one described here for the 5-HT_3 receptor. Competition studies between ethanol and octanol have shown the two sites to be separate (Wood et al., 1991).

The reduced enhancement of sub-maximal 5-HT-evoked currents and the reduced leftward shift in 5-HT EC_{50} values observed with the introduction of the h5-HT_{3B} subunit can result from a number of possibilities. (1) The number of enhancing sites is reduced in the heteromeric h5-HT_{3AB} receptor. (2) The number of enhancing sites remains the same but incorporation of the h5-HT_{3B} subunit renders the receptor unable to undergo the same degree of conformational change induced by anesthetics as that of a homomeric receptor. (3) The h5-HT_{3AB} receptor undergoes an increased desensitization rate in the presence of anesthetic and hence, the enhancement is underestimated. As seen in figure 4A, current amplitudes induced by high concentrations of 5-HT are identical in the absence and presence of chloroform. If chloroform were increasing the rate of desensitization, the maximal current amplitude at high agonist concentrations would be expected to decrease because the rate of desensitization would exceed the solution exchange time. This would produce a left shift in EC_{50} and a shallow Hill coefficient, none of which were observed. In addition, we have obtained analogous results using the whole-cell patch-clamp technique in combination with a rapid perfusion system that exchanges solutions in 1-3 ms. For h5-HT_{3AB} receptors, rate of desensitization is much slower than the rate of activation and the two can clearly be distinguished using this method (data not shown).

Recent work on $GABA_A$ and glycine receptors has mapped out a potential binding cavity for n-alcohols and volatile anesthetics. Residues within transmembrane domains TM1, TM2 and TM3 have been shown to be critical for volatile anesthetic and n-alcohol enhancement of these receptors (Jenkins et al., 2001; Mihic et al., 1997). These residues are thought to form a hydrophobic pocket into which volatile anesthetics bind and is separate from the agonist binding site. Although the size of this anesthetic binding site is calculated to be larger than the pocket in

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5-HT_3 and nicotinic receptors, the location within the receptor is thought to be similar with all the members of the Cys-loop ligand gated ion channel family.

Incorporation of the h5-HT_{3B} subunit may decrease the number of anesthetic binding sites in the heteromeric receptor. As mentioned above, it is commonly thought that anesthetics and alcohols occupy hydrophobic clefts in ligand-gated ion channel protein structure where they alter channel function by changing the channels' conformational flexibility. If such sites exist between adjacent h5-HT_{3A} subunits, then incorporation of the h5-HT_{3B} subunit would reduce the number of such sites. Alternatively, the binding site may be present within the h5-HT_{3A} subunit itself and may be absent within the h5-HT_{3B} subunit. A recent study described dramatic changes in the modulation of 5-HT-mediated currents by volatile anesthetics by mutating leucine (L) 270, the 15′ residue within TM2 of the murine 5-HT_{3A} receptor (Lopreato et al., 2003). The L270 residue is homologous to the glycine receptor α1 serine (S) 267 and $GABA_A$ α1 S270 residues that are known to be important for the enhancement by volatile anesthetics and alcohols on those receptors (Mihic et al., 1997). Lopreato et al (2003) proposed that L270 of the murine 5-HT_{3A} subunits is important for the effects of anesthetics and alcohols and may even form the hydrophobic pocket that is the binding site. Interestingly, the 15′ residue in the TM2 of h5-HT_{3A} is also a leucine whereas the h5-HT_{3B} subunits have an arginine. Further studies are needed to examine whether this change in residues is sufficient to change the pattern of channel modulation observed between homomeric and heteromeric receptors.

Although the modulation of 5-HT_3 receptors by volatile anesthetics is probably not the main mechanism by which these compounds produce clinical anesthesia, the role played by 5-HT_3 receptors in anesthesia and its side effects may not be completely insubstantial. There are many complicated components to the phenomenon of the anesthetized state (hypnosis, amnesia,

