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The 5-HT_3 receptor as a therapeutic target

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Abstract

The 5-HT_3 receptor is a neurotransmitter-gated ion channel. It is a member of the Cys-loop family of receptors, which also includes nicotinic acetylcholine, glycine and $GABA_A$ receptors. Each member of the family consists of an arrangement of five subunits surrounding a central ion-conducting pore. The 5-HT_3 receptor binding site is composed of six loops from two adjacent subunits, and the critical ligand binding residues within these loops are well documented. There are a range of 5-HT_3 receptor agonists and competitive antagonists, but it is the antagonists that dominate their clinical use. Studies have proposed a range of disease symptoms that might be amenable to 5-HT_3 receptor selective compounds; however, so far only the treatment of emesis and irritable bowel syndrome have been fully realised. In this review, the authors look at the structure, function and distribution of 5-HT_3 receptors and how this may influence their role in disease. The authors also describe the existing clinical applications of 5-HT_3 antagonists and the future potential of these drugs.

Keywords

5-HT_3; alosetron; Cys-loop receptor; dolasetron; emesis; granisetron; irritable bowel syndrome; ondansetron; palonosetron; ramosetron; tropisetron

1. Introduction

The 5-HT_3 receptor is an ionotropic ligand-gated ion channel (LGIC) and thereby differs from other serotonin receptors (5-HT_1 to 5-HT_7) whose actions are mediated via G-proteins. The structure and function of 5-HT_3 receptors shows they are members of the Cys-loop family of LGICs, which includes glycine, $GABA_A$ and nicotinic acetylcholine (nACh) receptors. Members of this family share a structure that is composed of five pseudo-symmetrically arranged subunits surrounding a central ion-conducting pore. Each subunit is composed of an extracellular, a transmembrane and an intracellular domain (Figure 1). The extracellular domain contains the binding site for agonists and competitive antagonists; it is the major therapeutic target in 5-HT_3 receptors and the site of action of all of the drugs discussed in this review. The binding site is formed at the interface of two adjacent subunits by the convergence of three amino acid loops (A – C) from one (the principal) subunit and three β-strands (D – F) from the adjacent (or complementary) subunit. The transmembrane region contains four membrane-spanning α-helices (M1 – M4) and a short C-terminus. M2 from each subunit lines the pore and contains regions responsible for channel gating and ion selectivity. In 5-HT_3 receptors, this pore is predominantly sodium and potassium selective, and its opening results in a rapidly activating and then desensitising inward current [1,2]. A

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large loop between M3 and M4 forms the intracellular domain and is involved in channel conductance and modulation. There is evidence that parts of the transmembrane and intracellular regions may be responsible for the aetiology of some 5-HT_3 related pathologies (e.g., alcohol [3]), but, so far, there have been no therapeutic developments that specifically target these regions.

2. Distribution of 5-HT_3 receptors

So far, genes for five 5-HT_3 subunits have been identified (A – E). 5-HT_{3A} receptor subunit mRNA is widely distributed in the adult human brain and internal organs, and has also been found in extraneuronal cells such as monocytes, T cells, synovial tissue and primary chondrocytes, which suggests a role in inflammation [4,5]. The distribution of 5-HT_{3B} receptor subunit mRNA is not as widespread but it is still detectable across a range of adult brain regions and kidney [6]. Immunochemical studies suggest that 5-HT_{3B} receptor subunits are either restricted to the peripheral nervous system (PNS), or exist in the CNS in low abundance or discretely localised cell populations [7-9]. A more recent study that used quantitative real-time PCR to analyse the tissue distribution of 5-HT_{3B} described similar results, but also reported two new 5-HT_{3B} splice variants that were almost exclusively found in the brain [10]. Interestingly, 5-HT_{3B} appears to be expressed in anatomical structures that are involved in drug-induced emesis, although there has been no direct link between heteromeric receptors and the effects of antiemetic drugs. 5-HT_{3C} receptor subunit mRNA also has a relatively wide distribution within adult brain, colon, intestine, lung, muscle and stomach, whereas 5-HT_{3D} mRNA is restricted to kidney, colon and liver and 5-HT_{3E} mRNA is restricted to the colon, intestine and stomach [9,11]. As yet, however, there is no published evidence that the genes for 5-HT_{3C}, 5-HT_{3D} and 5-HT_{3E} receptor subunits are transcribed, and thus it may be that 5-HT_3 receptors are homomeric A-only or heteromeric A +B receptors,. There is also some evidence that 5-HT_3 receptor subunits may co-express with subunits from other ligand-gated ion channels, such as the nACh α4 subunit [7,12].

