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# Effect of local anesthetics on serotonin$_{1A}$ receptor function

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ABSTRACT

The fundamental mechanism behind the action of local anesthetics is still not clearly understood. Phenylethanol (PEtOH) is a constituent of essential oils with a pleasant odor and can act as a local anesthetic. In this work, we have explored the effect of PEtOH on the function of the hippocampal serotonin$_{1A}$ receptor, a representative neurotransmitter receptor belonging to the G protein-coupled receptor (GPCR) family. Our results show that PEtOH induces reduction in ligand binding to the serotonin$_{1A}$ receptor due to lowering of binding affinity, along with a concomitant decrease in the degree of G-protein coupling. Analysis of membrane order using the environment-sensitive fluorescent probe DPH revealed decrease in membrane order with increasing PEtOH concentration, as evident from reduction in rotational correlation time of the probe. Analysis of results obtained shows that the action of local anesthetics could be attributed to the combined effects of specific interaction of the receptor with anesthetics and alteration of membrane properties (such as membrane order). These results assume relevance in the perspective of anesthetic action and could be helpful to achieve a better understanding of the possible role of anesthetics in the function of membrane receptors.



## 1. Introduction

Local anesthetics belong to a group of amphiphilic compounds which curb the feeling of pain, when applied in a particular part of the body by preventing the transmission of nerve impulse, thereby reducing the pain in that area. In spite of a large body of work, the molecular mechanism by which local anesthetics act is not understood. Two predominant models have been suggested to explain anesthetic action. The first model, the lipid hypothesis, attributes the anesthetic effect to variations in membrane physical properties. According to this model, changes in the physical (global) properties of the membrane (*e.g.*, membrane order) modulate membrane protein function (Rehberg et al., 1995). The second model, known as the protein hypothesis, attributes anesthetic effect to specific interaction of anesthetics with membrane proteins, thereby affecting membrane protein function (Arias, 1999). It is still not clear whether anesthetic action is an outcome of indirect anesthetic-lipid effect or a more direct anesthetic-protein interaction. Yet another way to understand the basis of anesthetic effects is to explore changes in the lateral pressure profiles in membrane bilayers due to addition of anesthetics (Cantor, 2001). In this overall scenario, a useful approach for understanding the molecular mechanism of local anesthetics is to identify specific targets of anesthetics.

Phenylethanol (PEtOH) (see inset of Fig. 1) is found in a variety of essential oils, it has a fragrant rose-like odor, and can act as a local anesthetic (Anbazhagan et al., 2010; Gray et al., 2013). It also possesses antibacterial activity (Corre et al., 1990). Interestingly, PEtOH has been reported to vary membrane order by altering the packing of lipid molecules (Anbazhagan et al., 2010; Jordi et al., 1990; Killian et al., 1992). We have recently shown that PEtOH causes disorder in various membrane phases (gel, fluid and liquid-ordered), although the disorder was found to be phase-specific (Shrivastava et al., 2016). Moreover, PEtOH has been shown to bring about translocation of the mitochondrial precursor protein apocytochrome *c* (Jordi et al., 1990), and modulate oligomerization of membrane proteins in *E. coli* by perturbing helix-helix interaction (Anbazhagan et al., 2010).

*Abbreviations:* 8-OH-DPAT, 8-hydroxy-2(di-*N*-propylamino)tetralin; BCA, bicinchoninic acid; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; GPCR, G protein-coupled receptor; GTP-$\gamma$-S, guanosine-5′-*O*-(3-thiotriphosphate); PEtOH, phenylethanol; *p*-MPPF, 4-(2′-methoxy)-phenyl-1-[2′-(*N*-2″-pyridinyl)-*p*-fluorobenzamido]ethyl-piperazine; PMSF, phenylmethylsulfonyl fluoride.

