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Distinct patterns of myogenic motor activity identified in isolated human distal colon with high-resolution manometry

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Abstract

Background—Colonic high-resolution manometry (HRM) has been used to reveal discrete, propagating colonic motor patterns. To help determine mechanisms underlying these patterns, we used HRM to record contractile activity in human distal colon *ex vivo*.

Methods—Surgically excised segments of descending (n = 30) or sigmoid colon (n = 4) were immersed in oxygenated Krebs solution at 36°C (n = 34; 16 Female; 67.6±12.4years; length 24.7±3.5cm;). Contractility was recorded by HRM catheters. After 30 minutes of baseline recording, 0.3mM lidocaine and/or 1mM hexamethonium were applied. Ascending neural pathways were activated by electrical field stimulation (EFS; 10Hz, 0.5ms, 50V, 5s duration) applied to the anal end before and after drug application.

Results—Spontaneous propagating contractions were recorded in all specimens (0.1–1.5 cycles/minute). Most contractions occurred synchronously across all recording sites. In five specimens, rhythmic antegrade contractions propagated the full length of the preparation. EFS evoked local contractions at the site of stimulation (latency 5.5±2.4s) with greater amplitude than spontaneous contractions (EFS; 29.3±26.9 vs 12.1±14.8mmHg; *P*=0.02). Synchronous or retrograde propagating motor patterns followed EFS; 71% spanned the entire preparation length. Hexamethonium and lidocaine modestly and only temporarily inhibited spontaneous contractions, whereas TTX increased the frequency of contractile activity while inhibiting EFS-evoked contractions.

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CONFLICTS OF INTEREST

John Arkwright is Managing Director of Arkwright Technologies, a company that manufactures fibre-optic sensors for a range of industrial applications. No conflicts of interest were reported by the other authors.

Conclusions & Inferences—Our study suggests that the propagated contractions recorded in the organ bath have a myogenic origin which can be regulated by neural input. Once activated at a local site, the contractions do not require the propulsion of faecal content to sustain long distance propagation.

Graphical Abstract

Introduction

Contractile activity in the human colon plays a critical role in the absorption of water and electrolytes and in the propulsion and excretion of colonic content. Recent development of high-resolution manometry catheters have enabled detailed descriptions of propagating motor patterns throughout the colon in healthy human adults^{1–3} and the identification of motor abnormalities in adults and children with severe constipation.^{3–6} The relationship between motor patterns recorded in conscious humans and those recorded in long isolated segments of colon^{7,8} remains to be established.

Using high-resolution manometry, at least six motor patterns have been recorded in human colon in vivo.^{1,3,4,9} Two of these motor patterns recorded in healthy adults correspond to cyclic propagating motor patterns¹ and pan-colonic pressurizations.^{2,3} The first of these comprise repetitive propagating pressure events with a cyclic frequency of 2–6 per minute. The second pattern is characterized by synchronous increases in pressure that span all recording sites throughout the colon. They occur at frequencies varying between one per minute to one every four minutes.^{2,3} The physiological roles of these two motor patterns remain uncertain. However, the cyclic motor pattern, found predominantly in the distal colon, has been proposed to act as a brake that controls rectal filling.^{10,11} It has been suggested that pan-colonic pressurizations are associated with the passage of gas and liquid.^{2,3}

The mechanisms underlying these motor patterns are incompletely understood. It is likely that the cyclic motor pattern is driven by the pacemaker networks of interstitial cells of Cajal (ICC) which have a similar slow wave frequency of 2–6 cycles per minute.¹² These slow waves have been temporally associated with small amplitude variations of intraluminal pressure.¹³ This myogenic cyclic activity appears to be influenced by extrinsic nerves; the cyclic motor pattern rapidly increases in amplitude and occurrence as soon as a subject consumes a high calorie meal.¹

The mechanisms underlying pan-colonic pressurizations are less well defined. Corsetti et al³ hypothesized that the motor pattern may require colonic distension. Organ bath studies using colonic specimens from guinea pigs or mice have shown that constant distension can induce synchronous colonic contractions at a similar frequency to pan-colonic pressurizations in human studies.^{14,15} Constant stretch, applied by force transducers attached to the serosa of human colon in an organ bath, can also initiate repetitive contractions that appear synchronously along the length of large intestine.⁷

In this study, using a technique initially developed by Spencer et al⁷ with the addition of high-resolution manometry,⁸ we provide a detailed characterisation of spontaneous motor patterns and their responses to pharmacological agents and electrical stimuli in *ex vivo* specimens of human colon.

