Mechanisms underlying distension-evoked peristalsis in guinea pig distal colon: is there a role for enterochromaffin cells?

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Submitted 11 March 2011; accepted in final form 20 June 2011

Mechanisms underlying distension-evoked peristalsis in guinea pig distal colon: is there a role for enterochromaffin cells? Am J Physiol Gastrointest Liver Physiol 301: G519–G527, 2011. First published June 23, 2011; doi:10.1152/ajpgi.00101.2011.—The mechanisms underlying distension-evoked peristalsis in the colon are incompletely understood. It is well known that, following colonic distension, 5-hydroxytryptamine (5-HT) is released from enterochromaffin (EC) cells in the intestinal mucosa. It is also known that exogenous 5-HT can stimulate peristalsis. These observations have led some investigators to propose that endogenous 5-HT release from EC cells might be involved in the initiation of colonic peristalsis, following distension. However, because no direct evidence exists to support this hypothesis, the aim of this study was to determine directly whether release of 5-HT from EC cells was required for distension-evoked colonic peristalsis. Real-time amperometric recordings of 5-HT release and video imaging of colonic wall movements were performed on isolated segments of guinea pig distal colon, during distension-evoked peristalsis. Amperometric recordings revealed basal and transient release of 5-HT from EC cells before and during the initiation of peristalsis, respectively. However, removal of mucosa (and submucosal plexus) abolished 5-HT release but did not inhibit the initiation of peristalsis nor prevent the propagation of fecal pellets or intraluminal fluid. Maintained colonic distension by fecal pellets induced repetitive peristaltic waves, whose intrinsic frequency was also unaffected by removal of the submucosal plexus and mucosa, although their propagation velocities were slower. In conclusion, the mechanoreceptors and sensory neurons activated by radial distension to initiate peristalsis lie in the myenteric plexus and/or muscularis externa, and their activation does not require the submucosal plexus, release of 5-HT from EC cells, nor the presence of the mucosa. The propagation of peristalsis and propulsion of liquid or solid content along the colon is entrained by activity within the myenteric plexus and/or muscularis externa and does not require sensory feedback from the mucosa, nor neural inputs arising from submucosal ganglia.

peristaltic reflex; serotonin; sensory neuron

Over the past few years, there has been a strong resurgence of interest in the role of enterochromaffin (EC) cells in the small and large intestine of a variety of mammals, including humans. This is most likely due to the fact that 5-hydroxytryptamine (5-HT) release from the mucosa has been thought to play a role in the control of complex gastrointestinal motor patterns, such as peristalsis (12, 15). EC cells in the intestinal mucosa synthesize and store the majority of serotonin in the body (2, 8, 10), in addition to other many substances, such as melatonin. More than 50 years ago, Büllbring and Lin (6) proposed a hypothesis that mechanical stimuli, such as luminal distension, caused release of 5-HT from EC cells, which then initiates peristalsis via intrinsic sensory nerve endings that lie in the mucosa (12, 14, 15). This hypothesis was based on an indirect observation that perfusion of exogenous 5-HT through the lumen of the guinea pig small intestine was able to evoke peristalsis (6), and removal of the mucosa abolished peristalsis. Recent amperometric studies on the guinea pig small intestine have indeed confirmed that 5-HT is released from EC cells in close temporal association with peristalsis (2). However, these recent studies have also shown that release of 5-HT from EC cells is potently induced by deformation of EC cells, as occurs during contraction of the gut wall, such as peristalsis. It is therefore not clear what is the precise role of 5-HT release from EC cells, if any, during peristalsis. Because a population of intrinsic sensory neurons have terminals that lie in intestinal mucosa (17), in close proximity to EC cells, it seems possible that release of 5-HT from EC cells may be involved in the initiation of peristalsis. This notion is further supported by the finding that exogenous 5-HT can activate intrinsic sensory neurons, which project into the mucosa (17). However, other laboratories have recently revealed that selective inhibitors of the enzyme tryptophan hydroxylase in EC cells leads to a significant reduction in serum 5-HT concentrations in vivo but does not have any significant effect on transit time, gastric emptying, or colonic motility in laboratory animals, such as mice (30).

