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Therapeutics of 5-HT₃ Receptor Antagonists: Current Uses and Future Directions

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Abstract

The 5-Hydroxytryptamine₃ (5-HT₃) receptor is a member of the cys-loop family of ligand gated ion channels, of which the nicotinic acetylcholine receptor is the prototype. All other 5-HT receptors identified to date are metabotropic receptors. The 5-HT₃ receptor is present in the central and peripheral nervous systems, as well as a number of non-nervous tissues. As an ion channel that is permeable to the cations, Na⁺, K⁺, and Ca²⁺, the 5-HT₃ receptor mediates fast depolarizing responses in pre- and post-synaptic neurons. As such, 5-HT₃ receptor antagonists that are used clinically block afferent and efferent synaptic transmission. The most well established physiological roles of the 5-HT₃ receptor are to coordinate emesis and regulate gastrointestinal motility. Currently marketed 5-HT₃ receptor antagonists are indicated for treatment of chemotherapy, radiation, and anesthesia-induced nausea and vomiting, as well as irritable bowel syndrome. Other therapeutic uses that have been explored include pain and drug addiction. The 5-HT₃ receptor is one of a number of receptors that play a role in mediating nausea and vomiting, and as such, 5-HT₃ receptor antagonists demonstrate greatest anti-emetic efficacy when administered in combination with other drug classes.

Keywords

5-Hydroxytryptamine₃; 5-HT; serotonin; antagonist; nausea and vomiting; irritable bowel syndrome

1. Introduction to the 5-HT₃ receptor, a ligand-gated ion channel

The 5-Hydroxytryptamine₃ (5-HT₃) receptor (Derkach et al., 1989; Maricq et al., 1991) is a member of the cys-loop family of ligand-gated ion channels, which include the excitatory nicotinic acetylcholine receptor and the Zn²⁺ activated cation-selective channel, as well as the inhibitory g-amino butyric acid A (GABA_A) and glycine receptors (Barnes et al., 2009). The 5-HT receptor primarily conducts the monovalent cations, Na⁺ and K⁺, and the divalent cation, Ca²⁺. At the resting membrane potential of most neurons in which 5-HT₃ receptors are localized, the electrochemical gradient favors Na⁺ and Ca²⁺ entry. Thus, 5-HT₃ receptors depolarize neurons and mediate fast, excitatory synaptic transmission. The highest number of 5-HT₃ receptor binding sites in the central nervous system occurs in the area postrema and solitary tract nucleus (Gehlert et al., 1991; Tecott, 1993; Morales and Wang,

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2002). In addition, large numbers of 5-HT₃ receptors are found in the peripheral nervous system and gut (Tyers, 1990; Gershon, 1999; Glatzle et al., 2002). The most well-documented actions of 5-HT₃ receptors are to alter gastrointestinal motility and to regulate the vomiting reflex (Sanger and Andrews, 2006), but they also appear to play a role in visceral pain and inflammation (Costall and Naylor, 2004). In addition, the 5-HT₃ receptor has been implicated in altering the voluntary intake of ethanol in humans (Johnson et al., 1993; Johnson et al., 2002; Johnson, 2004) and rodents (Knapp and Pohorecky, 1992; Kostowski et al., 1993; Hodge et al., 2004).

1.1 Structure and function of the 5-HT₃ receptor

To date, five subunits (A – E) of the 5-HT₃ receptor have been cloned (Mariq et al., 1991; Davies et al., 1999; Dubin et al., 1999; Karnovsky et al., 2003; Niesler et al., 2003), but only the A and B subunits have been studied extensively. In common with other members of the cys-loop superfamily, the 5-HT₃ receptor is an integral membrane protein that is pentameric in structure, with a central ion channel pore. Hydropathy plots of the deduced amino acid sequence of these receptor subunits suggest a putative long extracellular N-terminus, four transmembrane spanning domains (TM1-TM4), and a short C-terminus (Mariq et al., 1991). TM2 from each of the five subunits form the ion channel pore. A large intracellular loop between TM3 and TM4 is present, but is not required for expression of the homomeric A receptor in the plasma membrane or for its function (Jansen et al., 2008), based upon its substitution with a heptapeptide derived from the short intracellular loop between TM3 and TM4 in the prokaryotic homolog of the ion channel superfamily (Bocquet et al., 2007). The large intracellular loop of rodent A subunits is a site for alternative splicing (Isenberg et al., 1993; Hope et al., 1993; Lankiewicz et al., 1998). The large intracellular loops of the 5-HT₃ receptor subunits also contain multiple consensus sites for protein kinase dependent phosphorylation (Mariq et al., 1991; Davies et al., 1999; Dubin et al., 1999; Karnovsky et al., 2003; Niesler et al., 2003).

The N-terminal domain contains the ligand recognition site for 5-HT (Eiselé et al., 1993). The binding of 5-HT occurs at the interface of two subunits. With the homomeric A subunit, there are five possible binding sites, but the binding of two molecules of agonist are necessary and sufficient to open the channel. Within these subunits are amino acid domains, so called “loops”, which participate in binding. The binding interfaces in the receptor are inequivalent, and Loops A–C are located in the principal binding component or face, whereas Loops D–F are located in the complementary component. Binding of an agonist causes a conformational change in the receptor which results in channel opening and ion flux (reviewed in Barnes et al., 2009). Residues important in determining cation selectivity of the mouse 5-HT_{3A} receptor have been identified in the TM1-TM2 linker, as well as in two positions in TM2 (Gunthorpe and Lummis, 2001); a TM2 residue contributes to Ca²⁺ permeability (Livesay et al., 2008). The homomeric 5-HT_{3A} receptor differs from the heteromeric 5-HT_{3A/B} receptors in three key ways, lower single channel conductance, greater Ca²⁺ permeability, and slower kinetics of activation, deactivation, and desensitization (Davies et al., 1999; Hayrapetyan et al., 2005). Differences in desensitization kinetics may be observed in the representative tracings of mouse 5-HT_{3A} and 5-HT_{3A/B} receptors (Fig. 1). Chimeras of A/B or B/A subunits were utilized to identify the domain responsible for differences in biophysical properties. Three arginine residues in a domain of the large intracellular loop were determined to be important for both single channel conductance and Ca²⁺ permeability (Kelley et al., 2003; Livesay et al., 2008). This domain, present in each of the five subunits, is adjacent to TM4, is α helical, and is described as a membrane associated stretch that is a cytoplasmic extension of the conduction pathway.

