Lack of pyloric interstitial cells of Cajal explains distinct peristaltic motor patterns in stomach and small intestine

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Wang, Xuan-Yu, Wim J. E. P. Lammers, Premysl Bercik, and Jan D. Huizinga. Lack of pyloric interstitial cells of Cajal explains distinct peristaltic motor patterns in stomach and small intestine. Am J Physiol Gastrointest Liver Physiol 289: G539–G549, 2005. First published April 28, 2005; doi:10.1152/ajpgi.00046.2005.—The frequency and propagation velocity of distension-induced peristaltic contractions in the antrum and duodenum are distinctly different and depend on activation of intrinsic excitatory motoneurons as well as pacemaker cells, the interstitial cells of Cajal associated with Auerbach’s plexus (ICC-AP). Because ICC are critical for coordination of motor activities along the long axis of many regions in the gut, the role of ICC in antroduodenal coordination was investigated. We used immunohistochemistry, electron microscopy, simultaneous multiple electrical recordings in vitro, and videofluoroscopy in vivo in mice and rats. A strongly reduced number of ICC-AP with loss of network characteristics was observed in a 4-mm area in the rat and a 1-mm area in the mouse pyloric region. The pyloric region showed a slow wave-free gap of 4.1 mm in rats and 1.3 mm in mice. Between antrum and duodenum, there was no interaction of electrical activities and in the absence of gastric emptying, there was no coordination of motor activities. When the pyloric sphincter opened, 2.4 s before the front of the antral wave reached the pylorus, the duodenum distended after receiving gastric content and aboral duodenal peristalsis was initiated, often disrupting other motor patterns. The absence of ICC-AP and slow wave activity in the pyloric region allows the antrum and duodenum to have distinct uncoordinated motor activities. Coordination of aborally propagating peristaltic antral and duodenal activity is initiated by opening of the pylorus, which is followed by distention-induced peristaltic peristalsis. Throughout this coordinated motor activity, the pacemaker systems in antrum and duodenum remain independent.

pyloric sphincter; rat; mouse; gastroduodenal junction

ONE OF THE FASCINATING ASPECTS of gastrointestinal (GI) motility is that each organ has unique motor characteristics and motor control, yet each organ can synchronize its motor activity with the adjacent organ to allow for a smooth transfer of content. The recording of human gastroduodenal motility has shown that motor patterns from the stomach and duodenum are often distinct, but peristaltic contractions smoothly crossing the pylorus can also be observed. Complete understanding of control mechanisms underlying this coordination is important because abnormal gastroduodenal motor coordination is thought to be part of many motility disorders (37).

The coordination of motor activities of the stomach and small intestine was the subject of the earliest studies in GI motility. Canon (8) noted in 1911 that “gastric peristaltic waves do not pass on to the duodenum, but stop at the pylorus. This separation of the two regions is probably to be accounted for by the interruption in the continuity of the circular muscle fibers just beyond the pyloric sphincter.” Indeed, Cai and Gabella (7) noted that the guinea pig sphincter muscle was separated by a septum from the duodenal circular muscle but not from the gastric circular muscle. This was also observed in a recent study on the cat sphincter (31). Simultaneous recordings with large arrays of electrodes along the length of the stomach and proximal intestine of the cat showed that the slow wave activity was not continuous but abruptly blocked at the level of the pylorus (31). Because antral and duodenal motor activities are dominated by slow wave-driven peristalsis, the question arises how the interstitial cells of Cajal (ICC) network is organized at the pylorus, how slow wave activity is generated, and how the peristaltic activities are synchronized to allow for transport of content across the pylorus. In a landmark paper, Conklin and Du (10) implied that it was the network of ICC and not the musculature that was responsible for coordination of electrical activities along the long axis of the cat colon. This was confirmed in the canine colon (35, 44) and stomach (21).

