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Review

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Partial Agonism of 5-HT$_3$ Receptors: A Novel Approach to the Symptomatic Treatment of IBS-D

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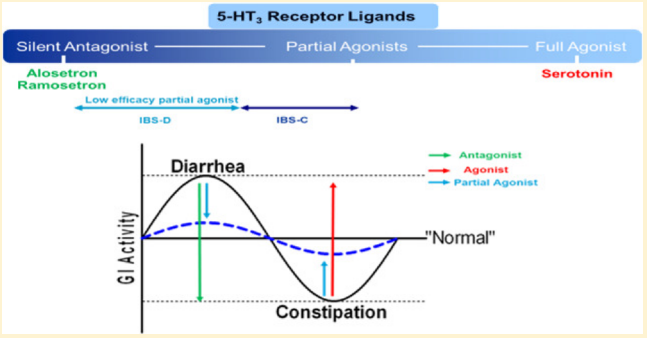
ABSTRACT: Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by abdominal pain, discomfort, and altered bowel habits, which have a significant impact on quality of life for approximately 10−20% of the population. IBS can be divided into three main types IBS-D (diarrhea predominant), IBS-C (constipation predominant), and mixed or alternating IBS. 5-HT$_3$ receptor antagonism has proved to be an efficacious treatment option for IBS-D. For example, alosetron displays efficacy in the treatment of multiple symptoms, including abdominal pain, discomfort, urgency, stool frequency and consistency. However, significant constipation occurred in approximately 25% of patients, leading to withdrawal of up to 10% of patients in clinical trials. Targeting compounds with partial agonist activity at the 5-HT$_3$ receptor represents a mechanistic departure from the classic 5-HT$_3$ receptor antagonist approach and should result in agents that are applicable to a broader array of IBS patient populations. Attenuation of the activity of the ion channel without completely abolishing its function may control or normalize bowel function without leading to a total block associated with severe constipation. We have identified a new class of selective, orally active 5-HT$_3$ receptor ligands with high 5-HT$_3$ receptor affinity and low partial agonist activity currently in preclinical development that should offer a significant advantage over existing therapies.



KEYWORDS: *5-HT$_3$ receptor, partial agonist, irritable bowel syndrome, IBS*

With the special edition *ACS Chemical Neuroscience* to mark the 25th anniversary of the Serotonin club, this is a good opportunity to revisit the role of 5-HT within the gastrointestinal tract, particularly with respect to the function of 5-HT$_3$ receptors. Many may think of the 5-HT$_3$ receptor as an "old" target that does not have the "sexy" appeal of a "novel" target, but there is still a lot to learn about 5-HT$_3$ receptors and many opportunities for it to be a valuable target for drug discovery. Although there is a lot of comment about the lack of innovation in the pharmaceutical industry, we would like to suggest that despite the claims that the "low hanging fruit" has been picked that there are still many opportunities to explore these "older/established" targets. We will discuss how using a partial agonist approach to this "established" target may lead to significant therapeutic advantages in the treatment of irritable bowel syndrome (IBS). Partial agonism as an approach is well established in other therapeutic areas; for example, aripiprazole is a dopamine receptor partial agonist for the treatment of schizophrenia,[1] and varenicline is a nicotinic partial agonist used for smoking cessation.[2] In this brief review we will discuss the background to the concept and some key data; this is not intended to be a definitive review of IBS and 5-HT$_3$, and there are numerous detailed reviews.[3−5] The concept of partial agonism as an approach will be expanded on later, but the key aim of this approach is to normalize GI function rather than completely shut down function which is achieved with a long acting antagonist.

