22.1 INTRODUCTION

The 5-HT₃ receptor is a Cys-loop ligand-gated ion channel and is structurally and functionally distinct from the other six classes of 5-HT receptors whose actions are mediated via G-proteins. 5-HT₃ receptors are pentamers, and five classes of subunit have been identified. These are widely distributed, both in the nervous system and in other tissues. 5-HT₃ receptor activation opens a cation-selective ion channel, and receptor function can be modulated by a wide range of compounds including anesthetics, opioids, and alcohols. 5-HT₃ receptors play a major role in the vomiting reflex; regulate gut motility, secretion, and peristalsis in the enteric nervous system; and are involved in information transfer in the gastrointestinal (GI) tract. Disturbances within the 5-HT₃ receptor system may contribute to the etiopathogenesis of a range of neurological, GI, and immunological disorders.

22.2 SUBUNIT DIVERSITY

22.2.1 5-HT₃A SUBUNITS

The first cDNA clone encoding a 5-HT₃ receptor subunit, the mouse 5-HT₃A receptor subunit, was isolated by functional screening of a mouse neuroblastoma (NCB20) cDNA library (Maricq et al., 1991). Subsequently, the full-length cDNAs for orthologous 5-HT₃A receptor subunits have been cloned from a range of species including human (Belelli et al., 1995; Miyake et al., 1995), guinea pig (Lankiewicz et al., 1998), ferret (Mochizuki et al., 2000), and dog (Jensen et al., 2006). The homology between 5-HT₃A receptor subunits and those from other Cys-loop receptors clearly indicates 5-HT₃ receptors are members of this family, although somewhat unusually, they can readily form functional homomeric receptors. This suggests they...
are perhaps evolutionarily ancient, but the lack of 5-HT₃ receptor homologues in invertebrates indicates they are more recently evolved than at least some other Cys-loop receptors, such as those for acetylcholine and GABA (Dent, 2006). 5-HT₃ receptors can also function as heteromeric proteins, and the presence of additional subunits was indicated from a range of studies some years before such subunits were identified, for example, when expressed in HEK-293 cells, 5-HT₃A receptors had a single channel conductance of <1 pS, while channel activity in rabbit nodose ganglion revealed a single-channel conductance of 19 pS (Gill et al., 1995). In 1999, a second subunit, the 5-HT₃B subunit, was identified (Davies et al., 1999; Dubin et al., 1999). Coexpression of this subunit with the 5-HT₃A subunit resulted in functional receptors with properties that more closely represented those found in some native receptors. Since then, three other subunits (5-HT₃C, 5-HT₃D, and 5-HT₃E; see Figure 22.1) have been identified (Niesler et al., 2003).

The repertoire of 5-HT₃ receptor subunits is increased by a number of different isoforms and promoters, alternative splicing, single nucleotide polymorphisms (SNPs), and posttranslational modifications (Figure 22.2) (Holbrook et al., 2009; Niesler et al., 2003, 2008). Alternative splicing of the transcript encoding guinea pig, mouse, and rat (but not dog, ferret, or human), 5-HT₃A subunits results in long (5-HT₃A(a)) and short (5-HT₃A(b)) isoforms, where the 5-HT₃A(b) isoform lacks 5 or 6 amino acid residues within the M3–M4 intracellular loop, resulting in some subtle differences in receptor properties (Hope et al., 1993; Lankiewicz et al., 1998). In the human 5-HT₃A subunit, three different splice variants have been described. Two of these (5-HT₃AL, 5-HT₃Rest) would result in larger receptors, while the other (5-HT₃AT) codes for a partial receptor containing only a single transmembrane domain (TMD). 5-HT₃Rest has not yet been functionally evaluated, but 5-HT₃AT and 5-HT₃AL, while not functional when expressed alone, form receptors with modified functional properties when coexpressed with canonical 5-HT₃A subunits (Bruss et al., 2000).

### 22.2.2 5-HT₃B, 5-HT₃C, 5-HT₃D, AND 5-HT₃E SUBUNITS

In the human HT3B gene, alternative tissue-specific promoters have the potential to create truncated versions of the canonical 5-HT₃B subunit (Figure 22.3) (Tzvetkov et al., 2007). One of these only has a few amino acids missing from the N-terminus, while the other is devoid of a large proportion of the extracellular domain (ECD). The functional significance of these isoforms has not yet been evaluated.

The 5-HT₃C subunits appear to have the largest number of isoforms with at least five homologous genes reported in humans, compared to two for the 5-HT₃D subunit and three for 5-HT₃E (Holbrook et al., 2009; Niesler et al., 2003, 2007, 2008). As yet many of these subunits have not been demonstrated to contribute to physiological receptors, so their significance is yet to be established.

Studies have indicated that microRNAs could also play a role in regulation of 5-HT₃R subunit expression. A mutation in the 3′UTR of the 5-HT₃E gene impairs binding of has-miR-510, resulting in enhanced expression (Kapeller et al., 2008). Further work is required to see if other genes are similarly influenced.