5-HT_3 receptors are located in many brain areas including the hippocampus, entorhinal cortex, frontal cortex, cingulate cortex, amygdala, nucleus accumbens, substantia nigra and ventral tegmental area, with highest levels in the brain stem, especially areas involved in the vomiting reflex such as the area postrema and the nucleus tractus solitarius. These brain regions are protected by the blood–brain barrier with the exception of the area postrema and the nucleus tractus solitarius. The area postrema is one of four structures in the ventricular system and, like the spinal cord, is surrounded by an ependymal layer. This layer lacks the tight junctions of the blood–brain barrier and allows the area postrema to fulfil a chemosensory role. 5-HT_3 receptors have also been detected in the dorsal horn and dorsal root ganglia of the spine and in combination with the area postrema are responsible for the vomiting reflex [13-15]. Interestingly, although the levels of 5-HT_3 receptors are highest in these regions, they are still low when compared with the densities of other serotonin receptors. 5-HT_3 receptors are found pre- and postsynaptically and activation can modulate the release of a variety of neurotransmitters, including dopamine, cholecystokinin, GABA, substance P and acetylcholine. There also appears to be differential cellular localisation of presynaptic and postsynaptic 5-HT_3 receptors within different central regions, depending on the nature of the neurons expressing them [13,16]. Consistent with their role in emesis, 5-HT_3 receptors are also involved in information transfer in the gastrointestinal tract, and in the enteric nervous system they regulate gut motility and peristalsis [17]. They also play an important role in the urinary tract, and expression of hypersensitive and constitutively active 5-HT_3 receptors in mice lead to excitotoxic neuronal cell death, resulting in their early death due to uropathy [18].

3. Structure of the 5-HT_3 receptor

Structural details of the 5-HT_3 receptor at the molecular level are unresolved, but a wealth of convergent evidence shows that the structure of these receptors is closely related to the structure of the nACh receptor (see [19,20] for reviews). Consequently, the 5-HT_3 receptor is thought to be well represented by cryo-electron microscope images of the nACh receptor and by crystal structures of the acetylcholine-binding protein (AChBP), a protein that is homologous with the extracellular domain of the nACh receptor [21] (Figures 1 and 2). Chimaeric receptors that combine AChBP with the transmembrane domain of the 5-HT_3 receptor can be activated by acetylcholine and further demonstrate the structural and functional similarity between these proteins [22].

The 5-HT_3 receptor is composed of five subunits that surround a central ion-conducting pore (Figures 1 and 2). The extracellular, N-terminal, domain contains the ligand binding site and crystal structures of AChBP have been used to create 5-HT_3 receptor homology models of this region [23-26]. These models indicate that the ligand binding site lies at the interface of two adjacent subunits and is formed by three loops (A – C) from the 'principal' subunit and three β-strands (D – F) from the adjacent or 'complementary' subunit. A number of studies have identified key residues that are involved in both agonist and antagonist binding. As many of the 5-HT_3 therapeutics are competitive inhibitors, these studies have been important in understanding the mechanisms of ligand binding. Comprehensive reviews of the 5-HT_3 ligand binding site can be found in Thompson *et al.* [27,28].

The transmembrane domain of each 5-HT_3 receptor subunit is primarily composed of four (M1 – M4) transmembrane α-helices (Figure 2) [2,29]. M2 α-helices from each subunit form an inner ring that is in direct contact with the permeating ions, and an outer ring consists of M1, M3 and M4. M2 residues that lie along one side of an α-helix line the water-accessible pore [30,31], and a kink at the centre of the M2 helices forms a hydrophobic constriction that represents the channel gate. Binding of 5-HT to its receptor causes movements within the extracellular domain that are translated to the M2 helices and open this gate. Studies of a conserved proline residue in the M2 – M3 loop of the 5-HT_3 receptor show that a transition between the *trans* and *cis* configuration of this residue may provide the molecular switch that is responsible for channel opening [32]. Compounds such as anaesthetics and *n*-alcohols may directly affect this region and alter the frequency of open time events (see below). Residues within M2 are also responsible for ion selectivity, as a ring of amino acids at the intracellular side of the M2 helices has been shown to influence selectivity properties in both 5-HT_3 receptor and other Cys-loop members [19]. In the 5-HT_3 receptor, mutations at the extracellular side of the channel have also been implicated in charge selectivity, and it is likely that charged amino acids at both the intracellular and extracellular sides of the pore concentrate the relevant ions before they pass through the channel (Figure 1) [33]. A possible therapeutic role for these charged residues is discussed in Section 5 of this review.

An increasing number of compounds are being identified which may act via the transmembrane domain of 5-HT_3 receptors. Picrotoxin, for example, a classic $GABA_A$ receptor antagonist, blocks the channel, and binding has been shown to be affected by mutations at a site close to the channel gate [34]. The hypertensive drug, diltiazem, which blocks voltage-gated calcium channels, is also known to block the 5-HT_3 receptor channel, highlighting the common mechanisms that many of these drugs share, and also the promiscuity that many of these compounds display. A wide range of substances, including alcohols, steroids and anaesthetics have also been reported to modulate 5-HT_3 receptors in a non-competitive fashion. Given their hydrophobicity, it is likely that the actions of these compounds are at binding sites located within the membrane, although their mechanisms of

action are largely unknown. Volatile anaesthetics and *n*-alcohols with small carbon chain lengths enhance the function of 5-HT_3 receptors and become more inhibitory with increasing carbon chain length. This dependency on molecular volume indicates that there is a binding pocket of limited size, and the similarities in their behaviour suggests that these agents act at the same site [35]. A study of residues in the M2 – M3 loop has attempted to identify this binding site. Although it was shown that the modulatory effects of *n*-alcohols and anaesthetics can be altered by mutations here, the authors concluded that they did not represent a binding site for these agents [36,37]. Interestingly, the effects of *n*-alcohols and anaesthetics are reduced in heteromeric 5-HT_{3AB} receptors [38].