To study a possible role of ICC in gastroduodenal coordination, measurements with arrays of electrodes were modified for use in the rat and mouse, structural studies were carried out using immunohistochemistry, and electron microscopy and motor patterns were analyzed based on videofluoroscopy and image analysis.

METHODS

Electrophysiology. Nine rats and eight mice (all males; weight 338 ± 37 and 35 ± 4 g, respectively) were used for the electrophysiological experiments, approved by the animal ethics committee at United Arab Emirates University. After an overnight fast, the animals were anesthetised with chloroform (rats) or urethane (mice, 2 mg/kg ip). The stomach and duodenum were rapidly removed, opened along the minor curvature and the mesenteric border, and positioned with the serosa side upward in a tissue bath. The preparations were superfused at a rate of 100 ml/min with a modified Tyrode solution kept at a constant pH (7.35 ± 0.05) and temperature (37 ± 0.5°C) and saturated with carbogen (95% O2-5% CO2) (31). The intracellular microelectrode recording displayed in Fig. 1 was obtained using methods described previously (34).

With the rat preparations, a row of 32 electrodes (Teflon-coated silver wire; 1-mm interelectrode distance) was carefully positioned on...
the serosal side of the preparation oriented in the longitudinal direction spanning the corpus, antrum, gastroduodenal junction, and the upper part of the duodenum. In the murine experiments, a row of 16 electrodes (0.37-mm interelectrode distance) was also positioned longitudinally on the serosal surface, and recordings were performed sequentially at three different locations: on the stomach, across the gastroduodenal junction, and on the duodenum. The recordings were made unipolarly with a large silver plate in the tissue bath acting as the indifferent pole. The electrodes were connected to alternating-current preamplifiers (gain 4,000), and the recorded signals were subsequently filtered (2–400 Hz), digitized (8 bits, 1 kHz sampling rate per channel), multiplexed, and stored.

After a 15- to 30-min control period, 1- to 2-min recordings of the spontaneous rhythm and propagation were performed every 10 min for 30 min. For the analysis, signals (duration of 60 s) were transferred to a personal computer, digitally filtered (20-point running average), and displayed on screen in groups of 16–32. From these signals, the spontaneous rate of the stomach and the duodenum was calculated by measuring the interval between sequential slow waves. The propagation of the slow wave in both organs was calculated by measuring the difference in time of activation between a proximal and a distal electrode. Finally, in the recordings that spanned the gastroduodenal junction, the number of electrodes that did not record a slow wave was noted.

To determine whether stomach activity had any effect on spontaneous duodenal rhythm, the following analysis was performed. Two electrogams, one from the antrum and one from the duodenum, located as close as possible to each other were used for this purpose.

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Table 1. Characteristics of slow wave activity at the gastroduodenal junction

<table>
<thead>
<tr>
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<th>Stomach</th>
<th>Duodenum</th>
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<tr>
<td></td>
<td>Width of Slow Wave-Free Zone, mm</td>
<td>Slow wave frequency, cycles/min</td>
</tr>
<tr>
<td>Mouse</td>
<td>1.3±0.4 (0.0–2.2)</td>
<td>6.8±0.6 (4.1–9.7)</td>
</tr>
<tr>
<td>Rat</td>
<td>4.1±1.0 (0.0–7.0)</td>
<td>3.07±1.04 (1.3–5.3)</td>
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Values are means ± SD with ranges in parentheses; n, no. of animals. Slow wave frequency and conduction velocity were statistically different between antrum and duodenum (P < 0.001) both in mice and rats.