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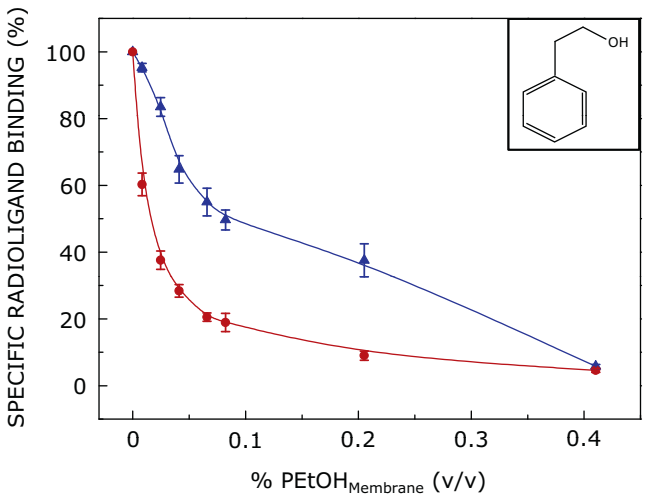


**Fig. 1.** Specific binding of the agonist [$^3$H]8-OH-DPAT (●), and the antagonist [$^3$H]*p*-MPPF (▲) to the serotonin$_{1A}$ receptor with increasing concentrations of the local anesthetic, PEtOH. The PEtOH concentration plotted here is the actual concentration of PEtOH partitioned into the membrane (see Table 1). Values are expressed as a percentage of the specific ligand binding obtained in the absence of PEtOH. Data points represent means ± S.E. of duplicate points from at least three independent experiments. The line joining the data points is provided merely as a viewing guide. The inset shows the chemical structure of PEtOH. See Section 2 for more details.

G protein-coupled receptors (GPCRs), which characteristically possess seven transmembrane domains, form the largest superfamily of membrane proteins implicated in information transfer from the extracellular region to the interior of cells (Chattopadhyay, 2014; Pierce et al., 2002; Rosenbaum et al., 2009). The total number of GPCRs is close to 800 belonging to different families (Fredriksson et al., 2003), and ∼5% of human genes encode them (Zhang et al., 2006). GPCRs play a central role in mediating diverse physiological processes and a wide array of ligands including light are responsible for their activation. As GPCRs are implicated in multiple physiological responses, they represent popular targets for currently prescribed drugs in all clinical areas and are useful for the development of novel drugs (Jacobson, 2015; Tautermann, 2014). Yet, new functions associated with GPCRs are still being explored. Serotonin receptors are an important class of GPCRs which bind the neurotransmitter serotonin (Nichols and Nichols, 2008). The serotonin$_{1A}$ receptor occupies a unique position among members of the serotonin receptor family for a number of reasons (Pucadyil et al., 2005). The serotonin$_{1A}$ receptor has emerged as a crucial target in developing new drugs to treat a range of diseases from anxiety and depression to cancer (Fiorino et al., 2014).

Among membrane proteins, ion channels appear to be the most common targets of anesthetic action (Arias, 1999; Fozzard et al., 2005; Franks and Lieb, 1997). On the other hand, involvement of GPCRs in anesthetics action is an emerging area. Although there are some reports on the interaction of GPCRs with anesthetics (Hollmann et al., 2005; Ishizawa et al., 2002; Kalipatnapu and Chattopadhyay, 2004; Matsunaga et al., 2015; Nakayama et al., 2005; Peterlin et al., 2005; Picardi et al., 2014), detailed information on interaction of GPCRs with local anesthetics with respect to affinity of binding and influence on membrane order, and their relative importance in anesthetic action, is lacking. Keeping this in mind, we have probed the effect of the local anesthetic PEtOH on serotonin$_{1A}$ receptor function. Our results show that PEtOH induces a decrease in specific ligand binding activity and G-protein coupling efficiency of the hippocampal serotonin$_{1A}$ receptor. There is a concomitant decrease in membrane order in presence of PEtOH. Our results show that both specific interaction of the receptor with anesthetics as well as alteration of global properties (such as membrane order) of the lipid environment could be involved in local anesthetic action.

