Molecular, functional, and pharmacological targets for the development of gut promotility drugs

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The science of gastrointestinal motility has made phenomenal advances during the last fifty years. Yet, there is a paucity of effective promotility drugs to treat functional bowel disorders that affect 10–29% of the U.S. population. A part of the reason for the lack of effective drugs is our limited understanding of the etiology of these diseases. In the absence of this information, mostly an ad hoc approach has been used to develop the currently available drugs, which are modestly effective or effective in only a subset of the patients with functional bowel disorders. This review discusses a grounds-up approach for development of the next generation of promotility drugs. The approach is based on our current understanding of (1) the different types of contractions that produce overall motility function of mixing and orderly net distal propulsion in major gut organs, (2) the regulatory mechanisms of these contractions, (3) which receptors and intracellular signaling molecules could be targeted to stimulate specific types of contractions to accelerate or retard transit, and (4) the strengths and limitations of animal models and experimental approaches that could screen potential promotility drugs for their efficacy in human gut propulsion in functional bowel disorders.

smooth muscle; slow waves; enteric neurons; excitation-contraction coupling; peristaltic reflex; irritable bowel syndrome; prokinetic agents; gastroparesis; functional bowel disorders

5-HT4 receptor agonists were tested in the 1980s based largely on the hunch that 5-HT plays a prominent role in the CNS. Although the CNS and the enteric nervous system (ENS) share several common neurotransmitters, their respective functions and networking have more dissimilarities than similarities. Further testing of 5-HT4 receptor agonists indicated that they stimulate the classic peristaltic reflex, i.e., ascending excitation and descending inhibition in in vitro experiments in rodents and guinea pigs (24). This observation was taken as supportive evidence of the suitability of these agonists as promotility drugs. However, in non-rodsent and non-guinea pig species, especially in humans, most postprandial propulsion of digesta does not occur by the classic peristaltic reflex, i.e., ascending contraction and descending inhibition; instead, it.
occurs by rhythmic phasic contractions (RPCs) that do not produce descending inhibition (15, 64). This ad hoc approach may be one of the reasons that the currently available 5-HT₄ agonists are modestly effective or effective only in a subset of patients with functional bowel disorders in which transit is delayed in specific organs. A complete understanding of the mechanisms by which existing promotility drugs work is also lacking. The understanding of these mechanisms can be helpful in modifying these drugs or in testing of alternate drugs to obtain greater efficacy in a broader population of functional bowel disorder patients. On the other hand, the lack of full understanding of the mechanisms of action may lead to serious untoward side effects.

The objective of this editorial is to present a grounds-up approach to developing promotility drugs that may yield more effective therapeutic effects for the treatment of motility disorders. These strategies are based on our current understanding of 1) the different types of contractions that produce overall motility function of mixing and orderly net distal propulsion in major gut organs, 2) the regulatory mechanisms of these contractions, 3) which receptors and intracellular signaling molecules could be targeted to stimulate specific types of contractions to accelerate transit, and 4) the strengths and limitations of animal models and experimental approaches that could screen potential promotility drugs for their efficacy in human gut propulsion. The focus of this review is on the development of the next generation of promotility drugs, rather than on the retrospective analysis of the existing drugs.

Types of Gut Contractions, Their Regulatory Mechanisms, and Roles in Motility Function

The circular smooth muscle cells in intact animals and human subjects generate three distinct types of contractions for distal propulsion of digesta (54): 1) RPCs (Fig. 1A), 2) giant migrating contractions (GMCs) (Fig. 1B), and 3) tonic contractions (TCs). The function, regulation, and spatial and temporal characteristics of these contractions differ.

RPCs. These contractions mix the ingested meal with exocrine, endocrine, and mucosal secretions and propel the digesta...
distally at relatively slow rates so that adequate time is available for digestion and absorption of nutrients. The RPCs are absent in the normal esophagus, because this organ does not require mixing, digestion, or absorption. The three important characteristics of these contractions that determine their propulsive efficacy are: 1) mean propagation distance in the distal direction, 2) mean amplitude, and 3) mean frequency of contractions (15). The distal propagation of RPCs is the most critical parameter that determines postprandial propulsion. Nonpropagating (randomly occurring) RPCs or RPCs that propagate very short distances cause mostly back and forth movements of digesta. The nonpropagating contractions frequently turn over the luminal contents, mix them with secretions, and expose the mixture uniformly to the absorptive mucosal surface. The propagation distance of RPCs depends on two factors: 1) the distance over which the slow waves are phase locked and 2) the contiguous distance over which the cholinergic motoneurons concurrently release ACh (57). The mean propagation distance of RPCs decreases steadily from the stomach to the rectum, which manifests as slower propulsion rates distally in the gut. This characteristic of propagated contractions is consistent with the digestive and absorptive functions of the stomach, small intestine, and colon. The maximum mean distance of propagation of RPCs in the stomach and the duodenum, where the slow waves are generally phase locked, is still only a few centimeters. The RPCs propagate very little in the colon (Fig. 1A).

