LEADING ARTICLE

Drugs 2001; 61 (3): 317-332
0012-6667/01/0003-0317/$27.50/0

Irritable Bowel Syndrome

New Agents Targeting Serotonin Receptor Subtypes

Fabrizio De Ponti[1] and *Marcello Tonini*[2]

1 Department of Pharmacology, University of Bologna, Bologna, Italy
2 Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy

Abstract

Although the past few years have seen an exponential growth of compounds of potential interest for the treatment of functional gastrointestinal (GI) tract disorders, the gap that still exists between basic and clinical research is easily noticed if one considers the relative paucity of drugs that have received marketing authorisation for the treatment of irritable bowel syndrome (IBS). Traditional efficacy outcomes in drug development for IBS include the ability of the compound to affect GI tract motility (i.e. to exert a prokinetic or an antispasmodic effect), which is thought to be of importance if a motor disorder is the underlying pathophysiological mechanism. More recently, altered visceral sensitivity to a distending stimulus has been suggested to be a key pathophysiological feature, at least in some patients, and has become a target for therapeutic interventions. However, there is now growing consensus that the primary outcome measure in the treatment of functional disorders are those that reflect overall control of the patient's symptoms (pain, diarrhoea, constipation) in everyday situations such as the clinical global improvement scales. Although, in general, guidelines on the design of treatment trials for functional GI tract disorders advise against subcategorisation of patients according to the main symptom (because of symptom instability), subcategorisation indeed makes sense especially in IBS (constipation- or diarrhoea-predominant). Compounds with a specific indication for each subpopulation of patients are now emerging.

The rationale for investigations on serotonin (5-hydroxytryptamine; 5-HT) receptor ligands in IBS rests mainly on the fact that serotonin, which may be released by enterochromaffin-like cells in the GI tract as well as from other sources, has a number of well documented motor effects on the GI tract and can produce hyperalgesia in several experimental models. Serotonin receptors belonging to the 5-HT_3 and 5-HT_4 subtype are the most extensively studied in gastroenterology, although hitherto 'orphan' receptor subtypes, such as the 5-HT_7 and the 5-$HT_{1B/D}$ receptors, are now emerging.

Among 5-HT_3 receptor antagonists, alosetron was recently approved for the treatment of diarrhoea-predominant IBS and is an example of a compound that, at least theoretically, may act at multiple levels: by inhibiting visceral sensitivity, by increasing compliance, and by inhibiting excitatory 5-HT_3 receptors located on both ascending and descending neuronal pathways involved in peristalsis. For this reason, 5-HT_3 receptor antagonists may slow transit, hence the specific indication of alosetron in diarrhoea-predominant IBS. However, alosetron has been recently withdrawn by the manufacturer because of safety concerns.

Hypomotility remains an attractive therapeutic target in IBS and the new gen-

eration of prokinetics includes several partial agonists at the 5-HT_4 receptor, such as tegaserod (HTF-919) and prucalopride (R0-93877). In addition, preliminary evidence suggests that 5-HT_4 receptors may also be involved in the modulation of visceral sensitivity. Second-generation 5-HT_4 receptor agonists seem to be devoid of the QT-prolonging effects observed in some clinical circumstances with cisapride and may be more active at the colonic level. Piboserod (SB-207266A) is a 5-HT_4 receptor antagonist under development for the treatment of diarrhoea-predominant IBS.

Finally, interest in 5-HT_7 and 5-$HT_{1B/D}$ receptor subtypes stems from the observation that the former receptors mediate smooth muscle relaxation (at least in the human colon), whereas sumatriptan (a 5-$HT_{1B/D}$ receptor agonist) can affect GI tract motility and visceral sensitivity.

1. Irritable Bowel Syndrome (IBS): A Functional Disorder

The irritable bowel syndrome (IBS) constitutes a major health problem with gastrointestinal (GI) symptoms that affect a substantial proportion (10 to 20%) of the general population in several parts of the world (North America, Europe, Japan and China).[1,2] The female preponderance among those who seek medical attention is approximately 2 : 1.

The pathophysiology of IBS is multifactorial and only partially understood. Diagnosis is made after exclusion of organic disease, on the basis of symptom clustering with criteria developed via expert consensus conferences (see the recent Rome II criteria[3]). The extent of diagnostic evaluation to rule out organic disease will depend on the characteristics of the patient.[1] Thus, IBS is a functional bowel disorder in which abdominal discomfort or pain (the key symptom that must be present to make the diagnosis) is associated with altered bowel habits (diarrhoea, constipation or alternating diarrhoea and constipation), and with features of disordered defecation.[4] It has a chronic relapsing course and, according to the Rome II criteria, diagnosis is made when the patient's complaints are at least 12 weeks (which need not be consecutive) in the preceding 12 months of abdominal discomfort or pain that has 2 of 3 features: (i) relieved with defecation; and/or (ii) onset associated with a change in frequency of stool; and/or (iii) onset associated with a change in form (appearance) of stool.[4] For entry into clinical trials, patients may be subclassified as having diarrhoea-predominant or constipation-predominant IBS. Symptoms that cumulatively support the diagnosis of IBS are summarised in table I.[4]

1.1. Pathophysiology of IBS

The pathophysiological hypotheses proposed to explain functional GI tract disorders include the following.

- GI tract dysmotility (hypomotility or hypermotility), which may lead to abnormal handling of intraluminal contents and generate symptoms; abnormal gas handling rather than presence of excessive gas may also represent a problem in some patients.[5] The hypothesis that a primary motor disorder underlies most functional GI

Table I. Symptoms supportive of irritable bowel syndrome (IBS): subclassification of patients with predominant diarrhoea or constipation for entry into clinical trials (adapted from Thompson et al.[4])

Supportive symptom of IBS
1. Fewer than 3 bowel movements a week
2. More than 3 bowel movements a day
3. Hard or lumpy stools
4. Loose (mushy) or watery stools
5. Straining during a bowel movement
6. Urgency (having to rush to have a bowel movement)
7. Feeling of incomplete bowel movement
8. Passing mucus (white material) during a bowel movement
9. Abdominal fullness, bloating or swelling
Diarrhoea-predominant IBS
One or more of 2, 4 or 6 and none of 1, 3 or 5
Constipation-predominant IBS
One or more of 1, 3 or 5 and none of 2, 4 or 6

Table II. Current and investigational therapeutic options in symptomatic patients with irritable bowel syndrome (IBS)

Therapeutic approach	Main symptom		
	Constipation	Diarrhoea	Pain/gas/bloating
Traditional approach	Review diet history Increase roughage (may be poorly tolerated by some patients) Mild osmotic laxative	Review diet history Antidiarrhoeal agent (e.g. loperamide)	Review diet history Antispasmodics/smooth muscle relaxants Low-dose tricyclic antidepressants
Key therapeutic target	Hypomotility	Motility/secretion	Visceral sensitivity, visceral tone and compliance
Investigational pharmacological approaches	5-HT_4 receptor agonists Neurotrophic factors (BDNF, NT-3)[13]	5-HT_3 receptor antagonists 5-HT_4 receptor antagonists	5-HT_3 receptor antagonists 5-HT_3/5-HT_4 receptor dual antagonists κ-Opioid receptor agonists Tachykinin receptor antagonists (NK_1, NK_2 and NK_3) M_3-Muscarinic receptor antagonists β_3-Adrenoceptor agonists (?) Somatostatin analogues α_2-Adrenoceptor agonists 5-HT_1 receptor agonists (?) 5-HT_7 receptor agonists (?) NMDA receptor antagonists (?)

BDNF = brain-derived neurotrophic factor; **NMDA** = *N*-methyl-D-aspartate; **NT-3** = neurotrophin-3; **(?)** indicates debate about clinical potential.

tract syndromes directed therapeutic efforts towards investigation of prokinetic[6] and antispasmodic agents.[7]

- Altered visceral sensitivity: in some patients, symptoms (pain, bloating) may originate from altered perception of normal motor events rather than from a primary motor disorder. Variations in sensory afferent activity could explain the occurrence of GI tract symptoms in the absence of other pathophysiological abnormalities, or in combination with other mechanisms in functional bowel disorders.[8] This hypothesis, which is supported by physiological and experimental data, has stimulated the development of novel pharmacological agents targeting visceral nociception.[9-12] This is the area that has received most attention recently, as can be noticed by considering the number of investigational agents listed in table II.
- Altered compliance (capacity of a hollow viscus to adapt its volume to imposed luminal pressures): this is an area of intensive research, now possible by resorting to GI tract distension studies with the barostat technique.[14] In some instances, the ability of a compound to decrease visceral perception may depend at least in part on its ability to increase compliance.[15]
- Minimal inflammation: a limited GI tract inflammatory process, leading to minimal changes at biopsy, may be the primary cause of disease and trigger symptoms. This hypothesis receives some substantiation from the clinical observation that a significant fraction of patients with IBS experienced episodes of infectious enteritis,[16,17] as well as from experimental evidence of increased susceptibility to irritant-induced reinflammation. Mast cell activity and neural modulation of GI tract immune system appear to be involved.[18,19]
- Psychological disturbances: this hypothesis prompted the use of anxiolytics and antidepressants for some functional disorders. Circumstantial evidence suggests that the latter agents act independently of their antidepressant action,[11,20] although controversy still exists on putative analgesic actions of antidepressants for visceral *vs* somatic pain.[21-23]

Since the various mechanisms postulated above are not necessarily mutually exclusive and we do not know their relation to the occurrence of symp-

toms, drug evaluation in patients with functional GI tract disorders may sometimes dismiss potentially useful agents as ineffective because of inappropriate selection among patients with the same complaint, but with a different pathophysiological base for symptom occurrence. In other words, the lack of efficacy of a new drug should not be confused with the inability of a trial to show its efficacy.