Methods

Specimen retrieval and initial handling

Segments of descending colon (n=40) or sigmoid colon (n=6) were obtained from 46 patients who underwent elective colorectal surgery (anterior resection) for non-obstructing distal large bowel cancer (n=43), or recurrent sigmoid diverticulitis (n=3), after obtaining prior informed consent. The length of colon resected was determined by the surgeon from the vascular territory of the inferior mesenteric artery, which spans from the splenic flexure to the upper rectum. The inferior mesenteric artery is routinely ligated in anterior resections, resulting in a length of macroscopically normal colon being resected along with the diseased segment.

To reduce time between specimen removal and motility recordings, an investigator (RMR or PH) was notified of surgery timing and immediately retrieved the specimen upon removal. The tumour with its mesentery and at least 5cm margin of normal colon was isolated and sent for histological review by a surgical pathologist. The remaining segment of normal colonic tissue proximal to the diseased segment (length = 24.7 ± 3.5 cm; range 15–36cm) was placed into warmed, pre-oxygenated Krebs solution (in mM: 118 NaCl, 4.7 KCl, 1.0 NaH_2PO_4 , 25.0 NaHCO_3 , 1.2 MgCl_2 , 11 D-glucose, 2.5 CaCl_2) and taken to the laboratory. Prior to placement in the organ bath, the specimen was cleared of residual blood and faecal content by washing with Krebs solution. In 10 additional experiments (n=8 descending colon; n=2 sigmoid colon) a 2cm wide ring was cut from the proximal end of the specimen. From these segments, circular muscle strips were excised from an inter-taenial region.

All specimens were placed in the organ bath within 20 minutes of removal from the patient. Following recordings, the intact tubular colonic specimens were required to be returned to the pathology department. Experimental recordings ranged from 1–5.5 hours (median = 2.13 hours). All specimens had normal colouration and did not appear to be cyanotic. The study was approved by the Flinders Clinical Research Ethics Committee no. 50/07.

Experimental set up

i) Intact colonic specimens (n=36)—Using an experimental setup similar to that described in previous studies (Figure 1),^{7,8,16} the specimen was placed into a water-jacketed organ bath filled with 2L of Krebs solution maintained at 36–37°C. Krebs solution was bubbled continuously with 95% O₂/5% CO₂. A tube attached to small aquarium pump was also position in the base of the organ bath. A high-resolution manometry catheter was taped with wax film (Parafilm 'M' laboratory film; Bemis, Neenah, WI, USA) to a Perspex rod which was inserted through the lumen of the specimen. The rod was fixed in the organ bath with the catheter abutting the mucosa on the lower side of the specimen. The oral and anal ends of the specimen were gripped by alligator clips, from which a thread ran to either end of the organ bath, anchoring the specimen (Figure 1). Either three or four isometric force transducers (depending on specimen length) were attached to the uppermost side of the specimen using alligator clips tied to silk threads and spaced evenly along the length of the specimen (~5–7 cm intervals; details below; Figure 1). The clips were attached to the serosa between the taenia coli. A resting tension of ~5g was applied to each transducer which lifted the colon up, ensuring close contact between the manometry catheter and the mucosa. The high-resolution manometry catheter and force transducers both recorded mechanical colonic activity, but data were analysed from the manometry catheter given the higher spatial resolution (1cm sensor spacing).

Specimens were equilibrated in the organ bath for 20 minutes. A control period was then recorded in which no drugs were applied, for a minimum of 30 minutes, to record spontaneous contractions. To establish the dependence of spontaneous activity on nicotinic synaptic transmission in the enteric circuits, we first added 1mM hexamethonium to 16 preparations. After hexamethonium, we tested further requirements for neural activity. Previously, the neurotoxin tetrodotoxin (TTX) has been used¹⁷ however, given the size of the 2L organ bath used for the intact tubular specimens, it was not feasible to use TTX for all studies on account of the volume required to achieve an effective concentration. In place of TTX, after the 30 minute control period, lidocaine (0.3mM), an alternative voltage-dependent sodium channel antagonist, was added to 16 preparations. TTX (0.6 µM) was only added to the final two preparations. Drugs were added along the length of the preparation. The vigorously bubbled Krebs solution, via the carbogen (95% O₂/5% CO₂) insufflation and the additional tube attached to the aquarium pump, ensured the drugs were rapidly mixed though the preparation.

To activate ascending neural pathways, electrical field stimulation (EFS; Grass SD9 Stimulator; Grass, Quincy, MA, USA; 2mm Pt electrodes, insulated up to the last 5mm of their length, spaced 2mm apart, 10Hz, 0.5ms pulse width, 50V, 5 seconds train duration) was applied at the anal end of the preparation. To avoid confusion between spontaneous and

EFS-evoked contractions, stimulation was applied after a spontaneous contraction, with a delay of half the average inter-contraction interval. Due to a technical fault, EFS was not applied to the preparation with TTX.