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Fig. 1. A: 4-mm gap in slow wave activity is observed in the pyloric region of the rat. There is no slow wave activity in the fundus (top part) because of a lack of interstitial cells of Cajal (ICC) associated with Auerbach’s plexus (ICC-AP). Starting from the corpus, stomach activity was characterized by rhythmic oscillatory discharges that propagated in the distal direction before terminating at approximately electrode 15. Each spindle represents a slow wave as observed previously using chronically implanted electrodes in the rat (18) and shown in B using the intracellular recording technique in the rat corpus. Duodenal slow waves originated from the upper part of the duodenum (at approximately electrode 22) and propagated also in the distal direction. Between the stomach and the duodenal activity, at least four electrodes (16–19) did not register any slow wave activity; accounting for a gap width of 4 mm. Occasional spike activities (smooth muscle action potentials) were visible in several electrograms.
With the use of the stomach activity as a reference point, the duodenal beat-to-beat intervals before, during, and after this stomach discharge were measured. This analysis was performed 18 times in rats and 18 times in mice. The calculated values were plotted on an individual basis (as shown in Fig. 4, C and D) and, after normalization, grouped together as shown in Fig. 4, E and F.

Immunohistochemistry. Twelve Sprague-Dawley rats, 250–350 g, and seven CD1 mice (25–35 g, 8–9 wk old) were used. Animals were handled according to McMaster University Animal Care Committee ethical guidelines, and the present study was approved by the Animal Ethics Research Board at McMaster University. After resection, the pyloric sphincter region was opened along the lesser curvature and washed in Krebs-Ringer bicarbonate solution. Both frozen sections and whole mounts were prepared for c-Kit immunohistochemical staining. For frozen section preparations, fresh tissues were directly embedded in Tissue-Tek and frozen with isopentane emerged in liquid nitrogen. Frozen longitudinal sections of 8 μm were cut from lesser to greater curvature sides with a cryostat. Musculature whole mounts were obtained by peeling away the mucosa and submucosa under the dissection microscope. Both the section and wholemount preparations were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 10 min at 4°C. After being washed for 30 min in 0.05 M PBS (pH 7.4, with 0.3% Triton X-100), the fixed tissues were processed for immunohistochemical staining (55). Sections from rat tissues were incubated with either polyclonal rabbit anti-c-Kit (DAKO, Denmark) or goat anti-c-Kit (Santa Cruz, CA) after endogenous peroxidase was quenched, whereas sections from mouse tissues were incubated with monoclonal rat anti-c-Kit (ACK4, Cedarlane, Canada). All the incubation times were 24 h at 4°C. Secondary immunoreactions were carried out with either Vectastain ABC kits (with biotinylated anti-rabbit, anti-goat, or anti-rat IgGs) or FITC-conjugated IgGs (Vector Laboratories, Burlingame, CA). 3,3-Diaminobenzidine was used as a peroxidase substrate for the ABC technique. Tissues were examined with either a conventional microscope with an attached digital camera (Sony 3CCD, model DXC-930) or confocal microscope (Zeiss LSM 510) with an excitation wavelength appropriate for FITC (494 nm).

Conventional electron microscopy. Tissues were fixed with 2% paraformaldehyde, 2.5% glutaraldehyde, 3% sucrose, and 1.25 mM
CaCl₂ in 0.05 M cacodylate buffer (pH 7.4) at 4°C for 2 h. They were then postfixied in 2% osmium tetroxide (OsO₄) for 1 h, stained en bloc with 2% aqueous uranyl acetate for 40 min, dehydrated, infiltrated, and embedded in Epon-Araldite resin. Ultrathin sections were cut parallel to the circular muscle layer and stained with lead citrate for 5 min before viewing with a transmission electron microscope (Jeol 1200EX Biosystem).

Quantification of Kit-positive cells within sections at the level of myenteric plexus of rat pylorus. Quantification of c-Kit positivity was performed with the KS400 program (Zeiss, Germany). Fifty longitudinal sections cut from lesser to greater curvature sides stained with c-Kit were chosen. Kit immunopositivity at the level of Auerbach plexus (mostly ICC-AP) at the points of the pyloric-duodenum junction as well as 1.5 and 3 mm oral and 0 and 1.5 mm caudal were identified and highlighted using density slicing on color-scale images. The area of immunopositive cells on each picture was measured and expressed as percentage of total area. Regions with increased background staining, occasionally found at the borders of the image, were excluded from the analysis. Due to stronger staining and different shape, mast cells were easily distinguished from ICC and excluded before the analysis.

