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MINIREVIEW

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5-HT_3 Receptors*

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5-Hydroxytryptamine type 3 (5-HT_3) receptors are cation-selective Cys loop receptors found in both the central and peripheral nervous systems. There are five 5-HT_3 receptor subunits (A–E), and all functional receptors require at least one A subunit. Regions from noncontiguous parts of the subunit sequence contribute to the agonist-binding site, and the roles of a range of amino acid residues that form the binding pocket have been identified. Drugs that selectively antagonize 5-HT_3 receptors (the "setrons") are the current gold standard for treatment of chemotherapy-induced and postoperative nausea and vomiting and have potential for the treatment of a range of other conditions.

5-Hydroxytryptamine (5-HT[2]; serotonin) is one of, if not the most, versatile of all neurohormones or neurotransmitters. Its diverse range of functions are due to a large family of receptors: 5-HT_1 to 5-HT_7 (1). The 5-HT_3 receptor is a Cys loop ligand-gated ion channel, most closely related to nicotinic acetylcholine (nACh) receptors, and it is structurally and functionally distinct from the other six classes of 5-HT receptor whose actions are mediated via G-proteins. The 5-HT_3 receptor is a cation-selective ion channel capable of mediating fast excitatory neurotransmission in the CNS and peripheral nervous system (PNS) (2). 5-HT_3 receptors are located in many brain areas, with the highest levels in the brainstem, especially areas involved in the vomiting reflex such as the area postrema and nucleus tractus solitarius (3, 4). The receptors are found both pre- and postsynaptically, and activation can modulate the release of a range of neurotransmitters, including dopamine, GABA, substance P, and acetylcholine (3, 5, 6). 5-HT_3 receptors also regulate gut motility, secretion, and peristalsis in the enteric nervous system and are involved in information transfer in the gastrointestinal tract (7).

Background

Serotonin was identified in the 1940s as a potent vasoconstrictor present in blood serum (8). The proposal of a specific receptor for this compound was first raised in the literature in 1953, when Rocha e Silva *et al.* (9) noticed that 5-HT had actions on guinea pig ileum and that its effects could be blocked by cocaine at micromolar concentrations. At approximately the same time, Gaddum (10) proposed that 5-HT acted on specific receptors, of which there were two types: one in smooth muscle, which was inhibited by LSD (lysergic acid diethylamide), and another in the nervous system, which was not inhibited by LSD. However, the classic "discovery" of the 5-HT_3 receptor is usually linked to the work of Gaddum and Picarelli in 1957 (11), who defined two classes of serotonin receptors in the ileum: M receptors, which were located primarily in the nervous system and inhibited by morphine, atropine, and cocaine, and D receptors, which were located mostly in muscle and blocked by dibenzyline. When the serotonin receptors were reclassified in 1986 (12, 13), the M receptor became the 5-HT_3 receptor, and the D receptor the 5-HT_2 receptor. These are just two of seven currently known 5-HT receptor families, most of which have a range of subtypes, in addition to splice variants and post-translationally modified receptors, creating one of the largest families of neurotransmitter receptors (13).

Despite being identified in 1957, it was not until the 1980s that the first selective 5-HT_3 antagonists were developed, MDL 72222 or bemesetron (14) and ICS 205-930 or tropisetron (15), and their antiemetic properties appreciated: MDL 72222 was found to be a potent antiemetic in cisplatin-treated ferrets (16, 17). Novel (second generation) antagonists were soon developed, including GR38032F (ondansetron) and BRL 43694 (granisetron). Use of these compounds, in addition to the older, nonselective, but still effective antagonists metoclopramide and cocaine, revealed a widespread distribution of 5-HT_3 receptors in the PNS. The presence of 5-HT_3-binding sites in the CNS was first established in 1987 using [^{3}H]GR65630 (18), and single-channel studies published in 1989 provided unequivocal evidence that 5-HT_3 receptors were indeed ligand-gated ion channels (19). In 1991, the first 5-HT_3 receptor subunit (5-HT_3A) was cloned (20). The homology between this subunit and those from other Cys loop receptors clearly indicated 5-HT_3 receptors were members of this family, but the 5-HT_3A subunit was a little unusual in that it could readily form functional homomeric receptors. The biophysical properties of the expressed homomeric receptors differed, however, from those observed in some native preparations. For example, when expressed in HEK 293 cells, 5-HT_3A receptors had a single-channel conductance of <1 picosiemen (pS), whereas channel activity in rabbit nodose ganglion revealed a single-channel conductance of 19 pS (21). This discrepancy was not explained until 1999, when a second subunit, the 5-HT_3B receptor subunit, was identified (22, 23). Coexpression of this subunit with the A subunit resulted in properties that more closely represented those found in some native receptors. Since then, three other subunits (C–E) have been identified, considerably expanding the known complexity of the 5-HT_3 receptor system (24). Over the last decade, much progress has been made in understanding the structure-function relationships of 5-HT_3 receptors and their pathophysiological relevance (see Refs. 6 and 25–28 for reviews). There is nevertheless much left to be

