Enteric locus of action of prokinetics: ABT-229, motilin, and erythromycin

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Sarna, Sushil K., Asensio Gonzalez, and Robert P. Ryan. Enteric locus of action of prokinetics: ABT-229, motilin, and erythromycin. Am J Physiol Gastrointest Liver Physiol 278: G744–G752, 2000.—We investigated the in vivo and in vitro locus of actions of prokinetics: motilin, erythromycin, and ABT-229. The test substances were infused close intra-arterially in short segments of the jejunum in the intact conscious state. Each prokinetic acted on a presynaptic neuron and utilized at least one nicotinic synapse to stimulate circular muscle contractions. The final neurotransmitter at the neuromuscular junction was ACh. Motilin and erythromycin, but not ABT-229, also released nitric oxide. Each prokinetic utilized somewhat different subtypes of muscarinic, serotonergic, tachykinergic, and histaminergic receptors, except for the M₃ receptor, which was common to all of them. In contrast, none of the prokinetics stimulated contractions in mucosa-free or mucosa-attached muscle strips, or rings, even though methacholine or electrical field stimulation induced phasic contractions in all of them. The prokinetics also did not release ACh in longitudinal muscle-myenteric plexus preparations. Each prokinetic, however, decreased the length of enzymatically dispersed single cells. In conclusion, each prokinetic may act on a different subset of presynaptic neurons that converge on the postsynaptic cholinergic and nonadrenergic noncholinergic motoneurons. The presynaptic neurons may be impaired in the muscle bath environment.

intestinal transit; gastric emptying; constipation; gastroesophageal reflux; enteric nervous system; smooth muscle

CLINICALLY SIGNIFICANT DELAY in gastric emptying and small intestinal and colonic transit occurs in a variety of diseases and conditions, including diabetes mellitus, anorexia nervosa, scleroderma, myotonic dystrophy, hypoparathyroidism, postvagotomy, postoperative ileus, and spinal cord injury. Delayed transit may also occur due to functional disorders of no known etiology, such as nonulcer dyspepsia, idiopathic intestinal pseudoobstruction, irritable bowel syndrome, and idiopathic constipation. Some therapeutic drugs (opiates, phenothiazines, tricyclic depressants, anti-Parkinson's disease medication, and ganglionic blockers) also delay transit. Some of the symptoms of delayed transit include bloating, early satiety, nausea and vomiting, gastroesophageal reflux, and constipation. Currently, there are few prokinetic agents available that can effectively normalize transit.

One class of prokinetic agents that has received attention during the last few years is motilides (28). These molecules, including ABT-229, are modifications of the macrolide molecule but are devoid of antibiotic activity. Whereas the molecular modification retains the ability of the new molecule to stimulate contractions, the efficacy and potency may be different from that of the original molecule. Moreover, it is not known whether even subtle molecular modification alters the site of action and receptors involved in stimulating gut contractions. Such alteration may account for differences in potency and efficacy and organ specificity of motilides.

It is now understood that the motility function of mixing and propulsion is regulated by a coordinated action of sensory and interganglionic neurons and motoneurons, neurotransmitters and their receptors, and smooth muscle cells (17). Pharmacological agents can stimulate contractions by acting on receptors located at a variety of locations on the neurons and smooth muscle cells. The aim of this study was to examine the in vivo and in vitro locus of action and types of receptors involved in the stimulation of canine jejunal circular muscle contractions by three potential prokinetic agents: motilin, erythromycin, and ABT-229. This information is important in targeting prokinetic molecules at specific receptor sites in the gut wall to normalize motility disorders. The studies were performed in vivo in intact conscious animals and in vitro in muscle bath and enzymatically dispersed single cells to examine whether the response of prokinetics and their locus of action differ among different experimental models.

EXPERIMENTAL METHODS

In vivo experiments. The experiments were performed on eight healthy conscious dogs of either sex, each weighing 20–26 kg (21.9 ± 0.81 kg). Dogs were anesthetized with pentobarbital sodium (30 mg/kg; Abbott Laboratories). Silastic catheters were implanted in two jejunal mesenteric arteries to infuse ~6-cm-long segments. Three strain gauge transducers were attached to the seromuscular layer in each infused segment to record circular muscle contractions. The catheters were tunneled subcutaneously to the subscapular region and exteriorized (8, 11, 18, 22). The catheters were housed in jackets that the dogs wore at all times. The lead wires of the transducers were brought out through an Amphe-
nol plug embedded in a stainless steel cannula (16). The dogs were allowed 1 wk to recover from surgery.