A larger amplitude of RPCs enhances their efficacy of propulsion by a greater or complete occlusion of the lumen so that the digesta does not escape through the partial opening of the lumen and get left behind during propulsion by a propagating RPC. The frequency of propagating RPCs determines how many times digesta is propelled per unit time and hence the total volume of propulsion in a given time period.

It is noteworthy that the RPCs do not produce the classic descending inhibition of spontaneous contractions and/or relaxation of tone in the distal segment (15, 64), which is a hallmark of peristaltic reflex (5). These contractions propel digesta relatively slowly in the distal direction and over short distances at a time. Therefore, the distal segment does not need to distend beyond its maximal filling to accommodate a large bolus. The luminal diameters of human small intestine and the colon are ∼2–4 cm. The luminal diameters size has to increase beyond these dimensions to produce distension. Therefore, the RPCs do not fit the criteria of classic peristaltic reflex as defined originally by Bayliss and Starling (5).

**GMCs.** GMCs are large-amplitude and long-duration ultrapropulsive contractions that strongly occlude the lumen and rapidly propagate over long distances in the esophagus, small intestine, and colon (11, 32, 41, 56). The GMCs do not occur in the stomach. In the esophagus, these contractions occur after each swallow; they also occur spontaneously in the distal esophagus to rapidly clear the refluxed acid. In the small intestine and colon, these contractions occur spontaneously in the terminal ileum and the proximal colon ∼2–5 times a day (1–3, 12, 32, 49, 51, 56). These contractions also precede defecation and provide the force for rapid evacuation of feces (Fig. 1B). The large lumen-occluding amplitude of GMCs, their long duration and rapid propagation over long distances produce mass movements of the digesta.

The strong compression of the gut wall by the large amplitude of GMCs activates the sensory receptors to trigger descending inhibition of spontaneously occurring RPCs and relaxation of muscle tone (4, 11, 49). This descending inhibition facilitates mass movements in two ways. The inhibition of spontaneous RPCs in the segment distal to a GMC reduces the resistance to rapid propulsion of digesta by the GMC. Furthermore, relaxation of the distal segment allows it to distend without an increase in tone to accommodate the large volume of digesta being propelled rapidly and without triggering nociceptors. The GMCs fit the criteria of the classic peristaltic reflex (5, 22, 23). The descending inhibition triggered by GMCs in respective organs also relaxes the lower esophageal sphincter (LES), ileocecal junction, and internal anal sphincter to let the luminal contents pass through without resistance (4, 40). The spontaneously occurring RPCs in the sigmoid colon/rectum and ileum do not produce descending inhibition to relax the internal anal and ileocecal sphincters, respectively. Note also that the descending relaxation of the LES may also contribute to impaired relaxation of the LES in these motility disorders.

The generation and distal propagation of GMCs do not depend on slow waves. The distance of their propagation, therefore, is not constrained by the distance over which the slow waves are phase locked, which is the case for RPCs (57). This distinction is critical for the production of mass movements by GMCs in the distal small intestine and the entire colon, where slow waves exhibit little or no phase locking and RPCs are unable to propel digesta rapidly. The descending distension due to the bolus being propelled by GMC is not in itself sufficient to propagate GMCs, because a transaction and reanastomosis in small intestine interrupts the distal propagation of GMCs, but it allows the bolus to pass beyond the reanastomosis (49). The precise mechanisms of generation of GMCs are not understood. However, the GMCs require intact interneurons to propagate. Available data show, however, that GMCs may be generated by large and sustained release of ACh at the neuroeffector junction (58). The sustained accumulation of ACh stimulates different signaling pathways for excitation-contraction coupling in circular smooth muscle cells than those stimulated by short-duration release of ACh that generates RPCs (6, 58). For example, neostigmine, a cholinesterase inhibitor, stimulates colonic GMCs by accumulating ACh at the neuroeffector junction (17, 32). In a physiological setting, the slow excitatory postsynaptic junctional potentials (sEPSPs) may release ACh at the neuromuscular junction for a longer duration resulting in its accumulation and stimulation of GMCs. In the small intestine, CGRP has been found to stimulate sEPSPs (50), and studies in intact animals show that it also consistently stimulates GMCs by release of ACh at the neuromuscular junction (55).

