The hungry stomach: physiology, disease, and drug development opportunities

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During hunger, a series of high-amplitude contractions of the stomach and small intestine (phase III), which form part of a cycle of quiescence and contractions (known as the migrating motor complex, MMC), play a "housekeeping" role prior to the next meal, and may contribute toward the development of hunger. Several gastrointestinal (GI) hormones are associated with phase III MMC activity, but currently the most prominent is motilin, thought to at least partly mediate phase III contractions of the gastric MMC. Additional GI endocrine and neuronal systems play even more powerful roles in the development of hunger. In particular, the ghrelin-precursor gene is proving to have a complex physiology, giving rise to three different products: ghrelin itself, which is formed from a post-translational modification of des-acyl-ghrelin, and obestatin. The receptors acted on by des-acyl-ghrelin and by obestatin are currently unknown but both these peptides seem able to exert actions which oppose that of ghrelin, either indirectly or directly. An increased understanding of the actions of these peptides is helping to unravel a number of different eating disorders and providing opportunities for the discovery of new drugs to regulate dysfunctional gastric behaviors and appetite. To date, ghrelin and motilin receptor agonists and antagonists have been described. The most advanced are compounds which activate the ghrelin and motilin receptors which are being progressed for disorders associated with gastric hypomotility.

Keywords: migrating motor complex, motilin, ghrelin, des-acyl-ghrelin, obestatin, stomach

INTRODUCTION

Read (1992) defined "hunger" as a word usually applied to the sensation of needing food, whereas in humans at least, the word "appetite" also encompasses the desire to eat and the enjoyment of certain foods. Hunger is not abolished by distension of the stomach by a balloon or by infusion of lipids into the bloodstream, but by a nutrient load within the small intestine (Read, 1992). This suggested that mechanisms within the gastrointestinal (GI) tract have the capacity to influence what we eat. A subsequent explosion in understanding how neuronal and hormonal communication from different regions of the GI tract can modulate food intake (Bray, 2000) and energy homeostasis (Murphy and Bloom, 2006) now places the gut, the largest endocrine organ of the body, at the center of an integrated gut–brain–energy axis, in which digestion is coordinated with appetite, satiation, and metabolism. It follows that disruption of this axis can be linked to early or late satiety, nausea, and to several conditions associated with eating and metabolic disorders and/or delayed gastric emptying.

During hunger, the muscle of the stomach and small intestine is largely quiescent. However, a regular cycle of contractions play a "housekeeping" role prior to the next meal and may contribute toward the development of hunger. This cycle of quiescence and contractions is known as the migrating motor complex (MMC), and particular phases of the MMC are closely associated with the release of certain GI hormones. **Figure 1**

illustrates the peak strength of the contractions which can begin in the middle part of the stomach and are temporally associated with hunger sensations. In addition, other GI endocrine and neuronal systems play even more powerful roles in the development of hunger.

The ways in which appetite can be modulated via mechanisms originating within the brain, have previously been described (Rolls, 1981; Cline et al., 2009). Here we focus on the role of the upper GI tract (and its large compliment of hormones) in the mechanisms of hunger, dividing the discussion into four key areas:

- The movements of the stomach and small intestine during hunger and their relationships with the release of GI hormones which are primarily involved in regulating GI motility, and which also assist in the development of hunger.
- The release of other GI hormones from the stomach during hunger, which although having the potential to affect gastric motility, appear to play greater roles in driving hunger and appetite, operating via GI, vagal, and central nerve mechanisms.
- The dysregulation of these processes during illness.
- The ways in which an understanding of the hungry stomach is leading to the discovery of new drugs to regulate dysfunctional gastric behaviors and appetite.
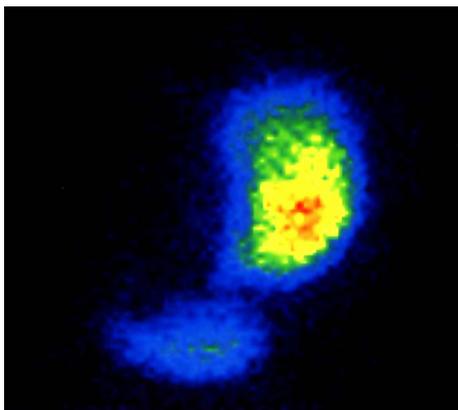
FIGURE 1 | Illustrates the strength of the contractions which begin in the middle part of the stomach and which are temporally associated with hunger sensations (image through courtesy of Per Grybäck).

MOVEMENTS OF THE HUNGRY STOMACH AND SMALL INTESTINE

THE MIGRATING MOTOR COMPLEX

In humans the stomach and small intestine adopt a cyclic pattern of motility and secretion during fasting, first noted in association with hunger by Boldyreff in St Petersburg, Russia, in 1905, and later rediscovered and described as the MMC by Szurszewski (1969) in the canine small intestine. MMCs are usually characterized by a relatively long period of near-quiescence (phase I), followed by a series of short, mostly non-propulsive contractions at irregular frequency (phase II) and then a short burst of high-amplitude propulsive contractions (phase III; Sarna, 1985; Nieuwenhuijs et al., 1998; Husebye, 1999). **Figure 2** shows an MMC trace taken from an 8-channel antroduodenal motility recording of a healthy test subject experiencing a hunger pang. In humans the MMC has a cycle time of between 80 and 120 min, during which phase III may last around 5–8 min. MMCs can occur during the day or night (when phase I occupies a greater time at the expense of phase II) and about 60% of phase IIIs begin in the stomach, the rest beginning at the ligament of Treitz on the border between the duodenum and jejunum (Kellow et al., 1986). MMCs move down the small intestine and its characteristic activity front (phase III) terminates in the distal small intestine. MMC activity is also accompanied by variations in splanchnic blood flow, gall-bladder emptying, and gastric and pancreatic secretions (Thollander et al., 1996; Nieuwenhuijs et al., 1998; Husebye, 1999). Ingestion of a meal replaces MMC activity with a "feeding pattern" of motility (Sarna, 1985).

