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The 5-HT_3 Receptor

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Summary

The 5-HT_3 receptor is a member of the Cys-loop neurotransmitter-gated ion channel family. It has five symmetrically placed subunits surrounding a central ion-conducting pore. Each subunit consists of an extracellular N-terminal ligand-binding domain, and C-terminal domain containing four transmembrane α helices (M1–M4); M2 lines the channel and controls ion selectivity and gating. A long intracellular loop between M3 and M4 is responsible for channel conductance and intracellular modulation. In this chapter we look at each of these regions, exploring the structure of the ligand binding site and its pharmacophore model, the importance of M2 and the complex modulatory mechanisms within the intracellular region that underlie the regulation, assembly, targeting, and trafficking of the 5-HT_3 receptor.

Key Words: Serotonin$_3$ receptor; 5-hydroxytryptamine$_3$ receptor; Cys-loop receptor; ligand-gated ion channel; ionotropic receptor; neurotransmitter binding site.

1. Introduction

The 5-hydroxytryptamine$_3$ (5-HT_3) receptor contains an integral, agonist-gated ion channel and in this way differs from all other known serotonin receptors whose actions are mediated via G proteins *(1)*. 5-HT_3 receptors were one of the original two classes of serotonin-activated receptors defined by Gaddum and Picarrelli *(2)*. Seven distinct classes have now been defined, but, to date, the 5-HT_3 receptor is the only vertebrate 5-HT-gated ion channel known; indeed, it is more closely related to the nicotinic acetylcholine (nACh) receptor than to any of these other classes of 5-HT receptor.

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The 5-HT_3 receptors are found in both the peripheral nervous system and central nervous system (CNS), where they mediate fast synaptic transmission at synapses *(3)*. In the CNS, they are located predominantly at interneurones, where they modulate the release of a range of neurotransmitters *(4–9)*. There is some evidence that 5-HT_3 receptors play roles in brain reward mechanisms and in neurological phenomena such as anxiety, psychosis, nociception, and cognitive function *(10,11)*, and in the first few years following the discovery of these receptors, there was also much interest in the therapeutic potential of 5-HT_3 receptor antagonists for antipsychotic, antinociceptive, and other psychiatric disorders *(12–15)*. This potential has not yet been realized, but there is still active research in this area *(16)*, and their current major therapeutic target is against emesis in cancer chemotherapy and irritable bowel syndrome *(17,18)*.

The cloning of cDNAs encoding 5-HT_3 receptor subunits over the last decade has taken the study of 5-HT_3 receptor pharmacology, physiology, and pathophysiology to the molecular level, although there is still much to be discovered. The availability of the acetylcholine-binding protein crystal structure has substantially enhanced our understanding of the ligand-binding domain *(19)*, and functional regions involved in receptor gating have been mapped (e.g., by electrophysiological analysis of mutated receptors expressed in heterologous cell systems) *(20)*. This chapter focuses primarily on the structural and functional insights of 5-HT_3 receptors revealed by molecular biological techniques, with particular attention being drawn to developments since 1990.

2. Receptor Subtypes

The first cDNA clone encoding the 5-HT_3 receptor A-subunit was isolated by screening a mouse neuroblastoma (NCB20) expression library for functional 5-HT-gated currents in *Xenopus* oocytes *(1)*. Subsequently, the full-length cDNAs for the orthologous 5-HT_{3A} subunit have been cloned from human *(21)*, rat *(22)*, guinea pig *(23)*, and ferret *(24)* by polymerase chain reaction (PCR) screening of several libraries. Sequence analysis of the 5-HT_{3A} subunit places it in the Cys-loop ligand-gated ion channel family because it has significant sequence and predicted structural similarity to other members of the family, which includes nACh, $GABA_A$, and glycine receptors. Topologically, these receptors consist of a large extracellular N-terminal and C-terminal domain, four transmembrane regions, M1–M4, of which M2 lines the pore, and a large intracellular loop between M3 and M4 (Fig. 1). Since 1999, four more genes encoding 5-HT_3 receptor subunits have been identified: 5-HT_{3B}, 5-HT_{3C}, 5-HT_{3D}, and 5-HT_{3E} subunits, although the latter two have not yet been expressed *(25,26)*. The 5-HT_{3B} and 5-HT_{3C} subunits have 45% and 39% sequence identities with their 5-HT_{3A} homologs, respectively, whereas the 5-HT_{3C} and 5-HT_{3D} predicted