All experiments were performed after an overnight fast. The test substances were infused at 1 ml/min during phase I or a quiescent period of phase II activity. One milliliter of 0.9% saline infused at the same rate was used as flush. Identical infusion of the vehicles was used as control. At least a 20-min rest period was allowed between two subsequent infusions. Preliminary experiments indicated that there was no refractoriness to any test agent after this period.

In vitro muscle bath experiments. Mucosa-free and mucosa-attached circular muscle strips were prepared from the canine and rabbit jejunum and mounted in 3-ml baths filled with oxygenated (95% O2-5% CO2) Krebs solution (in mM: 137.4 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgSO4, 134 Cl-, 15.5 HCO3, 1.2 H2PO4, and 11.5 glucose, pH 7.4) at 38 ± 0.5°C. The muscle strips were stretched to 1 g and allowed to equilibrate for at least 60 min. Then the muscle strips were stretched incrementally until the amplitude of spontaneous contractions was maximal. The bathing solution was changed every 10–15 min.

Intact segments (0.5–1 cm in length) from the rabbit jejunum, duodenum, and antrum were mounted in 10-ml baths. These were mounted also in oxygenated Krebs solution as described above to record circular muscle contractions.

A Silastic catheter was implanted surgically in a canine jejunal mesenteric artery to perfuse a 1- to 2-cm-long segment. The segment was removed along with the mesentery and mounted in a 10-ml muscle bath. The test substances were infused through the close intra-arterial catheter instead of adding them to the muscle bath. The contractile response for all above experiments was quantified as the area under the curves (AUC) for each agonist or antagonist.

In vitro single cell experiments. A 7- to 8-cm-long segment of the canine jejunum was removed under general pentobarbital sodium anesthesia (30 mg/kg; Abbott Laboratories). The segment was passed over a glass tube and scored along the longitudinal axis with a blunt blade. The longitudinal muscle layer was peeled off and discarded. The remaining tissue was scored deeper. The circular muscle layer was peeled off and collected in ice-cold HEPES buffer (pH 7.4).

Smooth muscle cells were isolated by two consecutive digestions with papain and collagenase, respectively (14, 23). The tissue was washed with Hanks’ solution (pH 7.2) for 15 min. Then, they were incubated in 20 ml of Hanks' solution containing 0.38 mg/ml papain and 0.3 mg 1,4-dithiothreitol until the tissue appeared loose and sticky (~10 min). The tissue was washed with HEPES buffer and further digested at 31°C with 0.2 mg/ml collagenase type IV (319 U/mg) and 0.1 mg/ml soybean trypsin inhibitor for 30–40 min. The digested tissue was washed three times with enzyme-free HEPES buffer, and the muscle cells were allowed to disperse spontaneously under gentle to-and-fro motion. Cells were harvested by filtration through a 500-µm Nitex mesh and collected by centrifugation at 350 g for 5 min.

Cell length was measured by scanning micrometry as described previously (14, 23). An aliquot (0.45 ml of 5 × 104 cells/ml) was exposed to 50 µl of test agent at 31°C for 40 s. The reaction in each case was terminated by adding acrolein to a final concentration of 1%. In other experiments, the cell samples were incubated with porcine [Phe3,Leu3]Motilin (a motilin antagonist) for 5 min before addition of the agonists. The lengths of 30 consecutive intact healthy cells were measured using a phase-contrast microscope (Nikon), fitted with a video camera (Javelin CCD) and connected to a Macintosh computer. The NIH Image 1.61 program was used to measure the length. The contractile response was expressed as percent cell shortening from vehicle control.