**TCs.** The increase of tone that decreases the luminal diameter, by itself, has little or no effect on mixing or propulsion in the small intestine and the colon. However, as a result of decrease in luminal diameter by increase in tone, the same amplitude of RPCs would occlude the lumen more effectively and therefore be more effective in propulsion. The tone of the small intestine and colon is increased after ingestion of a meal (13, 16, 30). The cellular signaling pathways for excitation-
contraction coupling to generate tone are different from those that generate RPCs and GMCs (27, 55).

**Strategies for the Design and Development of Gut Promotility Drugs**

According to the above, gut transit can be accelerated by enhancing the amplitude, frequency, and mean propagation distance of RPCs, by stimulating GMCs and by increasing smooth muscle tone. The two basic steps in the development of an effective promotility drug are 1) identify the type(s) of contractions that should be stimulated to accelerate transit in the desired gut organ and 2) identify the most suitable receptor and/or signaling molecule whose activation would preferably stimulate that contraction(s). The strategies for these steps are discussed below.

**Suitability of specific types of gut contractions to accelerate transit.** RPC. The postprandial transit of digesta may be delayed due to a decline in the overall incidence of RPCs resulting in a decrease in the mean amplitude and frequency of contractions. For this condition, the RPCs can be an effective target for stimulation by promotility drugs to restore normal transit. However, in several functional bowel disorders, the amplitude and/or frequency of postprandial RPCs may not be decreased or they may even be enhanced (8). The delay in transit in these conditions is due to a decrease in the mean distance of propagation of RPCs or near total absence of propagating RPCs (14). The stimulation of RPCs in these conditions may be ineffective in accelerating transit or it may further retard transit due to the stimulation of nonpropagating RPCs. As noted above, the propagation distance of RPCs depends on the distance over which slow waves are phase locked. Due to poor electrical coupling among circular muscle cells in the terminal ileum and colon, the slow waves are largely phase unlocked. Therefore, the stimulation of RPCs for accelerating transit in these parts of the gut may be counterproductive or marginally effective.

The stimulation of RPCs is an effective target for the acceleration of gastric emptying in conditions, such as diabetic or idiopathic gastroparesis. However, the regulation of gastric emptying is multifactorial and complex. It depends on coordination among several mechanisms, including pyloric contraction and tone, antro-pyloro-duodenal coordination, fundic adaptive relaxation followed by gradual increase in tone, and propagating RPCs in the body of the stomach (25, 46). It is, therefore, critical that the stimulation of gastric RPCs by promotility drugs does not adversely affect the other regulatory mechanisms so as to negate their beneficial effects (65). For example, concurrent stimulation of RPCs in the duodenum or increase of pyloric tone and RPCs may deteriorate antro-pyloro-duodenal coordination and adversely affect the rate of gastric emptying (25, 65). In this regard, erythromycin that accelerates the rate of gastric emptying stimulates the postprandial RPCs in the stomach but it suppresses them in the duodenum (65).

The stimulation of RPCs can also be effective in restoring the normal rate of transit in small intestine and colon when RPCs are suppressed in conditions such as idiopathic intestinal pseudoobstruction (33).

GMC. The stimulation of GMCs is an ideal target to accelerate colonic transit in functional bowel disorders, such as idiopathic constipation and constipation predominant irritable bowel syndrome (IBS-C). The advantages of stimulating colonic GMCs in these conditions are 1) they are very effective in mass propulsion over long distances because their generation and propagation do not depend on slow waves; therefore, any defect in the generation or phase locking of slow waves would not affect the rapid propulsion by GMCs, 2) the GMCs in the distal colon produce descending inhibition to relax the internal anal sphincter; this would facilitate defecation and partially or completely overcome outlet obstruction (4); the stimulation of RPCs would not achieve this effect, and 3) the strong propulsive force of a GMC can propel hardened or impacted feces due to constipation; the stimulation of RPCs may not achieve this effect.

The limitations of stimulating GMCs to accelerate transit are 1) overstimulation of GMCs could produce frequent mass movements and hence diarrhea; this limitation may be overcome by adjusting the dose and frequency of the promotility drug and 2) in patients with visceral hypersensitivity, the stimulation of GMCs may exacerbate the sensation of abdominal cramping; this may happen if the descending inhibition is defective (54).

The ultrarapid propulsion of bolus in the esophagus is produced by a GMC that follows a voluntary swallow. In conditions such as achalasia and diffuse esophageal spasm, the GMCs are replaced by simultaneous or randomly occurring smaller-amplitude RPCs that are ineffective in rapid propulsion of the swallowed bolus (9). In addition, the absence of spontaneous secondary GMCs in the distal esophagus in response to acid reflux may impair its rapid and effective clearance and contribute to the development of esophagitis. The stimulation of esophageal GMCs, therefore, would be an attractive target for relieving the symptoms of gastroesophageal reflux, achalasia, and diffuse esophageal spasm.

