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5-Fluorotryptamine is a partial agonist at 5-HT_3 receptors, and reveals that size and electronegativity at the 5 position of tryptamine are critical for efficient receptor function

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Abstract

Antagonists, but not agonists, of the 5-HT_3 receptor are useful therapeutic agents, and it is possible that partial agonists may also be potentially useful in the clinic. Here we show that 5-fluorotryptamine (5-FT) is a partial agonist at both 5-HT_{3A} and 5-HT_{3AB} receptors with an R_{max} (I_{max}/I_{max}5-HT) of 0.64 and 0.45 respectively. It is about 10 fold less potent than 5-HT: EC_{50}=16 and 27 μM, and K_i for displacement of [^{3}H]granisetron binding=0.8 and 1.8 μM for 5-HT_{3A} and 5-HT_{3AB} receptors respectively. We have also explored the potencies and efficacies of tryptamine and a range of 5-substituted tryptamine derivatives. At 5-HT_{3A} receptors tryptamine is a weak (R_{max}=0.15), low affinity (EC_{50}=113 μM; K_i=4.8 μM) partial agonist, while 5-chlorotryptamine has a similar affinity to 5-FT (EC_{50}=8.1 μM; K_i=2.7 μM) but is a very weak partial agonist (R_{max}=0. 0037). These, and data from 5-methyltryptamine and 5-methoxytryptamine, reveal the importance of size and electronegativity at this location for efficient channel opening.

Keywords: Ligand-gated ion channel; Cys-loop receptor; Serotonin receptor; Partial agonist, Binding site; Homology model

1. Introduction

The 5-HT_3 receptor is a member of Cys-loop family of ligand-gated ion channels, which also includes nicotinic acetylcholine, GABA and glycine receptors (Reeves and Lummis, 2002). These proteins are pentamers, and each subunit has a large extracellular N-terminal domain, four transmembrane helices (M1–M4) and an intracellular loop between M3 and M4. The binding site is located at the interface of two adjacent subunits and is formed by the convergence of three loops (A–C) from the principal subunit and another three loops (D–F) from the complementary subunit (Reeves and Lummis, 2002). Molecular details of the binding pocket have been extrapolated from the structure of the acetylcholine binding protein, which is homologous to the extracellular domain of Cys-loop receptors, and a range of amino acid residues that are important for agonist and antagonist binding have been identified (Reeves et al., 2003; Thompson et al., 2005). 5-HT_3 receptors can function as homopentamers of 5-HT_{3A} receptor subunits, or as heteropentamers of 5-HT_{3A} and 5-HT_{3B} receptor subunits (5-HT_{3AB} receptors). The incorporation of B subunits results in some changes in the biophysical characteristics of the receptor, but has little effect on the pharmacological profile (Brady et al., 2001; Davies et al., 1999; Dubin et al., 1999).

5-HT_3 receptor antagonists have been suggested to be potentially useful in treating inflammatory pain, anxiety, depression, schizophrenia, and drug abuse (Greenshaw and Silverstone, 1997), and are currently in clinical practice for the treatment of irritable bowel syndrome and emesis (Butler et al., 1988; Camilleri et al., 2000; Sanger and Nelson, 1989). It is therefore not surprising that many 5-HT_3 receptor antagonists have been developed. There are, however, fewer 5-HT_3 selective agonists. 2-methyl-5-HT and *m*CBPG have been widely used, and some novel compounds have been developed more recently such as benzoxazoles (Yoshida et al., 2005) and pyrroloquinoxaline-related compounds (Campiani et al., 1997). Here we explore the agonist properties of a compound closely related to 5-HT, 5-fluorotryptamine (5-FT), at

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both 5-HT_{3A} and 5-HT_{3AB} receptors, and compare them to the properties of 5-HT, *m*CBPG and tryptamine. We also explore several other 5-substituted tryptamine derivatives.

2. Materials and methods

2.1. Materials

All cell culture reagents were obtained from Gibco BRL (Paisley, U.K.), except foetal calf serum which was from Labtech International (Ringmer, U.K.). [^{3}H]granisetron (63.5 Ci mmol^{-1}) was from PerkinElmer (Boston, Massachusetts, USA). 5-FT, 5-chlorotryptamine (5-ClT), 5-methyltryptamine (5-MeT), 5-methoxytryptamine (5-MeOT) and tryptamine (Fig. 1) were from Sigma-Aldrich Co. Ltd. (Poole, Dorset, U.K.). All other reagents were of the highest obtainable grade.

2.2. Cell culture and oocyte maintenance

Human embryonic kidney (HEK) 293 cells were maintained in DMEM:F12 (Dulbecco's Modified Eagle Medium/Nutrient Mix F12 (1:1)) with GlutaMAX™ containing 10% foetal calf serum at 37 °C and 7% CO_2 in a humidified atmosphere. *Xenopus laevis* oocyte positive females were purchased from NASCO (Fort Atkinson, Wisconsin, USA) and maintained according to standard methods (Goldin, 1992).

