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Regional difference in intestinal contractile response to radial stretch in the human lower gastrointestinal tract

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ABSTRACT

Intestinal motility is governed by multiple overlapping mechanisms. Radial stretch is an important stimulus to evoke contractions in the lower gastrointestinal tract. Recent findings revealed that mechanosensory enteric neurons, which are located in the myenteric plexus, have pivotal mechanosensitive projections in the circular muscle. Distension or stretch of the colon activates these sensory neurons to initiate polarized neural pathways that result in oral contraction and anal relaxation. This study aimed to elucidate the differences in stretch reflexes according to their location in the human lower gastrointestinal tract using ex vivo techniques, including electrophysiological recordings from intestinal smooth muscle tissue excised during elective colon cancer surgery. Conventional microelectrode recordings from muscle cells and tension recordings of ileal or colonic segments were performed. Intestinal segments measuring 5×2 cm were obtained and immediately placed in an oxygenated Krebs-Ringer bicarbonate solution. To record the activity of muscular contractions, three stainless steel clips were attached to each lateral margin of the colon in a direction that was orthogonal to the segment. The intraluminal stretch model was applied using a fine 9-Fr Foley catheter. Radial stretches were evoked by 1.0-mL inflation of the balloon at the catheter tip for 10 min. Frequency (/min), amplitude (mV), and area under the curve (AUC, mV*min) of muscle contractions were recorded before and after stretch intervention at the proximal and distal sites of the segmented intestines. In intracellular recordings after the stretch intervention from the circular muscles, hyperpolarization (from -54.4 to -55.3 mV) was noted at the distal site of the sigmoid colon, in contrast to depolarizations at all other sites. Tension recordings of colonic segments were analyzed in 92 bowel samples (24 ileum, 23 transverse colon, and 45 sigmoid colon specimens). In the proximal sites of the ileum segment, there were no

significant changes in frequency, amplitude, and AUC after radial stretch (p=0.223, p=0.376, and p=0.568, respectively). In the proximal sites of the transverse colon segment, contractile activation was observed with statistically significant increases in frequency, amplitude, and AUC after radial stretch (p=0.003, p<0.001, and p<0.001, respectively). Similarly, this pattern of contractile excitation was also demonstrated in the sigmoid colon segment, in which frequency, amplitude, and AUC were significantly increased in response to radial stretch (p<0.001, p<0.001, and p<0.001, respectively). In the distal sites of the ileum segment, there were no significant changes in frequency, amplitude, and AUC after radial stretch (p=0.793, p=0.853, and p=0.862, respectively). Similar to the proximal sites, contractile activation was observed in the distal sites of the transverse colon segment, in which frequency, amplitude, and AUC after radial stretch were significantly increased (p<0.001, p=0.002, and p<0.001, respectively). However, in the distal sites of the sigmoid colon segment, a pattern of contractile relaxation was observed, and the parameters frequency, amplitude, and AUC were significantly decreased after radial stretch (p=0.022, p<0.001, and p=0.001, respectively). In the generalized linear mixed model, the changing patterns in frequency, amplitude, and AUC differed between the proximal and distal sites of the sigmoid colon. The slopes of frequency changes in the proximal and distal sites were 0.09 and -0.05, respectively, and the slope difference was 0.13 (p<0.001). The slopes of the amplitude changes in the proximal and distal sites were 4.60 and -4.16, respectively, and the slope difference was 8.76 (p<0.001). The slopes of the AUC changes in the proximal and distal sites were 152.80 and -83.51, respectively, and the slope difference was 236.31 (p<0.001). These results indicate that radial stretch in the human colon in vitro evokes excitatory contractions in both the proximal and distal sites of the transverse colon and the proximal site of the sigmoid colon but inhibits contractions in

the distal site of the sigmoid colon. This study draws attention to the stretch reflex as a potential determinant factor for the differences in functions between the right and left colon.

Keywords: radial stretch, colon, motility, enteric nervous system, mechanoreceptor

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INTRODUCTION

Gastrointestinal (GI) motility disorders are prevalent and have an immensely debilitating effect on the quality of life of the affected patients. These disorders have become a serious health and economic burden worldwide[1], and millions of hospital visits occur annually for the diagnosis and treatment of colonic motility disorders in the USA. However, some of these patients are dissatisfied with their treatment[2]. An attributable factor seems to be insufficient knowledge on human intestinal motility, preventing the development of efficient therapies.

There is considerable evidence that GI motility is mainly characterized by migrating motor complexes (MMCs) modulated by the enteric nervous system (ENS) and phasic contractions from slow waves mediated by the interstitial cells of Cajal (ICC)[3]. MMCs are well known as cyclic mass movements of the intestine that can move fecal material and are controlled by interactions of various neurotransmitters[4,5]. Phasic contraction is a brief rhythmic contraction originating from spontaneous activity caused by membrane potential changes in the ICC. Findings in various animal models suggest that phasic contractions may be associated with the mixing of food material in the intestine[6]. Colonic motility likely involves complex interactions between pacemaker activity and enteric neurons that cause the slow progression of fecal material along the colon[7,8].

Radial stretch is an important stimulus to evoke contractions in the lower GI tract. It is the mechanism by which distension of the colonic wall activates intrinsic neurons to generate peristalsis and propulsion of colonic contents. In various guinea pig models, intestinal motor patterns have been studied in isolated colon preparations using natural and artificial pellets[9,10].

The activation of polarized enteric neural pathways seems to be a basic electrophysiological mechanism of pellet propulsion. These move the pellet, activating another set of reflexes, to form a self-sustaining neuromechanical loop[11,12]. These recent findings revealed that mechanosensory enteric neurons located in the myenteric plexus have pivotal mechanosensitive nerve projections in the circular muscle (CM)[13]. Distension or stretch of the colon activates these sensory neurons to initiate polarized neural pathways that result in oral contraction and anal relaxation. These pathways do not require the mucosa but can be modulated by sensory nerve endings that project into the mucosa. The polarized enteric circuits form the basis of a neuromechanical loop that ensures that polarized enteric neural circuitry can efficiently propel content with a wide range of physical properties.

The GI tract in all mammalian species performs multifarious functions essential to the optimal handling of ingested material[13]. Although the regions of the lower GI tract have various contractile activities to perform their roles of storing, mixing, and expelling feces and absorbing fluid, there could be distinct functional variations based on their location[14]. It has been shown that the main role of the proximal colon is to mix and store liquid feces, facilitating the absorption of excess water and electrolytes, and stacking dried feces[15]. By contrast, the distal colon mainly expels the fecal material and exhibits a pattern of intense peristaltic movements[16]. The combination of these regional variations facilitates the balance among diverse colonic functions. Indeed, advanced imaging techniques of the human colon using high-resolution colonic manometry have recently revealed fundamental differences in motility between the right and the sigmoid colon[17]. As function varies from one part of the gut to another, so might its intrinsic innervation.

Many murine studies with electrophysiological design and immunohistochemical analysis

have established the temporal development pattern of the ENS, including the onset of spontaneous and induced electrical activity and site-specific motility patterns[6,18–21]. However, there has been a paucity of evidence regarding site-specific responses to radial stretch in the human lower GI tract, which is believed to be coordinated with electrical activity in the human ENS. Probably, findings on human GI motility will be similar to those in various preclinical studies, but the physiological motility characteristics of complex, large, and long human lower GI tract samples might be different from those derived from small-animal studies. The present study aimed to elucidate whether there are any differences in the stretch reflex according to locations in the human lower GI tract using ex vivo techniques, including electrophysiological recordings of intestinal smooth muscles excised during colon cancer surgery.

MATERIALS AND METHODS

1. Tissue acquisition

Human colon and ileum tissue samples were immediately acquired from patients undergoing radical surgery for nonobstructive colorectal cancer. Fresh isolated ileal or transverse colonic segments were obtained from right hemicolectomy specimens, whereas sigmoid colonic segments were obtained from anterior resection specimens.

After bowel resection in an elective surgery, 5×2-cm grossly normal ileal or colonic segments were removed from the resected sections. Wiped specimens were immediately placed in an oxygenated Krebs-Ringer bicarbonate (KRB) solution. The KRB solution contained (in mM) 120.4 NaCl, 15.5 $NaHCO_3$, 5.9 KCl, 11.5 glucose, 1.2 NaH_2PO_4, 2.5 $CaCl_2$, and 1.2 $MgCl_2$. This solution had a pH of 7.3–7.4 at 37.5℃ when bubbled to equilibrium with 97% O_2/3% CO_2[22].

2. Cross-sectional preparation for intracellular recordings

As in our previous studies[18,22], to detect membrane potential changes using intracellular recording, 1×1-cm intestinal segments were transferred into a Petri dish coated with Sylgard® (Dow Corning Co., Midland, MI, USA) and pinned down for dissection. The muscles were cut parallel to the longitudinal muscle (LM) fibers with a knife consisting of two sharp parallel scalpel blades 1.5 mm apart. The specimens were turned on the side to expose a cross-section of all muscle layers in an electrophysiological chamber[23] that was constantly perfused with prewarmed, preoxygenated KRB solution. The temperature was maintained at 37.5±0.5℃.

