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The binding orientations of structurally-related ligands can differ; A cautionary note

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ABSTRACT

Crystal structures can identify ligand-receptor interactions and assist the development of novel therapeutics, but experimental challenges sometimes necessitate the use of homologous proteins. Tropisetron is an orthosteric ligand at both 5-HT_3 and α7 nACh receptors and its binding orientation has been determined in the structural homologue AChBP (pdbid: 2WNC). Co-crystallisation with a structurally-related ligand, granisetron, reveals an almost identical orientation (pdbid; 2YME). However, there is a >1000-fold difference in the affinity of tropisetron at 5-HT_3 versus α7 nACh receptors, and α7 nACh receptors do not bind granisetron. These striking pharmacological differences prompt questions about which receptor the crystal structures most closely represent and whether the ligand orientations are correct. Here we probe the binding orientation of tropisetron and granisetron at 5-HT_3 receptors by *in silico* modelling and docking, radioligand binding on cysteine-substituted 5-HT_3 receptor mutants transiently expressed in HEK 293 cells, and synthetic modification of the ligands. For 15 of the 23 cysteine substitutions, the effects on tropisetron and granisetron were different. Structure-activity relationships on synthesised derivatives of both ligands were also consistent with different orientations, revealing that contrary to the crystallographic evidence from AChBP, the two ligands adopt different orientations in the 5-HT_3 receptor binding site. Our results show that even quite structurally similar molecules can adopt different orientations in the same binding site, and that caution may be needed when using homologous proteins to predict ligand binding.

1. Introduction

Tropisetron (e.g. Navoban®, ICS 205-930), granisetron (e.g. Kytril®, Sancuso®, Granisol™) and other structurally-related drugs are used to alleviate the symptoms of nausea and vomiting following general anaesthesia, cancer chemotherapy and radiotherapy (Thompson, 2013). The therapeutic effect of these is due to their high-affinity competitive block of 5-HT_3 receptors in the gut and brain stem.

5-HT_3 receptors belong to the Cys-loop family of transmembrane ligand-gated ion-channels that are responsible for fast synaptic neurotransmission in the central and peripheral nervous systems. All members of this family are composed of five subunits, each of which contains an extracellular, a transmembrane and an intracellular domain (Miller and Smart, 2012; Thompson et al., 2010). Binding of tropisetron and granisetron to extracellular binding sites blocks the action of the native agonist 5-HT. These binding sites are at the interface of two adjacent subunits and form a hydrophobic cavity that is composed of amino acids from loops A - C in the principal subunit interface and loops D - F in the complementary subunit interface (Fig. 1).

Abbreviations: 5-HT, 5-hydroxytryptamine; nACh, nicotinic acetylcholine; GABA, gamma-aminobutyric acid; HEK, human embryonic kidney; AChBP, acetylcholine binding protein; 5HTBP, an AChBP mutant modified to resemble the 5-HT_3R binding site; SAR, structure-activity relationship.

