Characterization of motor patterns in isolated human colon: are there differences in patients with slow-transit constipation?

Nick J. Spencer,1 Melinda Kyloh,1 David A. Wattchow,2 Anthony Thomas,3 Tiong Cheng Sia,2 Simon J. Brookes,1 and Sarah J. Nicholas1

1Discipline of Human Physiology and Centre for Neuroscience, 2Department of Surgery, and 3Department of Anatomical Pathology, Flinders Medical Center, Adelaide, Australia

Submitted 16 August 2011; accepted in final form 21 September 2011.

A number of recordings have now been made from the human colon in vitro and a variety of motor patterns have been described (1, 9, 23, 24). These motor patterns include high-amplitude propagating sequences, low-amplitude propagating sequences, colonic motor complexes (CMC), rectal motor complexes, and phasic or segmental-type contractions (see Ref. 23 for review). The mechanisms underlying the generation of each of these different patterns of activity is not entirely clear but is likely to involve, at least in part, the enteric nervous system. Extrinsic sympathetic and/or parasympathetic neural inputs are also thought to modulate or contribute to the control of colonic motor patterns. One way of testing whether colonic motor patterns recorded in vivo require inputs from extrinsic nerves is to determine whether these same motor patterns occur in vitro. However, because of difficulties in performing these experiments, these types of studies have not been performed.

Understanding the different types of motor activity of the human colon is essential if we hope to identify potential motility patterns that underlie impaired colonic transit. Slow-transit constipation (STC), for example, is one of many different types of constipation that is known to occur when the normal transit of colonic contents is impaired, leading to a buildup or blockage of fecal content in the large bowel (3, 5, 12, 13, 33). A number of studies have made attempts to identify mechanisms underlying the onset of STC, but no clear underlying mechanism has yet been determined. There is evidence that a population of patients with STC have reduced numbers of high-amplitude propagating contractions (2, 11). There is also evidence that the interval between CMCs is reduced in the transverse colon and splenic flexure (9). Other studies have demonstrated reductions in specific neurochemical classes of myenteric neurons in STC patients (4, 15, 21, 32), in addition to reduced density of pacemaker cells (16) or specific types of neuromuscular transmission (31). Whether anatomical or functional changes in the large intestine of STC patients represents a cause or an effect of STC is not fully clear.

To date, recordings have only been made from relatively small segments of isolated human colon (usually a few millimeters or centimeters in length) where different spontaneous mechanical and electrical activities have been characterized (14, 17). However, it is now understood that lesioning the colon into small segments disrupts long enteric neural pathways that are required to preserve a variety of neurogenic motor patterns (7). For example, when colonic motor complexes are recorded in vitro from the whole isolated colon of laboratory animals, lesioning the colon into smaller segments abolishes their activation (25, 27). This likely explains why CMCs have not been reported previously from small segments of human colon in vitro. Our relative lack of understanding of the different types of motor activity that occur in the isolated human colon is due, in large part, to the difficulty in obtaining and preserving large segments of human colon and, furthermore, how to study these large segments of bowel in isolation from the patient. In this study, we removed the whole colon from patients undergoing total colectomy for STC and developed an in vitro recording approach whereby the motor activity could be characterized along the full length of colon.
METHODS

Case Histories of Control Patients with No Histories of STC

In 17 patients, we (D. A. Wattchow) removed the descending and partial transverse colon from patients undergoing elective surgery for removal of rectal carcinomas, with prior written, informed consent. Under general anesthesia, a laparotomy was performed such that the bowel was mobilized and the inferior mesenteric vein, then artery, and finally the peripheral vessels were divided. Segments of colon removed from the patient were typically between 40–50 cm in length (see Fig. 1). For sigmoid cancers the time taken from removal to being placed in oxygenated Krebs solution was ~10 min and for rectal cancers ~20 min. As soon as each colon was removed from the patient, the whole preparation was immediately submerged into warm (35°C) oxygenated Krebs solution (see composition below). The colon was pink all throughout this time. No patient had colonic obstruction and all had bowel preparation prior to surgery. Once removed from the patient, colonic specimens were taken immediately to a neighboring laboratory, within the same building as the operating theatre. Data from these 17 patients was used as our experimental controls. In total, we made recordings in vitro from all specimens of human colon for a period of between 60–90 min, before these specimens had to be sent to pathology for further investigation. The procedure to study isolated segments of human colon was approved by the Flinders Clinical Research Ethics Committee; no. 50/07.