1.2 Subunit heterogeneity in and distribution of the 5-HT₃ receptor

1.2.1 A subunits of the 5-HT₃ receptor—To date, the 5-HT_{3A} receptor has been cloned from brain or other nervous system derived tissues and colonic tissues from mouse (Maricq et al., 1989), rat (Isenberg, et al., 1995), 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). All of these 5-HT_{3A} subunits form functional homomeric receptors in heterologous expression systems, such as *Xenopus laevis* oocytes and HEK 293 cells. Among them, amino acid identities vary from ~ 80 – 90%, with rat and mouse receptors having the greatest identity. Where studied, the receptors have similar ion permeabilities and current-voltage relationships. Although the receptor orthologs have similar agonist potencies (including 5-HT) and affinities for numerous competitive antagonists, there are some differences in ligand recognition. For example, the human 5-HT_{3A} receptor has ~ 1800 fold lower affinity for the competitive antagonist, curare, than the mouse receptor (Hope et al., 1999). In contrast, the agonist 2-methyl 5-HT has a greater efficacy at the human than mouse receptor (Werner et al., 1994; Miyake et al., 1995). Finally, 3-(2-hydroxy, 4-methoxybenzylidene)-anabaseine is a strong partial agonist at the mouse receptor and an apparent competitive antagonist at the human receptor (Zhang et al., 2006).

Distribution of the 5-HT_{3A} receptor subunit, as measured by mRNA levels and immunocytochemistry, correlates well with radio-ligand binding studies conducted with high affinity competitive antagonists of the 5-HT₃ receptor (Kilpatrick et al., 1987; Barnes et al., 1989; Laporte et al., 1992; Tecott et al., 1993; Morales et al., 1996; Morales and Bloom, 1997; Rosenberg et al., 1997). Expression of 5-HT_{3A} receptor mRNA has been measured in the rat forebrain, hippocampus, amygdala, spinal tract of the trigeminal nerve, facial nerve, and the spinal cord dorsal horn (Tecott et al., 1993; Morales and Bloom, 1997). Morales and co-workers developed a polyclonal antibody against the A subunit and observed intensely immunoreactive neurons in rat forebrain, brainstem, and spinal cord (Morales et al., 1998). More intense staining in the forebrain was observed in layers II and III of the neocortex, anterior olfactory complex, hippocampus, and amygdala. Many of the neurons in the telencephalon that express that A subunit are inhibitory GABA interneurons (Morales and Bloom, 1997). Some intensely stained neurons were also found in the striatum, and less robustly stained neurons were found in the nucleus accumbens (Morales et al., 1998). The trigeminal motor and facial nuclei in the brainstem were intensely labeled, and staining was also present in the dorsal and ventral horns of the spinal cord (Morales et al., 1998). Trigeminal ganglia, nodose ganglia, and superior cervical ganglia express the A subunit, with the highest concentration found in the nodose ganglia (Morales and Wang, 2002). Green fluorescent protein expression, controlled by the 5-HT_{3A} receptor promoter or the glutamic acid decarboxylase 67 promoter, has been used to demonstrate 5-HT₃ receptor expression in vasoactive intestinal peptide and neuropeptide Y containing interneurons in the somatosensory cortex (Vucurovic et al., 2010) and in GABAergic neurons in the spinal superficial dorsal horn (Fukushima et al., 2009), respectively. Immunolabeling of 5-HT_{3A} subunits in the gastrointestinal tract was observed in myenteric and submucosal neurons, interstitial cells of Cajal, and endocrine cells (Glatzle et al., 2002). Expression of the A subunit in virtually all tissues that express high affinity 5-HT₃ receptor binding sites, as well as the requirement of the A subunit for function of heterologously expressed heteromeric 5-HT₃ receptors as described below, underscores the dominance of the A subunit in the 5-HT₃ receptor.

1.2.2 B subunits of the 5-HT₃ receptor—The human B subunit maps to human chromosome 11q23, the same location as the A subunit, and has ~ 41% amino acid identity with the A subunit (Davies et al., 1999; Dubin et al., 1999). There are three known splice variants of the B subunit, with differences in the translation initiation site; one of the

variants lacks part of the N-terminus and is presumed to be non-functional (Tzvetkov et al., 2007). The B subunit does not assemble into receptors, but when co-expressed with the A subunit, forms functional heteromeric receptors (Davies et al., 1999; Dubin et al., 1999). In the absence of the A subunit, the B subunit is retained in the endoplasmic reticulum (Boyd et al, 2002, 2003). Rat and mouse (Hanna et al., 2000) B subunit cDNA's have also been cloned. Despite the fact that the 5-HT_{3A/B} receptor differs in biophysical properties compared to the 5-HT_{3A} receptor, as described above, pharmacological properties of drugs acting at the ligand binding domain demonstrate are very similar in homomeric and heteromeric receptors (Brady et al., 2001). Only drugs that are assumed to bind at sites other than the 5-HT recognition site, such as n-chain alcohols (Hayrapetyan et al., 2005; Stevens et al., 2005), volatile anesthetics (Stevens et al., 2005), and picrotoxin (Das and Dillon, 2003), have differential potencies in the homomeric and heteromeric receptor complexes.