Circular smooth muscle mechanical recordings – force transducers and high-resolution manometry (HRM)

Isometric force transducers (Grass FT-03C; Grass, Quincy, MA, USA) were connected to custom-made preamplifiers (Biomedical Engineering, Flinders University) and then to a PowerLab (model 16/35, AD Instruments, Bella Vista, NSW, Australia) and a Macintosh computer running Labchart version 6 (AD Instruments, NSW, Australia). Two high-resolution manometry catheters were used to record intraluminal muscle contractions. The first, used for 12 recordings, was a fibre-optic catheter with 72 pressure sensors spaced at 1cm intervals.¹⁸ The fibre-optic catheter was attached to a spectral interrogator unit (FBG-scan 804; FOS&S, Geel, Belgium) and pressures were recorded in real time on a custom-written LabVIEW program (National Instruments, Austin, TX, USA). The second catheter, used for 24 recordings, was a commercially available, solid state high-resolution impedance manometry catheter (Sandhill Scientific, Unisensor USA Inc.) with 32 pressure sensors spaced at 1cm intervals. Pressure data was acquired at 10Hz using InSIGHT™ (Sandhill Scientific, Unisensor USA Inc.). Impedance data was not used in this study. Pressure data acquired by either catheter was exported as text (*.txt) files for analysis in custom-made PlotHRM software developed by the authors (LW), written in Matlab (Mathworks, MA, USA) and Java (Sun Microsystems, CA, USA).¹⁹

ii) Muscle strip studies (n=10)—To further test the neural contributions to activity in colonic smooth muscles, studies of small muscle strips were conducted. In 10 specimens, the proximal disease-free end of the specimen was removed and placed in a petri dish containing Krebs solution (see above) warmed to 36–37.5°C and bubbled with 95% O₂/5% CO₂. The ring specimen was cut open along the taenia coli and pinned out as a flat sheet. Excess fat, mesocolon, and mucosa were excised via sharp dissection. Two inter-taenial, transmural tissue strips of 5mm width and 10–15mm length were cut parallel to the circular muscle fibres. These circular muscle strips were placed into a warm-jacketed 100mL organ bath filled with Krebs solution, as described above.

Each strip was passed through a pair of ring platinum electrodes. One end of the preparation was sutured to a rod at the base of the organ bath and the other end was attached to a force transducer. Each strip was then tensioned to 10g in the direction of orientation of muscle fibres. Contractile activity was recorded using LabChart software (ADInstruments) via force transducers. Each strip was passed through a pair of ring electrodes (Biomedical Engineering, Flinders University). One end of the preparation was sutured to a rod at the base of the organ bath and the other end was attached to a force transducer (Grass FT-03C; Grass, Quincy, MA, USA). Each strip was then tensioned to 10g in the direction of orientation of muscle fibres. Force transducers were connected to custom-made preamplifiers (Biomedical Engineering, Flinders University), a PowerLab (model 8/30, AD Instruments, Bella Vista, NSW, Australia), and a Macintosh computer running Labchart version 6 (AD Instruments, NSW, Australia).

After a 20–30 minute equilibration period, a 30 minute basal period of spontaneous contractile activity was recorded. During this period EFS (10Hz, 0.5ms, 20V, for a duration of 10s) was applied to the strips to induce contractile activity. TTX (0.6 μ M) was then applied in the organ baths.

Data analysis

i) Intact tubular preparations

Contractile activities were manually identified in PlotHRM. Individual events were characterized in terms of: i) the direction of propagation, antegrade, retrograde or “synchronous” (i.e. all contractions starting within a period of 1s at all points along the specimen); ii) extent (length) of propagation, expressed as a % of the length of the preparation; iii) amplitude of component pressure waves; iv) speed of propagation; v) frequency of propagating events per minute, (“cycles per minute”/cpm). These characteristics were obtained for the control period and for each period after administration of drugs.

For each of the electrical field stimulations (EFS), the following information was collected; i) time from the start of stimulation to the start of the next contraction adjacent to the stimulating electrode; ii) whether the contraction at the stimulation site was associated with a propagating contraction. If so, we then determined iii) the extent of propagation, expressed as a % of the length of the preparation); iv) the direction, v) speed of propagation, and; vi) amplitude of propagation.