The intracellular domain is formed by a loop of ~ 110 residues between M3 and M4. The structure of this domain remains uncertain, but functionally it has a role in channel conductance and receptor modulation. Homomeric 5-HT_3 receptors composed of A-subunits alone form functional channels with a conductance that is so small (sub-pS) that it cannot be resolved directly. Although the B-subunit cannot form homomeric channels, it can be combined with A-subunits to generate functional heteromeric receptors that display a much larger conductance (9 – 17 pS) [6,39]. This difference is the consequence of three arginine residues that lie within an α-helix in the M3 – M4 loop (Figure 1) [40]. The intracellular domain is also known to modulate 5-HT_3 receptor function as a result of post-translational modifications. The effects of these modifications have little therapeutic significance and are discussed in Thompson *et al*. [19]. Post-translational modification of the extracellular domain has been shown to be responsible for cell surface expression and calcium permeability, but it is unlikely to have an impact on future clinical developments [41,42].

4. Therapeutic uses of 5-HT_3 antagonists

Five 5-HT_3 antagonists are available for clinical use in Europe at present. These are tropisetron, ondansetron, granisetron, dolasetron and palonosetron. Others include azasetron and ramosetron, which are available in the Far East and alosetron, which has been approved by the FDA for the treatment of irritable bowel syndrome in the US (Table 1 and Figure 3). Owing to the unfavourable effects of 5-HT_3 agonists (e.g., nausea and anxiety) no clinical use of these is likely in the near future.

As a consequence of their potentially different subunit combinations and their varied tissue-specific distribution, it might be anticipated that 5-HT_3 receptors would provide a wide scope for novel therapeutic targets. Indeed, studies have revealed a diversity of potential disease targets that might be amenable to alleviation by 5-HT_3 receptor-selective compounds, the majority of which also have the advantage of being able to cross the blood–brain barrier [43,44]. Such disease targets include addiction, pruritis, emesis, fibromyalgia, migraine, rheumatic diseases and neurological phenomena such as anxiety, psychosis, nociception and cognitive function. Other possible targets are chronic heart pain and bulimia. Fortunately, despite a range of actions, 5-HT_3 receptor antagonists do not appear to alter normal behaviour in animal models, and the only typical physiological changes in clinical volunteers are mild effects on gut transit, constipation, headache, dizziness and clinically insignificant asymptomatic changes in cardiovascular behaviour [45]. All of these effects are reversible after termination of the drug. For further reading on a number of these therapeutic applications, a series of reviews can be found in [46]. Although these reviews were first published in 1994, many of the discussions still apply today.

4.1 Emesis

At present, 5-HT_3 antagonists are primarily used for controlling chemotherapy- and radiotherapy-induced nausea and vomiting (CINV) and in postoperative nausea and vomiting (PONV). In combination with substances such as corticosteroids (e.g.,

dexamethasone), they are important for treating acute and delayed symptoms of these therapies. The introduction of new, more potent, 5-HT_3 antagonists such as palonosetron, has further improved the treatment of these symptoms, and in combination with corticosteroids has been shown to have an improved long-term benefit compared with some of the established 5-HT_3 antagonists [47]. There is also clinical evidence that 5-HT_3 receptor antagonists could be useful for the alleviation of vomiting during pregnancy and following caesarean section [48,49]. It is believed that vomiting occurs because of the release of serotonin from enterochromaffin cells of the intestinal mucosa, which results in the stimulation of peripheral 5-HT_3 receptors in the adjacent vagal afferent neurons [50]. This effect is coincidental with a local release of 5-HT in the area postrema, located on the dorsal surface of the medulla elongata, and the actions at both locations triggers the vomiting reflex. The therapeutic effects result from inhibition of this vomiting reflex. Interestingly, as the area postrema lacks a blood–brain diffusion barrier, it is able to detect emetic toxins in the blood, as well as in the cerebrospinal fluid. However, circulating substances have not been shown to directly trigger the emetic response, which appears to be due to depolarisation of the vagal afferent nerves that terminate in this brainstem region [50]. For this reason, the use of 5-HT_3 antagonists for relieving vomiting caused by intoxication has not been pursued to any great extent. It has been suggested that the 5-HT_{3B} receptor subunit may play an important contribution to the effectiveness of these compounds and a study of polymorphisms has shown a positive link between a mutation in the promoter region of the 5-HT_{3B} gene and the frequency of vomiting [51]. However, it must be stressed that the pharmacology of homomeric and heteromeric receptors is not hugely different and other studies have found no link between polymorphisms and pharmacological responses [52-55]. It is also noteworthy that another potential postoperative use of 5-HT_3 receptor antagonists is for the prevention of pain during the injection of anaesthetics and for postoperative shivering. Studies have shown that dolasetron is as effective as the local anaesthetic lidocaine at preventing pain, but may not be as effective in preventing shivering [56,57]. Further information regarding the clinical application of 5-HT_3 antagonist in CINV and PONV can be found in [45], and guidelines for the clinical use of these compounds can be found in [58-60].

4.2 Irritable bowel syndrome and intestinal effects

The 5-HT_3 receptor antagonist alosetron is an effective treatment for irritable bowel syndrome as it decreases gut transit [61], increases fluid absorption [62] and reduces pain in irritable bowel syndrome patients [63-65]. Although desirable for irritable bowel syndrome patients, these effects probably contribute to the constipation experienced by some individuals undertaking 5-HT_3 antagonist-based therapy for other diseases. During clinical trials the use of alosetron was shown to be an efficient compound for the effective treatment of female patients with irritable bowel syndrome but post-marketing surveillance revealed several adverse reactions including reports of severe constipation or ischaemic colitis and even death. Consequently, the drug was voluntarily withdrawn in November 2000, before being reintroduced in 2002 for patients for whom the benefit-to-risk balance was favourable, and who did not respond adequately to conventional treatment [66]. This incident was unusual for 5-HT_3 antagonists which are usually well tolerated but highlights that if a symptom is not life threatening, the side effects must be resolved if the ameliorative capacity of these drugs is required for long-term use [67,68]. Future treatment of irritable bowel syndrome with 5-HT_3 antagonists may depend on combination with other therapies (e.g., tegaserod, a 5-HT_4 antagonist) in order to target multiple sites of action [69-71].