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immobility and analgesia) occurring at supraspinal and spinal regions (Campagna et al., 2003). It is well documented that anesthetics enhance $GABA_A$ receptor mediated synaptic currents, thus increasing the inhibitory drive in neuronal networks. However, it has to be remembered that 5-HT_3 receptors are located on some inhibitory GABAergic interneurons in the amygdala (Koyama et al., 2000), cortex (Puig et al., 2004; Zhou and Hablitz, 1999), hippocampus (McMahon and Kauer, 1997) and spinal cord (Alhaider et al., 1991; Tanimoto et al., 2004) and can control the release of GABA into the synapse, presumably through Ca^{2+} permeable homomeric 5-HT_{3A} receptors (Koyama et al., 2000). Hence, anesthetic modulation of 5-HT_3 receptors in these areas will affect GABA release and change the inhibitory drive. For example, anesthetics of low molecular volume can enhance presynaptic Ca^{2+} permeable homomeric 5-HT_{3A} receptors resulting in a greater release of GABA into the synapse. Anesthetic stimulation of GABA release and potentiation of postsynaptic $GABA_A$ receptors provide complementary actions to enhance inhibitory drive.

Recently, a study showed that 5-HT_3 receptor antagonists reduce the halothane-mediated inhibition of spinal dorsal horn sensory neuronal responses to noxious peripheral stimulation indicating that 5-HT_3 receptors are anesthetic targets for the reduction in nociception (Koshizaki et al., 2003). Small diameter (<25 μm) dorsal root ganglia (DRG) neurons innervating the dorsal horn of the spinal cord mainly expressing 5-HT_{3A} subunits are thought to be involved with the processing of nociceptive information whereas, co-expression of both 5-HT_{3A} and 5-HT_{3B} subunits are found in medium (26-40 μm) to large (>40 μm) DRG neurons and are believed to mediate proprioceptive as well as nociceptive information (Morales et al., 2001).

The major side effects of general anesthetic use include post-operative nausea and vomiting (PONV) and cardiopulmonary depression. 5-HT_3 receptors are known to regulate autonomic

reflexes within the nucleus tractus solitarius (NTS). Such autonomic reflexes include emesis (Hornby, 2001), blood pressure and heart rate (Comet et al., 2004). 5-HT_3 receptors within the NTS are mainly presynaptic and therefore would influence release of neurotransmitter (Huang et al., 2004). In addition to central innervations, the NTS receives input from the peripheral nodose ganglia (Nosjean et al., 1990). Some nodose ganglia neurons innervating the NTS express 5-HT_{3A} alone, while others express both 5-HT_{3A} and 5-HT_{3B} subunits (Morales and Wang, 2002). It remains to be seen whether anesthetic modulation of 5-HT_3 receptors in the NTS causes any unwanted side effects of anesthetic administration. The expression of various 5-HT_3 subunit combinations in different neurons innervating the spinal cord and NTS could result in a heterogenous response to anesthetics.

This is the first study on the modulation of human heteromeric 5-HT_{3AB} receptors by halogenated volatile anesthetics and n-alcohols. Given the 5-HT_3 receptors' role in neurotransmitter release, emesis, cardiovascular reflexes, nociception and addiction, they constitute an important class of target proteins for general anesthetics and alcohols. A clear dependence upon molecular volume of whether a volatile anesthetic is a potentiator or inhibitor of EC_{10} 5-HT-evoked current amplitudes is observed in both h5-HT_{3A} and h5-HT_{3AB} receptors. Current amplitude is only one part of a multi-faceted waveform. In the presence of both agonist and anesthetic, the apparent rate of desensitization appears to increase with anesthetics of small, intermediate and large molecular volumes. This suggests that the anesthetics may be having multiple effects on the kinetic gating process. Because of the slow solution exchange in the oocytes recording chamber (~500 ms) we are unable to accurately measure activation, desensitization or deactivation rates and therefore the effects of anesthetics upon them. Future

experiments will need to be performed using rapid solution exchange to examine these important components.

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Footnotes

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Figure Legends

Figure 1. Changes in the properties of 5-HT_3 receptors induced by the incorporation of 5-HT_{3B} subunit.

(A) Serotonin concentration-response relationships of homomeric h5-HT_{3A} (open circles) and heteromeric h5-HT_{3AB} receptors expressed in oocytes. The EC_{50} and Hill coefficients for h5-HT_{3A} and h5-HT_{3AB} receptors were 1.77 ± 0.05 μM, 2.9 ± 0.4 and 20 ± 3 μM, 1.2 ± 0.2 respectively.

