Peristalsis and fecal pellet propulsion do not require nicotinic, purinergic, 5-HT3, or NK3 receptors in isolated guinea pig distal colon

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Nicholas S. Spencer NJ. Peristalsis and fecal pellet propulsion do not require nicotinic, purinergic, 5-HT3, or NK3 receptors in isolated guinea pig distal colon. Am J Physiol Gastrointest Liver Physiol 298: G952–G961, 2010. First published April 1, 2010; doi:10.1152/ajpgi.00457.2009.—The neuronal mechanism by which distension of the colon triggers peristalsis and the propulsion of colonic contents is incompletely understood. In this study, we used video imaging and spatiotemporal mapping techniques to investigate the neuroneuronal mechanisms underlying peristalsis in isolated guinea pig distal colon. In direct contrast to previous studies, we found that hexamethonium (100 μM–1 mM) or mecamylamine (20 μM) never abolished peristalsis or fecal pellet propulsion, although a temporary blockade of peristalsis was common, giving the impression perhaps that peristalsis was blocked permanently. During the initiation of peristalsis, the intraluminal propulsive force applied to an inserted fecal pellet was significantly reduced by hexamethonium 100 μM, even though, once initiated, the propagation velocity of fecal pellets was never reduced by nicotinic antagonists. In the presence of hexamethonium or mecamylamine, further addition of PPADS (10 μM), ondansetron (1 μM), and SR 142801 (300 nM) had no inhibitory effect on the propagation velocity of fecal pellets. In these preparations, antagonists for nicotinic, purinergic (P2), serotonergic (5-HT3), or tachykinergic (NK3) receptors always abolished responses to the agonists for these receptors, confirming that when peristalsis occurred, nicotinic, P2, 5-HT3, and NK3 receptors were blocked. Tetrodotoxin abolished nonnicotinic peristalsis. In summary, nicotinic transmission contributes to excitatory neuroneuronal transmission underlying peristalsis and fecal pellet propulsion but is not required for peristalsis, nor fecal pellet propulsion, as once thought. These observations could be explained by an excitatory nonnicotinergic neuroneuronal pathway that can generate peristalsis and induce normal fecal pellet propagation velocities but does not require nicotinic, P2, 5-HT3, or NK3 receptors.

sensory neuron; enteric; reflex; mechanosensory; afferent; synaptic

IT HAS BEEN KNOWN FOR MORE THAN 100 years that the peristaltic reflex can be elicited in the large intestine by local distension of the colonic wall, which involves activation of both an ascending excitatory and descending inhibitory neural reflex pathway to the neighboring colonic smooth muscle layers (2, 4, 7, 11, 24). Originally, it was thought that the sensory neurons underlying the peristaltic reflex, which respond to luminal distension, lie outside the gut wall, in dorsal root ganglia. However, more recent studies have now confirmed that the peristaltic reflex in the small intestine (10, 12) and colon (4, 20, 27) is activated by a specific population of sensory neurons, whose cell bodies lie intrinsic to the gut wall. Activation of intrinsic sensory neurons is the first step in triggering the peristaltic reflex, which activates a local reflex arc, consisting of intrinsic sensory neurons, interneurons and motor neurons

(5, 6). The term peristalsis, on the other hand, refers to a propagating ringlike contraction of the gut wall, which requires the coordinated recruitment of a neuronal network, often described as a motor program (28). Unfortunately, the term peristaltic reflex and peristalsis are often used interchangeably, as if they are the same event or perhaps utilize the same neural pathways. However, there is gathering evidence to suggest that the neural pathways underlying the peristaltic reflex activated by local distension of the bowel are quite different from those pathways activated during peristalsis and the propulsion of luminal contents (28).

Much of our understanding about the mechanisms underlying peristalsis has arisen from studies on the guinea pig distal colon, which began nearly 40 years ago. Studies on this preparation revealed that insertion of an artificial fecal pellet into the oral end of an isolated segment of distal colon reproducibly elicits peristalsis leading to the propulsion of the fecal pellet along the colon (7). Since that time, the guinea pig distal colon has emerged as a model preparation to understand the mechanisms underlying peristalsis and fecal pellet propulsion.