1.2 Treatment of IBS

Treatment of IBS should be based on the nature and severity of the symptoms (diarrhoea, constipation or pain), the degree of physiological disturbance and functional impairment.[1] Presently available and investigational therapeutic options in IBS are outlined in table II.

2. Designing Clinical Trials for IBS

Although the past few years have seen an exponential growth of the number of compounds of potential interest for the treatment of functional GI tract disorders,[9] the gap still existing between basic and clinical research is easily noticed if one considers the relative paucity of drugs that have received marketing authorisation for the treatment of IBS. Designing clinical trials of new therapeutic agents for IBS indeed presents a considerable challenge.[24,25]

First, since diagnosis is made on the basis of symptom clustering, a great effort was made in the past decade to reach expert consensus on diagnostic criteria (now, the Rome II criteria[3]). Standardisation of inclusion criteria into clinical trials allows meaningful comparisons among multinational, cross-cultural studies. Still, the variable perception among practitioners of what constitutes a functional disorder creates a dichotomy between results obtained in clinical trials and in practice management. Moreover, the lack of a single coherent hypothesis to explain all symptoms has lead investigators in the field to subcategorise patients, although, in general, current guidelines[26] on the design of treatment trials for functional GI tract disorders advise against doing so because of symptom instability. Subcategorisation makes sense especially in IBS (constipation- or diarrhoea-predominant), although it obviously creates a problem with generalisability of results of a trial (the same patient may experience periods of alternating bowel habits with diarrhoea and constipation).

Secondly, in spite of many promising pharmacological and non-pharmacological treatments, no gold standard exists for comparison of efficacy/effectiveness. Thus, the placebo control group (or adequate control group) is an essential requirement, but the high placebo response (up to 70%) in several functional disorders makes it difficult to show superiority of a new treatment over placebo.

Finally, an aspect that needs careful consideration is definition of the desired outcome.[27] The primary end-point when testing new drugs should be symptom improvement rather than correction of a given functional parameter (for instance, a favourable motility change), unless this parameter clearly correlates with symptoms. Thus, the most important outcomes in the treatment of functional disorders are those that reflect the patient's symptoms. Since they vary among patients and over time, a measure of overall change in symptoms (such as the clinical global improvement scales[27]) should be a primary outcome criterion. Global assessment of multiple symptoms is now feasible by resorting to specifically designed scoring systems.[27] A recent trial in IBS developed an electronic data capture system using 'adequate relief' of IBS pain and discomfort as end-points. Responders for adequate relief were those patients who completed a 12-week study and responded that they had adequate relief for at least 6 of the weeks.[28] This approach best reflects the current shift from physician-oriented to patient-oriented scoring systems allowing global assessment of symptom severity or symptom change by the patient. IBS-specific quality of life questionnaires have also been applied,[27] although these remain secondary end-points, since they have not been sufficiently validated as an outcome measure.

Ideally, a primary clinical end-point in clinical trials of IBS should reflect a global improvement

of all the IBS clinical symptomatology used for inclusion criteria. Relief of constipation or diarrhoea without improvement of abdominal pain is not appropriate. Physiological measurements (e.g. motility studies) are important because they advance our understanding of the disorder and mechanism of drug action, but they should not be used as primary outcome measures.

3. Modulation of Intestinal Functions by Serotonergic Receptors

The rationale for investigations on serotonin (5-hydroxytryptamine; 5-HT) receptor ligands in IBS rests mainly on the fact that serotonin, which may be released by enterochromaffin-like cells in the GI tract as well as from other non-neuronal or neuronal sources, has a number of well documented motor effects on the GI tract and can produce hyperalgesia in several experimental models.[29] In mammalian species, the GI tract is indeed the largest source of serotonin. Serotonin may enhance the sensitivity of visceral neurones projecting between the GI tract and the central nervous system.

Serotonin receptors that are known to affect GI tract motor function are those belonging to the 5-HT_1, 5HT_2, 5-HT_3, 5-HT_4,[30] and[31]5-HT_7[32] subtype (for reviews, see ref.[33,34]). Among all serotonin receptors, those belonging to the 5-HT_3 and 5-HT_4 subtype are the most extensively studied in gastroenterology.

The involvement of different serotonin receptors in the regulation of GI tract motility is illustrated in figure 1. Neuronal serotonin receptors may enhance or inhibit transmitter release and include the 5-HT_{1A} (inhibitory[35,36]), and the 5-HT_3 and 5-HT_4 subtypes (both excitatory). Smooth muscle serotonin receptors may contract or relax the effector cells and belong to the 5-HT_{2A} (mediating contraction), the 5-HT_4 and the 5-HT_7 subtypes (both mediating relaxation). In the human small bowel, 5-HT_{2A} receptors mediating contraction and 5-HT_4-receptors mediating relaxation coexist on smooth muscle cells.[37]

5-$HT_{1B/D}$ receptors are now emerging as possible targets of drug action in the treatment of functional GI tract disorders.[38-42] A study carried out in the human ileum suggested that, in the circular muscle, 5-HT–induced contraction is mediated via a receptor of the 5-HT_{1D} subtype, whereas a receptor of the 5-HT_{2B} subtype mediates the contractile response to serotonin of longitudinal muscle layer.[40] However, selective 5-HT_{1D} receptor antagonists were not available at the time of this study to confirm the hypothesis.

Finally, besides these actions on GI tract motility, serotonin receptors (mainly the 5-HT_3 and 5-HT_4 subtypes) also affect secretory processes at the mucosal level.[42-44]

3.1 5-HT_3 Receptors

Both neurogenic contraction and relaxation can be induced *in vitro* by 5-HT_3 receptor activation in experimental animals[45,46](fig. 1). In rodents, the observation that serotonin- and restraint stress-induced increase in faecal pellet output were antagonised by the 5-HT_3 receptor antagonists ondansetron, granisetron, or YM-114 and by fabesetron (FK-1052; a mixed 5-HT_3/5-HT_4 receptor antagonist) was suggestive for a role for 5-HT_3 receptors in modulating colonic transit.[47,48] Autoradiographic studies indeed detected high densities of [125I](S)-iodozacopride (a 5-HT_3 receptor ligand) in the myenteric plexus of the human colon.[49,50]

From a functional standpoint, *in vitro* studies in the isolated ileum have repeatedly shown that 5-HT_3 receptor antagonists do not affect peristalsis when applied to the serosal side.[34] Conversely, they exert an inhibitory effect when applied intraluminally, suggesting blockade of 5-HT_3 receptors on intrinsic sensory neurones (fig. 1).[51,52] *In vivo*, more complex interactions seem to occur because of the multiple peripheral and central sites of action (table III).[9,34]

In humans, ondansetron has no effect on small bowel transit in healthy volunteers,[53] or in patients with diarrhoea-predominant IBS.[54] However, ondansetron slows colonic transit[55,56] and inhibits the colonic motor response to a meal[57] in healthy individuals. In a double-blind, placebo-controlled study on 50 patients with IBS, ondanset-