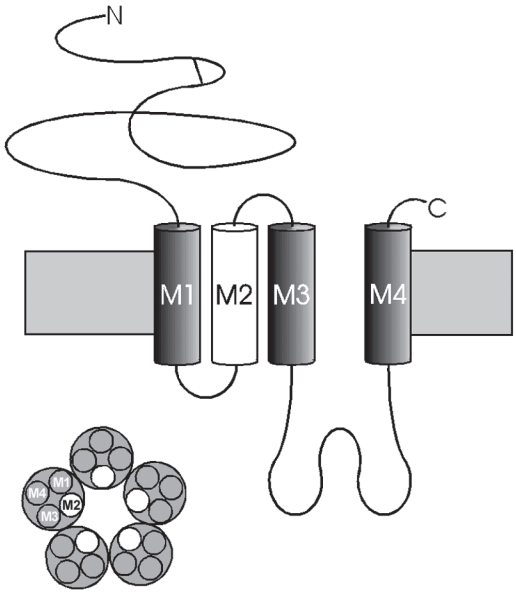

Fig. 1. Schematic representation of a typical Cys-loop ligand-gated ion channel subunit. The diagram at the lower left is a cross-section of the channel shown from above and demonstrates the association of the five subunits within the membrane. Attention is drawn to M2 (white circle), which has been shown to line the pore.

subunit sequences reveal overall identities of only 27% and 31% with the 5-HT$_{3A}$ subunit, respectively (Fig. 2).

Only the 5-HT$_{3A}$ subunit is able to form functional homomeric receptors, but some of their electrical properties, including channel rectification, desensitization, and single-channel conductance, do not resemble those of some native 5-HT$_3$ receptors. Studies of expressed heteromeric complexes of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits reveal a single-channel conductance that more closely resembles the conductance displayed by some native receptors *(25,27,28)*. In the absence of the B-subunit, 5-HT$_3$ responses have a conductance that is typically less than 1 pS *(28,29)*, but when the B-subunit is introduced, the conductance rises to around 20 pS. Specific amino acids that are responsible for this change in conductance have been identified *(28)*. In addition to low single-channel conductance, there are some differences in pharmacology between homomeric and heteromeric receptors. For example, heteromeric receptors have been shown to be less sensitive to *d*-tubocurarine *(25)*, picrotoxin *(30)*, alcohol *(105)*, and volatile anesthetics *(106)*. Additional biophysical differences include a relatively low permeability of the heteromeric 5-HT$_3$ receptor to calcium ions *(31)*, a linear