Measurement of release of [3H]ACh. The release of [3H]ACh from the longitudinal muscle-mycenteric plexus preparations was measured by the method described by Takahashi and Owyang (25) and Collins et al. (2). A 2- to 3-cm-long jejunal segment was passed over a glass tube. A dull scalpel blade was used to score the serosal surface longitudinally. The external longitudinal muscle layer was cut out and removed. The longitudinal muscle-mycenteric plexus was peeled off by using wet sponges. The longitudinal muscle-mycenteric plexus tissue was cut into 2 × 2-mm pieces for stimulation with the prokinetic agents. The tissue pieces were incubated at 37°C with 0.2 µM [3H]choline (80 Ci/mmol, New England Nuclear, Boston, MA) in 3 ml oxygenated Krebs buffer supplemented with 50 µM physostigmine for 1 h. After incubation, the longitudinal muscle-mycenteric plexus strips were washed by changing oxygenated Krebs bathing solution every 3 min. The strips were further equilibrated by adding Krebs solution supplemented with 50 µM physostigmine and 10 µM hemicholinium. At this time, the basal release of [3H]ACh reached a plateau.

For stimulation with the prokinetics, the strips were transferred to incubation baskets (5 strips in each basket). At least three consecutive superfusates were collected before the strips were exposed to a prokinetic agent. Another three consecutive 3-min samples were collected after the exposure. Previous chromatographic studies (2, 25) have established that [3H]ACh and not [3H]choline is released when stimulated as described above.

Materials. ABT-229 (8,9-anhydro-4-deoxy-3-N-ethylthyroymycin B-6,9-hemiacetal) and erythromycin lactobionate were obtained from Abbott Laboratories. Canine motilin, porcine [Phe3,Leu13]motilin, L-703606 oxalate, L-659877, and mepyramine were purchased from RBI (Natick, MA). All other materials were purchased from Sigma Chemical (St. Louis, MO) and dissolved or diluted in sterile water. Methoctramine tetrahydrochloride, ranitidine hydrochloride, hexamethonium bromide, and TTX were purchased from Sigma Chemical (St. Louis, MO) and dissolved or diluted in sterile water. Methoctramine tetrahydrochloride, 4-diphenylacetoxy-N-(2-chloroethyl)-piperidine hydrochloride (4-DAMP), tropisetone, NAN-190 hydrobromide, LY-53857 maleate, tropisetone, SDZ-205557, L-703606 oxalate, L-659877, and mepyramine were purchased from RBI (Natick, MA). All substances were dissolved in sterile water. NAN-190 hydrobromide was dissolved in ethanol and diluted in sterile water. [Trp3,8,Ala6]neurokinin A4-10 was purchased from Peninsula Labs and dissolved in sterile water. Atropine sulfate was purchased from Fujisawa (Deerfield, IL). Motilin antagonist porcine [Phe3,Leu3]Motilin was supplied by Dr. Theo Peeters.

Data and statistical analysis. Statistical analysis was performed by one-way ANOVA. Multiple comparisons were performed by Student-Newman-Keuls test. Data are expressed as means ± SE with n representing the number of experiments. P < 0.05 was considered statistically significant. This study was approved by the Animal Care Committee at the Zablocki Veterans Affairs Medical Center.

RESULTS

In vivo experiments. Shi and Sarna (22) reported recently that close intra-arterial infusion of 4 µM methacholine for 1 min stimulates nearly the maximum response to this agonist. This response was taken as 100%. All three prokinetics, motilin, ABT-229, and...
erythromycin, concentration-dependently stimulated phasic contractions (Fig. 1). These contractions resembled those seen during phase II activity of the migrating motor complex and the postprandial state (Fig. 2). The time lags between the arrival of the prokinetic in the gut wall after accounting for the dead space were significantly different for ABT-229 (63 ± 5 s), motilin (19 ± 2 s), and erythromycin (8 ± 1 s). The order of potency was motilin > ABT-229 > erythromycin (EC₅₀, 1.5 × 10⁻¹⁰ M, 4.1 × 10⁻⁹ M, and 9.3 × 10⁻⁹ M, respectively), and the order of efficacy was motilin = ABT-229 > erythromycin (EC₃₅ₐₓ, 294 ± 83%, 286 ± 45%, and 88 ± 15% of the response to methacholine for motilin, ABT-229, and erythromycin, respectively). Intravenous infusion of the maximum doses of the agonists given dose intra-arterially did not stimulate contractions in the small intestine.