The stoichiometry of heteromeric receptors is still not clear, although it has been established that only 5-HT₃A subunits can form functional homomeric 5-HT₃ receptors, and the presence of at least one 5-HT₃A subunit appears to be obligatory in heteromeric receptors (Holbrook et al., 2009; Niesler et al., 2008). 5-HT₃AB receptors were originally suggested to possess a 3B/2A subunit ratio, and atomic force microscopy with tagged subunits indicated a BABBAb arrangement (Barrera et al., 2005). However more recent data reveal a 3A/2B ratio with an ABAB arrangement (Miles et al., 2013; Lochner and Lummis, 2010; Thompson et al., 2011b), which is more consistent with the characteristics of these receptors (Thompson and Lummis, 2013). The arrangement and number of 5-HT₃C, 5-HT₃D, and 5-HT₃E subunits in functional receptors have not yet been determined, although there are many possibilities (Figure 22.2).

### 22.3 STRUCTURE

5-HT₃ receptors, like other Cys-loop receptors, are pentameric assemblies of five identical or nonidentical subunits that pseudosymmetrically surround the ion pore (Boess et al., 1992; Green et al., 1995). Each subunit has a large ECD, a TMD consisting of four membrane-spanning α-helices (M1–M4), and an intracellular domain (ICD) between M3 and M4 (Figures 22.1 and 22.4). The structure of the homomeric 5-HT₃A receptor only became available in 2014 (Hassaine et al., 2014), some years after the structures of related proteins were solved. Many of these related structures, which were derived using cryoelectron microscope and x-ray crystallography, are good structural representatives, and those that were used for templates before the structure became available include the nACh receptor, many acetylcholine-binding proteins (AChBPs), the invertebrate glutamate-gated chloride channel (GlutCl), and the bacterial homologues Erwinia and Gloeobacter ligand-gated ion channels (ELIC and GLIC). Examples of such homology models can be found in recent publications (e.g., Reeves and Lummis, 2002; Thompson et al., 2010; 2011a; Verheij et al., 2012).

#### 22.3.1 EXTRACELLULAR DOMAIN

Homology models of the ECD (Figure 22.4) are largely supported by experimental data, and thus we currently have a good understanding of many details of this region. The ECD contains the agonist-binding site, which is located at the interface of two adjacent subunits 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; key residues that contribute to the binding pocket in these loops have been identified from a range of studies (see Barnes et al., 2009; Thompson and Lummis, 2006; Thompson et al., 2010 for reviews). In loop A, substitutions in the sequence 128AsnGluPhe130 modify receptor function, although the only residue that has been extensively investigated is Glu129, which forms one or more hydrogen bonds critical for binding (Boess et al., 1997; Price et al., 2008; Steward et al., 2000; Sullivan et al., 2006). Loop B is very sensitive to modification, with substitution of many residues abating function; this region is an obligate rigid structure with an extensive hydrogen bond network (Hassaine et al., 2014;
Figure 22.1 5-HT$_3$ receptor subunit alignment. The approximate locations of the binding loops on the principal (Loops A–C) and complementary (Loops D–F) faces, the Cys loop, and the transmembrane $\alpha$-helices (M1–M4). Each subunit has multiple isoforms only one of which is shown here.

Thompson et al., (2008). The loop B Trp residue plays an especially critical role as it forms a cation–$\pi$ interaction with the primary amine of 5-HT (Beene et al., 2002; Spier and Lummis, 2000). Loop C shows the largest variability and is important in determining the species specificity of various ligands, with multiple regions of the loop being important. Loops C and D both contribute a Trp to the aromatic box found in all Cys loop receptors, and Loop D also contributes an Arg to the binding site. Many Loop E residues, and at least three loop F residues, have been shown to be important for ligand binding, although a role in
Figure 22.2 Molecular basis of the functional and pharmacological diversity of receptors in the 5-HT₃ receptor family. Receptor heterogeneity may be achieved at many levels in humans including (1) the existence of different subunits (5-HT₃A-5-HT₃E), (2) the use of alternative tissue-specific promoters driving differential expression, (3) alternative splicing (mostly SNPs), and (4) posttranslational modification (e.g., phosphorylation or glycosylation). The subunit composition of physiologically expressed heteromeric 5-HT₃ receptors is not yet known but there are many possibilities. (Reproduced from Walstab, J. et al., Pharmacol. Ther., 128, 146, 2010. With permission.)