The stimulation of GMCs in the small intestine would also accelerate transit. However, most absorption of nutrients occurs in this organ, and rapid mass movements produced by GMCs would deprive the digesta of adequate time required for digestion and absorption. The ultrarapid emptying of nutrients from the small intestine into the colon may also increase osmotic load and result in diarrhea. Therefore, stimulation of GMCs to accelerate postprandial transit in the small intestine may not be a desirable target, except on a short-term basis. The GMCs do not occur in gastric smooth muscle cells and, therefore, they cannot be the targets of promotility drugs to accelerate gastric emptying.

Several studies show that diarrhea in inflammatory bowel disease and diarrhea predominant IBS patients is due to an increased frequency of occurrence of GMCs (see Ref. 54 for full details). For these conditions, selective but partial inhibition of GMCs would have beneficial effects.

TC. The increase of tone only indirectly enhances the transit rate in the small intestine and the colon by enhancing the efficacy of RPCs. However, the increase of postprandial fundic tone is an effective target to enhance the rate of gastric emptying. The increase of postprandial fundic tone (or the blockade of adaptive relaxation) would transfer the fresh digesta more rapidly from the fundus to the body of the stomach in preparation of its emptying by gastric RPCs. Note, however, that an increase in postprandial tone may reduce food intake because of early satiety signals.
Selection of Enteric Neurons and/or Circular Smooth Muscle Cells as Targets of Promotility Drugs

The regulatory mechanisms of neurotransmitter release from the enteric neurons and excitation-contraction coupling in circular smooth muscle cells together determine the types of gut contractions generated and their spatiotemporal characteristics (Fig. 2). Obviously, therefore, they serve as the most suitable targets of promotility drugs. The reader is referred to other reviews for a complete discussion of these regulatory mechanisms (38, 39, 54, 57, 79). A brief summary of these regulatory mechanisms that is relevant to the design and development of promotility drugs follows.

The end point of enteric neural regulation of motility function is the release of ACh by cholinergic excitatory motoneurons and the release of nitric oxide (NO) and VIP by the nonadrenergic noncholinergic (NANC) inhibitory motoneurons. The motoneurons (S type) receive inputs from interneurons, intrinsic sensory neurons (ISNs), and intrinsic spontaneously active neurons (ISANs) at nicotinic receptors (Fig. 2A) (54). The ISANs spontaneously generate excitatory postsynaptic potentials (EPSPs). The cell bodies of these neurons are localized in the myenteric plexus and they innervate the motoneurons directly or through interneurons. On the other hand, the ISNs whose cell bodies are in the myenteric and submucosal plexi and sensory endings in the mucosal layer generate EPSPs largely in response to stimulation, such as that produced by nutrients in the lumen or mechanical stimulation of the mucosa (Fig. 2A). The phase II and phase III contractions of

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**Fig. 2.** A: organization of enteric neurons and circular smooth muscle cells to regulate the occurrence of contractions. The intrinsic sensory neuron (ISN) and intrinsic spontaneously active neuron (ISAN) both stimulate the excitatory and inhibitory motoneurons at nicotinic receptors (N). The neurotransmitter of the excitatory motoneuron is ACh and of inhibitory motoneuron nitric oxide (NO) and VIP. For the regulation of RPCs, the smooth muscle cell generates slow waves (right bottom). A contraction occurs only when there is the release of ACh during a slow-wave depolarization. The release of ACh increases the depolarization of the slow wave beyond excitation threshold to produce spikes. The spikes intermittently depolarize the membrane to the range of 10–30 mV, which causes $\text{Ca}^{2+}$ influx through the L-type calcium channels, and the intracellular excitation-contraction coupling is initiated as shown in Fig. 3. Note that the generation of GMCs and tonic contractions (TCs) does not require slow waves. B: units like those shown in A are arranged along the length and circumference of the gut and connected by interneurons. A network along the length of the gut is shown here. The interneurons can initiate both ascending and descending excitations and inhibitions. The smooth muscle cells are coupled electrically, but the strength of coupling decreases distally in the gut. For this reason the slow waves are phase locked in the stomach and the duodenum, and this phase locking deteriorates distally. The network of ganglia connected by interneurons and electrical coupling between adjacent circular smooth muscle cells are critical to produce propagated contractions (RPCs and GMCs).
the migrating motor complex in the fasting state result from spontaneous activity of ISANs (38, 43). The trigger for the release of neurotransmitters to stimulate phase II and phase III contractions in the interdigestive state does not come from the ISNs. There is no nutritional digesta in the gastric and small intestinal lumen during the interdigestive state.