(B) Example traces of maximal 5-HT-evoked current in oocytes expressing h5-HT_{3A} and h5-HT_{3AB} receptors. Serotonin was applied for 15 seconds (solid bar).

(C) Percentage decay of the maximal 5-HT-evoked current at the end of a 15 second 5-HT exposure. * $p < 0.0001$, n = 11-14 oocytes.

Figure 2. Modulation of 5-HT EC_{10}-evoked currents by volatile anesthetics.

(A) Example traces of the reversible modulation of currents evoked by an approximate EC_{10} concentration of 5-HT (2 μM). Compounds of a molecular volume smaller than 110 $Å^3$ (chloroform, top left; butanol, lower left) enhanced the sub-maximal current. Inhibition of the current was observed with larger compounds (sevoflurane, top right; octanol, lower right). Application of 5-HT represented by a solid line, anesthetic compound by the dashed line.

(B) Relationship between the modulation of sub-maximal 5-HT-evoked currents (2 μM, ~EC_{10}) and the molecular volume of anesthetic compound. A correlation analysis revealed a strong negative correlation (r_s = -0.962)) between modulation of current amplitude and molecular volume of n-alcohols and halogenated volatile anesthetics. All anesthetic compounds were used

at twice their anesthetic concentration. Data were collected using at least 3 oocytes for each compound and expressed as mean ± standard deviation.

Figure 3. Comparison of the modulation by volatile anesthetics and n-alcohols of h5-HT_{3A} and h5-HT_{3AB} receptor-mediated currents. Currents evoked by an EC_{10} concentration of 5-HT in oocytes expressing h5-HT_{3AB} receptors (solid bars) were differentially modulated compared to currents evoked from h5-HT_{3A} receptor (open bars) expressing oocytes, except for halothane, octanol and sevoflurane. All compounds were used at twice their anesthetic concentration. Data expressed as mean ± standard deviation from at least 3 oocytes. Each compound was compared using Student's *t*-test where * $p < 0.01$, ** $p < 0.005$ and *** $p < 0.0001$.

Figure 4. 5-HT concentration-response relationships in the absence (solid circles) and presence (open symbols) of volatile anesthetics Chloroform (A), isoflurane (B) and sevoflurane (C) at one (squares) and two (circles) times their anesthetic concentrations are shown. Control concentration-response curves were constructed by averaging all of the control experiments for a given drug concentration. Data expressed as mean ± standard deviation from at least 3 oocytes.

Figure 5. Effects upon 5-HT concentration-response relationships by n-alcohols of small (butanol, A) and large (octanol, B) molecular volumes. The alcohols were applied at one (squares) and two (circles) times their anesthetic concentrations. Control concentration-response curves were constructed by averaging all of the control experiments for a given drug concentration. Data expressed as mean ± standard deviation from at least 3 oocytes.

Figure 6. Summary of the changes in 5-HT EC_{50} and maximal 5-HT-evoked current by volatile anesthetics and n-alcohols (twice their anesthetic concentrations).

(A) Bar graph showing percent change in 5-HT EC_{50} in the presence of compound aligned on right. Chloroform and butanol caused a left shit of the concentration-response curve with both 5-HT_{3A} (open bars) and 5-HT_{3AB} receptors (closed bars). Sevoflurane and octanol caused a right shift, mainly with 5-HT_{3A} receptors and isoflurane only affected 5-HT_{3AB} receptors EC_{50}.

(B) Data expressed as a ratio of I_{max} in the presence of compound divided by I_{max} in the absence of compound for h5-HT_{3A} (open bar) and h5-HT_{3AB} (closed bar) receptors.