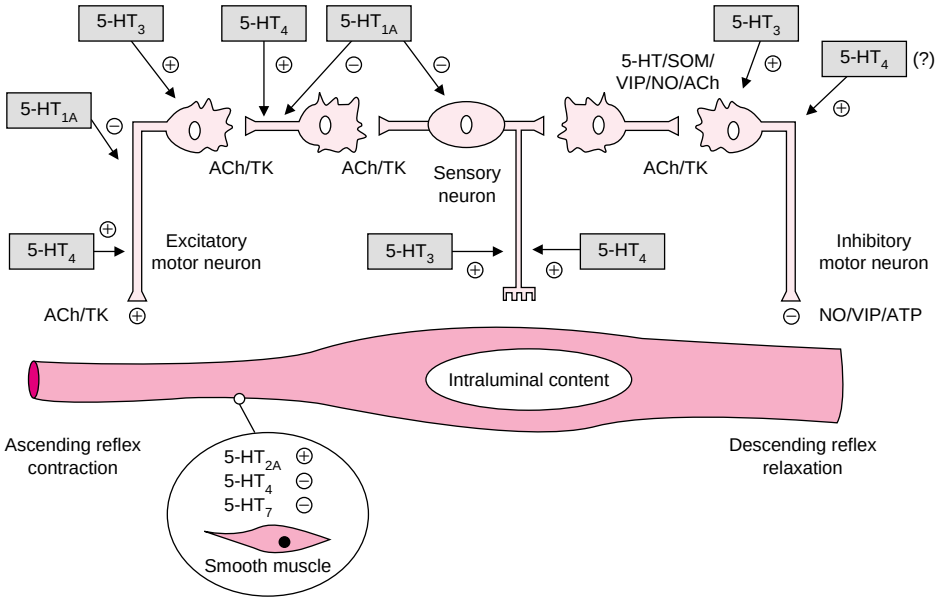


Fig. 1. Modulation of intestinal motility by serotonergic receptors. Distension by intraluminal contents stimulates sensory neurones (intrinsic primary afferent neurones), which trigger an ascending excitatory reflex (leading to contraction) and a descending inhibitory reflex (leading to relaxation). Transmitters released by interneurones in the ascending reflex include acetylcholine (ACh) and substance P, whereas descending interneurones belonging to different subpopulations may use serotonin (5-hydroxytryptamine; 5-HT), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), nitric oxide (NO), ACh and other mediators as transmitters. Excitatory motor neurones release ACh and tachykinins (TK) at the neuromuscular junction, whereas inhibitory motor neurones may release NO, VIP or adenosine triphosphate (ATP) depending on the gastrointestinal tract level and on the animal species. 5-HT_3 and 5-HT_4 receptors have an excitatory effect on enteric neurones, whereas 5-HT_{1A} receptors inhibit transmitter release. Serotonergic receptors may also directly contract or relax smooth muscle cells via 5-HT_{2A}, 5-HT_4 and 5-HT_7 receptors. **+** indicates stimulation; **–** indicates inhibition; **(?)** indicates limited data.

ron reduced bowel frequency and improved stool consistency in the diarrhoea-predominant subgroup (28 patients).[58]

Further evidence in favour of a role for 5-HT_3 receptors in humans was provided by Prior and Read,[59] who found a dose-dependent reduction of postprandial colonic motility with granisetron, and by von der Ohe et al.,[60] who reported a reduction of the postprandial colonic hypertonic response in carcinoid diarrhoea with ondansetron.

Recently, Sanger[61] reviewed the *pros* and *cons* of selective 5-HT_3 receptor antagonists for the treatment of functional bowel disorders. Table III provides a synopsis of putative actions of 5-HT_3 receptor antagonists in the GI tract. Apart from the well known involvement of 5-HT_3 receptors in mediating (centrally and peripherally) emesis of various origin, several animal models point to their role in modulating visceral sensitivity.[9] Granisetron and tropisetron (but not ondansetron) were found to inhibit the fall in blood pressure and intragastric pressure observed in rats after duodenal distension.[62] Whether the site of serotonin receptors modulating afferent information is on peripheral afferent nerve fibres or outside the GI tract, however, is unclear. For instance, alosetron, administered either centrally or peripherally in dogs, seems to modulate the visceral nociceptive effect of rectal distension in dogs.[63] In humans, reduced perception of colonic distension may also depend on an increased compliance of the colon to distension.[15]

An important issue when evaluating different studies with 5-HT_3 receptor antagonists is that, even in the same experimental model, differences

among different compounds[64] are not easily explained on the basis of current pharmacological knowledge. Granisetron was found to reduce rectal sensitivity in patients with IBS,[59] whereas ondansetron had no effect.[65] Interestingly, however, ondansetron reduced nausea and gastric sensitivity to distension during intraduodenal lipid infusion in healthy individuals.[66]

Another important aspect is whether or not these antagonists may affect normal bowel function. Recent studies with alosetron have shown that this compound delays colonic transit in patients with IBS, while having no effect on orocaecal transit time in healthy volunteers.[67-69]

3.2 5-HT_4 Receptors

5-HT_4 receptors mediate a number of responses in the GI tract (table IV).[9] Prokinesia may result from increased release of acetylcholine (and tachykinins) from excitatory neurones (fig. 1) and may operate in human small bowel and stomach,[70,71] whereas this pathway does not seem to operate in the human colonic circular muscle.[72,73]

In addition, it should be noted that, in contrast with what was observed in the one of the most widely used models (the guinea-pig colon, where neuronal 5-HT_4 receptors mediate contractile responses that are mainly cholinergic in nature[74,75]), human colonic circular muscle strips are endowed with 5-HT_4 receptors located on smooth muscle cells, where they mediate relaxation.[49,76,77] A recent report[78] suggests the presence of 5-HT_4 receptors on cholinergic neurons supplying the longitudinal muscle in the human colon. All these findings should be considered in the light of some clinical studies reporting a colonic prokinetic effect of cisapride, whereas, in others, cisapride was found to have no effect on stool frequency or transit time (for a review, see De Ponti and Malagelada[9]). These conflicting results are not surprising, if one considers that the net *in vivo* response to 5-HT_4 receptor stimulation is the result of a number of actions at different levels (table IV), that cisapride is a mixed 5-HT_4 receptor agonist/5-HT_3 receptor antagonist and, finally, that the underlying pathophysiology may strongly influence the clinical effect. The use of selective 5-HT_4 receptor ago-

Table III. Mechanisms by which serotonin 5-HT_3 receptor antagonists may influence gastrointestinal tract function

Function/location of receptors	Proposed mechanism	Expected effect
Motility		
Excitatory enteric neurones	Reduced transmitter release (e.g. acetylcholine, tachykinins)	Reduced ascending contraction (hence inhibition of peristalsis)
		Enhanced compliance?
Inhibitory enteric neurones	Reduced transmitter release	Reduced descending relaxation (hence inhibition of peristalsis)
Visceral sensitivity		
Sensory neurones (intrinsic and extrinsic)	Reduced transmitter release	Intrinsic neurones: peristalsis triggered at higher threshold
		Extrinsic neurones: reduced visceral nociception and inhibition of reflex behaviours (e.g. variations in blood pressure) induced by intestinal distension
Absorption/secretion		
Secretomotor neurones and enterochromaffin cells	Reduced transmitter/mediator release	Increased absorption/reduced secretion; inhibition of carcinoid-induced diarrhoea
Emesis		
Enteric and central nervous system neurones; enterochromaffin cells	Blockade of the effects of chemotherapy-induced release of serotonin	Antiemetic effect

Table IV. Mechanisms by which stimulation of serotonin 5-HT_4-receptors may influence gastrointestinal tract function

Function/location of receptors	Proposed mechanism	Expected effect
Motility		
Excitatory enteric neurones	Enhanced transmitter release (e.g. acetylcholine, tachykinins)	Facilitated contraction orad to an intraluminal bolus (hence prokinetic effect)
Inhibitory enteric neurones	Enhanced transmitter release	Facilitated relaxation anally to an intraluminal bolus (hence prokinetic effect)
Intrinsic primary afferent neurones	Enhanced transmitter release	Enteric reflexes triggered at lower threshold (hence prokinetic effect)
Smooth muscle cells	Relaxation	Increased compliance; indirect effect on visceral sensation
Absorption/secretion		
Enterocytes and secretomotor neurones	Increased intraluminal fluid content	Loose stools; diarrhoea

nists devoid of affinity for 5-HT_3 receptors will help to clarify this issue.

There are animal[79] and human[80] data suggesting that serotonin released by mucosal stimulation initiates a peristaltic reflex by activating 5-HT_4 receptors on sensory neurones containing calcitonin gene-related peptide (CGRP). These effects are mimicked by mucosal application of selective 5-HT_4 receptor agonists (prucalopride and tegaserod).[81] However, experimental evidence for this mechanism in humans is so far limited to the small bowel.

4. Emerging Serotonergic Agents for IBS

New and investigational drugs for the treatment of IBS are outlined in table V.

4.1 5-HT_3 Receptor Antagonists

Among 5-HT_3 receptor antagonists, alosetron was recently approved by the US Food and Drug Administration (FDA) for the treatment of diarrhoea-predominant IBS in female patients. However, only a few months after receiving marketing authorisation, alosetron was voluntarily withdrawn by the manufacturer because of safety concerns. Since the issue need extensive discussion, the reader is referred to the FDA dedicated internet address: http://www.fda.gov/cder/drug/infopage/lotonex/lotronex.htm.