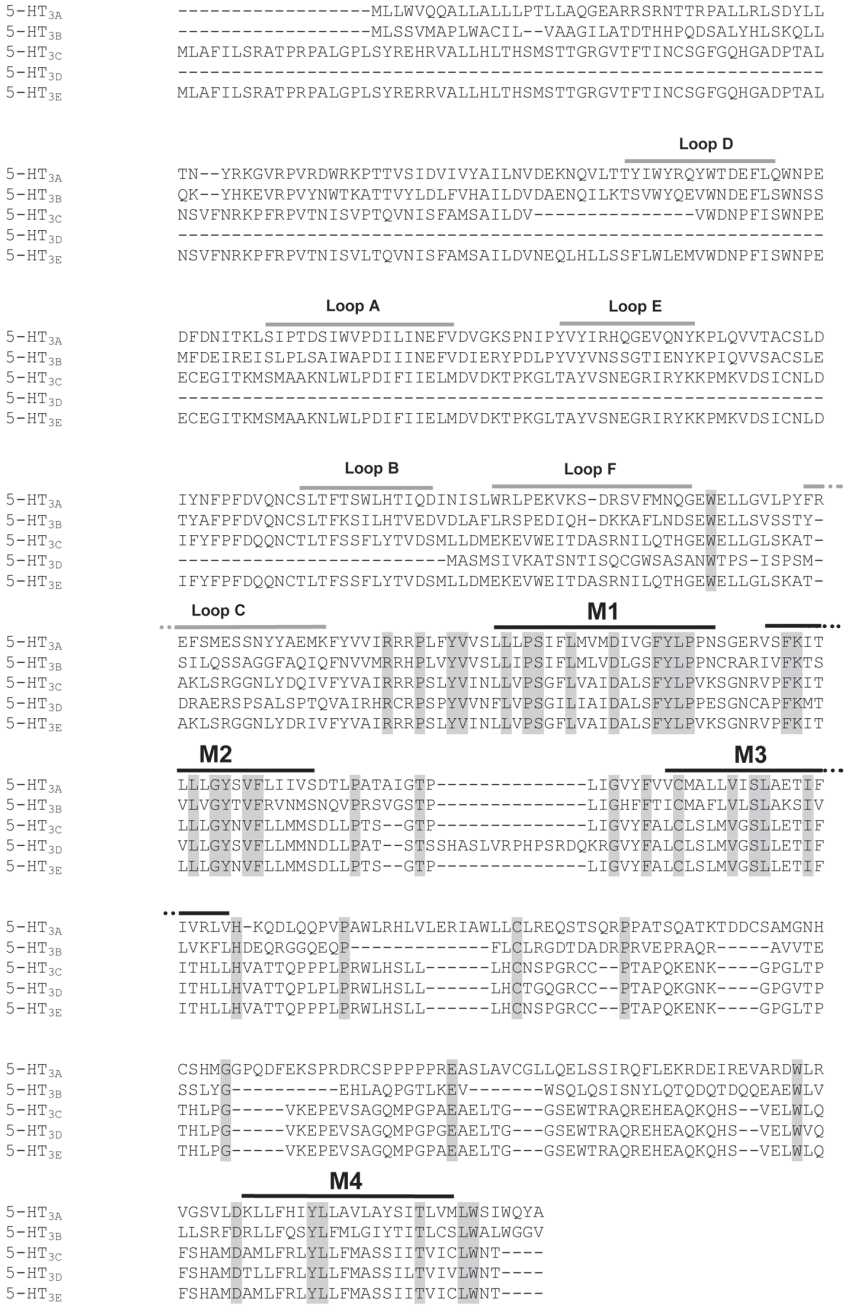

Fig. 2. Alignment of human 5-HT$_3$ receptor subunits. The binding loops and transmembrane (M1–M4) regions are highlighted by horizontal lines above the text. Conserved residues are highlighted in gray. Accession numbers for the alignment are as follows: 5-HT$_{3A}$, P46098; 5-HT$_{3B}$, O95264; 5-HT$_{3C}$, Q6V706. 5-HT$_{3D}$ and 5HT$_{3E}$ were taken from ref. *26*.

current–voltage relationship *(25)*, and complex changes in receptor desensitization *(32)*. The function of the 5-HT$_{3C}$, 5-HT$_{3D}$, and 5-HT$_{3E}$ subunits remains unknown, as they have not yet been characterized.

Structural heterogeneity resulting from alternative splicing has also been identified. A short form of the murine 5-HT$_{3A}$ receptors has been isolated in which five or six amino acid residues within the M3–M4 loop are absent *(33)*. The distribution of this short splice variant varies depending on its location in the adult animal *(34)* and during stages of embryonic development *(35)*, and there are some functional differences when compared to the long variant *(36–39)*. In humans, the splice acceptor site that is responsible for the long form of the receptor is missing *(36,40)* and, consequently, the long variant that is found in rodents is not expressed. However, in humans, a truncated (h5-HT$_{3AT}$) and an alternative long (h5-HT$_{3AL}$) form have been identified *(36)*. The truncated version consists of 238 amino acids and contains only a single transmembrane (M1) region, whereas the long form contains an additional 32 amino acids in the M2–M3 loop. Although these two subunit variants cannot form functional homomeric receptors when expressed alone, they are able to coassemble with 5-HT$_{3A}$ subunits and modulate the 5-HT response. Thus, the presence of splice variants might, in part, explain the functional variation seen in nature, but they are unlikely to account for all the functional diversity of native 5-HT$_3$ receptors.

