Interrelationship between colonic muscularis mucosae activity and changes in transmucosal potential difference

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PREVIOUS STUDIES (27) have demonstrated an association between colonic muscularis mucosae activity and changes in transmucosal potential difference. Spontaneous muscle contractions and potential difference oscillations occurred independently and were not neurally driven. 

Integrated function; afferent-efferent pathway

motility and secretion; luminal stimuli; submucosal plexus; integrated function; afferent-efferent pathway

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part, been due to the lack of a preparation in which the function of both systems could be measured concurrently. Because of this shortfall, the aims of the present study were to 1) develop an in vitro technique to simultaneously record colonic muscularis mucosae contractile activity and relaxation and transmucosal potential difference and 2) use this preparation to assess the nature of the relationship between muscularis mucosae motor activity and mucosal function under basal and stimulated conditions.

**METHODS**

**Preparation of Muscularis Mucosae-Mucosa Cylinder**

The technique used in this study was based on an early preparation for the investigation of muscularis mucosae contractile activity in vitro (15) and is also a modified version of a technique originally described by Goldhill and Percy (9) for the simultaneous measurement of rabbit distal colonic transmural potential difference and both longitudinal and circular muscle activity in vitro.

Male New Zealand White rabbits (2–3 kg) were euthanized by pentobarbitral overdose. After laparotomy, a segment of distal colon ~4 cm in length situated immediately proximal to the pelvic brim was removed and transferred to a dish coated with Sylgard (Dow Corning, Midland, MI) and filled with oxygenated Krebs solution. Under a low-power dissecting microscope, the longitudinal and circular muscle layers were removed en bloc by sharp dissection, leaving a cylinder composed of submucosa, submucosal plexus, muscularis mucosae, and mucosa. Throughout this procedure, the lumen was regularly perfused with fresh, oxygenated Krebs solution to maintain epithelial viability. The ability to maintain colonic mucosal integrity during extended periods of luminal perfusion with an oxygenated physiological solution has previously been noted (12). A polyethylene catheter with a flared end connected to a peristaltic pump was then tied securely into the proximal end of the cylinder.

The whole preparation was next transferred to a 50-ml organ bath as described previously by Goldhill and Percy (9). The distal end of the muscularis mucosae-mucosa cylinder was next attached to one arm of a “T” connector fixed in the base of the bath. The second arm of this connector was used as a luminal outflow. An agar-salt bridge electrode was inserted into the third arm of the T piece, bringing it into electrical contact with the luminal perfusion solution. For control experiments, this solution was oxygenated Krebs solution at 37 ± 0.5°C, at a flow rate of 12 ml/min, delivered via a Masterflex peristaltic pump (Cole-Parmer, Chicago, IL; model 7520-35). A second agar-salt bridge was then placed in the serosal bath to equilibrate for at least 30 min before the start of each experiment. All responses were recorded on a Grass model 7D polygraph. Under these recording conditions, movement artifacts were excluded from the potential difference recordings, because neither electrode was in contact with the preparation itself. By convention, increases in muscle tone and transmucosal potential difference (increased luminal negativity) were denoted by upward movements of the chart recorder pens.

**Muscle Strip Preparation**

Strips of muscularis mucosae attached to mucosa were prepared using the “sutured edge” technique originally described by Percy and Christensen (21). Briefly, strips of mucosa, muscularis mucosae, and submucosa 3 cm in length were tied in the middle with 5-0 surgical thread, and each end was tied in the serosal and luminal sides, respectively, with the oral and aboral ends, now side by side, were tied together to form a loop. Because curling of the tissue tends to expose the mucosal surface rather than the submucosal aspect, the vertical edges of the preparation were sutured at four points with 7-0 surgical thread. The tissue is anchored over a “T” piece. This serves as the outflow for the Krebs solution pumped through the lumen of the preparation at ~12 ml/min. The proximal end of the tissue is attached to a transducer that records muscularis mucosae motor activity. PD is measured between 2 agar-KCl electrodes: the first in the base of the T piece in contact with the luminal solution, the second in the serosal bathing solution. Movement artifacts are eliminated because neither electrode is in physical contact with the preparation itself. In addition to the serosal application of pharmacological agents, a variety of stimuli can be applied to the mucosal aspect of the preparation by using separate reservoirs containing solutions of different composition and opening and closing a 3-way connector (*). The Krebs solution bathing the tissue and that being used for luminal perfusion are constantly oxygenated and maintained at 37 ± 0.5°C.

