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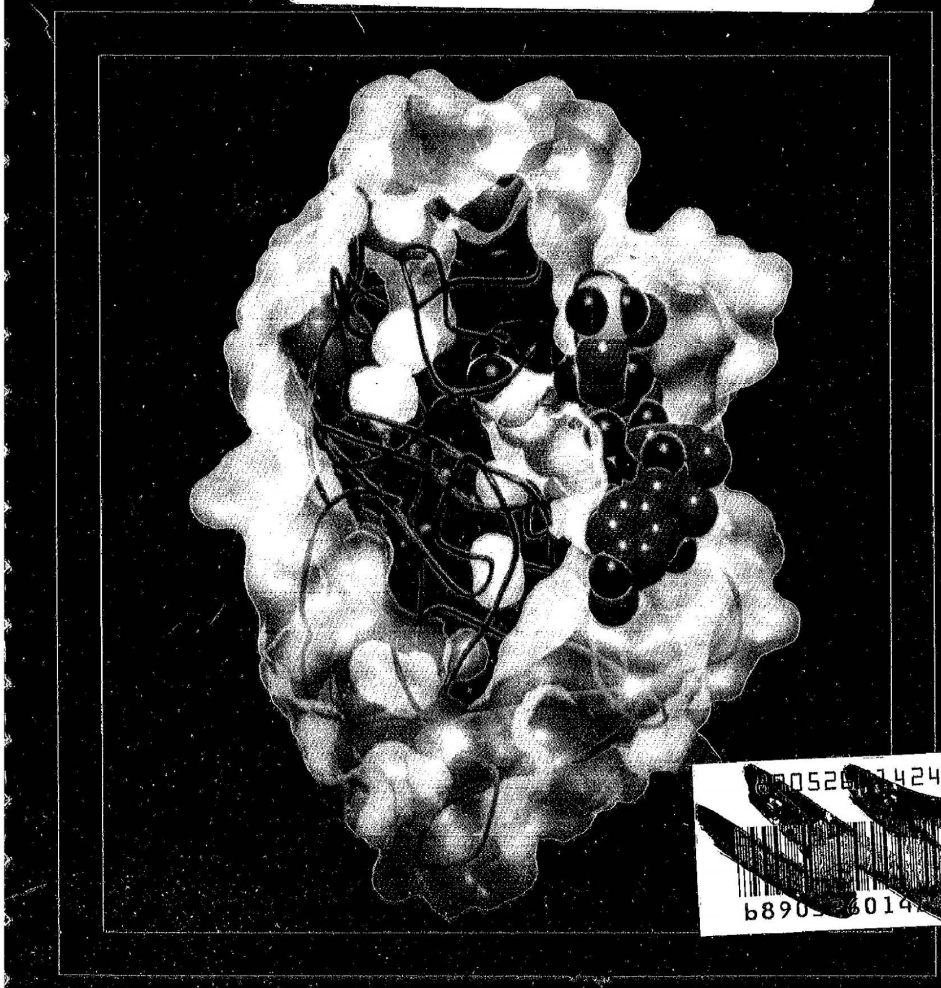
CURRENT PHARMACEUTICAL DESIGN



a timely review journal on recent advances in drug design

Aims and Scope

Current Pharmaceutical Design will publish timely in-depth reviews covering all aspects of current research in rational drug design. Each bi-monthly issue is devoted to a single major therapeutic area. A Guest Editor who is an acknowledged authority in a therapeutic field has solicited for each issue comprehensive and timely reviews from leading researchers in the pharmaceutical industry and academia. Each thematic issue of ***Current Pharmaceutical Design*** will cover all subject areas of major importance to modern drug design, including: medicinal chemistry, pharmacology, drug targets and disease mechanisms.

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5-Hydroxytryptamine (5-HT_3) Receptors: Molecular Biology, Pharmacology and Therapeutic Importance

R.M. Eglen* and D.W. Bonhaus

Institute of Pharmacology, Neurobiology Unit, Roche Bioscience, 3401, Hillview Ave., Palo Alto, CA 94304, USA.