Radiology and image analysis. Mice had access to food and water ad libidum, and tests were performed between 5:00 and 8:00 PM. Omnipaque 350 (0.2 ml/mouse; Nycomed Imaging AS, Oslo, Norway) was gavaged into the stomach, and mice were continuously fluoroscoped (Siemens, Polystar) for 3 min. Video images were stored on a VCR (JVC BR-S378U, Japan) for off-line analysis and then digitized by Power Macintosh G3 at a frequency of 10 images/s. Spatiotemporal maps were constructed and analyzed using National Institutes of Health (NIH) Image software (developed at NIH and available at http://rsb.info.nih.gov/nih-image) with self-routines as previously described (3, 13). Frequency and propagation velocity of contractions in the antrum and duodenum were evaluated. Peristalsis was defined as aborally propagating waves of muscle wall indentations corresponding to circular muscle contractions. Temporal correlation between peristaltic antral and duodenal contractions was assessed in spatiotemporal maps.

Data were expressed as means(SD). Statistical significance was determined by a two-tailed t-test. P values <0.05 were considered to indicate statistically significant differences. The number of animals is represented by N, and the number of tissue recordings is represented by n.

RESULTS

Electrical activities in the gastroduodenal region. The gastroduodenal junction of the rat showed a distinct region lacking any slow wave activity (Fig. 1A). When 32 electrodes were placed in one line on the stomach, across the pylorus, and into the small intestine, antral slow waves were seen to propagate toward the pylorus at a velocity of 1.5 mm/s and a frequency of 3 cycles/min (Fig. 1A and Table 1). The slow wave stopped abruptly in the pyloric region. Distally (4 mm), duodenal
Slow wave activity started at a propagation velocity of 1.0 mm/s and a frequency of 35 cycles/min (Table 1). Four electrodes in the pyloric region spaced 1 mm apart did not record any slow wave activity, although an occasional action potential was observed. The extracellular recording of the slow wave activity displayed short, spindle-shaped bursts of electrical oscillatory activity; each spindle representing one slow wave as shown using the intracellular recording technique in Fig. 1B.

Slow wave frequency and conduction velocity were statistically different between antrum and duodenum (*P* < 0.001) both in mice and rats [means (SD)].

The gastroduodenal junction of the mouse showed a similar distinct region without any slow wave activity, albeit shorter in length (Fig. 2 and Table 1). Slow wave activity of the stomach propagated distally at a velocity of 10 mm/s and a frequency of 7 cycles/min and stopped abruptly in the oral part of the sphincter region. After 1.3 mm, the duodenal slow waves started at 46 cycles/min and a conduction velocity of 15 mm/s.

Action potentials, originating on the antral slow waves, were seen to propagate into the sphincter region, on occasion farther than the slow wave activity (Fig. 3). On 24 occasions where action potential activity was seen in the pyloric region, 83% were associated with gastric slow wave activity, propagating for very short distances (1–3 mm) aborally (66%), orally (21%), or in both directions (14%).

The analysis of a possible temporal coordination between gastric and duodenal activity is shown in Fig. 4. If the gastric slow wave would influence the timing of the intestinal slow wave, a premature or delayed intestinal slow wave would produce shortening or lengthening of the duodenal slow wave interval. Duodenal slow waves that occurred before and after an antral slow wave were marked, and the intervals were calculated (*n* = 36 for both rats and mice). The duodenal intervals in both rats and mice were never significantly lengthened or shortened at any stage before, during, or after antral activity.
duodenum, was present in the sphincter region. With Kit
tivity was visible, no ICC-AP network, as in the antrum and
plexus (Figs. 5).

