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Motilin, ghrelin and related neuropeptides as targets for the treatment of GI diseases

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Motilin and ghrelin are released from the upper gut during fasting, to stimulate gastric motility. Additional actions of ghrelin (e.g. changes in appetite, nausea or endocrine functions) improve the possibility of using ghrelin receptor agonists to treat complex disorders such as functional dyspepsia. However, changes in endocrine functions increase the risk of unacceptable side effects. By comparison, the more restricted prokinetic activity of motilin limits the therapeutic possibilities but improves the risk:benefit ratio. Compounds targeting both receptors are in development. Recently, additional peptides have been identified from preproghrelin (obestatin) and prepromotilin. These exert biological activity but their pathophysiological significance is unknown.

Introduction

Motilin is a well-recognised peptide found in specific endocrine cells in the epithelia of the upper small intestine; it is released during fasting and in association with Phase III of the migrating motor complex (MMC), to act at its own receptor (motilin receptor, previously known as GPR38) [1,2]. MMCs begin in the upper regions of the gut. They involve secretory and motility functions, and are usually characterised by three distinct phases (I, a period of near-quiescence; II, short- or non-propulsive contractions at irregular frequency; III, a final burst of high-amplitude propulsive contractions), terminating within the distal regions of the small intestine [3]. Phase III activity may help clear the stomach and intestine from any undigested material, prevent bacterial overgrowth in the upper gut and perhaps develop the sensation of hunger [3]. The association of motilin release with this phase of the MMC, together with an ability of motilin to mimic the strong propulsive contractions which comprise Phase III, argues for an involvement of motilin in the regulation of certain functions of the gut during fasting [1]. Additionally, motilin receptor agonists can increase gastric emptying after ingestion of a meal. The antibiotic drug erythromycin, which also activates the motilin receptor [4], has been an especially useful tool to help elucidate the functions of motilin.

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Ghrelin (previously known as the growth hormone secretagogue receptor; GHSR) is also released by the gut during fasting and may be regarded as motilin's cousin. It is present as an acylated peptide, which binds to the ghrelin receptor (previously known as GHSR-1a), and as a des-acylated form (des-acyl ghrelin) which in certain experiments has been shown to have activity via a receptor that is distinct from the ghrelin receptor (e.g. increased feeding in rats after intracerebroventricular administration [5]). The ghrelin receptor shares a 52% amino acid identity with the motilin receptor (86% in the transmembrane domains) and was sufficiently similar to result in an original naming of ghrelin as the motilin-related peptide [6]. Although found within the brain and elsewhere, ghrelin is located predominantly in endocrine cells within the gastric mucosa and can increase gastric emptying and MMC activity [7]. However, compared with motilin, major differences exist. These include the absence of a meaningful ability of either peptide to modulate the receptor of the other [8] and the presence of a functional ghrelin system in rodents and in all species studied (see below for discussion on the absence of a functional motilin system in rodents). Further, ghrelin release is associated with stimulation of appetite and not MMC activity, and also with a wide array of additional actions which link an ability to increase gastric emptying to a coordinated modulation of appetite (or feeding) and metabolism (see below). This change in appetite may in turn be linked to an ability of ghrelin to inhibit emesis

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[9], contrasting with the induction of nausea by high doses of erythromycin (see below).

For both peptides, their attraction as potential drug targets for the treatment of GI and related disorders seems obvious. This review summarises the main advantages and disadvantages of each approach. At this stage of our understanding, it is also important to review the studies which highlight the unusually large length of peptide within the prepromotilin and preproghrelin genes, which do not generate motilin or ghrelin. Analysis of this region led to the discovery of obestatin within the preproghrelin gene and a similar peptide may also exist within the prepromotilin gene. As yet, the effects of these peptides on gastrointestinal functions are not clear. However, it is interesting to note that the unsuccessful pairing of obestatin to the orphan G-protein-coupled receptor GPR39, followed by a report on the characteristics of the GPR39 KO mouse, has indicated a possible involvement of GPR39 in GI biology.

Motilin

In terms of therapeutic intervention, the interest in motilin lies not in being able to generate a molecule which simply stimulates the Phase III component of the MMC, but in delivering an agent which increases gastric motility after feeding. To understand why this is possible, it is essential to make sense of a large and sometimes confusing literature on the biology of motilin.