Abstract: The 5-HT_3 receptor is unique among known monoamine receptors in that, rather than being a G-protein-coupled receptor, it forms a ligand-gated ion channel. In the CNS, this receptor is found in high density in nuclei of the lower brainstem, area postrema and nucleus of the tractus solitarius. Lower densities of the receptor are found in the cerebral cortex and limbic areas, including the hippocampus. In the periphery, 5-HT_3 receptors are located on pre- and postganglionic neurons of both sensory and enteric nervous systems. The receptor is a pentameric protein with multiple agonist and allosteric ligand binding sites. Thus it has structural and functional similarities with nicotinic, GABAergic and, other ligand gated ion channels.

5-HT_3 receptor antagonists have been shown to produce beneficial effects in animal models of cognitive and psychiatric disorders. Whether 5-HT_3 receptor antagonists may have similar profound effects in the treatment of anxiety, depression or psychosis will be determined by the outcome of ongoing clinical trials. However, it is in the treatment of cancer chemotherapy induced emesis that 5-HT_3 receptor antagonists have had their greatest impact. The cytotoxic agents used in cancer chemotherapy provoke the release of 5-HT from enterochromaffin cells in the gastrointestinal tract. This 5-HT acts on 5-HT_3 receptors in the central nervous system or on peripheral vagal afferent fibers to initiate vomit reflexes. 5-HT_3 receptor antagonists block this action and thereby greatly reduce the number of emetic episodes that occur during cancer chemotherapy. The marked clinical efficacy of 5-HT_3 receptor antagonists such as ondansetron, granisetron and tropisetron together with their lack of adverse side effects has revolutionized the treatment of cancer chemotherapy induced emesis.

Introduction

5-Hydroxytryptamine (5-HT, serotonin) exerts important modulatory effects on both central and peripheral nervous systems, via activation of several discrete receptor families. In general, 5-HT receptors structurally conform to the archetypal motif for G protein-coupled receptors and regulate cellular responses via modulation of adenylyl cyclase or phospholipase C activity. The one exception is the 5-HT_3 receptor subtype. This receptor, which bears significant sequence homology with the nicotinic receptor family, mediates a fast depolarization of central and peripheral neurons by increasing sodium and potassium permeability. In the last decade 5-HT_3 receptors have been the focus of extensive research efforts, not only because of their unique structure among monamine receptors, but also because of the clinical efficacy of selective antagonists in the treatment of emesis associated with cancer chemotherapy. 5-HT_3 receptor antagonists may also act as cognitive enhancers, anxiolytics, anti-psychotics and as analgesics, although, clinical validation of these putative applications is still lacking. This brief review summarizes some key features of the molecular structure and pharmacology of the 5-HT_3 receptor. It also addresses findings from recent clinical studies examining the therapeutic utility of 5-HT_3 receptor antagonists in the treatment of acute and delayed cancer chemotherapy induced emesis. Several reviews of 5-HT_3 receptor pharmacology and medicinal chemistry have recently been published [1-3]. Recent reviews of the clinical trials testing the efficacy of 5-HT_3 receptor antagonists have also been written [4-6]. The reader is referred to these reviews and the bibliographies cited therein for additional information on specific topics.

Molecular Biology and Structure

5-HT_3 receptors mediate rapid neuronal depolarization by increasing membrane permeability to sodium and potassium ions and in clonal cell lines they have also been shown to mediate calcium ion flux [7-11]. The rapid onset and offset of the 5-HT_3 receptor mediated responses led to the initial suggestion that this receptor may be an ion channel. However, only relatively recently, by the use of patch-clamp techniques, has the 5-HT_3 receptor been unequivocally shown to comprise an ion channel per se, rather than being a G-protein coupled receptor that activated ion channels via mobilization of a second messenger [12]. Thus, in isolated whole cells of guinea pig submucosal plexus, 5-HT_3 receptor-mediated currents were insensitive to *pertussis* toxin and independent of the presence of GTP in the intracellular solution. Moreover, in excised patches of cell membranes tropisetron-sensitive currents were evoked by extracellular 5-HT up to 5 hours following isolation of the patch. These data indicated that GTP or other soluble cytoplasmic proteins were not required for the currents evoked by 5-HT and convincingly argued that the receptor was an ion channel.