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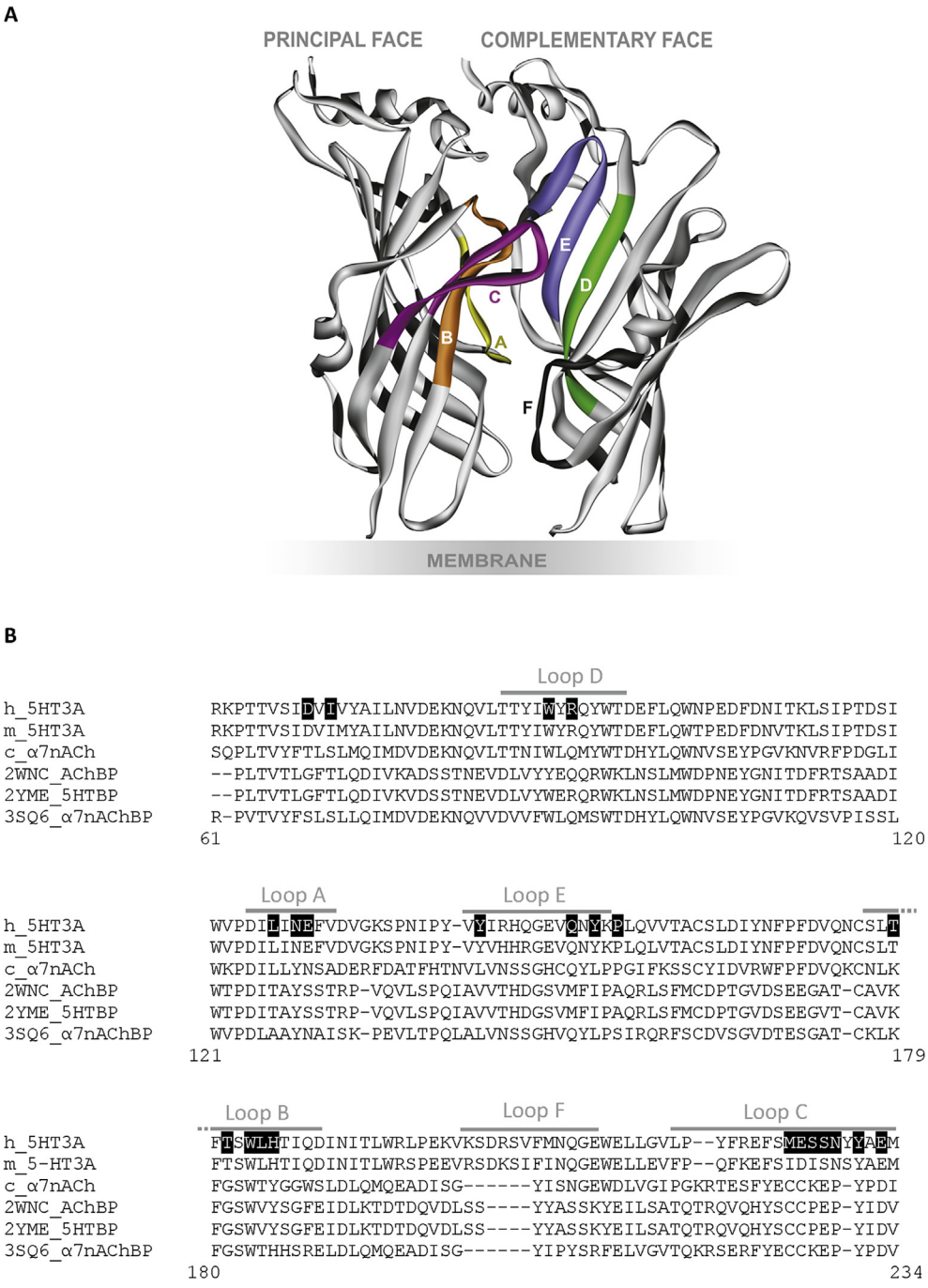

Fig. 1. Positions of the residues mutated in this study. (**A**) A cartoon showing the orthosteric binding site of the 5-HT$_3$ receptor formed by binding loops A–F. The extracellular binding site is found at the interface of two adjacent subunits. For clarity only two subunits are shown. (**B**) An amino acid sequence alignment showing the positions of mutated residues (white text, black boxes). The six recognised binding loops are shown as grey lines. The proteins are sequences from human 5-HT3A (P46098), mouse 5-HT3A (Q6J1J7), chick α7 nACh (F1P4Y5) and sequences taken from the AChBP crystal structures 2YME, 2WNC and 3SQ6. c_α7nACh and h_5HT3 are the sequences of receptors used in the binding studies presented here. EMBOSS Needle shows that the sequence of 2WNC_AChBP has a closer identity and similarity to c_α7nACh (Id = 27.4%; Sim = 41.8%) than to h_5HT3 (Id = 19.4%; Sim = 31.8%) (Rice et al., 2000). To facilitate comparisons with previous work, the numbering used throughout this manuscript refers to residues at equivalent positions of the mouse 5-HT3A subunit (Q6J1J7).

Crystallisation studies have attempted to evaluate the binding orientations of several 5-HT$_3$ receptor ligands, but all of these have been performed on a close structural homologue rather than the native receptor. For example, cocaine (pdbid: 2PGZ) and tropisetron (2WNC) were crystallised in the binding site of acetylcholine binding protein (AChBP), while granisetron (2YME) was crystallised with an AChBP mutant containing two loop D amino acids substitutions that increased its affinity for this ligand (Hansen and Taylor, 2007; Hibbs et al., 2009; Kesters et al., 2013). Other studies have sought to explore the binding orientation of granisetron using homology modelling and ligand docking, but the majority of these are over a decade old and do not benefit from our improved understanding of the receptor family or developments in the computer software used to model them (reviewed in Thompson et al.,