3. Distribution

The 5-HT$_{3A}$ subunits have been found to be located in many brain areas, including the cortex, hippocampus, nucleus accumbens, substantia nigra, and ventral tegmental area, although the highest levels are in the brainstem, especially the nucleus tractus solitarius and area postrema *(34,41,42)*. Localization of 5-HT$_{3A}$ receptors in cholecystokinin (CCK) and GABA-containing interneurons is consistent with their involvement in the regulation of GABA and CCK neurotransmission *(43)*. 5-HT$_3$ receptors have also been found to colocalize with the CB1 cannabinoid receptor in rat brain neurons *(44,45)*, and a high proportion of 5-HT$_{3A}$/CB1-expressing neurons contained the inhibitory neurotransmitter GABA, indicating a possible interactions between the CB1 and 5-HT$_{3A}$ receptors and their contributable roles to the regulation of GABA neurotransmission in the brain. At the subcellular level, there is strong evidence for differential subcellular localization of presynaptic and/or postsynaptic 5-HT$_{3A}$ receptors within different central regions, depending on the nature of the neurons containing 5-HT$_{3A}$ receptors *(46)*. For instance, 5-HT$_{3A}$ receptor immunoreactivity was most abundant in postsynaptic dendritic sites in the hippocampus, but it was primarily associated with presynaptic nerve endings in the amygdala *(34,46)*. There is also some evidence that 5-HT$_{3A}$ receptor subunits might coexpress with subunits from other ligand-gated ion channels such as the nACh α_4-subunit *(47)*.

Studies have described 5-HT_{3B} receptor mRNA and immunoreactivity being detected in human brain *(48)* and in rat hippocampal neurons respectively *(49)*, but recent evidence has strongly suggested that 5-HT_{3B} subunits are restricted to the peripheral nervous system *(50)*. Thus, if these subunits are in the CNS, it is likely that they are in low abundance and/or in small subpopulations of cells *(26,51)*.

4. 5-HT_3 Receptor Pharmacology

The 5-HT_3 receptor is unusual among ligand-gated ion channels in that there are many highly selective and potent compounds that act at this receptor. Early studies categorized the receptor using the nonselective compounds morphine and cocaine *(2)*, but using 5-HT as the origin, bemesitron and tropisetron were formulated, and it was also found that 2-methyl-5-HT was a more potent agonist than 5-HT. Later compounds that were developed include ondansetron, granisetron, and zacopride, which act at nanomolar concentrations, and there are now a wide range of similarly potent compounds. Although the range of selective antagonists is well represented, the number of selective agonists is more limited. These include 1-phenylbiguanide and chlorophenylbiguanide (mCPBG), which is the most potent 5-HT_3 agonist developed to date *(52)*.

Research into the design of novel compounds has allowed the development of pharmacophore models for the receptor (Fig. 3). 5-HT_3 receptor agonists have a common basic amine, an aromatic ring, a hydrophobic group, and two hydrogen-bond acceptors *(53)*. 5-HT_3 receptor antagonists share a basic amine, a rigid aromatic or heteroaromatic ring system, and a carbonyl group (or isosteric equivalent) that is coplanar to the aromatic system *(54–57)*, and here there are slightly longer distances between the aromatic and amine group when compared to the agonist pharmacophore. Further work has shown that the 5-HT_3 receptor can only accommodate small substituents on the charged amine, and a methyl group here appears to be optimal *(57)*. Most of the potent antagonists of 5-HT_3 receptors have 6.5 heterocyclic rings, and the most potent compounds contain an aromatic six-membered ring. The species differences in 5-HT_3 receptor pharmacology have identified the roles of particular amino acids and/or regions of sequence (discussed in more detail below), for example, a number of residues in the C loop are strongly implicated to interact with *d*-tubocurarine *(75)*. Docking of a range of antagonists into a model of the 5-HT_3 receptor-binding site shows reasonably good agreement with the pharmacophore model and the details that differ between species *(58)*.