Case Histories of Patients with STC

The patients with STC used in this study consisted of the following case histories.

Patient A. Female (59 years of age), ~40 year history of constipation, transit studies showed no passage of tracer past splenic flexure after 96 h. Procedure consisted of total colectomy and ileorectal anastomosis. Colon removed because of severe constipation.

Patient B. Female (41 years of age), longstanding severe constipation, transit studies revealed generalized slow transit with 100% of total activity remaining at 96 h. Procedure consisted of proctocolectomy and end ileostomy. Colon removed because of severe constipation.

Patient C. Female (61 years of age), longstanding severe constipation, transit studies revealed generalized slow transit with reduc-
classification of CMCs described in dog colon (22) and human colon (9, 23). CMCs in both dog and human colon in vivo occur repetitively and cyclically and can originate at any site along the colon, while being able to propagate in either an oral or anal direction. They can propagate varying distances along the bowel that may be less than or greater than half the length of colon.

**Measurements and Statistics**

In RESULTS, data are presented as mean values ± SE. The use of N in RESULTS refers to the number of colons on which observations were made. Measurements of peak contraction amplitude were made from baseline resting tension to the peak contractile force generated, represented in grams. The half-duration of contractions was made from the half-amplitude point on the rising phase to the half-amplitude point on the recovery phase of each contraction. The interval between each colonic motor complex was made from the half-amplitude point (on the rising phase of one contraction to the same point on the next successive contractions). Data sets were considered statistically significant if \( P \) values < 0.05 were reached. Unpaired two-tailed Student’s \( t \)-tests were used for comparison of data sets.

**Solutions**

The Krebs solution used contained (in mM) 118 NaCl, 4.7 KCl, 1.0 NaHPO\(_4\)·2H\(_2\)O, 25 NaHCO\(_3\), 1.2 MgCl·6H\(_2\)O, 11 D-glucose, 2.5 CaCl\(_2\)·2H\(_2\)O.

**RESULTS**

**Recordings from Control Segments of Descending/Transverse Colon**

In control segments of descending/transverse colon, mechanical recordings from the circular muscle layer revealed spontaneous contractions that were temporally uncoordinated at each recording site (Fig. 2). However, in most colonic specimens after a period of between 30–45 min, the resting tension of the circular muscle developed increasing tone (in the range of 5–10 g, Fig. 2), and this increase in tone was associated with the appearance of CMCs in 11 of 17 colons, which became temporally coordinated at each recording site (Fig. 2). These contractions fit the criteria of spontaneous colonic motor complexes (CMCs) as described previously (see above) and will be referred to as CMCs. The development of increased resting tone was not required for the generation of CMCs, because CMCs could be recorded as soon as the preparation was mounted in the organ bath, and before any changes in tone developed (Fig. 3). In the 11 preparations where CMCs were recorded, the mean interval between each CMC was 4.0 ± 0.6 min \((N = 11)\), where the mean amplitude of each contraction ranged between 9.7 and 12.3 g, with a mean half-duration ranging from 45.3 to 51.5 s \((N = 11)\); see Table 1).

In the 11 control colonic preparations in which spontaneous CMCs were recorded, they consisted of a cyclical contraction that occurred with a similar interval that could be temporally correlated at least three neighboring recording sites. CMCs were seen to propagate along the colon. However, as mentioned, determining a propagation velocity was not possible to ascertain, because of our inability to clearly determine the direction of propagation. In the remaining six specimens of colon, CMCs were not observed throughout any of the recording period. Instead, phasic random contractions occurred that did not clearly propagate between each recording site.

**Ascending Excitation and Descending Inhibition Activated During Spontaneous CMCs**

During many spontaneously occurring CMCs, a major observation was the presence of a descending inhibitory phase (relaxation) from recording sites located in the anal region of descending colon. When we investigated this more carefully, the presence of descending inhibition was only observed at the distal
recording sites of descending colon (see Table 2 and Fig. 4). Also, importantly, the onset of descending inhibition (at the anal end of descending colon) correlated simultaneously with the onset of the ascending contraction at the oral two recording sites (Table 2; Fig. 4). This strongly suggests that ascending excitatory and descending inhibitory nerve pathways are activated simultaneously along this region of colon, as occurs in laboratory animals.