4.3 Schizophrenia, anxiety and other neurological disorders

Serotonergic neurons have a regional distribution in brain areas implicated in a range of neurological phenomenon and there has been much interest in the therapeutic potential of 5-

HT_3 receptor antagonists for antipsychotic, antinociceptive and other psychiatric disorders. As 5-HT_3 antagonists freely pass the blood–brain barrier, these compounds appear to be ideal therapeutic candidates but, so far, this potential has not been realised [72]. The theory of 5-HT involvement in schizophrenia and bipolar disorder was first suggested in the mid-1950s, and proposed that there was a serotonergic deficiency in schizophrenic individuals [73,74]. Serotonin receptors have been implicated in many of the symptoms of schizophrenia and are prime candidates because of their functional diversity and their ability to modulate the release of other neurotransmitters such as dopamine, GABA, substance P and acetylcholine. So far, the focus of 5-HT and its impact on schizophrenia has largely been on the G-protein-coupled 5-HT_2 receptors, but the administration of selective 5-HT_3 antagonists such as ondansetron and tropisetron has been shown to improve P50 auditory gating in schizophrenic patients, and may also have a therapeutic use for the neurocognitive deficits of this disorder [75,76]. Further evidence comes from a recent study that identified two 5-HT_3 sequence variations (R344H and P391R) in a small group of patients with bipolar disorder and schizophrenia [77]. The two mutations were located in a functionally important region, the M3 – M4 loop, which contains a number of potential phosphorylation sites and also has an important role in channel conductance and ion selectivity. However, the rarity of these mutations (single mutations in 2 individuals from a study of 428) and emerging electrophysiological evidence indicates that they are unlikely to be a major contributor to schizophrenia [78,79]. Association analysis of other polymorphisms has also revealed no link between 5-HT_3 receptor genes and polymorphisms [54,55].

So far, the use of 5-HT_3 antagonists for the clinical treatment of other psychological disorders has also met with little success, although there is experimental evidence that the 5-HT_3 receptor plays a role, suggesting that further studies are worthwhile. For example, the deletion of the 5-HT_3 receptor gene creates knockout mice that exhibit anxiolytic behaviour [80,81] and the use of 5-HT_3 receptor antagonists has shown a range of anxiolytic effects [82]. Studies on 5-HT_3 receptors have shown that the actions of antagonists such as mirtazapine and clozapine are competitive, and it has been shown that the enrichment of non-competitive antidepressants in cell surface lipid microdomains may be crucial for their effects [83]. Some antipsychotic drugs, such as chlorpromazine and related phenothiazines, also appear to act directly at the 5-HT_3 receptor binding site [84]. However, many antipsychotic drugs are known to have a broad spectrum of activity, only some of which can be attributed to affects on the 5-HT_3 receptor. For example, clozapine affects dopaminergic transmission but its actions at the 5-HT_3 receptor may account for this drugs unique antipsychotic efficiency [85,86]. However, 5-HT_2, dopamine and serotonin-selective re-uptake inhibitors are among the most widely prescribed drugs for these disorders and it is likely that these antipsychotic drugs will continue to have a more significant impact. It is also possible that many of the ameliorative effects of the 5-HT_3 antagonists result from the serotonin-mediated dopamine, cholecystokinin or GABA responses, or through actions on other 5-HT receptor types. Indeed, a review by Olivier *et al.* [87] concluded that 5-HT_3 antagonists are active in a number of animal models, are well tolerated in the long-term and appear to have no appreciable side effects, but due to the large body of often contradicting results, it is often difficult to interpret their effectiveness. It may be that the benefit of these chemicals may only be realised when used as a combination therapy. Interestingly, a number of antidepressant drugs have also been used for gastrointestinal disorders and other uses may eventually come to light [88].

4.4 Cognitive function

Cognition describes aspects of behaviour such as awareness, perception, reasoning and memory. The cortex and dorsal hippocampus are both important memory-related structures and antagonism of the 5-HT_3 receptor at these locations inhibits the 5-HT modulated-release

of acetylcholine without affecting the steady-state release. 5-HT_3 receptor over-expressing mice have been shown to have enhanced learning, memory and attention, and ondansetron has been found to improve memory in patients > 50 years of age [89,90]. Interestingly, a polymorphism (C178T) in the regulatory region of the 5-HT_{3A} receptor subunit has been linked to reduced activity in the amygdale and dorsal and medial prefrontal cortices, and was associated with a reduced reaction time at face recognition [91]. The same mutation has also been associated with increased susceptibility to bipolar disorder [92]. However, memory appears to be multifactoral and, like other neurological disorders, is likely to involve a range of receptors that may require a cocktail of drugs to alleviate symptoms. For example, the administration of 5-$HT_{2A/2C}$ or 5-HT_4 receptor agonists or 5-HT_{1A} or 5-HT_3 and 5-HT_{1B} receptor antagonists retards memory impairment and promotes learning in tasks that require a high cognitive demand [93].