**Fig. 1. Schematic representation of the apparatus used for simultaneously recording muscularis mucosae contractile activity and changes in transmucosal potential difference (PD).**
nitude stretched this muscle to approximately the optimal length for the generation of active tension.

**Ussing Chamber Studies**

As previously described (10), segments of distal colon were opened along their mesenteric border and rinsed in Krebs solution to remove residual fecal material. The tissue was next pinned, mucosal surface down, in a petri dish in oxygenated Krebs solution. The muscularis propria was removed by sharp dissection, leaving a preparation consisting of mucosa, lamina propria, muscularis mucosae, and submucosal plexus. This was then mounted between two halves of an Ussing chamber. Both halves of the chamber were circulated with oxygenated Krebs solution. The potential difference was monitored using one pair of agar-salt bridges connected to this apparatus and short-circuit current automatically determined. Compensation for fluid resistance and potential difference arising from electrode asymmetry was made before mounting tissues.

**Effects of Pharmacological Agents on Cylinder Preparation**

ACh (10⁻⁹–10⁻³ M) was added to the serosal bathing solution to quantitate excitatory changes and to confirm tissue viability. All drugs were added to the bathing medium in amounts that did not exceed 10% of the total chamber volume, and they were removed by draining and refilling the bath. Concentration-response curves for each agonist were constructed utilizing noncumulative addition protocols, and the ensuing muscle and mucosal responses were expressed as a percentage of their respective maximum responses to ACh. To avoid tissue fatigue and to eliminate errors associated with tissue deterioration over time, these preparations were exposed to a maximum of three pharmacological agents in any one experimental protocol. When possible, the order in which these drugs were administered was varied from day to day.

**Statistical Analysis**

Statistical differences were assessed using either a paired Student’s t-test or, in the case of multiple group comparisons, ANOVA followed by an appropriate parametric or nonparametric post hoc test. *P* < 0.05 was considered to be significantly different. In all cases, *n* represents one preparation from one animal.

**Drugs and Solutions**

Experiments were performed using a Krebs solution of the following composition (in mM): 118.5 NaCl, 4.75 KCl, 2.54 CaCl₂, 1.19 NaH₂PO₄, 1.19 MgSO₄, 25 NaHCO₃, and 11 glucose. ACh chloride (Sigma, St. Louis, MO) was dissolved in 5% sodium phosphate and diluted in Krebs solution acidified to pH 4 with 0.1 N HCl. Atropine sulfate, 1,1-dimethyl-4-phenyl-piperazinium iodide (DMPP), and histamine (all from Sigma) were dissolved in and diluted with a modified Krebs solution (composition in mM: 143 NaCl, 4.75 KCl, and 2.54 CaCl₂). TTX (Sigma) was dissolved in a citrate buffer (composed of 50 mM citric acid and 48 mM NaH₂PO₄). Vasoreactive intestinal polypeptide (VIP; Sigma) was dissolved in and diluted with distilled water. VIP stock solutions were frozen at −5°C when not in use, and fresh dilutions were made daily. Indomethacin (Sigma) was initially dissolved in 10 ml of a 54 mM Na₂CO₃ solution and subsequently diluted with distilled water. Acetic acid, mannitol, oleic acid, taurocholic acid (all from Sigma), and 1-monooleoyl-rac-glycerol (monoolein; ICN Biomedical, Costa Mesa, CA) were added during preparation of the Krebs solution used for perfusing the lumen of the tissue.