Originally it was thought that acetylcholine acting via nicotinic receptors was the sole transmitter underlying fast synaptpic transmission within the enteric nervous system. Although nicotinic transmitter plays a major role (3, 6), only in the past decade has it become clear that in fact multiple neurotransmitters mediate fast synaptic transmission at different functional classes of myenteric neurons, including adenosine triphosphate (ATP) acting on P2 receptors and serotonin acting on 5-HT3 receptors (4, 9, 13, 14, 21, 22, 32). In the guinea pig distal colon, intracellular recordings from myenteric neurons have revealed that “fast excitatory postsynaptic potentials were completely blocked by the nicotinic receptor antagonists hexamethonium or mecamylamine in 62% of neurons” and the other neurons were also reduced (21). These findings are highly compatible with the work of Bian et al. (4), who concluded that “transmission between ascending or descending interneurons and from sensory neurons to descending interneurons is predominantly via nicotinic receptors.” Nurgali et al. (21) went on to find that the majority of nonnicotinic fast excitatory postsynaptic potentials in myenteric neurons of the guinea pig distal colon were dramatically reduced or abolished by the P2 receptor antagonist, pyridoxal phosphate-6-azo (benzene-2,4-disulfonic acid) tetrasodium salt hydrate (PPADS), leading them to conclude that “acetylcholine and ATP are the major fast excitatory neurotransmitters in guinea pig distal colon myenteric ganglia.” In addition to acetylcholine and ATP, there is also evidence that serotonin acting on 5-HT3 receptors also plays an important role in fast neuroneuronal transmission in the small and large intestine (13, 14, 16, 19, 32).
When peristalsis and the propulsion of fecal pellets have been studied in the past in isolated guinea pig distal colon, it has been suggested that nicotinic transmission is essential, since it was reported that hexamethonium (at a concentration of 100 μM) abolished fecal pellet propulsion (8, 16, 29). Following these studies, there have been remarkably few investigations on the effects of nicotinic transmission on peristalsis and fecal pellet propulsion in the colon.

In this study, we report major observations regarding peristalsis and the propulsion of fecal pellets in the isolated guinea pig distal colon that are highly inconsistent with previous studies on this preparation (8, 16, 29). In brief, we found that nicotinic receptors contribute but are not required for the neuroneuronal pathway underlying peristalsis in the guinea pig distal colon. This nonnicotinic neuroneuronal pathway can generate peristaltic contractions that propel fecal pellets anally at an undiminished speed but, interestingly, also do not require activation of P2 purinoreceptors, 5-HT3, or NK3 receptors. The identity of the neurotransmitters or receptors underlying this nonnicotinic pathway remain elusive and await further investigation.

METHODS

Preparation of tissues. Adult male guinea pigs, weighing between 200–350 g, were killed by a blow to the occipital region and exsanguinated, in a manner approved by the Animal Welfare Committee of Flinders University. The distal colon was removed and placed in warm Krebs solution that was constantly bubbled with carbogen gas (95% O2−5% CO2). After a period of time (usually <20 min), natural fecal pellets were expelled from the colon. A segment of distal colon (8 cm in length) was mounted in an organ bath and given a period of 30 min for equilibration to take place. After this time, video imaging or mechanical recordings from the circular muscle (see below) were made using the protocol described below.

Mechanical recordings from the circular muscle during peristalsis and fecal pellet propulsion. We recorded the relaxation underlying descending inhibition and contraction underlying ascending excitation of the circular muscle, both during fixed balloon distension-evoked reflexes and during the propulsion of fecal pellets along the colon, as originally described by Costa and Furness (7). To do this, we recorded circular muscle tension using two independent isometric recording transducers (Grass FT-03C; Grass, Quincy, MA) connected via fine suture thread to two spring stainless steel claws that anchored to the colon (Fig. 1A). Mechanical recordings were made under isometric conditions against a load of 300 ± 100 mg resting tension (see Fig. 1). The isometric force transducers were connected to two custom-made preamplifiers (Biomedical engineering, Flinders University) and then to a Powerlab (model 4/30; AD Instruments, Bella Vista, NSW, Australia). Labchart version 6.0 (AD Instruments, Australia) was used for analysis of data.

Balloon distension and fecal pellet propulsion. An intraluminal balloon (Fogarty, arterial embolectomy catheter, Edwards Life-science, Irvine, CA) was used to apply maintained balloon distensions (20-s duration) of 100-μl volume to the colonic wall. The maximal inflated balloon diameter (4 mm) closely mimicked the maximum diameter of the natural fecal pellet (4 mm) used for eliciting peristalsis and studying fecal pellet propulsion. The fecal pellet used for eliciting peristalsis was a natural fecal pellet, covered in resin to make a hard external surface.