**In Vitro Distension Responses in Control Colonic Preparations**

On 30 occasions from eight different descending/transverse colonic preparations, intraluminal distension stimuli were applied (10–15 ml balloon volume) to the colonic wall maintained for a period of 10 s. In only 8 of these 30 trials was a distension response elicited that consisted in three of eight occasions as a local ascending contraction that did not propagate further than the terminal descending colon (see Fig. 5), following distension of the terminal region of descending colon. The remaining responses consisted of an evoked CMC contraction in five of eight trials following distension of the descending colon (Table 3). Taken together, only three of eight specimens of colon responded to distension of the colonic wall (Table 3). We also applied transmural electrical stimuli (20 Hz, 5 s, 0.4 ms, 70 V) on six occasions to the serosal surface of three descending colon preparations. In four of these six occasions, a CMC was evoked whose characteristics were indistinguishable from spontaneously occurring CMCs. These stimuli clearly evoked orally migrating CMCs in response to stimuli applied to the terminal (anal) end of the descending colon (Table 3; Fig. 6). This was determined by the increasing temporal delay in onset of the CMC contraction at increasing distances from the stimulus site (Fig. 6).

**Recordings from Whole Isolated Colon from STC Patients**

In three patients with chronic STC, the entire colon was removed from each patient and mounted in an organ bath, by

**Table 1. Characteristics of colonic motor complexes recorded in isolated control descending/transverse colons compared with whole colons obtained from STC patients**

<table>
<thead>
<tr>
<th></th>
<th>Amplitude Oral Region, mN</th>
<th>Amplitude Mid Region, mN</th>
<th>Amplitude Anal Region, mN</th>
<th>Oral Half-Duration, s</th>
<th>Mid Half-Duration, s</th>
<th>Anal Half-Duration, s</th>
<th>Interval, min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means ± SE</td>
<td>12.3 ± 3.7</td>
<td>14.7 ± 2.6</td>
<td>9.7 ± 2.6</td>
<td>45.3 ± 9.3</td>
<td>47.1 ± 7.6</td>
<td>51.5 ± 15.1</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><strong>STC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means ± SE</td>
<td>7.9 ± 2.8</td>
<td>9.2 ± 4.8</td>
<td>10.9 ± 4.0</td>
<td>56.0 ± 8.7</td>
<td>65.3 ± 5.9</td>
<td>57.3 ± 10.9</td>
<td>6.8 ± 1.1*</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Amplitude and half-duration of cyclical motor complexes (CMCs) recorded in control colon was no different from colons obtained from slow-transit constipation (STC) patients. However, the interval between CMCs in patients with STC was significantly longer than control specimens. Control: all regions refer to descending colon. STC: oral region = ascending colon, mid region = transverse colon, and anal region = descending colon.
the identical approach described for the control recordings. All three whole colons were removed because of chronic constipation and were confirmed as having STC, based on transit studies. Interestingly, in these three whole colons, robust CMCs were recorded (Fig. 7 and 8). When the characteristics of CMCs in the descending colon of these three STC colons were compared with CMC recordings from descending colon from control colons, the mean interval between CMCs was significantly increased (unpaired two-tailed t-test, P < 0.05).

However, the amplitude and half-duration of CMC contractions in the descending colon of STC specimens was not significantly different from the descending colon of control colons (Table 1). As in control colon preparations, CMC contractions were initiated spontaneously at varying sites along the whole colon and could propagate significant distances. However, as with control preparations, it was not possible to determine propagation velocities because of the variability in the site of initiation and direction of propagation. It is particularly noteworthy that, unlike the control colons, in none of the three STC colons was a period of descending inhibition detected prior to any CMCs.

In Vitro Distension of the Ascending and Descending Colon from STC Patients

In the three patients with confirmed STC, 17 of 17 distension stimuli applied to the ascending and descending colon failed to evoke a distension response. Also, in eight of eight transmural electrical nerve stimuli applied to the ascending colon (6 trials) or descending colon (2 trials) failed to elicit a response (N = 8).

DISCUSSION

In this study, we recorded the spontaneous motor activity from the circular muscle layer of isolated descending and part transverse colon in 17 patients with no known histories of chronic constipation. The motor activities of these segments were compared with the motor activities recorded from the isolated intact whole colon removed of three patients with confirmed severe
STC. To date, we are not aware of any studies that have recorded the motor activity from large segments of isolated human colon. Even less common is the opportunity to study motor patterns from intact whole colons removed from patients with STC, since the total colectomy procedure is not commonly performed nowadays for STC.