**RESULTS**

**Baseline Activity**

Under resting conditions, the preparations exhibited atropine- (10⁻⁶ M; *n* = 8) and TTX-resistant (10⁻⁶ M; *n* = 8) spontaneous muscle contractions of 0.5–5 mN in amplitude and oscillations in potential difference that ranged from 0.5 to 1 mV (Fig. 2). The frequencies were 6.37 ± 0.73 and 6.48 ± 0.52 events/min, respectively (*n* = 15 each). Because the ranges of values for these parameters were similar, their respective mean frequencies did not differ significantly from each other (Fig. 3, left). However, when the frequency of spontaneous muscularis mucosae contractions was plotted against the corresponding frequency of ongoing potential difference oscillations in the same preparation, the resulting line was described by the equation \[ y = 0.46x + 3.54; r = 0.64 (n = 15) \] (Fig. 3, right). From the slope of this line (0.46), it may deduced that these events did not occur in a 1:1 ratio.

**MUSCULARIS MUCOSAE**

[Graph showing muscle activity]

**MUCOSA**

[Graph showing mucosal activity]

Fig. 2. Simultaneous recording of spontaneous events in the combined muscularis mucosae-mucosa cylinder preparation. Spontaneous contractile activity of the muscularis mucosae occurred in association with clearly defined oscillations in PD, but these were not temporally related. Note that this system can clearly discriminate events as small as 4.9 mN (0.5 g) in the muscularis mucosae and 0.5 mV across the mucosa. Increases in transmucosal PD (increased luminal negativity) are shown as an upward movement of the recorder pen.
Responses to Pharmacological Stimuli

ACh. Both the muscularis mucosae and the mucosa responded to ACh (10^{-9}–10^{-3} M) with, respectively, concentration-dependent increases in tension and potential difference. There was a clear distinction between basal and stimulated events, and the largest ACh-induced mucosal responses were up to 20–30 times larger than ongoing spontaneous potential difference oscillations (Fig. 4). Basal transmucosal potential difference in the rabbit distal colon is known to be ∼50% amiloride sensitive, reflecting a significant contribution from Na^+ absorption (8). However, stimulus-evoked increases in short-circuit current in this tissue have been shown to be due to Cl^- secretion (1), and in these experiments, agonist-induced increases in potential difference are considered to reflect luminally directed anion movement. It was also noted that although the time taken for the development of maximal transmucosal potential difference changes was similar to the time for peak muscle contraction to be achieved, mucosal responses persisted long after the muscle had returned to baseline tone following removal of agonists from the bathing medium. In many instances, 20 min and several additional washes were required for full mucosal recovery (Fig. 4).

In a second series of experiments, a submaximally effective concentration of atropine (10^{-7} M; 30-min exposure) was used to reduce the responses of both the muscularis mucosae and the mucosa to ACh (10^{-3} M) by ∼50% (Fig. 5). When these tissues were then exposed to TTX (3 × 10^{-6} M) for 5 min, the responses of the muscularis mucosae to the same concentration of ACh were unchanged, but the mucosal responses were reduced by a further 40% (Fig. 5).
untreated tissues exposed to this concentration of ACh over the same time course did not differ significantly from their respective controls (Fig. 5).

Histamine. The muscularis mucosae responded to histamine (10^{-9}–10^{-3} M) with concentration-dependent contractions that reached 113.8 ± 4.8% (n = 10) of the corresponding ACh maximum (Fig. 6A). Histamine-induced changes in transmucosal potential difference, however, plateaued at 10^{-5} M histamine and at 10^{-3} M were only 39.9 ± 13.7% (n = 10) of the ACh maximum (Fig. 6A). Under these conditions, the largest histamine-induced muscularis mucosae contractions were significantly larger than the corresponding maximal potential difference changes (P < 0.001; n = 10) when each was expressed as a percentage of their respective maximum responses to ACh.