ACh acts mainly on muscarinic M3 receptors on circular smooth muscle cells (72) and VIP acts on VPAC2 receptors (45), and NO permeates through the membrane to activate soluble guanylyl cyclase. The M3 and VPAC2 are G protein-coupled receptors through which they activate multiple intracellular signaling pathways (Fig. 3) (39, 54). The signaling pathways activated by these neurotransmitters are determined by their respective receptor subtypes and associated G proteins, as well as by the amount and duration of accumulation of the neurotransmitter at the neuroeffector junction (6, 58). NO activates its signaling pathways through cGMP. The end point of activation of intracellular signaling pathways by excitatory neurotransmitters is phosphorylation of 20-kD myosin light chain (MLC20), which initiates cross-bridge cycling and smooth-muscle contraction (Fig. 3). On the other hand, the end point of activation of signaling pathways by inhibitory neurotransmitters is dephosphorylation of MLC20 (44). The net intensity and duration of MLC20 phosphorylation determine the amplitude and duration of cell contraction. In a simplistic way, MLC20 is phosphorylated by myosin light chain kinase (MLCK) and dephosphorylated by myosin light chain phosphatase (MLCP). The net intensity and duration of phosphorylation of MLC20, therefore, depends on the relative intensities and time courses of MLCK and MLCP activities. The signaling pathways stimulated by ACh activate MLCK and inhibit MLCP concurrently to enhance the phosphorylation of MLC20 (73, 74). The signaling pathways stimulated by NO and VIP activate MLCP as well as decrease \([Ca^{2+}]_i\) and MLCK activity. In summary, therefore, the type of contraction stimulated by a promotility drug is determined by the kinetics of excitatory and inhibitory neurotransmitter release by the enteric motoneurons and by the activation of intracellular signaling pathways that compete for the phosphorylation of MLC20 in circular smooth muscle cells. The strategies that may help in selecting enteric neuronal receptors and intracellular signaling molecules in circular smooth muscle cells to stimulate the three types of contractions are discussed below.

Strategies in the Selection of Molecular and Pharmacological Targets of Promotility Drugs

Slow transit may occur due to a defect in the release of excitatory and inhibitory neurotransmitters from the enteric motoneurons and/or due to a defect in the excitation-contraction coupling in circular smooth muscle cells (Fig. 2A). The following strategies could be used in the development of an effective promotility drug to accelerate transit using the enteric neurons and circular smooth muscle cells as targets.

General strategies. In normal gut motor function, the motoneurons receive inputs directly from ISNs and ISANs, or indirectly through interneurons to release excitatory and inhibitory neurotransmitters. Together, these neurons are referred to as presynaptic neurons. Numerous types of receptors have been identified on presynaptic neurons by using in vitro pharmacological and electrophysiological approaches (7, 19, 79). However, the specific receptor types or subtypes on these neurons that mediate the in vivo postprandial release of ACh from the motoneurons remain unknown due to the limitations of in vivo measurements. Nevertheless, all those receptor types on presynaptic neurons that release ACh from the excitatory motoneurons in vitro are potential candidates for the stimulation of RPCs and GMCS and the increase of tone. Our current knowledge of the site of neuronal defects that retard gut transit in motility disorders, such as gastroparesis, idiopathic constipation, and constipation-predominant IBS, is severely limited. In the absence of this knowledge, it may be prudent to pick a...
neuronal receptor target that is located as close as possible to the motoneurons. If the neuronal defect is between the receptor to be stimulated and the motoneurons, then the defect is likely to impair the efficacy of the promotility drug in patients, even though it demonstrates efficacy in normal healthy subjects. In this regard, 5-HT₄ agonists that are thought to act on 5-HT₄ receptors on mucosal sensory nerve endings of ISNs may be at a disadvantage. These receptors are located farthest away from the motoneurons. This may be one of the reasons that 5-HT₄ agonists are effective only in a subset of IBS-C patients.

Although gut smooth muscle cells contract on the release of ACh from the excitatory cholinergic motoneurons, exogenous cholinergic agonists are ineffective in accelerating transit by the stimulation of RPCs. These drugs bypass the enteric nervous system and they act concurrently and directly on circular muscle cells everywhere, resulting in stimulation of simultaneous or nonpropagating RPCs. Furthermore, strong stimulation of muscarinic receptors on smooth muscle cells may uncouple slow waves at adjacent sites and suppress propagation of contractions that would further retard transit. Cholinesterase inhibitors that accumulate ACh at the neuromuscular junction also stimulate nonpropagating RPCs for the same reason. However, as noted above, the accumulation of ACh at the neuroeffector junction may also stimulate GMCs, which are highly propulsive irrespective of the slow waves (17, 32).