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[2] The abbreviations used are: 5-HT, 5-hydroxytryptamine; nACh, nicotinic acetylcholine; PNS, peripheral nervous system; pS, picosiemens; ECD, extracellular domain; TMD, transmembrane domain; ICD, intracellular domain; AChBP, ACh-binding protein; IBS, irritable bowel syndrome.

discovered, in particular in understanding the roles of the C, D, and E subunits, whose expression has only recently been confirmed (29); such studies may allow the development of novel agents to treat disorders such as anxiety, schizophrenia, and Alzheimer disease, which were originally postulated to be targets of 5-HT$_3$ receptor-specific compounds.

Receptor Structure

The functional 5-HT$_3$ receptor, like other Cys loop receptors, is a pentameric assembly of five identical or non-identical subunits that surround, in a pseudo-symmetric manner, a water-filled ion channel (30, 31). Each subunit has a large extracellular domain (ECD) that forms the ligand-binding site, a transmembrane domain (TMD) consisting of four membrane-spanning α-helices (M1–M4) that enable ions to cross the membrane, and an intracellular domain (ICD) formed by the large M3-M4 intracellular loop, which is responsible for receptor modulation, sorting, and trafficking, and which contains portals (openings) that influence ion conductance. The presence of portals has been deduced from nACh receptor data, but they are likely to exist, as these receptors are highly homologous (see Refs. 6, 20, 28, and 32 for reviews). The structural and functional similarity of these two receptors is such that chimeric receptors consisting of the ECD of the α7-nACh receptor and the TMD of the 5-HT$_3$A receptor can be activated by ACh and have the channel properties of the 5-HT$_3$A receptor (33). Thus, although there are no high resolution images of 5-HT$_3$ receptors, the currently available cryo-electron microscope- and x-ray crystallography-derived structures are likely to be structurally representative, and those that have been used as templates for homology models include the nACh receptor, many ACh-binding proteins (AChBPs), and the bacterial homologs ELIC and GLIC (*Erwinia* and *Gloeobacter* ligand-gated ion channels) (*e.g.* Refs. 32, 34, and 35). A model of one such subunit using the nACh receptor cryo-electron microscope structure as a template is shown in Fig. 1*A*; this reveals that the ECD (*blue*) is predominantly β-sheet and that the TMD (*purple*) is mostly α-helix, although most of the structure of the ICD, apart from a stretch of α-helix (*yellow*), is not currently known. Deletion studies revealed that this region is not essential for receptor expression, as the large intracellular loop of the mouse 5-HT$_3$A receptor subunit can be replaced by the heptapeptide M3-M4 linker of GLIC without loss of function (36). Homology models of the 5-HT$_3$ receptor ECD using the nACh receptor and AChBP as templates are broadly similar (Fig. 1*B*), although there are some subtle differences such as the orientation of the α-helix at the top of the receptor and the positions of some of the binding loops.

5-HT$_3$ Receptor Subunits

Five distinct 5-HT$_3$ receptor subunits (A–E) have been identified so far (Fig. 2), which is relatively few for a Cys loop receptor, although the repertoire is increased by a number of different isoforms (24, 37, 38). There are, for example, a long and short form of the human 5-HT$_3$A subunit that differ by 32 amino acids, three translational variants of the human 5-HT$_3$B subunit, and five isoforms of the 5-HT$_3$E subunit. The stoichiometry of heteromeric receptors is still not clear, although it