The 5-HT_{3B} subunit mRNA is present in spleen, colon, small intestine, kidney, and brain (Davies et al., 1999; Dubin et al., 1999), but the distribution of the subunit protein in the CNS is in dispute. Morales and Wang (2002) described a virtual absence of the 5-HT_{3B} receptor subunit in the rat CNS, except for the nerve terminals of the nodose ganglia that project to the dorsal vagal complex of the brainstem. In their studies, the 5-HT_{3B} subunit is predominately expressed in peripheral neurons, but not all of them, raising the possibility of distinct populations of 5-HT_{3A} and 5-HT_{3A/B} receptors. In contrast, other investigators found 5-HT_{3B} subunit staining in the hippocampus (Monk et al., 2001; Reeves and Lummis, 2006). Relatively large single channel conductances have been recorded in hippocampal 5-HT₃ receptors (Jones and Suprenant, 1994), which are similar to that obtained with recombinant 5-HT_{3A/B} receptors. It remains to be determined whether the B subunit or perhaps a C or E subunit combines with the A subunit to produce larger single channel conductances in native hippocampal receptors than that observed in heterologously expressed A homomers.

1.2.3 C, D, and E subunits of the 5-HT₃ receptor—The C, D, and E subunits of the receptor were cloned most recently (Karnovsky et al., 2003; Niesler et al., 2003; Holbrook et al., 2009). These three genes map to an ~100 kb region on human chromosome 3q27 and may represent gene duplication events that happened after the divergence of the A and B subunits (Niesler et al., 2003). The C, D, and E subunits are expressed in numerous mammalian species (Holbrook et al., 2009), but are absent in mice and rats (Niesler et al., 2003; Karnovsky et al., 2003; Holbrook et al., 2009). Multiple sub-types within each subunit have been identified, including several presumed pseudogenes in C and E subunits that lack the signature N-terminal cys-loop present in all subunits of the superfamily and/or that lack signal peptides or have stop codons in the open reading frames (Karnovsky et al., 2003; Niesler et al., 2003; Holbrook et al., 2009). The expression and functionality of the C, D, and E subunits have been studied, and the expression of C, D, and E subunits as homomers is equivocal, with Holbrook et al., 2009 reporting that expression, but no function was observed in CHO cells. In contrast, Niesler et al., 2007 reported no expression of the homomers in HEK 293 cells. Both groups reported that upon co-expression of the A subunit with the C, D, or E subunits, the tagged subunits co-localized with the tagged A subunit, as measured by immunocytochemistry or immunoprecipitation. Any of the subunits co-expressed with the A subunit produced functional receptors, but there was no apparent difference in radio-ligand binding, current voltage-relationships, and kinetics of whole-cell currents of the A homomer versus the presumed heteromers. Therefore, it is unclear whether the C, D, and E receptors do form heteromeric receptors with the A subunit. Initial studies suggested that the D and E subunits had a very restricted localization to the colon or liver, but that the C subunit was present in brain, colon, intestine, lung, and kidney (Niesler et al., 2003). Holbrook et al, 2009 have performed extensive RT-PCR studies on the C, D, and E subunit variants, and have discovered a widespread distribution of the E subunit, in

particular. Highest levels of C subunit mRNA was measured in lung, colon, and dorsal root ganglia and highest levels of D subunit transcripts were measured in the dorsal root ganglia, respectively.

1.3 Ligand-binding and initiation of channel gating in the 5-HT₃ Receptor

1.3.1 Gating in the 5-HT₃ receptor—Binding of an agonist causes a conformational change in the receptor which results in channel opening. In recent years, several advances have significantly impacted the cys-loop receptor field. The crystal structure of the molluscan acetylcholine binding protein (AChBP) (Brejc et al., 2001), a structural and functional homolog of the N-terminal domain of the nACh receptor, demonstrated that the ligand binding site is composed of loops from the principal face of one subunit and a series of β strands from the complementary face of an adjacent subunit. More recently, the extracellular domain of the $\alpha 1$ subunit of the nicotinic receptor was crystallized (Dellisanti et al., 2007). The crystal structures have provided the generalized three-dimensional configuration of the ligand binding domain, which has enabled investigators to model the N-terminal domains of all members of the cys-loop family. A second major advance occurred with electron microscopy studies of *Torpedo* nACh receptors at 4 angstrom resolution of the 1) TM domains (Miyazawa et al., 2003) and 2) the N-terminal domains, TM domains, and parts of the cytoplasmic loops (Unwin, 2005). Taken together, these findings have enabled investigators to begin mapping the ligand recognition site in the cys-loop family of receptors and to identify the intra-molecular movements in the receptors that couple ligand binding to channel gating.

1.3.2 Ligand binding in the 5-HT₃ receptor—Agonist binding to the ligand binding domain is thought to initiate movement by bringing Loop C of the ligand binding domain from an uncapped and disengaged state to the capped and engaged position (Celie et al., 2004; Gao et al., 2005; Law et al., 2005; Unwin, 2005). Experimental evidence from several groups suggests that Loop 2, Loop 7 (cys-loop), Loop 9 (Loop F from the opposing face of the ligand binding domain), and Loop 10 interact with the TM2 – TM3 linker region and play key roles in transmitting agonist binding to channel opening (Bouzat et al., 2004; Chakrapani et al., 2004; Kash et al., 2003; Law et al., 2005; Reeves et al., 2005). This is accomplished through electrostatic and/or hydrophobic interactions, such that movement of the TM2–TM3 linker in turn moves TM2, the channel lining element, which results in channel opening. Lummis et al. (2005) have provided evidence that a proline in the TM2–TM3 linker of the 5-HT_{3A} receptor undergoes *cis* - *trans* isomerization in the gating process. They propose that Trp183 in Loop B moves upon agonist binding and, in turn, moves the nearby Cys loop; movement of the Cys loop may provide the molecular switch to initiate isomerization and subsequent gating in members of the cys-loop family that contain proline at this key position. Lee and Sine (2005) have delineated another step in the pathway linking the binding of ligand to channel opening in *Torpedo* nACh receptors. They have identified an arginine in Loop 10, the domain between the ligand binding domain and TM1 that forms an electrostatic interaction with a glutamate in Loop 2; these residues are energetically coupled to another residue in Loop 2 and two residues in the TM2–TM3 linker previously identified as being critical in the gating process. A parallel pathway links the pre-TM1 domain and the TM2–TM3 linker via the cys-loop (Lee et al., 2009)