Alosetron is an example of a compound that, at least theoretically, may act at multiple levels[44,82,83]: by modulating visceral sensitivity,[84] by increasing compliance (i.e. increasing the ability of the colon to adapt to distension),[15] by blocking excitatory 5-HT_3 receptors located on sensory, ascending and descending neuronal pathways involved in peristalsis (fig. 1) and by increasing jejunal fluid absorption.[82] For this reason, 5-HT_3 receptor antagonists may slow transit, hence the specific indication of alosetron in diarrhoea-predominant IBS. Alosetron does not affect perception of gastric distension in volunteers,[85] but increases the compliance of the colon to distension and thus contributes to reduce perception of colonic distension and improve IBS symptoms.[15] A recent study,[86] which failed to observe a significant effect of alosetron on

Table V. Synopsis of new and investigational serotonergic drugs for irritable bowel syndrome

Drug class	Examples
5-HT_3 receptor antagonists	Alosetron (GR-68755), azasetron (Y-25130), cilansetron (KC-9946), dolasetron (MDL-73147)
5-HT_4 receptor agonists	Prucalopride (R0-93877), tegaserod (HTF-919)
5-HT_4 receptor antagonists	Piboserod (SB-207266A)
5-HT_7-receptor agonists	No selective compounds available for human use
5-HT_{1A} receptor ligands	Buspirone (?)
5-$HT_{1B/D}$ receptor agonists	Sumatriptan (?)

(?) indicates debate about clinical potential.

Table VI. Pharmacodynamic and pharmacokinetic data for some serotonergic agents

Parameter	Alosetron	Prucalopride	Tegaserod
Pharmacodynamics	5-HT_3 receptor antagonist[44,87]	5-HT_4 receptor partial agonist[88]	5-HT_4 receptor partial agonist[89]
Oral bioavailability (%)	≈60[82]	Not reported	11 (food reduces AUC by ≈50%)[90]
t_{max} (h) [oral]	≈1.5[82]	2.9-3.3[91]	1.2-1.8[92]
$t_{½β}$ (h)	1.5[82]	≈24[91]	8-18[92]
V_d (L/kg)	≈1[82]	Not reported	5.25[93]
CL (L/h)	37[82]	Not reported	77[93]

AUC = area under the plasma concentration time curve; **CL** = total body clearance; $\mathbf{t_{½β}}$ = elimination half-life; $\mathbf{t_{max}}$ = time to reach maximum plasma concentration; $\mathbf{V_d}$ = volume of distribution.

transit parameters, discusses important issues to optimise experimental design of trials designed to find mechanistic explanations for drug action in IBS. Pharmacocokinetic data for alosetron are summarised in table VI.

In a double-blind, placebo-controlled, parallel-group study,[94] a 12-week treatment period was carried out on a total of 462 patients with IBS (335 females) with twice daily doses of alosetron 0.1mg, 0.5mg and 2mg. In the total population and in the female subpopulation (but not in the males), alosetron 2mg twice daily significantly increased the proportion of pain-free days and decreased the visual analogue scale score for diarrhoea. It also led to a significant hardening of stool and a reduction in stool frequency in the total population.

In another study,[95] 623 nonconstipated females with IBS were randomised to receive alosetron 1mg twice daily or mebeverine 135mg 3 times daily for 12 weeks. The primary efficacy end-point was monthly responders for adequate relief of IBS-related abdominal pain and discomfort (defined as patients reporting adequate relief on at least 2 out of 4 weeks). There were significantly more responders in the alosetron group compared with mebeverine at months 2 and 3 ($p < 0.01$).

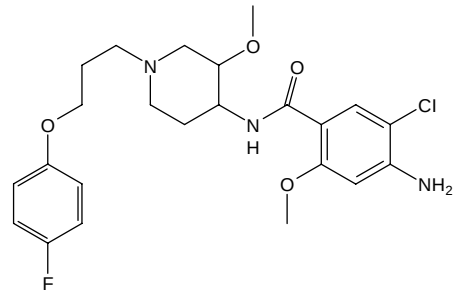
A recently published trial[96] studied 647 female patients with IBS with diarrhoea-predominant or alternating bowel patterns: 324 patients were assigned alosetron 1mg and 323 placebo orally twice daily for 12 weeks. Once again, adequate relief of abdominal pain and discomfort was the primary end-point. The drop-out rate was 24% in the alosetron group and 16% in the placebo group: the difference was mainly due to a greater occurrence of constipation in the alosetron group. Adequate relief for all 3 months of treatment was reported in a greater proportion of alosetron-treated patients (difference 12%). Alosetron also decreased urgency and stool frequency. Constipation occurred in 30 and 3% of patients in the alosetron and placebo groups, respectively.

4.2 5-HT_4 Receptor Agonists

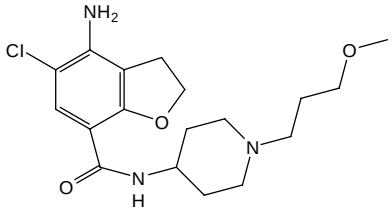
The chemical structures of some partial 5-HT_4 receptor agonists are illustrated in figure 2; available pharmacokinetic/pharmacodynamic data are reported in table VI.

Well known prokinetics such as cisapride are classified among 5-HT_4 receptor agonists since there is evidence that the prokinetic effect,[31] and at least some of the adverse effects (e.g. urinary urge[97,98]), are mediated by this receptor subtype. As regards the cardiac adverse effects of cisapride, it is well known that the drug may induce ventricular arrhythmias and prolongation of the QT interval through its class III antiarrhythmic properties[99,100] and not through activation of 5-HT_4 receptors. These are due to inhibition of a subset of K^+ channels involved in cardiac repolarisation and become clinically relevant especially in patients with mutations in the genes encoding cardiac cation channels (K^+, Na^+[101,102]), or in patients receiving concomitant medication with a variety of agents inhibiting drug metabolism (e.g. erythromycin, clarithromycin, ketoconazole, itraconazole, etc.).[100,103,104]

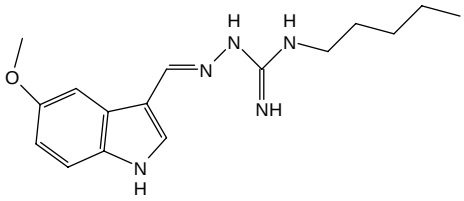
Second generation 5-HT_4 receptor agonists such as tegaserod, mosapride and ML-10302 seem to be



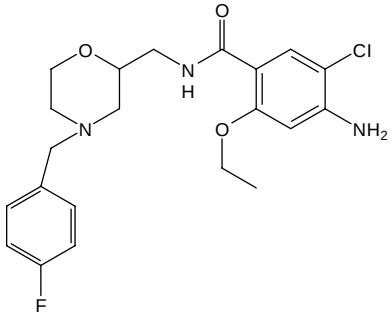
Cisapride



Prucalopride



Tegaserod



Mosapride

Fig. 2. Chemical structures of partial 5-HT_4 receptor agonists.

devoid of class III antiarrhythmic properties[100,105-108] and at least some of them (tegaserod,[109] prucalopride[88,110,111]) may be more active at the colonic level than cisapride. Interestingly, mosapride, whose main metabolite is a 5-HT_3 receptor antagonist,[112] displays little or no prokinetic activity in the colon,[113] similar to what is observed with the mixed 5-HT_4 receptor agonist/5-HT_3 receptor antagonist cisapride (see section 3.2 above).

Among 5-HT_4 receptor agonists, tegaserod, prucalopride and mosapride have undergone clinical trials.

Tegaserod has been recently reviewed in *Drugs*[89] and is currently undergoing evaluation by regulatory agencies. Since publication of the above review, a further study has appeared on the effects of tegaserod on gastric, small bowel and colonic transit in 24 patients with constipation-predominant IBS, who were randomised to 1 week of tegaserod (2mg twice daily) or placebo.[114] Interestingly, tegaserod accelerated orocaecal transit leaving gastric emptying unaltered and also tended to accelerate colonic transit. No serious adverse events were reported.

Oral tegaserod 4 and 12mg daily was also evaluated using the Subjects Global Assessment of Relief as the main efficacy variable in patients with constipation-predominant IBS: it was found to relieve key symptoms, with a sustained effect over at least 3 months.[115,116]

A recent study carried out in awake rats also suggests an effect of tegaserod on colorectal sensitivity not linked to alterations in compliance at the doses of 0.1 and 0.3 mg/kg intraperitoneally: tegaserod was found to increase pain threshold to colorectal but not to gastric distension.[117]

Prucalopride is being investigated for a range of conditions including constipation-predominant IBS and slow transit constipation. In a double-blind, crossover study in 24 healthy volunteers,[118] prucalopride 1 and 2mg for 1 week significantly increased the number of stools and the percentage of loose/watery stools compared with placebo. These parameters returned to baseline within 1 week after stopping prucalopride. Prucalopride also

significantly shortened mean colonic transit time and total GI tract transit time.