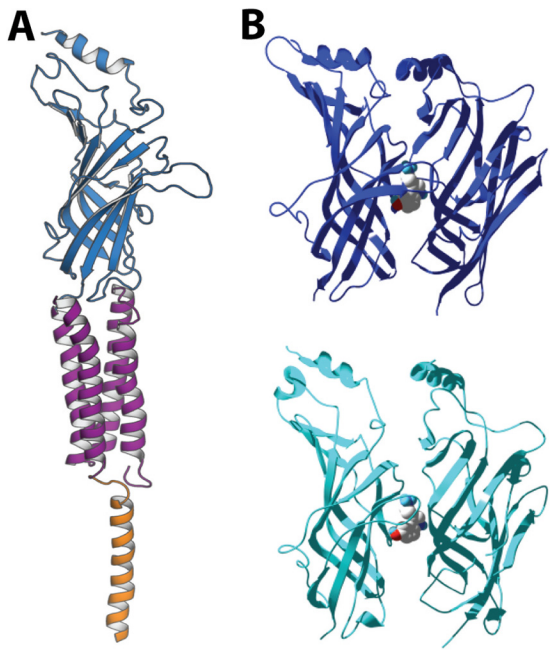

FIGURE 1. **Homology models of the 5-HT$_3$ receptor.** *A*, a single receptor subunit based on the nACh receptor structure (Protein Data Bank code 2BG9) showing the mostly β-sheet-containing ECD (*blue*), the four transmembrane α-helices (*purple*), and the α-helix that forms part of the ICD (*orange*). The structure of the remainder of this domain is not yet known. *B*, models of the 5-HT$_3$ receptor ECD based on AChBP (*blue*; code 1UV6) and the nACh receptor (*cyan*; code 2BG9), with 5-HT docked into the inter-subunit binding pocket.

has been established that only 5-HT$_3$A subunits can form functional homomeric 5-HT$_3$ receptors, and the presence of at least one 5-HT$_3$A subunit appears to be obligatory in heteromeric receptors (24, 37–39).

Localization of the subunits reveals considerable overlap. Distribution of 5-HT$_3$A receptor mRNA and protein is widespread and has been observed in many regions of the CNS (where it correlates well with radiolabeled antagonist binding studies (4, 18)), in peripheral and sensory ganglia, and in a wide range of other tissues, including the gastrointestinal tract (37, 40–43). 5-HT$_3$B subunit mRNA and protein were originally shown to be located in the spleen, colon, small intestine, and kidney, with some controversy as to their presence in the brain (22, 23, 44–46). Later studies showing that there are several 5-HT$_3$B receptor isoforms provided an answer to this conundrum: different tissue preferences of the different subunits. The longer B subunit variant is broadly expressed in many tissues, including the kidney, liver, brain, and gastrointestinal tract, whereas the shorter variant has a brain-specific expression pattern (37, 47). 5-HT$_3$ receptor C–E subunits were first identified in humans, and genes for these proteins have now been shown to exist in a range of species, although not in rodents (24, 37, 48). A recent study suggested that they all have a relatively widespread distribution, although initial studies suggested that the D and E subunits had a very restricted expression in the gastrointestinal tract. Studies examining protein levels have lagged behind the genetic work, but expression of the C–E subunits at the protein level in the gastrointestinal tract has recently been demonstrated (29).

FIGURE 2. **5-HT$_3$ receptor subunits.** Shown is a Clustal alignment of representative human (*h*) 5-HT$_3$ receptor A–E subunits, demonstrating the approximate locations of the binding loops on the principal (*red*) and complementary (*cyan*) faces, the Cys-Cys loop (*green*), and the transmembrane α-helices (*black*). Note the unusual sequence construction of the D subunit, which is missing a Cys-Cys loop and parts of loops D and E and which has a region of extra sequence in the M2-M3 loop. Accession numbers are as follows: NP_000860 (A subunit), NP_006019 (B subunit), NP_570126 (C subunit), NP_001157118 (D subunit), and NP_872385 (E subunit).

5-HT$_3$ Receptor Binding Pocket

The agonist-binding site lies at the interface of two adjacent subunits in the extracellular N-terminal domain and is formed by three loops (A–C) from one (the principal) subunit and three β-strands (referred to as loops D–F) from the adjacent or complementary subunit, as in other Cys loop receptors. Only a few

residues within each loop face into the binding pocket, with other residues having roles in maintaining the structure of the pocket and/or contributing to the conformational changes that result in channel opening (6). Evidence from AChBP structures suggests that the binding pocket contracts around agonists, which may initiate the conformational change that ultimately leads to channel opening, whereas antagonists tend to have little effect or may cause binding site expansion (32).