With the use of the sutured edge muscularis mucosae preparation, histamine elicited concentration-dependent contractions that reached 118.7 ± 5.6% (n = 7) of the ACh maximum (Fig. 6B). When studied using Ussing chambers, muscularis propria-free sheets of colonic mucosa were found to be refractory to histamine across the same concentration range (n = 6) (Fig. 6C).

DMPP and VIP. The ganglion-stimulating agent DMPP (10^{-9}–10^{-3} M) elicited concentration-dependent muscularis mucosae contractions and increases in transmucosal potential difference (Fig. 7A). In contrast to the effects of histamine, the largest DMPP-induced change in transmucosal potential difference (88.7 ± 7.1%) was significantly larger than the corresponding maximal muscularis contraction (56.5 ± 5.2) (P < 0.001; n = 19), when each was expressed as a percentage of the tissues’ maximum responses to ACh.

Exposure of this preparation to VIP (3 × 10^{-13}–3 × 10^{-7} M) produced large, concentration-dependent changes in transmucosal potential difference that reached a maximum of 109.1 ± 8.2% (n = 10) of the ACh maximum response, while causing small muscularis mucosae contractions (9.5 ± 2.7%; n = 10) only at the highest concentration utilized (Fig. 7B).
Responses to Luminal Stimuli

**Increased osmotic load.** With the use of the second luminal perfusion reservoir and the three-way stopcock (Fig. 1), the luminal perfusion solution was changed from normal Krebs solution to a Krebs solution containing 140 mM mannitol. Throughout a 30-min luminal perfusion with this solution, there was no measurable change in muscularis mucosae tone or transmucosal potential difference ($n = 8$).

**Increased intraluminal pressure.** Increased intraluminal pressure was achieved by occluding the perfusion outflow (Fig. 1) for a period of 10 min so that the inflow remained at 12 ml/min, but the outflow was reduced to 6 ml/min. Under these recording conditions, preparations were seen to “balloon” as the volume of fluid in the system increased, but no coordinated elevation of muscularis mucosae motor activity was noted in response to the change in luminal diameter. Distension was, however, associated with a small increase in muscularis mucosae resting tension (7 of 14 preparations) coupled to an attenuation or total loss of ongoing spontaneous motor activity (14 of 14 preparations) (Fig. 8). During this time, spontaneous transmucosal potential difference oscillations were lost (9 of 14 preparations), and the potential difference itself gradually declined (9 of 14 preparations) (Fig. 8). Preparations in which one of these mucosal phenomena was unchanged either lacked potential difference oscillations or had a small basal potential difference before the occlusion procedure. The characteristics of the occlusion-induced muscle and mucosal responses were not altered by pretreating a separate group of preparations with serosal TTX ($10^{-6}$ M; 5-min exposure; $n = 9$; Fig. 8).

Fig. 7. Contractile and transmucosal PD responses of the muscularis mucosae-mucosa preparation to the ganglion-stimulating agent 1,1-dimethyl-4-phenylpiperazinium iodide (A) and the putative neurotransmitter vasoactive intestinal polypeptide (B). Note that in both cases the relative change in PD exceeded the corresponding magnitude of muscularis mucosae contraction when each was normalized to its respective maximum ACh-induced response. Data are expressed as a % of each muscle and mucosal maximum response to ACh and are means ± SE of the no. of observations indicated. **P < 0.001, significantly larger than the corresponding %change in muscularis mucosae tone.