It is important to note that considerable species differences exist between the characteristics and mechanisms of MMCs. Carnivores such as dogs exhibit MMC activity with characteristics similar to that of humans (Sarna, 1985). Herbivores such as rabbits and rodents (including guinea-pigs, mice, and rats) exhibit different MMC patterns and mechanisms. In rabbits the stomach is never empty and phase III myoelectric activity can be detected even after feeding (Ruckebusch et al., 1985; Guerrero-Lindner et al., 1996). In rats, phase III-like MMC activity occurs at a much faster rate than in humans and dogs (12–15 min compared with 90–100 min; Argia et al., 2007). Further, the clear role of the GI hormone motilin in

the mechanisms of phase III MMC activity in the human and dog stomach (see Mechanisms of the MMC, below) and the absence of a functional motilin system in rats and mice (He et al., 2010) indicates that the mechanisms of gastric MMCs in these rodents are very different to those in humans and dogs. Perhaps in rats and mice the functions of motilin are unnecessary or are mimicked by those of ghrelin (Argia et al., 2007). Whatever the explanation, the differences indicate that in terms of human GI functions, the translational value of studies on MMC activity in rodents must be treated with caution. For this reason, this section focuses only on studies which use humans or dogs.

FUNCTIONS OF THE MMC

Phase III MMC activity is thought to help prevent bacterial overgrowth in the upper gut and clear the stomach and intestine from any undigested material not removed during normal gastric emptying (Vantrappen et al., 1977; Nieuwenhuijs et al., 1998; Husebye, 1999). MMCs may also help create the sensation of hunger. Itoh et al. (1975) first described phase III MMC contractions as "hunger contractions." More recently phase III activity of MMCs which spontaneously originate in the stomach were shown to correlate with the sensation of hunger – a similar correlation was not observed for MMCs originating within the small intestine (Ang et al., 2008a,b). Thus, hunger sensations seem to primarily emanate from the gastric antrum.

MECHANISMS OF THE MMC

Although phases II and III are mediated via the enteric nervous system (ENS), phase III is initiated via the vagus nerve. Thus in the canine stomach, phase III MMC activity has been prevented or reduced by vagotomy or other procedures which reduce vagal activity (Hall et al., 1984; Tanaka et al., 2001; Shibata et al., 2002). However, the mechanisms which regulate the periodicity of the MMC cycle are regionally specific and are thought to be governed by the local release of 5-HT and motilin from the mucosa of the upper gut.

Nakajima et al. (2010) proposed that MMCs begin with a build-up of 5-HT, released from enterochromaffin cells in the mucosa of the duodenum during phase I. The released 5-HT acts at 5-HT_4 receptors within the ENS to increase GI motility, representing phase II of the MMC. The mechanical activity caused by the increased motility is then argued to release more 5-HT from the enterochromaffin cells, which initiates phase III by activating 5-HT_4 receptors in the intestine and 5-HT_3 receptors in the stomach. Thus, phase III MMC activity can be attenuated by 5-HT_3 receptor antagonism when the MMC originates in the stomach (Yoshida et al., 1991; Wilmer et al., 1993; Nakajima et al., 2010) but not clearly in the intestine (Davidson et al., 1990). Instead, 5-HT_4 receptor antagonism was found to inhibit phase II and III MMC activity in the canine small intestine, a procedure which was similarly effective in the stomach (Nakajima et al., 2010).

The increased contractions also increase the release of motilin from endocrine cells in the mucosa to induce or re-enforce gastric phase III MMC activity (Sarna, 1985; Peeters, 1993). Thus, although the release of motilin is not prevented by vagotomy (Yoshiya et al., 1985) or by 5-HT_3 receptor antagonism (Yoshida et al., 1991),