Ever since the first discovery of enteramine (5-HT) in the gastrointestinal tract (GI) by Erspamer, it has been known that 5-HT plays a crucial role in GI function.[6] Ninety percent of all 5-HT in the body can be found in the GI tract. This 5-HT is principally found in enterochromaffin cells. Enterochromaffin cells respond to changes in luminal stimuli such as pressure, toxins, or nutrient changes by the release of serotonin which acts on nerve endings within the submucosa and mucosa via a range of serotonin receptor subtypes including the 5-HT$_3$ receptor. The level of released 5-HT is then controlled by the serotonin transporter (SERT) which removes 5-HT to mitigate the action. It is hypothesized that changes in serotonin signaling either by increased availability or a decrease in SERT can lead to a range of functional gastrointestinal disorders including IBS.[3,7,8] IBS affects about 10−20% of the adult population with symptoms ranging from constipation to diarrhea or a combination of the two accompanied by bloating, pain, and severe abdominal discomfort that can significantly affect the quality of life of subjects.[9] IBS is subdivided based on the predominant symptoms, for example, diarrhea predominant IBS (IBS-D) or constipation predominant IBS (IBS-C).

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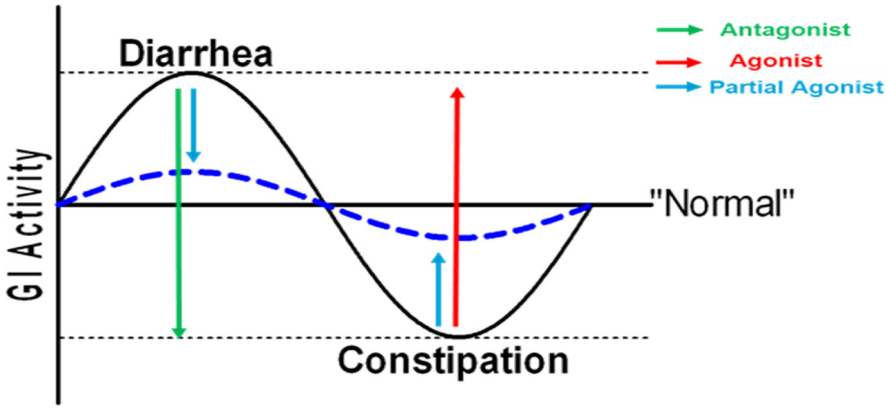

Figure 1. In IBS-D hyperactivity of the 5-HT system is thought to lead to enhanced GI activity and bouts of diarrhea and GI discomfort, whereas in IBS-C hypoactivity of the 5-HT system leads to bouts of constipation and GI discomfort. In IBS-D, a 5-HT_3 receptor antagonist will completely block the function, leading to constipation in a significant number of individuals. In the case of IBS-C, a full 5-HT_3receptor agonist overcomes the hypoactivity but rebounds into an hyperactive state leading to diarrhea and other agonist related AEs such as nausea, emesis, and pruritus. A weak partial agonist would normalize the activity rather than totally overriding activity.

At about the same time that 5-HT was identified as a key mediator of neuronal function, Rocha E Silva and colleagues reported that some of the actions of 5-HT within in GI tract were inhibited by cocaine.[10] They proposed that inhibition was mediated by the blockade of receptors located on post-ganglionic cholinergic neurons. Subsequently, Gaddum and Picarelli proposed that some of the actions of 5-HT on the GI tract are mediated via two types of 5-HT receptor, designated D and M. D receptors are located on the smooth muscle and blocked by dibenzyline, hence the D designation. The second 5-HT receptor mediated effect was produced via the indirect postganglionic cholinergic neurons reported in the Rocha E Silva paper and those were termed M receptors; the response mediated by this putative receptor could be blocked by cocaine, morphine, and methadone.[11] In 1986, Bradley et al. reclassified of the D receptor as the 5-HT_2-type receptor and the M receptor was renamed the 5-HT_3 receptor.[12]

Subsequent research has shown that the 5-HT_3 receptor is unique within the family of 5-HT receptors; all other 5-HT receptors exert their effects via G-protein coupled mechanisms and are part of the G-protein coupled receptor (GPCR) family. In contrast, the 5-HT_3 receptor is a member of the Cys-loop ligand gated ion channel family and is principally permeable to the cations sodium, potassium, and calcium. The receptor is composed of multiple protein subunits that form a pentameric structure around a central ion-conducting pore. The five subunits are proteins encoded by the *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D*, and *HTR3E* genes.[13] The 3A subunits form functional homomeric channels, whereas the B−E subunits only form functional heteromeric channels when coexpressed with the 3A subunits; the exact stoichiometry of the units in these heteromeric channels is not fully understood.