Key residues that contribute to the 5-HT$_3$ receptor ligand-binding site include one or more from each of the six binding loops (see Refs. 6, 25, and 49 for comprehensive reviews). In loop A, attention has focused on the sequence ^{128}Asn-Glu-Phe^{130}, as substitutions here have large effects on receptor function (50–53). Recent data indicate that only Glu-129 faces into the binding pocket, where it forms a hydrogen bond with the hydroxyl of 5-HT (54). Mutations in loop B show that many residues here are important for receptor function, and these data, when combined with modeling data, suggest that loop B is an obligate rigid structure (55–57). One residue plays an especially critical role, Trp-183, which forms a cation-π interaction with the primary amine of 5-HT (55, 58). Loop C shows the largest species variability and is important in determining the species specificity of various drugs (59). However, point mutations throughout the loop C region did not identify any residues that were essential for binding of the agonist *m*-chlorophenylbiguanide or the antagonist (+)-tubocurarine, suggesting that multiple regions of the binding site are important (60, 61). One residue that is critical for both agonist and antagonist binding is Tyr-234, which forms part of the aromatic box found in all Cys loop receptors (62). Loop D also contributes an aromatic residue (Trp-90) to the binding pocket, and double-mutant cycle analysis at Trp-90 and Arg-92 has indicated that the aromatic rings of the competitive antagonist granisetron are located close to Trp-90 and that the azabicyclic rings lie close to Arg-92 (63). Loop E residues Tyr-141, Tyr-143, Gly-148, Glu-149, Val-150, Gln-151, Asn-152, Tyr-153, and Lys-154 may all be important for granisetron binding and perhaps function, although it is not clear if some of these effects are due to alterations in the binding site structure (63–65). As yet, the structure of loop F is not well defined, although a study of granisetron binding has implicated Trp-195, Asp-204, and Ser-206 as potentially important residues for ligand binding; alternatively, these residues may influence conformational changes in or close to the binding pocket (49).

There are also binding sites for a range of other ligands and modulators (Fig. 3). Of these, the pore-binding sites are currently the best characterized, *e.g.* picrotoxin and the ginkgolides block the 5-HT$_3$ receptor channel by interacting with the 6′ residue (32, 66); there also may be specific binding pockets for many other compounds, including ions, steroids, alcohols, anesthetics, and a range of small molecules (6, 25, 67, 68).

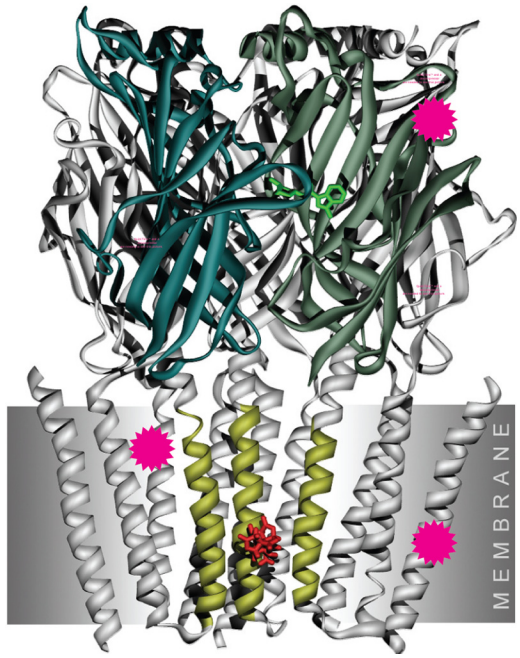

FIGURE 3. **5-HT$_3$ receptor binding sites.** Shown is a model of the 5-HT$_3$ receptor (with two subunits removed for clarity) showing granisetron (*green*) and picrotoxin (*red*) docked into their known binding sites in the ECD and in the pore, respectively. Other possible binding sites (*pink*), whose locations have not yet been confirmed, are an allosteric site in the ECD, an interhelical site in the TMD, and a lipid transmembrane site at the membrane-receptor boundary.

Receptor Function

Homomeric 5-HT$_3$A receptors mediate rapidly activating and desensitizing inward currents, which are carried primarily by Na^+ and K^+ ions (19). The receptors are also permeable to Ca^{2+} and other small organic cations (20, 69). As in other Cys loop receptors, the residues that line the ion-accessible inner face of the M2-generated pore are predominantly non-polar, and it is M2 residues that primarily control ion flux and size selection through the channel (70–72). Charge selectively is mediated predominantly via the Glu residue in the M1-M2 loop, at the so-called −1′ position, and when combined with the introduction of a positively charged residue at the 20′ position or insertion of a Pro at the −2′ position and a V9′T mutation, the channel conducts predominantly anions (73, 74). Residues in the α-helical stretch of the ICD and in the ECD also play roles in determining single-channel conductance and relative permeability to Ca^{2+} (75, 76).