Ultimately, the cloning and sequencing of a functional 5-HT_3 receptor demonstrated that the structure of the receptor had an amino acid sequence and topography homologous with other ligand gated ion channels, such as the nicotinic cholinergic or GABA receptors [13]. Recent studies using electron microscopy have shed light on the quaternary structure of this channel further confirming its inclusion in the family of ligand gated ion channels [14,15]. Platinum replica images of the channel as imaged by rotary shadowing of purified 5-HT_3 receptors show the receptor to be a rosette shaped particle, 8-9 nM in diameter,

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with a length of approximately 11 nM (Fig. **1**). This pentameric motif is, of course, similar to that determined for the nicotinic cholinergic [16] and GABAergic receptors [17].

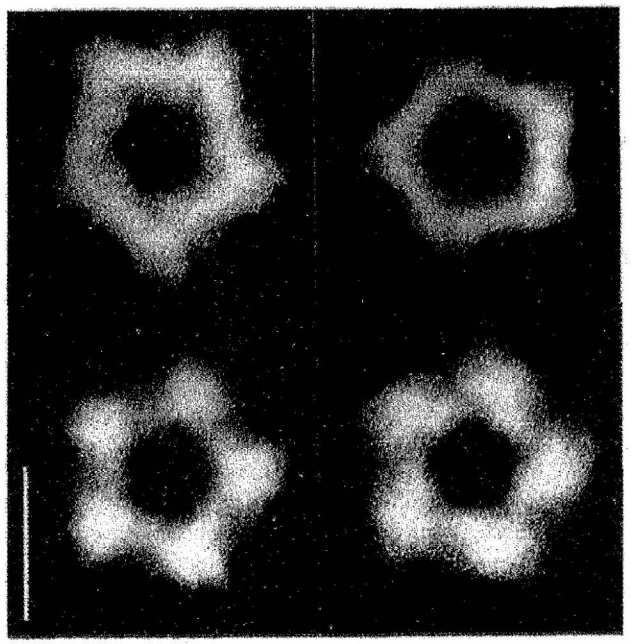

Fig. (1). Filtered electron microscopic images of four receptors demonstrating that the 5-HT_3 receptor is a symmetrical pentamer with a central core. This picture is taken, with permission, from Boess et al., 1995.

The similarities in primary and quaternary structure of the 5-HT_3, GABA and nicotinic receptors suggest that these receptors may share similar molecular pharmacology. Thus, it is unsurprising that the 5-HT_3 receptor possesses multiple agonist binding sites subject to allosteric regulation and susceptible to noncompetitive blockade by ligands which may bind in the pore of the channel. Based upon data from studies with nicotinic and GABAergic receptors, it would also be expected that the 5-HT_3 receptor would be a heteromeric structure with subtypes of the receptor being defined by the incorporation of different specific subunits into the protein. However, in this last regard at least, the 5-HT_3 receptor may differ from other ligand gated channels, since the existence of heteropentameric receptors has not been reported.

Ligand Interactions (Competitive, Non-competitive, Cooperative and Allosteric)

The presence of multiple, positively cooperative agonist binding sites on the receptor was first indicated by electrophysiological studies showing that the concentration-response curves had Hill slopes markedly greater than unity. Subsequent radioligand binding experiments, using radiolabeled agonists, show similar indications of positive cooperativity [18-24]. However, for reasons that are unclear, detection of this phenomenon by radioligand binding depends upon the specific radioligand, membrane preparation and ionic content of the buffer employed in the binding assay.

The interaction among agonist binding sites has also been demonstrated using kinetic binding methods in that rates of radioligand dissociation were found to be dependent upon the competing ligand [25]. This finding is inconsistent with simple competitive interactions in that it suggests that the "displacement" of one agonist molecule modifies the dissociation rate constants for the remaining bound agonist molecules. The results from these kinetic experiments also indicate that not all antagonists produce equivalent effects on dissociation rates suggesting that the binding of different antagonists can produce different conformations of the receptor. A final indication of multiple agonist binding sites comes from the results of saturation binding experiments conducted with different radiolabelled agonists. These studies have shown that the maximum number of detectable binding sites depends on the radioligand used to label the receptor [22,26]. One explanation for these data is that the binding of certain ligands to one site on the receptor precludes occupancy of adjacent sites either by a steric hinderence or by allosterically modifying the affinity of adjacent binding sites on the receptor. Since heterogeneity in these agonist binding sites has not been detected, these multiple binding sites may be pharmacologically equivalent. Taken together, the findings from radioligand and electrophysiological studies suggest a model of agonist interactions that are similar to those which have been established for the nicotinic acetylcholine receptor. Namely, that occupancy of multiple binding sites is required for receptor activation and that the binding of one molecule of agonist facilitates the binding of subsequent molecules [27].