The concentration of Kit-positive cells at the level of Auerbach's
muscle layers. In the pyloric sphincter region, there was no
ICC were mainly found concentrated at the level Auerbach's
proximal duodenum, except the first millimeter, Kit-positive

tions and connecting with each other to form a dense network. In contrast,
the sphincter). In both regions, there were abundant ICC-AP (arrows) that were
antrum (b: 3 mm oral to the sphincter) and the duodenum (d: 3 mm caudal to
the sphincter). In both regions, there were abundant ICC-AP (arrows) that were
triangular/multipolar in shape, with processes proceeding in different direc-
tions and connecting with each other to form a dense network. In contrast,
there were almost no ICC-AP in the sphincter region (c). Instead, many
bipolar-shaped ICC-IM running parallel to the pyloric sphincter (PS; c, left)
were observed. The right portion of c shows a loose network of ICC in the very
beginning of the proximal duodenum.

Morphological analysis of ICC in the pylorus. The distribu-
tions of ICC in the gastroduodenal regions were similar in rat
(Figs. 5 and 6) and mouse tissues (Fig. 7) based on c-Kit
immunohistochemistry. ICC-AP in the pyloric sphincter region
were markedly decreased in a 4-mm region from lesser to
greater curvatures compared with the adjacent antrum and
duodenum. In the distal antrum, Kit-positive ICC were distrib-
uted evenly in both muscle layers. In addition a concentration
of cells was observed at the level of Auerbach’s plexus and at
the submucosal border of the circular muscle layer. In the
proximal duodenum, except the first millimeter, Kit-positive
ICC were mainly found concentrated at the level Auerbach’s
plexus (ICC-AP) as well as in the deep muscular plexus area
(ICC-DMP). In the first millimeter caudal to the pylorus-
duodenum junction, ICC were diffusely distributed in both
muscle layers. In the pyloric sphincter region, there was no
concentration of Kit-positive cells at the level of Auerbach’s
plexus (Figs. 5A and 7A). Although sparse Kit immunoreac-
tivity was visible, no ICC-AP network, as in the antrum and
duodenum, was present in the sphincter region. With Kit
antibodies from different sources, similar results were ob-
tained; Kit immunoreactivity at the level of myenteric plexus
was virtually absent. The gap had a width of ~4 mm for rats
and ~1.2 mm for mice in unstretched tissues, determined from
sections that were taken from the lesser curvature and the
greater curvature. Quantification based on Kit immunoreactiv-
ity and image analysis confirmed the significant decrease in
ICC-AP in the pyloric region (Fig. 8). Assessment of whole
mount preparations confirmed the remarkable reduction in
ICC-AP and interruption of ICC-AP networks at the region of
the pyloric sphincter in rat (Figs. 5, B-D) and mouse (Fig. 7B).

Electron microscopic (EM) examination of the sphincter
region rarely encountered ICC-AP cell bodies (Fig. 6B). In
contrast, in the antrum (Fig. 6A) and duodenum (Fig. 6C), a
large number of ICC-AP was found around and in between
myenteric ganglia. Connections including gap junctions be-
tween ICC-AP were commonly observed in antrum and duo-
denum, indicating their network structure (insets in Figs. 6, A
and C). In the pyloric region (Fig. 6B), only occasional ICC
processes were found between the profiles of nerve bundles.
Ganglia were not commonly present in the myenteric plexus of
the pyloric sphincter region. Instead, many large enteric nerve
bundles with varying density of varicosities were present. In
contrast to the sparse number of ICC-AP, intramural ICC
(ICC-IM) were found in abundance and in close contact with
enteric nerves and smooth muscle cells within the sphincter
(Fig. 6D).