Specific strategies for stimulation of RPCs and GMCs. RPCs are highly propulsive irrespective of the slow waves (17, 32). The latter probability is consistent with the data that the interneurons, ISNs, and ISANs provide inputs to both the excitatory and inhibitory motoneurons at nicotinic receptors. Accordingly, a preferred target of promotility drugs would be those presynaptic neurons that innervate both the excitatory and inhibitory motoneurons. The simulation of RPCs at their maximal rate by targeting only the excitatory motoneurons may be counterproductive.

Concurrent recordings of EPSPs from the enteric neurons and the contractions that they stimulate are not available. However, it seems likely that RPCs, whose duration is short when compared with those of GMCs or TCs are stimulated by a single or a group of fast EPSPs. The S type interneurons, but not the S type motoneurons are therefore the attractive targets of promotility drugs to stimulate intermittently propagating RPCs of variable amplitude (Fig. 2B). The presynaptic locus of action of a potential promotility drug, therefore, must be ascertained.

Second, the intermittently occurring phase II RPCs of the migrating motor complex are nearly as effective in propulsion as the postprandial contractions (64, 75). Therefore, a drug that stimulates phase II-like or phase III-like contractions in the interdigestive state likely acts on presynaptic ISANs to stimulate RPCs and is, therefore, likely to accelerate postprandial transit in the stomach and small intestine. Note that phase III-like contractions do not occur in the postprandial state. The stimulation of phase III-like contractions by a potential promotility drug during the interdigestive state is only an indication of its locus of action on presynaptic ISANs. In this regard, motilin and erythromycin that stimulate phase II-like and phase III-like contractions in the interdigestive state have demonstrated potential in accelerating postprandial transit in the stomach and small intestine. In the postprandial state, the phase II-like contractions triggered by ISANs are potentiated by additional input to the motoneurons by the activation of ISNs by the digesta.

Third, the RPCs result from competing inputs to the smooth muscle cells for excitation-contraction coupling by the excitatory (ACh) and inhibitory neurotransmitters (NO/VIP) (Fig. 2A). Theoretically, the RPCs can be enhanced either by the release of ACh or inhibition of NO and VIP. However, most studies show that inhibition of the inhibitory nitricergic neurons retards gastric emptying, although it stimulates the RPCs (47). This is due to the stimulation of pyloric and duodenal contractions by unopposed action of ACh, resulting in the impairment of antro-pyloro-duodenal coordination (47, 48). The blockade of inhibitory neurons, thus, may not be an effective target of promotility drugs.

Fourth and finally, slow waves determine the timing, maximum frequency, and maximum propagation distance of RPCs (Fig. 2B). Electrical stimulation of slow waves by implanted electrodes has been reported to accelerate the rate of gastric emptying in normal animals (10). However, this method may not work if the delayed gastric emptying is due to the impaired release of ACh or due to a defect in excitation-contraction coupling. Slow waves, by themselves, do not generate contractions. It requires the release of ACh during membrane depolarization to generate an RPC (57). Furthermore, electrical stimulation can increase slow-wave frequency only marginally, and when the slow-wave frequency is increased, the distance over which they are phase locked decreases in the small intestine (61, 62), resulting in reduced distance of propagation of RPCs. On the contrary, reversing the direction of propagation of slow waves in the stomach or the duodenum or by stimulating them in pyloric smooth muscle cells to disrupt antro-pyloro-duodenal coordination can effectively retard the rate of gastric emptying (35, 80). This may have beneficial effects by inducing early satiety in obese patients (81).

Concurrent electrical stimulation of enteric neurons and slow waves has been largely unsuccessful in accelerating intestinal transit because ACh release by electrical stimulation of neurons is not similar to its spontaneous intermittent release under physiological conditions. Therefore, the spatial pattern of RPCs produced by concurrent electrical stimulation of slow waves and enteric neurons does not mimic the intermittent spatiotemporal pattern of postprandial contractions. Furthermore, electrical stimulation of enteric neurons can be accomplished only at a few discrete locations, not over the entire organ.