5. 5-HT_3 Receptor Structure: The N-Terminal, Extracellular Domain

There are currently no high-resolution structural data available for the 5-HT_3 receptor or, indeed, any ligand-gated ion channel, but the extracellular N-terminal

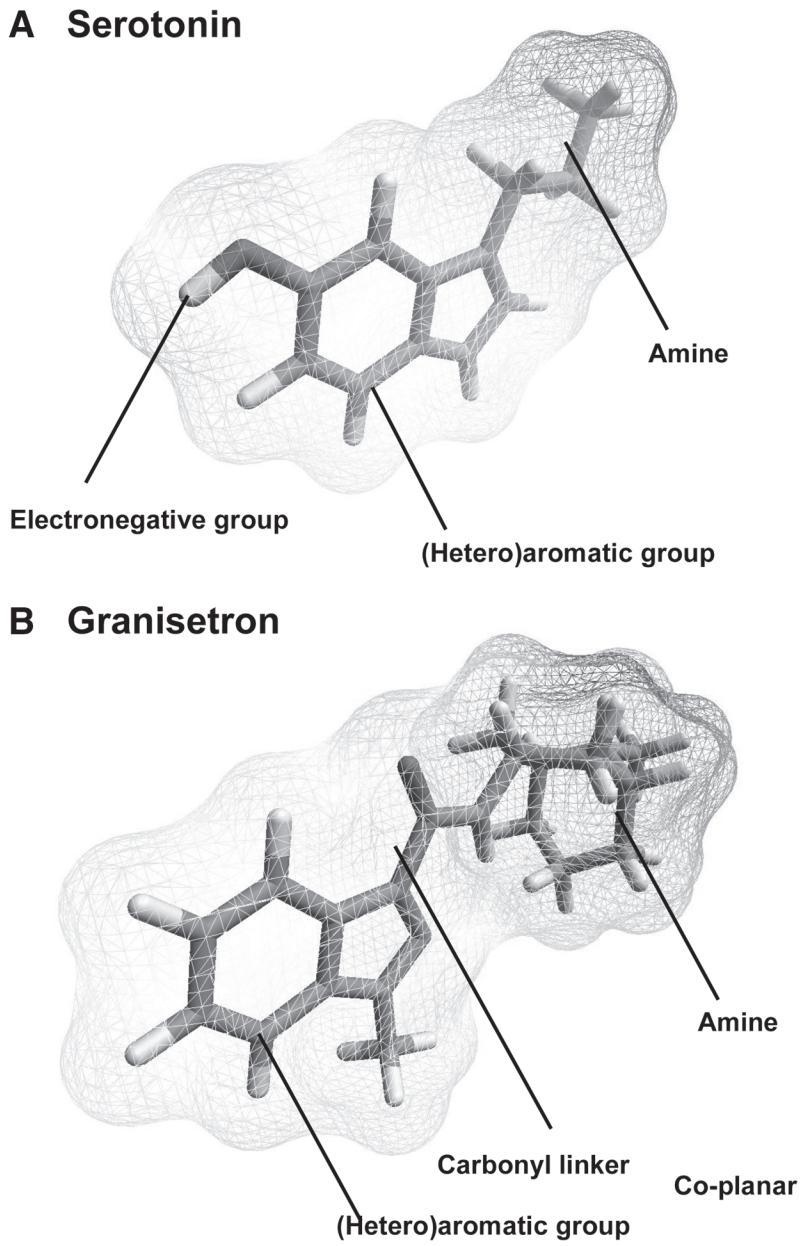

Fig. 3. Examples of 5-HT$_3$ agonist and antagonist pharmacophores. Serotonin **(A)** and granisetron **(B)** are shown as examples of 5-HT$_3$ receptor agonists and antagonists. Both molecules are shown as stick models. Electrostatic potential is displayed in wire frame. Attention has been drawn to the important features of each pharmacophore.

domain of these receptors is homologous to the acetylcholine-binding protein (AChBP), whose structure has been resolved to 2.1 Å *(59)*. The molecular details obtained from AChBP have been used to create homology models of the extracellular domains of a range of Cys-loop receptors, including the 5-HT_3 receptor. Although some structural details of specific regions appear to differ from those of AChBP *(60)*, the creation of a functional chimaeric receptor containing AChBP and the transmembrane domain of the 5-HT_3 receptor demonstrates that there is considerable structural and functional similarity between AChBP and the extracellular domain of the 5-HT_3 receptor. *(61)*.