Fig. 8. Responses of 2 separate muscularis mucosae-mucosa cylinder preparations to increased luminal perfusion pressure that was achieved by occluding (but not closing) the luminal outflow. The period of occlusion begins at the arrows. Note that this stimulus evoked a concurrent, TTX ($10^{-6}$ M)-insensitive inhibition of muscularis mucosae motor activity that was preceded by a small increase in resting tone and a decline in transmucosal PD. Increases in transmucosal PD (increased luminal negativity) are shown as an upward movement of the recorder pen; 10 mN = 1.02 g.
**Bile salt-lipid mixture.** When the luminal perfusion solution was changed to Krebs containing taurocholic acid (15 mM), oleic acid (10 mM), and monoolein (5 mM), there was an increase in muscularis mucosae resting tone and an increase in the amplitude of spontaneous contractions (18 of 21 preparations). The increase in muscle tone was small relative to the amplitude of spontaneous contractions (Fig. 9). This was associated with an initial concurrent increase in transmucosal potential difference (16 of 21 preparations) that declined over the ensuing 20 min. In 2 of 21 preparations, the increase in potential difference was sustained, and in an additional 3 of 21 preparations, the decline in potential difference was not preceded by an increase. The muscle response was attenuated by a 5-min pretreatment with serosal TTX (10^{-6} M; 5-min exposure; n = 6; Fig. 9). The responses of the muscularis mucosae to luminal perfusion with this bile salt-lipid mixture were also attenuated after a 30-min pretreatment with serosal atropine (10^{-6} M; 30-min exposure; n = 6; not shown). The characteristics of the mucosal response to the bile salt-lipid mixture were unchanged by either TTX or atropine at these concentrations.

When the same bile salt-lipid mixture was applied to the serosal aspect of the preparation (n = 8), it elicited an immediate inhibition of muscularis mucosae motor activity and a loss of resting tone. This was associated with a concurrent ballooning of the cylinder and a decline in the transmucosal potential difference (not shown).

**Decreased intraluminal pH.** When the pH of the luminal perfusion solution was reduced to 4 by the addition of acetic acid, the transmucosal potential difference began a decline to zero immediately on its entry to the lumen (6 of 21 preparations); in the remaining 15 preparations, the reduction in potential difference was interrupted by a transient increase before the decline was complete (Fig. 10). However, there was a delay of 1–2 min before the muscularis mucosae exhibited a contractile response to this stimulus (19 of 21 preparations) (Fig. 10). In an additional two preparations, the onset of the muscle and mucosal responses appeared to be concurrent. In contrast to the effects of the bile salt-lipid perfusion, acetic acid-induced responses of the muscularis mucosae were large compared with spontaneous motor activity. Neither the muscle nor the mucosal responses were modified by serosal pretreatment with TTX (10^{-6} M; 5-min exposure; n = 16; Fig. 10), atropine (10^{-6} M; 30-min exposure; n = 6), indomethacin (10^{-6} M; 30-min exposure, n = 6), or pyrilamine (10^{-6} M; 30-min exposure; n = 5).

**DISCUSSION**

The results of the present study demonstrate that the cylinder preparation utilized in this investigation provides a viable method for the concurrent recording and analysis of the relationship between muscularis mucosae motor activity and transmucosal potential difference in vitro. Under basal conditions, it was possible to discriminate muscular events as small as 4.9 mN (0.5 g) and transmucosal potential difference changes of 0.5 mV. In response to pharmacological stimuli, muscular contractions in excess of 100 mN and transmucosal potential differences of 10–20 mV were
seen routinely. Thus the recording system is capable of measuring responses that vary by between one and two orders of magnitude, while maintaining a large signal-to-noise ratio across this range. The data obtained under these experimental conditions also show that the system is capable of distinguishing serosally initiated responses from those that are mucosal in origin. Furthermore, with the appropriate pharmacological tools, this preparation can be used to determine the relative contributions of neural, muscular, and epithelial events to the overall responses obtained after different stimuli.