Enteric locus of action. ABT-229, motilin, and erythromycin infusions at 6, 0.2, and 16 µM, respectively, for 1 min were used to determine their locus of action. These concentrations produced the nearly maximum response (Fig. 1). The previously established effective concentration of atropine (30 µM for 1 min) was used to block muscarinic receptors, hexamethonium (70 µM for 1 min) to block nicotinic receptors, and TTX (25 µM for 1 min) to block Na⁺ channel enteric neural conduction (22). The infusion of prokinetics was started 1 min after the end of infusion of the antagonists. The response to each prokinetic agent was blocked almost completely by atropine, hexamethonium, and TTX (Fig. 3), indicating that they acted at a presynaptic receptor site, involved at least one nicotinic synapse, and induced release of ACh at the neuroeffector junction to stimulate contractions.

The inhibition of nitric oxide synthase (NOS) by prior close intra-arterial infusion of 10 mM nitro-L-arginine methyl ester (L-NAME) for 1 min had no effect on the response to ABT-229, but it enhanced the response to

Fig. 1. Concentration-response curves of ABT-229 (A), motilin (B), and erythromycin (C). All responses are expressed as %response to methacholine infused at 4 nmol/ml·min for 1 min (n = 6).

Fig. 2. Each prokinetic agent stimulated a series of phasic contractions when infused close intra-arterially. Contractions were similar to those seen during phase II activity of the migrating motor complex cycle or during the postprandial state.
motilin and erythromycin (n = 5 each; Fig. 3). The above dose of L-NAME has been reported previously to block NOS (8).

Types of receptors mediating the responses to ABT-229, motilin, and erythromycin. Each of the following antagonists was infused for 5 min at 1 ml/min. The prokinetics were coinfused for 1 min at the beginning of the third minute of infusion of each antagonist. The effective concentration of each of the antagonists has been established previously (8, 21, 22, 27).

Pirenzepine, methoctramine, 4-DAMP, and tropicamide were used to antagonize M₁, M₂, M₃, and M₄ receptors, respectively. Shi and Sarna (22) reported that infusing 4-DAMP at 2 µM for 5 min was effective in blocking M₃ receptors. Other muscarinic receptor antagonists were used at the same concentration. At higher concentrations, M₁, M₂, and M₄ antagonists exhibit nonspecific effects (22).

The responses to all three prokinetics were blocked almost completely by 4-DAMP (Fig. 4). The responses to ABT-229 and motilin were blocked partially but significantly by pirenzepine. In addition, the response to ABT-229 was also partially blocked by methoctramine. The response to erythromycin was not affected significantly by M₁, M₂, and M₄ receptor antagonists (Fig. 4).

NAN-190 hydrobromide (1.2 µM for 5 min), LY-53857 (2 µM for 5 min), tropisetron (2 µM for 5 min), and SDZ-205557 (2 µM for 5 min) were used to block 5-HT₁A, 5-HT₂/5-HT₁C, 5-HT₃, and 5-HT₄ receptors, respectively. The response to ABT-229 was blocked partially by the 5-HT₂/5-HT₁C receptor antagonist LY-53857 but was not affected by other 5-HT receptor antagonists (Fig. 5). The responses to motilin and erythromycin were not affected by any of the 5-HT receptor antagonists.

Fig. 3. Blockade of muscarinic receptors with atropine, nicotinic receptors with hexamethonium, and Na⁺ channel enteric neural conduction with TTX in the infused segment almost completely blocked contractile responses to ABT-229 (A), motilin (B), and erythromycin (C). Blockade of nitric oxide synthase by nitro-L-arginine methyl ester (L-NAME) enhanced the response to motilin and erythromycin but not ABT-229. In each case, the control response to the prokinetic alone was taken as 100%.

Fig. 4. M₃ muscarinic receptor antagonist 4-DAMP almost completely blocked the response to ABT-229 (A), motilin (B), and erythromycin (C). M₁ receptor antagonist pirenzepine blocked the response partially but significantly only to ABT-229 and motilin. M₂ receptor antagonist methoctramine partially blocked the response to ABT-229 only. M₄ receptor antagonist tropicamide had no significant effect on the response to any of the prokinetic agents.
L-703606, L-659877, and [Trp',β,Ala³]neurokinin A 4–10 (each at 1.6 µM for 5 min) were used to block NK₁, NK₂, and NK₃ receptors (27). The response to motilin was not affected by NK₁, NK₂, or NK₃ receptor antagonists (Fig. 6). In contrast, the response to ABT-229 was blocked by almost 50% by each of the three tachykinin receptor antagonists (Fig. 6). The response to erythromycin was only partially blocked by NK₂ and NK₃ receptor antagonists (P < 0.05, n = 6).