The muscles were equilibrated for at least 2 h before the start of the experiments. Conventional intracellular microelectrode recordings were performed by impaling the tissue with a sharp glass microelectrode filled with 3 mol/L KCl (Fig. 1). The isolated tissue segment was connected via a string sutured to the lateral side of the bowel to a pulley system to which a weight of 1 g was applied to evoke radial stretch. Transmembrane potentials were measured with a high-input resistance electrometer, and outputs were displayed on an oscilloscope[24]. Resting membrane potential (RMP; mV), as well as amplitude (mV) and frequency (/min) of the slow waves, were directly recorded. Outputs were measured and analyzed using Origin software (MicroCal Software Inc., Northampton, MA, USA) and pClamp software R (version 9.0. Axon Instruments, Foster City, CA, USA).

3. Preparations for tension recordings of bowel segments

As in our previous studies[22,23], whole ileal or colonic segments were prepared by cutting the whole layer of a segment parallel to the LM. The dimension of the segments that included a taenia coli was 5 cm in length and 2 cm in width. CM tension was recorded at three sites (proximal, middle, and distal) at 2 cm apart by perpendicular traction using sutures placed at each site (Fig. 2). The intraluminal radial stretch model was applied by using a fine 9-Fr Foley pediatric urinary catheter (Uro Technology Sdn. Bhd., Johor, Malaysia), which was inserted into the colonic segment through the aboral ending, and the balloon was positioned in the middle segment without any mucosal stimulation. Radial stretches were evoked by 1.0-mL inflation of the balloon at the catheter tip for 10 min.

4. Tension recordings of bowel segments

Ileal or colonic segments were suspended in a tissue chamber perfused with a prewarmed, preoxygenated KRB solution. Each clip was attached to an isometric force transducer (TST125C; Biopac Systems Inc., Goleta, CA). The temperature was maintained at 37.5±0.5℃. The muscles were equilibrated for at least 2 h before the start of the experiments. A resting force of 9.8 mN (1 g) was applied to each measuring site. The tension of each measuring site was detected using an isometric strain gauge. Frequency (/min), amplitude (mN), and area under the curve (AUC; mN*min) of contractions were recorded. The AUC was defined as the integrated area under slow waves for 10 min and was used to assess myogenic contractility. The mechanical signals of contractions were digitized using an MP150 interface and recorded using AcqKnowledge software (Biopac Systems Inc.). Data were analyzed offline using Clampfit 10.2 (Molecular Devices, Sunnyvale, CA, USA).

5. Drug administration

After regular waves were detected in each type of experiment, drugs affecting the ENS were perfused into the tissue chamber. After 10 min, a stretch stimulus was evoked, and the changes in the measured parameters were assessed. The following drugs were used: tetrodotoxin (1 μM TTX) to block sodium channels, atropine (1 μM) as an anticholinergic drug, and N^{w}-oxide-L-arginine (NOLA, 100 μM), as a nitric oxide synthase inhibitor. All drugs have been purchased from Sigma Chemical Co. (St Louis, MO, USA).

6. Statistical analysis

The results of each experimental group are expressed as the mean ± standard deviation or the median with range, where appropriate. The Wilcoxon signed-rank test was used to

compare data between baseline and stretch responses in the same group. Linear mixed model and generalized linear mixed model analyses were employed to estimate the changes in electrophysiological data after radial stretch and to compare the results between groups as the mean and slope difference. The model included coefficients for the random intercept for each group. To determine the drug-induced difference in tension recording data, we calculated fold changes and applied Student's t-test. All statistical analyses were performed by an independent experienced biostatistician (JH Lee) using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R 3.6.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The graphics were plotted using GraphPad Prism 5.0 (GraphPad Software Inc., USA). All statistical tests were two-sided, and a p-value <0.05 indicated a statistically significant difference between sets of observations.

7. Ethics statement

Obtainment and use of human intestinal specimens were approved by the institutional review board of the Seoul National University Hospital (IRB approval number, H-0603-071-170). No personal information was collected, all samples used in the experiments were de-identified and discarded in accordance with the guidelines of the board, and there was no secondary use of the samples.

RESULTS

1. Intracellular recordings of the membrane potential

Intracellular recordings of the membrane potential were successfully performed in 16 intestinal tissue samples. The transmembrane potentials were detected immediately after the impalement of the CM by intracellular recordings in ileum and colon specimens. Table 1 presents the detailed analyses of the intracellular recordings of the membrane potential before and after radial stretch (Table 1).

1) Radial stretch-induced responses of the membrane potential at the proximal site

At the proximal sites of the specimens, changes in membrane potentials after radial stretch were detected in all bowel segments. Electrophysiological recordings in the proximal end of the bowel specimens revealed the membrane potential changes caused by radial stretch (for representative traces, see Fig. 3). The RMP depolarizations in both the right and the sigmoid colon were statistically significant (from -55.0±13.8 to -51.2±15.4 mV, p=0.038 and from -56.3±10.8 to -52.9±12.2 mV, p=0.042, respectively). However, the mean change observed in the ileum was not statistically significant (from -47.4±6.0 to -45.7±4.8 mV, p=0.066). The changing patterns in RMP, amplitude, and frequency did not differ among the three bowel locations (p=0.144, p=0.466, and p=0.557, respectively; Fig. 4).

2) Radial stretch-induced responses of the membrane potential at the distal site

At the distal site of the bowel samples, changes in membrane potentials after radial stretch

were also discovered in all bowel segments (Fig. 5). Depolarizations of the RMP were observed in the ileum and the transverse colon (from -51.3±4.9 to -49.2±4.1 mV, p=0.066 and -49.8±9.3 to -47.2±10.9 mV, p=0.043, respectively). However, in the sigmoid colon, hyperpolarization was found (from -54.4±2.8 to -55.3±3.8 mV, p=0.197). Thus, the changing patterns in RMP, amplitude, and frequency were significantly different among the three locations (p=0.035, p=0.010, and p=0.001, respectively; Fig. 6)

3) Radial stretch-induced responses of the membrane potential in the sigmoid colon

In the sigmoid colon, the membrane potential responses to stretch differed between the recording sites (Fig. 7). In the proximal segment, stretch responses induced a depolarization, changing the RMP from -56.3 to -52.9 mV (p=0.042), the amplitude from 39.8 to 44.7 mV (p=0.046), and the frequency from 12.6 to 20.8/min (p=0.028). In the distal site, the RMP was inversely changed from -54.4 to -55.3 mV (p=0.197), the amplitude from 49.8 to 43.3 mV (p=0.068), and the frequency from 15.6 to 6.8/min (p=0.043). The slopes of the differences in RMP, amplitude, and frequency between the proximal and distal sites were 4.50 (p=0.044), 11.43 (p=0.004), and 17.47 (p=0.002), respectively.

2. Tension recordings of the bowel segments

A total of 127 intestinal samples were tested. After a period of equilibration lasting 1–3 h, 92 samples (yield, 72.4%) showed interpretable slow waves. The intestinal samples used for further analyses comprised the ileum (n=24), transverse colon (n=23), and sigmoid colon (n=45). Table 2 presents the detailed analyses of the tension recordings before and after radial stretch. Figures 8–10 show representative changes in segmental contractility in tension

recordings after radial stretch in the ileum, transverse colon, and sigmoid colon, respectively.

1) Radial stretch-induced contractility responses at the proximal site

In the ileum, there were no significant changes in frequency, amplitude, and AUC after radial stretch ($p=0.223$, $p=0.376$, and $p=0.568$, respectively; Table 2). In the transverse colon, contractile activation was observed, in which frequency, amplitude, and AUC were significantly increased after stretch ($p=0.003$, $p<0.001$, and $p<0.001$, respectively). Similarly, in the sigmoid colon, this pattern of contractile excitation was also demonstrated, and radial stretch increased the frequency, amplitude, and AUC values significantly ($p<0.001$, $p<0.001$, and $p<0.001$, respectively). Additionally, radial stretch-induced fold changes in the parameters assessed in tension recordings were calculated. In the ileum, fold changes in frequency, amplitude, and AUC after radial stretch were 1.0 ± 0.2, 1.0 ± 0.5, and 1.1 ± 0.5, respectively (Fig. 11). In the transverse colon, fold changes in frequency, amplitude, and AUC after radial stretch were 1.3 ± 0.4, 1.9 ± 0.8, and 2.0 ± 1.0, respectively (Fig. 12). In the sigmoid colon, fold changes in frequency, amplitude, and AUC after radial stretch were 1.3 ± 0.3, 1.3 ± 0.4, and 1.7 ± 1.0, respectively (Fig. 13). Thus, the parameters measured in tension recordings were stationary in the ileum following radial stretch but were increased in the right and the sigmoid colon.

2) Radial stretch-induced contractility responses at the distal site

In the ileum, there were no significant changes in frequency, amplitude, and AUC after radial stretch ($p=0.793$, $p=0.853$, and $p=0.862$, respectively). In the distal site of the transverse colon, contractile activation after stretch was observed as indicated by significant

increases in frequency, amplitude, and AUC ($p<0.001$, $p=0.002$, and $p<0.001$, respectively). However, in the distal site of the sigmoid colon, a pattern of contractile relaxation was observed, and radial stretch significantly decreased the frequency, amplitude, and AUC values ($p=0.022$, $p<0.001$, and $p=0.001$, respectively). In the ileum, fold changes in frequency, amplitude, and AUC after radial stretch were 1.0 ± 0.2, 1.1 ± 0.6, and 1.1 ± 0.4, respectively (Fig. 11). In the transverse colon, fold changes in frequency, amplitude, and AUC after radial stretch were 1.3 ± 0.3, 2.7 ± 5.2, and 1.7 ± 0.8, respectively (Fig. 12). In the sigmoid colon, fold changes in frequency, amplitude, and AUC after radial stretch were 0.9 ± 0.2, 0.8 ± 0.2, and 0.8 ± 0.3, respectively (Fig. 13). These findings show that following radial stretch, the measured parameters in tension recordings were stationary in the ileum, increased in the transverse colon, but decreased in the sigmoid colon.