Video imaging of peristalsis and generation of spatiotemporal maps. The propagation of natural fecal pellets was recorded via the Gastrointestinal Motility Monitoring system (GIMM; Med-Associates, Saint Albans, VT). The colon was illuminated from beneath, and a digital video camera was used to record the propagation of the fecal pellet along the colon. Fecal pellets were inserted into the oral end of the colon, and the time taken for them to be expelled was determined by use of software developed for the GIMM. In each isolated segment of colon, between five and nine individual propulsive runs of single fecal pellets were made in control solutions and following each drug application. A minimum period of 3–5 min was allowed for the preparation to recover between each insertion of the fecal pellet. Propagation velocities were compared between control groups and those exposed to the antagonists described below. Spatiotemporal maps were constructed from the digital videos that were acquired from individual runs. To do this, changes in colonic diameter were plotted over time (vertical axis). The image of the colon in each video frame was converted to a silhouette and the diameter along the entire length was calculated and converted into a grayscale. The smallest diameter, or fully contracted segment, is represented in white, whereas the largest diameter, or dilated, region is represented in black. Each frame of video produced is represented by a single row of pixels, corresponding to the diameter of each segment along the entire length of the isolated colon. Each computed image has a calibration scale that indicates the change in diameter in grayscale (mm), time (s), and length (mm) for each spatiotemporal map produced for each experiment (GIMM software, Med-Associates).

Measurements and statistics. In RESULTS, data are presented as means ± SE. The use of n in RESULTS refers to the number of animals on which observations were made. Measurements of peak relaxation and peak contraction were made during the descending inhibitory and ascending excitatory phases of peristalsis that occurred as single fecal pellets were propelled freely in an anal direction along the colon (7). Data sets were considered statistically significant if P values < 0.05 were reached. Paired Student’s t-tests or ANOVA were used with post hoc tests where required.

Drugs and solutions. The Krebs solution used contained (in mM) 118 NaCl; 4.7 KCl, 1.0 NaHPO4·2H2O, 25 NaHCO3, 1.2 MgCl·6H2O, 11 D-glucose, 2.5 CaCl2·2H2O. The NK3 antagonist SR 142801 was provided by Sanofi Recherche, Paris, France, and the NK3 agonist senktide was obtained from Auspep, Parkville, Australia. Hexamethonium bromide, ondansetron hydrochloride, mecamylamine hydrochloride, 2-methyl-5-hydroxytryptamine, 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP), PPADS, and d-α-methylene ATP were all obtained from Sigma Chemical, St. Louis, MO. All antagonists were made up fresh on the day of use.

RESULTS

When a natural fecal pellet was inserted into the oral end of an isolated segment of guinea pig distal colon, a peristaltic wave was reliably elicited, consisting of ascending contraction behind the fecal pellet and descending relaxation in front of the pellet, which led to the propulsion of the fecal pellet along the colon, as previously described (7). The mean anal propagation velocity of single fecal pellets along the full 8-cm length of colon was 1.8 ± 0.03 mm/s (range: 0.2–3.4 mm/s; 205 runs, n = 22).

Effects of hexamethonium and mecamylamine on peristalsis and fecal pellet propulsion. It has been suggested previously that hexamethonium (100 μM) abolishes fecal pellet propulsion in the isolated guinea pig distal colon (8, 16, 29). With this in mind, we applied hexamethonium (100 μM) to the isolated guinea pig distal colon, to determine the effects on pellet propulsion. We found that after perfusion of hexamethonium (100 μM) for 30–45 min, in 13 of 22 animals, the first time the fecal pellet was reinserted back into the oral end of colon the pellet failed to move anally, giving the impression that hexamethonium had abolished peristalsis and pellet propulsion. However, if the pellet was left in the oral end of colon, after a
period of time (typically between 2 and 30 min), the pellet began to move in a staggered irregular fashion analy along the colon, until it was finally expelled. In these preparations, this first peristaltic run could last up to 30 min before the pellet was expelled from the colon. In all of these 13 animals, the second time the pellet was reinserted into the oral end (second peristaltic run), the pellet resumed peristalsis leading to normal propagation velocities, as seen in control solution. When DMPP $10^{-20}$ M/H$_{9262}$ was applied in the presence of hexamethonium, where peristalsis had recovered and pellets were propelled at normal velocities along the colon. In the presence of these antagonists, 2-methyl 5-HT (10 M) and DMPP (10 M) failed to evoke a contraction. D: in a separate preparation from the same colon as shown in B and C, video imaging was performed of control fecal pellet propulsion. E: spatiotemporal map of control peristalsis. F: hexamethonium (Hex) upon initial application temporarily arrested peristalsis, leading to slow irregular pellet propulsion. G: however, the 2nd peristaltic run recovered with normal peristalsis despite maintained perfusion of hexamethonium. H: further addition of ondansetron (1 M) and PPADS (10 M) had no effect on slowing pellet propulsion or peristalsis.

