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The 5-HT_3 receptor - the relationship between structure and function

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Abstract

The 5-hydroxytryptamine type-3 (5-HT_3) receptor is a cation-selective ion channel of the Cys-loop superfamily. 5-HT_3 receptor activation in the central and peripheral nervous systems evokes neuronal excitation and neurotransmitter release. Here, we review the relationship between the structure and the function of the 5-HT_3 receptor. 5-HT3A and 5-HT3B subunits are well established components of 5-HT_3 receptors but additional *HTR3C*, *HTR3D* and *HTR3E* genes expand the potential for molecular diversity within the family. Studies upon the relationship between subunit structure and the ionic selectivity and single channel conductances of 5-HT_3 receptors have identified a novel domain (the intracellular MA-stretch) that contributes to ion permeation and selectivity. Conventional and unnatural amino acid mutagenesis of the extracellular domain of the receptor has revealed residues, within the principle (A-C) and complementary (D-F) loops, which are crucial to ligand binding. An area requiring much further investigation is the subunit composition of 5-HT_3 receptors that are endogenous to neurones, and their regional expression within the central nervous system. We conclude by describing recent studies that have identified numerous *HTR3A* and *HTR3B* gene polymorphisms that impact upon 5-HT_3 receptor function, or expression, and consider their relevance to (patho)physiology.

Keywords

5-Hydroxytryptamine type-3 (5-HT_3) receptor; Ion selectivity; Single channel conductance; Ligand binding domain; 5-HT_3 subunit distribution; *HTR3A* and *HTR3B* polymorphisms

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1. Introduction

The 5-hydroxytryptamine type-3 (5-HT_3) receptor is a cation-selective ligand-gated ion channel mediating neuronal depolarization and excitation within the central and peripheral nervous systems. The cloning of the first 5-HT_3 receptor subunit, termed 5-HT3A (note the nomenclature employed in this article adopts the recommendations of NC-IUPHAR; Collingridge et al., 2009), placed the 5-HT_3 receptor within the Cys-loop superfamily of ligand-gated ion channels (LGICs). The structural cousins of the 5-HT_3 receptor in vertebrates are the cation-selective nicotinic acetylcholine (nACh) receptors, the anion selective gamma-aminobutyric acid-type-A ($GABA_A$) and strychnine-sensitive glycine receptors (Maricq et al., 1991; Peters et al., 1997, 2005; Reeves and Lummis, 2002; Lester et al., 2004; Sine and Engel, 2006). A far less extensively studied Zn^{2+}-activated cation-selective channel is an additional member of the Cys-loop superfamily (Davies et al., 2003; Houtani et al., 2005). In common with all members of the Cys-loop family, 5-HT_3 receptors are assembled as a pentamer of subunits that surround, in pseudo-symmetric manner, a central ion channel (Boess et al., 1995; Green et al., 1995; Barrera et al., 2005). The individual subunits comprise a large extracellular N-terminal domain (ECD), four transmembrane domains (M1-M4) connected by intracellular (M1-M2 and M3-M4) and extracellular (M2-M3) loops, of which the M3-M4 linker is the most extensive, and an extracellular C-terminus (Karlin, 2002; Reeves and Lummis, 2002; Lester et al., 2004; Peters et al., 2005; Unwin, 2005; Sine and Engel, 2006). M2 lines the intramembraneous portion of the ion conduction pathway, surrounded by M1, M3 and M4 that partition it from the membrane lipid (Miyazawa et al., 2003). Amino acids within an α-helical portion of the large intracellular loop (the MA-helix) also contribute to the ion permeation pathway (Peters et al., 2005). However, the latter is not essential to receptor function. Indeed, the large intracellular loop of the mouse 5-HT3A subunit can be replaced by the short heptapeptide M3-M4 linker of the Cys-loop receptor homologue Glvi (a proton-gated channel from the cyanobacterium *Gloebacter violaceus*, Bocquet et al., 2007) without loss of function (Jansen et al., 2008).

2. Molecular composition

2.1. The 5-HT3A subunit

The 5-HT3A receptor subunit (Maricq et al., 1991) was initially isolated using an expression cloning strategy employing the NCB-20 murine hybridoma cell line that expresses functional 5-HT_3 receptors at high density (Peters and Lambert, 1989; McKernan, 1992). Critical to the success of this approach 5-HT3A subunits assemble into functional homopentamers upon heterologous expression in mammalian cell hosts and *Xenopus laevis* oocytes. 5-HT3A subunit species orthologues have been cloned from rat superior cervical ganglia and brain (Isenberg et al., 1993; Miyake et al., 1995; Akuzawa et al., 1996) human hippocampus, amygdala and colon (Belelli et al., 1995; Miyake et al., 1995; Lankiewicz et al., 1998), guinea pig small intestine (Lankiewicz et al., 1998), ferret colon (Mochizuki et al., 2000) and dog brain (Jensen et al., 2006) (Table 1). Inclusive of a predicted signal peptide of 23 residues, canonical 5-HT3A subunit polypeptides identified to date range

between 478 and 490 amino acids in length and exhibit a high degree of conservation (83-87% sequence identity, excluding the signal peptide).

The human *HTR3A* gene comprises 9 exons and 8 introns and is located upon chromosome 11 (Weiss et al., 1995) (Table 1). Alternative splicing of the primary RNA transcript encoding mouse, rat and guinea pig, but not human, dog, or ferret, 5-HT3A subunits results in 'long' (5-HT3A(a)) and short (5-HT3A(b)) isoforms (Hope et al., 1993; Isenberg et al., 1993; Werner et al., 1994; Belelli et al., 1995; Lankiewicz et al., 1998; Mochizuki et al., 2000; Jensen et al., 2006). The 5-HT3A(b) isoform lacks 6 (mouse and guinea pig), or 5 (rat) amino acid residues within the large intracellular loop linking M3 and M4. Differences in the efficacy of certain 5-HT_3 receptor agonists at the two isoforms have been noted along with differential modulation by activators of protein kinase A (PKA) and protein kinase C (PKC) (Downie et al., 1994; van Hooft et al., 1997; Niemeyer & Lummis, 1999; Hubbard et al., 2000). The human isoform corresponds to the 5-HT3A(b) subunit (Belelli et al., 1995; Miyake et al., 1995). Additional truncated and extended splice variants of the human 5-HT3A subunit, unofficially dubbed 5-HT3AT and 5-HT3AL, respectively, are not functional when expressed alone, however, upon co-expression with canonical 5-HT3A subunits receptors with modified functional properties are formed (Bruss et al., 2000).

2.2. The 5-HT3B subunit

The isolation of the second member of the 5-HT_3 receptor subunit family (i.e., 5-HT3B) occurred in 1999 *via* the screening of human genomic sequence data (Davies et al., 1999; Dubin et al., 1999). The human *HTR3A* and *HTR3B* gene loci are in close proximity on the long arm of chromosome 11 (Table 1) and it is likely that these members of the family arose from a local duplication event (Davies et al., 1999). The human 5-HT3A and 5-HT3B subunits share 41% amino acid sequence identity. Mouse and rat orthologs of the 5-HT3B subunit have only 73% identity to the human sequence, but their gene structure is conserved with *HTR3A*. The human 5-HT3B subunit was initially reported to comprise 441 amino acids (Davies et al., 1999; Dubin et al., 1999), but latterly amended to 436 due to a revised prediction of the sequence at which translation is initiated (Hanna et al., 2000). Furthermore, tissue specific alternative promoters in the *HTR3B* gene result in different transcription start sites in the intestine (the canonical form) *versus* the brain. One brain transcript (unofficially dubbed BT-1) predicts a translation product differing from the canonical form in only the signal peptide. However, the second (BT-2) lacks the coding sequence for a substantial portion of the N-terminal domain (Tzvetkov et al., 2007). It remains to be shown if the predicted proteins are present in brain tissue.