A number of ligand binding and electrophysiological approaches have been used to assess the roles of Loops A–F of the 5-HT₃ receptor in ligand recognition. Homology models, using the AChBP and/or the N-terminal of the $\alpha 1$ subunit of the nicotinic receptor, have been constructed to orient ligands with their presumed contact points with amino acids in the agonist binding domain (Thompson et al., 2006; Zhang et al., 2007; Joshi et al., 2008; Nyce et al., 2010) and presented in Fig. 2. In this context, the three dimensional space of the

ligand binding domain is being defined. Numerous tyrosine and tryptophan residues in Loops A–E have a major impact on binding and/or gating, and some differentially interact with agonists (m-chlorophenylbiguanide (m-CPBG)) and antagonists (granisetron and GR65630) (Yan et al., 1999; Spier and Lummis, 2000; Venkataraman et al., 2002; Price and Beene et al., 2004; Lummis, 2004; Yan and White, 2005). For example, W183 in Loop B forms a critical cation- π interaction with the primary amine of 5-HT (Beene et al., 2002). Mutation of a series of residues in Loop A alters affinity of antagonists and/or agonists (Boess et al., 1997; Steward et al., 2000), and mutant-thermodynamic cycle analysis strongly suggests that 2' N of curare interacts directly with N128 (Yan et al., 2006). Substitution of E129 with unnatural amino acids strongly points to a hydrogen bond forming between this amino acid and the –OH group of 5-HT (Price et al., 2008). Mutagenesis of six Loop C residues has resulted in reduced affinity or elimination of [3 H]-granisetron binding (Suryanarayanan et al., 2005; Thompson et al., 2005), reduced affinity of [3 H]mCPBG and [3 H]GR65630 (Schreiter et al., 2003) and altered relative efficacies of the partial agonist 2-Methyl 5-HT and/or 5-HT (Suryanarayanan et al., 2005). Loop C is highly variable between species and thus using interspecies chimeras, four groups identified Loop C as contributing to differential drug action at the in human, mouse, rat, and guinea pig 5-HT_{3A} receptors (Lankiewicz et al., 1998; Hope et al., 1999; Mochizuki et al., 1999; Zhang et al., 2007). In Loop D, W90 strongly influences ligand binding (Spier and Lummis, 2000), whereas R92 likely interacts directly with the indazole ring of granisetron (Yan and White, 2005). In Loop E, mutation of G143 and Y143 to alanine completely eliminated granisetron binding (Venkataraman et al., 2002). Finally, the crystal structure of Loop F was poorly resolved in the AChBP, and thus, its structure in the ligand binding domain is uncertain. This loop has been studied the least. Thompson et al. (2006) investigated 21 mutations inclusive of and near Loop F and found that two regions displayed increases in K_d values for [3 H]-granisetron. Two residues in Loop F of the human receptor, K195 and V202, account for the contribution of Loop F to the differential curare potency of human and mouse receptors (Zhang et al., 2007).

2. Therapeutic uses of 5-HT₃ receptor antagonists

2.1 Available 5-HT₃ receptor antagonists for gastrointestinal disorders

The 5-HT₃ receptor antagonists currently available for therapeutic use in the U.S. are ondansetron (Zofran), granisetron (Kytril), dolasetron (Anzemet), palonosetron (Aloxi), and alosetron (Lotronex), Table 1. Tropisetron and ramosetron are marketed outside the U.S. Ondansetron, granisetron, dolasetron, and palonosetron are used for chemotherapy-induced and post-operative nausea and vomiting (PONV), whereas alosetron is available for irritable bowel syndrome (Aapro, 2005; Fayyaz and Lackner, 2008; Walstab et al., 2010). Ondansetron, granisetron, dolasetron, and alosetron are competitive antagonists of the 5-HT₃ receptor, with structural similarity to 5-HT, and with carbazole, indazole, indole, and imidazole main-chains, respectively. In contrast, palonosetron is a non-competitive antagonist of the 5-HT₃ receptor, with a fused tricyclic ring and a quinuclidine side chain (Rojas et al., 2008).

2.2 Treatment of nausea and vomiting with 5-HT₃ receptor antagonists

2.2.1 Physiology of nausea and vomiting—Vomiting is coordinated by neurons in the medulla oblongata. This complex process requires stepwise gastric fundus relaxation, intense contraction of abdominal, intercostal, and diaphragmatic muscles, closing of the glottis, and relaxation of the upper esophageal sphincter prior to the expulsion of gastric contents. These physiological responses are the result of the activation of numerous efferent neurons whose nuclei are spread throughout the medulla oblongata. This region of the brainstem that controls the motor outflow is called the vomiting center or more recently, the

central pattern generator (Hornby, 2001). Nausea and vomiting may be initiated by a number of factors. Noxious chemicals or toxins may act directly on the gastric mucosa to stimulate abdominal vagal afferents, which project to the dorsal vagal complex that contains the nucleus of the solitary tract, the dorsal motor nucleus of the vagal nerve, and the area postrema. Circulating noxious chemicals act directly at the chemoreceptor trigger zone (CTZ) of the area postrema. The CTZ is located on the floor of the fourth ventricle, an area of the CNS not bound by the blood-brain-barrier. Motion sickness and other vestibular disturbances stimulate nausea and vomiting. Changes in intracranial pressure, trauma to the brain, or lesions in or near the vomiting center can evoke vomiting, as can extreme emotion, bad odors, or hormonal changes associated with pregnancy. Thus, inputs from the meninges and higher brain centers, e.g., the cortex, thalamus, and hypothalamus, are thought to innervate the vomiting center. Finally, gastrointestinal dysfunction, such as gastroparesis, can produce nausea and vomiting (Hornby, 2001; Costall and Naylor, 2004; Sanger and Andrews, 2006).