4.5 Substance abuse and addiction

Using 5-HT_3 antagonists for the alleviation of substance abuse has had some success. Antagonists are particularly effective at reducing ethanol and morphine self-administration but are less effective at reducing the self-administration of psychostimulants such as cocaine [94-96]. It has been shown that with the administration of ondansetron, alcohol craving is significantly reduced in early onset alcoholics but increases craving in late onset alcoholics [97]. It is believed that this effect may be the result of altered 5-HT_3 modulation of dopamine release. Interestingly, substance abuse is particularly high among patients suffering from schizophrenia, suggesting a possible link between the systems that modulate these responses [98]. Co-expression of the B-subunit has been shown to reduce alcohol sensitivity in recombinant expressed 5-HT_3 receptors [99].

4.6 Bulimia

It has been shown that increased vagal afferent nerve activity is associated with binge-eating and vomiting and can be suppressed by the use of the 5-HT_3 antagonist ondansetron [100]. The depressive symptoms of these patients were also reduced. These findings are supported by evidence that the 5-HT_3 antagonist *m*-chlorophenylpiperazine has also been shown to improve mood and patient perception of body image, although these affects may be compounded by actions at other 5-HT receptors [101].

4.7 Pruritis

Pruritis is the medical term for itching and can be the result of rashes caused by burns, infection and other local irritations, or can display systemic symptoms as a consequence of renal, hepatic, hematopoietic or endocrine pathologies. It has been suggested that 5-HT_3 antagonists may have an antipruritic effect but current research reveals mixed reports in this area, and the effectiveness of treatment may vary according to the type of pruritus studied [102]. For example, in patients with cholestatic itch, either some or no benefit has been reported [103,104], whilst only marginal or no relief has been reported for haemodialysis-related pruritus [105,106]. However, the underlying mechanisms of this disorder are still poorly understood and will need further work if a therapeutic potential is to be realised [107].

4.8 Analgesics and anti-inflammatory actions

Pain results from the activation of sensory nociceptors (sensory pain), or as the consequence of damage to peripheral and central nerves (neuropathic pain). 5-HT_3 receptors are located in pain-related regions and research has shown their involvement in pain processing and inflammation. Following tissue injury, the mechanism of pain and inflammation appears to be complex. Symptoms have been attributed to the 5-HT-mediated release of neuropeptides

such as substance P, and 5-HT_3 receptors are expressed in the immune system where activation leads to T cell activation and the secretion of cytokines and prostaglandins [5,108].

Fibromyalgia is a chronic pain illness characterised by widespread aches, pain, stiffness, tenderness, general fatigue and sleep disturbances. There is clinical evidence that the 5-HT_3 antagonists granisetron, ondansetron and tropisetron can significantly reduce the effects of fibromyalgia when administered systemically [109-113]. Studies have also been performed on symptoms such as chronic lower back pain and arthritis [114,115]. A localised injection of tropesetron has also been shown to reduce pain, and the effect was longer lasting than a comparable injection of local anaesthetic [116]. The use of the 5-HT_3 antagonist dolasetron has also been used for the reduction of local pain during the injection of anaesthetics [57]. The mechanisms for these local and systemic effects have been reviewed in Riering *et al.* [117] and Giordano *et al.* [118].

As well as its use in chemotherapy, methotrexate is used to treat several different types of rheumatic disease. However, as the effects of this drug can only be seen 3 – 12 weeks after first use, the emergence of nausea in some patients is of importance. Suppression of this side effect could potentially be accomplished using 5-HT_3 receptor antagonist in the same way as they are used for CINV and PONV [119]. The effects of 5-HT_3 antagonists on the pain relieving properties of acetylsylic acid (aspirin or acetosal), acetaminophen (paracetamol) may also be important. For example, co-administration of tropisetron or granisetron with acetaminophen completely blocks the analgesic effect of acetaminophen but ondansetron does not affect the actions of acetylsylic acid [120-122].

5. Expert opinion

So far, 5-HT_3 receptor-based therapy has depended entirely on high-affinity competitive antagonists. The two main therapeutic applications for these have included their use as antemetics and for relieving the symptoms of irritable bowel syndrome. Other applications have been considered and a number of clinical trials have been conducted to assess their potential. However, the complex nature of some of the pathological symptoms, the difficulty in assessing patient benefit and the presence of established alternative drugs has limited their use in the clinic.

An interesting and potentially widespread application for 5-HT_3 receptor antagonists in the future is their capacity to reduce pain. It has been shown that the systemic administration of the compounds has beneficial affects for patients suffering from fibromyalgia and the side effects of these compounds are few and often inconsequential. However, their effect at both central and peripheral 5-HT_3 receptors introduces complex pharmacokinetic variability and may limit their clinical use. A more exciting development is the local administration of these drugs by injection or cream, both of which have been shown to have a measurable impact on pain reduction. This may include applications as diverse as alleviating the pain-related symptoms of tissue injury or arthritis. Whether or not these applications are successful will largely depend on further research to prove their effectiveness and the cost savings that these drugs can provide.

