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The muscarinic antagonists scopolamine and atropine are competitive antagonists at 5-HT_3 receptors

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ABSTRACT

Scopolamine is a high affinity muscarinic antagonist that is used for the prevention of post-operative nausea and vomiting. 5-HT_3 receptor antagonists are used for the same purpose and are structurally related to scopolamine. To examine whether 5-HT_3 receptors are affected by scopolamine we examined the effects of this drug on the electrophysiological and ligand binding properties of 5-HT_3A receptors expressed in *Xenopus* oocytes and HEK293 cells, respectively. 5-HT_3 receptor-responses were reversibly inhibited by scopolamine with an IC_{50} of 2.09 μM. Competitive antagonism was shown by Schild plot ($pA_2 = 5.02$) and by competition with the 5-HT_3 receptor antagonists [^{3}H]granisetron ($K_i = 6.76$ μM) and G-FL ($K_i = 4.90$ μM). The related molecule, atropine, similarly inhibited 5-HT evoked responses in oocytes with an IC_{50} of 1.74 μM, and competed with G-FL with a K_i of 7.94 μM. The reverse experiment revealed that granisetron also competitively bound to muscarinic receptors ($K_i = 6.5$ μM). In behavioural studies scopolamine is used to block muscarinic receptors and induce a cognitive deficit, and centrally administered concentrations can exceed the IC_{50} values found here. It is therefore possible that 5-HT_3 receptors are also inhibited. Studies that utilise higher concentrations of scopolamine should be mindful of these potential off-target effects.

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1. Introduction

Scopolamine is a high-affinity (nM) muscarinic antagonist that is used to treat post-operative nausea and vomiting, and motion sickness. As a research tool it is often administered to induce cognitive dysfunction. At higher doses it can also produce amnesia and compliance (Klinkenberg and Blokland, 2010). Atropine is a related muscarinic antagonist from the same biosynthetic pathway as scopolamine and is used as a cycloplegic and mydriatic in ophthalmology, and for the treatment of bradychardia.

Scopolamine readily passes the blood brain barrier and it is believed that inhibition of muscarinic receptors in the central nervous system causes a cholinergic deficit that impairs memory (Klinkenberg and Blokland, 2010). As an age-related deterioration in cognitive function is thought to be predominantly related to a decline in cholinergic neurotransmission, scopolamine administration has often been used to model dementia (Bartus, 2000). Scopolamine has therefore been extensively used for preclinical and clinical testing of treatments for cognitive impairment (Bartolomeo et al., 2000; Blin et al., 2009; Liem-Moolenaar et al., 2011).

In the clinic, 5-HT_3 antagonists are mainly used for the treatment of nausea and vomiting following cancer therapy and general anaesthesia (Thompson, 2013; Walstab et al., 2010). Experimentally, they can also be administered to reverse scopolamine-evoked learning and memory deficits (Barnes et al., 1990; Chugh et al., 1991; Carli et al., 1997). In the brain 5-HT_3 receptors are widely distributed in the amygdala and hippocampus, regions of critical importance in memory and spatial navigation, and involved in the control of emotional responses and their associated disorders such as anxiety and depression (Gulyas et al., 1999; Thompson and

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Lummis, 2007; Walstab et al., 2010). It is thought that the reversal of scopolamine-induced cognitive dysfunction by 5-HT_3 receptor antagonists occurs by inhibiting pre-synaptic 5-HT_3 receptors that modulate the functions of other neurotransmitters such as acetylcholine, dopamine, γ-aminobutyric acid and glutamate in this region (Seyedabadi et al., 2014). A similar mechanism is thought to underlie the anti-anxiolytic and anti-depressive actions of 5-HT_3 antagonists.

5-HT_3 receptors are members of the Cys-loop family of ligand-gated ion channels (LGIC). These are responsible for fast excitatory and inhibitory neurotransmission in the central and peripheral nervous systems. The family includes nicotininc acetylcholine (nACh), γ-aminobutyric acid ($GABA_A$) and glycine receptors, which are all cell-surface, transmembrane ion channels. They consist of five subunits that surround a central ion-conducting pore, and each subunit contains three distinct functional regions that are referred to as the extracellular, transmembrane and intracellular domains. The orthosteric binding site (that occupied by the endogenous agonist) is located between the extracellular domains of adjacent subunits, and is formed by the convergence of three amino acid loops from the principal subunit (loops A − C) and three β-sheets (loops D − F) from the complementary subunit (Thompson et al., 2008). Agonist binding results in the opening of a central ion-conducting pore that is located within the transmembrane domain (Peters et al., 2010; Hassaine et al., 2014). Ligands bind to both domains, but the orthosteric binding site is the main drug target. These 5-HT_3 receptor competitive antagonists have high affinities (nM) and conform to a pharmacophore that consists of an aromatic group coupled to an azabicyclic ring via a carbonyl linker (Fig. 1). Both atropine and scopolamine also have these structural features, suggesting that these muscarinic antagonists could also bind at 5-HT_3 receptors (Thompson, 2013).