2.2.2 Discovery of antiemetic properties of 5-HT₃ receptor antagonists—The development of 5-HT₃ receptor antagonists and the recognition of the 5-HT₃ receptor's role in nausea and vomiting preceded the receptor's identification as a ligand-gated ion channel. In the 1950's, two laboratories identified the 5-HT₃ receptor through its contraction of guinea pig ileum. It was initially given the name "5-Hydroxytryptamine M" receptor because of morphine's ability to block the contractile response (Rocha et al., 1953; Gaddum, 1953; Costall and Naylor, 2004). Twenty-five years later, Fozard and co-workers (Fozard and Mobarok, 1978) identified a 5-HT-M receptor in isolated rabbit heart and began investigating it pharmacologically. Their observation that the dopamine receptor antagonist, metoclopramide, blocked the action of 5-HT at the M receptor led to the development of early M-selective agents, such as MDL 72222. The observation by Florczyk et al., 1982 that high dose metoclopramide relieved cisplatin-induced vomiting in ferrets led two groups of investigators to demonstrate that MDL 72222 is very effective in reducing cisplatin-induced vomiting in ferrets (Costall et al., 1986; Miner and Sanger, 1986).

2.2.3 Possible mechanisms of 5-HT₃ receptors in mediating nausea and vomiting—The mechanism(s) through which 5-HT₃ receptor activation initiates nausea and vomiting may involve both a peripheral and central component. In the gut, enterochromaffin cells release 5-HT in response to gastric irritation or cellular damage. Released 5-HT binds to 5-HT₃ receptors on vagal afferents in the gastric mucosa, which project to the dorsal vagal complex of the vomiting center (Andrews et al., 1990; Hillsley and Grundy, 1998). 5-HT₃ receptors are on the nerve terminals of the projecting vagal afferents to the dorsal vagal complex. Post-synaptic 5-HT₃ receptors may also be present in the dorsal vagal complex (Glaum et al, 1992). It is important to point out that other neurotransmitter systems are important in initiating and coordinating nausea and vomiting. Besides the 5-HT₃ receptor, three other major classes of neurotransmitters receptors have been identified as targets for antiemetic drugs: the dopamine D2 receptor, muscarinic M1 cholinergic receptor, and histamine H1 receptor (Scuderi, 2003). Antiemetic drugs have also been developed that antagonize the cannabinoid receptor and neurokinin₁ (NK1) receptor, which is activated by Substance P. That so many drugs are marketed highlights the fact that no single drug class can successfully block nausea and vomiting produced by any possible cause. Accordingly, 5-HT₃ receptor antagonists are limited to the treatment of nausea and vomiting associated with chemotherapy or radiation treatment and surgery, since they have little or no efficacy in treating other causes of emesis (Scuderi, 2003, Sanger and Andrews, 2006).

2.2.4 Efficacy of 5-HT₃ receptor antagonists in chemotherapy and radiation induced nausea and vomiting—

The introduction of 5-HT₃ receptor antagonists into clinical use revolutionized the treatment of nausea and vomiting in cancer patients receiving chemo- or radiation therapy (Aapro, 1991). At the time of ondansetron's release, dopamine D2 receptor antagonists, anti-histamines, and anti-muscarinics were the primary antiemetic pharmacotherapies. None of these three classes was as efficacious in decreasing emesis associated with cancer treatment as ondansetron proved to be. The relative risk for developing nausea and vomiting with chemotherapy ranges from 30 – 90% and is dependent upon the chemotherapeutic agent used. Relative risk for nausea and vomiting with radiation therapy is ~ 40% (Kovac, 2003). All currently marketed 5-HT₃ receptor antagonists have good efficacy in reducing acute emesis associated with chemo- and radiation therapy, and in fact are generally well tolerated with limited side effects. Most common side effects are headache, dizziness, constipation, diarrhea, fever, malaise, and transient increases in serum transaminases (Hesketh, 2000). Cardiovascular effects, primarily changes in ECG recordings, have been reported and are dose related; these are considered to be clinically insignificant, as they are small in magnitude and are transient (Jantunen et al., 1996; Keefe, 2002). However, 5-HT₃ receptor antagonists must be used judiciously in patients who have long QT syndrome.

Early on, it was observed that ondansetron's therapeutic effect was limited to the acute phase of nausea and vomiting and had little effect on the delayed phase occurring > 24 hours after treatment (Scuderi, 2003; Sanger and Andrews, 2006). The combination of a 5-HT₃ receptor antagonist and a corticosteroid, such as dexamethasone or methylprednisolone, increases the antiemetic efficacy of both drugs in the early and delayed phases. The drug regimen includes intravenous therapy prior to and during the cancer treatment, followed by oral administration of dexamethasone for two to four days. The mechanism by which corticosteroids exert their antiemetic effect is unknown. The NK₁ receptor antagonist, aprepitant, is effective in the delayed phase of nausea and vomiting and is currently used in combination with a 5-HT₃ receptor antagonist and a corticosteroid for treatment of delayed nausea and vomiting for highly emetogenic chemotherapy (Kris et al., 2006).