In patients with inflammatory bowel diseases, such as Crohn’s disease and ulcerative colitis, there are changes in the numbers of EC cells that populate the intestine (5). It has been suggested that changes in EC cells, 5-HT release, or 5-HT reuptake may underlie some of the alterations in motility and sensation that these patients experience. In combination, this accumulated evidence suggests that EC cells may be a potentially attractive target for therapeutic intervention in a range of motility or sensory disorders.

If 5-HT from EC cells does play a major role in gut motility, removal of the mucosa would be expected to cause major disruption of motor patterns, including peristalsis. Previous studies in the colon have shown that activation of the peristaltic reflex (ascending excitation and descending inhibition), following radial stretch, is preserved after removal of the mucosa and submucosal plexus (24). However, it is known that there are major differences between the mechanisms underlying local peristaltic reflex circuitry and the mechanisms underlying peristalsis and the propulsion of content (26). Therefore, it remains unclear what role EC cells play in the generation and propagation of colonic peristalsis.

Until we have a clear understanding of the role of 5-HT release from EC cells in the generation of peristalsis in the...
healthy intestine, it is difficult to interpret the significance of changes that occur to EC cells during any gastrointestinal disorder. The major aim of the current study was to develop an isolated preparation of distal colon in which it is possible to record, for the first time, the dynamic release of 5-HT from EC cells during colonic peristalsis to determine whether 5-HT release from EC cells is required for distension-evoked peristalsis.

**METHODS**

**Preparation of tissues.** Guinea pigs (350–500 grams) of either sex were killed humanely by stunning with a blow to the head followed by severing of the carotid arteries, approved by the animal welfare committee at Flinders University. An 8-cm-long segment of distal colon was removed from guinea pigs (taken 4–6 cm from the anus) and placed in cold Krebs solution, which was constantly bubbled with carbogen gas (95% O2-5% CO2).

**Dissection to remove the mucosa and submucosa in tubular preparations of colon.** To preserve peristalsis in tube preparations of colon with the mucosa and submucosal plexus removed, it was necessary to retain the circumferential integrity of the colon. We developed a technique in which we could reliably invert the distal colon (see Fig. 1A) so that the mucosa faced outermost. This allowed us to sharply dissect off the mucosa and submucosal plexus (Fig. 1B) and then reinvert the preparation so that the colon could be studied in its normal orientation (i.e., serosa outermost).

**Amperometric measurements of serotonin release from enterochromaffin cells.** Amperometric recordings from the distal colon were made using the previously described method (16). In brief, currents due to the oxidation of 5-HT were recorded using an EPC-7 amplifier (List Medical, Darmstadt, Germany) and Pulse software (HEKA Electronic), sampled at 10 kHz, using a low pass filter of 1 kHz, using an ITC-18 A-D interface (Instrutech). Carbon fiber electrodes were mounted on an electronic micromanipulator (Sutter MP-285; Sutter Instruments). The whole colon was placed in a Sylgard-lined organ bath and continuously perfused with oxygenated Krebs solution that was temperature controlled at 35–37°C, using an automatic temperature controller (TC-344B; Warner Instrument). A carbon-fiber electrode (5 mm diameter, ProCFE; Dagan) was placed within 100 μm of the epithelial layer at the opening of the anal end of the intact distal colon tube. This allowed us to avoid contact with the mucosal or tissue surface during peristaltic contractions and ensured no 5-HT release or signal artifacts were evoked by the electrode compressing the tissue. At this electrode location, we were able to measure any 5-HT ejected from the anal end of the colon during peristalsis. A +375-mV holding potential was applied to the electrode, under voltage-clamp conditions. This potential is most selective to sensing 5-HT release rather than melatonin, which can also be released from the mucosa and which has an oxidation peak at approximately +700 mV (20). The use of SERT inhibitors to increase these signals (3) also indicates the sensitivity of this approach to measuring 5-HT. To compensate for any decreased sensitivity due to fouling during an experiment, electrode calibrations were taken at the beginning and end of experiments and the average of the two measurements used.

**Amperometric recordings during peristalsis.** To correlate direct release of 5-HT from EC cells with the initiation of peristalsis, it was necessary to record the propulsive force generated by the colon, on the inserted fecal pellet. To do this, we used an isometric force transducer connected via fine suture thread to a natural fecal pellet covered in epoxy resin (see Fig. 4). As soon as peristalsis was triggered, the string attached to the pellet prevented the pellet from being expelled.