Mepyramine (15 µM for 5 min) and ranitidine (150 µM for 5 min) were used to block H₁ and H₂ receptors, respectively (27). H₁ and H₂ receptor antagonists had no significant effect on the response to motilin (Fig. 7). However, the response to ABT-229 was blocked partially by the H₂ receptor antagonist ranitidine and that to erythromycin by the H₁ receptor antagonist mepyramine (P < 0.05, n = 6).

In vitro muscle bath experiments. In contrast to the in vivo experiments, none of the prokinetic agents (10⁻² M ABT-229, 10⁻⁶ M motilin, and 10⁻⁵ M erythromycin) stimulated a contractile response in mucosa-free or mucosa-attached circular muscle strips from the jejunum of five dogs. Methacholine (10⁻⁶ M), however, contracted the muscle strips as has been reported previously (22). In one dog, 0.5-cm-wide rings of jejunal segments with mucosa attached also produced no response to 10⁻⁶ to 10⁻⁴ M ABT-229 and 10⁻⁵ M erythromycin. These rings exhibited spontaneous contractions.

Fig. 5. Only 5-HT₂/5-HT₄ receptor blockade with LY-53857 partially blocked the response to ABT-229. Antagonism of 5-HT₁, 5-HT₃, and 5-HT₄ receptors had no significant effect on the response to any of the prokinetics. A: ABT-229. B: motilin. C: erythromycin.

Fig. 6. Each of the NK₁, NK₂, and NK₃ receptor antagonists partially inhibited the responses to ABT-229 (A). None of these antagonists had any effect on the response to motilin (B). Only NK₂ and NK₃ receptor antagonists partially blocked the response to erythromycin (C).
and responded to $10^{-6}$ M methacholine. In one additional dog, a dose intra-arterial catheter was implanted in a mesenteric artery, and the perfused area was identified. This perfused segment was removed along with the mesenteric blood vessels and mounted in a 10-ml muscle bath. The segment exhibited spontaneous contractions and contracted in response to methacholine infused for 1 min at 4 µM. However, 5 or 10 µM ABT-229 infused for 1 min or 10$^{-2}$ M erythromycin infused for 1 min produced no response.

Next, we investigated whether the prokinetic agents would enhance the response when endogenous ACh was already being released by electrical field stimulation (EFS). EFS (75 V, 0.75 ms, 10 Hz for 5 s) consistently and reproducibly contracted jejunal circular muscle strips. Prior addition of 10$^{-4}$ M ABT-229 or 10$^{-4}$ M erythromycin had no significant effect on the response to EFS (data not shown). Similar results were obtained from antral and duodenal circular muscle strips (data not shown).

Circular muscle strips and segments were also obtained from the jejunum and duodenum of eight rabbits. This tissue exhibited a response to the first dose of 10$^{-8}$ M motilin and 10$^{-7}$ M to 10$^{-5}$ M ABT-229, but subsequent doses after a wash had little or no effect or the response was significantly attenuated. All strips and segments contracted to 10$^{-4}$ M methacholine.

Single dispersed cells. Each of the three agonists decreased the cell length concentration dependently in single cells. The EC$_{50}$ values were not different from one another, but the EC$_{max}$ of ABT-229 was significantly less than that of motilin (Table 1). Motilin antagonist porcine [Phe$^3$,Leu$^{13}$]motilin concentration-dependently inhibited the response to ABT-229, motilin, and erythromycin (Fig. 8). The IC$_{50}$ values of 4.3 ± 3.2, 1.7 ± 0.81, and 5.1 ± 0.45 × 10$^{-8}$ M, respectively, were not different from each other. The IC$_{max}$ values of 81 ± 12%, 80 ± 5%, and 53 ± 15% were also not different for the three agonists. However, we found that atropine also concentration-dependently reduced the responses to ABT-229, motilin, and erythromycin (Fig. 8). This raised the possibility that, in single cells, the agonists may act on muscarinic receptors and the [Phe$^3$,Leu$^{13}$]motilin antagonist may also partially block the muscarinic receptors.