1. *Rodents do not have a functional motilin system*. Most studies have failed to demonstrate an ability of motilin to affect gut function in many different models using rats, mice or guinea-pigs [10]. Further, neither the peptide nor its gene or functional rodent orthologues of the human motilin receptor [11] have been identified. A minority of studies do claim to detect an ability of motilin to stimulate rat gastric motility and brain function (e.g. [12]), but these reports must be treated with caution. Speculations on the reason for the loss of the motilin system from rodents focus on the marked differences which exist between rodent and human gastric anatomy and physiology and in particular on their inability to vomit. This conclusion means that in drug-discovery, it is necessary to use non-rodent animal species, such as the rabbit, which has an 84% protein identity to the human receptor polypeptide and similar pharmacology to the human orthologue [8].
2. *Low concentrations of motilin preferentially stimulate neuronal functions within the upper regions of the gut, whereas higher concentrations are required to directly contract the muscle*. This is an important conclusion, because of the large volume of literature which looks at only the ability of motilin receptor agonists to act directly at the muscle and evoke contraction [13]. Although a focus on the smooth muscle activity of motilin would be consistent with the high expression of motilin receptors in the muscle itself, especially in the upper

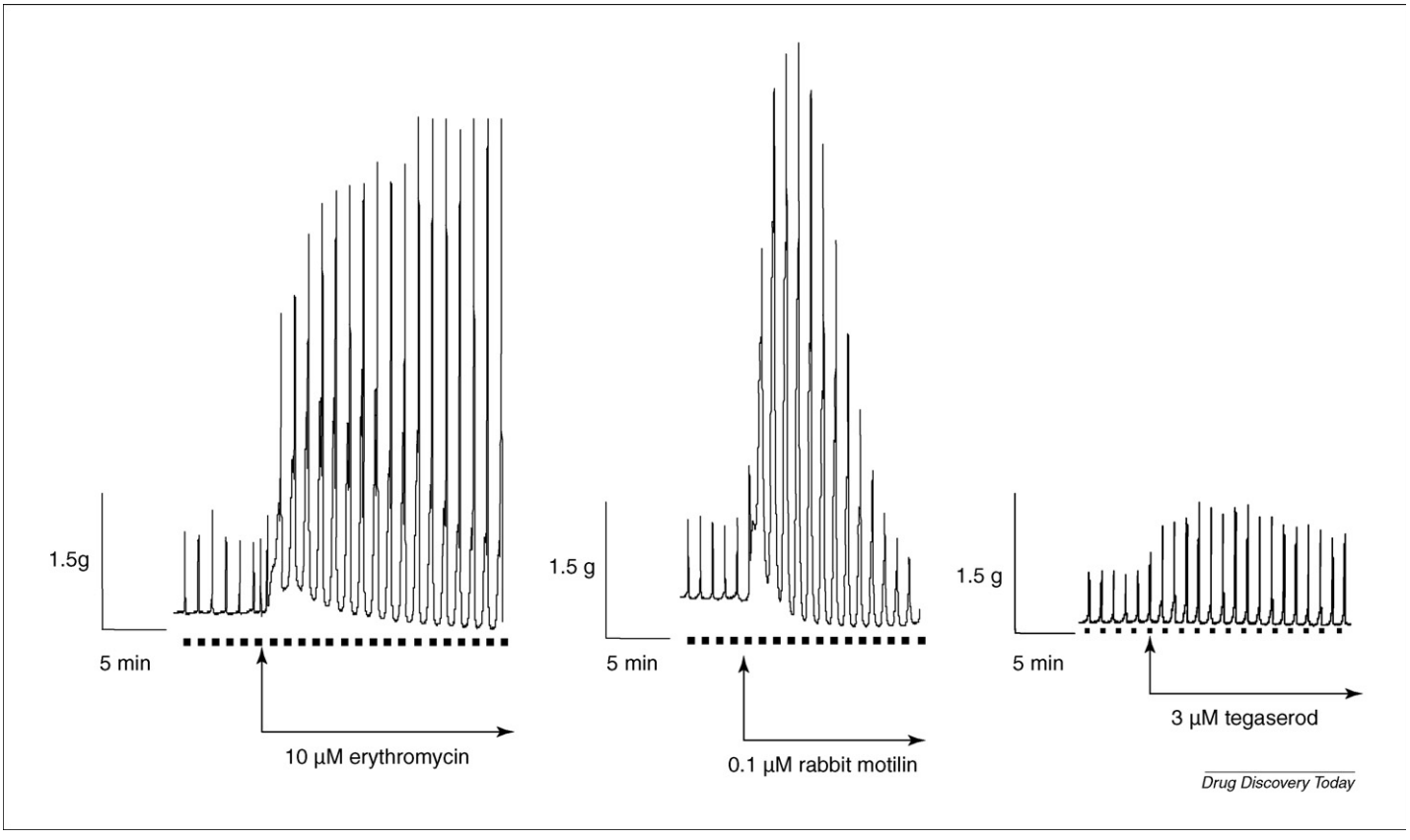


FIGURE 1

Comparison of the maximum-effective abilities of erythromycin, motilin and tegaserod to facilitate cholinergically mediated contractions in rabbit isolated gastric antrum. Illustration derived from Jarvie *et al.* [17]. In brief, strips of stomach were suspended in isolated tissue baths and cholinergically mediated contractions were evoked by electrical field stimulation. After obtaining consistent responses, erythromycin, the rabbit form of motilin or tegaserod were each added to the bathing solution. The responses shown are representative of those obtained using the maximally effective concentrations of each substance.