Hopefully, future studies will give us a better understanding of the promiscuous nature of some of the existing 5-HT_3 antagonists, as their targeting of multiple receptors can produce complex behaviours, the effects of which can be counterproductive. The development of more specific ligands may also allow a more directed approach, while further improvements in drug half-life should enhance their long-term effectiveness. At present, little is known about the physiological role of the five 5-HT_3 receptor subunits, and research in this area may lead to novel therapeutic interventions, particularly if subunit-specific antagonists can

be found. It also seems increasingly likely that developments, particularly in the treatment of psychological disorders, will include combination therapies in which 5-HT_3 antagonists are only a part of the overall treatment.

So far, there has not been any development of compounds that modulate receptor function by intracellular modulation and it is hard to imagine how this approach could be accomplished without having detrimental affects on other cellular responses. However, as the development costs of new therapeutics continue to rise, we may find new uses for existing compounds. For example, there is evidence that antimalarial compounds such as quinine, chloroquine and mefloquine are antagonists at a number of Cys-loop receptors [123,124]. These drugs have a good clinical record and they may turn out to have measurable benefits in the treatment of 5-HT_3 receptor-related disorders. Future therapeutic applications might also use parts of the 5-HT_3 receptor, although this approach has not been pursued as yet. For example, Broughman *et al.* [125,126] has found that M2 segments of the glycine receptor spontaneously self assemble to form chloride-permeable channels when exposed to the cell surface and could be used an effective means of channel replacement therapy by restoring chloride permeability in cystic fibrosis patients. Charged residues close to M2 are known to be responsible for ion selectivity in the 5-HT_3 receptor and manipulation of these, or similar peptides, may allow the development of novel therapeutics tailored for specific clinical uses.

5-HT_3 receptors are well understood in terms of their distribution, structure, function and pharmacology. However, there is still some way to go in order to understand their roles in both the CNS and the PNS, and a better knowledge of this might lead to more areas for therapeutic intervention by 5-HT_3 receptor agonists, antagonists and modulators. At present, 5-HT_3 receptor antagonists are proving to be useful agents for controlling chemotherapy-induced emesis and in irritable bowel syndrome, but as studies suggest there is considerable potential for therapeutic intervention in other areas, the authors anticipate that there will be further developments in their clinical use.

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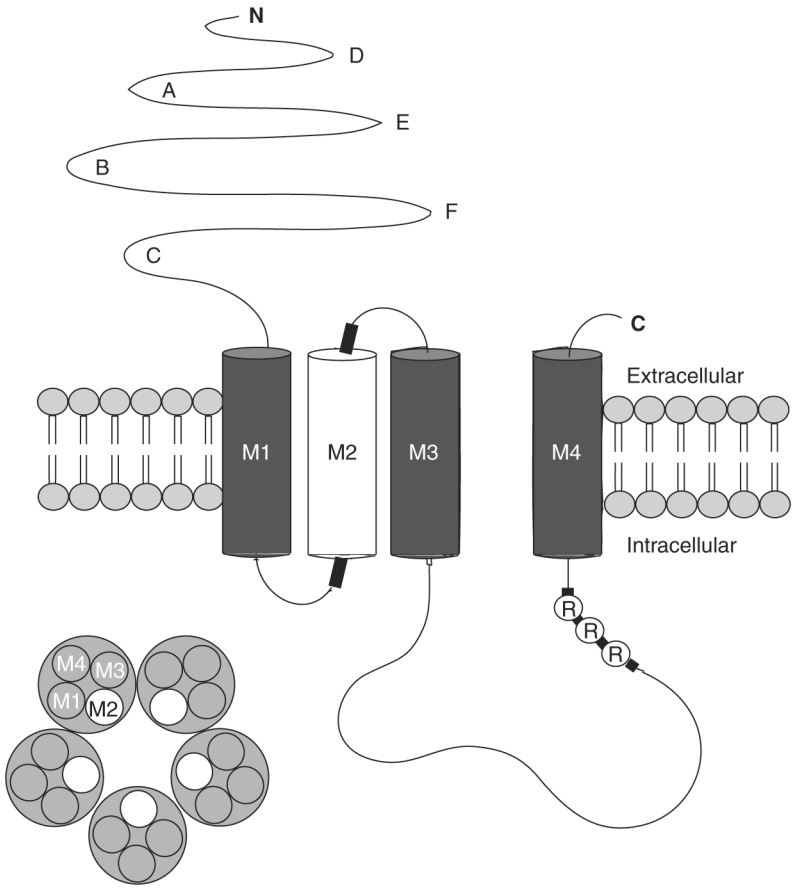

Figure 1. A schematic representation of a typical Cys-loop receptor subunit

The diagram at the lower left is a cross-section of the transmembrane region shown from above and demonstrates how five subunits associate to form a central ion-conducting pore that is lined by M2 α-helices. Attention is drawn to six loops that form the binding ligand binding site (A – F), regions associated with ion-selectivity (dark lines either side of M2) and the region that has been shown to influence ion conductivity (R-R-R).