![Fig. 1. Technique to remove the mucosa and submucosal plexus from intact tube preparations of distal colon. A: normal intact colon. Ai, intact colon stained for human neuronal protein (Hu) shows myenteric and submucosal plexus. B: fully inverted colon with half mucosa removed. C: fully inverted colon with mucosa and submucosal plexus completely removed. D: partially reinverted colon with all mucosa and submucosal plexus removed. E: fully reinverted colon so that serosa is now outermost, with mucosa and submucosal plexus removed; a dissected preparation, in which Hu staining revealed only myenteric ganglia and no submucosal plexus. Ei, Hu staining shows myenteric ganglia present but submucosal ganglia absent.](http://ajpgi.org/)

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and the propulsive force generated by the colon that was exerted on
the pellet could be accurately recorded by the tension transducer. The
propagation of natural fecal pellets was later recorded on the same
piece of tissue using the gastrointestinal motility monitoring system
(Med-Associates, Saint Albans, VT). This allowed us to make spatiotemporal maps and accurately determine propagation velocity, as
previously described.

Fluid-infused peristalsis. Intact preparations and preparations with
mucosa and submucosal plexus removed (6 cm long) were mounted in
an organ bath constantly perfused with Krebs solution at 36°C. The
lumen of the colon was also perfused with Krebs solutions at 36°C
with a flow rate of 0.24 μl/min. The propagation velocity and
frequency of peristaltic waves were determined by video imaging of
colic wall movements using spatiotemporal maps.

Measurements and statistics. Measurements of the half-duration and peak amplitude of each peristaltic wave were measured from
tension recordings, as was the interval between each cyclical perilastic
contraction. The propagation velocity of inserted pellets was
determined from spatiotemporal maps. In these cases, the propagation
of pellets along the colon was characterized as either continuous (i.e.,
did not cease propagation at any point) or showed a staggered
propagation, where pellets remained stationary for periods ≥5 s or
where inserted pellets did not propagate at all once inserted. Only
preparations that showed continuous propagation of pellets were
included in analysis of propagation velocity. Data in the results
section are presented as means ± SE. The use of “n” in the results
section refers to the number of animals on which observations were
made. Data sets were considered statistically significant if P values
<0.05 were reached. Student’s unpaired t-test were used for compar-
ison of data.

Drugs and solutions. The Krebs solution used contained (in mM): 118 NaCl, 4.7 KCl, 1.0 NaHPO4·2H2O, 25 NaHCO3, 1.2 MgCl2·6H2O,
11 d-glucose, and 2.5 CaCl2·2H2O. Tetrodotoxin was obtained from
Sigma Chemical and made up fresh on the day of use in deionized
water.

RESULTS

Effects of removal of the mucosa and submucosal plexus on
peristalsis and pellet propulsion. We used a tubular prepara-
tion of distal colon in which the circumferential integrity of the
colon was preserved to test whether the initiation and propaga-
tion of peristalsis would still occur if the mucosa and
submucosal plexus were removed (see Fig. 1, A–E). In 60 out of
74 trials (n = 25 animals) insertion of natural fecal pellets
into the oral end of intact control preparations (i.e., with
mucosa and submucosa present) reliably elicited peristalsis
such that pellets were propelled anally at 2.5 ± 0.1 mm/s (62
trials, n = 25), as previously described (7). In another 12 trials,
pellets did not propagate smoothly along the colon and showed
a staggered propagation (periods ≥5 s stationary), whereas
in two trials the inserted pellet did not move from the oral end of
the colon for the entire duration of the experiment.

In dissected colon preparations that had their mucosa and
submucosa removed, insertion of the same fecal pellets also
reliably elicited peristalsis, and pellets ran continuously along
the colon in 34 out of 41 trials (n = 14, Fig. 2, A–C). The
propagation velocity of these continuously running pellets was
1.7 ± 0.2 mm/s (33 trials, n = 14), which was significantly
slower than velocity of continuously propagating pellets in
intact preparations (P < 0.001; Student’s unpaired 2-tailed
t-test, Fig. 2D). In six trials, pelletspropelled in a staggered
fashion along the colon, whereas, in one trial, inserted pellets
did not move from the oral end of the colon once inserted.