Administration of prucalopride 1 and 2mg for 1 week in healthy volunteers significantly increased number of stools and the percentage of loose/watery stools.[119] Prucalopride accelerated orocaecal and whole GI tract transit, while having no effect on GI tract sensitivity to distension and electrical stimulation.

In a randomised, double-blind study in 50 healthy volunteers, prucalopride 0.5 to 4mg daily for 7 days significantly accelerated colonic transit at 4, 8, 24 and 48 hours and proximal colonic emptying, while having no significant effects on gastric emptying or small bowel transit.[88]

In a multicentre, randomised, double-blind study in 251 patients with chronic constipation, prucalopride 0.5 to 2mg twice daily for 12 weeks significantly increased stool frequency and consistency throughout the study period, with a dose-dependent increase in the number of responding patients.[120]

Diarrhoea is the most common adverse effect reported with prucalopride in healthy volunteers and in patients with constipation.

Mosapride has been recently approved in Japan,[121] but it is targeted for the treatment of upper GI tract disorders, such as gastro-oesophageal reflux disease.[122]

4.3 5-HT_4 Receptor Antagonists

Because of the different locations of 5-HT_4 receptors in the GI tract (table III), it is difficult to predict the net effect of a selective antagonist *in vivo*. 5-HT_4 receptor antagonists do not seem to affect normal bowel motility in animals[123] or humans,[124] although they may antagonise both the ability of serotonin to sensitise the peristaltic reflex and 5-hydroxytryptophan-induced defecation/diarrhoea, at least in animals.[61,123,125]

Clinical data on the possible role of selective 5-HT_4 receptor antagonists in the treatment of functional gastrointestinal disorders are now becoming available. Piboserod (SB-207266A) is one of the best characterised 5-HT_4 receptor antagonists so far. It displays subnanomolar affinity (pK_B value: 9.98) in the human intestine[123] and, at single oral doses of 0.5 to 5mg in healthy male volunteers, significantly and dose-dependently antagonised the effects of cisapride in a pharmacodynamic model of 5-HT_4 receptor activation (increase in plasma aldosterone levels).[126] Dynamic modelling in this study predicted that a dose of ≈1mg piboserod would block 90% of the cisapride-induced aldosterone response.

Piboserod prolongs orocaecal transit time in patients with diarrhoea-predominant IBS,[127] hence the proposed indication in this subset of patients. The ability of piboserod to affect visceral sensitivity is still under investigation. At variance with 5-HT_3 receptors, only limited data are available to support a role for 5-HT_4 receptors in controlling visceral sensitivity.[10] Although oral piboserod 20mg daily for 10 days tended to increase the distension volume required to induce the sensation of discomfort in diarrhoea-predominant IBS patients, this effect did not reach statistical significance.[127] Interestingly, in a rat model of intestinal hyperalgesia,[128] piboserod *per se* had no effect, but potentiated the effects of submaximal doses of granisetron, suggesting that 5-HT_4 receptors may cooperate with 5-HT_3 receptors in inhibiting intestinal hyperalgesia. This observation poses a rationale for the development of dual antagonists (5-HT_3/5-HT_4 receptor antagonists)[48]

4.4 Other Serotonin Receptor Ligands

The observation that the antimigraine agent sumatriptan, currently classified as a 5-$HT_{1B/D}$ receptor agonist, delays gastric emptying[39,129] prompted a number of studies which showed that sumatriptan inhibits postprandial fundic tone and antral motility (hence, the delay in gastric emptying), inhibits meal-induced satiety in healthy humans, and reduces perception of gastric distension in patients with functional dyspepsia.[41,130] In addition, sumatriptan relaxes the descending colon (without changing colonic compliance), thus allowing larger volumes to be accommodated before threshold for perception is reached.[131] Interestingly, buspirone (a 5-HT_{1A} receptor agonist) is also

reported to decrease fundic tone, reducing perception of gastric distension in humans,[132] but it is still premature to draw conclusions on receptors and pathways involved in these responses. At the present state of knowledge, involvement of 5-$HT_{1B/D}$ receptors is assumed on the basis of the action of sumatriptan, but no firm conclusions can be drawn until selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists (now available for preclinical studies) are specifically tested. In any case, gastric relaxation by sumatriptan is reversed *in vivo* by GR-127935 (a 5-$HT_{1B/D}$ receptor antagonist), at least in the dog.[133]

The same caveats hold true for the possible involvement of 5-HT_{1A} receptors. Firstly, it is not clear whether central or peripheral neuronal receptors are involved.[134] In addition, after the discovery of the 5-HT_7 receptor (which mediates relaxation at least in human colonic smooth muscle),[32] some investigators suggest caution in ascribing a response to 5-HT_{1A} receptors, since 8-OH-DPAT, a compound previously considered a selective 5-HT_{1A} receptor agonist, is also a partial agonist at the 5-HT_7 receptor.[135] However, to the best of our knowledge, no selective 5-HT_7 receptor ligands are available for clinical use.

5. Conclusions

In the present review, we have focused on investigational agents that target serotonin receptors for the treatment of IBS. On the basis of currently available information, 5-HT_3 receptor antagonists may indeed be a first-line approach for patients with diarrhoea-predominant IBS, who may also benefit from the reduction of visceral sensitivity. Future studies will determine whether other functional GI tract disorders or symptom complexes are responsive to these agents. Because accelerated delivery of colonic contents into the rectum with reduced compliance is not specific for IBS (it may occur in inflammatory conditions or radiation-induced colonic damage), 5-HT_3 receptor antagonists may turn out to be useful even in some organic conditions with altered bowel habits and lower abdominal pain.

Selective 5-HT_4 receptor agonists and antagonists are likely to become new classes of drugs with colonic prokinetic or antiprokinetic effect. However, their role in IBS still needs to be fully characterised, especially as regards the control of visceral sensitivity.

Finally, other possible candidate mediators of motor derangement/altered visceral sensitivity are being actively investigated (see table II). In particular, clinical trials are now under way with tachykinin receptor antagonists (in particular, NK_2 receptor antagonists), which may affect concomitantly colonic motor activity and visceral sensitivity.[136-138]

References

1. Drossman DA, Whitehead WE, Camilleri M. Irritable bowel syndrome: a technical review for practice guideline development. Gastroenterology 1997; 112: 2120-37
2. Anonymous. American Gastroenterological Association medical position statement: irritable bowel syndrome. Gastroenterology 1997; 112: 2118-9
3. Drossman DA. The functional gastrointestinal disorders and the Rome II process. Gut 1999; 45 Suppl. 2: II1-II5
4. Thompson WG, Longstreth GF, Drossman DA, et al. Functional bowel disorders and functional abdominal pain. Gut 1999; 45 Suppl. 2: II43-II47
5. Serra J, Azpiroz F, Malagelada JR. Intestinal gas dynamics and tolerance in humans. Gastroenterology 1998; 115: 542-50
6. Tonini M. Recent advances in the pharmacology of gastrointestinal prokinetics. Pharmacol Res 1996; 33: 217-26
7. Klein KB. Controlled treatment trials in the irritable bowel syndrome: a critique. Gastroenterology 1988; 95: 232-41
8. Mayer EA, Gebhart GF. Basic and clinical aspects of visceral hyperalgesia. Gastroenterology 1994; 107: 271-93
9. De Ponti F, Malagelada JR. Functional gut disorders: from motility to sensitivity disorders: a review of current and investigational drugs for their management. Pharmacol Ther 1998; 80: 49-88
10. Sanger GJ. Hypersensitivity and hyperreactivity in the irritable bowel syndrome: an opportunity for drug discovery. Dig Dis 1999; 17: 90-9
11. Pandolfino N, Howden C, Kahrilas P. Motility-modifying agents and management of disorders of gastrointetsinal motility. Gastroenterology 2000; 118: S32-S47
12. Bueno L, Fioramonti J, Garcia-Villar R. Pathobiology of visceral pain: molecular mechanisms and therapeutic implications. III. Visceral afferent pathways: a source of new therapeutic targets for abdominal pain. Am J Physiol 2000; 278: G670-G676
13. Coulie B, Szarka LA, Camilleri M, et al. Recombinant human neurotrophic factors accelerate colonic transit and relieve constipation in humans. Gastroenterology 2000; 119: 41-50
14. Whitehead WE, Delvaux M, and the Working Team. Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. Dig Dis Sci 1997; 42: 223-41