In the absence of an external stimulus, there were measurable spontaneous contractions of the muscularis mucosae that occurred in association with clearly defined oscillations in transmucosal potential difference. The higher rate of spontaneous muscle activity in individual preparations may be related to the observation that the time taken for mucosal events to occur is greater than that required for similar degrees of muscle contraction and relaxation. This was particularly obvious during large agonist-induced responses. The resistance of spontaneous muscularis mucosae and mucosal events to both atropine and TTX suggests that neither is neurally driven nor dependent on spontaneous ACh release. In contrast, sutured edge muscularis mucosae preparations have been found to contract spontaneously at a higher frequency that can be increased or decreased by treatment with eserine or atropine, respectively (24). This implies that cutting strips of muscularis mucosae elicits continuous ACh release from submucosal neurons impinging on the muscle, whereas in the cylinder preparation the submucosal plexus is better preserved and this does not occur. Although spontaneous muscularis mucosae contractions occurred at a lower frequency under these experimental conditions, the rate of spontaneous potential difference oscillations lay within a range of values previously obtained (9) utilizing Ussing chamber techniques and also a cylinder preparation including the muscularis propria. This suggests that, as for the muscularis mucosae, a tonic release of ACh does not modulate spontaneous mucosal events in the cylinder preparation and is consistent with the observation that a significant component of the innervation of this colonic mucosa is noncholinergic (1). The data obtained with ACh and histamine illustrate several important points. First, although both agents elicited muscle contraction by a direct action, their effects on the mucosa were predominantly indirect. This was demonstrated by the TTX sensitivity of the mucosal response to ACh and also by the lack of mucosal sensitivity to histamine in the Ussing chamber studies. It is tempting to infer from these data that changes in potential difference elicited by muscularis mucosae contraction may be partly neurally in origin. This is based on the observations that ≧50% maximal histamine-induced muscle contractions elicited ≈40% maximal increases in potential difference, but in the presence of TTX, 70% maximal ACh-induced contractions produced only ≈15% maximal increase in potential difference. This hypothesis is further supported by our (22) recent discovery that, under the same experimental conditions, maximal bradykinin-induced changes in transmucosal potential difference are reduced by ≈80% in the presence of TTX, although con-
traction of the muscularis mucosae are unchanged in amplitude by this procedure. These data support the belief that the in vivo motor activity of the muscularis mucosae does modulate mucosal function and further suggests that this is, in part, coordinated via the submucosal plexus.

Although histamine elicited large muscularis mucosae contractions but relatively small changes in transmucosal potential difference, DMPP-induced mucosal responses were significantly larger than those of the muscle, when both were expressed as a percentage of their respective maximal responses to ACh. This is consistent with the observations that this mucosa has a substantial excitatory (secretomotor) innervation (1), whereas neurally driven muscularis mucosae excitation is small and compounded by a concurrent inhibition (24). Similarly, VIP evoked large increases in transmucosal potential difference but only trivial responses from the muscularis mucosae. Again, this is consistent with the known actions of VIP on the mucosa (1) and the muscularis mucosae (24). Thus under certain conditions large changes in transmucosal potential difference can be achieved in the absence of maximal muscularis mucosae contractions.

From these observations, it can be seen that the properties of the combined muscularis mucosae-mucosal cylinder closely resemble the known behavior of the individual components of this preparation. Therefore, this strongly suggests that in terms of serosal stimuli the responses seen in the present study are a true reflection of the nature of the relationship between the muscle and the mucosa and their modulation by the submucosal plexus in the intact organ in vivo.

In dog small intestine, the mucosal application of a bile salt-lipid mixture elicits an atropine-sensitive increase in villous motor activity (18), which cannot be reproduced with saline or acidified predigested food (38). In the present study, a similar atropine- and TTX-sensitive, muscularis mucosae motor response to the presence of a bile salt-lipid mixture in the colonic lumen was found. Although luminally applied bile salts stimulate colonic afferent fibers (17, 36), the muscularis mucosae responses obtained previously (18) and in the present study were atropine sensitive, implying that the increase in motor activity was not the result of the release of substance P from sensory neurons. Release of this peptide from afferent fibers has previously (28) been suggested to contribute to the excitatory responses of opossum esophageal muscularis mucosae to electrical field stimulation. Therefore, the present data suggest the existence of a specific afferent-efferent pathway that ultimately leads to the activation of cholinergic submucosal motoneurons. Selective neural mediation of this motor phenomenon is further supported by the inhibitory actions of the same bile salt-lipid mixture when applied to the serosal aspect of the preparation. It should be noted, however, that luminally applied bile salts stimulate colonic mucosal generation of prostaglandins and nitric oxide (4), and the potential contribution of these to the responses seen in the present study warrants further investigation.