GMC. First, most evidence indicates that excessive release or accumulation of ACh at the neuroeffector junction stimulates GMCs (17, 32, 55). The excessive release of ACh may be due to the stimulation of slow EPSPs that can generate a long series of action potentials lasting longer than 10 s (50, 79). Close intra-arterial administration of CGRP that stimulates sEPSPs consistently generates GMCs in the small intestine (55). Systemic administration of guanethidine also transiently generates GMCs by blocking the inhibitory effect of norepinephrine on.
the release of ACh at the presynaptic terminals (77). Likewise, systemic or close intra-arterial administration of anticholines- terase neostigmine stimulates GMCs (17, 32). In all these cases, the GMCs propagate distally and produce mass movements. The receptors that stimulate GMCs are not the same in the small intestine and the colon. For example, CGRP stimulates GMCs in the small intestine, but not in the colon (55, 77). On the other hand, close intra-arterial administration of substance P stimulates GMCs in the colon, but not in the small intestine (31). Substance P acts directly on smooth muscle cells to stimulate colonic GMCs (77), whereas CGRP acts on presynaptic neurons to release ACh and hence stimulate small intestinal GMCs. As noted earlier, the propagation of GMCs does not depend on slow waves. Therefore, either smooth muscle or enteric neural receptors are effective targets to stimulate them to enhance transit. In the neurons, the drugs that stimulate slow EPSPs may be more effective in generating GMCs. The stimulation of GMCs by a direct action of promotility drugs on circular smooth muscle cells is an attractive option in cases where the delayed transit is due to neural defects. Furthermore, a GMC can enhance propulsion regardless of whether the delayed transit is due to the suppression of RPCs or enhancement of nonpropagating RPCs.

Second, accumulating evidence over the last two decades shows that the signaling pathways for excitation-contraction coupling in smooth muscle cells differ for the generation of the three types of gut contractions (27, 55). This opens up tremendous opportunities for targeted activation of intracellular signaling molecules by promotility drugs to selectively stimulate a specific type or types of contractions, particularly the GMCs that do not depend on slow waves for their generation or propagation. As noted above, the targeting of intracellular signaling molecules for excitation-contraction coupling is particularly attractive if slower transit is due to impaired neurotransmitter synthesis or release from the motoneurons. In this case, pharmacological stimulation of receptor on presynaptic neurons may yield limited beneficial effect in patients. The potential of promotility drugs to enhance excitation-contraction coupling in smooth muscle cells has not been tapped yet.

Third, recent studies (37, 69–71) show that the expression of receptors, G proteins, ion channels, and signaling molecules for neurotransmitter release and excitation-contraction coupling in smooth muscle cells is highly plastic. For example, the expression of the pore-forming subunit of L-type Ca$^{2+}$ channels is suppressed in colonic inflammation and this contributes to the suppression of RPCs and tone (36, 69). More important for the design of promotility drugs, the expression of key signaling molecules for excitation-contraction coupling can be enhanced by neurotransmitters, such as VIP and ACh (67, 68), and presumably by sustained exposure to other pharmacological compounds that can stimulate appropriate transcription factors in circular smooth muscle cells. The treatment of human colonic circular smooth muscle cells with VIP induces gene expression of the $\alpha_{1C}$ subunit of L-type of Ca$^{2+}$ channels, whereas the treatment of these cells with ACh enhances the expression of MLC$_{20}$. In both cases, the contractile response of muscle strips incubated with VIP or ACh is enhanced compared with strips incubated with medium only. VIP induces the gene expression of $\alpha_{1C}$ by activating adenyl cyclase, synthesizing cAMP, and phosphorylating PKA, which phosphorylates transcription factor cAMP response element binding (CREB) protein (67). The promoter ($\alpha_{1C}$) of the $\alpha_{1C}$ gene that is 5’ to exon 1b has two binding sites for CREB (67, 69). These findings indicate that the impairment of ACh release from the enteric neurons may be compensated by enhancing the expression of key signaling molecules for excitation-contraction coupling in smooth muscle cells. The targeting of specific molecules for excitation-contraction coupling in smooth muscle cells may allow for selective enhancement of the three types of gut contractions in different organs.

Strengths and Limitations of Experimental Models for Screening and Preclinical Testing of Promotility Drugs

The ultimate test of the efficacy of a promotility drug is its clinical trial in patients with delayed transit. However, prior to this expensive undertaking, the potential compounds can be tested in several experimental models to evaluate their chances of success. Furthermore, an understanding of the mechanisms of action of potential promotility drugs and their locus of action would help in modifying them if the final tests do not meet clinical expectations. Some of the information that can be sought in these models include the following: 1) Does the compound stimulate or inhibit circular smooth muscle contractions? 2) Which type(s) of contraction does it stimulate? 3) Does it accelerate transit in normal gut as well as in an animal model of delayed transit? 4) What is the locus of action (postsynaptic or presynaptic neurons, or circular smooth muscle cells) of the compound, and which receptors and intracellular signaling molecules mediate its effects. A brief summary of some of the commonly used experimental models and their strengths and limitations is given below.