15. Delvaux M, Louvel D, Mamet JP, et al. Effect of alosetron on responses to colonic distension in patients with irritable bowel syndrome. Aliment Pharmacol Ther 1998; 12: 849-55
16. Gwee KA, Leong YL, Graham C, et al. The role of psychological and biological factors in postinfective gut dysfunction. Gut 1999; 44: 400-6
17. Gwee KA, Graham JC, McKendrick MW, et al. Psychometric scores and persistence of irritable bowel after infectious diarrhoea. Lancet 1996; 347: 150-3
18. Bueno L, Fioramonti J, Delvaux M, et al. Mediators and pharmacology of visceral sensitivity: from basic to clinical investigations. Gastroenterology 1997; 112: 1714-43
19. Collins SM. The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. Gastroenterology 1996; 111: 1683-99
20. Farthing MJ. Irritable bowel syndrome: new pharmaceutical approaches to treatment. Baillieres Best Pract Res Clin Gastroenterol 1999; 13: 461-71
21. Mertz H, Fass R, Kodner A, et al. Effect of amitriptyline on symptoms, sleep, and visceral perception in patients with functional dyspepsia. Am J Gastroenterol 1998; 93: 160-5
22. Peghini PL, Katz PO, Castell DO. Imipramine decreases oesophageal pain perception in human male volunteers. Gut 1998; 42: 807-13
23. Gorelick AB, Koshy SS, Hooper FG, et al. Differential effects of amitriptyline on perception of somatic and visceral stimulation in healthy humans. Am J Physiol 1998; 275: G460-G6
24. Malagelada JR. Review article: clinical pharmacology models of irritable bowel syndrome. Aliment Pharmacol Ther 1999; 13 Suppl. 2: 57-64
25. Camilleri M. Ten secrets for development of drugs for functional gastrointestinal diseases. Gastroenterology 2000; 118: 653
26. Veldhuyzen van Zanten SJ, Talley NJ, Bytzer P, et al. Design of treatment trials for functional gastrointestinal disorders. Gut 1999; 45 Suppl. 2: II69-II77
27. Klein KB. Assessment of treatment outcome in the functional gastrointestinal disorders. In: Corazziari E, editor. Approach to the patient with chronic gastrointestinal disorders. Milan: Messaggi, 1999: 545-56
28. Mangel AW, Northcutt AR. Review article: the safety and efficacy of alosetron, a 5-HT3 receptor antagonist, in female irritable bowel syndrome patients. Aliment Pharmacol Ther 1999; 13 Suppl. 2: 77-82: 77-82
29. Gershon MD. Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel. Aliment Pharmacol Ther 1999; 13 Suppl. 2: 15-30
30. Read NW, Gwee KA. The importance of 5-hydroxytryptamine receptors in the gut. Pharmacol Ther 1994; 62: 159-73
31. Briejer MR, Akkermans LM, Schuurkes JA. Gastrointestinal prokinetic benzamides: the pharmacology underlying stimulation of motility. Pharmacol Rev 1995; 47: 631-51
32. Prins NH, Briejer MR, Van Bergen PJ, et al. Evidence for 5-HT7 receptors mediating relaxation of human colonic circular smooth muscle. Br J Pharmacol 1999; 128: 849-52
33. Galligan JJ. Electrophysiological studies of 5-hydroxytryptamine receptors on enteric neurons. In: Gaginella TS, Galligan JJ, editors. Serotonin and gastrointestinal function. Boca Raton (FL): CRC Press, 1995: 109-26
34. Tonini M, De Ponti F. Serotonin modulation of gastrointestinal motility. In: Gaginella TS, Galligan JJ, editors. Serotonin and gastrointestinal function. Boca Raton (FL): CRC Press, 1995: 53-84
35. Galligan JJ, Surprenant A, Tonini M, et al. Differential localization of 5-HT1 receptors on myenteric and submucosal neurons. Am J Physiol 1988; 255: G603-G611
36. Dietrich C, Kilbinger H. 5-HT1A receptor-mediated inhibition of acetylcholine release from guinea pig myenteric plexus: potential mechanisms. Neuropharmacology 1996; 35: 483-8
37. Kuemmerle JF, Murthy KS, Grider JR, et al. Coexpression of 5-HT2A and 5-HT4 receptors coupled to distinct signaling pathways in human intestinal muscle cells. Gastroenterology 1995; 109: 1791-800
38. Coulie B, Tack J, Sifrim D, et al. Role of nitric oxide in fasting gastric fundus tone and in 5-HT1 receptor-mediated relaxation of gastric fundus. Am J Physiol 1999; 276: G373-G7
39. Coulie B, Tack J, Maes B, et al. Sumatriptan, a selective 5-HT1 receptor agonist, induces a lag phase for gastric emptying of liquids in humans. Am J Physiol 1997; 272: G902-G8
40. Borman RA, Burleigh DE. 5-HT1D and 5-HT2B receptors mediate contraction of smooth muscle in human small intestine. Ann NY Acad Sci 1997; 812: 222-3
41. Tack J, Coulie B, Wilmer A, et al. Influence of sumatriptan on gastric fundus tone and on the perception of gastric distension in man. Gut 2000; 46: 468-73
42. Borman RA, Burleigh DE. Evidence for the involvement of a 5-HT4 receptor in the secretory response of human small intestine to 5-HT. Br J Pharmacol 1993; 110: 927-8
43. Stoner MC, Arcuni JC, Lee J, et al. A selective 5-HT_4 receptor agonist induces cAMP-mediated Cl^- efflux from rat colonocytes [abstract]. Gastroenterology 1999; 116: A648
44. Humphrey PP, Bountra C, Clayton N, et al. Review article: the therapeutic potential of 5-HT3 receptor antagonists in the treatment of irritable bowel syndrome. Aliment Pharmacol Ther 1999; 13 Suppl. 2: 31-8
45. Miyata K, Kamato T, Nishida A, et al. Pharmacologic profile of (R)-5-[(1-methyl-3-indolyl) carbonyl]-4,5,6,7-tetrahydro-1H- benzimidazole hydrochloride (YM060), a potent and selective 5-hydroxytryptamine3 receptor antagonist, and its enantiomer in the isolated tissue. J Pharmacol Exp Ther 1991; 259: 15-21
46. Messori E, Candura SM, Coccini T, et al. 5-HT3 receptor involvement in descending reflex relaxation in the rabbit isolated distal colon. Eur J Pharmacol 1995; 286: 205-8
47. Miyata K, Kamato T, Nishida A, et al. Role of serotonin$_3$ receptor in stress-induced defecation. J Pharmacol Exp Ther 1992; 261: 297-303
48. Kadowaki M, Nagakura Y, Tomoi M, et al. Effect of FK1052, a potent 5-hydroxytryptamine$_3$ and 5-hydroxytryptamine$_4$ receptor dual antagonist, on colonic function *in vivo*. J Pharmacol Exp Ther 1993; 266: 74-80
49. Sakurai-Yamashita Y, Yamashita K, Yoshimura M, et al. Differential localization of 5-hydroxytryptamine3 and 5-hydroxytryptamine4 receptors in the human rectum. Life Sci 2000; 66: 31-4
50. Sakurai-Yamashita Y, Yamashita K, Kaibara M, et al. Differential distribution of 5-hydroxytryptamine3 receptor in the colon between human and guinea pig. Chin J Physiol 1999; 42: 195-8
51. Tuladhar BR, Kaisar M, Naylor RJ. Evidence for a 5-HT_3 receptor involvement in the facilitation of peristalsis on mucosal application of 5-HT in the guinea pig isolated ileum. Br J Pharmacol 1997; 122: 1174-8
52. Jin JG, Foxx-Orenstein AE, Grider JR. Propulsion in guinea pig colon induced by 5-hydroxytryptamine (5HT) via 5-HT4 and 5-HT3 receptors. J Pharmacol Exp Ther 1999; 288: 93-7