Significant species differences exist in subunit expression: In the rodent, 5-HT_3 receptors are limited to the 5-HT3A and 5-HT3B subunits.[13] In man and other nonrodent species, all five subunits exist, with relatively high expression levels of 5-HT3C, 5-HT3D, and 5-HT3E subunits in the GI tract.[13−15] The species specific expression of the subtypes also questions the utility of rodent GI models for compound development.[15]

5-HT_3 RECEPTOR ANTAGONIST AND THE TREATMENT OF IBS

In the mid-1980s, a number of groups identified potent and selective antagonists of the 5-HT_3 receptor. Two of the first to be reported were MDL72222 (bemesetron) and ICS205-930 (tropisetron) (for review, see ref 16). Subsequently, Glaxo and Beecham reported on the discovery of GR38032 (ondansetron) and BRL43694 (granisetron). Tropisetron, ondansetron, and granisetron were all marketed for the prevention of acute chemotherapy induced nausea and emesis (CINV).[16]

In addition to the clinical studies for CINV, ondansetron was also shown to have beneficial effects in both IBS-D and carcinoid diarrhea. Studies with ondansetron demonstrated that the compound suppressed the reflex activation of colonic motor function in response to food ingestion in both normal subjects and patients with IBS-D. The postprandial activation of colonic function is a prominent feature of normal post ingestive behavior. However, in some disease states (e.g., IBS-D) the response tends to be exaggerated, leading to abdominal cramping, increased urgency, bloating, abdominal discomfort, and diarrhea in patients with IBS-D.[17] Subjects suffering from carcinoid diarrhea also display enhanced colonic motility during the early postprandial period and ondansetron was shown to be effective in normalizing function, demonstrating that 5-HT acting via 5-HT_3 mechanisms plays a key role in disease states characterized by enhanced colonic motility.[17]

Shortly after the initial reports relating to the pharmacology of ondansetron,[18] Glaxo scientists published on a follow up selective long acting 5-HT_3 receptor antagonist, alosetron (GR68755).[19] Alosetron was shown to have rapid onset and long duration of action in the 5-HT_3 mediated Bezold-Jarisch reflex in the cat.[19] Although ondansetron was the first 5-HT_3 receptor antagonist shown to be effective in IBS-D, most clinical studies have been performed with alosetron.[3] Alosetron was subsequently marketed for the treatment of IBS-D. Alosetron is about 10 times more potent than ondansetron and appears to have a longer duration of action in man. Clinical trials demonstrated that alosetron was efficacious in the symptomatic treatment of IBS-D leading to improved stool consistency, reduced urgency, and relief of abdominal discomfort. A recent meta-analysis reported on 14 trials of 5-HT_3 receptor antagonists in IBS-D, including 3024 patients

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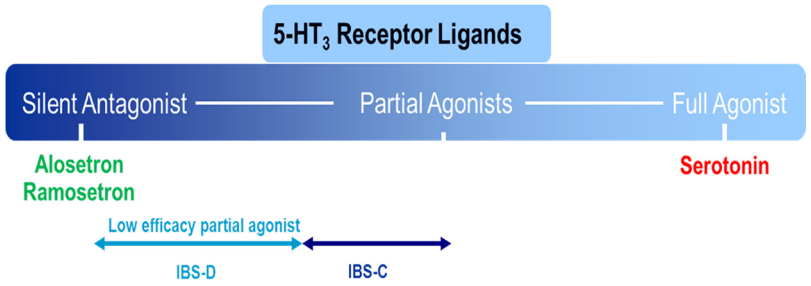