3) Differences in site-specific responses to radial stretch in the sigmoid colon

Figure 14 shows the differences in site-specific responses to radial stretch in the sigmoid colon. In the generalized linear mixed model, the changing patterns in frequency, amplitude, and AUC were distinct for proximal and distal sigmoid colon sites. The slopes of frequency changes in the proximal and distal sites were 0.09 and -0.05, respectively, and the slope difference was 0.13 ($p<0.001$). The slopes of the amplitude changes were 4.60 for the proximal and -4.16 for the distal sites, and the slope difference was 8.76 ($p<0.001$). The slopes of the AUC changes in the proximal and distal sites were 152.80 and -83.51, respectively, with a slope difference of 236.31 ($p<0.001$).

4) Tension recordings in response to radial stretch following drug administration

A total of 64 intestinal samples were used to test drug effects on the stretch response. The intestinal samples used for analysis comprised the ileum (n=17), transverse colon (n=18), and sigmoid colon (n=29). Table 3 presents the changes in segmental tension recordings in response to radial stretch after administration of TTX (1 μM). In the presence of TTX, there was no significant change in tension recording data in response to stretch stimuli. Table 4 shows the inhibitory changes in all segmental tension recordings following radial stretch after administration of atropine (1 μM). Regardless of the bowel segment, there was a lack of stretch responses. After perfusion with NOLA (100 μM), the distal responses to colonic stretch were all characterized by contractions (Table 5). Figures 15 and 16 illustrate the NOLA-specific responses to radial stretch in the sigmoid colon. In the proximal sigmoid colon, the fold changes in frequency, amplitude, and AUC in response to radial stretch were after NOLA administration 1.6±1.0, 2.3±0.8, and 2.1±1.1, respectively (Fig. 15). The changing patterns in frequency, amplitude, and AUC were different between drug-naïve stretch and drug-perfused stretch ($p=0.071$, $p<0.001$, and $p=0.285$, respectively). In the distal segment of the sigmoid colon, the fold changes in frequency, amplitude, and AUC in response to radial stretch were after NOLA administration 1.9±1.4, 2.9±2.2, and 1.9±0.8, respectively (Fig. 15). These changing patterns in frequency, amplitude, and AUC were all significantly different between drug-naïve stretch and drug-perfused stretch ($p<0.001$, $p<0.001$, and $p<0.001$, respectively). Table 6 demonstrates the regional differences in intestinal contractile responses to radial stretch in the human lower GI tract with and without drug perfusion.

DISCUSSION

To the best of knowledge, this is the first study to evaluate regional differences in the stretch reflex according to the location in the human lower GI tract using ex vivo techniques, including electrophysiological recordings of intestinal smooth muscles. In intracellular recordings of the transmembrane potential, depolarizations after radial stretches were observed in the proximal and distal sites of the ileum and transverse colon, as well as the proximal site of the sigmoid colon, whereas hyperpolarization was detected in the distal site of the sigmoid colon. In the tension recording experiment, radial stretch-induced contractions were observed in both sites of the transverse colon and the proximal site of the sigmoid colon but in the distal site of the sigmoid colon, relaxation was noted. The measured parameters in the ileum were not substantially influenced by radial stretch.

Intestinal motility is considered to result from the physiological harmonization of multiple overlapping, coordinated mechanisms. The electrical activities of ICCs, including changes in membrane potential following the activation of various ion channels, provoke slow waves in smooth muscle cells that are connected with the ICCs by gap junctions[21]. The ICCs act as pacemakers for the generation of phasic contractions in smooth muscles. However, MMCs are thought to be controlled by the ENS, not by ICCs[18]. MMCs are subjected to cholinergic agents or neuronal blockers[3,4]. Normal colonic motility reflects the interplay of these overlapping control mechanisms including the ENS, ICCs, and autonomic innervation[19,25]. These mechanisms pattern a range of contractions, which may be static or move in retro- or antegrade directions, propagate over short or long distances, and present with a variety of

amplitudes[26]. Together, these patterns contribute to the regulation of critical colonic functions, including transit time, absorption, stool consistency and frequency, meal responses, and continence.

It is generally accepted that different locations in the GI tract have different functions. The main function of the proximal colon comprises anterograde and retrograde movements that facilitate mixing and absorptive functions. The distal colon and rectum usually have a thicker external muscular layer, facilitating the propulsive function of this region, and play a unique role in both storage and evacuation of feces[20]. Recently, advanced imaging techniques of the human colon using high-resolution colonic manometry have revealed fundamental motility differences between the right and the sigmoid colon[17]. Since gut functions vary between GI parts, it can be hypothesized that their intrinsic innervation also differs. The potential of "regional variation" adds a dimension of complexity to the ENS and its quantitative characterization[27]. Regarding the colon, differences in autonomous nerve regulation could also be addressed. Siri et. al. recently reported differential biomechanical properties of the mouse colon and rectum innervated by the splanchnic and pelvic afferents[28]. Their study showed a progressive increase in tissue compliance and prestress from colonic to rectal segments, which supports prior electrophysiological findings of distinct mechanical neural encodings by afferents in the lumbar splanchnic and pelvic nerves that dominate colonic and rectal innervations, respectively.

Propulsion of luminal contents can also be modified by differences in their consistency[13]. In tension recordings of the present study, ileal segments were not affected by radial stretch. The ileum usually passes semifluid contents, not hard feces; therefore, in this segment, stationary responses to radial stretch can be expected. The colon is necessary for the reabsorption of

water and electrolytes from semifluid contents resulting from digestion and secretions in the small intestine that is connected to the colon through the ileocecal valve[8]. The human microbiota breaks down carbohydrates and processes vitamins for absorption in the colon which also stores and accommodates fecal contents before evacuation through the anal sphincter. To do this, the colon relies on a series of functionally different compartments that eventually convert this liquid chyme into stool or discreet fecal pellets in some mammals, e.g., guinea pigs, mice, and rats[29,30].

Differences in the nitric oxide (NO) distribution might be responsible for the decrease in GI motility in the distal site of the sigmoid colon observed in the current study. NO contributes to the maintenance of mucosal integrity, which might be mediated by vasodilatation and increased blood flow or by inhibition of neutrophil-platelet interactions that can lead to thrombosis formation and mucosal hemorrhage[23]. NO has restrictive effects on GI smooth muscle contractions by reducing non-cholinergic and non-adrenergic neurotransmitter release and altering calcium concentrations in ICCs and smooth muscle cells[31]. NO can be synthesized by three isoforms of NO synthase (NOS) of which the neuronal and endothelial NOS participate in normal physiological responses, whereas the inducible NOS is expressed during inflammation and injury[32,33]. Interestingly, the NOS expression patterns differ in various parts of the gut [34]. This probably reflects differences in functions. For instance, the low NOS expression in the proximal colon may contribute to its storage function, whereas the high expression levels in the distal colon may correlate with its propulsive function[35]. Wattchow et. al. reported regional variations in the neurochemical coding of the myenteric plexus of the human colon in patients with slow-transit constipation[20]. In their study, they observed that the percentage of NOS immunoreactive

nerve cells was lower in the transverse colon than in the sigmoid colon. Our drug perfusion data indicate that the blocking effect by NOLA on stretch responses in the S-colon is related to its high NOS expression. The results of this study combined with those of previous studies in humans and animals provide evidence for the complexity of the role played by nitrergic innervation in the control of radial stretch-associated contractile responses.

Several limitations of this study should be acknowledged. First, because of inadequate laboratory facilities, we could not perform simultaneous microelectrode recordings from CM cells at both the oral and aboral ends, which would effectively have shown the polarized reflex. Second, the inherently small sample size of human specimens may have limited the statistical power to detect significant differences, especially in the electrophysiological study. Third, this is an ex vivo study using bowel segments mimicking the intact colon. However, it is difficult to ascertain how much of the normal human physiology is reflected in isolated specimens. Finally, we cannot demonstrate the mechanisms underlying regional differences. This remains a fertile area of research that requires a combination of recordings at both the cellular and organ levels to identify stretch response mechanisms in the human gut. Therefore, further basic studies are warranted.

CONCLUSION

The results of this ex vivo study indicate that radial stretch in the human colon introduces excitatory contractions in both the proximal and distal sites of the transverse colon and the proximal site of the sigmoid colon but inhibits contractions in the distal site of the sigmoid colon. This study draws attention to the stretch reflex as a potential determinant factor for functional differences in the human lower GI tract.

Table 1. Intracellular recordings of membrane potential changes before and after radial stretch. Data are presented as the mean ± standard deviation. Sample numbers were as follows: Ileum (n=5), transverse colon (n=5), and sigmoid colon (n=6).