53. Talley NJ, Phillips SF, Miller LJ, et al. Effect of selective $5HT_3$ antagonist (GR 38032F) on small intestinal transit and release of gastrointestinal peptides. Dig Dis Sci 1989; 34: 1511-5
54. Steadman CJ, Talley NJ, Phillips SF, et al. Selective 5-hydroxytryptamine type 3 receptor antagonism with ondansetron as treatment for diarrhea-predominant irritable bowel syndrome: a pilot study. Mayo Clin Proc 1992; 67: 732-8
55. Talley NJ, Phillips SF, Haddad A, et al. GR 38032F (ondansetron), a selective $5\text{-}HT_3$ receptor antagonist, slows colonic transit in healthy man. Dig Dis Sci 1990; 35: 477-80
56. Gore S, Gilmore IT, Haigh CG, et al. Colonic transit in man is slowed by ondansetron (GR38032F), a selective 5-hydroxytryptamine receptor (type 3) antagonist. Aliment Pharmacol Ther 1990; 4: 139-44
57. von der Ohe M, Hanson RB, Camilleri M. Serotonergic mediation of postprandial colonic tonic and phasic responses in humans. Gut 1994; 35: 536-41
58. Maxton DG, Morris J, Whorwelll PJ. Selective 5-hydroxytryptamine antagonism: a role in irritable bowel syndrome and functional dyspepsia? Aliment Pharmacol Ther 1996; 10: 595-9
59. Prior A, Read NW. Reduction of rectal sensitivity and post-prandial motility by granisetron, a 5 HT_3-receptor antagonist, in patients with irritable bowel syndrome. Aliment Pharmacol Ther 1993; 7: 175-80
60. von der Ohe MR, Camilleri M, Kvols LK. A $5\text{-}HT_3$ antagonist corrects the postprandial colonic hypertonic response in carcinoid diarrhea. Gastroenterology 1994; 106: 1184-9
61. Sanger GJ. 5-Hydroxytryptamine and functional bowel disorders. Neurogastroenterol Motil 1996; 8: 319-31
62. Moss HE, Sanger GJ. The effects of granisetron, ICS 205-930 and ondansetron on the visceral pain reflex induced by duodenal distension. Br J Pharmacol 1990; 100: 497-501
63. Miura M, Lawson DC, Clary EM, et al. Central modulation of rectal distension-induced blood pressure changes by alosetron, a 5-HT3 receptor antagonist. Dig Dis Sci 1999; 44: 20-4
64. Banner SE, Sanger GJ. Differences between $5\text{-}HT_3$ receptor antagonists in modulation of visceral hypersensitivity. Br J Pharmacol 1995; 114: 558-62
65. Goldberg PA, Kamm MA, Setti-Carraro P, et al. Modification of visceral sensitivity and pain in irritable bowel syndrome by 5-HT3 antagonism (ondansetron). Digestion 1996; 57: 478-83
66. Feinle C, Read NW. Ondansetron reduces nausea induced by gastroduodenal stimulation without changing gastric motility. Am J Physiol 1996; 261: G591-G597
67. Foster JM, Houghton LA, Whorwell PJ. Alosetron slows colonic transit in patients with irritable bowel syndrome (IBS) [abstract]. Gastroenterology 1997; 112: A732
68. Gunput MD, Sohail S, Frith L, et al. Alosetron, a 5-HT3 receptor antagonist, has no effect on oro-cecal transit time in man [abstract]. Dig Dis Sci 1996; 41: 1896
69. Houghton LA, Foster JM, Whorwell PJ. Alosetron, a 5-HT3 receptor antagonist, delays colonic transit in patients with irritable bowel syndrome and healthy volunteers. Aliment Pharmacol Ther 2000; 14: 775-82
70. Schuurkes JAJ, Meulemans AL, Obertop H, et al. $5\text{-}HT_4$ receptors on the human stomach [abstract]. J Gastrointest Motil 1991; 3: 199
71. Sakurai-Yamashita Y, Takada K, Takemura K, et al. Ability of mosapride to bind to 5-HT4 receptor in the human stomach. Jpn J Pharmacol 1999; 79: 493-6
72. Burleigh DE, Trout SJ. Evidence against an acetylcholine releasing action of cisapride in the human colon. Br J Clin Pharmacol 1985; 20: 475-8
73. Burke TA, Sanger GJ. Regionally selective cholinergic stimulation by BRL 24924 in the human isolated gut. Br J Clin Pharmacol 1988; 26: 261-5
74. Briejer MR, Akkermans LM, Meulemans AL, et al. Cisapride and a structural analogue, R 76,186, are 5-hydroxytryptamine4 (5-HT4) receptor agonists on the guinea-pig colon ascendens. Naunyn Schmiedebergs Arch Pharmacol 1993; 347: 464-70
75. Wardle KA, Sanger GJ. The guinea-pig distal colon--a sensitive preparation for the investigation of 5-HT4 receptor-mediated contractions. Br J Pharmacol 1993; 110: 1593-9
76. Tam FS, Hillier K, Bunce KT, et al. Differences in response to 5-HT4 receptor agonists and antagonists of the 5-HT4-like receptor in human colon circular smooth muscle. Br J Pharmacol 1995; 115: 172-6
77. McLean PG, Coupar IM. Stimulation of cyclic AMP formation in the circular smooth muscle of human colon by activation of 5-HT4-like receptors. Br J Pharmacol 1996; 117: 238-9
78. Prins NH, Akkermans LMA, Lefebvre RA, et al. $5\text{-}HT_4$ receptors on cholinergic nerves involved in contractility of canine and human large intestine longitudinal muscle. Br J Pharmacol 2000; 131: 927-32
79. Grider JR, Kuemmerle JF, Jin JG. 5-HT released by mucosal stimuli initiate peristalsis by activating $5\text{-}HT_4$/$5\text{-}HT_{1p}$ receptors on sensory CGRP neurons. Am J Physiol 1996; 270: 778-82
80. Foxx-Orenstein AE, Kuemmerle JF, Grider JR. Distinct 5-HT receptors mediate the peristaltic reflex induced by mucosal stimuli in human and guinea-pig intestine. Gastroenterology 1996; 111: 1281-90
81. Grider JR, Foxx-Orenstein AE, Jin JG. 5-Hydroxytryptamine4 receptor agonists initiate the peristaltic reflex in human, rat, and guinea pig intestine. Gastroenterology 1998; 115: 370-80
82. Gunput MD. Review article: clinical pharmacology of alosetron. Aliment Pharmacol Ther 1999; 13 Suppl. 2: 70-6
83. Balfour JA, Goa KL, Perry CM. Alosetron [see guest commentaries]. Drugs 2000; 59: 511-8
84. Kozlowski CM, Green A, Grundy D, et al. The $5\text{-}HT_3$ receptor antagonist alosetron inhibits the colorectal distention induced depressor response and spinal c-fos expression in the anaesthetised rat. Gut 2000; 46: 474-80
85. Zerbib F, Bruley d, V, Oriola RC, et al. Alosetron does not affect the visceral perception of gastric distension in healthy subjects. Aliment Pharmacol Ther 1994; 8: 403-7
86. Thumshirn M, Coulie B, Camilleri M, et al. Effects of alosetron on gastrointestinal transit time and rectal sensation in patients with irritable bowel syndrome. Aliment Pharmacol Ther 2000; 14: 869-78
87. Clayton NM, Sargent R, Butler A, et al. The pharmacological properties of the novel selective $5\text{-}HT_3$ receptor antagonist, alosetron, and its effects on normal and perturbed small intestinal transit in the fasted rat. Neurogastroenterol Motil 1999; 11: 207-17
88. Bouras EP, Camilleri M, Burton DD, et al. Selective stimulation of colonic transit by the benzofuran $5HT_4$ agonist, prucalopride, in healthy humans. Gut 1999; 44: 682-6
89. Scott LJ, Perry CM. Tegaserod. Drugs 1999; 58: 491-6
90. Zhou H, Khalilieh S, Lau H, et al. Effect of meal timing not critical for the pharmacokinetics of tegaserod (HTF 919). J Clin Pharmacol 1999; 39: 911-9
91. Mannaert E, Van Bortel I, Woestenborghs R, et al. Single-dose and once-daily pharmacokinetics and effects of food on prucalopride in humans [abstract]. Gut 1999; 45 Suppl. 5: A137
92. Appel S, Kumle A, Hubert M, et al. First pharmacokinetic-pharmacodynamic study in humans with a selective 5-