Finally, using an analysis technique similar to one we have published previously¹⁷, we determined if EFS could re-set the timing of spontaneous propagating contractile activity. This was achieved by determining the time interval between; i) spontaneous propagating contractions prior to EFS; ii) the last spontaneous propagating contraction prior to EFS and next spontaneous propagating contraction following EFS, and; iii) the EFS induced propagating contraction and the next spontaneous propagating contraction.

ii) Muscle strips preparations

The frequency of spontaneous contractions and resting tone was calculated during the basal period and after application of TTX. In addition, the amplitude of EFS-induced contractile activity was calculated prior to and after TTX. To quantify the response to EFS, the change in force was derived from mean force during stimulation minus the mean force in the 1-minute prior to stimulation.

Drugs—Lidocaine (0.3mM) and Hexamethonium (1mM) were purchased from Sigma-Aldrich, Castle Hill, Australia. These drugs were dissolved in Krebs solution. TTX was purchased from Alomone Labs. Jerusalem, Israel, and dissolved in de-ionized water.

Statistical analysis—For the intact tubular preparations, comparisons of characteristics (numbers, amplitude, velocity, extent of propagation) for propagating events before and after drug applications were made with a non-parametric Wilcoxon matched-pairs signed rank test.

The interval between electrical field stimulation (EFS) and the next contraction were compared to the average intervals between the five preceding spontaneous contractions at the same location using a non-parametric Wilcoxon matched-pairs signed rank test. The characteristics of the event evoked by EFS were also compared to the five preceding propagating events using the same test. In addition, the characteristics of the evoked propagating events were compared before and during exposure to lidocaine or hexamethonium. A one-way ANOVA with Tukey's range test post-hoc analysis was used to determine if there was a significant difference between the time interval between spontaneous propagating contractions prior to EFS and the time interval between EFS induced propagating contraction and the next spontaneous propagating contraction.

For the muscle strip studies, statistical comparisons of the spontaneous contractile frequency, tone, and EFS-induced contraction amplitude prior to and after TTX were made using with a non-parametric Wilcoxon matched-pairs signed rank test.

Statistical analysis was performed using IBM SPSS (Version 19.0, Released 2010; IBM Corp., Armonk, New York, USA).

Results

As there was no significant difference between the recording pressures captured by the two manometry catheters, the data recorded by the fibre-optic and solid state catheters were combined.

Mechanical recordings of colonic contractions during control period

Spontaneous contractile events were recorded in every specimen during control periods. Most contractions propagated the full length of the specimen and occurred at a frequency of 0.1–1.5 cpm (median 0.6 cpm). In 24 specimens, contractions appeared to occur simultaneously across most or all of the recording sites (Figure 2A). In five of these specimens, propagating contractions were in an unequivocal, antegrade direction (Figure 2B). The characteristics of the propagating activity during the control period are shown in table 1.

Electrical Field Stimulation (EFS) during the control period

EFS was applied to the distal end of 19 specimens (Figure 3). In 18 of these, a local contraction was observed with short latency after the onset of EFS (5.5 ± 2.4 s). In one specimen, no local contraction occurred. An attempt was made to apply EFS mid-way between contractions, with a delay of about half the interval between spontaneous contractions. Thus, we could test whether electrical stimulation modified the timing of the next contraction. This was indeed the case; the interval between the onset of EFS and the following contraction was significantly less than the average interval between the preceding five spontaneous contractions at the same location (5.5 ± 2.4 s vs 173 ± 154 s; range 31 – 600s; $P < 0.0001$). This indicates that the next contraction was advanced by EFS. The interval between the spontaneous contractions prior to EFS and the delay from the EFS-induced contraction and the next spontaneous propagating contraction were similar (173 ± 154 s vs 198 ± 137 s; NS). This indicate that the EFS reset the rhythm of the propagating

contractions throughout the preparation, and that selective stimulation of the enteric nervous system can interfere with the intrinsic pacemaker frequency.

In all cases, contractions evoked by EFS became the start of a propagating event. Most of these evoked propagating events (79%) spanned the entire length of the preparation (up to 30cm). In the remaining specimens, propagating contractions were observed over at least 19% of the length. Overall, the length of propagation after EFS did not differ from the preceding spontaneous propagating contraction (EFS; 17.9 ± 5.7 v spontaneous 17.4 ± 7.3 cm). Following EFS, 85.8% of the evoked propagating contractions travelled in a retrograde direction at an average velocity of 44.2 ± 39.9 mm/s. This contrasts with spontaneous contractions prior to stimulation which mostly appeared simultaneously along the length of colon.