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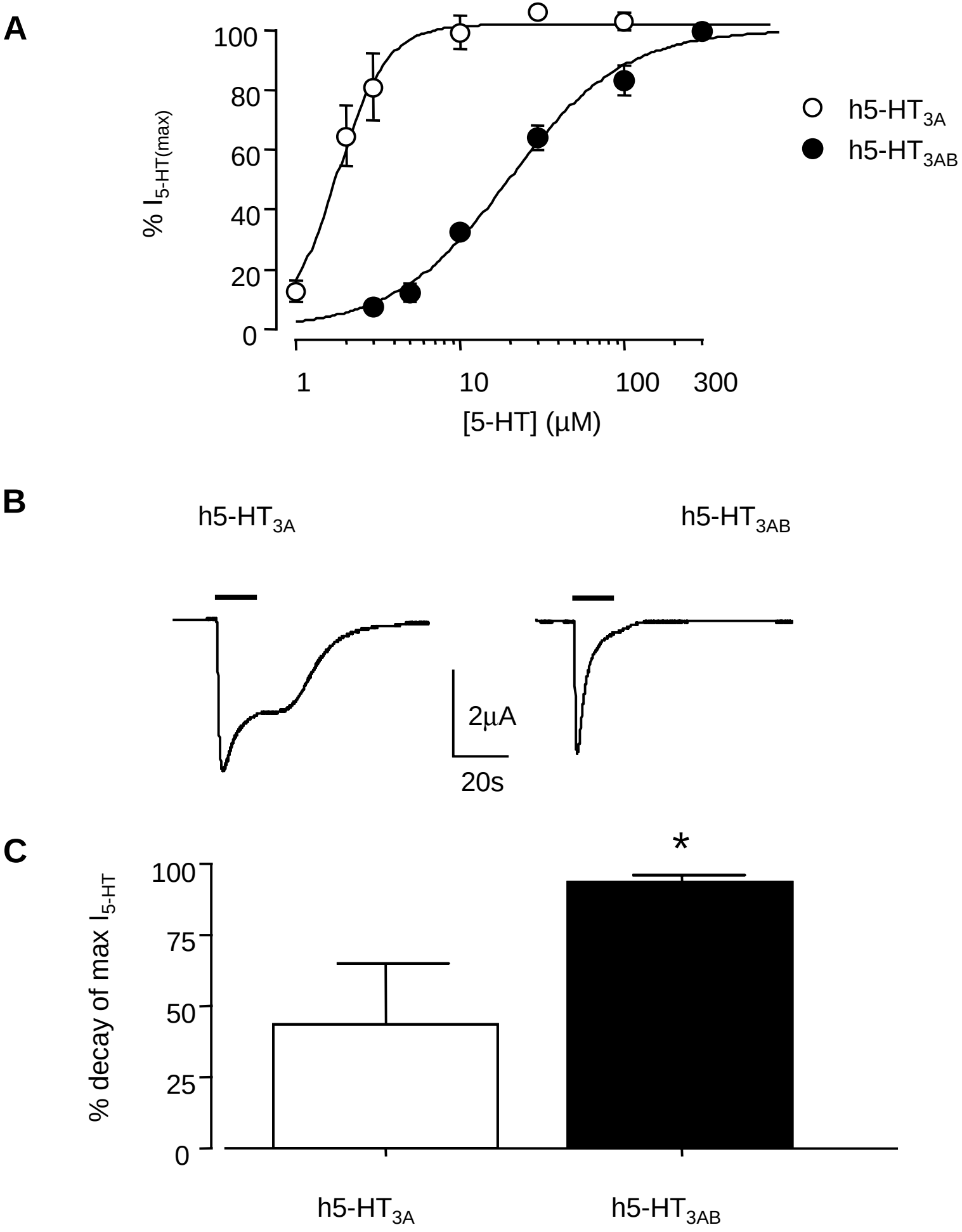