hydroxytryptamine4 receptor agonist. J Clin Pharmacol 1997; 37: 229-37
93. Appel-Dingemanse S, Lemarechal MO, Kumle A, et al. Integrated modelling of the clinical pharmacokinetics of SDZ HTF 919, a novel selective 5-HT4 receptor agonist, following oral and intravenous administration. Br J Clin Pharmacol 1999; 47: 483-91
94. Bardhan KD, Bodemar G, Geldof H, et al. A double-blind, randomized, placebo-controlled dose-ranging study to evaluate the efficacy of alosetron in the treatment of irritable bowel syndrome. Aliment Pharmacol Ther 2000; 14: 23-34
95. Jones RH, Holtmann G, Rodrigo L, et al. Alosetron relieves pain and improves bowel function compared with mebeverine in female nonconstipated irritable bowel syndrome patients. Aliment Pharmacol Ther 1999; 13: 1419-27
96. Camilleri M, Northcutt AR, Kong S, et al. Efficacy and safety of alosetron in women with irritable bowel syndrome: a randomised, placebo-controlled trial. Lancet 2000; 355: 1035-40
97. Pillans PI, Wood SM. Cisapride increases micturition frequency. J Clin Gastroenterol 1994; 19: 336-8
98. Tonini M, Messori E, Franceschetti GP, et al. Characterization of the 5-HT receptor potentiating neuromuscular cholinergic transmission in strips of human isolated detrusor muscle. Br J Pharmacol 1994; 113: 1-2
99. Puisieux FL, Adamantidis MM, Dumotier BM, et al. Cisapride-induced prolongation of cardiac action potential and early afterdepolarizations in rabbit Purkinje fibres. Br J Pharmacol 1996; 117: 1377-9
100. Tonini M, De Ponti F, Di Nucci A, et al. Review article: cardiac adverse effects of gastrointestinal prokinetics. Aliment Pharmacol Ther 1999; 13: 1585-91
101. Chiang CE, Roden DM. The long QT syndromes: genetic basis and clinical implications. J Am Coll Cardiol 2000; 36: 1-12
102. Napolitano C, Schwartz PJ, Brown AM, et al. Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. J Cardiovasc Electrophysiol 2000; 11: 691-6
103. De Ponti F, Poluzzi E, Montanaro N. QT interval prolongation by non-cardiac drugs: lessons to be learned by recent experience. Eur J Clin Pharmacol 2000; 56: 1-18
104. Michalets EL, Williams CR. Drug interactions with cisapride: clinical implications. Clin Pharmacokinet 2000; 39: 49-75
105. Carlsson L, Amos GJ, Andersson B, et al. Electrophysiological characterization of the prokinetic agents cisapride and mosapride *in vivo* and *in vitro*: implications for proarrhythmic potential? J Pharmacol Exp Ther 1997; 282: 220-7
106. Crema F, Modini C, Croci T, et al. Intestinal prokinesia by two esters of 4-amino-5-chloro-2- methoxybenzoic acid: involvement of 5-hydroxytryptamine-4 receptors and dissociation from cardiac effects *in vivo*. J Pharmacol Exp Ther 1999; 288: 1045-52
107. Drici MD, Ebert SN, Wang WX, et al. Comparison of tegaserod (HTF 919) and its main human metabolite with cisapride and erythromycin on cardiac repolarization in the isolated rabbit heart. J Cardiovasc Pharmacol 1999; 34: 82-8
108. Whorwell PJ, Krumholz S, Muller-Lissner S, et al. Tegaserod has a favorable safety and tolerability profile in patients with constipation predominant and alternating forms of irritable bowel syndrome (IBS) [abstract]. Gastroenterology 2000; 118 Suppl. 2: A1204
109. Nguyen A, Camilleri M, Kost LJ, et al. SDZ HTF 919 stimulates canine colonic motility and transit *in vivo*. J Pharmacol Exp Ther 1997; 280: 1270-6
110. Emmanueal AV, Nicholls T, Roy AJ, et al. Prucalopride (PRU) improves colonic transit and stool frequency in patients (pts) with slow and normal transit constipation [abstract]. Gastroenterology 2000; 118 Suppl. 2: A846
111. Camilleri M, McKinzie S, Burton D, et al. Prucalopride accelerates small bowel and colonic transit in patients with chronic functional constipation (FC) or constipation-predominant irritable bowel syndrome (C-IBS) [abstract]. Gastroenterology 2000; 118 Suppl. 2: A845
112. Yoshida N, Omoya H, Kato S, et al. Pharmacological effects of the new gastroprokinetic agent mosapride citrate and its metabolites in experimental animals. Arzneimittelforschung 1993; 43: 1078-3
113. Mine Y, Yoshikawa T, Oku S, et al. Comparison of effect of mosapride citrate and existing 5-HT4 receptor agonists on gastrointestinal motility *in vivo* and *in vitro*. J Pharmacol Exp Ther 1997; 283: 1000-8
114. Prather CM, Camilleri M, Zinsmeister AR, et al. Tegaserod accelerates orocecal transit in patients with constipation-predominant irritable bowel syndrome. Gastroenterology 2000; 118: 463-8
115. Lefkowitz MP, Rueegg PC, Shi Y, et al. Validation of a global relief measure in two clinical trials of irritable bowel syndrome with tegaserod [abstract]. Gastroenterology 2000; 118 Suppl. 2: A145
116. Mueller-Lissner S, Fumagalli I, Bardhan KD, et al. Tegaserod, a 5-HT_4 receptor partial agonist, relieves key symptoms of irritable bowel syndrome (IBS) [abstract]. Gastroenterology 2000; 118 Suppl. 2: A175
117. Coelho AM, Rovira P, Fioramonti J, et al. Antinociceptive properties of HTF 919 (tegaserod), a 5-HT_4 receptor partial agonist, on colorectal distension in rats [abstract]. Gastroenterology 2000; 118 Suppl. 2: A835
118. Poen AC, Felt-Bersma RJ, Van Dongen PA, et al. Effect of prucalopride, a new enterokinetic agent, on gastrointestinal transit and anorectal function in healthy volunteers. Aliment Pharmacol Ther 1999; 13: 1493-7
119. Emmanuel AV, Kamm MA, Roy AJ, et al. Effect of a novel prokinetic drug, R093877, on gastrointestinal transit in healthy volunteers. Gut 1998; 42: 511-6
120. Otten M, Schneider H, Wurzer H, et al. A double-blind, placebo controlled evaluation of safety and efficacy of 12-week, twice-daily treatment with prucaloprid in patients with chronic constipation. Gastroenterology 1999; 116: 1055
121. Anonymous. Mosapride price-listed in Japan. Scrip 1998; 2374: 26
122. Ruth M, Hamelin B, Röhss K, et al. The effect of mosapride, anovel prokinetic, on acid reflux variables in patients with gastro-oesohageal reflux disease. Aliment Pharmacol Ther 1998; 12: 35-40
123. Sanger GJ, Banner SE, Smith MI, et al. SB-207266: 5-HT4 receptor antagonism in human isolated gut and prevention of 5-HT-evoked sensitization of peristalsis and increased defaecation in animal models. Neurogastroenterol Motil 1998; 10: 271-9
124. Bharucha AE, Camilleri M, Haydock S, et al. Effects of a serotonin 5-HT_4 receptor antagonist SB-207266 on gastrointetsinal motor and sensory function in humans. Gut 2000; 47: 667-74
125. Sanger GJ, Yoshida M, Yahyah M, et al. Increased defecation during stress or after 5-hydroxytryptophan: selective inhibition by the 5-HT4 receptor antagonist, SB-207266. Br J Pharmacol 2000; 130: 706-12

126. Cooper SM, Haydock SF, Tompson DJ, et al. A pharmacodynamic model of 5-HT4 receptor activation in man: antagonism by the 5-HT4 receptor antagonist SB-207266 [abstract]. Gastroenterology 1999; 116: A598
127. Houghton LA, Jackson NA, Whorwell PJ, et al. 5-HT4 receptor antagonism in irritable bowel syndrome: effect of SB-207266-A on rectal sensitivity and small bowel transit. Aliment Pharmacol Ther 1999; 13: 1437-44
128. Smith MI, Banner SE, Sanger GJ. 5-HT4 receptor antagonism potentiates inhibition of intestinal allodynia by 5-HT3 receptor antagonism in conscious rats. Neurosci Lett 1999; 271: 61-4
129. Houghton LA, Fowler P, Keene ON, et al. Effect of sumatriptan, a new selective 5HT1-like agonist, on liquid gastric emptying in man. Aliment Pharmacol Ther 1992; 6: 685-91
130. Tack J, Piessevaux H, Coulie B, et al. Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 1998; 115: 1346-52
131. Coulie B, Tack J, Gevers A, et al. Influence of sumatriptan-induced colonic relaxation on the perception of colonic distention in man [abstract]. Gastroenterology 1997; 112: A715
132. Coulie B, Tack J, Janssen J. Influence of buspirone-induced fundus relaxation on the perception of gastric distention in man [abstract]. Gastroenterology 1997; 112: A715
133. De Ponti F, Crema F, Nardelli G, et al. Blockade by the selective 5-$HT_{1B/D}$ receptor antagonist GR127935 of the effect of sumatriptan on canine gastric compliance [abstract]. Neurogastroenterol Motil 2000; 12: 383
134. Croci T, Landi M, Bianchetti A, et al. Drug-induced defaecation in rats: role of central 5-HT_{1A} receptors. Br J Pharmacol 1995; 115: 203-9
135. Vanhoenacker P, Haegeman G, Leysen JE. 5-HT_7 receptors: current knowledge and future prospects. Trends Pharmacol Sci 2000; 21: 70-7
136. Bueno L. New and future drugs in nerve-gut dysfunction. Ital J Gastroenterol Hepatol 1999; 31: 794-801
137. Toulouse M, Coelho AM, Fioramonti J, et al. Role of tachykinin NK_2 receptors in normal and altered rectal sensitivity in rats. Br J Pharmacol 2000; 129: 193-9
138. Onori L, Aggio A, Taddei G, et al. Contribution of NK(2) tachykinin receptors to propulsion in the rabbit distal colon. Am J Physiol Gastrointest Liver Physiol 2000; 278: G137-G47

Correspondence and offprints: Dr *Fabrizio De Ponti*, Department of Pharmacology, Via Irnerio, 48, I-40126 Bologna BO, Italy.
E-mail: deponti@biocfarm.unibo.it