$[^3]$H]ACh release from longitudinal muscle-myenteric plexus preparations. 10$^{-6}$ M ABT-229 or 10$^{-6}$ M erythromycin did not significantly increase the release of $[^3]$H]ACh from longitudinal muscle-myenteric plexus preparations (47 ± 25% and 78 ± 28%, respectively). In contrast, Shi and Sarna (21) have reported that 10$^{-4}$ M veratridine, an Na$^+$ channel activator, significantly increases $[^3]$H]ACh release from the canine jejunal longitudinal muscle-myenteric plexus preparations by 200 ± 55%.

DISCUSSION

Our findings show that in the intact conscious state, each of the three prokinetic agents acts on presynaptic neurons to stimulate phasic contractions. These contractions resemble those seen during phase II activity or the postprandial state but not during phase III activity. In our study, we infused the prokinetic agents close intra-arterially in a short segment of the jejunum. The doses were so small that they affected only the infused segment. This method allows investigations of the enteric neural and smooth muscle function in the intact conscious state without the modulating effects of the

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<th>Prokinetic</th>
<th>EC$_{50}$, M</th>
<th>EC$_{max}$, %</th>
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<tr>
<td>ABT-229</td>
<td>1 ± 0.18 × 10$^{-9}$</td>
<td>20.6 ± 2.4*</td>
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<tr>
<td>Motilin</td>
<td>1.2 ± 0.54 × 10$^{-8}$</td>
<td>28.7 ± 2.2</td>
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<tr>
<td>Erythromycin</td>
<td>5.3 ± 1.9 × 10$^{-8}$</td>
<td>22.9 ± 3.3</td>
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Values are means ± SE; n = 6. EC$_{max}$, maximal effective concentration. *P < 0.05 vs. motilin.
drugs on other organs, such as the central nervous system and the spinal cord. Also, in the intact conscious state, all elements of the enteric nervous system are fully operational. As noted later, the presynaptic neurons may be impaired in the muscle bath. In previous studies (10, 26, 30), when some of the prokinetic agents were infused intravenously, they acted preferentially on the duodenum and the antrum to stimulate phase III activity, which then migrated distally.

The response to each agent was blocked by atropine and hexamethonium, indicating that it utilizes at least one nicotinic synapse and the release of ACh at the neuromuscular junction. The nicotinic receptor may not be in parallel with any other receptor because its blockade inhibited the response almost completely. There was little or no direct effect of any prokinetic on circular smooth muscle cells in the intact conscious state since the responses were blocked almost completely by TTX. Intravenous infusion of motilin and erythromycin in humans and dogs has been reported also to stimulate phasic contractions or phase III activity by acting at a presynaptic neuronal site and utilizing at least one nicotinic synapse (1, 19).

Our findings also show that molecular modification of erythromycin that eliminates its antibiotic activity significantly alters its potency, efficacy, the time lag for onset of contractions, and the types of receptors that are involved in the stimulation of jejunal contractions by the new molecule, ABT-229. Many of the above characteristics are also different between motilin and erythromycin or ABT-229. It seems that each of the above prokinetic agents may act on a different subset of presynaptic neurons. The synapses in each subset contain different subtypes of serotonergic, tachykinergic, and histaminergic receptors. The subset of neurons for each prokinetic agent converges on postsynaptic cholinergic excitatory motoneurons. M3 receptors located on smooth muscle cells (7) mediate the final response to all prokinetics, but the involvement of M1 and M2 receptors may be specific to each prokinetic agent. These findings explain the previous observations of Haba and Sarna (9) that in intact dogs motilin and erythromycin stimulate different spatial and temporal characteristics of gastro-duodenal contractions in the postprandial state by acting on different subsets of presynaptic neurons.

The subset of presynaptic neurons associated with motilin and erythromycin but not ABT-229 also synapse on nitroreceptor inhibitory neurons. The inhibition of NOS enhanced the response to motilin and erythromycin, but it had no effect on the response to ABT-229.

Whereas all three prokinetic agents consistently and reproducibly contracted the canine jejunal circular muscle when infused close intra-arterially in the intact conscious state, none exhibited a response in circular muscle strips taken from nearly the same location in the jejunum. These muscle strips responded normally to methacholine that acts directly on muscarinic receptors on smooth muscle cells (22) and to EFS that releases ACh from the postsynaptic neurons (21, 22).