Antral and duodenal peristaltic activities in mice in vivo.
Videofluoroscopic image analysis showed that immediately
after contrast material was introduced into the stomach,
aborally directed peristaltic contractions occurred in the
stomach at a frequency of 5.1 ± 1.5 cycles/min and propa-
gated at a velocity of 1.02 ± 0.16 mm/s (Fig. 9). Concom-
antly, contrast material entered the duodenum in a pulsating
manner. On its entry into the small intestine, contrac-
tions occurred at a frequency of 44.5 ± 2.9 cycles/min; they
propagated in an aboral direction at a velocity of 10.4 ± 2.1
mm/s and orally at 4.9 ± 3.0 mm/s. Regular aborally
directed peristalsis prevailed in the first minute after gavage
of contrast material into the stomach (Fig. 9, left). Thereaf-
ner, regular aboral peristalsis in the stomach continued, but
segmental contractions and retroperistalsis dominated the
motor patterns of the duodenum, interrupted at regular
intervals by one or more aborally propagating contractions
(Fig. 9, middle). This allowed us to answer the question
whether a temporal relationship between the antral and
duodenal peristaltic contractions existed. Video images and
spatiotemporal maps showed that one to several duodenal
aborally propagating peristaltic contractions appeared most
of the time temporally related to the antral peristaltic wave
(Fig. 10). On average, duodenal contractions started 2.4 ±
2.8 s before the front of an antral contraction reached the
pylorus, pushing content ahead of them. This coincided with
the opening of the pylorus and passage of the gastric luminal
contents into the proximal duodenum (Fig. 10). On rare
occasions, independent and noncoordinated patterns coex-
isted in antrum and duodenum (Fig. 9, right). This corre-
sponded to periods when the pylorus remained closed and
there was no mechanical communication between the stom-
ach and duodenum.
DISCUSSION

The present study used narrowly spaced surface electrodes to monitor for the first time simultaneously the electrical activity of the distal antrum, pylorus, and proximal small intestine in the mouse and rat. Slow wave activity was not generated in and did not spread into the pylorus; consistently, we show that no pacemaker ICC network was present. The absence of slow wave activity in the pyloric region allowed for distinct independent slow wave activities in stomach and duodenum with distinct frequencies and propagation velocities and hence independent motor activities. Indeed, our in vivo experiments showed predominantly independent motor activities, in particular during periods of pyloric closure. However, when a single antral wave of contraction was followed by opening of the pylorus, gastric content arrived into the duodenum a few seconds ahead of the antral contraction reaching the pylorus, and a few synchronized aborally propagating contractions in the duodenum were evident. Presumably, the luminal content evoked peristaltic contractions in the duodenum either by distending the muscle wall (5, 29) or by shear stress induced by luminal flow (15, 28, 38, 39). Distention and/or shear stress cause activation of enteric excitatory motoneurons providing the final stimulus for contraction (11). The slow waves allow the musculature to be depolarized only periodically such that contractions are periodic and propagate aborally. The role of the pylorus is not to transmit electrical slow waves or action potentials to the duodenum.
potentials from the stomach to the duodenum but to control the flow of luminal contents into the duodenum, also observed in humans (47). The pylorus opens according to complex regulatory systems involving extrinsic and intrinsic nerves. Antral contractile activity can result in the opening of the pylorus (42) mediated by intrinsic descending inhibitory nerves (1, 33). On arrival of each antral peristaltic wave in the pyloric region, there are 6–8 duodenal slow waves in the mouse and 10–14 slow waves in the rat available for coordinated motor activity; hence, the smooth transition of contents into the small intestine. Recently, simultaneous assessment of antral and duodenal peristalsis and video analysis as performed in the present study were carried out in humans, whereas for each antral slow wave, three to five slow waves occur in the duodenum (4).