Overall, there was no difference in the proportion of peristaltic
waves that showed continuous, staggered, or nonpropagating
pellets between intact preparations of colon and those prepa-
rations with their mucosa and submucosalplexus removed (Fig. 2E).

Effects of mucosa and submucosal plexus removal on fluid-
induced peristalsis. Because preparations with mucosa re-
moved had very different intraluminal surfaces from intact
preparations with mucosa present, we tested whether the re-
duced propagation velocity observed (Fig. 2D) is possibly due
to these differences. To do this, we infused warm Krebs solution at 0.24 ml/min into the oral end of the intact colon.
This was found to reliably initiate peristalsis in both intact
preparations and dissected preparations with mucosa and sub-
mucosal plexus removed. Peristaltic waves were initiated in the
oral region, which then propagated anally, propelling fluid in
an anal direction until the fluid was expelled from the lumen.

The propagation velocity of peristalsis evoked in preparations
void of mucosa and submucosal plexus was still consistently
and significantly slower (2.1 ± 0.1 mm/s; n = 7) than the
velocity of fluid propelled in intact preparations (4.9 ± 0.9
mm/s; P = 0.006; n = 5; Fig. 3). These peristaltic waves
occurred with a mean interval between contractions of 3.6 ±
0.5 mm/s (n = 6) in intact preparations, which was similar to
the interval in dissected tube preparations with mucosa and
submucosal plexus removed (3.1 ± 0.3 mm/s; n = 5; P =
0.41). Infusion of tetrodotoxin (1 μM) through the lumen
abolished peristalsis in both mucosa-free and intact prepara-
tions (n = 3). We found thresholds too difficult to measure
with respect to fluid volumes because it was not possible to
control the amount of residual fluid remaining in the colon
before infusion of fluid.

Effects of removal of the mucosa and submucosal plexus on
5-HT release and on the pattern generator underlying the
generation of cyclical perilastic waves. We next recorded
5-HT release from the mucosa, in real time, to determine
whether release of 5-HT from this layer is required for
peristalsis, as has been previously suggested (12, 14, 15). In
intact preparations of colon, amperometric recordings re-
vealed a basal release of 5-HT of 22.2 ± 3.7 μM (n = 5).
This release ceased when the holding potential of the elec-
trode was shifted to 0 mV, at which point 5-HT is no longer
oxidized (2, 16). When a pellet was inserted into the oral
end of the colon and peristalsis was initiated, there was no
consistent increase in release of 5-HT (Fig. 4B). As the
pellet moved anally, tension in the fixed pellet was recorded
while amperometric recordings were made from the anal end
of the colon (Fig. 4).

Maintained colonic distension by a fecal pellet is known to
generate cyclical perilastic waves (19). In intact preparations
when the pellet was held at a fixed location between the oral
and anal regions of the colon, cyclical perilastic waves were
generated at a mean interval of 2.7 ± 0.3 min (range: 1.5–4.9
min; n = 7; Fig. 5) and with a peak amplitude and half-
duration of each perilastic wave of 8.8 ± 0.3 g (range:
4.2–13.4 g; n = 7) and 43.1 ± 1.8 s (20–65 s; n = 7),
respectively. We simultaneously measured 5-HT release from
the mucosa at the anal end of the tube preparation and
found inconsistent temporal correlation between the release of
5-HT and the occurrence of peristalsis. Each transient
release of 5-HT had a mean half-duration of 3.4 ± 0.3 s and
mean peak 5-HT concentration of 250.2 ± 28.5 μM (n = 5; Fig. 4, C and D).

When a pellet was held fixed in preparations free of mucosa and submucosal plexus, the cyclical generation of peristaltic waves persisted remarkably unaltered, suggesting that the pattern generator does not require the mucosa, submucosal plexus, or release of 5-HT from the mucosa. We confirmed that there was no detectable release of 5-HT during the initiation of these cyclical peristaltic waves (0.0 μM; n = 7; Fig. 5B). In these dissected preparations, the peak force generated during peristalsis (mean: 8.1 ± 0.5 g; range: 4.0–12.4 g; n = 7; P = 0.3; Fig. 5C), the half-duration of peristaltic waves (39.9 ± 3.1 s; range: 16–85 s; n = 7; P = 0.4; Fig. 5D), and the interval between cyclical peristaltic waves (mean: 3.7 ± 0.6 min; range: 1.7–6.5 min; n = 7; P = 0.11) were not different from those in the intact preparations. We additionally confirmed following these experiments that peristalsis of a free-moving pellet could still occur in these same preparations devoid of mucosa and submucosal plexus (Fig. 5F).