Figure 22.3 Protein sequences predicted from the alternative 5-HT₃B transcripts. The N-terminal localization signal and the β1–β2 loop structure mediating channel gating are missing in the Brain-2 isoform. The part of the localization signal that differs between the intestinal and Brain-1 form is marked by hatching. The N-terminal sequences expected to differ between the isoforms are shown in the lower part of the figure. The signal sequence (underlined) and the cutting site of the signal peptidase (arrow) are shown for the canonical 5-HT₃B form. The six amino acids of the N-terminus of the Brain-1 isoform that differ from the canonical form are shaded. The two β-sheets are boxed and the potential N-glycosylation sites are shown in bold. (Reproduced from Tzvetkov, M.V. et al., Gene, 386, 52, 2007. With permission.)
22.3 STRUCTURE

The TMD of each of the 5-HT₃ receptor subunits is composed of four transmembrane α-helices (M1–M4), with short loops between M1 and M2 (intracellular) and M2 and M3 (extracellular). The M2 α-helices line the ion pore and extrapilation from nACh receptor data indicates that the α-helices of M1 and M2 project above the level of the membrane (Figure 22.5). M1, M3, and M4 protect M2 from the surrounding membrane lipids, although various mutations in these α-helices have been shown to modify channel function, suggesting that some residues or regions in these α-helices are involved in receptor activation. The conserved proline in M1, for example, is essential for activation, and the receptor is expressed but cannot function when this proline is replaced by alanine, glycine, or leucine (Dang et al., 2000). However, substitution with trans-3-methyl-proline, pipercolic acid, or leucic acid yields active channels similar to wild-type receptors. The commonality between these residues and proline is the lack of hydrogen bond donor activity; thus, the data suggest this is a key element in channel gating, possibly because of the resulting flexibility in secondary structure in this region of M1. Some regions, however, are probably purely structural; a minimum number of C-terminal residues in M4, for example, are essential for the expression of 5-HT₃ receptor on the cell surface (Butler et al., 1990).

### 22.3.2 TRANSMEMBRANE DOMAIN

The TMD of each of the 5-HT₃ receptor subunits is composed of four transmembrane α-helices (M1–M4), with short loops between M1 and M2 (intracellular) and M2 and M3 (extracellular). The M2 α-helices line the ion pore and extrapilation from nACh receptor data indicates that the α-helices of M1 and M2 project above the level of the membrane (Figure 22.5). M1, M3, and M4 protect M2 from the surrounding membrane lipids, although various mutations in these α-helices have been shown to modify channel function, suggesting that some residues or regions in these α-helices are involved in receptor activation. The conserved proline in M1, for example, is essential for activation, and the receptor is expressed but cannot function when this proline is replaced by alanine, glycine, or leucine (Dang et al., 2000). However, substitution with trans-3-methyl-proline, pipercolic acid, or leucic acid yields active channels similar to wild-type receptors. The commonality between these residues and proline is the lack of hydrogen bond donor activity; thus, the data suggest this is a key element in channel gating, possibly because of the resulting flexibility in secondary structure in this region of M1. Some regions, however, are probably purely structural; a minimum number of C-terminal residues in M4, for example, are essential for the expression of 5-HT₃ receptor on the cell surface (Butler et al., 1990).

### 22.3.3 INTRACELLULAR DOMAIN

The ICD is formed by the large M3–M4 intracellular loop, and is responsible for receptor modulation, and possibly also plays a role in trafficking. Deletion studies reveal the ICD is not essential, as the mouse 5-HT₃A receptor subunit ICD can be replaced by the heptapeptide M3–M4 linker of GLIC without loss of function (Jansen et al., 2008). Further evidence that the ICD can function as a separate domain comes from studies where it was added to the GLIC linker peptide, resulting in modification of GLIC function by the intracellular protein RIC-3 (Goyal et al., 2011).

ICD structural details are sparse, but each subunit is known to possess an α-helix that contributes to openings, known as portals, just below the level of the membrane. The residues that line these portals are important for ion conductance: when the 5-HT₃A subunit residues are replaced with those found in the 5-HT₃B subunit, the single-channel conductance, which is very low in the homomeric 5-HT₃A receptor, is increased to that of the heteromeric 5-HT₃Aβ receptor (Kelley et al., 2003; see Section 22.5 for more details).

**Figure 22.5** Sequence alignment of the M1, M2 (indicated by bars), and flanking regions of mouse 5-HT₃ and other representative Cys-loop receptor subunits. The white sections indicate regions of the structure that protrude above the membrane. The dashed line is the M1–M2 loop. The conserved proline residue in M1 is highlighted, as are the two residues in the 5-HT₃ receptor that result in a reversal of ion selectivity when mutated. The prime (’) labeling system in M2 is also shown. (Reproduced from Lummis, 2004. With permission.)
22.4 PHYSIOLOGICAL ROLES AND EXPRESSION

5-HT₃ receptors are located in many brain areas including the hippocampus, entorhinal cortex, frontal cortex, cingulated cortex, dorsal horn ganglia, amygdala, nucleus accumbens, substantia nigra, and ventral tegmental area (Barnes et al., 2009; Pratt et al., 1990). The dorsal vagal complex in the brainstem, which is key to the vomiting reflex and contains the area postrema and nucleus tractus solitarius, has the highest levels, consistent with the potent antiemetic properties of 5-HT₃ receptor antagonists (Pratt et al., 1990).