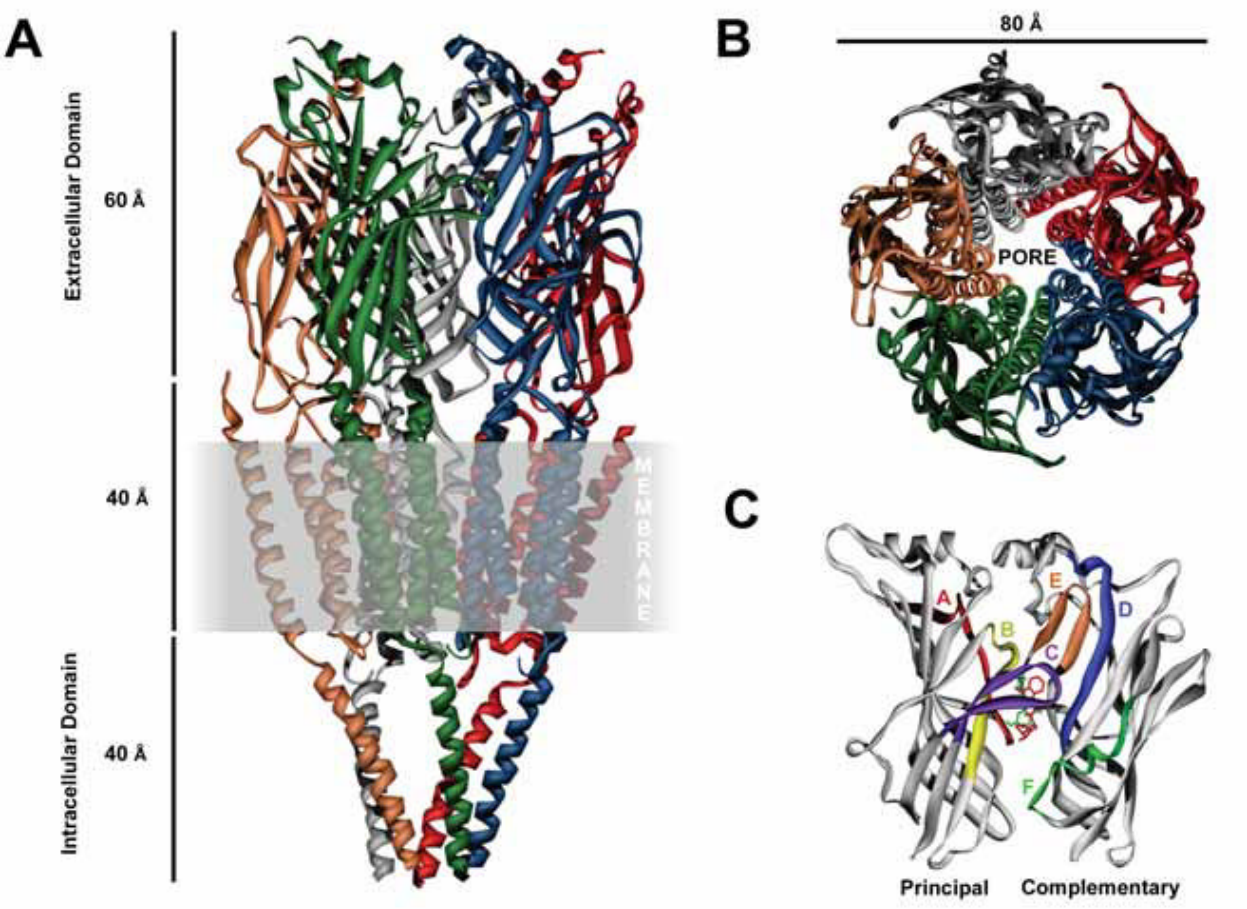

Figure 2. A homology model of the extracellular, transmembrane and intracellular domains of the 5-HT$_3$ receptor

A. The receptor is shown from the side and the position of the membrane is shown as a grey box. So far, the only resolved structure within the intracellular domain of each subunit is an α-helix. **B.** The receptor is shown from above, looking down towards the membrane and through the central ion-conducting pore. **A.** and **B.** are homology models based on cryo-electron microscopy images of the nACh receptor at 4 Å resolution (PDB ID; 2bg9). **C.** A homology model of the extracellular domains of two adjacent subunits (principal and complementary). This model was based on the crystal structure of AChBP at 2.7 Å (PDB ID; 1i9b) and highlights the six loops that converge to form the ligand binding site. Only two of the five subunits have been shown for ease of viewing. 5-HT (green) and granisetron (red) are docked into the binding site. The positions of these ligands is based upon the most likely orientations taken from [23] and [24].

AChBP: Acetylcholine binding protein; 5-HT: 5-Hydroxytryptamine; nACh: Nicotinic acetylcholine receptor; PDB: Protein DataBank.