Positive allosteric interactions among ligands binding to sites distinct from the agonist recognition sites have been suggested on the basis of findings made with ethanol and ketamine [28,29]. Ethanol, a drug that modifies the function of a number of ligand-gated ion channels, augments 5-HT_3 receptor-gated ion currents [30] and 5-HT_3 receptor mediated [^{14}C]guanidinium ion influx [31]. The concentrations of ethanol required to achieve this action, while high, are not outside the range of those achieved in humans; thus augmentation of 5-HT_3 receptor mediated neurotransmission may occur during alcohol intoxication. Whether this interaction plays a role in the psychobehavoral effects of alcohol is unclear. However, 5-HT_3 receptor antagonists have been shown to reduce the voluntary ethanol consumption in rats [32] and humans [33] and to disrupt ethanol state-dependent learning [34] thus raising the possibility that some of the reinforcing actions of ethanol are a consequence of 5-HT_3 receptor activation. The potentiating actions of ketamine were detected at micromolar concentrations and were reversible upon washout of the drug. Importantly the effects of ketamine were not mimicked by a 5-HT uptake blocker. The site of action of this compound is not known but the mechanism may involve a reduction in the rate of desensitization of the channel.

While these findings of positive modulatory actions strongly suggest direct actions on the receptor through allosteric ligand binding sites, these interactions have, for the most part not yet been confirmed with ligand binding studies or isolated tissue patches. For this reason the possibility of indirect interactions underlying these apparent allosteric effects cannot be excluded. In this regard, the finding that 5-HT_3 receptor mediated currents can be enhanced by activators of protein kinase C underscores the potential for indirect interactions via activation or inhibition of cellular kinases [35].

The precedent established by NMDA, nicotinic and GABAergic ligand gated ion channels suggests that 5-HT_3 receptors should also be susceptible to blockade by

noncompetitive antagonists such as channel binding ligands. And indeed, such interactions are now being found. Ifenprodil, a non competitive blocker of NMDA receptor mediated currents, also blocks 5-HT$_3$ receptor mediated currents in NG108-15 cells via a reversible, noncompetitive mechanism [36]. The most likely site of interaction would seem to be the pore of the channel itself, although voltage dependence of the blockade has not been shown. A second compound which may block 5-HT$_3$ receptor mediated currents via an action in the channel is curare [37-39]. This compound blocks other ligand-gated ion channels and its block of 5-HT$_3$ receptor gated channels shows both competitive and noncompetitive components. Curare is interesting in its own right in that, its potency for species variants of the 5-HT$_3$ receptor varies by nearly 2000 fold.

Other ligands, which interact with undefined binding sites on the 5-HT$_3$ receptor at relatively low affinities are some local anesthetics [40,41] and cannabinoids [42]. The finding that local anesthetics interact with the channel, at concentrations similar to those at which they interact with sodium channels, has implications for studies attempting to elucidate the mechanism by which this class of compounds produces analgesia. The data showing that cannabinoids, including the endogenous ligand anadamide, block 5-HT$_3$ receptors, not only suggests a possible mechanism by which these compounds mediate their antiemetic actions, but also raises the possibility of endogenous antagonists for this receptor.

In summary the presence of multiple ligand binding sites on the 5-HT$_3$ receptor greatly enriches the pharmacology of the receptor and provide mechanisms of regulation not found with the G-protein coupled 5-HT receptors. Additional studies are clearly needed to address these intriguing interactions and to further define the therapeutic potential of noncompetitive 5-HT$_3$ receptor antagonists.