## 2. Materials and methods

### 2.1. Materials

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), EDTA, EGTA, $MgCl_2$, $MnCl_2$, $Na_2HPO_4$, iodoacetamide, PEtOH, 4-(2′-methoxy)-phenyl-1-[2′-(*N*-2″-pyridinyl)-*p*-fluorobenzamido]ethyl-piperazine dihydrochloride (*p*-MPPF), PMSF, polyethylenimine, serotonin hydrochloride, sodium azide, sucrose and Tris were purchased from Sigma Chemical Co. (St. Louis, MO). [$^3$H]8-hydroxy-2(di-*N*-propylamino)tetralin ([$^3$H]8-OH-DPAT) (specific activity 187.4 Ci/mmol) and [$^3$H]*p*-MPPF (specific activity 74.2 Ci/mmol) were purchased from MP Biomedicals (Santa Ana, CA). Bicinchoninic acid (BCA) assay reagent for protein estimation was from Pierce (Rockford, IL). GF/B glass microfiber filters were from Whatman International (Kent, UK). GTP-γ-S (guanosine-5′-*O*-(3-thiotriphosphate)) was purchased from Roche Applied Science (Mannheim, Germany). DPH was purchased from Molecular Probes/Invitrogen (Eugene, OR). The concentration of a stock solution of DPH prepared in methanol was calculated using its molar extinction coefficient (ε) of 88,000 $M^{-1}$ $cm^{-1}$ at 350 nm in methanol. All other chemicals used were of the highest purity available. Solvents used were of spectroscopic grade. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout. Stock solution of PEtOH (2% (v/v)) was prepared in 50 mM Tris buffer (pH 7.4) and used for experiments. Fresh bovine brains were procured from a local slaughterhouse within 10 min of death, and the hippocampal region was cautiously cut out. The hippocampi were immediately flash frozen in liquid nitrogen and stored at −80 °C till further use.

### 2.2. Methods

#### 2.2.1. Preparation of native hippocampal membranes

Native hippocampal membranes were prepared as described previously (Pucadyil and Chattopadhyay, 2004a). Briefly, hippocampal tissue (∼50 g) was homogenized as 10% (w/v) in a polytron homogenizer in 2.5 mM Tris, 0.32 M sucrose, 5 mM EDTA, 5 mM EGTA, 0.02% sodium azide, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4 buffer. The homogenate was centrifuged at 900 × *g* for 10 min at 4 °C. The resultant supernatant was filtered through three layers of cheesecloth and centrifuged at 50,000 × *g* for 20 min at 4 °C. The pellet obtained was suspended in 10 vol. of 50 mM Tris, 1 mM EDTA, 0.24 mM PMSF, 10 mM iodoacetamide, pH 7.4 buffer using a hand-held Dounce homogenizer and centrifuged at 50,000 × *g* for 20 min at 4 °C. This procedure was repeated until a clear supernatant was obtained. The final pellet was suspended in a minimum volume of 50 mM Tris (pH 7.4) buffer, homogenized using a hand-held Dounce homogenizer, flash frozen in liquid nitrogen and stored at −80 °C. Protein concentration was assayed using BCA reagent with bovine serum albumin as standard (Smith et al., 1985).

#### 2.2.2. Radioligand binding assays

Receptor binding assays were conducted as described previously (Harikumar and Chattopadhyay, 1999; Pucadyil and Chattopadhyay, 2004a). Briefly, tubes in duplicate with ∼1 mg protein in a total volume of 1 ml of buffer (50 mM Tris, 1 mM EDTA, 10 mM $MgCl_2$, 5 mM $MnCl_2$, pH 7.4) were incubated with the radiolabeled agonist [$^3$H]8-OH-DPAT for 1 h at room temperature (25 °C). For antagonist binding with [$^3$H]-*p*-MPPF, the buffer did not contain $MgCl_2$ and $MnCl_2$. The final concentrations of both agonist and