Despite the efficacy of 5-HT₃ receptor antagonists of drugs as a whole, some patients respond to this class of drugs better than others. In addition, some patients are resistant to the antiemetic effects of ondansetron, but are successfully treated with granisetron (Kovac, 2003; de Wit et al., 2005). With regard to patients who do not respond well to the class of drugs, two hypotheses have been put forward. First, ondansetron is primarily metabolized by CYP2D6, and patients with allelic variants of the enzyme that cause ultra-rapid metabolism may have reduced efficacy (Kaiser et al., 2002). Second, there are allelic variants of 5-HT₃ receptor subunits that have been identified that may play a role (Niesler, 2008). The 5-HT_{3B} receptor has several polymorphisms, one of which is a deletion in the promoter that has been associated with reduced efficacy of ondansetron and tropisetron in moderate to highly emetogenic chemotherapy (Tremblay et al., 2003). Polymorphisms in the 5-HT_{3C} subunit may have predictive value in determining the relative risk for nausea and vomiting in moderately emetogenic chemotherapy (Fasching et al., 2008). The intriguing finding that some patients do not respond to ondansetron, but are treated successfully with granisetron may, in part, depend on the properties of the drug and genetic variability in the patient population (de Wit et al., 2005). Ondansetron has a shorter duration of action and a lower affinity and selectivity in comparison with granisetron. Thus, patients who are rapid metabolizers, or who have the short serotonin transporter polymorphism resulting in reduced activity and expression (Lesch et al., 1996) and increased lifetime of 5-HT in the gut, might be predicted to have a poorer outcome with ondansetron.

2.2.5 Treatment of post-operative nausea and vomiting with 5-HT₃ receptor antagonists—Post-operative nausea and vomiting have been reported to range from 12 – 40% and even higher in patients who are at greater risk. Patients at greater risk are those who have a previous history of PONV or motion sickness, who are female, and who are non-smokers. Risk is also associated with specific anesthetics drugs, such as nitrous oxide and volatile anesthetics, as well as opiates. Type and length of surgery also play a factor (Gan, 2007). Although the mechanism(s) through which PONV occurs has not been elucidated, it may be due to anesthetic-induced release of 5-HT in the gut and activation of 5-HT₃ receptors locally; a direct action on the vomiting center may also occur. Interestingly, volatile anesthetics stimulate 5-HT₃ receptor mediated currents (Machu and Harris, 1994), but propofol has been reported to have no effect on (Machu and Harris, 1994) or to inhibit (Rusch et al., 2007) 5-HT₃ receptor function. Propofol is an intravenous general anesthetic that produces far less nausea and vomiting than volatile anesthetics. Similar to chemotherapy induced nausea and vomiting, 5-HT₃ receptor antagonists appear to most effective in the prophylaxis of nausea and vomiting and in the treatment of the early phase of PONV. Accordingly, the efficacy of 5-HT₃ receptor antagonists in the early and late phases of PONV is enhanced when given in combination with dexamethasone or in a triple combination, with the addition of aprepitant (Scuderi, 2003; Yang and Scott, 2009).

2.3 Treatment of irritable bowel syndrome with 5-HT₃ receptor antagonists and 5-HT₄ receptor partial agonists

2.3.1 Irritable bowel syndrome: features of disease and prevalence—Irritable bowel syndrome (IBS) is a disorder that is characterized by periods of intense abdominal symptoms, followed by periods in which there are no symptoms. Severe intestinal cramping or pain, in the absence of any organic disease, is the hallmark of IBS. It may be accompanied by diarrhea, constipation, or diarrhea that alternates with constipation. The Rome guidelines, developed and refined over a period of years (Drossman, 1999; Drossman and Dumitrascu, 2006), define criteria by which IBS is diagnosed. In a period of 12 months, the patient must experience a minimum of 12 weeks of abdominal pain or discomfort, with two out of three of the following features. Abdominal distress is 1) relieved by defecation, 2) is associated with a change in stool frequency, or 3) is associated with change in appearance of stool. The sub-type of IBS, constipation or diarrhea predominant, mixed, alternating, or unspecified, is based upon stool consistency. IBS is one of the most common disorders that gastroenterologists treat, with an incidence of 10 – 20 % worldwide (Fayyaz and Lackner, 2008). It has ~ a two-fold greater incidence in females than in males (Sandler, 1990). The frequency and severity of IBS symptoms and even its onset may be influenced by the patient's medical history, such as emotional stress or psychiatric disorder. However, the presence of a psychosocial disorder is not a requirement for diagnosis of IBS (Drossman and Dumitrascu, 2006).

2.3.2 Irritable bowel syndrome: possible mechanisms and involvement of the 5-HT₃ and 5-HT₄ receptors—Gastrointestinal function is controlled by intrinsic and extrinsic factors, including endocrine and paracrine mediators, as well as the sympathetic, parasympathetic, and enteric nervous systems. Nerve plexuses in the enteric nervous system form reflex arcs that coordinate activity in the GI tract. The enteric nervous system can function independently of the sympathetic and parasympathetic nervous systems, but is normally modulated by their input (Furness et al., 1998; Goyal, 2000). IBS has features of both altered motility and enhanced visceral sensitivity (Drossman, 1999). Given the large number of hormones and neurotransmitters involved in GI motility and in transmitting sensory information in the gut, including pain, it is not surprising that IBS has been treated with so many different classes of drugs, including anti-cholinergics, tricyclic antidepressants, anti-diarrheal drugs or laxatives, fiber, pro-biotics, peppermint oil, non-

absorbable antibiotics, selective C-2 chloride channel activators, 5-HT selective re-uptake inhibitors, 5-HT₄ receptor agonists, and 5-HT₃ receptor antagonists (Mertz, 2003; Brandt et al., 2009).

The mechanisms underlying irritable bowel syndrome are poorly understood. They are likely multi-factorial and may differ depending upon the sub-type of IBS that the patient displays (Brandt et al, 2009). The therapeutic efficacy that some anti-cholinergics (anti-spasmodic agents) demonstrate in a sub-set of patients lent early support to the idea that autonomic dysregulation may contribute to the syndrome. Tricyclic antidepressants have efficacy at doses that are anti-muscarinic, but that are not sufficient to produce anti-depressant activity. Tricyclic antidepressants also have analgesic and neuromodulatory effects. In general, most studies suggest that there is an increase in sympathetic versus parasympathetic activity in IBS patients. In these studies, cardiovascular function, e.g., heart rate variability, blood pressure, and fingertip blood flow, were the primary measures (Manabe et al., 2009). Aggarwal et al. (1994) compared the autonomic function in the IBS sub-groups and found vagal dysfunction and increased sympathetic tone in constipation predominant and diarrhea predominant IBS, respectively.