FIGURE 2 | Propagated phase III from antrum through jejunum, preceded by phase II and followed by phase I, of the migrating motor complex of a healthy subject experiencing hunger.
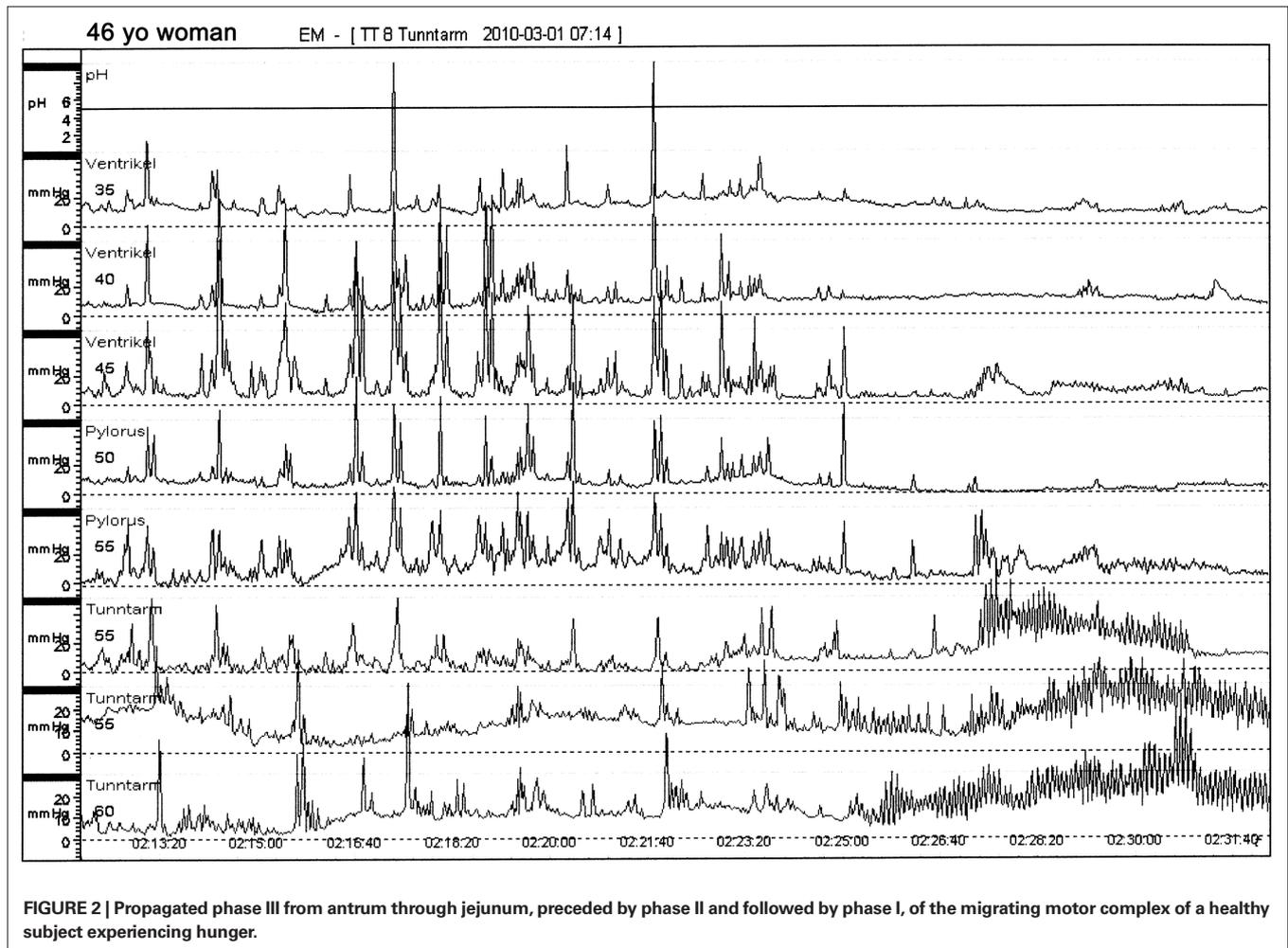
phase III MMC activity within the stomach can be attenuated by motilin antiserum (Lee et al., 1983). Motilin acts by strongly facilitating gastric cholinergic motor nerve activity in a short-lasting manner (Dass et al., 2003a; Jarvie et al., 2007), consistent with its role in mediating the short-lived phase III contractions. The released motilin may also activate the vagus nerve. This has been demonstrated in conscious dogs, where motilin evoked a transient increase in blood plasma concentrations of pancreatic polypeptide (Mochiki et al., 1997) and insulin (Suzuki et al., 1998) via vagal muscarinic pathways; these effects are consistent with the increase in plasma concentrations of both hormones in association with phase III gastric MMC activity (Suzuki et al., 1998). Acute vagal blockade has also been reported to inhibit the increase in canine gastric motility caused by low but not by high doses of motilin (Inatomi et al., 1996). Interestingly, induction of a gastric phase III-like activity by administration of the motilin receptor agonist erythromycin was recently shown to correlate with the sensation of hunger (Ang et al., 2008a,b). These observations reinforce the suggestion of a role for motilin, released in association with phase III of the gastric MMC, to promote hunger either directly (e.g., by activating the vagus nerve) or indirectly by promoting the release of ghrelin (Zietlow et al., 2010; see below for discussion on the role of ghrelin in hunger).

The reasons why both 5-HT and motilin are involved in regulating the mechanisms which support phase III MMC activity are not clear.

ASSOCIATIONS BETWEEN MMCs AND THE RELEASE OF OTHER GI HORMONES

In addition to motilin, other peptides are associated with phase III MMC activity. One of the most recently studied is ghrelin, a 28-amino acid peptide first derived from rat stomach and shown to be the natural ligand for the growth hormone (GH) secretagogue receptor 1a (Kojima et al., 1999), now renamed as the ghrelin receptor (Davenport et al., 2005). Ghrelin has several actions, among which are the ability to release GH and other hormones into the circulation, stimulate appetite, and modulate fat utilization (Williams and Cummings, 2005; Huda, et al., 2006; Murphy and Bloom, 2006). In humans ghrelin can increase the rate of gastric emptying (e.g., Levin et al., 2006) via vagal nerve activation (Asakawa et al., 2001; Date et al., 2002; Sakata et al., 2003) and to a lesser extent via receptors within the ENS (Dass et al., 2003b; Edholm et al., 2004). This prokinetic activity may even induce a premature phase III of the MMC (Tack et al., 2006). Nevertheless, the gastric prokinetic properties of ghrelin are usually apparent only when high blood concentrations of ghrelin are achieved by the use of exogenously

administered ghrelin or ghrelin receptor agonists (Camilleri et al., 2009). Further, there have been no studies demonstrating a cyclical release of ghrelin which coincides with the phases of the MMC. Instead, ghrelin levels display a diurnal rhythm in humans, with a peak at night during fasting, and lowest concentrations in the morning after breakfast (Cummings et al., 2001). It is, therefore, unlikely that ghrelin is involved in the physiological regulation of MMCs in man.

