Neuropharmacology 98 (2015) 31–40

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Invited review

A review of fluorescent ligands for studying 5-HT$_3$ receptors

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ARTICLE INFO

Article history:
Available online 16 April 2015

Keywords:
Fluorescent ligand
5-HT$_3$ receptor
Fluorescence polarisation
Imaging
Synthesis
Flow cytometry

ABSTRACT

The use of fluorescence is a valuable and increasingly accessible means of probing the pharmacology and physiology of cells and their receptors. To date, the use of fluorescence-based methods for 5-HT$_3$ receptor research has been quite limited and, although a variety of approaches have been described, these are broadly distributed throughout the literature. In this review we condense these findings into a single, accessible source of reference with the hope of promoting the use of these valuable molecular probes.

This article is part of the Special Issue entitled 'Fluorescent Tools in Neuropharmacology'.

Contents

1. Introduction

Fluorescence can be used to directly label receptors or the ligands that bind to them. For example, it has become common practice to visualise receptors by genetically fusing green fluorescent protein (GFP) and its engineered homologues (Giepmans et al., 2006; Shaner et al., 2005). This has allowed scientists to visualise receptor biogenesis, membrane targeting and ligand-mediated receptor internalisation in real-time (Ilegems et al., 2004). The application of these fluorescent proteins in Förster resonance energy transfer (FRET) has also allowed the study of agonist-induced

Abbreviations: 5-HT, 5-hydroxtryptamine; BHK, baby hamster kidney; BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene; Cou, coumarin; Cy, cyanine; Dns, dansyl; Flu, fluorescein; FRET, Förster resonance energy transfer; GFP, green fluorescent protein; HEK, human endothelial kidney; NBD, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl); Rho, rhodamine; SAR, structure–activity relationship; SiRho, Silarhodamine; TAMRA, tetramethyl rhodamine; TrCou, triazole coumarine.

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http://dx.doi.org/10.1016/j.neuropharm.2015.04.002
0028-3908/

conformational changes, ligand binding and receptor stoichiometry (Ilegems et al., 2005; Miles et al., 2013). However, because of their size (e.g. GFP 238 aa, 27 kDa) it can sometimes be challenging to insert a fluorescent protein without significantly altering the expression and function of the receptor (Andresen et al., 2004). Small-molecule fluorophores provide an alternative approach that allows the physical environments of the fluorophores to be monitored and the function, pharmacology and physiology of the receptor to be measured (Pantoja et al., 2009; Tairi et al., 1998).

Traditionally high-affinity, radiolabelled ligands were used to characterise ligand binding at 5-HT_3 receptors, but fluorescent ligands are providing new opportunities for quantifying binding interactions. In contrast to traditional radioligand methods, fluorescent approaches can be non-destructive and readily adapted to high-throughput methods that provide fast, economical and information rich outputs without the generation of radioactive waste. These direct measurements of quantitative pharmacological parameters rely upon the creation of fluorescent tracers that have a sufficiently high affinity for the protein target and give suitable fluorescent signals. 5-HT_3 receptors provide excellent opportunities for creating these ligands as established high-affinity ligands already exist and have synthetically accessible regions that permit the addition of large substituents. With continued interest in the associations of 5-HT_3 receptors with psychiatric and neurological disorders and recent reports of novel 5-HT_3 ligands and allosteric modulators, fluorescent 5-HT_3 receptor ligands are likely to find further utility in the assessments of *in vitro* and *in vivo* pharmacology (Machu, 2011; Thompson, 2013; Walstab et al., 2010).