Complementary DNA encoding the 5-HT3B subunit introduced into mammalian cells, or *Xenopus* occytes, does not direct the formation of functional receptors, or specific 5-HT_3 binding sites (Davies et al., 1999; Dubin et al., 1999; Hanna et al., 2000; Boyd et al., 2002). However, when co-expressed with the 5-HT3A subunit the 5-HT3B subunit assembles into heteromeric receptors that display distinct biophysical characteristics as described in Section 5 (Davies et al., 1999; Dubin et al., 1999; Boyd et al., 2002; Reeves and Lummis, 2006). Atomic force microscopy applied to human epitope-tagged receptor subunits heterologously expressed in tsA-201 cells indicates the heteromeric 5-HT_3AB receptor contains two 5-

HT3A and three 5-HT3B subunits assembled with the order B-B-A-B-A (Barrera et al., 2005). Whether the same, fixed, stoichiometry holds for 5-HT_3AB receptors native to neurones remains to be addressed.

2.3. The 5-HT3C, D and E subunits

Three additional human 5-HT_3 receptor subunit genes occur as a close cluster on human chromosome 3 at 3q27 (Niesler et al., 2008). Unfortunately, two provisional gene nomenclatures arose in parallel namely: *HTR3C* (aka *HTR3C3*), *HTR3D*, *HTR3E* (aka *HTR3C1long*), *HTR3C2* and *HTR3C4* (Karnovsky et al., 2003; Niesler et al., 2003). However, *HTR3C2* and *HTR3C4* are considered to be pseudo-genes in man (Karnovsky et al., 2003) and do not require further consideration. Here, we adopt the HUGO Gene Nomenclature Committee (HGNC) names *HTR3C*, *HTR3D* and *HTR3E* (Table 1). The 279 residue 5-HT3D subunit predicted by Niesler et al. (2003) lacks a signal peptide and most of the ECD including the signature Cys-Cys loop. However, an unprocessed precursor predicted to encode a 454 amino acid polypeptide has also been reported (Jensen et al., 2008). The predicted products of the putative *HTR3C* and *HTR3E* genes (Table 1) share 36% and 39% amino acid identity, respectively, with the human 5-HT3A subunit (Karnovsky et al., 2003). A splice variant of the 5-HT3E subunit, dubbed 5-HT3E(a) (aka 5-HT3C1short), differs from the former in the N-terminal and signal peptide sequences (Karnovsky et al., 2003; Niesler et al., 2007). The 5-HT3C, 5-HT3D, or 5-HT3E subunits do not traffic to the cell surface, or form a ligand binding site, when singularly expressed in HEK-293 cells (Niesler et al., 2007). However, their trafficking to the cell surface is facilitated by the 5-HT3A subunit with which they co-immunoprecipitate. Co-expression of the 5-HT3A subunit with any one of the 5-HT3C, 5-HT3D, or 5-HT3E subunits does not result in receptors with substantially altered pharmacological profiles (Niesler et al., 2007, 2008). It remains to be tested whether the biophysical properties of heteromeric receptors incorporating the 5-HT3C, 5-HT3D, or 5-HT3D subunits are distinct from those of either the 5-HT_3A, or 5-HT_3AB receptors.

3. Post-translational modifications, protein associations and biogenesis

It is increasingly appreciated that common mechanisms exist to promote and regulate the functional expression of the various members of the LGIC family within the cell membrane. These mechanisms range from post-translational modifications to chaperone proteins that facilitate the fidelity of receptors trafficked to the cell membrane; a prerequisite for their function.

Atypically for Cys-loop LGIC subunits, heterologous expression of the 5-HT3A subunit efficiently generates a functional cell surface receptor. This contrasts with the 5-HT3B subunit which when expressed alone fails to exit the endoplasmic reticulum (ER) due, at least in part, to a retention motif (sequence CRAR) within the short first intracellular loop between M1 and M2 (Boyd et al., 2003). The co-expression of the 5-HT3A with the 5-HT3B subunit may shield this ER retention motif allowing export of the heteromeric 5-HT_3AB receptor to the cell membrane. Remarkably, co-expression of the 5-HT3A and 5-HT3B subunits in tsA-201 cells fails to generate detectable homomeric 5-HT3A receptors

indicating that, when expressed, the 5-HT3B subunit forces a preference for expression of the heteromeric 5-HT_3 receptor (Barrera et al., 2005). The underlying mechanism for the selective expression of heteromeric receptors in this heterologous system is unknown and it is uncertain whether this observation can be extrapolated to 5-HT_3 receptors in a neuronal environment.

N-glycosylation of at least the 5-HT3A subunit has a direct impact upon the formation of a receptor that binds radioligand and the ability to export from the ER and hence expression at the cell surface (Boyd et al., 2002; Monk et al., 2004; Quirk et al., 2004). Within the h5-HT3A subunit, each of the four consensus sequence *N*-glycosylation sites within the N-terminal ECD domain is *N*-glycosylated (Monk et al., 2004). Interestingly, the three *N*-glycosylation sites conserved between various species investigated to date (i.e., N104, N170 and N186; numbering in human including the signal peptide) display a more pronounced effect on the formation of a radioligand binding site and expression in the cell membrane compared to the most N-terminal *N*-glycosylation site (human numbering N28), which is absent in rodents (Monk et al., 2004). However, the presence of sugar residues is not required to preserve a ligand binding site (see Section 4) once the receptor has matured (Green et al., 1995).

5-HT_3 receptors also undergo post-translational modification through phosphorylation at kinase consensus sites primarily in the large cytoplasmic M3-M4 loop. The guinea pig 5-HT3A subunit is phosphorylated at S409 but a functional correlate of this modification remains to be shown (Lankiewicz et al., 2000). The function of native 5-HT_3 receptors is modulated by activators of PKA (Yakel et al., 1991) and PKC (Zhang et al., 1995) or by kinases such as casein kinase II introduced into the cell interior (Jones and Yakel, 2003). However, although the large intracellular loop of the 5-HT3A subunit contains the appropriate consensus sequences for such kinases, there is no proof that their action is direct. Indeed, potentiation of responses mediated by the mouse 5-HT3A receptor by activators of PKC persists when all PKC consensus sequences are mutated from the M3-M4 linker. Instead, enhancement of macroscopic current responses evoked by 5-HT results from increased cell surface expression of the receptor due to structural rearrangements of F-actin with which the receptor clusters (Sun et al., 2003).

A number of ER chaperone proteins associate with the 5-HT_3 receptor (e.g., BiP, calnexin, RIC-3) and, as is typical for such proteins, they likely promote correct folding, oligomerisation, post-translational modification and export from the ER (Boyd et al., 2002). The actions of RIC-3, however, appear dependent upon the receptor isoform and species origin of the transfected cells (see Castillo et al., 2005 *versus* Cheng et al., 2005, 2007). Within transfected mammalian cells, a promotion of homomeric (h5-HT_3A) and an inhibition of heteromeric (h5-HT_3AB) receptor expression within the cell membrane are apparent (Cheng et al., 2007). Other proteins have also been shown to influence cell surface 5-HT_3 receptor expression and their clustering. For instance, the cytoplasmic cyclophillin A, reportedly *via* an integral peptidyl prolyl isomerase activity, promotes expression in the cell membrane (Helekar et al., 1994; Helekar and Patrick, 1997).