It has been reported that increases in blood concentrations of somatostatin occur in association with phase III MMC activity originating in the stomach and duodenum (Peeters et al., 1983; Chung et al., 1995; Tomita, 2009) although this was not found in two other studies where duodenal MMCs were recorded (Näslund et al., 1998; Medhus et al., 1999); nevertheless, increased duodenal intraluminal concentrations were observed (Näslund et al., 1998). The exact role of the released somatostatin is not clear, since intravenous-administration of somatostatin or the somatostatin SST2 receptor agonist octreotide may abolish all activity within the stomach, delay small intestinal transit and inhibit intestinal phase II activity while initiating phase III-like activity (Peeters et al., 1983; Von der Ohe et al., 1995). In addition to somatostatin, peak intraduodenal levels of VIP and substance P have also been associated with human duodenal phase III activity (Näslund et al., 1998), suggesting an ability of certain neuropeptides to modulate GI functions during this phase of the MMC, after diffusion into local neurocircuits. Increases in blood concentrations of xenin (found in specific endocrine cells of duodenal mucosa) have also been found in association with phase III MMC activity and when given IV initiate phase III-like activity in intestine (Feurle et al., 2001). Finally, relationships between MMC activity and blood plasma concentrations of other gut hormones such as gastrin, glucagon, gastric inhibitory polypeptide (Rees et al., 1982), vasoactive intestinal peptide, or substance P, have not been found (Näslund et al., 1998).

GI HORMONES, HUNGER, AND APPETITE

Fasting decreases the availability of hormones such as leptin and glucagon-like peptide-1, which are released from the gut after eating meals to reduce appetite and help control the flow of nutrients down the GI tract (Näslund and Hellström, 2007). However the availability of other GI hormones is increased. In addition to motilin these include ghrelin and orexin, which are not released in close association with MMC activity, but can influence hunger and metabolism.

PRODUCTS OF THE PREPROGENE GHRELIN GENE

This extraordinary gene generates three different peptides, des-acyl-ghrelin, ghrelin, and obestatin, which may each have their own functions but which also interact to regulate the functions of ghrelin. Each is described separately.

Ghrelin

The release of this hormone from endocrine cells within the stomach (where the largest amounts are found) increases plasma ghrelin concentrations in humans during fasting. The fact that plasma concentrations rise just before mealtimes and drop after eating, prompted Cummings et al. (2001) to suggest a role for ghrelin in meal initiation. Later studies showing that the magnitude of

suppression of ghrelin release may be dependent on the type of food (the rank order for suppression was protein > carbohydrate > lipid) suggests a finer control than previously appreciated (Foster-Schubert and Cummings, 2006). The released ghrelin may promote appetite in humans by activation of vagal nerve pathways (le Roux et al., 2005) or after release into the blood and transport across the blood–brain barrier to act centrally (though active transport systems also exist to remove ghrelin from the brain; Banks et al., 2002). Interestingly, the sensitivity of ghrelin receptors may be increased during fasting (Scott et al., 2007). Further, an ability of ghrelin to inhibit emesis (Rudd et al., 2006), prompts the idea that appetite and nausea are opposite ends of the same pathway, so that hormones such as ghrelin which increase appetite also reduce nausea, and *vice versa* (Sanger and Lee, 2008). This activity of ghrelin may contribute to the reduction in vomiting observed in patients with diabetic gastroparesis following intravenous-administration of the ghrelin receptor agonist TZP-101 (Wo et al., 2010). Also, the orally active compound TZP-102 is reported to improve symptoms within 1 week of treatment initiation with significant reductions in symptom scores for nausea, early satiety, excessive fullness, postprandial fullness, and the total Gastroparesis Cardinal Symptom Index. In addition, improvement of upper and lower abdominal pain and discomfort has been noted[1] (Presentation by John Wo at ACG 2010, October 20, 2010).

Des-acyl-ghrelin

Ghrelin is formed from the non-acylated peptide (des-acyl-ghrelin) by a unique post-translational acylation of the third serine residue. This modification is mediated by ghrelin *O-acyl* transferase (GOAT) and can be readily reversed by endogenous esterases (Gutierrez et al., 2008). GOAT is primarily localized to the stomach and intestine, tissues from which active ghrelin is secreted (Yang et al., 2008). Since acylation may be at least partly regulated by nutrient availability in the gut (Liu et al., 2008), it is becoming clear that the ratio of ghrelin to des-acyl-ghrelin may be under physiological and/or pathological control. Such control may be important in tissues where des-acyl-ghrelin has biological activity.

In blood plasma from healthy volunteers the concentration of des-acyl-ghrelin greatly exceeds that of acylated ghrelin (Chen et al., 2009) and an ability of this peptide to affect GI function is increasingly recognized (Fujimiya et al., 2010). For example, in rats, intraperitoneal administration of des-acyl-ghrelin decreased food intake in free-feeding rats during the dark, but not the light phase of food intake (Chen et al., 2005). However, in another example, systemically administered des-acyl-ghrelin did not affect appetite in rats and mice but stimulated feeding when administered via the intracerebroventricular route, an activity also present in ghrelin receptor knock-out mice (Toshinai et al., 2006). These data contribute to the hypothesis that des-acyl-ghrelin can act via binding sites that are distinct from the ghrelin receptor (Lear et al., 2010). Finally, central and systemic administration of des-acyl-ghrelin may also disrupt fasted but not fed motility in the gastric antrum, an activity apparently dependent on activation of CRF_2 receptors in the brain (after the peptide had crossed the

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[1]http://www.news-medical.net/news/20100629/Tranzyme-reports-positive-results-from-TZP-102-Phase-2-study-in-diabetic-patients-with-gastroparesis.aspx

blood–brain barrier) but curiously, not on vagal nerve activity (Chen et al., 2005). Together, these data indicate a need to further explore the GI roles of des-acyl-ghrelin.