Pharmacology

The discovery of the 5-HT$_3$ receptor is extensively documented elsewhere. However, it is worth noting that the pharmacological characterization of 5-HT$_3$ receptors began forty years ago with the recognition of a distinct 5-HT "M' receptor [43]. Interestingly, the ligand first used in the characterization of the "M" receptor, namely cocaine, served as a lead for the one of the first selective antagonists, MOL 72222. This research extensively used tissue based functional assays such as those based on the guinea-pig isolated ileum or rabbit nodose ganglion to identify selective 5-HT$_3$ receptor antagonists. One in vivo assay step that has been extensively used in studies of the 5-HT$_3$ receptor is the von Bezold Jarisch reflex. This is a vago-vagal reflex, observed in in rats, dogs, ferrets or man, is mediated by afferent nerve terminals in the heart. Taken together, it was only relatively late in the discovery process that radioligands and expression cloning techniques were applied to the characterization of 5-HT$_3$ receptors.

Agonists

The 5-HT$_3$ receptor is uniquely sensitive to the agonist 2-methyl 5-HT, and relatively insensitive to indoles such as 5-methoxytryptamine or 5-carboxamidotryptamine. Indeed, the response to 2-methyl 5-HT is so selective that it is diagnostic of activation of 5-HT$_3$ receptors. Other agonists for the receptor include several phenylbiguanides, notably *meta*chlorophenylbiguanide. A feature of these latter compounds is that, although they possess relatively high potency, they are partial agonists with respect to 5-HT. Therefore, the potency of these agonists depends on tissue factors such as the receptor reserve and or efficiency of stimulus response coupling. Different effects on desensitization rates may also contribute to the appearance of partial efficacy. A lack of response to these agonists in a specific tissue may not therefore necessarily indicate a lack of involvement of the receptor.

Antagonists

Extensive research has centered around the identification of silent, surmountable, antagonists for the 5-HT$_3$ receptor (see below). The medicinal chemistry in this area has been extensively reviewed previously and will be briefly summarized here. Current 5-HT$_3$ receptor antagonists comprise two major structural classes: the benzamides or benzoate esters, and the 6,5-heteroaromatics, of which MDL 72222 and tropisetron are prototypic compounds. In the benzamide series, incorporation of the 2-methoxy group and a 4-amino-5-chloro substitution results in compounds with high affinity. Several aromatic nuclei have been incorporated in this molecule including benzofurans, benzothiophenes, quinolines and benzoxazines or pyridines. In terms of the 6,5-heteroaromatics, a large number of high affinity antagonists utilize this substitution. Granisetron, for example, possess an indazole nucleus that acts as a bioisostere for the indole ring. Indeed, a large number of nuclei may act as a biosteric replacement of the indole. The position of the basic nitrogen to the carbonyl linker is important in determining affinity, particularly with the groups orientated close to planarity. Several antagonists are now available of exceptionally high affinity i.e. pKi values greater than 9.0 [44-46]

Receptor Subtypes

Pharmacological evidence from tissue-based functional studies have, for several years, suggested the presence of receptor subtypes. The principal finding being that tissues from guinea-pig have lower affinity for nearly all 5-HT$_3$ receptor antagonists in comparison to equivalent tissues from rat, mouse or man. Similar differences have been reported using high affinity radioligands to label 5-HT$_3$ receptors in guinea-pig and other species. Differences in antagonist affinity between 5-HT$_3$ receptors in rat, rabbit or mouse, as well as between different strains of mice have also been reported (see Table 1) [47-49]. The molecular basis for these affinity differences are unknown, and cloning of a guinea-pig 5-HT$_3$ receptor α subunit has not yet been reported. However, the sequence homology between the cloned rat and rabbit 5-HT$_3$ receptors is only 80 %. It is also possible, though unproven, that these interspecies differences in affinity reflect different subunit assemblies.

Interestingly, several studies also report that intraspecies differences in 5-HT$_3$ receptors may exist. However, the pharmacological and electrophysiological data indicating intraspecies differences are sparse and the differences in ligand affinity small. Nonetheless, there are unconfirmed reports of differences in ligand affinity at 5-HT$_3$ receptors in mouse cerebral cortex and ileum [50]. Electrophysiologically,

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