The amplitude of the local contraction evoked by EFS was significantly greater than the amplitude of spontaneous contractions at the same location (EFS 29.3 ± 26.9 v spontaneous 12.1 ± 14.9 mmHg; $P = 0.02$). The contractions 10 & 20mm oral to the site of the EFS that formed part of the initiated propagating contract were also of significantly greater amplitude than spontaneous contractions at those locations (10mm, EFS 23.6 ± 19.9 V spontaneous 54.7 ± 42.6 ; $P = 0.003$; 20mm, EFS 24.6 ± 20.1 V spontaneous 47.3 ± 43.4 ; $P = 0.03$). However, when the average amplitude of the EFS contractions throughout the entire propagating event were compared to the average amplitude of the spontaneous propagating contractions, there was no significant difference (EFS; 17.2 ± 16.2 v spontaneous; 21.1 ± 22.7 mmHg). This indicates that the EFS induced a large local contraction before triggering a propagating contraction that was indistinguishable from spontaneous propagating contractions.

Effects of nerve conduction blockade on colonic motility using lidocaine (n=14)

In 14 specimens, lidocaine (0.3 mM) was added after the control period. In another four specimens, it was added after hexamethonium (1 mM). In two of the 14 (where lidocaine was added first), there was a brief (<5min) interruption of the spontaneous propagating contractions. This was followed by a return of propagating activity in the continuing presence of lidocaine (Figure 4). Apart from this brief interruption, lidocaine had no effect upon the characteristics of the propagating activity compared to control (Table 1). Lidocaine applied after hexamethonium also had no additional effects on spontaneous propagating contractions.

Electrical field stimulation was applied to six specimens in the presence of lidocaine (Figure 4). In five of these, EFS was followed by a contraction at the site of stimulation with an average delay of 14 seconds (7.7 ± 2.9 s). This interval was significantly shorter than the interval between spontaneous propagating contractions recorded in the presence of lidocaine (74.8 ± 57.3 s; $P = 0.03$). Similar to the control period, EFS in the presence of lidocaine evoked a local contraction that was significantly larger than spontaneous contractions at the same site (EFS; 23.4 ± 14.1 vs spontaneous; 9.2 ± 8.3 mmHg; $P = 0.03$). In the presence of lidocaine, electrically evoked local contractions initiated a propagating event that spanned the length of the preparation, on each occasion. The majority of these evoked events (78%) propagated in a retrograde direction at 44.2 ± 30 mm/s. The characteristics of the EFS-

evoked propagating events during lidocaine did not differ from those stimulated during the control period (Table 1).

Effects of nicotinic receptor blockade on colonic motility using hexamethonium (n=16)

Nicotinic receptor antagonist hexamethonium (1mM), produced complete or near complete inhibition of spontaneous propagating contractions in 3/16 specimens tested. In six other preparations, hexamethonium was associated with a brief (<5 min) inhibition of spontaneous propagating activity, followed by a return of activity (Figure 5). In the remaining seven preparations, hexamethonium had no apparent effect on contractility. Where they occurred, spontaneous propagating contractions in hexamethonium did not show significant differences in their characteristics, compared to the control period (Table 1).

EFS was applied to all 16 specimens in the presence of hexamethonium (Figure 5). In all preparations, a contraction at the stimulation site was recorded within 14s of the start of the stimulus (mean $\pm$ SD; 5.6 ± 2.4 s). This delay was significantly shorter than the interval between spontaneous propagating events in hexamethonium preceding EFS (145.4 ± 57.6 s; $P < 0.0001$). EFS after hexamethonium induced a local contraction with a similar amplitude to spontaneous contractions (EFS; 23.2 ± 19.5 v spontaneous; 16.1 ± 17.5 mmHg; $P = 0.3$). The EFS-evoked local contraction developed into a propagating contraction in all but one instance; 63% of these propagating contractions spanned the full length of the colonic segment. The remainder spanned at least 18% of the colon length. The majority (77%) of these propagating events travelled in a retrograde direction at 31.3 ± 27 mm/s. In the three preparations in which spontaneous propagating contractions were inhibited by hexamethonium, EFS still evoked both local and propagating contractions. The characteristics of the EFS-evoked propagating events during hexamethonium did not differ from EFS-evoked propagating events during the control period (Table 1).

Effects of nerve conduction blockade on colonic motility using TTX (n=2)

The addition of TTX to two preparations did not inhibit spontaneous propagating contractions. To the contrary, TTX dramatically increased the frequency of these contraction (Fig 6) and, in one preparation, increased the basal tone (Fig 6A).

Muscle strip studies; effects of nerve conduction blockade on colonic motility using TTX (20 preparations from 10 patients)

As with the intact tubular preparation, application of TTX to muscle strips did not inhibit spontaneous contractions. Instead, TTX induced a significant increase in their frequency (basal; 1.7 ± 0.9 vs TTX; 3.2 ± 1.7 cpm; paired t-test $t(19) = -4.75$, $p < 0.001$). (Fig 7) A small increase in basal tone after TTX did not reach significance (basal 14.2 ± 5.6 vs TTX 15.7 ± 8.8 g; Wilcoxon signed ranks test $Z = -0.97$, $p = 0.33$).