Once formed in the ER and Golgi, elegant studies from Vogel's group indicate an efficient folding and assembly of the receptor, which is then trafficked in vesicle-like structures along microtubules to the cell surface (Ilegems et al., 2004). Following insertion into the cell membrane, it is evident that 5-HT_3A receptors form clusters in F-actin-rich regions in various cells including hippocampal neurones (Emerit et al., 2002; Grailhe et al., 2004; Ilegems et al., 2004). Furthermore, F-actin depolymerisation disrupts cluster formation supporting the anchoring of the 5-HT_3A receptor to specific subcellular sites (Emerit et al., 2002), conceptually consistent with the precise anatomical location needed by a receptor known to mediate fast synaptic neurotransmission. Agonist interaction with the 5-HT_3 receptors at the cell surface evokes their internalisation (Ilegems et al., 2004; Freeman et al., 2006). Although the mechanisms have yet to be studied in detail, the process probably involves clathrin-coated early endosomes (Freeman et al., 2006).

4. The 5-HT_3 receptor ligand binding site

The 5-HT_3 receptor has strong structural and functional homology with the nicotinic ACh receptor (see Reeves and Lummis, 2002; Peters et al., 2005; Thompson and Lummis, 2006, 2007 for reviews). Indeed, chimeric receptors combining the ECD of the α7 nicotinic ACh receptor subunit and the transmembrane domains of the 5-HT_3A receptor are activated by ACh and have channel properties approximating to those of the 5-HT_3A receptor (Eiselé et al., 1993). Thus, while there are no high resolution images of these proteins, a number of homology models have been created from the structure of the acetylcholine binding protein (AChBP) (Brejc et al., 2001; Schapira et al., 2002; Maksay et al., 2003; Reeves et al., 2003). Importantly, the similarity between the AChBP and the ECD of Cys-loop receptors has been confirmed by crystallisation of a nicotinic ACh α-subunit monomer and a related prokaryotic channel (Dellisanti et al., 2007; Hilf and Dutzler, 2008). As the accuracy of the AChBP structures is now established, we can be reasonably confident that structural details based on AChBP are broadly correct. Thus the ligand binding site lies at the interface of two adjacent subunits and is formed by three loops (A-C) from the 'principal' subunit and three β-strands (D-F) from the adjacent or 'complementary' subunit (Fig. 1). A number of studies have identified key residues that are involved in both agonist and antagonist binding and these are described below (see Thompson and Lummis, 2006, 2007 for comprehensive reviews). In the following discussion, we employ the amino acid numbering of the mouse 5-HT3A subunit, including the signal peptide.

4.1. Loops A-C

Loop A (Fig. 1) contributes only a single amino acid residue to the binding pocket, and early models indicated this was N128 (Maksay et al., 2003; Reeves et al., 2003). However subsequent data revealed that mutations of N128 did not significantly alter ligand binding parameters (Sullivan et al., 2006) and a recent, more comprehensive, study using both natural and unnatural amino acids, concluded that E129, rather than N128, or F130, faces into the binding pocket (Price et al., 2008). The data specifically indicate a critical hydrogen bond between the E129 and the hydroxyl group of 5-HT. Other loop A residues, such as N128 and F130, play a role in receptor gating, but are not directly involved in binding ligands (Price et al., 2008). Loop A may also be involved in receptor assembly because

mutations of W121 and P123 result in receptors that do not traffic to the cell surface (Spier and Lummis, 2000; Deane and Lummis, 2001).

Loop B (Fig. 1) contains a critical tryptophan ligand binding residue (W183) that contributes to a cation-π interaction between the pi electron density of tryptophan and the primary amine of 5-HT (Spier and Lummis, 2000; Beene et al., 2002). The equivalent tryptophan residue in the nicotinic ACh receptor α1 subunit (W149) also forms such an interaction (Zhong et al., 1998) and molecular details from AChBP have confirmed that this tryptophan (147 in AChBP) is a key component of both nicotine and carbamylcholine binding (Brejc et al., 2001).

The most important aromatic residue within loop C (Fig. 1) is probably Y234 that lies opposite to the loop B tryptophan in the ligand binding pocket. Y234 is involved in ligand binding whilst mutation of the closely positioned Y240 does not have any significant effects (Price and Lummis, 2004). Unnatural amino acid mutagenesis has revealed that an aromatic residue at position 234 is essential for both binding and function and a group at the 4 position of the same size as a hydroxyl is important for efficient receptor function (Beene et al., 2004). Of all 5-HT3A subunit binding loop residues, those of loop C are the most divergent between species. They have thus been considered as candidates for the differing pharmacology of rodent and human 5-HT_3 receptors. However, point mutations throughout the loop C region did not identify any single residues that were essential for binding of the agonist *m*-chlorophenylbiguanide (*m*CPBG), or the antagonist (+)-tubocurarine (+-Tc), suggesting multiple regions of the binding site are important (Hope et al., 1999; Mochizuki et al., 1999).

4.2. Loops D-F

These ‘loops’ are in fact β-strands, although for historical reasons they are still often referred to as loops. A number of residues in loop D (Fig. 1) are important for binding and/or receptor function: W90, for example, is critical for ligand binding, whilst W95 plays a role in cell surface expression (Spier and Lummis, 2000; Thompson et al., 2005). Antagonists may directly contact R92, and double-mutant cycle analysis at W90 and R92 has indicated that the azabicyclic ring of the competitive antagonist granisetron is located close to R92 and the aromatic rings lie close to W90 (Yan and White, 2005). Aromatic contacts have also been demonstrated in AChBP between the residue that is equivalent to W90 (W53) and the agonist nicotine (Xie and Cohen, 2001; Celie et al., 2004). The equivalent residues in nicotinic ACh (γW55, δW57, εW55) and $GABA_A$ (αF64) receptors are also important in binding, indicating that this position is functionally similar among many members of the LGIC family (Akk, 2002; Holden and Czajkowski, 2002; Celie et al., 2004).

Sequence variability in loop E (Fig. 1) can be seen both between subunits of the same species and the same subunit in different species, suggesting that the structure in this region may differ according to the stoichiometry of the receptor and/or the species. Scanning alanine mutagenesis of loop E in the mouse 5-HT3A receptor subunit has revealed that Y143, G148, E149, V150, Q151, N152, Y153 and K154 may be important for granisetron binding, and indeed mutation of G148 and V150 completely abolished binding

(Venkataraman et al., 2002a). The two tyrosine residues Y143 and Y153 have been further studied using unnatural amino acid mutagenesis which has shown that they both play roles in function, and Y153 also has a role in ligand binding (Beene et al., 2004; Price and Lummis, 2004).

The structure of loop F (Fig. 1) has yet to be clarified as this region was poorly resolved in the AChBP crystal structure (Brejc et al., 2001). Thus, its current location on the homology model on the 5-HT_3A receptor is only tentative. A study of granisetron binding implicated W195 and S206 in the mouse 5-HT3A receptor subunit as potentially important residues (Thompson et al., 2005) and a more recent and comprehensive mutagenesis and modelling study has shown that amino acid residues in this loop, and in particular those centered around W195 and D204, are critical for ligand binding, and may also influence conformational changes in or close to the binding pocket (Thompson et al., 2006a).