Obestatin

This peptide was identified through sequence analysis of the ghrelin-precursor gene, proghrelin (Zhang et al., 2005). Initially, obestatin was thought to be the endogenous ligand for the orphan G protein-coupled receptor GPR39, and when systemically administered was shown to suppress feeding and weight gain in mice in a time- and dose-dependent manner, as well as reduce rat gastric emptying (Zhang et al., 2005). Unfortunately the proposal that obestatin represented the endogenous ligand for GPR39 is no longer accepted (Wigglesworth et al., 2006; Holst et al., 2007; Zhang et al., 2007). Nevertheless, the ability of obestatin to inhibit food intake has been replicated (Zhang et al., 2007) and confirmed or partly confirmed (Bresciani et al., 2006; Sibilia et al., 2006; Lagaud et al., 2007; Chen et al., 2009), although not when administered centrally (albeit using a different protocol; Seoane et al., 2006). However, it is worth noting that although obestatin was shown to have a considerable effect on food intake, there was no actual reduction in body weight but rather a slower weight gain; changes in fat mass were not measured (Zhang et al., 2005). Another study suggests that obestatin may increase thirst (Samson et al., 2007). Finally, serum leptin levels were not affected by either ghrelin (Zhang et al., 2005; Falkén et al., 2010) or obestatin (Zhang et al., 2005), suggesting that both these effects were outside of adipose tissue and most likely confined to the GI tract (Nogueiras and Tschöp, 2005).

Several studies have failed to demonstrate significant effects of obestatin on GI motility when using *in vitro* models, when measuring MMC activity or gastric emptying of a non-nutrient meal (Gourcerol et al., 2006; Bassil et al., 2007; De Smet et al., 2007). Ataka et al. (2008) confirmed these observations but then showed that the gastro-duodenal motility of rats after eating standard chow was inhibited 30–90 min after intravenous-administration of obestatin. This inhibitory action appeared to be dependent on CRF_1 and CRF_2 receptor activity within the brain, and for the duodenum but not the antrum, was also prevented by capsaicin pretreatment. From these and other data, the authors suggested that obestatin acts centrally (after crossing the blood–brain barrier) and at the vagus, to inhibit rat upper GI motility, but only after feeding (Ataka et al., 2008; Fujimiya et al., 2010).

The studies with obestatin and GPR39 raise important questions. Firstly, since obestatin has undoubted biological activity and since immunoreactivity for obestatin has been detected in the rat gastric mucosa and in myenteric plexus (Dun et al., 2006), what is the role of this source of obestatin and what receptor does it act on? One possibility is suggested by the fact that obestatin appears to act in a manner which is opposite to that of ghrelin – reducing appetite and gastric emptying after meals, and antagonizing certain functions of ghrelin (Zhang et al., 2005; Bassil et al., 2007; Fujimiya et al., 2010; Kumar et al., 2010). Such activity points toward a role in the mechanisms of nausea, contrasting to the anti-emetic activity of ghrelin (Rudd et al., 2006). Another question is created by reports that GPR39 is upregulated in rat adipose tissue during fasting (Egerød et al., 2007) and that mice lacking GPR39 appear to have normal food intake and body weight gain (Tremblay et al., 2007) but

increased gastric emptying (Moechars et al., 2006) and a changed glucose-dependent insulin secretion (Tremblay et al., 2009). Do these observations suggest that the cognate ligand for GPR39 will have profound effects on GI functions? Clues may be provided by the suggestion that GPR39 is the receptor which mediates the ability of free zinc ions (Zn^{2+}) to affect cell functions (Egerød et al., 2007). GPR39 receptors are found throughout the GI tract (Egerød et al., 2007; Yamamoto et al., 2009) raising the possibility that these receptors mediate the ability of Zn^{2+} to modulate colonocyte functions such as proliferation, ion transport, and hormone transport, reduce epithelial erosion and inhibit diarrhea (Azriel-Tamir et al., 2004; Hershfinkel et al., 2007; Sharir et al., 2010). A role for Zn^{2+}-sensitive receptors in upper GI functions is not clear but now needs to be investigated.

OREXIN

Plasma concentrations of orexin also increase during fasting (Komaki et al., 2001). This hormone, present as orexin A and B in the myenteric plexus and endocrine cells of the human GI tract (and pancreas; Nakabayashi et al., 2003) has been reported to depolarize enteric neurons and facilitate gastric nitrergic activity in rodents (Katayama et al., 2005; Baccari et al., 2009), inhibit rat small intestinal MMC activity, stimulate rat gastric emptying and increase acid secretion (Näslund et al., 2002; Bülbül et al., 2010). However, in healthy human volunteers, infusion of orexin caused only a small inhibition of gastric emptying and failed to affect appetite (Ehrström et al., 2005), suggesting that the release of orexin from endocrine cells of the gut during fasting may play only a minor role in the control of human gastric emptying during hunger. Instead, the decrease in plasma concentrations of leptin and increase in insulin suggest a possible role in metabolic regulation (Ehrström et al., 2005).

DYSREGULATION OF THE HUNGRY HUMAN STOMACH
GASTROINTESTINAL MOTILITY

Disorders associated with disrupted MMC activity are not common, although clearly MMCs are absent during the paralytic ileus which follows abdominal and other types of surgery. MMCs reoccur in the proximal small intestine within 3 h after the surgery but initially with a quicker cycle time and reduced Phase II activity (Benson et al., 1994; Husebye, 1999). Interesting, patients undergoing gastrectomy for gastric cancer who then had Roux-en-Y reconstruction (the stomach is removed and to achieve continuity to the intestine the proximal jejunum is divided and the aboral jejunum anastomosed to the esophagus – the divided oral part of the jejunum is reconnected to the jejunum distal to the esophageal anastomosis so bile and pancreatic secretions can be directed into the jejunum and the shape of a Y is created), could be divided into those with intestinal MMC activity and those without (Tomita et al., 2003). Compared to the latter, patients with phase III MMC activity tended to have more appetite and greater food intake, as well as fewer symptoms associated with dumping, reflux, nausea or lower bowel symptoms. These observations show the importance of the MMC activity in eliciting hunger sensations.