Prior to TTX, EFS induced contractions in all specimens (force pre-TTX = 10.2 ± 6.7 g). TTX abolished the response to EFS in 16 of 20 preparations tested (80%). In the remaining n=4 preparations, the response to EFS was significantly diminished by TTX but a contraction of reduced amplitude persisted (force after-TTX = 0.7 ± 4.6 g; Wilcoxon signed

ranks test $Z=-3.55$, $p<0.001$). These results indicate that TTX, a low molecular weight water-soluble drug, penetrates colonic tissue effectively in the majority of preparations.

Discussion

The primary aim of this study was to provide a detailed description of a distinctive propagating motor pattern observed in long specimens (15 – 36 cm) of excised, intact human descending or sigmoid colon. In each preparation, we recorded spontaneous semi-regular propagating contractions spanning the length of the colonic specimen. These propagating events either occurred simultaneously at all recording sensors, or in some specimens, propagated in an antegrade direction. These findings confirm and extend the results of two previous studies.^{7,8} In one study, motor patterns were recorded with widely spaced force transducers which prevented accurate determination of the direction of propagation.⁷ The second study focused primarily on excised colons from four patients with slow transit constipation.⁸ Here, our aim was to provide a detailed account of these events and identify some of the underlying mechanisms.

Identity of low frequency propagating contractions, *ex vivo*

In most experimental animals investigated, neurally-dependent repetitive motor complexes have been observed in isolated *ex vivo* preparations of distal bowel.^{20–24} In rabbit and rat, repetitive myogenic slow contractions extended over large proportions of the colon, becoming most prominent after pharmacologically blocking neural activity.^{21,23,24} How these relate to the contractions in the present study is an important question. In the majority of our human specimens, pressure waves occurred simultaneously across all recording channels. The frequency of these events varied, but by the end of the 30-minute equilibration period, they occurred at close to 1cpm. Spontaneous contractions at similar frequencies have been recorded in human circular^{17,25–29} and longitudinal^{27,29–33} muscle strips, and in short (4cm) tubular segments, *ex vivo*.²⁹ This slow phasic activity is tetrodotoxin-resistant^{17,26,28,32,33} and present in aganglionic segments,³⁰ suggesting that it is fundamentally myogenic. However, these myogenic contractions appear to be modulated by neural pathways, as activation of ascending myenteric neural pathways triggered premature contractions with similar time course and amplitude¹⁷ (also see section on Drug Resistance below).

Similar motor patterns have also been recorded during *in vivo* colonic manometry recordings. Corsetti et al,³ defined “pan-colonic pressurizations” which occurred in healthy adults at close to 1 minute intervals, a finding supported Chen et al.⁹ Corsetti et al³ proposed that these motor patterns require colonic distension. Previous studies in animals have shown that constant distension of excised animal colon can result in contractile events of a similar frequency and appearance of the human pan-colonic pressurizations.^{15,34} In our previous *ex vivo* studies of excised, intact human colon^{7,8} as well as this current study, clips were attached to the serosa to apply constant tension to the gut wall; a stimulus that mimics distension. In all of these studies, regular synchronous contractions were observed.

Pan-colonic pressurizations have not been reported in all *in vivo* manometry recordings of humans.^{1,6,8,35} This inconsistency may be explained by differences in the protocols. In

studies where pressurizations were present, either tap water enemas had been given³ or recordings were made with water-perfused catheters.⁹ Tap water enemas often fail to remove stool from the entire colon, so a degree of content-distension persists. In the study using water perfused manometry,⁹ up to four litres of water was introduced into the colon over a seven-hour period which may have also resulted in a degree of distension. Studies in isolated mouse and guinea pig colon have shown that colonic motor complexes occurring at ~1cpm are generated by distension.^{14,20} In contrast, in human *in vivo* studies where pan-colonic pressurizations were not regularly observed, subjects underwent a full bowel preparation and a solid state catheter was used; these recordings were made in an empty colon.^{1,8,35}