5. Determinants of ion conduction

Structural models of the nicotinic ACh receptor, the prototypical Cys-loop receptor, reveal that the N-terminal extracellular residues form a wide outer vestibule that funnels into a narrow conduit through the membrane formed by a rosette of five α-helical M2 domains (Miyazawa et al., 1999; Unwin, 2005). The exact location of the channel gate in Cys-loop receptors is disputed. In nicotinic ACh receptors it has been placed either midway across the membrane (Miyazawa et al., 2003; Unwin, 2005), or deep within the channel pore which subsequently opens into an intracellular vestibule (Wilson and Karlin, 2001). Studies in which cysteine residues were systematically introduced into the M2 domain of the 5-HT3A subunit and subsequently probed with sulphydryl-modifying agents, or Cd^{2+}, in the closed and open states of the channel are most consistent with a centrally located gate (Reeves et al., 2001; Panicker et al., 2002, 2004, Table 2). The latter is compatible with the 'hydrophobic girdle' model of channel gating which places the closed gate of the *Torpedo* nicotinic ACh receptor approximately midway across the cell membrane between the 9′ and 14′ residues (Miyazawa et al., 2003; Unwin, 2005). Substitution of hydrophobic V13′ residues in the 5-HT_3A receptor by threonine or, serine, causes an increase in agonist potency (Dang et al., 2000), or spontaneous channel openings (Bhattacharya et al., 2004), respectively. Substitutions of L9′ by several amino acids (Yakel et al., 1993) affected agonist potency and desensitization (Table 2). Collectively, these observations are also consistent with the 5-HT_3A receptor channel gate being located centrally within the M2 domain.

Several structure-function studies demonstrate that determinants of ion conduction (single channel conductance and ionic selectivity) reside within the amino acid sequences of the M2 domains of Cys-loop receptors. Amino acids in M2 influence the ion conduction of Cys-loop receptors gated by ACh, 5-HT, GABA and glycine (reviewed by Keramidas et al., 2004 and Peters et al., 2005). Rings of acidic amino acids at extracellular, intermediate and cytoplasmic locations within M2, residues 20′, -1′ and 4′, respectively, act as determinants of ion conduction in cation selective channels.

The recently refined 4-Å resolution structural model of the *Torpedo marmorata* nicotinic ACh receptor reveals cytoplasmic extensions of the conduction pathway with five lateral openings (dubbed "portals") formed by α-helical stretches of amino acids (termed either helical amphipathic (HA)- or membrane associated (MA)-stretches) located within the large M3-M4 loops of adjacent subunits (Finer-Moore and Stroud, 1984; Unwin, 2005). Recent studies, predominantly of the 5-HT_3A receptor, reveal that the intracellular portals also contain determinants of ion conduction (Kelley et al., 2003; Hales et al., 2006; Deeb et al., 2007; Livesey et al., 2008).

5.1. Single channel conductance

A reduction in the number of negative charges in the intermediate ring of nicotinic ACh receptor subunits causes a corresponding reduction in single channel conductance (Imoto et al., 1988; Konno et al., 1991). Furthermore, the presence of voluminous residues at the 2′ position of the M2 domain also reduces single channel conductance (Imoto et al., 1991; Villarroel et al., 1991).

Homomeric 5-HT_3A receptors have a single channel conductance below the resolution of single channel recording. Estimates of single channel conductance derived by variance analysis of macroscopic currents evoked by 5-HT range from ~0.40 to ~1 pS (Brown et al., 1998; Gunthorpe et al., 2000; Hales et al., 2006). Incorporation of the 5-HT3B subunit into heteromeric 5-HT_3AB receptors causes at least a ~16-fold increase in single channel conductance, in the case of human receptors, enabling direct observation of 16 pS conductance single channels in recordings from excised outside-out patches (Davies et al., 1999). Neither a change in residue charge (at M2 -1′), nor volume (at M2 2′), can explain this striking effect of the 5-HT3B subunit. The 5-HT3B subunit lacks the negatively charged intermediate residue (M2 E-1′) and has a larger residue at M2 2′ (valine *versus* serine) than is found in the 5-HT3A subunit. Furthermore, insertion of the M2 sequence of the 5-HT3B subunit into 5-HT3A/B chimeras did not increase single channel conductance (Kelley et al., 2003). Instead, replacement of the cytoplasmic α-helical MA-stretch within the M3-M4 loop of the 5-HT3A subunit with that of the 5-HT3B subunit caused a large increase of conductance. The key residues responsible for this effect are located at the MA -4′, 0′ and 4′ positions (i.e., R432, R436 and R440 in the human 5-HT3A subunit sequence; Kelley et al., 2003; Hales et al., 2006). Homology models of the 5-HT_3A receptor generated using the refined 4-Å resolution nAChR model suggest that the three periodically spaced arginine residues within the MA-stretches lie at the mouths of the cytoplasmic portals.

Within the MA-stretch, replacement of the 0′ residue has the greatest impact on single channel conductance (Kelley et al., 2003; Hales et al., 2006; Deeb et al., 2007) (Table 3). By replacing the MA 0′ arginine by cysteine and covalently modifying the latter with basic, acidic and non-polar methanethiosulfonate (MTS) reagents the charge of the cytoplasmic MA 0′ residue was identified as the principle determinant of the single channel conductance of 5-HT_3A receptor (Deeb et al., 2007).

Taken together, these studies of the determinants of single channel conductance of the 5-HT_3 receptor support the hypothesis that cytoplasmic portals form obligate pathways through which ions must navigate during their passage across the membrane. Furthermore,

introduction of arginine at either the MA -4′ or 0′ positions of nicotinic ACh α4β2 receptors halved the single channel conductance when the mutation was present in both the α4 and β2 subunits (Hales et al., 2006). This observation suggests that portals may participate in ion conduction through nicotinic ACh receptors also.

5.2. Ionic selectivity

Like nicotinic ACh receptors, 5-HT_3 receptors have a negligible permeability to anions. The source of their cation selectivity appears to reside primarily within the M2 domain (Table 2). A combination of mutations resulting in the neutralization of the intermediate ring, insertion of a proline between the -1′ and -2′ residues, and the replacement of a 13′ valine by threonine led to the creation of an anion permeable mouse 5-HT_3A receptor (Gunthorpe and Lummis, 2001). However, subsequent studies showed that the replacement of only two residues (E-1′, S19′R) was needed to invert ion selectivity to mildly favour anions (P_{Cl}/P_{Na} = 2.7), and changing 1′E alone resulted in non-selective channels indicating that the rings of charge at either end of M2 charge make the most critical contribution (Thompson and Lummis, 2003) (Table 2). Equivalent residues participate in the selectivity filters of other Cys-loop receptors (Keramidas et al., 2004; Peters et al., 2005).