Muscle bath. The stimulation of contractions in muscle strips is one of the most frequently used method for in vitro investigitations. The use of circular muscle strips is preferable because their contractions are more relevant to propulsion than those of longitudinal muscle strips. The circular muscle contractions constrict the gut lumen, which causes mixing and propulsion, whereas the longitudinal muscle contractions shorten the gut length, which has minimal effect on mixing and propulsion. The experiments in muscle strips can provide data on the types of contractions stimulated by the drug and its locus of action. The human and canine muscle strips in vitro generate only the tonic contractions and RPCs. On the other hand, the rodent circular muscle strips generate all three types of contractions in vitro (21). The limitations of the muscle bath method are that this model cannot predict whether the RPCs would propagate. This limitation can be overcome by follow-up transit studies in intact animals or in ex vivo segments. There is evidence that some of the enteric neurons, particularly the cholinergic neurons, are impaired in in vitro preparations (63). Thus the test drug may seem to act directly on smooth muscle receptors in muscle strips, whereas, in vivo, it acts preferentially on enteric neurons (34). Both of the above limitations of the in vitro muscle bath method can be overcome by the use of close intra-arterial infusions of test drugs in intact conscious animals. However, this method works primarily in large animals such as dogs (58).

Single dispersed cells and primary cultures of circular smooth muscle cells and enteric neurons. The freshly dispersed cells or primary cultures of circular smooth muscle cells and enteric neurons are ideal for the study of intracellular signaling...
pathways for excitation-contraction and excitation-inhibition couplings and neurotransmitter release, respectively. Numerous studies have shown that the primary cultures of circular smooth muscle cells and neurons maintain their phenotype (52). Also, the dispersed or cultured smooth muscle cells contract and, therefore, the effects of stimulation of specific signaling molecules can be related to contractile function. The major limitation of dispersed cells is that they generate initial and sustained contractions lasting about 30 s and 4 min or longer, respectively. The precise relationship between these contractions and those occurring in integrated systems (RPCs, GMCs, and TCs) are not known. However, studies of signaling molecules for excitation-contraction coupling by close intrarterial infusions of test substances indicate the similarities of utilization of the signaling molecules for in vitro and in vivo contractions (55, 58). The use of pure single dispersed cells and their primary cultures ensures the correct localization of proteins and mRNA of interest in these cells in molecular and genomic studies. The enteric neurons from adult animals do not proliferate in cultures, which limits the quantities of proteins and mRNAs that can be extracted from them for molecular biology experiments. It must be emphasized that the findings in single smooth muscle cells and enteric neurons must be confirmed in integrated systems, such as muscle strips and whole animals, for them to have relevance to drug development.

Transit studies. Several methods of measuring small intestinal, colonic, and whole gut transit as well as gastric emptying rate in animal models are available that can enhance confidence in the potential efficacy of new prokinetic drugs. Some of the important considerations in the use of these methods are that 1) the gastric emptying of a nutrient meal would give more relevant information than that of a nonnutrient meal. 2) The propulsion of artificial pellets in ex vivo segments is easy to measure and quantitate the transit rates. This method is adequate for compilation purposes. However, propulsion in ex vivo segments occurs without autonomic reflexes. 3) The propulsion of fecal contents in rodent colons occurs by GMCs, whereas in humans it occurs largely by RPCs with infrequent GMCs. The extrapolation of transit data from rodents to human colon merits further consideration. 4) Premature stimulation of phase II or phase III contractions of MMCs in the interdigestive state in intact animals by a promotility drug is only an indication that IS ANs are the locus of action of the drug. The phase III contractions do not occur in the postprandial state in nonruminants, and their premature stimulation cannot be taken as evidence of efficacy of a potential prokinetic drug.

In conclusion, a ground-up approach to developing new promotility drugs consists of 1) identification of the organ in which the transit rate needs to be accelerated and the most suitable type of contraction to be stimulated, 2) selection of appropriate receptors on presynaptic neurons and/or intracellular signaling molecules on circular smooth muscle cells as the targets of the drug, and 3) determination of whether the desired action is pharmacological or transcriptional (genomic). These steps are likely to result in more effective and specific promotility drugs. A pharmacological effect appears soon after the binding of the compound to its receptors or activation of an intracellular signaling pathway. A genomic effect is delayed because it requires transcriptional and translational processes to alter the expression of a receptor or a signaling protein to achieve the desired effect (67, 68). The transcriptional effects can be much longer-lasting when compared with pharmacological effects. The types of contractions generated in gut organs and their broad regulatory systems comprised of enteric neurons and excitation-contraction coupling in smooth muscle cells are similar except that all major organs do not generate all three types of contractions. However, some of the intracellular signaling molecules for neurotransmitter release and excitation-contraction and excitation-inhibition couplings as well as the receptors on presynaptic neurons differ among the major organs for stimulation of the same type of contraction. It is, therefore, unlikely that a given promotility drug would be equally effective in accelerating transit throughout the gut. On the other hand, these differences in signaling molecules can be used to develop promotility drugs targeted at a single organ and to stimulate a single type of contraction.

GRANTS

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REFERENCES