In contrast to the actions of the bile salt-lipid mixture, other luminal stimuli failed to elicit neurally mediated motor responses from the muscularis mucosae. Increased intraluminal pressure attenuated the motor activity of the muscle and elicited a decline in the transmucosal potential difference in a TTX-insensitive manner. Furthermore, distension of the cylinder preparation by this procedure did not elicit any observable coordinated muscle activity analogous to a "peristaltic reflex," suggesting that luminal clearance is not a physiological function of the muscularis mucosae in this region of the gut.

The resistance of the acetic acid Krebs-induced muscle response to a variety of pharmacological agents demonstrates that it was not neural in origin and did not arise from prostaglandin production or the release of histamine from mast cells. Interestingly, although short-chain fatty acids have been reported (5) to decrease colonic motility in vivo via the release of polypeptide YY, their excitatory in vitro actions result from direct stimulation of intestinal smooth muscle, supporting the findings of the present study. The lag time between the onset of the mucosal response and muscularis mucosae contraction to acetic acid suggests that this is a measure of the time taken for it to diffuse across the mucosa (19) and act on the muscle. The effects of the various luminal stimuli on the transmucosal potential difference may be explained either by a transient, distension-induced decrease in mucosal integrity or the high concentration of bile salts and charged ions overwhelming the ionic gradient that forms the basis of the potential difference being measured.

On the basis of these observations, it may be concluded that the neural pathways stimulated by the presence of the bile salt-lipid mixture are highly selective. This would suggest that neurally coordinated muscularis mucosae motor activity and mucosal function occur in response to specific luminal stimuli and that other mechanisms, such as release of paracrine substances (22), may facilitate absorption/secretion at other times. Given the varied pharmacological properties of the muscularis mucosae throughout the intestinal tract (e.g., Refs. 23–25), the possibility exists that different luminal stimuli elicit coordinated muscularis mucosae and mucosal activity in sequential regions of the gut.

Although it could be argued that radial distension in the absence of structural support from the muscularis propria, a bile salt-lipid mixture, or acidified Krebs solution are not truly physiological stimuli in the distal colon, their use in this instance was not designed to mimic in vivo conditions. Rather, these were chosen as tools having the potential to reveal mucosal-to-muscularis mucosae pathways available to coordinate the activity of these two structures. Thus the demonstration of muscularis mucosae contraction in response to irritant or potentially damaging luminal contents suggests that this may be one component of a mucosal
protective response, because muscularis mucosae contraction reduces the mucosal surface area on which such substances can act. The nature of the in vivo physiological stimuli that initiate activity within the pathways now shown to be present requires further investigation.

The results of these experiments clearly demonstrate that the muscularis mucosae-mucosa cylinder in vitro exhibits basal and stimulated responses that do not differ significantly from several of the known properties of the individual tissues that comprise this system. However, the data from the present study greatly extend these observations by allowing for an evaluation to be made of the temporal relationships between these events and for the quantitative assessment of the consequences of activity in one structure on the function of the other structure. This novel in vitro technique significantly advances our ability to determine the nature of the relationship between the muscularis mucosae, the mucosa, and the various mechanisms by which the activities of these companion structures are coordinated throughout the gastrointestinal tract. By using this technique in conjunction with organ bath and Ussing chamber technology, it will now be possible to begin a determination of the function of the muscularis mucosae and its relationship to the mucosa throughout the gastrointestinal tract.

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