5-HT$_3$B receptor subunits do not form functional homomeric receptors but can coexpress with A subunits to yield heteromeric 5-HT$_3$AB receptors that differ from 5-HT$_3$A receptors in their EC$_{50}$, Hill slope, desensitization kinetics, calcium permeability, shape of current-voltage relationship, and, most noticeably, single-channel conductance, which is much larger: ~16 pS in 5-HT$_3$AB receptors compared with <1 pS in 5-HT$_3$A receptors (22, 23). Despite these biophysical differences, the pharmacology of the orthosteric sites of 5-HT$_3$A and 5-HT$_3$AB receptors is almost identical, and to date only one compound has been identified that can distinguish between them (77). This is consistent with the action of agonists and competitive antagonists being at an AA interface, as has been described for both human and mouse receptors (78, 79), but conflicting with data suggesting a BABBA arrangement determined using atomic force microscopy (80).

There have been only two studies to date examining the functional effects of roles of the C–E subunits (37, 39). None of these subunits form functional homomers but do result in functional

receptors when coexpressed with the A subunit, although there is no reported difference in radioligand binding, current-voltage relationships, or kinetics of whole cell currents of the presumed heteromers compared with the homomer. The physiological roles of these subunits are therefore still unknown. The role of the 5-HT$_3$D subunit is particularly tantalizing; there are two variants, one of which is missing a significant proportion of the ECD, whereas the other is lacking a Cys-Cys loop, both critical features of the vast majority of functionally characterized Cys loop receptor subunits. Perhaps these subunits can modify receptor function when in a very specific stoichiometry or are involved in receptor expression and trafficking. More studies are needed on these potentially interesting subunits.

Therapeutic Use and Potential

There are currently a range of 5-HT$_3$ antagonists available for clinical use in Europe, including tropisetron (Navaban®), ondansetron (Zofran®, Emetron®), granisetron (Kytril®), dolasetron (Anzemet®), and palonosetron (Aloxi®). These drugs have revolutionized the treatment of nausea and vomiting in cancer patients receiving chemotherapy or radiation therapy, which is their largest therapeutic use (81). The efficiency of these drugs may depend on the particular variants of 5-HT$_3$ receptors expressed by the patient; , for example, one isoform of the 5-HT$_3$B receptor has a promoter deletion that is associated with reduced efficacy of tropisetron and ondansetron (82–84).

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder affecting 10–15% of adults and is another major therapeutic area for 5-HT$_3$ receptor-selective compounds, perhaps not surprisingly as these receptors have roles in gastrointestinal motility, sensation, and secretion. Alosetron (Lotronex®, Lotronox®), a 5-HT$_3$ receptor antagonist, has been approved for the treatment of IBS, but there have been problems with constipation and, more rarely, ischemic colitis, and it is now less frequently used (primarily in female patients suffering from IBS with diarrhea). A range of other 5-HT$_3$-selective compounds, including some partial agonists, may prove more successful and are currently being explored (26, 85).

An important consideration is that the 5-HT$_3$ receptor-related actions of all drugs now on the market have been determined using homomeric 5-HT$_3$A receptors. This may not prove to be the most useful testing protocol given that other subunits may play important roles. Emerging studies suggest that alterations in a number of 5-HT$_3$ receptor subunits contribute to a range of disorders. Thus, mutations in the A, B, D, and E subunits have been associated with bipolar disorder, depression, anxiety, IBS, and anorexia (see Ref. 84 for a recent review). A greater understanding of the roles of C–E subunit-containing heteromeric receptors may therefore allow a wide range of other diseases to be treated with 5-HT$_3$ receptor-selective drugs, potentially including addiction, pruritis, emesis, fibromyalgia, migraine, chronic heart pain, bulimia, and neurological phenomena such as anxiety, psychosis, nociception, and cognitive function (26, 84, 85).

Conclusions

The 5-HT$_3$ receptor is a widely expressed, cation-selective member of the Cys loop receptor family. A range of studies, in particular heterologous expression and molecular modeling, have revealed many molecular details of its distribution, structure, function, and pharmacology. Nevertheless, information on receptor stoichiometry and the roles of these receptors in the CNS and PNS is still limited, suggesting there is much potential for therapeutic intervention in areas beyond those for which 5-HT$_3$ receptor-specific drugs are proving highly successful.

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