We finally made amperometric recordings from dissected preparations as pellets propagated freely along the colon (Fig. 6). Similar to our results using a fixed pellet, under no conditions did we record release of 5-HT when pellets were fixed or propelled along the colon in dissected preparations.

**DISCUSSION**

It is well known that endogenous 5-HT (and many other substances) are released from EC cells in both the small intestine (2, 6) and colon (9, 12) following mechanical stimulation of the mucosa. It is also known that exogenous 5-HT can initiate peristalsis. These findings have led to the suggestion that release of 5-HT from EC cells following luminal distension plays an important role in the initiation of peristalsis in small intestine (6) and colon (12, 14, 15). We sought to investigate this hypothesis more deeply using amperometry, which enables real-time dynamic changes in 5-HT release to be recorded. Our study shows that, when the colonic mucosa and submucosal plexus is removed from tubular preparations of colon, all detectable release of 5-HT from EC cells ceases, but this does not prevent peristalsis evoked by either solid pellets or fluid distension. In fact, although the rate of propagation of peristalsis was slower following removal of the mucosa and submucosal plexus, all the other major parameters of peristalsis were unaffected, including the force generated by the muscularis externa and the frequency of peristaltic waves. How these observations can be reconciled with previous reports is important for our understanding of the genesis of gastrointestinal motility and mechanisms underlying distension-evoked peristalsis. 

[^1]: http://ajpgi.physiology.org/}

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**Fig. 2.** The initiation and propagation of peristalsis is unaffected by removal of the mucosa and submucosal plexus. **A:** video imaging set up used to record the diameter of the colon during peristalsis and propagation of fecal pellets. **B:** spatiotemporal map showing the propagation of a fecal pellet in a preparation devoid of mucosa and submucosal plexus. **C:** sequential photomicrographs of the tube preparation used in B whereby the propagation of a fecal pellet from the oral to anal end is shown. **D:** the propagation velocities of fecal pellets was significantly slower in preparations with mucosa and submucosal plexus removed. **E:** table showing the proportion of colonic preparations that showed continuous, intermittent, or nonpropagating pellet propulsion. Following removal of the mucosa and submucosal plexus, there was no overall difference in the proportion of preparations that showed continuous, staggered, or nonpropagating peristalsis. 

**N:** No. of animals on which observations were made.
future strategies for the development of drugs targeting
motility.

5-HT acts at multiple sites. Luminal distension of the
intestinal wall is not the only stimulus associated with 5-HT
release from EC cells. Mechanical distortion of the mucosa
(shear) as well as chemical stimuli, including acids, bases,
short-chain fatty acids, glucose, various tastants and olfac-
tants (22), and cholera toxin, all trigger EC cell 5-HT release
(28). In addition, a large number of neurotransmitters and
receptor agonists increase mucosal 5-HT release (21). It is
also now clear that endogenous 5-HT is released during
peristalsis in response to muscle contraction (2, 16) or
increased intraluminal pressure (4, 6) and that exogenously
applied 5-HT can stimulate intrinsic sensory nerve endings
in the mucosa (4) and accelerate colonic transit (27).

Peristalsis and release of 5-HT. Given these roles of
endogenous mucosal 5-HT in controlling gut function, the
results of the present study are, at first sight, surprising.
Removal of the mucosa had minor effects on peristalsis,
only affecting the propagation velocity and was without any
effect on the intervals between peristaltic waves or the
propulsive force exerted on pellets. If 5-HT release from EC
cells was required for peristalsis, removal of the mucosa
would be expected to abolish the motor pattern. Clearly, this
did not happen. We confirmed EC cells were removed by the
dissection, since immunohistochemical staining confirmed
that the entire submucous plexus had been excised and 5-HT
release could not be detected with carbon fiber amperomet-
ry.