The primary motor activity in control specimens consisted of spontaneous colonic motor complexes that occurred approximately every 4 min and that could propagate over significant lengths of colon in vitro. In colonic specimens obtained from patients with STC, we anticipated observing major differences in the patterns of motor activity. Surprisingly, we did not observe major differences, at least in terms of the amplitude and duration of spontaneous CMCs in STC patients. The intervals between CMCs were longer in STC patients. However, it is not clear whether this difference is a cause or an effect of STC. Or, alternatively, whether this difference is simply due to differences in the length of colon studied between control and STC specimens (i.e., whole colons from STC patients vs. descending/transverse colons from control preparations). Interestingly, although we only studied three whole colons from patients with STC, we failed to elicit any distension or electrical nerve stimulated responses, indicating perhaps failure in enteric neural transmission.

Comparison of the Characteristics of CMCs Recorded in Human Colon in Vivo with Those Recorded in Vitro

The frequencies of CMCs in human colon in vivo has been reported to vary considerably with an incidence every 0.2–0.6 per hour and duration of 6–8 min (23). In the study by Hagger and colleagues (9), CMCs were reported to occur in human

Table 3. Responses to intraluminal distension and electrical stimulation of the ascending and descending colon from non-STC patients

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Distension Oral Region of Descending Colon</th>
<th>Distension Anal Region of Descending Colon</th>
<th>Elect Nerve Stimulation of Oral Region of Descending Colon</th>
<th>Elect Nerve Stimulation of Anal Region of Descending Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive response</td>
<td>No response</td>
<td>Positive response</td>
<td>No response</td>
</tr>
<tr>
<td>Specimen 1</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Specimen 2</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Specimen 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Specimen 4</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Specimen 5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Specimen 6</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Specimen 7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Specimen 8</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total no. of responses</td>
<td>12</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

Proportion of distension responses that lead to a response being elicited. The majority of stimuli delivered by balloon distension or electrical stimulation did not evoke a response. However, in a proportion of trials, balloon distension or electrical stimuli did elicit a response.
colon in vivo every 30–40 min in healthy colon. In our study, the mean interval between CMCs was ~4 min in control specimens and approximately every 7 min in STC colons. Clearly, in the human colon in vivo CMCs have been described to occur at much slower frequencies and have considerably longer durations. The faster occurrence of CMCs recorded in our study in vitro could be due to many factors, one of the more likely being the loss of extrinsic sympathetic inhibitory influences on the enteric nervous system, which would normally be expected to slow colonic motility in vivo.

Rectal motor complexes have also been recorded by Orkin and colleagues (18) in human patients in vivo, with a mean interval of 0.8 per hour and mean duration of 9.5 min. These contractions are also much less frequent and are of significantly...
longer duration compared with the CMCs we recorded in vitro. It is not clear whether the rectal motor complexes recorded by Orkin and colleagues in vivo, are related to the cyclical motor pattern we recorded in the human colon, in vitro. Our specimens of human colon were evacuated of feces prior to in vitro recordings being made. In the study of Orkin and colleagues, the recordings of rectal motor complexes in vivo were found to occur in the fed or fasted state (18).

Interestingly, in the study of King and colleagues (11), it was identified that in children with STC there was a significant impairment in antegrade propagating activity, with no change in the frequency of high-amplitude propagating sequences. Also, King et al. reported deficient or-absent responses to physiological stimuli. In our study, we were not able to elicit any distension or electrical nerve stimulation response, as was possible in some, but not all, control (non-STC) specimens. Also, in the study by Dinning et al. (6), it was found that in vivo there was a disorganization among consecutive propagating sequences and reduced responses to physiological stimuli. In support of this, we found no responses were evoked to intraluminal balloon distension or electrical stimulation of STC colons. However, it is important to acknowledge that these responses were also difficult to evoke and not consistent in control colons. Nevertheless, it is suggestive that the lack of stimulus response in STC colon may be deficit that is intrinsic to the colon of STC patients.

Direction of Propagation of Colonic Motor Complexes

In control and STC colonic specimens, the direction of propagation of cyclical CMCs was not possible to ascertain, because of the large spacings (10 cm) between each recording site. Similar sentiments were published in the study Hagger et al. (9), for recordings of CMCs in vivo. Nevertheless, it was clear that many CMCs appeared to originate in the ascending or transverse colon and propagate anally, in an antegrade direction, whereas others appeared to originate in the descending colon and propagate orally in a retrograde direction. This is highly analogous to our previous recordings of colonic migrating motor complexes in isolated whole mouse colon (25, 26), which were also found to originate in the proximal, mid, or distal colon in vitro and migrate either orally or anally. Interestingly, when transmural electrical stimulation was applied to the descending colon in this study on human colon (Fig. 6), a clear orally migrating CMC was identified. This is again remarkably similar to the electrically evoked colonic migrating motor complexes we reported previously in isolated mouse colon, which also migrated orally, following electrical stimulation of the distal colon (26).