The homology model of the homomeric 5-HT_{3A} receptor (Fig. 4) shows the ligand-binding site that lies between the faces of two adjacent subunits (as in AChBP) and is formed by three loops (A–C) from the "principal" subunit and three (D–F) from the adjacent or "complementary" subunit. The residues identified as being less than 5 Å from 5-HT are shown in Fig. 4B, and the proposed orientation of 5-HT in this binding pocket in Fig. 4C. As in all Cys-loop receptors, the binding pocket contains a large proportion of aromatic residues, many of which have roles in the binding of agonists or antagonists and/or in receptor gating. These are discussed in more detail below.

The model proposed by Reeves et al. *(62)* suggests that Asn128 is the only loop A residue that is within 5 Å of 5-HT. There are currently no studies to confirm this, but mutation of neighboring residues Glu129 and Phe130 significantly alters the binding efficiency of 5-HT_3 antagonists *(63,64)*. In addition, changing Phe130 to tyrosine resulted in the receptor being activated by ACh *(64)*. This residue is homologous to the AChBP residue Tyr89 that has been identified as a key binding residue *(19)*. There might also be a role of loop A in the structure and/or assembly of the receptor; mutation of residues Trp121 and Pro123 results in receptors that are expressed but no longer reach the cell surface *(65,66)*.

Trp183, in loop B, is critical for both ligand binding and function and has been shown to form a cation–π bond with the primary amine of 5-HT *(67)*. This residue is equivalent to Trp149 in the nACh α_1-subunit, which also forms a cation-π interaction with acetylcholine *(68)*. The equivalent tryptophan residue in AChBP has been found to be a key component of binding of both nicotine and carbamylcholine *(59)*. Equivalent aromatic residues in $GABA_A$ and glycine receptors have also been shown to be vital for ligand binding *(69–71)*.

Fig. 4. (A) 5-HT_3 homology model based on the AChBP crystal structure (1I9B) showing the binding loops within two of the five subunits that make up the extracellular domain of the 5-HT_3 receptor. The binding site is formed from loops A–C within the principal subunit and loops D–F in the complementary subunit. **(B)** Locations of the amino acids that are proposed to be within 5 Å of the ligand-binding site.