Vantrappen et al. (1977) hypothesized that a dysfunctional MMC would lead to bacterial overgrowth within the upper gut, in an environment favored by accumulation of food remnants,

desquamated cells, and secretions. Such patients were detected using a $^{14}CO_2$ bile acid breath test and treated successfully with antibiotic drugs. The number of patients studied were, however, too small to speculate on the causes of the dysfunctional MMC.

Phase III-like activity persists in mechanically ventilated patients with critical illness, following perfusion of the small intestine with liquid nutrients (Chapman et al., 2005). Plasma motilin concentrations may be increased during such infusions, rather than decreased as in healthy volunteers (Nguyen et al., 2010). The reasons for these differences are not clear.

Migrating motor complex activity has often been studied in patients with functional bowel disorders, especially irritable bowel syndrome. Small variations have been observed, critically discussed by Husebye (1999). The role of any changes in the etiology of the disorders is, however, far from clear, being most likely caused by the disorder itself. Perhaps the most interesting are a group of patients with an idiopathic delay in gastric emptying where MMC activity was reduced or absent (Labò et al., 1990).

Finally, although not a disorder of hunger itself, gastric phase III MMC activity may lead to increased gastro-esophageal reflux during the night (Gill et al., 1987). This activity, observed in healthy individuals, has clear consequences for patients with hiatus hernia.

HORMONAL STATUS
There are physiological and pathological conditions characterized by high circulating ghrelin levels. These include starvation, anorexia nervosa (Otto et al., 2001), bulimia (Tanaka et al., 2002), and cachexia (Nagaya et al., 2001), in which ghrelin values are doubled, compared with normal controls. Binge eating has been associated with a dysfunction in the ghrelin signaling system and with a reduction in post-prandial satiety cues arising from an increase in gastric capacity (Hellström et al., 2004).

In patients with anorexia nervosa, plasma concentrations of ghrelin are paradoxically higher (Nakai et al., 2003) but return to normal as patients increase in weight; it has been demonstrated that there is a specific reduction in the GH response to ghrelin, despite the hyper-responsiveness to administration of GH releasing hormone. The impaired GH response to ghrelin could reflect desensitization of the ghrelin receptor induced by chronic elevation of ghrelin levels in this pathological state (Broglio et al., 2004). Interestingly there is one report of a patient with a ghrelin-producing neuroendocrine tumor who had circulating plasma concentrations of ghrelin of 12000 pM. There is no report regarding hunger sensations in this patient but she was obese (BMI 29.9 kg/m²) and had type 2 diabetes mellitus (Corbetta et al., 2003).

Clinical conditions associated with changes in blood concentrations of des-acyl-ghrelin and obestatin are as yet, unclear. Nevertheless, important biological functions are suggested by close links between ghrelin and obestatin (both are co-produced and their ligand and receptor sequences are highly conserved throughout mammalian evolution, along with the functions; Kaiya et al., 2008) and ghrelin and des-acyl-ghrelin (Kumar et al., 2010). The effects of these peptides now need to be studied in man, especially in patients with obesity and/or nausea.

TRANSLATION TO DRUGS
Increased understanding of the different roles played by hormones released from the gut during fasting has provided new opportunities for the development of new drugs. To date, motilin and ghrelin are the hormones which have received the most attention.

MOTILIN RECEPTOR ANTAGONISTS
Recent progress in identification of selective antagonists has been described by Westaway and Sanger (2009). Oral dosing of the motilin receptor antagonist MA-2029 to rabbits reduced motilin-induced colonic contractions (Sudo et al., 2008). MA-2029 also reduced abdominal muscle contractions induced by administration of motilin in combination with colorectal distension, indicating a reduction in the visceral pain experienced by these animals that had been exacerbated by motilin (Sudo et al., 2008). Similarly, MA-2029 inhibited motilin-induced increases in gastric fundus tone, GI transit, and diarrhea in conscious dogs without effects on basal GI tone or motility (Ozaki et al., 2009). However, although these experiments demonstrate the pharmacodynamic characteristics of MA-2029 (i.e., they establish that the antagonist can block the effects of exogenously administered motilin *in vivo*) they do not demonstrate a pathophysiological role for motilin. Evidence for such a role is provided by the macrocyclic peptidomimetic and motilin receptor antagonist TZP-201, which reduced the incidence of diarrhea in a dog model of chemotherapy-induced diarrhea providing a superior level of efficacy compared to loperamide and octreotide (Thomas et al., 2007). If confirmed, these data represent an important breakthrough in establishing a potential therapeutic use for motilin receptor antagonists. TZP-201 was also shown to antagonize motilin-induced contractile activity of the colon of the musk shrew (Venkova et al., 2009a). Although this is again a simple pharmacodynamic study, it is nevertheless, an important observation because these animals represent a small laboratory animal that actually possesses a functional motilin receptor.

MOTILIN RECEPTOR AGONISTS
In addition to its antibiotic use, erythromycin is used clinically as a gastric prokinetic agent. The latter has been described for a number of different disorders associated with delayed gastric emptying (Sanger, 2008), including patients in critical care receiving enteral feeding (given via a naso-gastric tube; enhanced gastric emptying improves delivery of nutrients, reduces the time needed for intubation, and the incidence of complications such as pulmonary aspiration or infection, improving survival), diabetic patients needing better control of blood glucose, and patients with diabetic- or non-diabetic gastroparesis where improved gastric emptying can reduce symptoms. Nevertheless, the ability of erythromycin to inhibit the cytochrome P450 enzyme CYP3A4 and cause drug–drug interactions (Zhou et al., 2004), together with the use of erythromycin as a gastric prokinetic agent in an age when antibiotic resistance continues to rise, highlights the pressing need for alternative medication. The use of erythromycin also highlights the extraordinary lack of safe and effective gastroprokinetic drugs (Sanger and Alpers, 2008). An improved, selective motilin receptor agonist would, therefore, have potential use in each of the conditions described above. A role for motilin receptor agonists in

reducing post-operative ileus is also suggested by the involvement of motilin in phase III of the gastric MMC, the return of which is used a marker of recovery.