During this study, we recorded transient increases in local
intraluminal 5-HT concentrations. The basis for these tran-
sient peaks is not clear but may reflect local contractions of
the colon, leading to release, as has been well described in
the small intestine (5). Evidence that these peaks in 5-HT
release were arising from the mucosa was supported by the
observation that, when the mucosa and submucosa were
removed, peristalsis persisted, but 5-HT release was no
longer measurable. Although the basis for these rises in
5-HT are not entirely clear, what is clear is that EC cell-
derived 5-HT release does not initiate colonic peristalsis,
nor is it required for their propagation. Recently, we re-
ported similar observations in our study of the mouse colon
that showed that 5-HT release could be released from EC
cells at the same time as many, but not all, spontaneous

Fig. 3. Effects of removal of the mucosa and submucosal plexus on fluid-evoked peristalsis. A: schematic of the preparation used to evoke peristalsis by fluid
infusion while making video recordings of circular muscle diameter during constant fluid infusion and peristalsis (spatiotemporal D-map). B: hematoxylin and
evans (H&E) stain of intact distal colon showing the presence of the mucosa and muscularis mucosa. C: spatiotemporal map of an intact colon showing cyclical
peristaltic waves occur rhythmically in response to a slow constant infusion of warm Krebs solution (at 240 μl/min). D: H&E stain from a dissected preparation
confirming removal of the mucosa and submucosal plexus. E: a spatiotemporal map showing removal of the mucosa and submucosal plexus did not prevent the
initiation of peristalsis by fluid infusion nor the propagation of fluid content. F: the propagation velocity of peristaltic waves evoked by fluid infusion were
significantly slower in preparations in which the mucosa and submucosal plexus were removed. *Significantly different from the other data.
colonic migrating motor complexes (CMMCs) (16). However, removal of the mucosa and submucosal plexus abolished all release of 5-HT without preventing generation or propagation of spontaneous CMMCs (16). Put together, these observations suggest that 5-HT release occurs as a result of motor activity, rather than acting as a trigger for it.

Exactly how motility is coupled to EC cell activation is unclear. Distortion of the EC cell itself may directly cause 5-HT release, or transmitters released by enteric neurons may act as this trigger (2, 21).

Intrinsic sensory neurons and 5-HT. Since experiments carried out early in the last century (1), it is clear that nerve cells within the gut wall can activate enteric motor reflexes in response to mechanical stimuli, without extrinsic circuitry. Several types of mechanosensitive enteric neurons exist, including myenteric and submucosal Dogiel type II neurons (11) and different classes of Dogiel type I neurons (18, 25). Of these putative enteric mechanosensors, Dogiel type II cells project to the lamina propria and mucosa (23) and are potently activated by exogenous 5-HT applied to their cell bodies (29) and mucosal terminals, largely via 5-HT3 receptors (4). Surprisingly, there is rather less direct evidence that afferent neurons with mucosal terminals are activated by endogenous 5-HT released in response to physiological stimuli. The results of the present study suggest that submucosal intrinsic primary afferent neurons either play no role or only a minor role in the initiation and propagation of colonic peristalsis in the guinea pig. It is possible that they are more closely involved in reflexes activated by nutrients (13) or in secretomotor reflexes. The sensory neurons that are activated by distension must be located within the outer muscle layers (myenteric plexus) of the colon and are sufficient to trigger and maintain fully functional peristalsis. While these myenteric neurons may

Fig. 4. The measurement of 5-hydroxytryptamine (5-HT) release during peristalsis using amperometry in a colonic tube preparation. In an intact colon, real-time amperometric recordings of 5-HT release were made from a population of enterochromaffin (EC) cells at the same time as cyclical peristaltic waves were initiated in response to a fixed pellet. A: photomicrograph showing the preparation used, showing the location of the carbon fiber electrode and fixed pellet. Amperometric recordings were made at 2–3 mm anal to the fixed pellet. B: a simultaneous recording of 5-HT release at the same time as cyclical peristaltic waves in response to a fixed pellet. Left, insertion of fecal pellet and initiation of cyclical peristaltic waves. Transient 5-HT release events occurred, which were uncorrelated with peristaltic contractions. Release of the pellet (arrow) induced propagation of the pellet to the anal end of the colon as shown in A. At this site, there was an increased amplitude of 5-HT release events, some of which occurred at the same time as peristalsis and other release events that had no temporal correlation with peristalsis (B, right). CM, circular muscle. C: a frequency histogram of the concentrations of 5-HT release events. D: a frequency histogram of the duration of 5-HT release transients.
be activated by endogenous 5-HT released from EC cells and many project to the mucosa and express 5-HT receptors, our results suggest such activation is clearly not involved in the initiation of peristalsis. These conclusions are consistent with recent work which has shown that selective inhibition of the enzyme that synthesizes 5-HT in EC cells does not lead to any significant change in transit time, gastric emptying, or colonic motility in mice (30).