Serotonin has multiple roles in regulation of GI function (Fayyaz and Lackner, 2008; Sikander et al., 2009). It is released by enterchromaffin cells and binds to multiple 5-HT receptor subtypes to directly stimulate excitatory (acetylcholine) or inhibitory (nitric oxide) motor neurons that regulate smooth muscle tone. Serotonin also binds to 5-HT_{1p} receptors on intrinsic primary afferent neurons (IPANs), which are the primary sensors and regulators of the enteric nervous system. IPANs secrete acetylcholine and calcitonin gene related peptide, which stimulate interneurons that control motor or sensory output. 5-HT₃ receptors play a relatively minor role in enhancing GI motility via activation of IPANs and other neurons. A greater role of 5-HT₃ receptors is to transmit sensory information to the central nervous system, including pain via spinal afferents and non-painful sensory information, such as the sensation of bloating, via parasympathetic afferents (Gershon, 2004). Thus, the 5-HT₃ receptor antagonist, alosetron, has been marketed to reduce the visceral sensation associated with IBS that is diarrhea predominant. Indeed, antagonism of 5-HT₃ receptors on sensory nerve endings or on pre-synaptic nerve terminals of Substance P containing neurons may play a role in reducing pain associated with fibromyalgia and other chronic pain disorders (Faerber et al., 2007). The 5-HT₄ receptor is present on distal terminals of IPANs and its stimulation by 5-HT or a partial agonist, such as tegaserod, augments 5-HT_{1p} mediated release of acetylcholine and calcitonin gene related peptide (Gershon, 2004). As such, tegaserod has been marketed for IBS that is constipation predominant.

Several meta-analyses have supported the efficacy of alosetron, tegaserod, and the investigational 5-HT₃ receptor antagonist, cilansetron, in the treatment of IBS (Jones et al., 2002; Lesbros-Pantoflickova et al., 2004; Evans et al., 2007; Rahimi et al., 2008; Ford et al., 2009). However, alosetron produced severe constipation and ischemic colitis in two subsets of patients, respectively. It was removed from the market and later re-introduced with tightly controlled regulation by the FDA. Tegaserod produced cerebrovascular or cardiovascular ischemia in a small subset of patients and was also removed from the market. It is now only available for emergency use as an investigational drug (Ford et al., 2009).

3. Investigational uses of 5-HT₃ receptor antagonists

3.1 5-HT₃ receptor antagonists and ethanol and other drugs of abuse

The function of the 5-HT_{3A} (Lovinger and White, 1991; Machu and Harris, 1994; Jenkins et al., 1996), but not the 5-HT_{3A/B} (Hayrapetyan et al., 2005) receptor, is enhanced by pharmacologically relevant concentrations of ethanol. In common with positive allosteric

modulation by other drugs, ethanol only enhances 5-HT₃ receptor mediated currents at low gating concentrations of agonist. Ethanol does not alter the affinity of the 5-HT₃ receptor for either partial agonists (Loving et al., 2000) or competitive antagonists (Hellevuo et al., 1991). The use of single channel measurements to determine the mechanisms of ethanol's effects on the 5-HT_{3A} receptor is not feasible, given the low conductance (~ 0.5 pS) of the homomer (Kelley et al., 2003). Likewise, the larger conducting 5-HT_{3A/B} receptor is relatively insensitive to ethanol (Hayrapetyan et al., 2005). Therefore, measurements of kinetics of whole cell currents with rapid application methods have been performed to identify mechanisms through which alcohols enhance current (Zhou et al., 1998). Ethanol likely enhances 5-HT₃ receptor mediated currents by stabilization of the open channel state through at least three ways. Ethanol and other alcohols increase the activation rate, i.e., increase the rate of opening the receptors. Ethanol and other alcohols reduce the intrinsic desensitization rate of the receptor. Finally, deactivation rate, or unbinding of 5-HT is slowed.

Both neurochemical and behavioral data support the idea that 5-HT₃ receptors play a role in alcohol intake. Dopaminergic neurons in the ventral tegmental area that project to the nucleus accumbens, amygdala, and the pre-frontal cortex are part of the "reward pathway", i.e., the areas of the brain that are stimulated when drugs of abuse, such as alcohol, are ingested or taken through other routes of administration (Dworkin and Smith, 1987). 5-HT₃ receptors are located on nerve terminals of the dopaminergic neurons in the nucleus accumbens and amygdala (Morales et al., 1998). Ethanol enhances dopamine release, and 5-HT₃ receptor antagonists have been demonstrated to reduce the evoked release, and either inhibit or have no effect on basal release (Carboni et al., 1989; Wozniak, et al., 1990, Campbell et al., 1996). Likewise, in the absence of alcohol, 5-HT stimulated dopamine release was blocked by selective 5-HT₃ receptor antagonists (Campbell et al., 1996). Thus, ethanol-induced activation of the 5-HT₃ receptor may, in part, provide a cellular basis for enhanced dopamine release by ethanol.