The propagating contractions recorded in the present study at approximately 1cpm do not resemble “high-amplitude propagating contractions” recorded in human colon *in vivo*, which have a much lower frequency, a higher amplitude and propagate much more slowly (0.4–0.1 cm/s) than the events recorded in the present study (4.4 ± 4.0 cm/s).^{1,36–38} Another colonic motor pattern has been described; the cyclic propagating motor pattern, is also commonly recorded in human distal colon *in vivo*. It comprises pressure waves at 2–6 cycles per minute (cpm) which often propagate in a retrograde direction.¹ This pattern was not observed prior to the addition of the drugs or after application of hexamethonium or lidocaine, nor was it present in other studies of isolated large tubular preparations.^{7,8} However, in the present study, contractile events with a frequency of 2–6 cpm did emerge after application of TTX in both tubular and muscle strip preparations. The frequency of cyclic propagating motor pattern (2–6/min) is the same as the dominant type of slow waves generated by interstitial cells of Cajal in the colon.^{12,39} The cyclic propagating motor pattern is present *in vivo* before a meal and is greatly enhanced (within a minute) after consumption of a meal.¹ Previously, we had hypothesized that the amplitude of contractions at slow wave frequency is modulated by extrinsic neural input after eating via activation of enteric excitatory neurons. Extrinsic neural pathways to the colon are obviously not functional in resected specimens.⁸ This hypothesis could explain the conspicuous absence of 2–6 cpm cyclic propagating motor patterns throughout most of the present study. However, the appearance of this pattern after TTX suggests that this motor pattern may normally be suppressed by ongoing inhibitory motor activity. Application of TTX to segments of the proximal colon of mice and dogs also causes a significant increase in contractile activity^{40,41}, indicating the presence of ongoing inhibitory drive to the muscle. Human colonic manometry studies have also shown that proximal colonic propagating contractions increase after partial removal of tonic nitrenergic inhibition⁴². It should also be noted that repetitive contractions and underlying electrical activity in the 2–6/min range have previously been recorded from small muscle strips of human colon *ex vivo*.^{27,28}

Drug effects

In this study our specimens were largely unaffected by high concentrations of the nerve blocker lidocaine and the nicotinic channel blocker hexamethonium. There are two possible explanations. Either large rapidly propagating contractions do not require neural activity for their expression, or the drugs failed to penetrate the tissue. Electrophysiological recordings from human colonic circular muscle have shown, in some preparations, intermittent long depolarisations (at 1–3 minute intervals) with superimposed action potentials^{26,28} which are

not blocked by TTX. Similarly, Carbone et al¹⁷ recorded slow, intermittent phasic spontaneous contractions that were also resistant to TTX. In our current study, application of TTX to both muscle strips and tubular preparations caused an increase in contractile frequency and, in the latter, it also failed to block propagating contractions. Since lidocaine also works by blocking neural voltage-sensitive sodium currents, this suggests that the large phasic contractions do not require neural activity.

Electrical stimulation potently evoked premature contractions that propagated rapidly over long distances in our large preparations. This is consistent with fast neurotransmission in ascending neural pathways. Therefore, neural input may not be required, but neural inputs can affect the muscle activity, activating a premature contraction. Surprisingly, the effects of the electrical stimulation persisted after the addition of lidocaine or hexamethonium. This led us to question whether small molecular weight drugs could penetrate into the tissue during our experiments. However, in the muscle strip studies, TTX blocked the response to EFS. This strongly suggests that water soluble low molecular weight drugs do penetrate the tissue specimens. Lidocaine has been shown to inhibit motor patterns in several studies of colonic motility in ex vivo preparations from animals.^{23,24,43} However, in the current study, it seems likely that lidocaine did not fully access the myenteric plexus to cause complete neuronal blockade. This may be because lidocaine is lipid-soluble,⁴⁴ so it may also have been absorbed into the large fatty epiploic appendages along the colonic specimen which we could not trim, as the specimen had to be returned intact at the end of the recording to the surgical pathologist.

Arguments about fat solubility do not apply to hexamethonium, which also failed to block the simultaneous appearance of contractions over many centimetres of tissue. In some preparations, hexamethonium had no effect at all; in others it caused a temporary inhibition, but contractions reappeared after a few minutes. This suggests that hexamethonium did access nicotinic receptors within the enteric plexuses. However, it is possible that it was unable to access some ganglia, perhaps those located under the thick taenia coli. Such hexamethonium-inaccessible neural pathways (which would also be lidocaine-inaccessible) may suffice to trigger spontaneous myogenic contractions all along the entire preparation, which then appear to be tightly coordinated. Alternatively, it is possible that hexamethonium has a limited effect upon motor patterns in the distal colon as reported in the rabbit.⁴⁵ The effects of TTX, on the other hand, may have been mediated primarily by blockade of neural activity outside the ganglionated plexuses; for example in the axons of motor neurons.