The ICC-AP are the pacemaker cells that generate the primary dominant pacemaker activity (25, 49). Tissues that are rich in ICC-IM but lack ICC-AP, such as the esophagus and the fundus, do not generate omnipresent slow wave activity. When pacemaker ICC are removed from tissues in the intestine (24, 56) or stomach (2, 21) or colon (34, 48), the primary slow wave activity disappears. It is likely, however, that the ICC-IM do play a role in modifying and propagating slow waves once they are generated. In the mouse antrum, the ICC-IM contribute to the secondary regenerative component of the slow wave (14) and may be involved in slow wave propagation (20). Furthermore, they can provide secondary peristalsis in the absence of the primary pacemaker activity (22, 24, 40). The present study shows that slow waves are not generated in the pylorus despite the presence of ICC-IM. Similarly, strong slow wave activity in the very distal part of the canine antrum quite suddenly disappears when entering the pylorus (45). Hence, ICC-IM may not be capable of regenerating slow wave activity that originates from ICC-AP over long distances without specific stimulation.

Action potentials were observed in the pyloric region, both independent of and associated with gastric slow wave activity. Contractile activity in the pylorus related to the slow wave activity of the stomach or duodenum will result from action potentials originating at the crest of a stomach or duodenal slow wave being able to propagate into the pyloric muscle as shown in this study and in the cat sphincter (30). This indicates that lack of slow wave propagation into the pylorus is not due to a barrier to electrical conduction but likely due to the strongly reduced number of ICC-AP. In the canine pylorus, one
region close to the antrum had variable intrinsic oscillatory activity that could be paced by antral slow waves (45). The results from the present study make it likely that this region in the canine pylorus with variable slow wave-like activity is a region with an irregular and sparse ICC network. Some motor patterns originating from this region might be referred to as intrinsic pyloric activity. Interestingly, this region was generally more excitable consistent with distinct neural input (12, 27). It is clear that the pylorus does not have a primary pacemaker system in connection with the networks of ICC-AP in stomach and duodenum. Pyloric phasic contractile activities (52) rely on 1) excitation of the pyloric muscle cells and ICC-IM by efferent nerve supply specific to the pylorus (59) or 2) propagation of action potentials from the gastric or duodenal

Fig. 9. In vivo motor patterns in mice. Spatiotemporal maps of contractions in the stomach and duodenum (x-axis = position along the gut, y-axis = time). Darker regions contain more contrast medium and represent relatively relaxed areas, whereas lighter regions are associated with contractions. Any propagating contraction appears in a map as an oblique light line due to changes in optical density associated with displacement of contrast material. Antral peristaltic contractions (double arrows) propagated up to the pylorus (dotted line). Duodenal peristaltic contractions (single arrows) appeared either continuously (left) or coordinated with (but preceding) the antral contractions (middle; most common). Occasionally, there was continuous retroperistalsis in the duodenum (single arrow, right) despite the presence of regular aborally propagated contractions in the antrum (double arrows). This appeared on occasions when the pylorus remained closed and no gastric contents entered the duodenum.

Fig. 10. Temporal association between antral and duodenal peristaltic contractions in the mouse. A: video images of mouse stomach and duodenum showing an antral contraction (arrow) that propagates from the proximal antrum (image 2) to the pylorus (image 5). The pylorus opens (arrowhead) when the antral contraction reaches midantrum (image 3). B: a spatio-temporal map constructed from the above video sequence is shown. The numbers indicate time points corresponding to single video images. Time lag between the end of an antral contraction, defined as the contraction reaching the pylorus (dotted line) and the beginning of a duodenal contraction, was measured. Note that opening of the pylorus (arrowhead) with passage of gastric content coincides with the origin of the duodenal contraction. C: most frequently, duodenal contractions started a few seconds before the arrival of the antral contraction at the pylorus.
musculature, which occur in short bursts associated with gastric or duodenal slow wave activity. In a previous study (57) on the mouse pylorus, ICC-AP were identified in young mice (20–30 days old), but a gap in this network was not described. In the canine pylorus, a high density of ICC-IM was found using EM (12) but the study did not focus on ICC-AP. ICC-AP were detected in the human pylorus (32, 51, 53) but a quantitative study searching for a possible gap in the network has not yet been conducted. In our present study, the combination of immunohistochemistry and EM showed that a normal ICC-AP network at the pylorus was absent and that only scattered ICC-AP were present. The Auerbach’s plexus in the stomach and intestine is characterized by a very dense aggregation of large ganglia. This was interrupted at the pylorus, and the dominant nerve structures were large groups of nerve varicosities, many in contact with ICC-IM. In the stomach and duodenum, ICC-AP are prominent in between and surrounding ganglia; hence, the following question arises: Are ICC-AP reduced in number at the pylorus as a consequence of loss of ganglia? The latter is not likely, because complete absence of ganglia in total aganglionosis can be accompanied by normal development of ICC (23). Furthermore, animal models indicate separate development of ICC and enteric nerves (58).