Effects of ondansetron and PPADS on hexamethonium-resistant peristalsis. Since experiments above showed that peristalsis and fecal pellet propulsion were either unaffected or recovered in the presence of hexamethonium, we investigated whether other known blockers of neuroneuronal transmission
would prevent fecal pellet propulsion. In the continued presence of hexamethonium, we applied a combination of ondansetron (1 μM) and PPADS (10 μM) for at least 45 min before reinserting the fecal pellet. Overall, there was no significant difference in the mean propagation velocity of fecal pellets in the combined presence of all three antagonists, compared with hexamethonium alone. To confirm that 5-HT3 receptors and P2 purinoceptors were blocked, we applied 2-methyl 5-HT (10 μM) and \( \text{α,β}-\)methylene ATP (100 μM) before and after the antagonists were applied. In the absence of PPADS (10 μM), \( \text{α,β}-\)methylene ATP (100 μM) caused a relaxation of the circular muscle, which was not detected in the presence of PPADS. When 2-methyl 5-HT (10 μM) was applied before ondansetron, and it was found that a circular muscle contraction was evoked (4.4 ± 0.1 mN orally; 5.3 ± 0.1 mN anally; \( n = 9 \)). No responses were seen when 2-methyl 5-HT was applied after ondansetron (1 μM; \( n = 9 \)).

Effects of a NK3 receptor antagonist on fecal pellet propulsion that persisted in the presence of PPADS, hexamethonium and ondansetron. Intrinsic sensory neurons in the guinea pig small intestine (17) and proximal colon (20) have been shown to communicate to other enteric neurons via NK3 receptors (15). We tested whether NK3-mediated neuroneuronal transmission may underlie nonnicotinic peristalsis described above. In four separate experiments, we applied SR 142801 (300 nM) to the colon, after hexamethonium (500 μM), PPADS (10 μM), and ondansetron (1 μM) had already been present and fecal pellets were reliably propelled along the colon. SR 142801 (300 nM; \( n = 4 \)) had no inhibitory effect on fecal pellet propulsion, even up to 1 μM (\( n = 1 \)). In separate preparations of colon, from these same four animals, we confirmed that the concentrations of antagonists used blocked NK3 receptors, by applying senktide (50 nM) to the colon. No response to senktide was obtained when applied after SR 142801 (1 μM; \( n = 2 \)), whereas the circular muscle contracted to senktide, in the absence of SR 142801. We could not use NK1 antagonists to discern their role in neuroneuronal transmission, because NK1 receptors are also prolific on smooth muscle (23).

Effects of hexamethonium on balloon distension-evoked ascending excitatory nerve pathways. Nicotinic transmission has been shown to play a major role in the ascending excitatory reflex contraction of the guinea pig distal colon evoked by fixed local balloon distension (7). In light of this, we tested whether hexamethonium would inhibit the ascending excitatory reflex evoked by balloon distension, in the same preparations of distal colon where we could demonstrate that peristalsis and fecal pellet propulsion still occurred in hexamethonium. To do this, an intraluminal balloon was inserted into the distal colon at a fixed distance (30 mm) from the oral and anal cut ends of the distal colon. Inflation of the intraluminal balloon to a volume of 100 μl generated an intraluminal distension of 4 mm, which closely mimicked the diameter of the artificial fecal pellet used to elicit peristalsis. When the intraluminal balloon was inflated for 20 s, it reproducibly elicited an ascending contraction of 7.3 ± 3.4 mN and anal relaxation of 0.4 ± 0.6 mN (Fig. 5E). Hexamethonium (100 μM; \( n = 4 \)) or
mecamylamine (20 μM; n = 3) abolished the ascending excitatory reflex contraction (n = 4; Fig. 5E), with no effect on the descending relaxation (Fig. 3C). In these same preparations, while in the presence of hexamethonium, we removed the balloon from the distal colon and inserted a fecal pellet into the oral end of the colon. In four of four animals tested, we found that the fecal pellet was immediately propelled along the colon at normal propagation velocities, even though in these same preparations the balloon distension-evoked ascending contraction was abolished. When this same experiment was repeated in three different animals, but using mecamylamine (20 μM) instead of hexamethonium, the same result was obtained (see Fig. 4). The amplitude of the ascending contraction and descending relaxation recorded during the propulsion of fecal pellets was not reduced (Fig. 3B) when the balloon distension-evoked ascending reflex contraction was abolished (Fig. 3C).