Direction	Bowel	Parameter	Baseline	Stretch	p-value
Proximal					
	Ileum	RMP	-47.4±6.0	-45.7±4.8	0.066
		Amp	32.2±9.0	35.1±8.6	0.042*
		Freq	8.8±1.3	15.6±6.7	0.066
	transverse colon	RMP	-55.0±13.8	-51.2±15.4	0.038*
		Amp	25.4±3.2	30.7±5.7	0.043*
		Freq	8.6±3.8	12.0±4.9	0.041*
	sigmoid colon	RMP	-56.3±10.8	-52.9±12.2	0.042*
		Amp	39.8±11.8	44.7±10.9	0.046*
		Freq	12.2±6.8	20.8±9.3	0.028*
Distal					
	Ileum	RMP	-51.3±4.9	-49.2±4.1	0.066
		Amp	26.1±10.7	30.3±9.1	0.104
		Freq	12.5±6.1	21.8±6.0	0.042*
	transverse colon	RMP	-49.8±9.3	-47.2±10.9	0.043*
		Amp	40.2±9.3	47.3±6.6	0.028*
		Freq	12.8±9.9	20.5±8.6	0.028*
	sigmoid colon	RMP	-54.4±2.8	-55.3±3.8	0.197
		Amp	49.8±6.2	43.3±5.5	0.068
		Freq	15.6±10.0	6.8±1.1	0.043*

RMP, resting membrane potential (mV); Amp, amplitude (mV); Freq, frequency (/min)

*p<0.05

Table 2. Changes in segmental tension recordings before and after radial stretch. Data are presented as median (range). Sample numbers were as follows: Ileum (n=24), transverse colon (n=23), and sigmoid colon (n=45).

Direction	Bowel	Parameter	Baseline	Stretch	p-value
Proximal					
	Ileum	Freq	3.0 (1.2–5.0)	2.9 (1.0–4.4)	0.223
		Amp	10.4 (1.7–29.1)	10.8 (1.3–24.3)	0.376
		AUC	220.8 (42.6–546.1)	198.3 (37.6–566.5)	0.568
	transver	Freq	0.4 (0.2–0.8)	0.6 (0.3–0.9)	0.003*
		Amp	16.6 (4.5–50.5)	24.4 (8.2–91.8)	<0.001
		AUC	349.4 (66.2–913.0)	646.7 (225.1–2045.9)	<0.001
	sigmoid	Freq	0.1 (0.1–0.7)	0.4 (0.1–1.1)	<0.001
		Amp	12.0 (4.1–51.0)	16.9 (5.0–55.0)	<0.001
		AUC	262.0 (27.6–1327.2)	411.1 (36.2–1554.9)	<0.001
Distal					
	Ileum	Freq	2.7 (1.3–4.8)	2.7 (1.3–4.5)	0.793
		Amp	10.8 (5.1–40.9)	12.3 (1.3–36.6)	0.853
		AUC	205.5 (46.6–764.4)	196.0 (35.6–875.7)	0.853
	transver	Freq	0.4 (0.2–0.8)	0.5 (0.3–0.9)	<0.001
		Amp	18.1 (2.1–92.4)	32.4 (0.9–106.8)	0.002*
		AUC	364.9 (107.5–1241.7)	765.4 (47.5–2011.2)	<0.001
	sigmoid	Freq	0.4 (0.2–0.9)	0.3 (0.1–0.9)	0.022*
		Amp	23.1 (6.0–71.6)	16.7 (4.0–58.0)	<0.001
		AUC	355.8 (66.5–1099.1)	306.6 (24.7–965.7)	0.001*

Freq, frequency (/min); Amp, amplitude (mN); AUC, area under the curve of contractile waves

for 10 min (mN*min)

*p<0.05

Table 3. Changes in segmental tension recordings in response to radial stretch after administration of TTX (1 μM). Data are presented as median (range). Sample numbers were as follows: Ileum (n=7), transverse colon (n=5), and sigmoid colon (n=8).

Direction	Bowel	Parameter	Baseline	Stretch	p-value
Proximal					
	Ileum	Freq	2.2 (1.6–3.2)	1.6 (1.2–2.8)	0.034*
		Amp	9.7 (4.7–30.4)	11.8 (6.4–24.2)	0.345
		AUC	100.9 (43.3–236.3)	113.7 (39.5–257.2)	0.115
	transver	Freq	0.6 (0.3–0.7)	0.5 (0.3–0.8)	0.893
		Amp	13.3 (10.8–25.1)	13.0 (10.5–20.1)	0.224
		AUC	71.7 (53.9–1212.1)	58.1 (45.1–1708.9)	0.685
	sigmoid	Freq	0.6 (0.3–4.4)	0.5 (0.2–5.2)	0.347
		Amp	13.7 (9.1–25.0)	15.9 (8.7–31.2)	0.236
		AUC	73.9 (30.7–199.0)	58.1 (17.3–236.5)	0.735
Distal					
	Ileum	Freq	1.9 (1.4–2.9)	1.8 (1.1–2.2)	0.058
		Amp	10.5 (5.0–17.7)	11.0 (7.4–28.0)	0.248
		AUC	110.0 (8.1–805.4)	166.4 (12.6–865.8)	0.345
	transver	Freq	0.6 (0.3–1.1)	0.5 (0.2–0.8)	0.336
		Amp	28.7 (12.2–37.0)	29.4 (16.5–37.6)	0.043*
		AUC	102.2 (60.5–1641.7)	90.9 (32.5–1566.5)	0.138
	sigmoid	Freq	0.5 (0.2–1.3)	0.6 (0.2–1.8)	0.665
		Amp	26.2 (4.4–38.6)	17.4 (4.0–34.4)	0.236
		AUC	51.7 (21.7–128.2)	56.6 (16.5–105.6)	0.865

Freq, frequency (/min); Amp, amplitude (mN); AUC, area under the curve of contractile waves

for 10 min (mN*min)

*p<0.05

Table 4. Changes in segmental tension recordings in response to radial stretch after administration of atropine (1 μM). Data are presented as median (range). Sample numbers were as follows: Ileum (n=5), transverse colon (n=7), and sigmoid colon (n=11).

Direction	Bowel	Parameter	Baseline	Stretch	p-value
Proximal					
	Ileum	Freq	18.0 (5.0–21.0)	11.0 (2.0–14.0)	0.043*
		Amp	12.3 (3.1–22.6)	8.9 (3.0–16.0)	0.345
		AUC	89.4 (20.3–570.9)	77.5 (14.8–593.1)	0.043*
	transverse	Freq	7.0 (2.0–10.0)	7.0 (2.0–8.0)	1.000
		Amp	12.5 (5.5–28.8)	9.0 (3.7–23.1)	0.018
		AUC	326.2 (19.7–850.8)	296.3 (19.8–764.6)	0.236
	sigmoid	Freq	6.0 (2.0–13.0)	5.0 (2.0–12.0)	0.069
		Amp	20.0 (14.2–38.2)	10.5 (7.0–28.4)	0.004*
		AUC	80.0 (38.3–817.9)	51.8 (17.9–586.7)	0.004*
Distal					
	Ileum	Freq	19.0 (6.0–40.0)	10.0 (3.0–33.0)	0.136
		Amp	6.2 (2.5–10.4)	3.4 (2.1–8.4)	0.043*
		AUC	47.0 (12.5–365.9)	30.0 (12.4–306.0)	0.043*
	transverse	Freq	7.0 (4.0–16.0)	7.0 (2.0–15.0)	0.373
		Amp	10.8 (5.4–72.4)	8.0 (2.8–63.4)	0.028*
		AUC	711.1 (20.0–1485.6)	493.4 (11.3–1286.7)	0.018*
	sigmoid	Freq	5.0 (2.0–12.0)	4.0 (2.0–12.0)	0.141
		Amp	22.9 (12.8–47.4)	14.9 (5.6–42.9)	0.004*
		AUC	85.6 (34.9–990.7)	71.3 (31.3–555.4)	0.004*

Freq, frequency (/min); Amp, amplitude (mN); AUC, area under the curve of contractile waves

for 10 min (mN*min)

*p<0.05

Table 5. Changes in segmental tension recordings in response to radial stretch after administration of N^{w}-oxide-L-arginine (NOLA, 100 μM). Data are presented as median (range). Sample numbers were as follows: Ileum (n=5), transverse colon (n=6), and sigmoid colon (n=10).