Figure 2. Preferred functional activity of 5-HT_3 agonists for IBS.

receiving alosetron and 1116 receiving cilansetron (a 5-HT_3 receptor antagonist under development by Solvay in 2005[20]) compared to 3043 on placebo.[21] This analysis clearly demonstrated that both alosetron and cilansetron showed significant global benefits in IBS-D.[21] Similar findings were reported in a subsequent meta-analysis of 5-HT_3 receptor antagonists and a 5-HT_4 receptor agonist.[22] In both meta-analyses, the predominant adverse effect (AE) reported for 5-HT_3 receptor antagonists was constipation. In the Ford et al. analysis, 25% (n = 716) of patients on active treatment reported experiencing constipation whereas only 6% (n = 96) of placebo treated subjects reported this AE; constipation was one of the main reasons for discontinuation of alosetron treatment.[22] Alosetron use has been limited to women with severe IBS-D following the observation of a higher rate of ischemic colitis (IC) in patients taking alosetron. A recent review by Chang and colleagues reports that the incidence of IC is about 0.95 cases per 1000 patient year.[23] IC incidence is much lower than the incidence of constipation and also seen in subjects who displayed no constipation.[24] The exact pathophysiological cause of alosetron-induced IC remains unclear,[24] and animal studies have failed to replicate the condition.[24,25] Interestingly, the 5-HT_3 receptor antagonist, ramosetron, is marketed for IBS-D in Japan and there have been no reports of IC.[26]

WHY WOULD A PARTIAL AGONIST OFFER ADVANTAGES OVER A SILENT ANTAGONIST FOR THE TREATMENT OF IBS-D?

Antagonists act by blocking the receptor and preventing an agonist or endogenous transmitter from stimulating the receptor and producing a downstream event. In situations where there is a need for some tonic activity within the system a full antagonist may be disadvantageous since it functions to completely block the receptor's response rather than normalize the response. In contrast, a weak partial agonist behaves as an antagonist in the presence of a higher intrinsic activity agonist while retaining some residual agonist activity and therefore would normalize the action (Figure 1). Thus, a high affinity, low intrinsic activity 5-HT_3 receptor partial agonist would attenuate 5-HT_3 receptor function in the presence of excessive endogenous 5-HT, yet maintain a basal level of receptor activity, thus reducing the risk of constipation and other AEs. Further, by tuning the intrinsic activity of a partial agonist it may be possible to identify compounds that could treat a range of IBS symptoms (Figure 2). Recently, Dynogen took pumosetrag (DDP733/MKC733) into clinical development for IBS-C and nocturnal gastresophageal reflux disease (NGERD).[27] The compound did show some signs of efficacy, but a number of 5-HT_3 receptor agonist-like AEs were noted, particularly nausea, flushing, and pruritus at the highest dose tested, suggesting that the intrinsic activity of this compound may be toward the upper end of the acceptable spectrum.[27]

In the case of ligand gated ion channels such as 5-HT_3 receptors, agonists increase the probability of channels being in the open-state. The duration of channel opening is not just dependent on agonist concentration; distributions of open and closed states depend on individual agonist attributes. Each agonist produces a unique pattern of channel openings; partial agonists tend to evoke fewer openings of shorter duration.[28,29] Another consideration is receptor desensitization; channels can adopt a closed state despite the presence of the agonist.[30] Partial agonists may be less likely to cause desensitization because of fewer total channel openings at any given time. Although rapid desensitization has been reported both in vitro and in vivo; for example, in vivo in the von Bezold-Jarisch bradycardia model, 5-HT administered at very short intervals (every 5 min) produced tachyphylaxis that the authors concluded was due to receptor desensitization.[31] In contrast, in our studies, no reduction in response was observed when a longer 30 min pretreatment was utilized, suggesting the desensitization may be transient (unpublished observation).