The enteric nervous system provides the dominant stimulus for generation of contractile activity. The unique motor activity of the pylorus is determined by extrinsic and intrinsic innervation specific to the pylorus. In contrast to adjacent areas, the Auerbach’s plexus of the pylorus is devoid of large ganglia, but the numbers of nerve bundles and nerve axons and density of varicosities are higher (7). Nerve varicosities are distant from smooth muscle cells as shown in the guinea pig pylorus (7) but form synapse-like connections with ICC-IM, which form gap junctions with the adjacent smooth muscle cells as shown in our present study. This indicates a rich innervation of the smooth muscle cells in this special region despite the lack of local myenteric enteric ganglia. The high density of ICC-IM and their close innervation suggest a regulatory function for ICC-IM in pyloric innervation (54, 57). Both extrinsic and intrinsic nerves supply the pylorus where they function to regulate gastric emptying and prevent reflux of duodenal content (59). The enteric nerves can also provide gastroduodenal coordination; an example is the orchestration of the migrating myoelectric complex (MMC) (46) that can propagate from the stomach into the duodenum. When the wave of excitation reaches the gastroduodenal junction, the omnipresent slow waves contribute to the organization of the contractile activity associated with the MMC (19). The present study focuses on distension-evoked motor patterns. Distension activates enteric motor nerves that provide the stimulus (depolarization) for smooth muscle contraction, whereas the myogenic slow waves impose restrictions related to timing, duration, frequency, and propagation. Propagation does not occur through the circular muscle layer of the antrum, because it is not continuous with that of the duodenum; a band of connective tissue forms a distinct separation (Ref. 7 and present study). Furthermore, because of the organization of circular muscle in lamellae, it does not provide a continuous pathway for propagation in any case (35). Electrical propagation could potentially be provided by the longitudinal muscle layer that is continuous (Ref. 7 and present study). It was shown in the colon that slow wave propagation does not occur through longitudinal muscle but through the ICC pacemaker network (10). The present study shows that slow wave propagation does not happen through the pylorus despite a continuous longitudinal muscle, clearly due to the absence of a pacemaker ICC network.

The human pylorus has Kit-positive ICC-associated Auerbach’s plexus, which has extensions that are short and branched (53) and possess ICC-IM, scattered throughout the musculature that are bipolar with relatively long branches (53). Ultrastructurally, the ICC of the human stomach have typical features (50) and have close contact with smooth muscle cells and nerve varicosities (17) very similar to the rat and mouse (26, 43). The electrical pacemaker activities in both human (16) and mouse (6, 14) stomach and human (9) and mouse (36) small intestine are not fully elucidated but at this point do not appear to be fundamentally different. Hence, the rat and mouse appear to be suitable models to gain insight into human gastroduodenal motility (43).

In conclusion, the motor patterns of the stomach and duodenum are largely independent due to absence of slow waves and absence of an ICC-AP network in the pyloric region. Aborally propagating peristaltic contractions of the stomach are coordinated with peristaltic contractions of the duodenum on flow of luminal content through a transiently relaxed pyloric sphincter. These results have implications for development of new therapies designed to improve gastropyloroduodenal coordination, which could focus on pyloric function (41) and duodenal mechanosensitivity.

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GRANTS

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