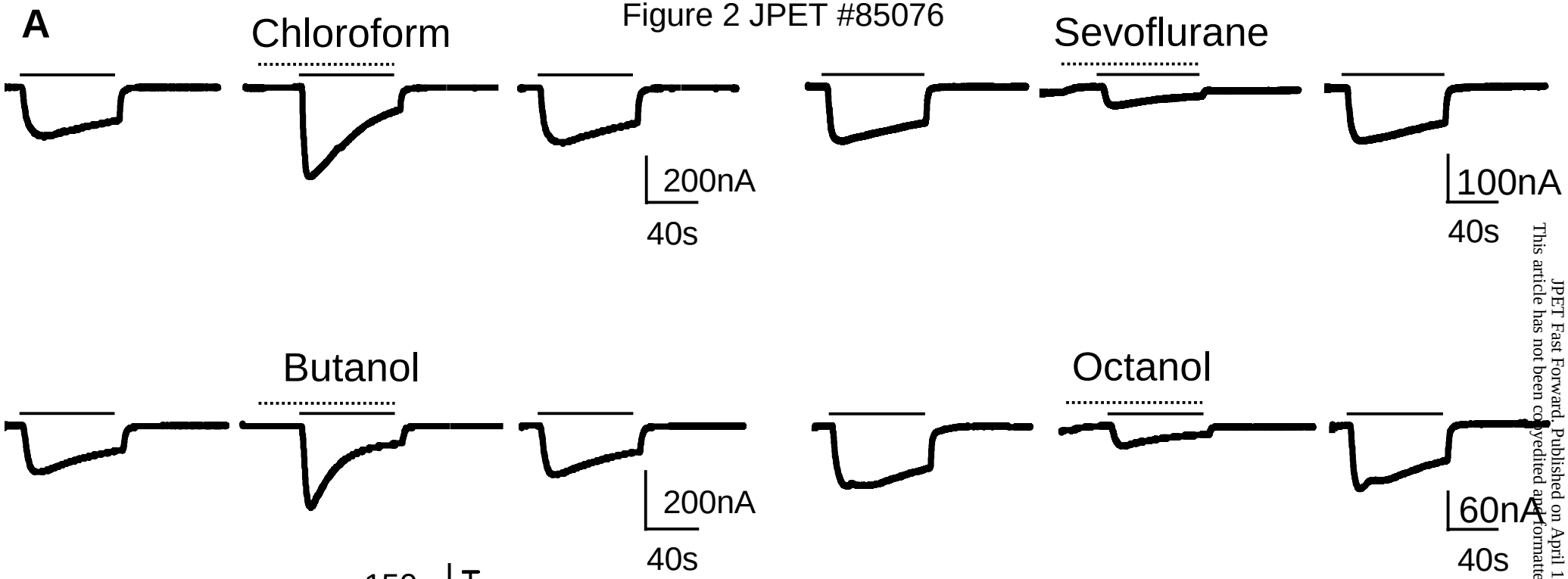
Figure 1 JPET #85076