**Mucosal release of 5-HT is not required for the initiation or propagation of peristalsis.** We recently reported that hexamethonium, a nicotinic antagonist, did not block peristalsis nor reduce the propagation velocity of fecal pellets along the guinea pig colon. However, it did significantly reduce the propulsive force exerted on a fixed pellet during peristaltic contractions (19). This suggests that propulsive force is a useful measure of the functioning of the underlying circuitry, in addition to propagation velocity. In our present study, a major observation was that the intervals between bouts of peristaltic contractions and propulsive force evoked by a fixed pellet were not affected by removal of the mucosa and submucosal plexus. However, propagation velocity of both pellets and fluid was reduced. There may be several explanations for this. One possibility could include any number of substances secreted from EC or epithelial cells in the mucosa or neurons within the submucosal plexus, and identifying this regulatory mechanism remains an area of further investigation.

**Identification of the pattern generator underlying cyclical peristaltic waves.** The mechanism and identity of the pattern generator that underlies the cyclical generation of peristalsis has remained elusive for some time. A major observation in this study was that, in response to maintained distension by a fixed pellet, the cyclical generation of peristalsis persisted when the mucosa and submucosal plexus were removed from the colon and all detectable release of 5-HT was prevented. Thus the pattern generator underlying the cyclical generation of peristaltic waves cannot require EC cells, epithelial cells, 5-HT release from EC cells, or activity from within submucosal ganglia. This leaves us with the inescapable conclusion that the pattern generator activated by maintained distension to generate repetitive peristaltic waves must lie in the myenteric plexus and/or muscularis externa (Fig. 7). In strong support of this latter conclusion, we recently showed using intracellular electrophysiology...
that circumferential stretch of the guinea pig distal colon by fecal pellets potently activated intracellularly recorded peristaltic reflex circuitry, which was unaffected by removal of the mucosa and submucosal plexus (24).

In conclusion, the findings of the current study show that, in response to physiological distension of the distal colon, the mechanisms that initiate and propagate peristalsis do not require any release of 5-HT from EC cells, submucosal neurons, or activation of sensory nerve endings in the mucosa. We also show that, following maintained radial distension, the pattern generator that underlies the cyclical initiation of peristaltic waves must lie in myenteric ganglia and/or the muscularis externa. Whether EC cells and serotonergic mechanisms play a direct role in inducing changes in motility following inflammatory bowel disease states requires further careful and systematic experimentation.

Fig. 6. Real-time amperometric recordings of 5-HT release during the propagation of a fecal pellet in a dissected preparation, free of mucosa and submucosal plexus. A: preparation used showing the location of the carbon fiber electrode at the anal end and the pulley system to record force generated by the circular muscle during peristalsis. Arrow 1 indicates the tightly contracted circular muscle during peristalsis. B: as the string is cut, the fecal pellet is propelled anally, without any detectable basal or transient release of 5-HT.

Fig. 7. Diagrammatic representation of the location of the sensory neurons and mechanoreceptors required for the initiation of peristalsis evoked by solid or liquid content. The mechanoreceptors and sensory nerve cell bodies that respond to radial distension and initiate peristalsis lie in the myenteric plexus and/or muscularis externa. Submucosal neurons and nerve endings in the mucosa are not required for the initiation or propagation of peristalsis.
GRANTS

The experiments carried out in this study were funded by grants to N. J. Spencer (Grant nos. 535034 and 535033) from the National Health and Medical Research Council of Australia. D. J. Keating was supported by a Future Fellowship from the Australian Research Council and Bioinnovation.

DISCLOSURES

No conflicts of interest are declared by the authors.

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