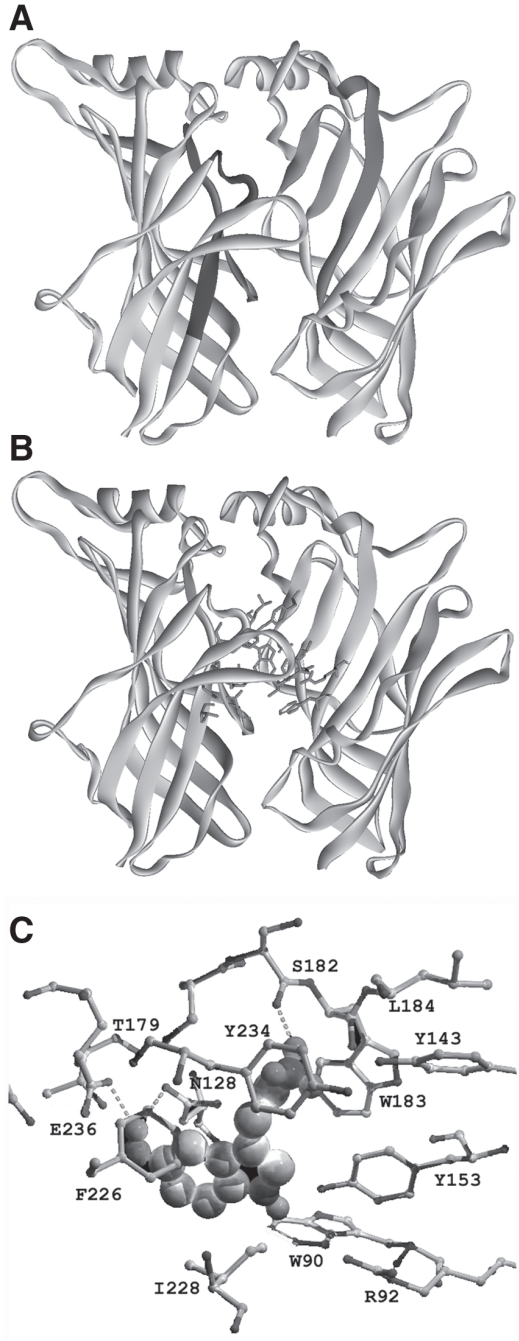

Fig. 4. *(continued)* Residues (black) are superimposed upon two adjacent subunits from the extracellular domain. Data from ref. *62*. **(C)** The proposed orientation of 5-HT in the 5-HT_3 receptor ligand-binding site. Modified from ref. *62*.

The loop C region has been extensively investigated by a number of groups. Studies have revealed that the aromatic group of Tyr234 is essential for both binding and function, whereas the hydroxyl group, or indeed any group at the 4-position, is required for efficient function *(72)*. Glu236 is also an important ligand-binding residue and might form a hydrogen bond with the agonist *(73)*. The C loop has been found to be critical in controlling the potency of both the 5-HT_3 receptor agonist mCPBG *(74)* and the antagonist *d*-tubocurarine *(75)*. However, a single amino acid responsible for the difference could not be identified, indicating that a number of other residues and/or binding regions might also play a role.

A considerable number of residues in loop D have been implicated in binding, showing that this region is important for agonist and antagonist interactions. An aromatic residue is required at both Trp90 and Trp95; Trp90 has a role in ligand binding, whereas Trp95 controls localization of the receptor to the cell surface *(76)*. Aromatic contacts have been demonstrated in AChBP between the residue equivalent to Trp90 (Trp53) and nicotine, and similar contacts probably occur in the 5-HT_3 receptor. There is also evidence that equivalent residues in nACh and $GABA_A$ receptors are important in binding, indicating that this position is functionally similar among many members of the ligand-gated ion channel family *(76)*. Other amino acids that have been studied include Tyr91, Arg92, and Tyr94. Mutation of these residues to alanine altered antagonist-binding affinity depending on the antagonist studied, indicating that different ligands have different points of interaction with the binding site *(77)*.

Loops E and F are considerably more varied than the above-described loops, and interestingly, the sequence variability seen between subunits of the same family suggests that the structures in these regions might differ according to the stoichiometry of the receptor. Scanning alanine mutagenesis of the E loop have revealed that Tyr143, Gly148, Glu149, Val150, Gln151, Asn152, Tyr153, and Lys154 might be important for granisetron binding *(78)*. In particular, mutation of Gly148 and Val150 completely abolished radioligand binding, although it is currently difficult to assign a particular role to these residues. Tyr143 and Tyr153 have been further studied using unnatural amino acid mutagenesis *(72,79)*. These studies have shown that whereas Tyr153 is involved in both binding and gating, the role of Tyr143 is primarily in gating, and it has been proposed to be involved in initiating the conformational changes that lead to channel opening *(79)*.

The role of loop F residues have yet to be elucidated. In the AChBP crystal structure, the loop F region was poorly resolved *(19)*; thus, its current location on the homology model on the 5-HT_3 receptor is only tentative. We await further studies to reveal the importance of this region.

6. 5-HT$_3$ Receptor Structure: The Transmembrane Region

The transmembrane region of the 5-HT$_3$ receptor consists of four transmembrane-spanning segments (M1–M4) that are linked by loops (Fig. 1). The structure of the M1–M4 segments is believed to be similar to that of the nACh receptor, which has been resolved to a resolution of 4 Å *(60)*. Consequently, the 5-HT$_3$ transmembrane segments are thought to be α-helical, an observation that is in agreement with predictions using hydrophobicity analysis, infrared spectroscopy, and circular dichroism *(1,80)*.