Is There a Peristaltic Reflex in Human Colon?

We were particularly interested in whether acute luminal distension could reliably elicit a peristaltic reflex in human colon, as occurs reliably in other laboratory animals (30). In general, we found the isolated descending and ascending human colon responded poorly to even intense acute luminal distension stimuli. Only a small proportion of control preparations of colon studied ever responded to luminal distension with a balloon. We were able to demonstrate an ascending excitatory reflex to acute intraluminal distension or local transmural electrical stimulation of the ascending, transverse, or descending colon, as occurs in laboratory animals (28, 29). A previous study suggested an ascending excitatory and descending inhibitory neural reflex could be readily elicited following distension of isolated segments of human intestine (8). Although we were unable to reliably demonstrate a polarized intrinsic neural reflex, we did find that it was possible to evoke a CMC (in 8 of 30 trials from 8 independent segments of colon), which could propagate significant lengths along the colon in vitro. Also, these spontaneous and distension-evoked CMCs consisted of an ascending excitatory and descending inhibitory reflex.
phase, which strongly suggested that the intrinsic nerve pathways in human colon are indeed polarized (19), as in other mammals. But they are simply more difficult to activate following distension. Immunohistochemical studies have shown that polarized ascending excitatory and descending inhibitory neural motor neuron projections innervate the circular muscle of human colon (20).

Differences in CMC Pacemaker Frequencies in Colons from STC Patients?

Overall, CMCs recorded from whole colons obtained from STC patients were significantly less frequent than CMCs recorded from control colonic preparations. It is not clear whether the CMC pattern of motor activity recorded in this study has any role in normal transit in human colon. In this regard, the true function of the CMC is not clear. They are presumed to underlie the propulsion of colonic content, but this has not been demonstrated. It is important to note that a previous study of human colon found CMCs also occurred less frequently (9). Whether the CMCs we recorded in vitro are the same phenomena as the CMCs recorded in vivo is not known.

Identification of Polarized Intrinsic Neural Pathways in Human Colon: Mechanisms Underlying CMC Generation

We believe one of the major findings of the present study is the presence of a period of relaxation of the circular muscle that occurred prior to the CMC contraction, specifically at the anal end of the descending colon (Fig. 4C). When the timing of onset of these periods of relaxation were investigated more closely, it was clear that the onset of each relaxation occurred at the same time as the contraction phase of CMC in the oral region of descending colon (compare site 1 with site 3 recording in Fig. 4, B and C). Since there are no myogenic mechanisms to spontaneously relax smooth muscle, this observation strongly suggests that ascending excitatory and descending inhibitory neural pathways are activated simultaneously to the circular muscle during CMCs, in exactly the same way that this mechanism underlies activation of colonic migrating motor complexes in laboratory animals (27). In this regard, the mechanisms underlying CMC generation in isolated human colon do not appear to be any different from those identified for migrating complexes in other species (27, 33). We suggest, then, that the generation of CMCs in human colon is likely to involve simultaneous activation of ascending excitatory and descending inhibitory neural pathways that lie within the myenteric plexus (see Ref. 27). It is of particular interest that no descending inhibition was detected in any of the CMCs recorded from the three STC patients. Loss of descending inhibition could easily lead to impaired colonic transit; however, larger data sets would be needed to determine whether this is a cause of STC. At present, the nature of the pacemaker that underlies CMC generation in human colon is not clear but must lie intrinsic to the colon wall and also likely resides in the enteric nervous system, and does not require the presence of the mucosa (10).

Conclusions

The major findings of the present study reveal the presence of rhythmic CMCs in isolated human colon that occur in patients with or without STC. The characteristics of spontaneous CMCs in isolated human colon are considerably different from CMCs described in human patients in vivo, with the primary difference in their frequencies of occurrence. The observation that CMCs still occur in whole colons obtained from patients with STC suggests that despite impaired colonic transit in these patients in vivo, the mechanisms underlying CMC generation are preserved in this region, at least in vitro.

GRANTS

The experiments carried out in this study were funded by grants to N. J. Spencer (grant nos. 535034 and 535033) from the National Health and Medical Research Council of Australia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