Effects of hexamethonium on the propulsive force on exogenous fecal pellets during the initiation of peristalsis. Our results above show that nicotinic transmission is not required for peristalsis, nor the propulsion of exogenous fecal pellets introduced into the colon. However, it is possible that the propulsive force applied to propel the pellet is reduced by hexamethonium, but that this reduction in synaptic transmission is not reflected in a reduction in the propagation velocity of introduced exogenous fecal pellets. Therefore, we tested whether blockade of nicotinic neural pathways would reduce the maximal propulsive force applied to an introduced fecal pellet during the initiation of peristalsis. To do this an isometric force transducer was connected via fine cotton thread to an exogenous fecal pellet that was then inserted into the oral end of the isolated intact segment of colon (8 cm in length). We found that during the initiation of peristalsis, hexamethonium (100 μM) significantly reduced the peak tension applied to the fecal pellet from a control of 8.9 ± 1.1 to 4.1 ± 0.25 mN in hexamethonium (n = 5; P < 0.05; Fig. 5). Similarly, the half duration of the peristaltic contraction that was exerted onto the pellet was also significantly reduced from 32.9 ± 5.3 to 12.3 ± 1.5 s (n = 5; P < 0.05). However, the rate of rise of the tension developed by the nonnicotinic pathway on the fecal pellet was not significantly different in the presence of hexamethonium (control: 0.49 ± 0.2 g/s to 0.42 ± 0.1 g/s in hexamethonium; n = 5; P > 0.05; Fig. 6).

Effect of hexamethonium on the ejection of endogenous fecal pellets. It is well known that the isolated guinea pig distal colon will expel natural endogenous fecal pellets, when the isolated colon is placed into warm Krebs solution (7). Since our data above showed that nicotinic antagonists did not block the propulsion of exogenous fecal pellets, we were particularly interested in whether nicotinic receptors were required for the expulsion of natural endogenous pellets. To test this hypothesis, we removed a segment of distal colon (8 cm long) from guinea pigs and placed this preparation (with endogenous fecal pellets) immediately into warm (36°C) Krebs solution containing hexamethonium (500 μM). Under these conditions, no pellets were naturally expelled from the colons of five animals, suggesting that background activity in nicotinic pathways is required for natural expulsion of endogenous pellets. Interestingly, in these same preparations in which endogenous pellet propulsion was prevented by hexamethonium, if an exogenous pellet was inserted into these same segments of colon, peristalsis was activated in the presence of hexamethonium, leading to the propulsion of exogenous pellets along the bowel. These exogenous pellets propagated consistently along the colon in hexamethonium, until they collided with the endoge-
nous pellets (Figs. 7 and 8). The collision between the exogenous pellet and endogenous pellet(s) usually triggered peristalsis that led to the propulsion and expulsion of endogenous pellets (Fig. 7 and 8).

DISCUSSION

One of the major findings of this study is that the nicotinic receptor antagonists hexamethonium, or mecamylamine, do not abolish peristalsis, nor the propulsion of fecal pellets along the isolated guinea pig distal colon. This is in direct contrast to the literature over the past 40 years, which has consistently stated that peristalsis and the propulsion of fecal pellets in the guinea pig distal colon is abolished by hexamethonium (8, 16, 29). Since peristalsis was resistant to either mecamylamine or hexamethonium but was abolished by tetrodotoxin, our data suggest that a nonnicotinic neuroneuronal pathway exists in the guinea pig distal colon, which is responsible for the generation of a peristaltic wave that leads to normal propagation velocities of fecal pellets along the colon. Surprisingly, nonnicotinic peristalsis was also resistant to antagonists of NK3 receptors, P2 purinoceptors, and 5-HT3 receptors.