5-HT₃ receptor activation in the CNS can modulate the release of a variety of neurotransmitters, including dopamine, cholecystokinin, GABA, substance P, and acetylcholine (Barnes et al., 2009). In the rat brain, 5-HT₃ receptors are expressed by subsets of inhibitory interneurons in the CA1 area and dentate gyrus of the hippocampus and layer I of the cerebral cortex (Kawa, 1994; Ropert and Guy, 1991; Zhou and Hablitz, 1999). Presynaptic 5-HT₃ receptor activation at these interneurons likely results in sufficient Ca²⁺ entry to influence GABA release and cause an increase in the frequency of GABAergic receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs). Presynaptic 5-HT₃ receptors also facilitate the release of glutamate onto dorsal vagal preganglionic, nucleus tractus solitarius, and area postrema neurons (Glaum et al., 1992; Jeggo et al., 2005).

Postsynaptic 5-HT₃ receptor activation contributes to fast excitatory synaptic transmission in a range of locations including the lateral amygdala, nucleus tractus solitarius, visual cortex, and neocortical GABAergic interneurons that contain cholecystokinin and vasoactive intestinal peptide (Ferezou et al., 2002; Glaum et al., 1992; Koyama et al., 2000; Sugita et al., 1992). Indeed, 5-HT₃ receptors are often colocalized with cholecystokinin and also with central CB1 cannabinoid receptors: in neurons of the rat telencephalon, anterior olfactory nucleus, cortex, hippocampus, dentate gyrus, and amygdala, 37%–53% of all neurons expressing the 5-HT₃A receptor subunit also expressed CB1 transcripts, and 5-HT₃A/CB1-expressing neurons also contained GABA (Morales et al., 2004).

5-HT₃ receptors have long been known to play a role in the emetic response, and thus it is not surprising that they are involved in information transfer in the GI tract, and in the enteric nervous system they regulate gut motility and peristalsis (Galligan, 2002). They also play an important role in the urinary tract, and expression of constitutively active hypersensitive 5-HT₃ receptors in mice leads to excitotoxic neuronal cell death, resulting in fatal uropathy (Bhattacharya et al., 2004).

The functional studies are supported by expression studies, with 5-HT₃A receptor mRNA and protein being observed in regions known to have 5-HT₃ receptors. Such studies also indicate 5-HT₃ receptor expression in a wide range of tissues other than the CNS, including peripheral and sensory ganglia, and the GI tract (Barnes et al., 2009). In addition, expression of the 5-HT₃A subunit has been reported in immune cells such as monocytes, chondrocytes, T cells, synovial tissue, and platelets (Fiebich et al., 2004; Stratz et al., 2008).

There was initial controversy as to the presence of 5-HT₃B subunits in brain, but later studies show it is expressed here, with a preference for distinct brain-type isoforms. The longer canonical B subunit is broadly expressed in many tissues including the kidney, liver, and the GI tract, with relatively high levels in the spleen, colon, small intestine, and kidney (Holbrook et al., 2009; Tzvetkov et al., 2007).

5-HT₃C, 5-HT₃D, and 5-HT₃E receptor 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 (Holbrook et al., 2009; Niesler et al., 2008). Initial studies suggested that the 5-HT₃D and 5-HT₃E subunits had a very restricted expression in the GI tract, but more recent data suggest all these subunits have a widespread distribution. Studies examining protein levels have lagged behind the genetic work, but expression of 5-HT₃C, 5-HT₃D, and 5-HT₃E subunits at the protein level in the GI tract has been confirmed (Kapeller et al., 2011).

22.5 BIOPHYSICAL PROPERTIES

22.5.1 RECEPTOR ACTIVATION

5-HT₃A receptors mediate rapidly activating and desensitizing inward currents, which show inward rectification. Concentration response studies from many groups indicate multiple agonist-binding sites, and occupation of at least two binding sites is needed for maximal activation (Jackson and Yake, 1995). Detailed studies using a high conductance mutant of the 5-HT₃A receptor show that full activation arises from receptors with three agonist molecules bound (Corradi et al., 2009). The kinetic model derived from these data shows that a conformational change of the fully liganded receptor occurs while the channel is still closed, and the receptor subsequently enters an open–closed cycle involving three open states.

22.5.2 IONIC SELECTIVITY

The 5-HT₃ receptor pore is a relatively nonselective cation channel, constructed of five pseudosymmetrically arranged M2 α-helices (one from each of the five subunits). The residues that line the ion-accessible face of M2 are predominantly nonpolar and are the major controlling influence on ion flux (McKinnon et al., 2011; Panicker et al., 2002; Reeves et al., 2001). Currents are primarily carried by Na⁺ and K⁺ ions, although divalent and small organic cations are also permeable (Derkach et al., 1989; Maricq et al., 1991; Yang, 1990).