The permeability of human 5-HT_3 receptors to Ca^{2+} is dependent on subunit composition (Davies et al., 1999). 5-HT_3A receptors are essentially equally permeable to monovalent and divalent cations (P_{Ca}/P_{Cs} = 1.0-1.4; Brown et al., 1998; Davies et al., 1999; Livesey et al., 2008). By contrast, the incorporation of the 5-HT3B subunit causes a reduction in the permeability of heteromeric 5-HT_3AB receptors to Ca^{2+} relative to monovalent cations (P_{Ca}/P_{Cs} = 0.62; Davies et al., 1999). The human 5-HT3B subunit contains an M2 20′ asparagine at the outer ring position, whereas an acidic aspartate occupies this position in the human 5-HT3A subunit. The absence of an acidic residue at the outer ring position of the β2 subunit of $(\alpha4)_2(\beta2)_3$ nicotinic ACh receptors has been implicated in the reduced Ca^{2+} conductance observed in receptors with this stoichiometry compared to $(\alpha4)_3(\beta2)_2$ nicotinic ACh receptors that have acidic residues at three of five outer ring locations (Tapia et al., 2007). Furthermore, replacement of the 20′ aspartate of the human 5-HT_3A receptor by alanine results in a significant reduction in relative permeability to Ca^{2+} (P_{Ca}/P_{Cs} = 0.44; Livesey et al., 2008) (Table 2). Therefore, it is likely that the absence of the 20′ aspartate from the 5-HT3B subunit contributes to the low relative Ca^{2+} permeability of 5-HT_3AB receptors. Furthermore, 5-HT3B subunits lack an acidic M2 -1′ residue, while 5-HT_3A receptors have a -1′ glutamate. The replacement of the -1′ glutamate within homomeric α7 nicotinic ACh receptors by alanine (the equivalent residue within the human 5-HT3B subunit) abolished permeability to Ca^{2+} (Bertrand et al., 1993; reviewed by Keramidas et al., 2004). Therefore, the absence of an acidic residue at the -1′ position in the 5-HT3B subunit is also likely to decrease Ca^{2+} flux through 5-HT_3AB receptors.

However, the TM2 domain is not the sole determinant of ionic selectivity in 5-HT_3 receptors (Table 3). A recent study demonstrated that cytoplasmic MA-stretch residues, which line putative intracellular portals of the 5-HT_3 receptor, also contribute to ionic selectivity (Livesey et al., 2008). Replacement of MA -4′, 0′ and 4′ arginines by glutamine, aspartate

and alanine, respectively, caused an approximately 3-fold increase in the permeability of Ca^{2+} relative to Cs^{+}.

6. Distribution and relationship to neuronal activity

6.1. Dorsal vagal complex

A number of studies using various radioligands, oligonucleotides and antibodies have mapped the distribution of 5-HT_3 receptor expression in the CNS. A consistent finding from the species investigated so far, including human, is that highest levels of 5-HT_3 receptor binding sites and immunoreactivity are within the dorsal vagal complex in the brainstem (e.g., for review see Pratt et al., 1990; Doucet et al., 2000). This region comprises the nucleus tractus solitarius (NTS), area postrema and dorsal motor nucleus of the vagus nerve, which are key to the initiation and coordination of the vomiting reflex. Antagonism of the 5-HT_3 receptors in these nuclei is therefore likely to contribute to the anti-emetic action of 5-HT_3 receptor antagonists in the amelioration of chemotherapy- and radiation-induced nausea and vomiting and post-operative nausea and vomiting. A comparison of 5-HT_3 receptor radioligand binding, immunohistochemical and *in situ* hybridisation studies (detecting the mRNA for the 5-HT3A and 5-HT3B subunits) highlights a notable absence of mRNA expression within the dorsal vagal complex. Such data are consistent with the 5-HT_3 receptor being expressed on the vagal terminals within the dorsal vagal complex, with their cell bodies within the nodose ganglion (where 5-HT3A and 5-HT3B subunit mRNA and immunoreactivity, 5-HT_3 receptor binding sites and functional responses are readily detected (Hoyer et al., 1989; Peters et al., 1993; Morales and Wang, 2002). Consistent with this distribution of receptor expression, removal of the nodose ganglion, and the subsequent neurodegeneration of the vagus nerve, is associated with a loss of 5-HT_3 receptor binding sites within the dorsal vagal complex (e.g., Pratt and Bowery, 1989).

Consistent with the anatomical expression studies, *in vivo*, or *in vitro*, electrophysiological recordings have demonstrated that the release of glutamate onto dorsal vagal preganglionic, nucleus tractus solitarius and area postrema neurones is facilitated by presynaptic 5-HT_3 receptors (Wang et al., 1998; Funahashi et al., 2004; Jeggo et al., 2005). However, another electrophysiological study has presented evidence for both presynaptic and post-synaptic 5-HT_3 receptors within the nucleus tractus solitarius (Glaum et al., 1992). Interestingly, nodose ganglion neurones projecting to the NTS possess mRNA encoding both the 5-HT3A and 5-HT3B subunit, or only the 5-HT3A subunit, potentially giving rise to a mixture of presynaptic 5-HT_3A and 5-HT_3AB receptors upon distinct terminals (Morales and Wang, 2002). This may have considerable functional significance in view of the differing permeabilities of such receptors to Ca^{2+} (Davies et al., 1999).

6.2. Forebrain

Relative to the dorsal vagal complex, 5-HT_3 receptor expression in the forebrain is low. There is general consistency in regional 5-HT_3 receptor expression within a species between radioligand binding, *in situ* hybridisation and immunohistochemical data indicating that the majority of receptor expression in the forebrain is by local neurones as opposed to the terminals of projection neurones. However, it is noteworthy that there are marked

interspecies differences in the expression of the 5-HT_3 receptor at both the regional and cellular level. For example, within the human forebrain, relatively high levels of 5-HT_3 receptor recognition sites have been located within the caudate nucleus and putamen (collectively the striatum), whereas relatively low levels are detected within cortical regions (Abi-Dargham et al., 1993; Bufton et al., 1993; Parker et al., 1996). This relative expression pattern is reversed in rodents, where 5-HT_3 receptor binding sites are undetectable in the striatum yet relatively high levels are associated with the superficial layers of the cerebral cortex (e.g., Barnes et al., 1990; Laporte et al., 1992; Steward et al., 1993a,b).

The use of tissue from patients with neurodegenerative disorders involving the human basal ganglia has indicated that the striatal 5-HT_3 receptors are associated with neurones that degenerate in Huntington's disease (Steward et al., 1993a,b); characteristically neurones that have their cell bodies within the striatum, which include the GABAergic projection neurones (Sieradzan and Mann, 2001). In contrast, the striatal 5-HT_3 receptors would not appear to be expressed by dopaminergic neurone terminals since their density is not influenced by the neurodegeneration associated with Parkinson's disease (Steward et al., 1993a,b).

The majority of species investigated so far (e.g., mouse, rat, marmoset, man) express high levels of 5-HT_3 receptors within the amygdala and hippocampus relative to other forebrain regions (e.g., Barnes et al., 1989, 1990; Waeber et al., 1989; Jones et al., 1992; Tecott et al., 1993; Parker et al., 1996). Within the hippocampus, 5-HT_3 receptor expression has been studied in more detail. Thus, consistent with the presence of 5-HT_3 receptor binding sites, 5-HT3A subunit mRNA and protein are evident within rodent hippocampi (e.g., Tecott et al., 1993; Morales and Bloom, 1997), where expression appears to be exclusive to GABAergic interneurones (e.g., Morales and Bloom, 1997). High resolution immunohistochemical studies report the rodent 5-HT3A subunit within cell bodies, dendrites and varicose axons (Spier et al., 1999; Miquel et al., 2002). In contrast to the rodent 5-HT3A subunit, the expression of the 5-HT3B subunit in rodent hippocampus is controversial (see van Hooft and Yakel, 2003; Jensen et al., 2008). Despite evidence that rodent hippocampal neurones possess relatively high single channel conductance 5-HT_3 receptors (Jones and Surprenant, 1994; reviewed by Fletcher and Barnes, 1998) which would be consistent with the expression of 5-HT_3AB receptors (see Section 5), 5-HT3B subunit transcripts appear absent (van Hooft and Yakel, 2003; Dover and Barnes, unpublished observations). To add to the inconsistency, three independent studies using different antisera have identified 5-HT3B-like immunoreactivity in rat hippocampus that appear to be expressed by a similar pattern of neurones to those expressing 5-HT3A subunits (Monk et al., 2001; Reeves and Lummis, 2006; Doucet et al., 2007). The above discrepancies remain to be resolved.