Identification of a nonnicotinic neuroneuronal pathway that underlies peristalsis and fecal pellet propulsion. In the past, at least three independent studies have all concluded that hexamethonium abolishes peristalsis that underlies fecal pellet propulsion in the isolated guinea pig distal colon (8, 16, 29). Our results are highly inconsistent with these previous studies. We found that nicotinic receptor antagonists never abolished peristalsis, nor fecal pellet propulsion. What we did find was that in about half of all animals tested (13 of 22) hexamethonium (100 \mu M) temporarily arrested peristalsis and fecal pellet propulsion, but this inhibitory effect was temporary. In these animals, when hexamethonium was applied to the colon, initially the pellet failed to move, giving the impression that nicotinic receptors were essential for the propulsion of the inserted exogenous pellet. However, if time was allowed for the pellet to remain in the oral end of the colon, the pellet gradually propelled analy, until finally being expelled (Fig. 1).
This restoration of peristalsis in hexamethonium was not due to unblocking, or desensitization of the nicotinic receptors, or possibly the hexamethonium loosing its efficacy, because DMPP never gave a response in the presence of hexamethonium 100 \( \mu M \), whereas in the absence of hexamethonium a robust contraction of the colon occurred to DMPP. It also was not due to an insufficient concentration of hexamethonium because hexamethonium at 500 \( \mu M \) had no different effect on peristalsis compared with 100 \( \mu M \).

**Hexamethonium reduces the propulsive force applied to an exogenous fecal pellet but does not the propagation velocity of pellets.** Although the mean propagation velocity of fecal pellets along the colon was often faster in the presence of hexamethonium compared with control Krebs solution, the propulsive force applied to the fecal pellet was always significantly reduced by hexamethonium, as was the duration of each peristaltic contraction. This result shows that under normal circumstances in control solution, nicotinic pathways do have a major role in the neural circuitry activating intrinsic excitatory motor neurons during peristalsis, but that blockade of these receptors does not lead to a reduction in the propagation velocity of fecal pellets (Fig. 2). It was also of particular interest that in the same preparations of colon in which hexamethonium abolished the ascending excitatory neural reflex to transient balloon distension, peristalsis and the propagation of fecal pellets persisted without any reduction in velocity. This also showed that nicotinic receptors are required for the ascending excitatory reflex, but, again, are not required for the generation or propagation of peristalsis.

**Plasticity in the enteric nervous system that underlies peristalsis.** The first time an exogenous pellet was introduced into the colon after application of hexamethonium or mecamylamine, it did not move anally (in 13 of 22 animals), giving the impression that peristalsis was blocked. However, if the same pellet was left in the colon, it was always ultimately expelled. In these same preparations, where peristalsis was temporarily inhibited, we found that peristalsis always recovered in the second and successive peristaltic runs, whereby pellets propagated along the colon, without any reduction in velocity. This observation can be explained by facilitation of release of nonnicotinic neurotransmitter(s), which, following acute blockade of nicotinic receptors with hexamethonium, require time to develop sufficiently intensity to restore peristalsis. It is not clear to us why our results with hexamethonium are different from those of other...
laboratories, which suggested that peristalsis was blocked by hexamethonium. It is possible that the temporary blockade of pellet propulsion we noted in the first peristaltic run in hexamethonium may have been interpreted as a permanent blockade by other investigators, leading to the premature conclusion that nicotinic receptors are essential for peristalsis. Or, alternatively, nonnicotinic pathways in other strains of guinea pigs may simply be insufficient to generate peristalsis.

Interestingly, in other animals (9 of 22), the first peristaltic run in hexamethonium propagated completely uninhibited along the colon, as if nicotinic antagonists have no effect on neuroneuronal transmission. Our observation that peristalsis can recover in the presence of hexamethonium is conceptually similar to the observations of Bartho et al. (1) in the guinea pig small intestine, where fluid-induced peristalsis could also recover in the presence of hexamethonium.