Ionic selectivity is predominantly mediated via residues in M2: a triple mutant receptor with a proline insertion at –1′ and the substitutions E→V₃ and V₃′→I resulted in an anion permeable mouse 5-HT₃A receptor (Gunthorpe and Lummis, 2001), although subsequent studies showed that the replacement of only two residues (E→I, S₁₉′R) was needed to invert ion selectivity (Figure 22.3) (Thompson and Lummis, 2003). Changing –1′E alone to Ala resulted in nonselective channels indicating that the rings of charge at either end of M2 charge make the most critical contribution (Thompson and Lummis, 2003). Equivalent residues participate in the selectivity filters of other Cys-loop receptors (Thompson et al., 2010).
5-HT₃A receptors are almost 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., 2011). However, human 5-HT₃AB receptors have lower Ca²⁺ permeability (P_Ca/P_Cs = 0.6 [Davies et al., 1999]), possibly the consequence of the 20′ residue being neutral (Asn and not Asp) in human 5-HT₃B subunits. Consistent with this, a D20′A substitution in 5-HT₃A subunits reduces Ca²⁺ permeability (P_Ca/P_Cs = 0.4) (Livesey et al., 2008). Studies suggest that the ICD may also play a role in Ca²⁺ permeability as substitutions of charged residues in this region can have a major effect on Ca²⁺ permeability (Livesey et al., 2011).

### 22.5.3 SINGLE-CHANNEL CONDUCTANCE

The single-channel conductance of the homomeric receptor is low: values of 0.4–0.76 pS have been reported (Davies et al., 1999; Gunthorpe et al., 2000; Kelley et al., 2003). The presence of Lys in the M2 region was originally considered a possible explanation, but substitutions here revealed this was not the case (Gunthorpe et al., 2000). Subsequent work revealed that the low conductance was due to residues located in the amphipathic helix of the M3–M4 loop, which forms portals through which ions can cross between the intracellular vestibule of the receptor and the cell interior (Kelley et al., 2003).

### 22.5.4 HETEROmeric RECEPTOR PROPERTIES

Heteromeric 5-HT₃AB receptors show a range of distinct biophysical characteristics when compared to 5-HT₃A receptors: their desensitization is more rapid, and concentration response curves reveal lower E_C₅₀ and Hill slopes (Figure 22.6); their voltage dependence is linear, and their divergent cation permeability is much reduced; most noticeable, however, is their large single-channel conductance (13–16 pS) (Peters et al., 2005). This has been shown to be due to the substitution of Arg residues in the M3–M4 helical region of the 5-HT₃A subunit by neutral or negatively charged residues in the 5-HT₃B subunit (Kelley et al., 2003).

### 22.5.5 CHANNEL GATE

The gate of the 5-HT₃A receptor channel is located centrally within M2 (Panicker et al., 2002), consistent with the hydrophobic girdle model of channel gating, the hydrophobic girdle being a region in the center of M2 that is less than 3.5 Å in diameter over a distance of ~8 Å; the residues that face the pore here are hydrophobic (13′Val and 9′Leu) making it effectively impermeable to ions in the closed conformation (Miyazawa et al., 2003). Data consistent with this hypothesis are substitutions of Val13′ residues in the 5-HT₃A receptor by threonine or serine, which causes an increase in agonist potency (Dang et al., 2000), or spontaneous channel openings (Bhattacharya et al., 2004), and substitutions of Leu9′ by a range of amino acids, which affects agonist potency and desensitization rates (Yakel et al., 1993).

### 22.6 PHARMACOLOGY

#### 22.6.1 5-HT₃ RECEPTOR AGONISTS

There are currently many selective and potent compounds that act at the 5-HT₃ receptor (Figure 22.7). 5-HT₃ receptor agonists have in common a basic amine, an aromatic ring, a hydrophobic group, and two hydrogen bond acceptors, and active compounds include 2-methyl-5-HT, phenylbiguanide, and m-chlorophenylbiguanide (Cockcroft et al., 1995; Kilpatrick et al., 1990). Data from AChBP suggest that agonists are relatively small compounds that cause the C-loop to close over the binding site, initiating the gating process, and the structure of 5-HTBP, which is a modified version of AChBP that binds serotonin and granisetron, supports a similar mechanism of action for the 5-HT₃ receptor (Kesters et al., 2013; Tsetlin and Hucho, 2009).

#### 22.6.2 5-HT₃ RECEPTOR ANTAGONISTS

5-HT₃ receptor competitive antagonists, which bind at the orthosteric (agonist) binding site, are usually larger than agonists; they require an aromatic part, a basic moiety, and an intervening hydrogen bond acceptor. For most antagonists, these are a rigid aromatic or heteroaromatic ring system, a basic amine, and a carbonyl group (or isosteric equivalent) that is coplanar to the aromatic system (Evans et al., 1991), and there are slightly longer distances between the aromatic and amine groups when compared to the agonist pharmacophore. Only small substituents, such as a methyl group, can be accommodated on the charged amine (Schmidt and Peroutka, 1989). Many potent antagonists of 5-HT₃ receptors have 6.5 heterocyclic rings, and the most potent compounds contain an aromatic 6-membered ring. Morphine and cocaine were the first antagonists used to characterize the 5-HT₃ receptor (Gaddum and Picarelli, 1957), with more selective 5-HT₃ antagonists being developed in the 1980s: MDL72222 or bemestron (Fozard, 1984) and ICS 205–930 or tropisetron (Donatsch et al., 1985). Later compounds that were developed include ondansetron, granisetron, and zacopride, which act at nanomolar concentrations, and there is now a wide range of similarly potent compounds, with many containing...
5-HT<sub>3</sub> receptors

Figure 22.7 Schematic representation of the 5-HT<sub>3</sub> receptor. One subunit has been removed to reveal the cation-selective channel. The binding sites of agonist, antagonist, and modulators are shown. The setrons include ondansetron, granisetron, tropisetron, bemesetron, dolasetron, and palonosetron. (Reproduced from Walstab, J. et al., Pharmacol. Ther., 128, 146, 2010. With permission.)