Here we use a combination of electrophysiology, radioligand binding, flow cytometry and *in silico* ligand docking to provide evidence that, in addition to its block of muscarinic receptors, scopolamine is also a competitive antagonist of 5-HT_3 receptors.

2. Materials and methods

2.1. Materials

Atropine and scopolamine were from Sigma-Aldrich (St. Louis, MO, USA). [^{3}H]*N*-methylscopolamine (84 Ci/mmol) was from Perkin Elmer (Boston, MA, USA). Human 5-HT3A (Accession: 46,098) subunit cDNA was kindly provided by J. Peters (Dundee University, UK).

2.2. Oocyte maintenance

Xenopus laevis oocytes were purchased from EcoCyte Bioscience (Castrop-Rauxel, Germany) and maintained according to standard methods (Goldin, 1992) in ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.4).

2.3. Cell culture

Human embryonic kidney (HEK) 293 cells were grown on 90 mm round tissue culture plates as monolayers in DMEM/F12 (Gibco, Life Technologies, CA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma Aldrich) at 37 °C in a moist atmosphere containing 5% CO_2.

2.4. 5-HT_3 receptor expression

5-HT3A subunit cDNA was cloned into pGEMHE for oocyte expression. cRNA was *in vitro* transcribed from linearised plasmid cDNA template using the mMessage mMachine Ultra T7 Transcription kit (Ambion, Austin, Texas, USA). Stage V and VI oocytes were injected with 50 nl of 100–600 ng/μl cRNA (5–30 ng injected), and currents were recorded 1–3 days post-injection.

5-HT3A subunit cDNA was cloned into pcDNA3.1 for expression in HEK 293 cells. Cells were transiently transfected with this cDNA using polyethyleneimine (PEI: 25 kDa, linear, powder, Polysciences Inc., Eppelheim, Germany). 30 μl of PEI (1 mg ml^{-1}), 5 μg cDNA and 1 ml DMEM were incubated for 10 min at room temperature, added drop wise to a 90 mm plate, at 80–90% confluency, and incubated for 2–3 days before harvesting.

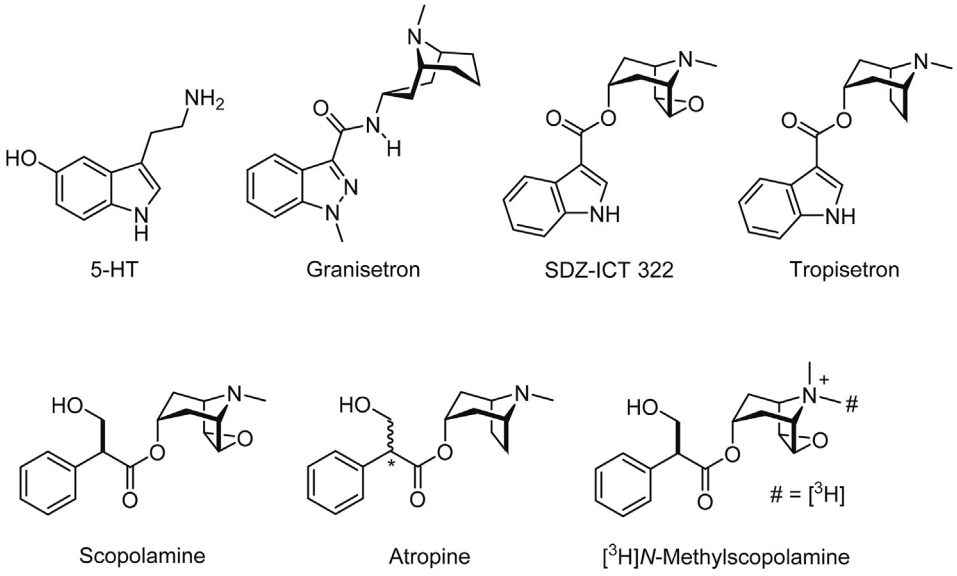

Fig. 1. Chemical structures of endogenous agonist 5-HT, 5-HT_3 receptor antagonists granisetron, tropisetron and SDZ-ICT 322, scopolamine, atropine and the radioligand [^{3}H]*N*-methylscopolamine. Note that scopolamine is a single enantiomer whereas atropine is a mixture of epimers at the indicated (asterisk) carbon atom.

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