Direction	Bowel	Parameter	Before	After	p-value
Proximal					
	Ileum	Freq	26.0 (5.0–39.0)	17.0 (13.0–43.0)	0.587
		Amp	14.5 (4.5–34.6)	15.2 (3.4–27.1)	0.892
		AUC	402.9 (39.8–1344.4)	419.7 (65.3–911.6)	0.892
	transver	Freq	5.5 (3.0–6.0)	5.5 (4.0–8.0)	0.201
		Amp	6.9 (4.5–39.1)	12.1 (5.8–26.4)	0.463
		AUC	343.6 (38.6–923.9)	371.6 (43.8–996.0)	0.137
	sigmoid	Freq	5.5 (2.0–18.0)	11.0 (2.0–25.0)	0.039*
		Amp	9.9 (3.0–35.0)	24.6 (5.2–76.4)	0.005***
		AUC	272.7 (34.1–704.2)	470.5 (127.5–1047.0)	0.013*
Distal					
	Ileum	Freq	19.0 (5.0–32.0)	21.0 (11.0–36.0)	0.176
		Amp	9.7 (2.2–11.5)	10.6 (3.8–12.8)	0.345
		AUC	433.2 (38.9–582.0)	377.8 (47.1–592.2)	0.225
	transver	Freq	4.5 (3.0–12.0)	6.0 (3.0–16.0)	0.034*
		Amp	14.3 (5.5–36.3)	16.9 (10.1–26.7)	0.463
		AUC	463.8 (36.7–1376.0)	554.5 (52.3–2014.2)	0.028*
	sigmoid	Freq	5.5 (1.0–11.0)	8.0 (2.0–27.0)	0.052
		Amp	10.2 (3.6–24.3)	22.1 (9.9–74.8)	0.007*
		AUC	313.0 (24.0–583.0)	601.9 (62.3–954.9)	0.007*

Freq, frequency (/min); Amp, amplitude (mN); AUC, area under the curve of contractile waves

for 10 min (mN*min)

*p<0.05

Table 6. Summary of the regional differences in intestinal contractile response to radial stretch in the human lower gastrointestinal tract.

	Ileum		Transverse colon		Sigmoid colon	
	Proximal	Distal	Proximal	Distal	Proximal	Distal
EPS (stretch)	+	+	++	++	++	-
Tension (stretch)	±	±	++	++	++	--
Tension (TTX + stretch)	±	±	±	±	±	±
Tension (atropine + stretch)	--	--	±	--	--	--
Tension (NOLA + stretch)	±	±	±	++	++	++

EPS, Electrophysiological study; TTX, tetrodotoxin; NOLA, N^{w}-oxide-L-arginine.
+: excitatory response, -: inhibitory response

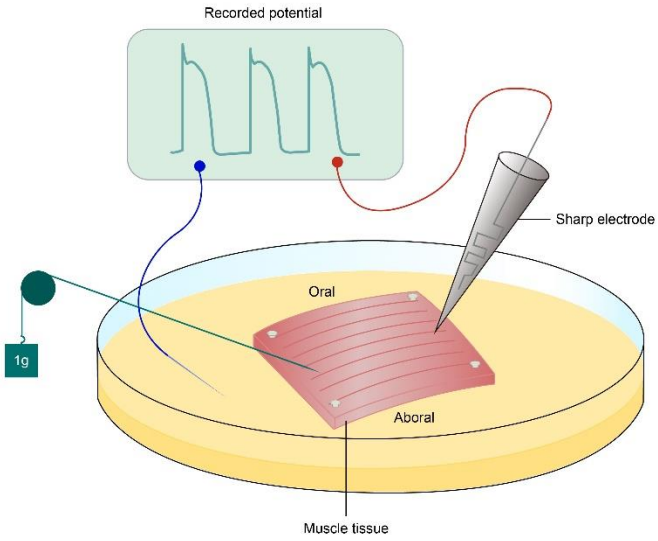

Figure 1. Schematic representation of intracellular microelectrode recordings in the intestinal circular muscle strip. The bowel tissue was pinned down in a dish, with the circular muscle side facing upward. Conventional intracellular microelectrode recordings were performed by impaling the tissue with a sharp glass microelectrode filled with 3 mol/L KCl. This isolated segment was connected via a string sutured to the lateral bowel side to a pulley system to which a weight of 1 g was applied.

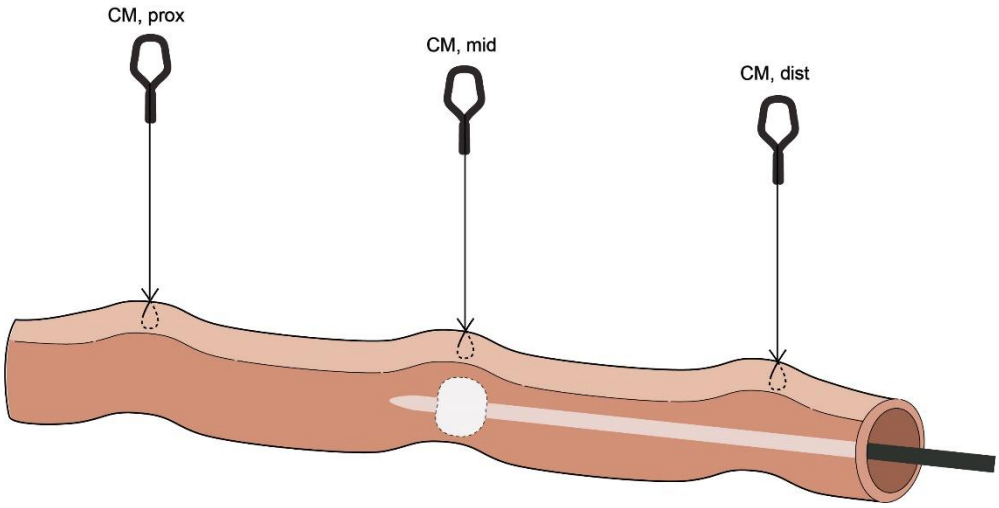

Figure 2. Schematic representation of the mechanical tension recordings. Bowel contractions were recorded at three sites with stainless steel clips and silk strings connected to a force transducer. Ballooning of a Foley catheter in the middle of an isolated segment induced radial stretch.

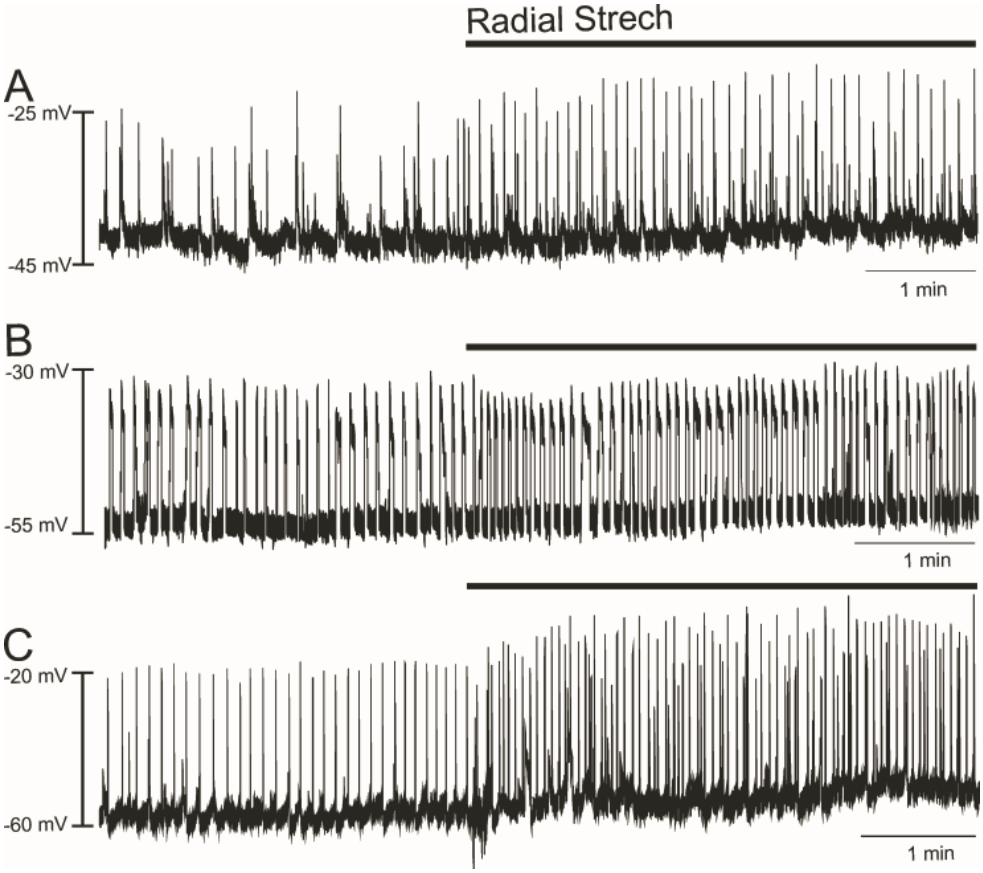

Figure 3. Representative changes in membrane potentials. Electrophysiological recordings before and after radial stretch in the proximal site of A) the ileum, B) the transverse colon, and C) the sigmoid colon.

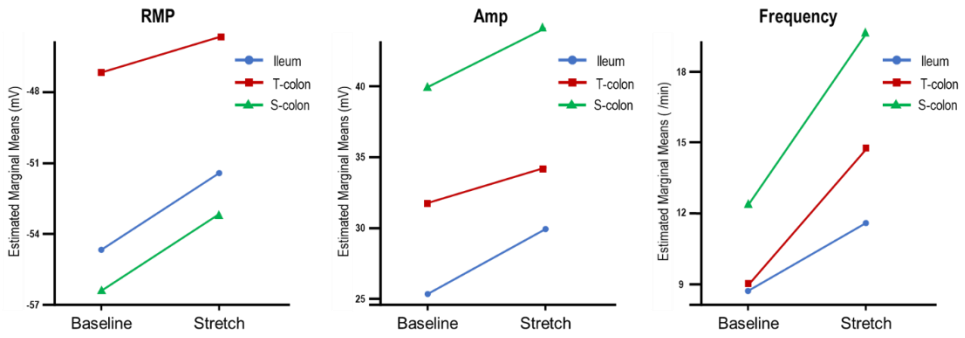

Figure 4. Comparison of the changing patterns in membrane potential after radial stretch. The electrophysiological parameters were recorded in the proximal site of the indicated bowel segments. The changing patterns in resting membrane potential (RMP), amplitude (Amp), and frequency were not different among the three bowel locations (p=0.144, p=0.466, and p=0.557, respectively).