Figure 22.8 The 5-HT<sub>3</sub> receptor–binding pocket. (a) The orientation of 5-HT and granisetron has been revealed in 5-HT3B, a version of AChBP whose binding site has been modified to resemble that in the 5-HT<sub>3</sub> receptor. The equivalent residues found in the 5-HT3A subunit are shown in grey. (Reproduced from Kesters, K. et al., 2013 with permission.) (b) A homology model of the 5-HT<sub>3</sub> receptor bound to the antagonist tropisetron reveals that water is integral to ligand binding. (Reproduced from Verheij, M.H. et al., J. Med. Chem., 55, 8603, 2012. With permission.)

bicyclic heteroaromatic structures, that is, quinazolines, quinolines, or quinolones (Verheij et al., 2012). Data indicate that these compounds, and therefore possibly all ligands that bind to the orthosteric site, are stabilized in the binding pocket by interaction with water molecules (Figure 22.8).

Antagonists may also act in 5-HT<sub>3</sub> receptor channel. Picrotoxin, which was originally considered to be relatively specific as a GABA<sub>A</sub> receptor noncompetitive inhibitor, blocks the 5-HT<sub>3</sub> receptor channel; the binding of picrotoxinin (the active component of picrotoxin) has been localized to the 6′ position of M2 (Das and Dillon, 2003; Thompson et al., 2011a). Compounds structurally similar to picrotoxin, such as the ginkgolides and bilobalide, act similarly (Thompson et al., 2011a). Diltiazem, which blocks voltage-gated calcium channels, is also known to block the 5-HT<sub>3</sub> receptor pore and act close to the 7′ and/or 12′ residues in homomeric receptors (Gunthorpe and Lummis, 1999; Thompson et al., 2011a). Morphine and its analogue methadone and the antimalarial compounds quinine and mefloquine may also exert their inhibitory effects via binding to the pore, highlighting the common mechanisms that many of these drugs share, and also the promiscuity that many of these compounds display (Baptista-Hon et al., 2012; Brady et al., 2001; Deeb et al., 2009).

### 22.6.3 5-HT<sub>3</sub> RECEPTOR MODULATORS

There are a number of allosteric modulators that effect 5-HT<sub>3</sub> receptor function including n-alcohols, anesthetics, antidepressants, cannabinoids, opioids, steroids, and natural compounds; these can inhibit or enhance receptor activity and many also modulate other Cys-loop receptors, although not always in the same direction (see reviews by Davies, 2011; Machu, 2011; Walstab et al., 2010). Specific binding sites for these compounds have mostly not yet been confirmed, although some may bind in...
an intersubunit binding cavity at the top of the TMD (Trattnig et al., 2012). The effects of these compounds have mostly been studied on 5-HT$_3$A receptors to date, although alcohols and inhaled anesthetics have been shown to have reduced sensitivity at 5-HT$_3$AB receptors, while the effects of etomidate, propofol, and pentobarbital are similar at 5-HT$_3$A and 5-HT$_3$AB receptors (Rusch et al., 2007; Solt et al., 2005; Stevens et al., 2005).

## 22.7 GATING MECHANISMS AND SUBUNIT CONTRIBUTIONS

### 22.7.1 GATING MECHANISMS

The 5-HT$_3$ receptor is an allosteric protein, and extrapolation of data from nACh receptors suggests that binding of agonists causes a structural change in the ECD that is transduced through the protein to the TMD, opening the integral pore. Structural data suggest the ECD links to the TMD via the β1–β2 and β8–β9 loops and the β10 strand, which are close to residues in the M2–M3 linker, regions known to be important for receptor function (Thompson et al., 2010; Tsetlin and Hucho, 2009). In support of this mechanism, coupling of binding to gating in a chimeric AChBP (ECD)/5-HT$_3$ (ICD) receptor could only be achieved when three amino acid regions from the ECD of the 5-HT$_3$ receptor were substituted into the corresponding regions of AChBP (Bouzat et al., 2004). There is, however, a critical M1–M2 proline but no ECD/TMD salt bridge in the 5-HT$_3$ receptor in contrast to nACh and GABAC receptors (Lee and Sine, 2005; Lummis et al., 2005; Price et al., 2007; Wang et al., 2007), although it is clear that charged residues are important (Dougherty, 2008; Xiu et al., 2005). Thus, it may be that specific details of channel gating are subtly different in different Cys-loop receptors.