Harvested stage V–VI *Xenopus* oocytes were washed in six changes of ND96 (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.5), de-folliculated in 1.5 mg ml^{-1} collagenase Type 1A for approximately 2 h. Enzyme treatment was terminated by washing in six changes of ND96 and oocytes were stored in ND96 containing 2.5 mM sodium pyruvate, 50 mM gentamicin and 0.7 mM theophylline.

2.3. Receptor expression

Mouse 5-HT_{3A} (accession number: AY605711) or 5-HT_{3B} (accession number: NM_020274, kindly provided by Ewen Kirkness) subunit cDNAs were cloned into pGEMHE for oocyte expression (Liman et al., 1992). cRNA was in vitro transcribed from linearised (NheI) plasmid cDNA template using the mMessage mMachine T7 kit (Ambion, Austin, Texas, USA). Stage V and VI oocytes were injected with 50 ng–100 ng cRNA, and recorded from 1–4 days post-injection. For expression in HEK 293 cells, 5-HT_3 receptor subunit cDNAs were cloned into pcDNA3.1 (Invitrogen Ltd., Paisley, UK.). Mutagenesis reactions were performed using the Kunkel method and confirmed by DNA sequencing. Cells were transfected using calcium phosphate precipitation at 80–90% confluency (Jordan et al., 1996). Following transfection cells were incubated for 3–4 days before assay.

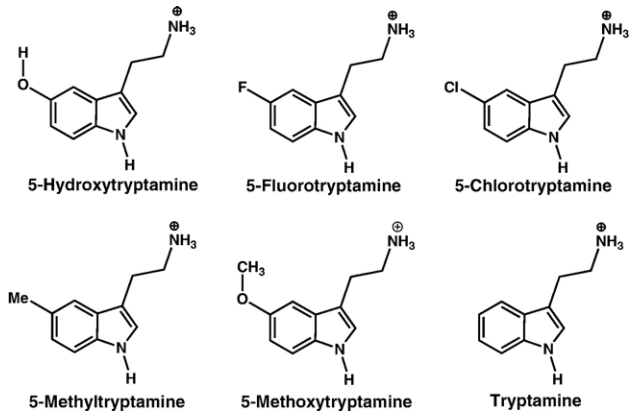

Fig. 1. Structures of the 5-HT_3 receptor agonists used in this study.

2.4. Radioligand binding

This was undertaken in HEK 293 cells which provide an established and robust method of studying ligand binding. Methods were as previously described (Lummis et al., 1993), with minor modifications. Briefly, transfected HEK 293 cells were washed twice with phosphate buffered saline (PBS) at room temperature and scraped into 1 ml of ice-cold HEPES buffer (10 mM, pH 7.4) containing the following proteinase inhibitors (PI): 1 mM EDTA, 50 μg ml^{-1} soybean trypsin inhibitor, 50 μg/ml bacitracin and 0.1 mM phenylmethylsulphonyl fluoride. After thawing, they were washed with HEPES buffer, resuspended, and 50 μg of cell membranes incubated in 0.5 ml HEPES buffer containing 0.5 nM [^{3}H]granisetron (a concentration approximately equivalent to the K_d); non-specific binding was determined using 10 μM quipazine. Competition binding was performed using ligand concentrations from 0.1 μM–10 mM. Reactions were incubated for at least 1 h at 4 °C and terminated by vacuum filtration using a Brandel cell harvester onto GF/B filters presoaked in 0.3% polyethyleneimine. Radioactivity was determined by scintillation counting using a Beckman LS6000SC (Fullerton, California, USA). Competition binding data were analyzed by iterative curve fitting (GraphPad Prism v3.02, GraphPad Software, San Diego, California, USA), according to the equation:

$$y = B_{\min} + \frac{B_{\max} - B_{\min}}{1 + 10^{[L] - \log IC_{50}}}$$

where B_{min} is the lowest total binding, B_{max} is the maximum specific binding at equilibrium, $[L]$ is the concentration of competing ligand and IC_{50} is the concentration of competing ligand that blocks half of the specific bound radioligand. K_i values were estimated from IC_{50} values using the Cheng–Prusoff equation:

$$K_i = \frac{IC_{50}}{1 + [L]/K_d}$$

where K_i is the equilibrium dissociation constant for binding of the unlabeled antagonist, $[L]$ is the concentration of radioligand and K_d is the equilibrium dissociation constant of the radioligand.

2.5. Electrophysiology

Agonist-induced currents were recorded at 22–25 °C from individual oocytes using the OpusXpress system (Molecular Devices Axon Instruments, Union City, CA). 5-HT, *m*-chlorophenylbiguanide (*m*CPBG), 5-FT and tryptamine