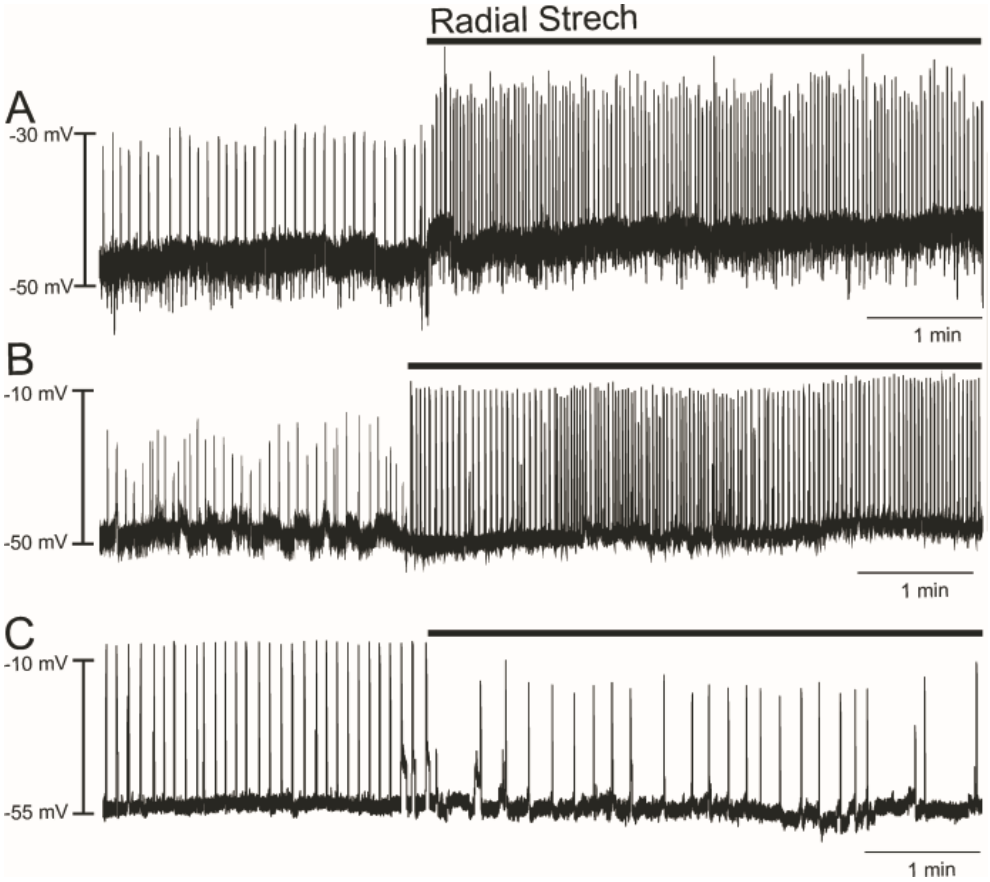

Figure 5. Representative changes in membrane potentials. Electrophysiological recordings before and after radial stretch in the distal site of A) the ileum, B) the transverse colon, and C) the sigmoid colon.

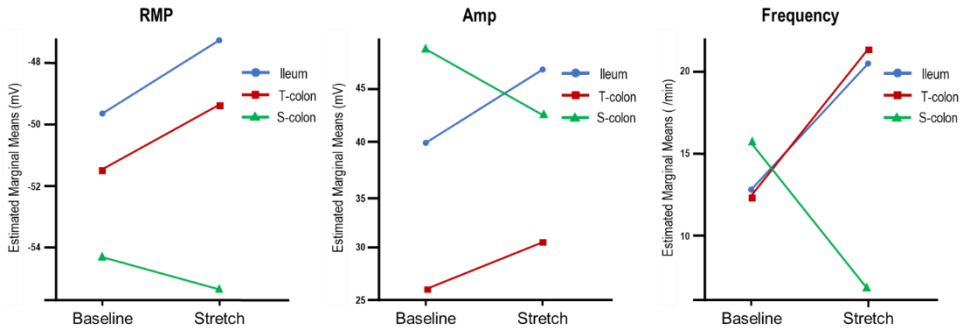

Figure 6. Comparison of the changing patterns in membrane potentials after radial stretch. The electrophysiological parameters were recorded in the distal site of the indicated bowel segments. The changing patterns in resting membrane potential (RMP), amplitude (Amp), and frequency were significantly different among the three groups (p=0.035, p=0.010, and p=0.001, respectively).

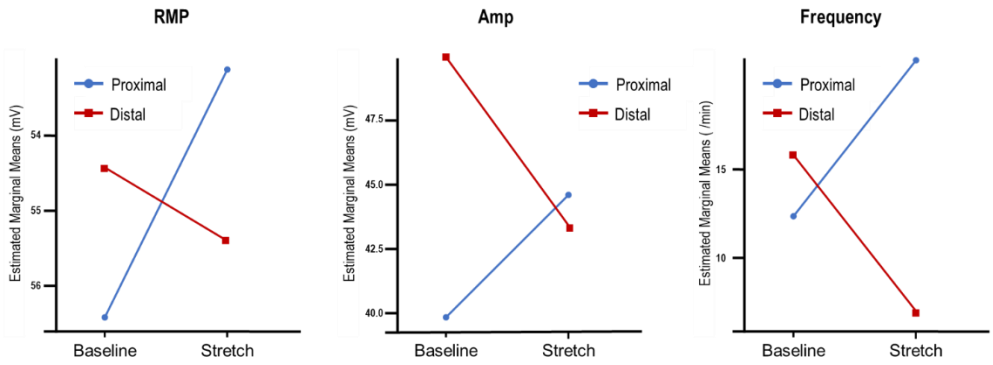

Figure 7. In the sigmoid colon, the membrane potential responses to stretch differed between proximal and distal sites. In the proximal site, stretch-induced depolarization was observed. The resting membrane potential (RMP) changes from -56.3 to -52.9 mV (p=0.042), the amplitude (Amp) increases from 39.8 to 44.7 mV (p=0.046), and the frequency rises from 12.6 to 20.8/min (p=0.028). In the distal site, the RMP decreases from -54.4 to -55.3 mV (p=0.197), Amp from 49.8 to 43.3 mV (p=0.068), and the frequency from 15.6 to 6.8/min (p=0.043). The slope differences in RMP, Amp, and frequency between proximal and distal sites are 4.50 (p=0.044), 11.43 (p=0.004), and 17.47 (p=0.002), respectively.

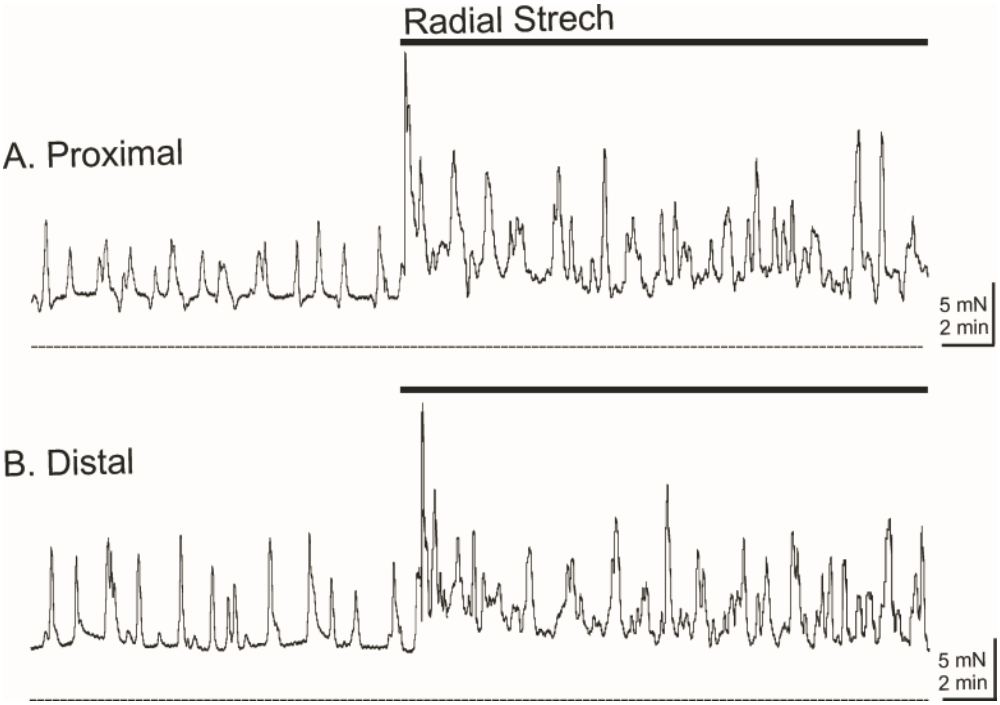

Figure 8. Representative changes in segmental contractility in tension recordings before and after radial stretch in the A) proximal and B) distal ileum.