### 22.7.2 SUBUNIT CONTRIBUTIONS

5-HT$_3$B receptor subunits do not form functional homomeric receptors, but can coexpress with 5-HT3A subunits to yield heteromeric 5-HT$_3$AB receptors that differ from 5-HT$_3$A receptors in a range of properties (EC$_{50}$, Hill slope, desensitization kinetics, calcium permeability, shape of current–voltage relationship, and single-channel conductance) as described in Section 22.5. Despite these biophysical differences, the pharmacology of the orthosteric site of 5-HT$_3$A and 5-HT$_3$AB receptors is almost identical. 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 (Lohner and Lummis, 2010; Thompson et al., 2011b), but conflicting with older data suggesting a BABBA arrangement determined using atomic force microscopy (Barrera et al., 2005).

There have been only two studies to date examining the functional effects of subunits 5HT3C, 5HT3D, and 5HT3E (Holbrook et al., 2009; Niesler et al., 2007). None of these subunits form functional homomers, but they do form functional receptors when coexpressed with 5-HT3A subunits, although there is no reported difference in radioligand-binding characteristics, current–voltage relationships, or kinetics of whole-cell currents of the heteromers compared to homomers. Thus, although we are aware of their sequences (Figure 22.9), the physiological roles of these more recently identified subunits are still unclear.

## 22.8 REGULATION

5-HT$_3$ receptors can undergo posttranslational modification through phosphorylation at kinase consensus sites that have been identified in the ICD. While phosphorylation has been observed on a putative protein kinase A (PKA) site at S409 in the guinea pig 5-HT$_3$ receptor (Lankiewicz et al., 1998), most studies have been indirect. Thus, activation of PKA substantially accelerates the desensitization kinetics of 5-HT$_3$ receptors in mouse neuroblastoma and HEK-293 cells, and activators of protein kinase C (PKC) increase the amplitude of 5-HT-activated currents (Hubbard et al., 2000; Shao et al., 1991; Yakel et al., 1991; Zhang et al., 1995). Unexpectedly, point mutations of putative PKC sites did not affect the sensitivity of the mutant receptors to PKC potentiation (Coultrap and Machu, 2002; Sun et al., 2003). Instead, the authors propose that enhancement of 5-HT-elicited responses 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). This suggests that PKC modulation of 5-HT$_3$A receptor function occurs via an F-actin-dependent mechanism, a conclusion that is consistent with observations that 5-HT$_3$A receptors colocalize with F-actin-rich membrane domains. As neurotransmitter release can be regulated through an actin-dependent mechanism, and 5-HT$_3$A receptors can modulate neurotransmitter release, it seems possible that PKC enhancement of 5-HT$_3$ receptor function may play a role in modulating the efficacy of 5-HT$_3$ receptor–elicited transmission.

5-HT$_3$A receptors can also specifically interact with the light chain of microtubule-associated protein 1B (MAP1B-LC1) altering receptor desensitization (Sun et al., 2008). This may play a role in shaping the efficacy of 5-HT$_3$ receptor-mediated synaptic transmission.

## 22.9 CELL BIOLOGY

5-HT$_3$ receptors have been followed from birth to death in elegant studies from Vogel’s group (Ilegems et al., 2004). 5-HT$_3$A receptors formed in the endoplasmic reticulum (ER) and Golgi are trafficked in vesicle-like structures along microtubules to the plasma membrane. They aggregate in specific subcellular sites that can be disrupted by F-actin depolymerization (Emerit et al., 2002). These aggregations have been identified in a range of cells, including neurons, consistent with the precise anatomical location that is required to mediate fast synaptic neurotransmission (Emerit et al., 2002; Grailhe et al., 2004; Ilegems et al., 2004). Agonist interaction with cell surface 5-HT$_3$ receptors results in their internalization and destruction or recycling (Ilegems et al., 2004).

The other subunits have been less well studied but it has been shown that 5-HT3B receptor subunits, when expressed alone, fail to exit the ER, providing a possible explanation as to why these subunits cannot form functional homomeric receptors. ER retention is due, at least in part, to a CRAR retention motif that forms part of the M1–M2 intracellular loop (Boyd et al., 2003). Coexpression with the 5-HT3A subunit may shield this ER retention motif allowing heteromeric 5-HT$_3$AB receptors to reach the cell surface. There is some evidence that the 5-HT3B


**Figure 22.9** Schematic diagram of the different HTR3 isoforms in their respective genomic contexts. Exons are indicated as light grey boxes and introns as black lines connecting the exons, and the untranslated regions (UTRs) are depicted as white boxes. Conserved domains of the respective 5-HT₃ subunits are indicated in black (transmembrane region) and dark grey (Cys-loop) boxes. The genes are not drawn to scale. (Reproduced from Niesler, 2011. With permission.)

Subunit forces a preference for expression of the heteromeric 5-HT₃ receptor, as coexpression of the 5-HT₃A and 5-HT₃B subunits in tsA-201 cells did not indicate the presence of homomeric 5-HT₃A receptors (Barrera et al., 2005), although the physiological relevance of this finding is not yet clear.