Hexamethonium-resistant, long duration large contractions that occurred spontaneously and which could also be triggered prematurely by electrical stimulation were described in human colonic muscle strips by Carbone et al.¹⁷ There, as in the present study, the mechanism underlying activation is unlikely to be due to direct stimulation of excitatory motor neurons, which have orally-directed projections up to 10–12 mm.⁴⁶ Ascending interneurons can project up to 30mm up the gut and descending interneurons have even longer projections and can be activated antidromically by electrical stimulation.⁴⁷ If either pathway impinged on circular muscle motor neurons it could then trigger the contractions.¹⁷ It was speculated that neural stimulation worked via non-nicotinic pathways. This, together with poor drug penetration by lidocaine, may explain the present results.

Conclusion

Large segments of *ex vivo* human distal colon show spontaneous, organised propagating contractions that were detectable using high-resolution manometry. These contractions resembled previously described low frequency, myogenic phasic contractions. Nevertheless, electrical nerve stimuli potently evoked premature propagating contractions over long distances of gut, suggesting smooth muscle excitation via neural release of excitatory neurotransmitters from ascending neural pathways. The preparations used in the present study appeared resistant to hexamethonium and lidocaine, showing only temporary inhibition of spontaneous contractions while EFS-evoked propagating activity persisted. In the presence of TTX, contractile activity increased in both tubular and muscle strip studies and effects of EFS were blocked or greatly reduced. These data suggest that spontaneous slow phasic contractions are fundamentally myogenic but can be initiated by electrically induced local neurotransmitter release.

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Key Points

- Distinct propagating motor patterns have been identified in the human colon in vivo using high resolution manometry. To investigate mechanisms underlying these motor patterns, we used manometry in this study to record propagating motor patterns in excised human distal colon.
- Regular propagating motor patterns were recorded in all specimens. These motor patterns were only temporarily inhibited by hexamethonium or lidocaine. Electrical field stimulation evoked synchronous or retrograde propagating contractions which also persisted after application of hexamethonium or lidocaine. Tetrodotoxin increased the frequency of spontaneous propagating contractions.
- These findings suggest that the propagating contractions recorded in the organ bath have a myogenic origin which can be regulated by neural input. Once activated at a local site, the contractions do not require the propulsion of faecal content to sustain long distance propagation.

Figure 1.

A) Ex vivo setup of excised human descending colon. The preparation was immersed in an organ bath filled with Krebs solution, bubbled with 95% O₂/5% CO₂. The manometric catheter, bonded to a steel rod, was passed through the colon so that the sensors detected contractions in the gut wall. Force transducers applied tension to the wall, holding the bowel firmly against the recording catheter. B) Shows a schematic of the set up with the electrical field stimulation (EFS) applied at the anal end of the specimen via platinum electrodes insulated to within 2mm of their tips. C) Shows a profile of the setup.

Figure 2.

Spontaneous cyclical contractile events during control period occurred with two distinguishable patterns. (A) contractions were synchronous along the preparation (n=24) or, (B) contractions showed a clear antegrade propagation (blue hatched arrows denotes direction; n=5).

Figure 3.

EFS applied to the anal end of the specimen elicited propagating contractions that invaded the 25cm length of the preparation. The bottom image contains an expanded image of the region within the black rectangle. EFS caused a propagating contraction that travelled in a retrograde direction at 45mm/s.

Figure 4.

Application of lidocaine (0.3mM) caused a brief cessation of spontaneous activity in 2 specimens, followed by a return of contractions. In another 12 preparations, lidocaine had no detectable effect. As shown in the expanded section of the trace, EFS still elicited retrograde propagated contractions after lidocaine.

Figure 5.

(A) In this specimen, application of hexamethonium (1mM) caused a cessation of the regular spontaneous activity. After a brief period of inhibition propagating contractions recommenced. EFS was still able to induce propagating contractions that spanned the length of the preparation. B and C show expanded sections of the trace from the box outlined in A. Note that prior to hexamethonium (B) propagating contractions travelled in an antegrade direction. After hexamethonium (C) the propagating contractions travelled in a retrograde direction. In 7 preparations hexamethonium had no detectable effect at all on spontaneous contractions.

Figure 6.

The two preparations in which TTX was added. In A (top) TTX resulted in a dramatic increase in the frequency of the contractile activity occurring at 2–4cpm and basal tone. The post-TTX contractions regularly propagated along the segment of colon. Some examples are shown by blue arrows in expanded selection of trace. B. TTX resulted in an increase in propagating contractions (blue hatch arrow).

Figure 7.
A and B show examples of the muscle strip studies. In both A and B, TTX was only added to the top trace (red dashed line). Application of TTX blocked responses to EFS, while increasing contractile frequency.

Characteristics of propagating contractions

Characteristics of propagating contractions