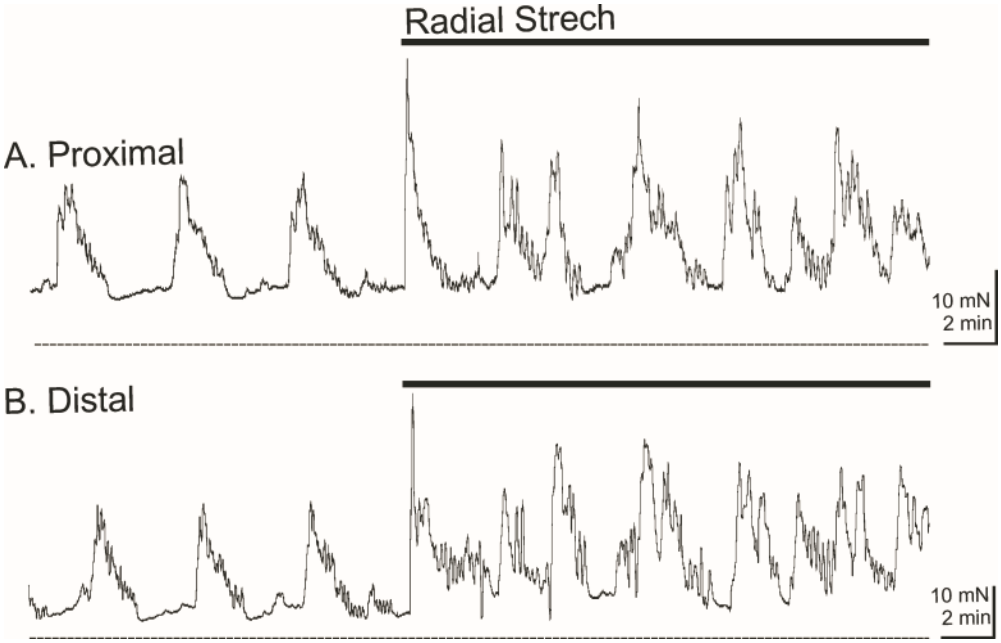

Figure 9. Representative changes in segmental contractility in tension recordings before and after radial stretch in the A) proximal and B) distal transverse colon

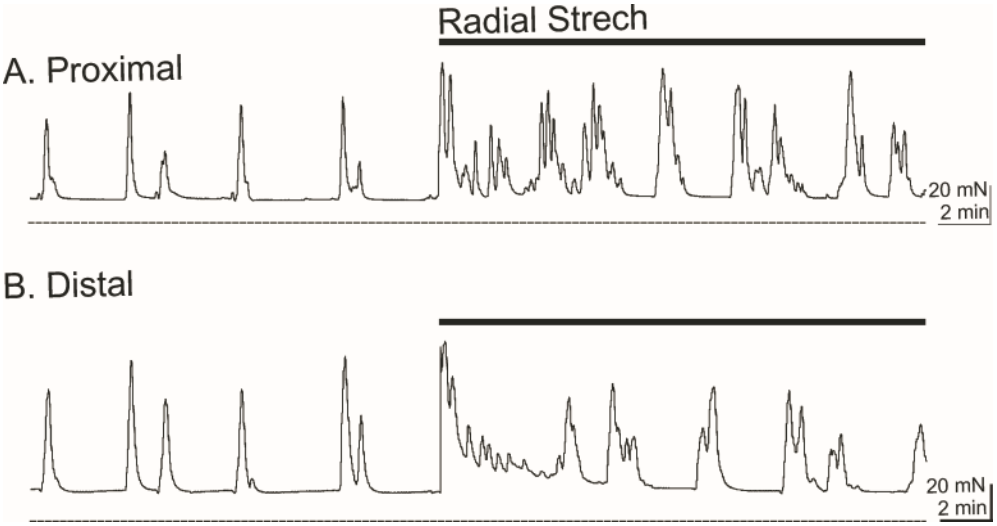

Figure 10. Representative changes in segmental contractility in tension recordings before and after radial stretch in the A) proximal and B) distal sigmoid colon.

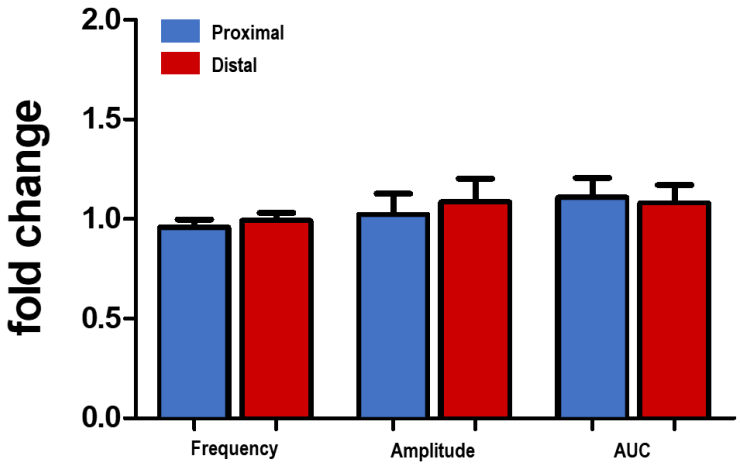

Figure 11. Changing patterns in segmental contractility in tension recordings after radial stretch of the ileum. Fold changes after radial stretch in proximal and distal sites are depicted. After radial stretch, all parameters are unchanged.

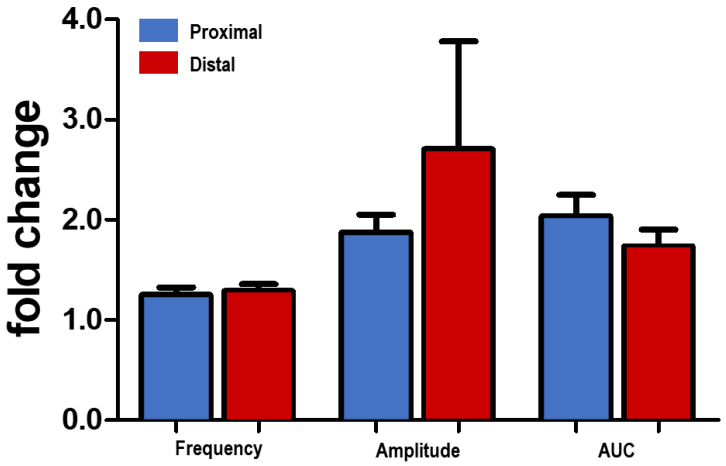

Figure 12. Changing patterns in segmental contractility in tension recordings following radial stretch of the transverse colon. Fold changes after radial stretch in proximal and distal sites are shown. After radial stretch, the tension recording parameters are increased in the proximal and distal segments of the transverse colon.

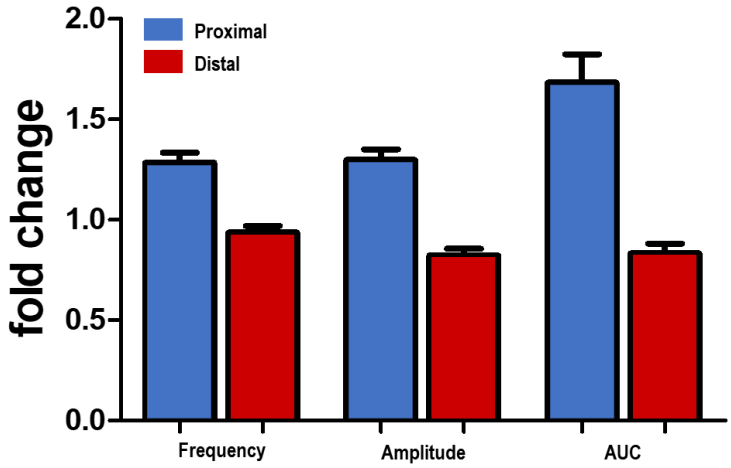

Figure 13. Changing patterns in segmental contractility in tension recordings after radial stretch of the sigmoid colon. Fold changes after radial stretch in the proximal and distal sites are displayed. In response to radial stretch, tension recording parameters are increased in the proximal site but decreased in the distal site of the sigmoid colon.

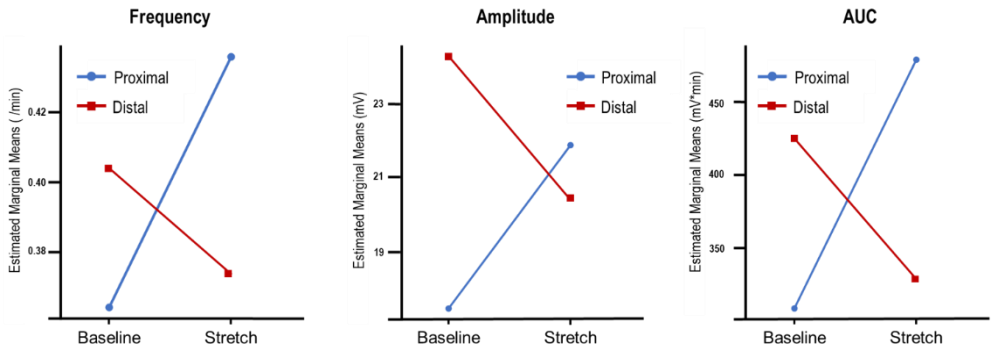

Figure 14. Differences in site-specific responses to radial stretch in the sigmoid colon. In the generalized linear mixed model, the changing patterns in frequency, amplitude, and area under the curve (AUC) differed between proximal and distal sites.