The above anomaly may be due to the following reasons: 1) the prokinetics act on mucosal sensory neurons that are removed in the preparation of mucosa-free strips; 2) the prokinetics do not diffuse to the ganglia in the muscle bath environment, but with intra-arterial infusion they are transported close to them by the blood vessels; or 3) the presynaptic neurons are impaired in the muscle bath environment. We ruled out the first two possibilities because the prokinetics had no effect on mucosa-attached muscle strips and rings or when they were infused close intra-arterially in ex vivo segments.
Both veratridine and EFS induce the release of ACh from the longitudinal muscle-myenteric plexus preparations of the dog small intestine (21). These responses are not affected by hexamethonium and atropine but are blocked by TTX, indicating that these stimuli release ACh by their action on postsynaptic neurons. In our study, the prokinetic agents did not release ACh in longitudinal muscle-myenteric plexus preparations. This confirms the above findings that the presynaptic neurons that are the site of action of prokinetics may be impaired in the muscle bath environment. However, electrophysiological studies (5, 6, 13, 24, 31), mostly in the guinea pig model, show that these neurons are excitable. However, concurrent recordings of contractile activity and electrophysiological tracings have not been made to show that presynaptic neuronal excitation results in contraction in a muscle bath environment. The mechanisms of impairment or dysfunction of the presynaptic neurons in the in vitro environment, therefore, remains unknown.

In contrast to the canine jejunal muscle strips, the rabbit jejunal, duodenal, and antral muscle strips did respond to ABT-229. However, the response was a tonic contraction, not phasic contractions, as seen in vivo. In addition, we found that the strips became refractory after the first application of ABT-229. In previous studies (12, 15), the concentration-response curves to motilin in rabbit muscle strips have been determined by cumulative addition of the agonist. Muscarinic agonists did not exhibit such refractoriness. Also, such refractoriness was not observed in intact conscious dogs. Satoh et al. (20) also reported that motilin and another motilide, EM574, stimulate in vitro segments of rabbit small intestine but not those of rat and guinea pig.

The responses in the rabbit muscle strips were not blocked by TTX, hexamethonium, or atropine, indicating that they are mediated by smooth muscle receptors (3, 12, 15). It seems that the direct activation of smooth muscle receptors by these prokinetic agents increases tone, whereas the activation of neuronal receptors stimulates phasic contractions.

Van Assche et al. (29) reported that motilin enhances the twitch responses to EFS in rabbit antral circular muscle strips. The EFS response is mediated by the release of ACh. However, motilin did not enhance the response to carbachol that acts directly on smooth muscle muscarinic receptors. In our study, ABT-229 or erythromycin did not enhance the response to EFS in canine muscle strips. Presumably, this is due to the lack of release of ACh by these agents in the muscle bath environment.

Whereas none of the prokinetic agents indicated a response by direct action on smooth muscle cells in the intact conscious state or in muscle bath, they decreased the cell length concentration dependently in single dispersed cells. There was no difference in potency or efficacy among the three compounds, whereas such differences existed in vivo. The decrease in cell length in response to each agonist was inhibited concentration dependently by motilin antagonist porcine [Phe3,Leu13]-motilin. Farrugia et al. (4) reported also that motilin decreases cell length in single dissociated jejunal circular muscle cells. Surprisingly though, the response to each prokinetic was also inhibited concentration dependently by atropine. This suggested that the prokinetics may act on muscarinic receptors in single cells.

In conclusion, all three prokinetic agents act on presynaptic neurons in the canine jejunum to stimulate phasic contractions. Erythromycin is less potent than ABT-229, which is less potent than motilin. Each prokinetic agent may act on a different subset of presynaptic neurons, and its response is mediated by somewhat different muscarinic, serotonin, tachykinin, and histamine receptors. It may be possible to utilize these differences to design motilides that target specific organs or suborgans of the gastrointestinal tract, such as fundus, gastro-pyloro-duodenal junction, small intestine, and colon, to achieve specific prokinetic activity. The presynaptic neurons on which the prokinetics act converge on postsynaptic cholinergic motor excitatory neurons. The final neurotransmitter at the neuromuscular junction is ACh. Also, the action of all prokinetics involves at least one nicotinic synapse that is not in parallel with another receptor. The presynaptic neurons seem to be impaired in the muscle bath environment.

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REFERENCES