The human 5-HT₃A subunit has four consensus sequence \(N\)-glycosylation sites in the N-terminal ECD domain and all can be \(N\)-glycosylated (Monk et al., 2004). \(N\)-glycosylation is essential for export from the ER, cell surface expression, and radioligand binding, although it is not necessary to preserve a ligand-binding site once the receptor has matured (Boyd et al., 2003; Green et al., 1995; Quirk et al., 2004). Three of the four \(N\)-glycosylation sites are conserved between a range of species (N104, N170, and N186) and appear to be critical, while the \(N\)-glycosylation site at residue 28 is less important and indeed absent in rodents.

BiP, calnexin, and RIC-3 have been identified as ER chaperone proteins that associate with the 5-HT₃ receptor and are likely to promote correct folding, oligomerization, posttranslational modification, and/or export from the ER (Boyd et al., 2003). RIC-3 has been the most widely studied but has different effects depending on the subunits, the species they originated from, and the expression system (Castillo et al., 2005; Cheng et al., 2005, 2007). Expression of human homomeric 5-HT₃A receptors in transfected mammalian cells, for example, is enhanced by RIC-3, but it causes inhibition of heteromeric (5-HT₃AB) receptor expression, and mouse 5-HT₃A receptor expression in oocytes is completely abolished (Cheng et al., 2005). This apparent discrepancy may be due to other proteins that could influence 5-HT₃ receptor expression. Cyclophilin A, for example, promotes 5-HT₃A receptor expression in the cell membrane via an integral peptidyl prolyl isomerase activity (Helekar and Patrick, 1997), and there may be a range of other proteins yet to be identified that can modify 5-HT₃ receptor expression.

### 22.10 CHANNELOPATHIES AND THERAPEUTIC POTENTIAL

Studies implicate the malfunction of 5-HT₃ receptors in a range of neurological and GI disorders (Niesler, 2011; Walstab et al., 2010). The identification of 5-HT₃ receptors in immune cells also suggests a possible role of 5-HT₃ receptors in immunological processes and inflammation and suggests that they may plausibly be involved in diseases like atherosclerosis, tendinopathies, and fibromyalgia (Fiebich et al., 2004; Stratz et al., 2008). Some SNPs have been identified in patients with bipolar affective disorder (BPAD) or schizophrenia, disorders that...
segregate with cytogenetic abnormalities involving a region on chromosome 11 that harbors the HTR3A gene (Weiss et al., 1995). Further studies are needed with two SNPs found in schizophrenic patients (R344H and P391R) to determine if they contribute to disease in these patients, but a significant association was found in BPAD with a P16S mutation in the 5-HT3A subunit, with reporter constructs indicating this mutant could modulate expression levels (Krzywkowski et al., 2007; Niesler et al., 2001; Thompson et al., 2006b). Additional SNPs in the HTR3A gene result in the 5-HT3A<sup>33T</sup> and 5-HT3A<sup>25S</sup> subunit variants, both of which are associated with reduced levels of cell surface expression, and the 5-HT3A<sup>25S</sup> variant that does not appear to compromise plasma membrane expression (Krzywkowski et al., 2007). The significance of these is yet to be determined.

In the 5-HT3B subunit, there has been an extensive investigation into a very common SNP, Y129S, which is linked both to BPAD and major depression in women (Hammer et al., 2012; Krzywkowski, 2006). Unusually, the Y129S variant is a gain of function mutation, as 5-HT<sub>3</sub>AB<sup>129S</sup> receptors have an increased maximal response to 5-HT, decreased desensitization and deactivation kinetics, and a sevenfold 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 is apparent for receptors assembled from a mixture of wild-type 5-HT<sub>A</sub>, wild-type 5-HT<sub>B</sub>, and 5-HT<sub>B</sub>(Y129S) subunits, suggesting that signaling via the 5-HT<sub>3</sub>AB receptor in heterozygous, as well as homozygous, individuals is altered by this SNP (Krzywkowski et al., 2008).

Studies in the more recently discovered HTR3C, HTR3D, and HTR3E genes also indicate possible involvement in disease. An SNP in the 5-HT<sub>3</sub>C gene (N163K) has been correlated with irritable bowel syndrome (IBS), and expression studies suggest it causes an increase in receptor density (Kapeller et al., 2011). Increased expression has also been associated with an SNP in the 3′UTR of the HTR3E gene, also associated with IBS, which inhibits the binding of a microRNA as described earlier (Kapeller et al., 2008).

IBS is one of the disorders in which 5-HT<sub>3</sub> receptor antagonists can be effective therapeutic agents, although their major use is to ameliorate nausea and vomiting in cancer patients receiving chemo- or radiation therapy, consistent with the role of 5-HT<sub>3</sub> receptors in the vomiting center. Studies suggest that a wide range of other diseases have the potential to be treated with 5-HT<sub>3</sub> receptor–selective drugs, including addiction, pruritus, emesis, migraine, chronic heart pain, bulimia, and neurological phenomena such as anxiety, psychosis, nociception, and cognitive function (Thompson and Lummis, 2007; Walstab et al., 2010).

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Ion channel families