The M2 region of the 5-HT$_3$ receptor has been extensively explored using mutagenesis. Studies using the substituted cysteine scanning method (SCAM) have identified residues that run along the ion-accessible inner face of the channel *(83,89)*. These residues are predominantly nonpolar except for rings of charged amino acids. Surprisingly, scanning histidine accessibility mutagenesis (SHAM) on the 5-HT$_3$ receptor has suggested some differences in these water-accessible residues *(81),* which cannot yet be explained. SCAM analysis has also shown that movement in the center of M2 coincides with channel activation, indicating that this is the location of the channel gate *(82)*.

The part of M2 that controls ion selectivity, however, appears to be quite distinct and might involve several regions. Studies on the α7 nACh receptor *(83)* showed that changing only three amino acids could convert this channel from cationic to anionic, albeit with a substantial change in receptor properties. Comparable mutations in the 5-HT$_3$ *(84)*, glycine *(85),* and GABA ρ1 *(86)* receptors demonstrated that changing the equivalent amino acids in these channels also resulted in a change in ion selectivity, although, again, the mutant receptors had significant changes in some of their properties. These changes might be the result of insertion or removal of a proline residue, which was considered essential *(87)*. However, more recent work on the 5-HT$_3$ receptor indicates that this is not the case: Here, neutralizing a single charged ring at the cytoplasmic end of the pore yields a nonselective receptor, which can then be converted to an anion-preferring channel by insertion of a positively charged ring at the extracellular end *(88)*. These changes in selectivity were made without any changes in other channel properties and, therefore, suggest that ion selectivity is largely controlled by the presence of charged amino acids at one or both ends of M2.

7. Posttranslational Modulation of 5-HT$_3$ Receptors

The function of the 5-HT$_3$ receptors has been shown to be regulated by various protein kinases, probably via its large cytoplasmic domain, which contains a cluster of potential phosphorylation sites *(1)*. Activation of protein kinase A (PKA) substantially accelerates desensitization kinetics of 5-HT$_3$

receptors *(38,90,91)*, and activators of protein kinase C (PKC) increase the amplitude of 5-HT-activated currents *(92,93)*. In addition, the PKC activator PMA is reported to regulate the probability of occurrence of certain conductance levels of 5-HT-activated single-channel currents in N1E-115 cells *(94)* and there is some evidence that modulation of receptor responses by casine kinase II and tyrosine kinases might also occur *(95)*. A serine (S409) in the large intracellular loop of 5-HT_{3A} receptor has been found to be critical for PKA-induced phosphorylation of the receptor protein expressed in HEK293 cells *(96)*, but identifying a PKC phosphorylation site has proved more elusive. Neither single mutations or combinations of known sites significantly affected the sensitivity of the mutant receptors to PKC activation *(93,95)*.

There is, however, increasing evidence that PKC modulates 5-HT_3 receptor trafficking. Activation of PKC rapidly increases surface expression of 5-HT_{3A} receptors, which might occur via an F-actin-dependent mechanism; 5-HT_{3A} receptors are colocalized and coclustered with F-actin-rich membrane domains such as lamellipodia and microspikes *(93,97–100)*. In addition, preapplication of phalloidin, which stabilizes the actin polymerization, significantly inhibited PMA potentiation of 5-HT-activated responses, and latrunculin-A, which disrupts F-actin cytoskeletons, altered the topology and the size of 5-HT_{3A} receptor clusters *(97)*. Given that neurotransmitter release can be regulated through an actin-dependent mechanism in the CNS *(101)* and that 5-HT_{3A} receptors can modulate the release of dopamine and GABA in some important brain areas, it is possible that the enhancement of 5-HT_3 receptor function and trafficking by PKC activation might play an important role in modulating the efficacy of serotonergic synaptic transmission.

Other processes of posttranslational modulation such as protein glycosylation and palmitorylation have also been described in the studies of 5-HT_{3A} receptors *(102–104)*. The exact roles of these processes in the regulation of 5-HT_{3A} receptor assembly, targeting, and trafficking is yet to be determined.

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