At first, the clinical efficacy of erythromycin as a prokinetic agent may seem surprising in view of the short-lived action of motilin itself; indeed tolerance to repeat-dose erythromycin has been observed (Richards et al., 1993; see below). To understand these apparently conflicting observations, Dass et al. (2003a) and Jarvie et al. (2007) conducted a series of experiments with rabbit isolated stomach, in which the pharmacology of motilin receptor agonists were examined for their abilities to facilitate cholinergically mediated contractions evoked by electrical field stimulation. This model is argued to provide a more physiological representation of their abilities to facilitate on-going gastric emptying (in which the ENS plays a key role), than alternative models which simply investigate their effects on resting muscle tension. Using this technique, low concentrations of motilin were found to greatly facilitate cholinergic activity but in a short-lived manner, consistent with a role for motilin in the short-lasting gastric phase III MMC activity. However, low concentrations of erythromycin induced long-lasting increases in cholinergic activity which did not rapidly fade; higher concentrations of erythromycin evoked greater but shorter-lived responses and also increased muscle tension. Similar observations have now been made using human isolated stomach in which contractions were evoked by electrical field stimulation (Broad et al., 2010). Together, these observations are consistent with the finding that low doses of erythromycin (50–100 mg 3× daily and at bedtime) reduced symptoms in patients with gastroparesis (Dhir and Richter, 2004) whereas higher doses may tolerate with repeat dosing (250–400 mg 4× daily; Richards et al., 1993). Similarly, symptoms associated with gastroparesis may be improved by repeated intravenous-administration of erythromycin, provided the dose was titrated to achieve efficacy and tolerance in each patient (Dibaise and Quigley, 1999). Further, in human volunteers, a low dose of erythromycin (40 mg) evoked an increase in gastric propulsive activity which could be prevented by atropine (Coulie et al., 1998), whereas a higher dose (200 mg) evoked atropine-resistant, non-propulsive contractions (Coulie et al., 1998) and an increase in meal-induced satiety (Cuomo et al., 2006). Thus, low doses of erythromycin appear to behave in a manner which differs from that of motilin or high doses of erythromycin. The explanation is not clear and speculation centers on possible different binding sites for the peptide and non-peptide structures (Matsuura et al., 2006). The isolated tissue results are also consistent with observations that repeat dosing with low doses of erythromycin may increase gastric emptying whereas higher doses can induce nausea and stomach cramping (Boivin et al., 2003).

The experiments described above argue that it is possible to identify alternative molecules which mimic the actions of erythromycin but in a more selective manner, without the antibiotic activity or ability to inhibit P450 activity. Until recently, these molecules were based on the complex macrolide structure of erythromycin, where small changes in structure removed antibiotic activity but additional changes to optimize the "drug-like" properties of the molecule in an iterative manner are extremely difficult; to date, most have not progressed far in the clinic (Westaway and Sanger, 2009). **Table 1** lists the motilin receptor agonists recently described.

The most advanced is mitemcinal, the remaining macrolide still in clinical development (Takanashi and Cynshi, 2009), which has shown some ability to improve symptoms in a subset of patients with diabetic gastroparesis, good glycemic control and a body mass index of <35 kg/m^2 (McCallum et al., 2007). Interestingly, attempts to profile the desensitization liability of mitemcinal *in vitro* illustrate the fact that several assays commonly used to examine the functions of motilin *in vitro*, actually lack translational value. For example, the abilities of mitemcinal and other motilin receptor agonists to cause contraction of rabbit isolated duodenal muscle were reported to undergo rapid tachyphylaxis (Carreras et al., 2004). A desensitization liability was also noted using CHO cells expressing the human motilin receptor (Takanashi and Cynshi, 2009). By contrast, in human volunteers, the ability of mitemcinal to increase gastric emptying was maintained over a 28-day repeat-dose schedule (McCallum et al., 2007). This anomaly led to the suggestion (Sanger, 2008; Westaway and Sanger, 2009) that a more meaningful *in vitro* assay was to examine the abilities of motilin receptor agonists to facilitate on-going gastric neuronal activity, argued to represent a more physiological model of gastric motility. Using this model, GSK962040, the most advanced non-motilide small molecule motilin receptor agonist, has been shown to act in a manner similar to that of erythromycin, facilitating cholinergic contractile activity in rabbit (Sanger et al., 2009) and human (Broad et al., 2010) isolated stomach, in a prolonged manner. GSK962040 is currently being evaluated in healthy volunteers, where both singly administered (Dukes et al., 2009) repeated doses (Dukes et al., 2010) accelerated gastric emptying in a maintained manner.

Finally, RQ-00201894 has been described as a non-motilide, small molecule, potent, and selective motilin receptor agonist. However, its structure is currently unknown. This molecule is said to increase gastric emptying in dogs and monkeys, as well as induce gastric Phase III-like activity in dogs (Takahashi et al., 2010).

GHRELIN RECEPTOR ANTAGONISTS

The use of ghrelin-specific antibodies, immunization against endogenous ghrelin, "RNA Spiegelmers" such as l-NOX-B11 (oligonucleotides made stable by placing unnatural l-enantiomers of ribose in the sugar–phosphate backbone) and even a vaccine has shown that in adult animals (usually rodents), inhibition of on-going ghrelin function can reduce food intake and body weight (Foster-Schubert and Cummings, 2006; Zorrilla et al., 2006; Becskei et al., 2008; Depoortere, 2009). These data indicate the potential value of a small molecule ghrelin receptor antagonist (Moulin et al., 2007). Several have been shown to be active against the receptor that is naturally expressed (e.g., in rat isolated pituitary cells) and some have demonstrated an ability to reduce food intake and body weight in rodents as well as improve glucose tolerance (e.g., Esler et al., 2007, who used mice for the feeding experiments and rats for the glucose tolerance test). However, as yet, none have progressed into the clinic.