The human hippocampus apparently differs from that of rodents in an additional aspect. 5-HT3A and 5-HT3B subunit mRNA and protein are expressed in human hippocampus (Davies et al., 1999; Dubin et al., 1999; Brady et al., 2007; Tzvetkov et al., 2007; although see Niesler et al., 2003) by principle (excitatory glutamatergic) neurones (Brady et al., 2007). By contrast, in rodent hippocampus 5-HT3A subunit expression is associated with GABAergic interneurones (Tecott et al., 1993; Morales and Bloom, 1997). However, there is one report that supports the association of 5-HT3A subunit immunoreactivity with presumed principle neurones in the CA1 field of the rat hippocampus (Miquel et al., 2002).

In agreement with immunocytochemical and *in situ* hydridization studies in rodent brain, electrophysiological studies of single neurones in rat brain slices indicate that 5-HT_3 receptors are expressed by subsets of inhibitory interneurones in the CA1 area (Ropert and Guy, 1991; McMahon and Kauer, 1997) and dentate gyrus (Kawa, 1994) of the hippocampus and layer I of the cerebral cortex (Zhou and Hablitz, 1999). 5-HT_3 receptor activation at such interneurones causes a large increase in the frequency of $GABA_A$ receptor-mediated spontaneous inhibitory post-synaptic currents (sIPSCs) recorded from their post-synaptic targets (Ropert and Guy, 1991; Kawa, 1994; Zhou and Hablitz, 1999; Turner et al., 2004). A variety of evidence indicates that the release of GABA is, at least in part, mediated by Ca^{2+} influx into presynaptic terminals and that 5-HT_3 receptor channels at this location provide a significant pathway for Ca^{2+} entry (Nichols and Mollard, 1996; Rondé and Nichols, 1998; Koyama et al., 2000; Katsurabayashi et al., 2003; Turner et al., 2004).

5-HT_3 receptors also occupy a post-synaptic location on central neurones. In brain slices, synaptically released 5-HT contributes a component to fast excitatory synaptic transmission that is 5-HT_3 receptor-mediated in the rat lateral (but not basolateral) amygdala (Sugita et al., 1992; Koyama et al., 2000), ferret visual cortex (Roerig et al., 1997) and rat neocortical GABAergic interneurones that contain cholecystokinin and vasoactive intestinal peptide (Férézou et al., 2002). The rapid temporal characteristics of the rat 5-HT_3 receptor-mediated evoked excitatory post-synaptic current (eEPSC) recorded by Férézou et al. (2002) are markedly different from the relatively slowly activating and decaying 5-HT_3 receptor-mediated eEPSCs recorded from neurones of the ferret visual cortex or rat lateral amydala. In addition, the 5-HT_3 receptor-mediated eEPSCs in rat neocortical GABAergic interneurones activate and deactivate much more rapidly than the currents mediated by heterologously expressed homomeric 5-HT_3A receptors under conditions that closely mimic synaptic delivery and removal of agonist (Mott et al., 2001; Solt et al., 2007). Expression of the 5-HT3B subunit cannot explain this discrepancy, because currents mediated by heterologously expressed heteromeric 5-HT_3AB receptors also display comparatively slow kinetics (Krzywkowski et al., 2008). Moreover, the mRNA encoding the 5-HT3B subunit cannot be detected in rat neocortical interneurones by single cell RT-PCR (Férézou et al., 2002). There is at present no explanation for this conundrum.

In contrast to the debate regarding central expression of the 5-HT_{3B} subunit (van Hooft and Yakel, 2003; Jensen et al., 2008), *in situ* hybridisation histochemistry indicates that rat peripheral neurones, including dorsal root, superior cervical and nodose ganglion cells have the potential to express both the 5-HT3A and 5-HT3B subunits, or solely, the 5-HT3A subunit (Morales et al., 2001; Morales and Wang, 2002). Rat and mouse trigeminal ganglion neurones also express 5-HT3A and 5-HT3B subunits on the basis of immunohistochemistry (Doucet et al., 2007). Accordingly, rat superior cervical and rabbit nodose ganglion neurones express 5-HT_3 receptors with a single channel conductance that is compatible with that of 5-HT_3AB receptors (Yang et al., 1992; Peters et al., 1993). By contrast, electrophysiological recordings performed on rat dorsal root ganglion neurones are suggestive of the expression of homomeric, 5-HT_3A, constructs (Robertson and Bevan, 1991). Immunohistochemical studies support the expression of 5-HT3A and 5-HT3B subunits in human submucous plexus neurones (Michel et al., 2005). Similarly, a subpopulation of mouse myenteric neurones in culture is immunoreactive for the 5-HT3A subunit and expresses mRNA encoding the 5-

HT3B subunit (Liu et al., 2002). The single channel conductance of 5-HT_3 receptors endogenous to guinea pig myenteric and submucous plexus neurones is also compatible with the expression of 5-HT_3AB receptors (Derkach et al., 1989; Zhou and Galligan, 1999).

Information concerning the regional expression of the human 5-HT3C, 5-HT3D and 5-HT3E subunits, and hence their impact upon the potential generation of 5-HT_3 receptor isoforms is relatively sparse (Niesler et al., 2003; Karnovsky et al., 2003). No evidence has yet been forwarded concerning the molecular stoichiometry of human native central 5-HT_3 receptors, although the expression of 5-HT3A, 5-HT3B and possibly 5-HT3C subunits, but not 5-HT3D, or 5-HT3E subunits, suggests the potential for considerable molecular diversity. Greater complexity may apply in the case of the intestine, where all five 5-HT_3 receptor subunits have the potential for expression (Niesler et al., 2003; Jensen et al., 2008).

7. Polymorphisms and (patho)physiology

Several polymorphisms of the *HTR3A* and *HTR3B* genes have been described and case *versus* control studies suggest a role for 5-HT_3 receptors in psychiatric disorders (Table 2). Pharmacogenetic studies also implicate the 5-HT_3 receptor in adverse effects of drugs, such as paroxetine, that act as selective inhibitors of the serotonin transporter (i.e., SSRIs) (reviewed by Krzywkowski, 2006 and Niesler et al., 2008).