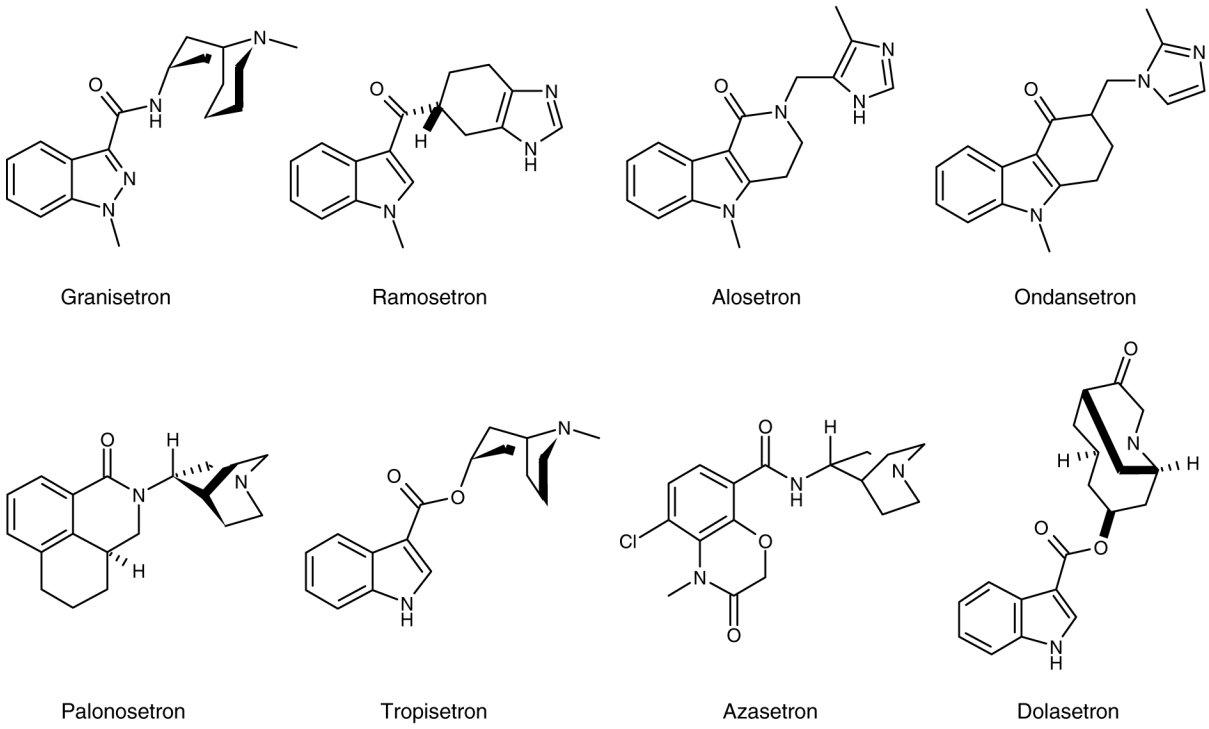

Figure 3. Molecular structures of commercially available 5-HT$_3$ receptor antagonists

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Table 1

K_d, K_i and IC_{50} values for a range of 5-HT_3 receptor antagonists

Antagonist	K_d, K_i or IC_{50}	Species	Reference
In present clinical use:			
Granisetron	230 pM	N1E-115	[127]
Granisetron	2.01 nM*	Mouse	[128]
Granisetron	2.43 nM	NG 108-15	[128]
Granisetron	410 pM	N1E-115	[129]
Granisetron	1.44 nM*	Human	[130]
Granisetron	5.13 nM	Rat brain homogenate	[131]
Tropesetron	3.80 nM*	Mouse	[128]
Tropisetron	3.85 nM	NG 108-15	[128]
Tropisetron	46.0 pM§	Rabbit nodose ganglion	[132]
Tropisetron	4.90 nM	Rat brain homogenate	[131]
Tropisetron	11.0 nM§*	Human	[53]
Ondansetron	34.3 pM	NG 108-15	[128]
Ondansetron	4.03 nM	N1E-115	[129]
Ondansetron	4.90 nM*	Human	[130]
Ondansetron	57.0 pM§	Rabbit nodose ganglion	[132]
Ondansetron	7.40 nM	N1E-115	[127]
Ondansetron	46.8 nM	Rat brain homogenate	[131]
Palonosetron	31.6pM	NG 108-15	[133]
Palonosetron	31.6 pM	Rat brain	[133]
Palonosetron	5.01 nM	Guinea-pig ileum	[133]
Dolasetron	20.0 nM	NG 108-15	[134]
Azasetron	0.33 nM	Rat small intestine	[135]
Alosetron	3.16 nM	Rat brain homogenate	[131]
Alosetron	398 pM*	Human	[136]
Alosetron	158 pM	Rat brain homogenate	[136]
Ramosetron	0.15 nM*	Human	[137]
Not in present clinical use:			
Bemesetron	328 pM§	Rabbit nodose ganglion	[132]
LY-278,584	5.00 nM§*	Human	[53]
Y-25130	36.0 nM§*	Human	[53]
MDL-72222	16.0 nM	N1E-115	[127]
MDL-72222	30.2 nM	Rat brain homogenate	[131]
BRL-46470	150 pM	NG 108-15	[128]
BRL-46470	1.58 nM	Rat brain homogenate	[131]
ICS-205-930	640 pM	N1E-115	[127]
Quipazine	510 pM‡	N1E-115	[129]

Antagonist	K_d, K_i or IC_{50}	Species	Reference
Quipazine	1.00 nM[≠]	N1E-115	[127]
Quipazine	1.10 nM[≠]	Rat brain homogenate	[131]
GR-65630	2.50 nM	N1E-115	[127]
SDZ 206-830	871 pM	Rat brain homogenate	[131]
(S)-zacopride	955 pM	Rat brain homogenate	[131]
(R)-zacopride	11.0 nM	Rat brain homogenate	[131]
Renzapride	67.6 nM	Rat brain homogenate	[131]
Clozapine	269 nM	Rat brain homogenate	[131]
2-(4-methyl-1-piperazine)cyclohexa[c] quinoline	230 pM	Rat brain homogenate	[138]
Indisetron	1.70 nM	Rat brain homogenate	[139]
Lerisetron	0.80 nM[*]	Mouse	[140]
Cilansetron	0.19 nM	Rat brain homogenate	[141]

[*] Recombinantly expressed in cells.

[≠] Note that quipazine has been classified as both an agonist and antagonist.

[§] IC_{50} values, calculated using electrophysiological techniques.