Recently, Manning and colleagues[32] reported on a number of 5-HT_3 receptor ligands that exhibit low intrinsic activity both in vitro and in vivo. These compounds showed relatively weak activity in vitro in cells expressing 5-HT_{3A} receptors; interestingly, the agonist-like response could be enhanced by the addition of the allosteric modulator, 5-chloroindole (5-CI), whereas full antagonists failed to show any agonist-like properties in the presence of 5-CI.[32] In the von Bezold-Jarisch bradycardia model in which 5-HT induces a transient fall in heart rate mediated by 5-HT_3 receptors located on vagal afferents, these compounds displayed antagonist-like properties when administered orally 30 min before the full agonist 5-HT. However, when given alone by the intravenous route, some compounds also showed an agonist response. These results are significant because they show these agents can display intrinsic activity in vivo without the need for 5-CI (Figures 3 and 4).[33]

CONCLUSIONS

Weak partial 5-HT_3 receptor agonism appears to be an attractive alternative to a silent antagonist for the treatment of IBS-D; the approach potentially offers the benefits of an antagonist while reducing the AE liability by not fully blocking the receptor. The data presented above demonstrates that it is possible to develop compounds with a range of agonist intrinsic activities that may offer advantages over current 5-HT_3 receptor antagonist treatment for IBS-D. One of the key challenges is identifying the degree of agonism which will prevent the AEs associated with antagonist treatment while maintaining the clinical efficacy. The degree of agonism also needs to be low

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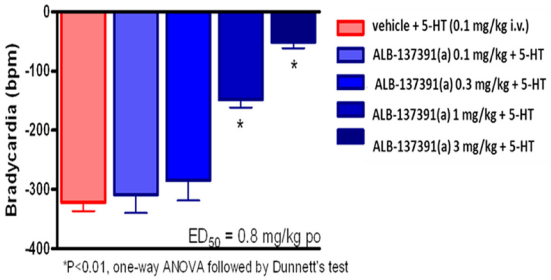

Figure 3. Inhibition of 5-HT induced transient bradycardia by oral administration of ALB-137391(a) in mice (reduction in heart rate SEM). Antagonist properties are demonstrated by oral administration of ALB-137391(a) to mice (n = 8 per group) 1 h prior to a challenge dose of 5-HT administered iv.[33]

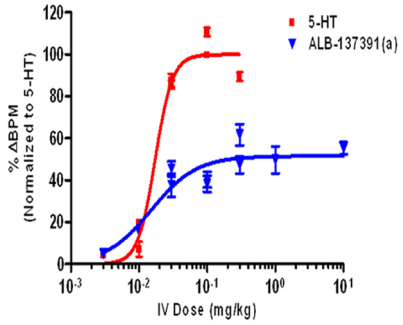

Figure 4. Agonist dose response data in Bezold-Jarisch model for 5-HT and ALB-137391(a). Agonist response following intravenous compound administration in mice. (n = 8 per group, SEM). ALB-137391(a) exhibited an E_{max}= 52% (ED_{50} = 0.015 mg/kg iv) relative to the full agonist 5-HT. (ED_{50} = 0.017 mg/kg iv, E_{max} = 100%.)[33]

enough to avoid agonist-induced AEs such as nausea, flushing, and pruritus. Given the species differences in 5-HT_3 receptor subunit expression and possible differences in the tonic activity of the 5-HT system, it would appear that the hypothesis will only be tested in carefully designed and controlled clinical trials. In early clinical testing it should be relatively easy to establish (a) if the compound produces less constipation than a full antagonist and (b) whether it produces agonist-like AEs. A 5-HT_3 partial agonist that produces less constipation than a full antagonist and no agonist-like side effects while normalizing bowel function in IBS would be a significant advance over existing treatments.

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Notes

The authors declare no competing financial interest.

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