Psychiatric disorders such as schizophrenia and bipolar affective disorder, segregate with cytogenetic abnormalities involving a region on chromosome 11 that harbours the *HTR3A* gene (Weiss et al., 1995). Niesler et al. (2001b) identified, in separate schizophrenic patients, two single nucleotide polymorphisms (SNPs) that result in missense mutations (R344H and P391R) within the large intracellular loop of the 5-HT3A subunit. These residues are located N-terminal to the α-helical MA-stretch (see Section 5). Although these were initially stated to occur at an allelic frequency too low to be a significant factor in the aetiology of the disorder (Niesler et al., 2001b), the genotyping of additional ethnic populations has shown that the R344H variant is not uncommon and occurs with a minor allele frequency (MAF) of 0.038-0.108. The functional properties of homomeric receptors assembled from the 5-HT3A(R344H) subunit variant are unperturbed (Thompson et al., 2006b; Krzywkowski et al., 2007) and its total abundance and level of expression at the plasma membrane have been reported to be unchanged (Thompson et al., 2006b), or reduced (Krzywkowski et al., 2007), in comparison to the wild-type receptor. Similarly, the 5-HT3A(P391R) mutation confers no striking changes in receptor properties (Kurzwelly et al., 2004; Thompson et al., 2006b), and is in one study (Krzywkowski et al., 2007), but not another (Thompson et al., 2006b) associated with reduced cell surface expression. Additional SNPs of the *HTR3A* gene result in the 5-HT3A(A33T) and 5-HT3A(M257I) subunit variants, both of which are associated with reduced levels of cell surface expression in comparison to the wild-type 5-HT_3A receptor, and the 5-HT3A(S253N) variant which does not compromise plasma membrane expression (Krzywkowski et al., 2007). The S253N and M257I substitutions occur in M1 and cause a profound reduction in the maximal response to 5-HT in Ca^{2+} imaging and fluorescent membrane potential assays in comparison to the wild-type receptor. It is likely that the S253 N and M257I substitutions impair signal transduction (Krzywkowski et al., 2007) but this remains to be confirmed by direct electrophysiological recordings.

A very common SNP (386AC) of the *HTR3B* gene (MAF = 0.433) results in the 5-HT3B subunit variant Y129S within the β5 strand of the ECD. This polymorphism occurs at a reduced frequency in female patients manifesting major depression and also in patients with bipolar affective disorder, suggesting a protective influence of the variant allele (Krzywkowski, 2006). Unusually, the Y129S variant is a gain of function mutation. When combined with the wild-type 5-HT3A subunit in heteromeric receptors, the 5-HT3B(Y129S) variant confers an increased maximal response to 5-HT, decreased desensitization and deactivation kinetics and a 7-fold increase in mean channel open time in comparison to heteromeric receptors containing the wild-type 5-HT3B subunit (Krzywkowski et al., 2008). An intermediate effect upon the maximal response to 5-HT in Ca^{2+} imaging and fluorescent membrane potential assays is evident for receptors assembled from a mixture of wild-type 5-HT3A, wild-type 5-HT3B and 5-HT3B(Y129S) subunits. Hence, the Y129S variant has been hypothesised to affect signaling *via* the 5-HT_3AB receptor in heterozygous, as well as homozygous, individuals (Krzywkowski et al., 2008).

Polymorphisms outwith the coding region of the *HTR3A* and *HTR3B* genes have also been proposed to be functionally important (Niesler et al., 2008). The relatively common polymorphism C178T (aka -42C>T) within the 5′ untranslated region of the *HTR3A* gene results in increased translation of a luciferase reporter construct (Niesler et al., 2001a). This polymorphism appears to be associated with bipolar affective disorder (in Caucasians), and the personality trait of lower harm avoidance in women (reviewed by Krzywkowski, 2006). Intriguingly, functional magnetic resonance imaging indicates that the less common C/T allele, *versus* C/C allele, is associated with a reduced level of neuronal activation in the right amygdala and prefrontal cortex during a face recognition task (Iidaka et al., 2005). It has been speculated that the cellular basis of this effect is enhanced 5-HT_3 receptor expression on inhibitory GABAergic interneurones and as a consequence enhanced release of GABA (Iidaka et al., 2005). Such speculation gains credence from the observation that 5-HT_3 receptors are frequently expressed by GABAergic interneurones within the CNS and that their activation (by exogenous 5-HT) increases the frequency of minature inhibitory post-synaptic currents mediated by GABA (reviewed by Chameau and van Hooft, 2006; see Section 6).

A -100_-102AAG deletion in the promoter region of the *HTR3B* gene has been associated with an increased frequency of chemotherapy-induced nausea and vomiting in patients receiving 5-HT_3 receptor antagonists as anti-emetic therapy (Tremblay et al., 2003). Specifically, patients homozygous for the deletion had the highest score for nausea and vomiting of all groups studied. However, a definitive link between the -100_-102AAG deletion and a reduced efficacy of anti-emetic therapy with 5-HT_3 receptor antagonists remains to be established (Tremblay et al., 2003) and a subsequent study found no significant correlation between the deletion and the frequency of vomiting in a patient group undergoing chemotherapy for breast cancer (Fasching et al., 2008). Nonetheless, it is interesting that the same deletion also associates with an increased incidence of nausea in response to paroxetine, a selective serotonin reuptake inhibitor (Tanaka et al., 2008). The -100_-102AAG deletion has recently been shown to increase the promoter activity of the *HTR3B* gene, *in vitro* (Meineke et al., 2008). Whether increased expression of the 5-HT3B subunit occurs *in vivo* as a consequence of -100_-102AAG deletion remains to be assessed,

but it is interesting that the gain of function Y129S variant is also associated with an increased frequency of parotexine-induced nausea (Sugai et al., 2006). Finally, the -100_-102AAG deletion variant has been found to be underrepresented in a sample of patients suffering from bipolar depression (Frank et al., 2004).

8. Concluding remarks

Much is now known about the structure and function of 5-HT_3 receptors but there are still a number of conundra and controversies that we have alluded to. It is, however, becoming increasingly clear that a better knowledge could have a significant impact on our understanding of a range of clinical conditions in which the 5-HT_3 receptor is involved, in addition to contributing to the design of more selective and potent potentially therapeutic compounds. In this respect, determining the roles of the 5-HT3C, D and E subunits in 5-HT_3 receptor function at the molecular level is a goal for the future.

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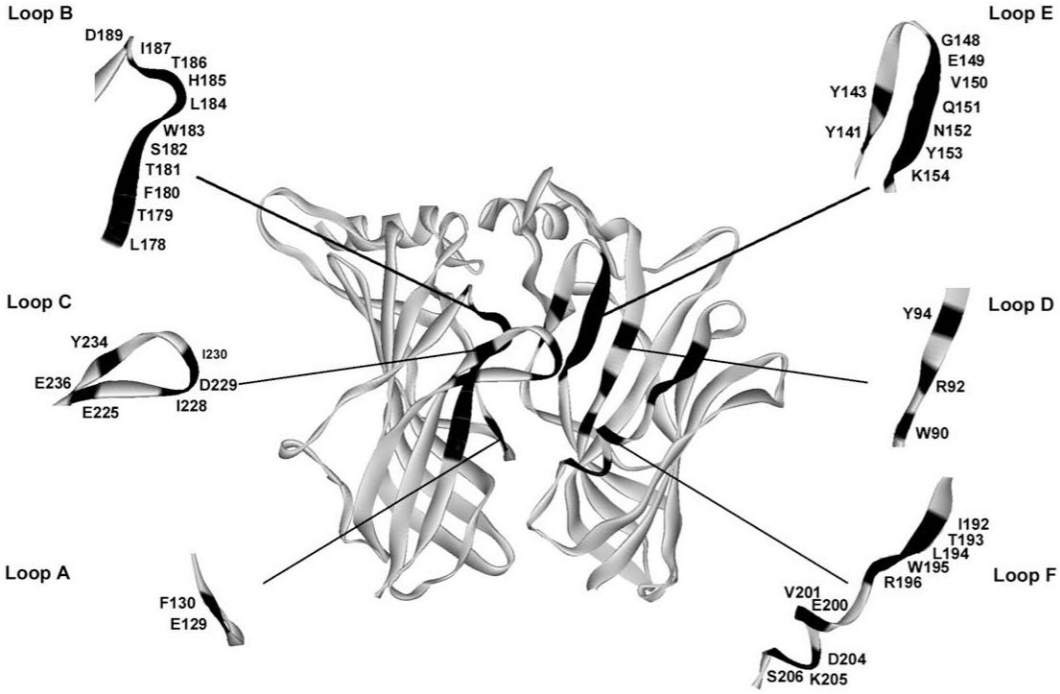

Fig. 1.