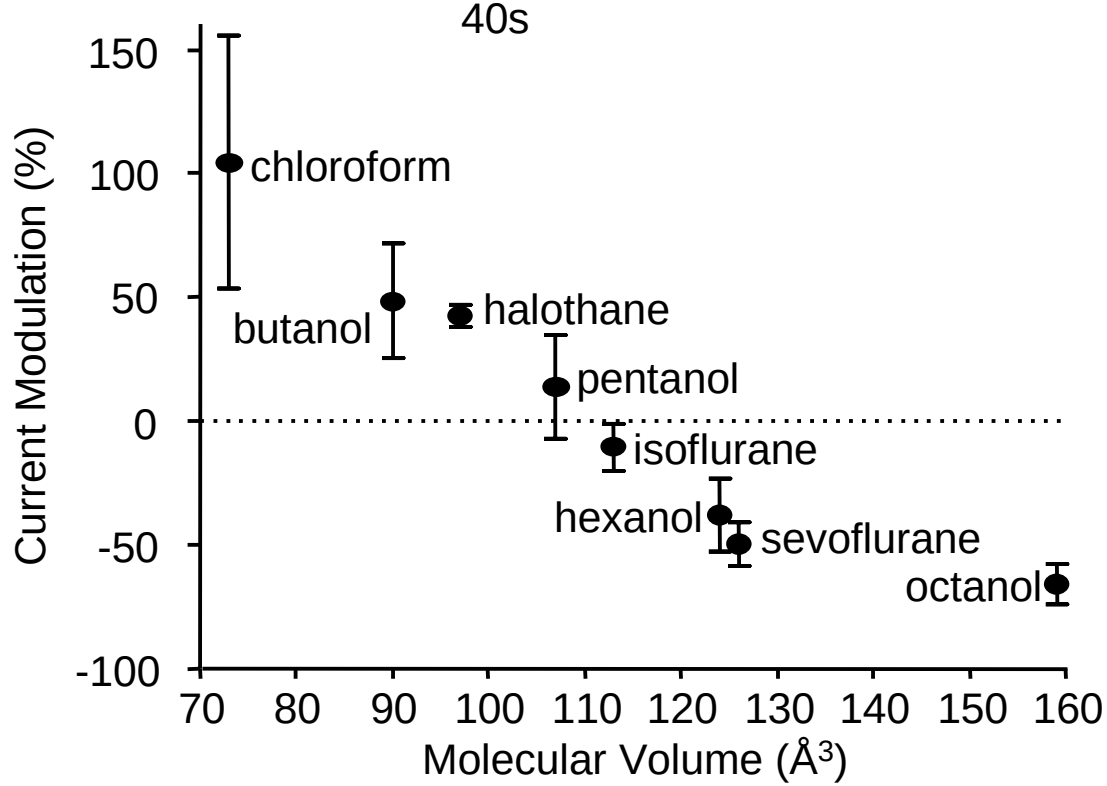


Figure 3 JPET #85076

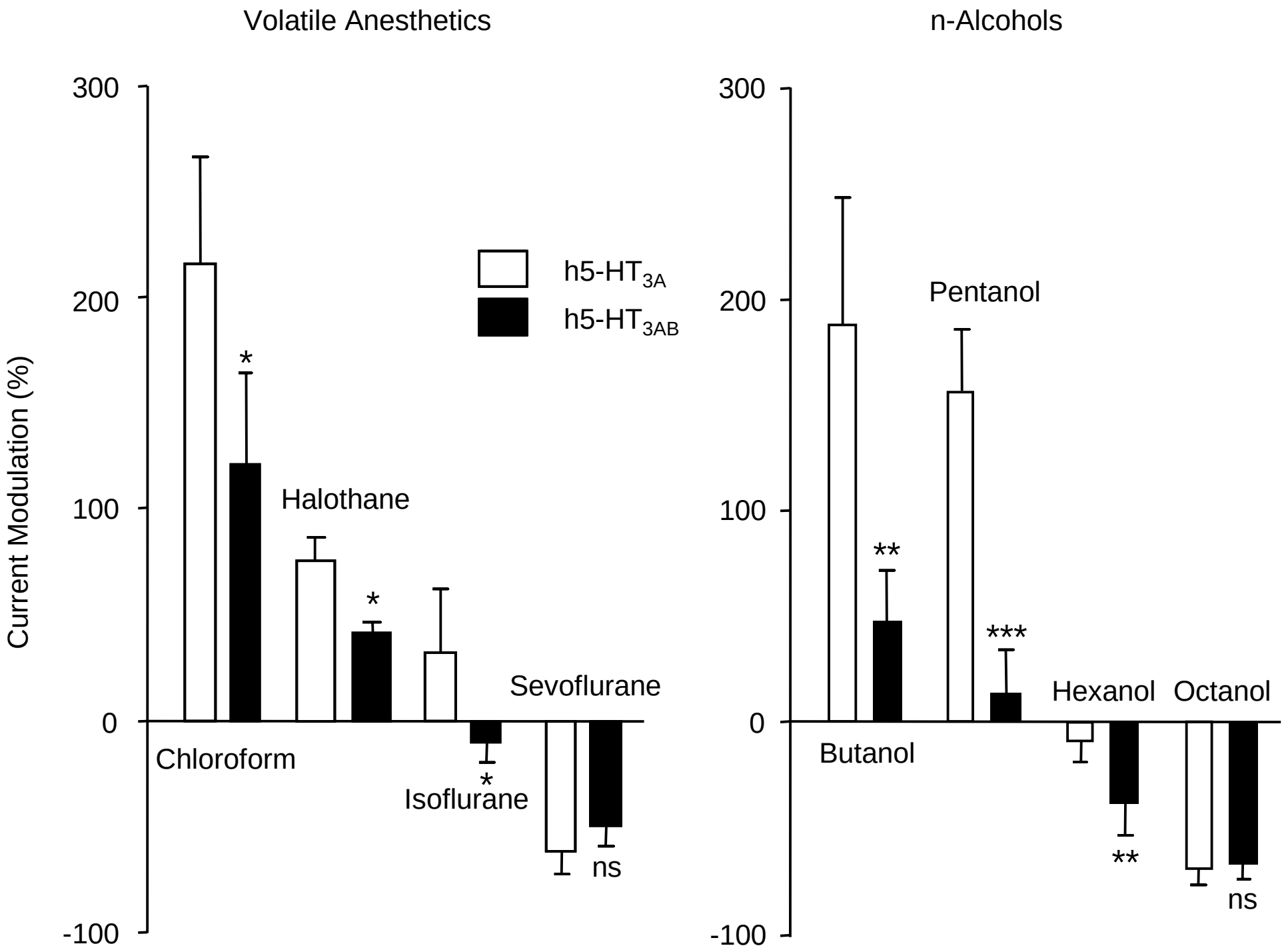


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Figure 