TABLE 1

Clinical usage of motilin receptor agonists

Drug	Indication	Comment	Refs
Erythromycin	Enteral feeding		[23]
	Helping to control blood glucose levels in diabetic patients		[60]
	Diabetic- or non-diabetic gastroparesis	Importantly, symptoms associated with gastroparesis may be improved by repeated intravenous administration of erythromycin, provided the dose was titrated to achieve both efficacy and tolerance in each patient [26]	[28,61]
	Clearing of gastric contents before emergency surgery or endoscopy for acute upper GI bleeding		[62]
	Facilitation of rapid intubation in critically ill patients		[63]
Mitemcinal	Gastroparesis	Reduce symptoms in patients with gastroparesis	[58]
ABT-229	Functional dyspepsia or gastroparesis associated with type 1 diabetes mellitus	Failed to improve symptoms	[31,32]

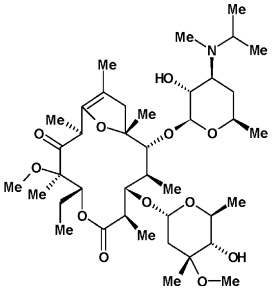
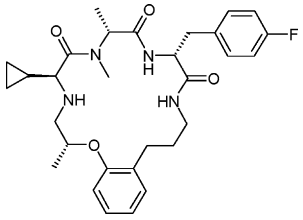
regions of the gut, it ignores the influence of the motilin receptors present within the enteric nervous system [8,14] and on the peripheral terminals of the vagus [15]. Thus, functional studies, both in animals and humans, clearly demonstrate a major influence of neuronally located receptors in mediating the ability of motilin receptor agonists to increase gastric emptying in a coordinated manner. This apparent imbalance between receptor number and function may be explained by the fact that receptor functions are governed not just by their density within a tissue but also by the efficiency with which they couple to their effector mechanism. Studies using rabbit isolated gastric antrum illustrate the point. In these experiments, low concentrations of motilin or erythromycin greatly increase the amplitude of electrically evoked, cholinergically mediated contractions whereas higher concentrations were required to directly contract the muscle [8,16,17]. This ability of erythromycin to facilitate cholinergic activity may be considerably greater than that caused by the gastroprokinetic agent and 5-HT_4 receptor agonist tegaserod (Fig. 1) [17], an observation consistent with at least some clinical studies where the two pharmacologies have been compared [18,19]. Further evidence to suggest that facilitation of cholinergic function is more closely related to gastric prokinetic activity comes from studies in which healthy volunteers were given a low (40 mg) and a high (200 mg) dose of erythromycin; the propulsive activity evoked by the low dose was reduced by atropine, but when the higher dose was given, a non-propulsive, atropine-insensitive excitatory activity was observed [20], as well as an increase in meal-induced satiety [21]. Finally, repeat dosing with low doses of erythromycin has been found to increase gastric emptying whereas higher doses can induce nausea and stomach cramping [22]. It is tempting to speculate that the increase in satiety, the nausea and the stomach cramping observed when using high doses of erythromycin are at least partly related to the ability of high concentrations of erythromycin to directly contract gut muscle, and that the many studies which focus on this type of activity have doubtful physiological or therapeutic relevance.

3. *Responses to motilin receptor agonists can be long-lasting*. In the rabbit gastric antrum experiments described above, the cholinergic activity of erythromycin appeared to be long-lasting, whereas the direct contractile activity observed at higher concentrations was short-lived. By contrast, the ability of [Nle^{13}]-motilin (a more stable analogue of the peptide) to excite cholinergic activity was not as long as that caused by erythromycin and again, the direct muscle contraction was short-lived. The difference in duration of cholinergic facilitation did not appear to be because of obvious peptide degradation. Speculation on a possible reason for the different kinetic actions of erythromycin and motilin centre on the existence of different binding sites for the peptide and non-peptide structures [8,17]. Whatever the reason, such short-lived but intense activity of motilin, finds consistency with the hypothesis that motilin might be involved in mediating Phase III of the MMC. For erythromycin, a longer lasting activity again finds consistency with clinical observations in which low doses of erythromycin have been shown to increase gastric emptying of meals in humans [22,23]. Interestingly, high doses of erythromycin may indeed tolerate with repeat dosing (250–400 mg, 4× daily) [24], whereas lower doses (50–100 mg, 3× daily and at bedtime) may not [25].
4. *As yet there are no clear functions for motilin in the brain*. There are several reports which suggest that motilin receptor activation may affect brain function in rats and mice, but the absence of a functional motilin system in this species, strongly questions the conclusions drawn. Small amounts of motilin have been reported as mRNA or after detection by immunohistochemistry in the brains of several species, including humans and monkeys [26,27].