A homology model of the 5-HT_3A receptor binding domain, showing 2 of the 5 subunits. The binding site is located at the interface of the two subunits that provide principal (loop A-C) and complementary (loops D-F) components to binding. Binding site substitutions that cause significant changes in the binding affinity of granisetron at the 5-HT_3A receptor are shown. The data were taken from Beene et al. (2004), Boess et al. (1997), Joshi et al. (2006), Schreiter et al. (2003), Spier and Lummis (2000), Sullivan et al. (2006), Thompson et al. (2006a), Venkataraman et al. (2002a,b), Yan et al. (1999) and Yan and White (2005).

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Table 1

Species orthologues of 5-HT_3 receptor subunits

Subunit	Species	AA	Accession number	Chromosomal location	References
5-HT3A	Human	478	NP_000860	11q23.1	Belelli et al. (1995), Miyake et al. (1995)
	Rat	483	NP_077370	8q23	Miyake et al. (1995)
	Mouse	487	NP_038589	9 A5.3	Maricq et al. (1991), Hope et al. (1993)
	Dog	483	NP_001041584	5	Jensen et al. (2006)
	Guinea pig	490			Lankiewicz et al. (1998)
	Ferret	483			Mochizuki et al. (2000)
5-HT3B	Human	441	NP_006019	11q23.1	Davies et al. (1999), Dubin et al. (1999)
	Rat	437	NP_071525	8q23	Hanna et al. (2000)
	Mouse	437	NP_064670	9 B	Hanna et al. (2000)
5-HT3C	Human	447	NP_570126	3q27.1	Niesler et al. (2003), Karnovsky et al. (2003)
5-HT3D	Human	279	NP_872343	3q27.1	Niesler et al. (2003), Karnovsky et al. (2003)
5-HT3E	Human	471	NP_872395	3q27.1	Niesler et al. (2003), Karnovsky et al. (2003)

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Table 2

Mutations within the M2 of the 5-HT_3A receptor that affect function

Original M2 residue	Replacement residue(s)	Effect of mutation	References
E-1′	A	More rapid desensitization and reduced ionic selectivity towards cations. When combined with V130T and a Pro insertion between -1′ and -2′ the channel exhibits selectivity to anions. Anion selectivity also evident when E-1′A is combined with S19′R.	Gunthorpe and Lummis (2001), Thompson and Lummis (2003)
S2′	A/C	Ala decreases 5-HT EC_{50}. Cys increases 5-HT EC_{50}.	Panicker et al. (2002), Das and Dillon (2005)
K4′	R/Q/S/Q/G	All mutations reduce 5-HT EC_{50} to the same degree and increase desensitization in the order Arg > Gly > Ser > Gln.	Gunthorpe et al. (2000)
T6′	F/C	Phe decreases 5-HT EC_{50}. Cys increases 5-HT EC_{50}.	Panicker et al. (2002), Das and Dillon (2005)
L7′	T/C	Thr increases 5-HT EC_{50}. Cys decreases 5-HT EC_{50}.	Panicker et al. (2002), Das and Dillon (2005)
L9′	F/Y/A/T/C	Tyr, Thr and Ala increase agonist potency. Phe, Tyr, Ala cause increased desensitization. Thr reduces desensitization. Cys caused decreased 5-HT EC_{50}. MTSET modification of Cys elicits a standing current.	Yakel et al. (1993), Reeves et al. (2001), Panicker et al. (2002)
V13′	S/T	Ser decreases 5-HT EC_{50} and produces spontaneous gating. When V13′T is combined with E-1′A and a Pro insertion between -1′ and -2′ an anion selective channel is formed.	Dang et al. (2000), Gunthorpe and Lummis (2001), Bhattacharya et al. (2004)
F14′	A	Reduces 5-HT EC_{50}.	Lopreato et al. (2003)
L15′	A/F/S/V/Y/C	All except Cys reduce 5-HT EC_{50}. MTSES modification of Cys elicits a standing current.	Reeves et al. (2001), Panicker et al. (2002), Lopreato et al. (2003)
I16′	T/L/C	Thr reduces 5-HT EC_{50} and increases desensitization. Leu increases 5-HT EC_{50}. Cys reduced 5-HT EC_{50} and MTSES modification of Cys causes standing current.	Reeves et al. (2001), Panicker et al. (2002), Sessoms-Sikes et al. (2003)
S19′	R	Faster desensitization. Remains cation selective. When combined with E-1′A a anion selective channel is formed.	Thompson and Lummis (2003)
D20′	A/R	Ala and Arg reduced 5-HT EC_{50} and increased rates of both desensitization and deactivation. D20′A reduced Ca^{2+} permeability and conductance of 5-HT_{3A}(QDA) mutant.	Hu and Lovinger (2005), Livesey et al. (2008)

The prime numbering scheme for M2 residues was described previously (Miller, 1989). Panicker et al. (2002) also demonstrated that F3′C and S12′C reduce 5-HT EC_{50}, while I5′C, I17′C and V18′C decrease 5-HT EC_{50}. To reduce complexity within the table, the effects of Cys modification by MTS reagents are only included when application of the reagent alone causes the appearance of a standing current. For a more detailed review of Cys accessibility see Peters et al. (2005). Also omitted are the effects of mutations on pharmacology of 5-HT_3A receptors (Sessoms-Sikes et al., 2003; Das and Dillon, 2005) and the function of 5-HT_3AB receptors (Bhattacharya et al., 2004; Das and Dillon, 2005).

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Table 3

Mutations within the MA-stretch of the 5-HT_3A receptor that affect function

Original MA-residue	Replacement residue(s)	Effect of mutation	References
R-4′	Q	When R-4′Q is combined with R0′D and R4′A it contributes to a 35-fold increase in single channel conductance.	Kelley et al. (2003), Hales et al. (2006)
R0′	D/E/Q/FC	R0′D causes a 10-fold increase in single channel conductance. When combined with R-4′Q and R4′A, R0′D contributes to a 35-fold increase in conductance. The charge of the 0′ residue is a determinant of both conductance and relative permeability to Ca^{2+}. Negative charges favour enhanced conductance and relative Ca^{2+} permeability.	Kelley et al. (2003), Hales et al. (2006), Deeb et al. (2007), Livesey et al. (2008)
R4′	A/D/K/N/E/W/H/G/L	R4′A causes a 5-fold increase in single channel conductance alone and, when combined with R0′D and R-4′Q, it contributes to a 35-fold increase in conductance. Residues affect desensitization kinetics according to their hydrophobicity and polarity. R4′L exhibits slowest desensitization of all listed.	Kelley et al. (2003), Hales et al. (2006), Hu et al. (2006)

The prime numbering scheme was described previously (Hales et al., 2006). The combined R-4′Q, R0′D and R4′A mutations in the 5-HT_3A receptor also decrease the 5-HT EC_{50} (Livesey et al., 2008).