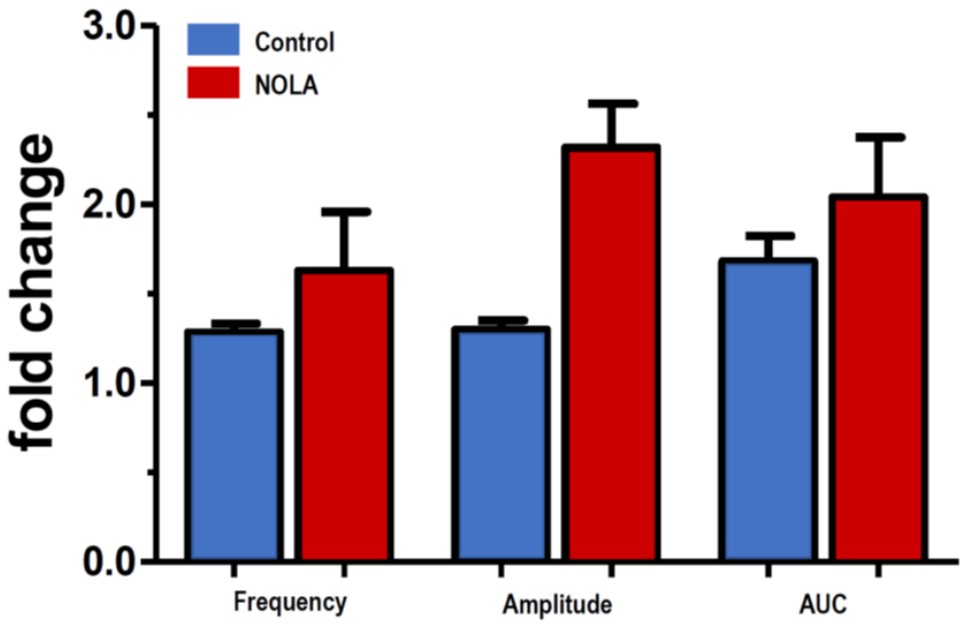

Figure 15. N^{w}-oxide-L-arginine (NOLA) specific responses to radial stretch in the proximal segment of the sigmoid colon. Fold changes in frequency, amplitude, and area under the curve (AUC) between drug-naïve stretch and drug-perfused stretch are compared (1.3±0.3 vs. 1.6±1.0, p=0.071; 1.3±0.4 vs. 2.3±0.8, p<0.001; and 1.7±1.0 vs. 2.1±1.1, p=0.285; respectively).

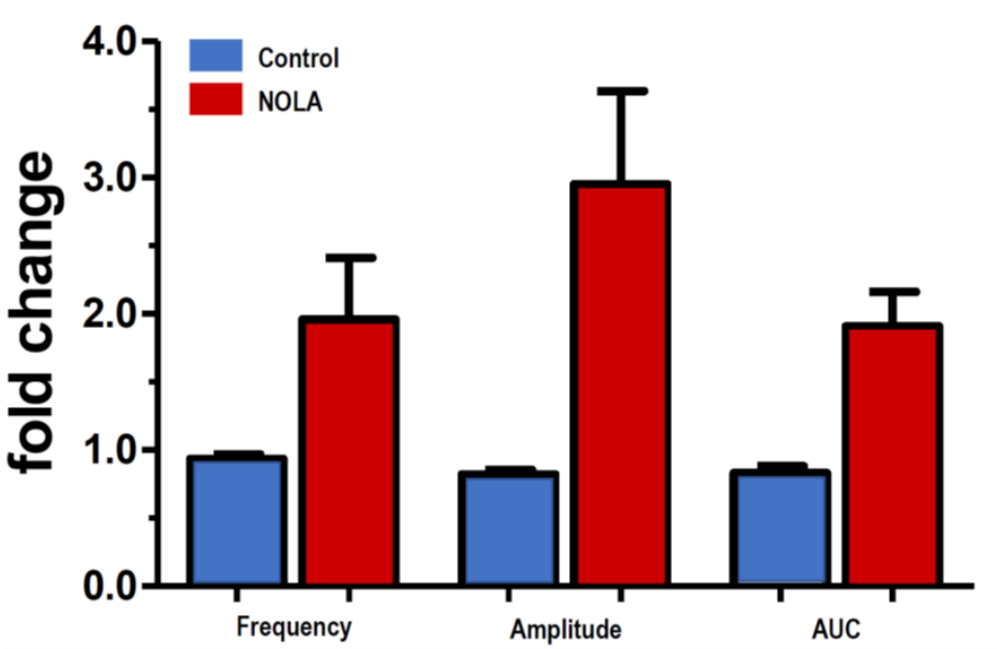

Figure 16. N^{w}-oxide-L-arginine (NOLA) specific responses to radial stretch in the distal site of the sigmoid colon. Fold changes in frequency, amplitude, and area under the curve (AUC) between drug-naïve stretch and drug-perfused stretch are significantly different (0.9±0.2 vs. 1.9±1.4, $p<0.001$; 0.8±0.2 vs. 2.9±2.2, $p<0.001$; and 0.8±0.3 vs. ±0.8, $p<0.001$; respectively).

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국문 초록

장관운동은 다양한 기전의 중첩으로 좌우된다. 신전자극은 하부위장관에서 수축을 촉발하는 중요한 자극이다. 장간막 신경초(Mesenteric plexus)에 위치하는 기계감각적 장관 뉴론이 장 평활근 중 윤상근(circular muscle)에 중추적인 기계감각적 돌기를 가지고 있다는 것이 알려져 있는데, 대장의 확장과 신전은 근위부 수축과 원위부 이완을 초래하는 분극된 신경 경로를 시작하기 위해 이러한 감각 뉴런을 활성화시킨다. 이 연구는 결장암 수술로 절제된 장 평활근을 이용하여 전기생리학적 계측을 통해, 하부위장관의 위치에 따라 신전자극의 대한 반응이 어떤 차이가 있는지를 살펴보는 것을 목적으로 한다.

대장 절제 표본들에서 소장이나 대장 분절들의 근육 세포들의 기존의 미세 전극 기록들과 장력 기록들을 수행하였다. 측정된 소장 분절은 5×2 cm로 얻어졌고, 즉시 산화된 Krebs-ringer bicarbonate 용액에 보관하였다. 근육 수축의 활동을 기록하기 위해 분절의 직각 방향으로 세가지 스테인레스 클립을 대장의 각각 외측에 부착했다. 실험적 신전 자극은 9Fr 폴리 카테터를 원위부 끝을 통해서 대장 분절 안으로 삽입하여, 점막의 자극없이 풍선을 분절의 가운데에 위치하게 하였다. 신전 자극은 10분동안 카테터 끝에 있는 1 mL의 풍선을 확장시켜서 촉발하였다. Frequency (/min), amplitude (mV), area under the curve (AUV, mV*min)은 분절들의 근위부와 원위부의

신전 자극 전후에 기록되었다. 윤상근으로부터 신전 자극 후 세포내 기록들에서 과분극은(from -54.4 to 55.3 mV) 좌측 결장의 원위부에서 기록되었다. 이와 대조적으로 탈분극은 그 외 모든 다른 부분에 있었다.

대장 분절의 장력 측정은 92개의 샘플들로 분석되었다. 회장 분절의 근위부에서는 frequency, amplitude, and AUC에 저명한 변화는 없었다(p=0.223; p=0.376; p=0.568). 우측 결장 분절의 근위부에서 수축활동이 관찰되었고, frequency, amplitude, and AUC가 유의미하게 상승하였다(p=0.003; p<0.001; p<0.001). 좌측 결장 분절에서도 수축 활성 패턴이 또한 관찰되었지만, 회장 분절의 원위부에서는 frequency, amplitude, and AUC에는 저명한 변화는 없었다(p=0.793; p=0.853; p=0.862). 우측 대장 분절의 원위부에 수축활동이 관찰되었다. 그러나 좌측 대장 분절의 원위부에는 수축 이완 패턴이 관찰되었고, frequency, amplitude, and AUC는 유의미하게 감소하였다(p=0.022; p<0.001; p=0.001). Generalized linear mixed model에서 frequency, amplitude, AUC의 변화된 패턴은 좌측 결장의 근위부와 원위부 사이에서 분극되었다. 근위부와 원위부의 빈도 변화의 경사는 각각 0.09와 -0.05 이었고, 경사의 차이는 0.13 (p<0.001) 이었다. 근위부와 원위부 사이의 진폭 변화의 기울기는 4.60와 -4.16 이었다. 각각 기울기의 차이는 8.76 (p<0.001) 이었다. 근위부와 원위부에서 AUC 변화의 기울기는 각각, 152.80와 -83.51 이었고, 기울기 차이는 236.31 (p<0.001) 이었다.

이 실험의 결과로 인체 대장에서 신전 자극은 회장과 우측 대장의 근위부와

원위부 모두와 좌측 결장의 근위부에서는 흥분성 수축들을 유발하지만 좌측 결장의 원위부에서는 이완이 관찰되는 것을 보여주었다. 이 연구는 신전 자극에 대한 반응의 차이가 우측과 좌측 결장 사이의 기능적인 차이를 만드는 잠재적이고 결정적인 요소일 수 있음을 보여주었다.

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