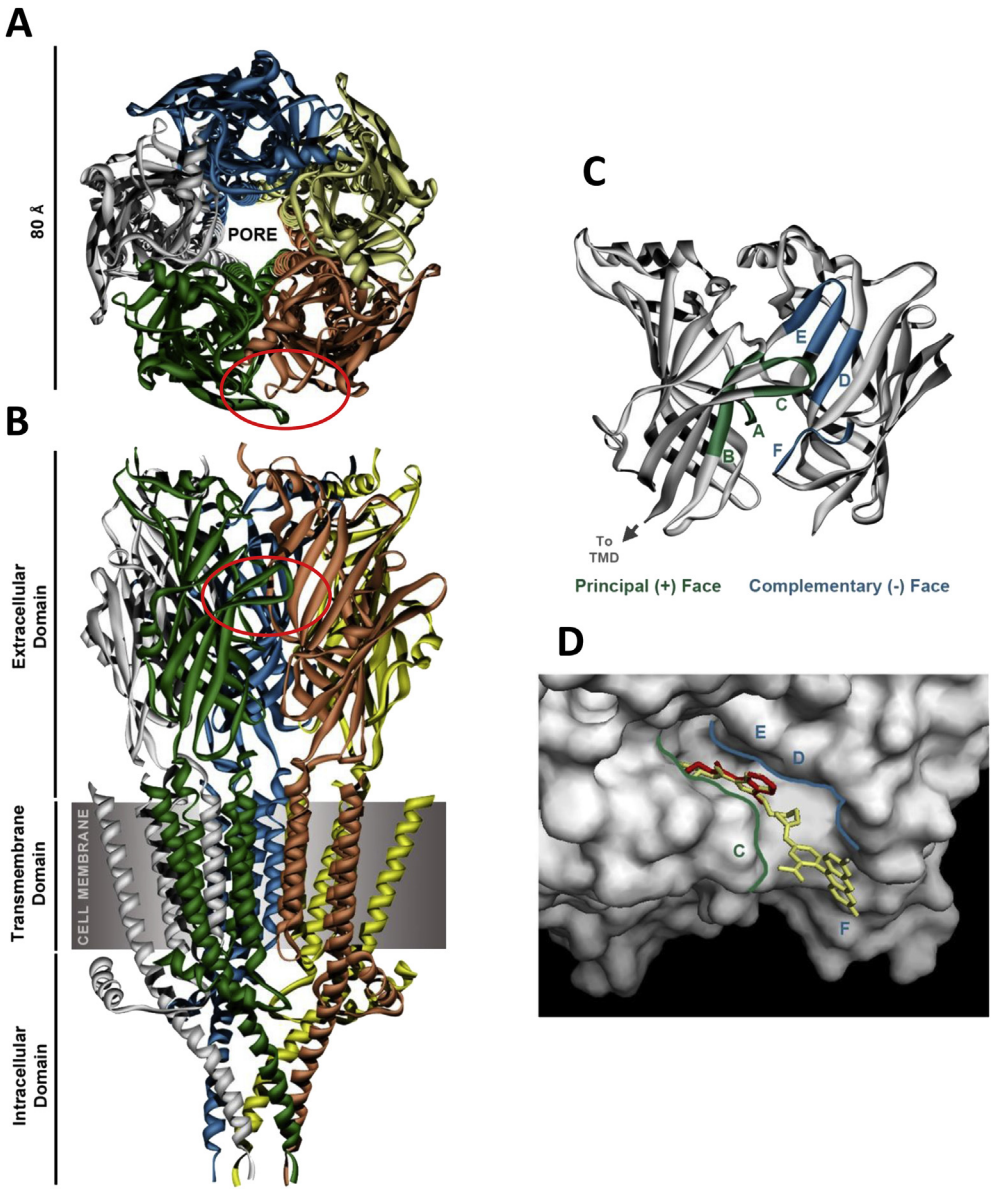

Fig. 1. The structure of a 5-HT_3 receptor. The 5-HT_3 receptor seen from, (A) the extracellular side and, (B) parallel to the plane of the plasma membrane (shown in grey). Agonists and competitive antagonists bind to the orthosteric binding sites that are located in the extracellular domain at the interface of adjacent subunits (*circled*). (C) The extracellular domains of only two subunits are shown to highlight the main protein loops that interact with ligands in the orthosteric binding site. Loops A–C (green) are found on the principal face and loops D–F (blue) are on the complementary face of the binding site. The importance of residues within these loops is reviewed in Thompson et al. (2010). The structure is the mouse 5-HT_3 receptor (PDB: 4PIR). The five stabilising nanobodies whose recognition loops interact strongly with residues in the receptor orthosteric binding sites have been removed for clarity. (D) A model for **Gran-Flu** binding to the 5-HT_3 receptor. **Gran-Flu** (yellow) was aligned with granisetron (red) in complex with 5HTBP (PDB: 2YME). The labelling and colour code of binding loops is the same as in panel C.