Erythromycin currently finds clinical use in the treatment of patients requiring rapid intubation or endoscopy, as well as the removal of gastric contents before endoscopy or surgery. The drug is also used to facilitate enteral feeding, to help control blood glucose levels in diabetic patient and to treat symptoms in patients with diabetic- or non-diabetic gastroparesis (Table 1). Importantly, in the study by DiBiase and Quigley [28], erythromycin was given by repeated intravenous administration, with the dose titrated to achieve both efficacy and tolerance in each patient. However, although an effective agent, the continued use of this antibiotic drug in such patients continues to exacerbate the rise in antibiotic

TABLE 2

Motilin and ghrelin receptor agonists in development as gastroprokinetic agents

Compound	Structure	Status	Comments
Motilin receptor agonist			
Mitemcinal (GM-611)		Phase II	May improve symptoms in patients with diabetic gastroparesis, compared to placebo [58]
PF-04548043 (KOS-2187)	Unknown motilide structure	Phase I	Effective as a gastroprokinetic in dogs [59]
Ghrelin receptor agonist			
TZP-101 (IV) and TZP102 (oral)	Tranzyme patent example (WO2006/009674A1) 	TZP-101: Phase II; TZP102: Preclinical	Pilot study showing symptom improvement in diabetic gastroparesis after intravenous administration [64]. TZP-101 effective in a rat model of post-operative ileus [65]

resistance [29,30]. There is, therefore, an urgent need for a non-antibiotic motilin receptor agonist. Among the non-antibiotic, motilin receptor agonists currently in development (Table 2), the most advanced is the motilide, mitemcinal, reported to reduce symptoms in patients with gastroparesis. An earlier motilide, ABT-229, failed to improve symptoms in patients with functional dyspepsia [31] or with type 1 diabetes mellitus [32]. These data argue against the use of such drugs in the treatment of these disorders or alternately, given the potential worsening of symptoms in these trials, questions the selectivity of action of this compound and the doses used. ABT-229 is, for example, reported to be effective in rats [33], a species where a functional motilin receptor is not thought to exist, and in spite of having a 20 h plasma half-life, ABT-229 was given *b.i.d.* [34].

The rationale for the development of motilin receptor antagonists [35] is unclear, because a need for a drug which may reduce MMC activity is not obvious. Nevertheless, if endogenous motilin is found to play a role in excessive rates of gastric emptying or intestinal transit, then perhaps an indication may become apparent.

Ghrelin

Ghrelin is now well established as being able to increase appetite or food intake, modulate fat utilisation and increase the release of different hormones into the circulation [36]. In the gut of different species, including humans [37], ghrelin is thought to increase gastric motility and emptying mostly by acting on the gastro-vagal innervation, though receptors within the enteric nervous system may also play a role [38,39]. Vagal and central (arcuate nucleus) pathways are also implicated in the control of appetite in humans [40]. There is an additional possibility that ghrelin released from the gut can increase gastric emptying and feeding by acting centrally, after being transported across the blood–brain barrier, though active transport systems also exist to remove ghrelin from the brain [41]. Elsewhere in the gut, ghrelin may protect against ethanol- or stress-induced gastric damage [42] by acting via enteric and/or vagal pathways, or increase defecation, by acting on receptors within the spinal cord [43].

In terms of GI disorders, the interest in ghrelin is derived not just from its prokinetic activities (suggesting utility in a variety of different GI disorders) but from the idea that because ghrelin receptor agonists exert multiple activities on upper gut functions, this approach might represent a more complete treatment of complex disorders such as functional dyspepsia. This is illustrated by various animal models. For example, treatment with ghrelin has been shown to offer improvements in post-operative gastric ileus [44] or septic ileus [45]. In other animal models, ghrelin has been shown to have the potential to alleviate symptoms of dyspepsia such as delayed gastric emptying, anorexia and vomiting [46,9]. In cancer patients with impaired appetite, ghrelin may increase energy intake and meal appreciation during a buffet meal [47]. Together, these observations suggest that ghrelin receptor agonists may be a useful therapeutic target for the treatment of a range of gastrointestinal disorders. However, to counter-balance this enthusiasm, it needs to be remembered that ghrelin has a widespread ability to increase endocrine release [36,48]. Whilst acute administration of a ghrelin receptor agonist may present little issue, a long-term exposure to ghrelin created by the repeated doses necessary to treat many of the upper GI disorders may present a problem. The need for this caution is exemplified by a study which showed that constant intravenous infusion of ghrelin may provoke a fall in insulin sensitivity [48].