**Effects of hexamethonium on the propulsion of endogenous fecal pellets.** When the distal colon was removed from guinea pigs and placed into warm Krebs solution containing hexamethonium, pellets were not expelled, even if extended periods of time were given (Figs. 7 and 8). However, in these same preparations, insertion of an exogenous fecal pellet was always able to trigger peristalsis that led to the propulsion of the exogenous pellets (Fig. 7) and endogenous pellets (Fig. 8). These results suggest that, in the presence of hexamethonium, the maintained natural distension applied to the colon wall by endogenous fecal pellets is, on its own, insufficient to trigger the nonnicotinic neural pathway and peristalsis. However, when a peristaltic wave was evoked by an exogenous pellet, we found that the collision between the exogenous and endogenous pellets could readily evoke peristalsis in the endogenous pellet, leading to the independent propulsion of both exogenous and endogenous pellets (Fig. 8). This strongly suggests that for both endogenous and exogenous (inserted) pellets, activation of nonnicotinic peristalsis requires a stimulus. In the case of the exogenous pellets, the stimulus is the acute distension applied to the gut wall, whereas the stimulus for endogenous pellet propulsion is the collision from the exogenous pellet.

**Nonnicotinic peristalsis and fecal pellet propulsion.** In the guinea pig distal colon, extensive intracellular recordings have been made from myenteric neurons (18, 21, 27, 30, 31) and the
smooth muscle layers (4, 25, 26). In all of these studies there has been consistent agreement that nicotinic transmission provides the major excitatory neuroneuronal synaptic input to most myenteric S neurons (interneurons and motor neurons). Our study shows that in the presence of high concentrations of hexamethonium or mecamylamine, where all nicotinic receptors are abolished, the nonnicotinic pathway is sufficient to generate peristalsis and propel fecal pellets anally. Although this result was most unexpected, it was perhaps even more surprising to us that PPADS, ondansetron, and SR 142801 also

![Image of propulsion mechanisms](https://example.com/image)

Fig. 7. Effects of hexamethonium on the propulsion of endogenous and exogenous fecal pellets. A: photomicrograph showing the location of 2 endogenous fecal pellets in a segment of distal colon immediately after the guinea pig was euthanized and the distal colon removed from the animal. B: a spatiotemporal map showing that when the distal colon with the endogenous pellets (shown in A) is placed immediately into hexamethonium (500 µM) the endogenous pellets fail to be expelled. However, when an exogenous fecal pellet (see arrow) was inserted into the oral end of this same segment of colon, a peristaltic wave was evoked that propelled the exogenous pellet anally, until it collided with the endogenous pellets. In this preparation, the collision between the exogenous and endogenous pellets was insufficient to trigger peristalsis and the endogenous pellet failed to be expelled. C: photo sequence of the peristaltic wave evoked by the exogenous pellet and the collision with the endogenous pellets. D: location of the endogenous and exogenous pellets within the colon, while in the maintained presence of hexamethonium.

![Image of peristaltic wave propagation](https://example.com/image)

Fig. 8. Propulsion of endogenous fecal pellets in hexamethonium following collision with an exogenous pellet. 1. Endogenous fecal pellets within the colon failed to be propelled along the colon in the presence of hexamethonium 500 µM. 2. However, insertion of an exogenous fecal pellet triggered peristalsis that lead to the propulsion of the exogenous pellet. 3. Collision of the exogenous pellet with the endogenous pellet. 4. The collision was a sufficient stimulus to trigger an independent peristaltic wave that lead to the propulsion of the endogenous pellet. Now both the endogenous and exogenous pellet were propelled independently of each other in the presence of hexamethonium.
failed to inhibit the nonnicotinic pathways that underlie pellet propulsion. Interestingly, in the study of Bian et al. (4), distension-evoked excitatory junction potentials in the guinea pig distal colon were only reduced by 30% by nicotinic antagonists. Thus, although some transmission was via nicotinic receptors, the majority was through nonnicotinic receptors. Consistent with our results, they also found that PPADS, the 5-HT3 antagonist, granisetron, and the NK3 antagonist also had very little effect on nonnicotinic transmission (4). This raises the fundamental question as to what is the neuroneuronal transmitter(s) underlying the generation and propagation of peristalsis and movement of luminal contents in the colon. The identity of this major nonnicotinic transmitter(s) or receptor(s) is unknown and awaits further investigation. Preliminary observations suggest that glutamatergic NMDA receptors are not involved (data not presented).

Conclusions. In summary, we show that peristalsis and the propulsion of fecal pellets in the isolated guinea pig distal colon do not require nicotinic receptors, P2 purinoceptors, 5-HT3 receptors, or NK3 receptors but are abolished by tetrodotoxin. This suggests that a nonnicotinic excitatory neuroneuronal pathway exists in the guinea pig distal colon and that this pathway is capable of not only generating peristalsis but also coordinating the propulsion of fecal pellets along the colon at normal velocities.

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