GHRELIN RECEPTOR AGONISTS

The multiple activities of ghrelin make this an interesting drug target. Its ability to increase appetite suggests a role in treatment of cachectic patients (Ashitani et al., 2009), as exemplified by

Table 1 | Motilin and ghrelin receptor agonists under development for the treatment of GI disorders.

Compound	Status	Structure
MOTILIN RECEPTOR AGONISTS		
Mitemcinal (GM-611)	Phase II; diabetic gastroparesis	
RQ-00201894	Preclinical	Small molecule, non-macrolide; structure not yet disclosed
GSK962040	Phase I	
GHRELIN RECEPTOR AGONISTS		
Ulimorelin (TZP-101)	Entering phase III as IV formulation for GI dysmotility conditions in acute care	
TZP-102	Phase II for gastroparesis	Orally active macrocyclic peptidomimetic
Ipamorelin	Phase II; for opioid-induced bowel dysfunction	
EX-1314 (previously known as BMS-604992)	Preclinical	dimethyl glycyl-*O*-benzyl-serine
ST-1141	Phase I; for opioid-induced bowel dysfunction	Structure not yet disclosed
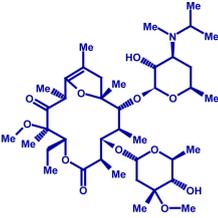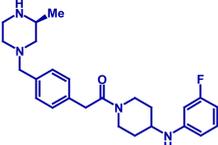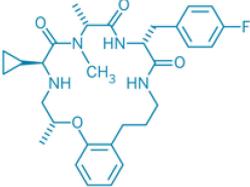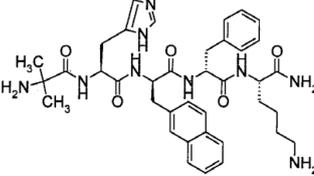
studies in cancer patients with impaired appetite where ghrelin increased energy intake and meal appreciation during a buffet meal (Neary et al., 2004). The ability of ghrelin to increase gastric emptying also suggests a role in treatment of disorders associated with delayed gastric emptying, as exemplified by animal models of post-operative gastric ileus (Trudel et al., 2003) or septic ileus (De Winter et al., 2004). Finally, an anti-emetic activity of ghrelin (Rudd et al., 2006), combined with the ability to increase appetite and gastric emptying suggests a potential to treat more complex conditions such as dyspepsia, as exemplified in an animal model of this condition (Liu et al., 2006) and as suggested by raised plasma ghrelin levels in patients with functional dyspepsia (Nishizawa et al., 2006).

Table 1 lists the ghrelin receptor agonists being evaluated for a GI disorder. In general, these compounds were first characterized using recombinant receptors, followed by studies *in vivo*, where their ability to increase gastric emptying and stimulate feeding may be dependent on vagal nerve activity (Page et al., 2007; Sanger, 2008). Success in the development of such an agent may lie in selecting a patient group where the new drug is only required on an acute basis (e.g., post-operative ileus), as a potential difficulty with a long-term exposure is the widespread ability of ghrelin to increase endocrine release (Leite-Moreira and Soares, 2007). The need for this caution is exemplified by a study which shows that constant intravenous infusion of ghrelin may provoke a fall in insulin sensitivity (Vestergaard et al., 2007).

Currently the most advanced is TZP-101 (ulimorelin), an intravenously administered macrocyclic peptidomimetic (Fraser et al., 2005) reported to improve GI recovery in patients with post-operative ileus after abdominal surgery (Popescu et al., 2009) and improve symptoms (loss of appetite, vomiting, post-prandial fullness) 4 days after treatment in patients with diabetic gastroparesis (Wo et al., 2010). A second molecule, TZP-102, is said to be orally available and have gastric prokinetic properties[2], but details have not been published

for critical review. Ipamorelin, a pentapeptide originally identified as a GH secretagogue (Raun et al., 1998) has been shown to be effective in a rat model of post-operative ileus when given by intravenous infusion (Venkova et al., 2009b). Other molecules include EX-1314, a GI prokinetic able to stimulate food intake in rodents (Charoenthongtrakul et al., 2009) and ST-1141, said to be an orally active for treatment of opioid-induced bowel dysfunction[3].

CONCLUSIONS

Recognition that the GI tract represents the largest endocrine organ in the body provides a wealth of opportunities for understanding the physiological control of GI functions and its pathophysiology. Not surprisingly, this increased understanding also creates new opportunities for drug discovery.

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[2]http://www.tranzyme.com/pipeline.html

[3]http://www.helsinn.com/

In this review we have focused on what happens to the stomach during hunger. It is becoming clear that phase III MMC contractions in the gastric antrum are involved in signaling feelings of hunger, pointing to a role for motilin as well as ghrelin in promoting this sensation. The absence of a functional motilin system in rodents has hampered investigations into possible roles for this hormone, but the availability of selective motilin receptor agonists suitable for human studies, now promises to open up this area of research. Finally, the post-translational processing of ghrelin, and its interactions with des-acyl-ghrelin and obestatin, reveals a complex physiology which now needs to be studied in patients with different disorders.

In terms of new drugs, the biology of the "hungry stomach" is already beginning to generate interesting investigational compounds which interact with both the motilin and the ghrelin receptors. Their progression through clinical trials is awaited with great interest.

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