Several ghrelin receptor agonists are currently in development for a range of non-GI conditions, including cachexia and growth-related conditions [8]. Only one chemical series is focussed towards the treatment of GI disease (Table 2). The most advanced is TZP-101, an intravenously administered compound, which has been shown in a pilot study, to improve symptoms in diabetic gastroparesis.

Ghrelin- and motilin-associated peptides

The precursors of many neuropeptides or hormones contain additional regions of peptide which lack known biological function but may facilitate recognition of cleavage sites by proteases. The recent identification of obestatin within such a region of the preproghrelin gene suggests that the biology of ghrelin and possibly motilin, is more complex than previously realised.

1. *Obestatin and GPR39*. Obestatin was identified from the ghrelin-precursor gene, proghrelin. This ghrelin-associated peptide, was originally thought to be the endogenous ligand for the G-protein-coupled receptor GPR39 and was shown to suppress feeding and weight gain in mice [49] as well as reduce rat gastric emptying and spontaneous contractile activity in the jejunum. The ability of obestatin to reduce food intake has been replicated and confirmed by others [50], and the findings extended to include an ability of obestatin to increase thirst [51]. However, several other studies have failed to demonstrate significant effects of obestatin on GI motility [52,53] or shown an interaction with GPR39 [50]. A number of interesting problems are thrown up by this research. Firstly, since obestatin immunoreactivity has been detected in the rat gastric mucosa and in choline acetyltransferase-positive nerves of the myenteric plexus [54], what is the role of this peptide and what receptor does it act on? Secondly, mice lacking the GPR39 receptor appear to have an increased rate of gastric emptying [55]. These data are consistent with a high level of GPR39 receptor expression in the stomach and small intestine, liver, pancreas and adipose tissue [56] and as such, leave open the possibility that the cognate ligand for GPR39 may have profound effects on GI functions.
2. *Motilin-associated peptide*. The motilin-associated peptide (MAP) at the carboxy-terminal of the prepromotilin gene has been thought to play a role in protein degradation and post-translational processing of motilin [57]. However, following the identification of obestatin, we re-examined the motilin precursor protein using similar techniques, to see if a functionally active peptide could be identified. One peptide (H-Leu-Thr-Ala-Pro-Leu-Glu-Ile-Gly-Met-Arg-Met-Asn-Ser-Arg-Gln-Leu-Glu-OH), similar in length to obestatin, was found to partly mimic the ability of motilin to increase cholinergically mediated contractions in rabbit isolated gastric antrum. These findings demonstrate the existence of a small MAP with potential to operate in the gastric nervous system (S Topp, V Bolton, J Brown, E Jarvie, GJ Sanger, unpublished).

Conclusions

1. Motilin and ghrelin are related peptides (structural, gastrointestinal location and functions) which are both released from the upper gut during fasting. However, whereas the functions of motilin are largely restricted to the upper gut, ghrelin released from the gut and elsewhere, can have profound actions on appetite, fat metabolism and endocrine function. These functions of ghrelin place the gut at the centre of a coordinated control of eating, digestion and metabolism.
2. In terms of drug development, the additional actions of ghrelin (e.g. improvements in appetite or nausea) improve the possibility of using ghrelin receptor agonists to treat complex disorders such as functional dyspepsia. However, changes in endocrine functions (insulin sensitivity, for example) increase the risk of unacceptable side effects.
3. The more restricted prokinetic activity of motilin limits the therapeutic possibilities (e.g. improvements in enteral feeding, control of glucose levels in diabetics and gastroparesis) but improves the risk:benefit ratio relative to that of ghrelin receptor agonists.
4. Motilin and ghrelin receptor agonists are currently in development as potential new therapeutic agents for the treatment of gastrointestinal disorders.
5. Additional peptides have now been identified from preproghrelin and prepromotilin. The most noteable of these is obestatin. Such peptides may exert biological activity but their pathophysiological significance is unknown. Nevertheless, research into obestatin has highlighted the potential of the orphan G-protein-coupled receptor GPR39 in the control of gastrointestinal function.

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