Studies in both rodents and humans have demonstrated the effectiveness of 5-HT₃ receptor antagonists in reducing alcohol intake. However, there are inconsistencies in rodent studies regarding 5-HT₃ receptor antagonist efficacy and access to alcohol. Some studies demonstrated that 5-HT₃ receptor antagonists were only effective in reducing alcohol intake under limited access conditions (Dyr and Kostowski, 1995; Tompkins et al., 1995), whereas another study showed that these drugs reduced intake under 24 hour alcohol access (Knapp and Pohorecky, 1992). The differences in experimental findings may be explained by strain or line of rat or the antagonist itself, some of which are not that selective for the 5-HT₃ receptor (Engleman et al., 2008). In a 5-HT_{3A} receptor knock-out study, the presence of the 5-HT_{3A} receptor was required for the selective 5-HT_{3A} receptor antagonist, LY-278-584, to reduce alcohol consumption, underscoring the importance of this receptor in alcohol consumption (Hodge et al., 2004). Transgenic mice overexpressing the 5-HT_{3A} receptor drank less alcohol than wild-type mice (Engel et al., 1998), but these mice had increased sensitivity to the low dose effects of alcohol (Engel and Allan, 1999). Decreased sensitivity to ethanol in humans has been shown to correlate with a higher alcohol consumption and greater risk for alcoholism (Schuckit, 1994). Thus, increased sensitivity to alcohol may result in reduced consumption. In humans, ondansetron reduces alcohol intake and the pleasurable effects of alcohol (Johnson et al., 1993; Swift et al., 1996). Johnson and co-workers' studies suggest that ondansetron has its greatest efficacy in early onset alcoholics (Johnson, 2000; Johnson et al., 2002), that is, those that are at greatest risk of severe abuse of alcohol, because of genetic vulnerability and possible serotonergic hypo-function accompanied by an up-regulation of 5-HT₃ receptors.

There is less than compelling evidence that 5-HT₃ receptor antagonists alter the use of other drugs of abuse. In rats, 5-HT₃ receptor antagonists had no effect on heroin (Higgins et al., 1994), nicotine (Corrigall and Coen, 1994), or cocaine (Walsh and Cunningham, 1997; Engleman et al., 2008) self-administration. In contrast, morphine self-administration was reduced by ondansetron or tropisetron (Hui et al., 1993). In humans, ondansetron did not alter methamphetamine use, withdrawal, or craving (Johnson et al., 2008).

4. Conculsions and future directions for 5-HT₃ receptor antagonists

The most beneficial therapeutic use of 5-HT₃ receptor antagonists to date is for nausea and vomiting. Ondansetron revolutionized the treatment of radiation and chemotherapy-induced nausea and vomiting. The use of alosetron in diarrhea predominant irritable bowel syndrome has been hampered by the life-threatening side effect of ischemic colitis in a very small number of patients. It is likely that treatment for IBS could be improved by development of 5-HT₃ receptor antagonists with less propensity for producing severe constipation and colitis. Further classification of IBS subtypes and development of biomarkers for the subtypes may also play a future role in predicting patients that could most likely benefit from 5-HT₃ receptor antagonist therapy. The presence of 5-HT₃ receptors on sensory nerve endings and on the nerve terminals of neurons that release pain mediators, such as Substance P, has generated interest in the use of 5-HT₃ receptor antagonists in diseases that cause chronic pain, such as fibromyalgia and peripheral neuropathies. The use of 5-HT₃ receptor antagonists in the treatment of alcoholism has been explored, but they are likely to benefit, at best, only a subpopulation of patients with the disease. The disease states or conditions in which 5-HT₃ receptor antagonists have been shown to be beneficial are complex and are likely mediated through multiple pathways. Therefore, therapies employing 5-HT₃ receptor antagonists in combination with agents from other drug classes may be a common theme in therapeutics, as is already the case in the treatment of nausea and vomiting. Pharmacogenomics, whether through the classification of a patient's polymorphisms of 5-HT₃ receptor subunits or his polymorphisms of cytochrome p450 enzymes responsible for metabolism of 5-HT₃ receptor anatagonists, are expected to help predict the patient population that will benefit most from treatment with this class of drugs.

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Abbreviations

ACh	acetylcholine
5-HT	5-Hydroxytryptamine
GABA	γ-amino butyric acid
IBS	irritable bowel syndrome
IPANs	intrinsic primary afferent neurons
PONV	post-operative nausea and vomiting
TM	transmembrane

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Fig. 1.

Representative traces of inward currents evoked by 25 mM 5-HT in mouse 5-HT_{3A} and 5-HT_{3A/B} receptors. Patch clamp electrophysiological recordings were performed in HEK 293 cells transfected with mouse 5-HT_{3A} or 5-HT_{3A} plus 5-HT_{3B} subunit cDNA's. Addition of the B subunit markedly enhanced desensitization relative to that in the homomeric receptor.

Fig. 2.

Molecular model of the mouse 5-HT_{3A} receptor N-terminal domain in the unliganded/antagonist bound conformation. The model was created using MODELLER (www.saliab.org) with antagonist-bound forms of the AChBP as the templates (Nyce et al., 2010). Residues that are important in ligand recognition in each of the six loops of the ligand binding domain of the receptor are indicated: Loop A: N128 (Yan et al., 2006), Loop B: W183 (Thompson et al., 2005), Loop C: Y234 (Thompson et al., 2005), Loop D: R92 (Yan and White, 2005), Loop E: Y143 (Beene et al., 2004), and Loop F: I207 (Zhang et al., 2007).

Table 1**5-HT₃ Receptor Antagonists Available in the United States**

Name	Chemical Class	Mechanism of Action	Therapeutic Use	Side Effects
Ondansetron (Zofran™)	Carbazole	Competitive Antagonist	CTNV & PONV*	headache, dizziness, & constipation
Granisetron (Kytril™)	Indazole	Competitive Antagonist	CTNV & PONV	headache, dizziness, & constipation
Dolasetron (Anzemet™)	Indole	Competitive Antagonist	CTNV & PONV	headache, dizziness, & constipation
Alosetron (Lotronex™)	Imidazole	Competitive Antagonist	IBS**, Diarrhea Predominant-restricted use by the FDA	headache, dizziness, & constipation risk of severe constipation, ischemic colitis
Palonosetron (Aloxi™)	Isoquinoline	Non-competitive Antagonist	CTNV & PONV	headache, dizziness, & constipation

* CTNV & PONV: Chemotherapy-Induced Nausea and Vomiting and Post-Operative Nausea and Vomiting.

** Irritable Bowel Syndrome