4 JPET #85076

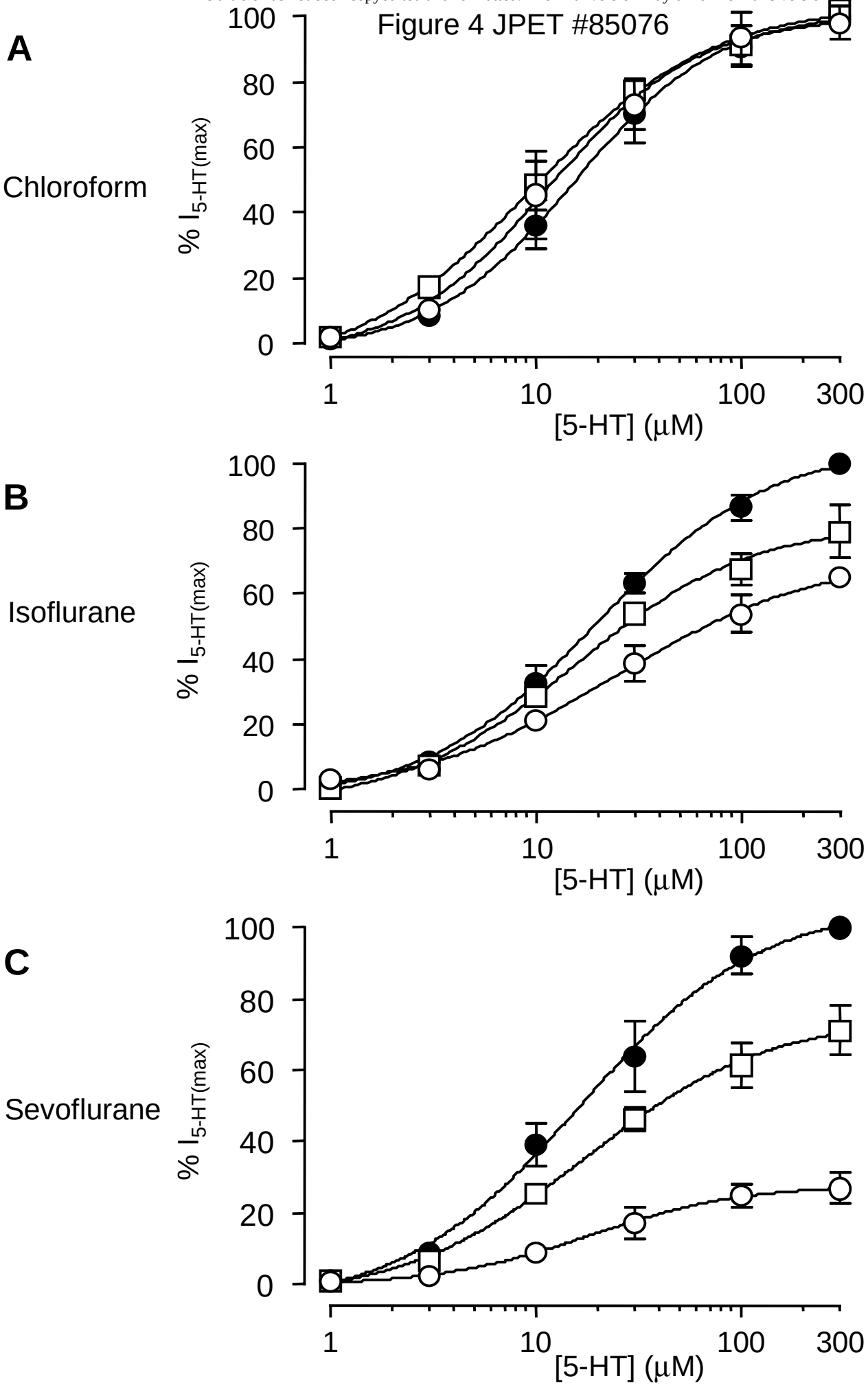

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Figure 5 JPET #85076