Mechanisms underlying nutrient-induced segmentation in isolated guinea pig small intestine

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Gwynne RM, Bornstein JC. Mechanisms underlying nutrient-induced segmentation in isolated guinea pig small intestine. Am J Physiol Gastrointest Liver Physiol 292: G1162–G1172, 2007. First published January 11, 2007; doi:10.1152/ajpgi.00441.2006.—Mechanisms underlying nutrient-induced segmentation within the gut are not well understood. We have shown that decanoic acid and some amino acids induce neurally dependent segmentation in guinea pig small intestine in vitro. This study examined the neural mechanisms underlying segmentation in the circular muscle and whether the timing of segmentation contractions also depends on slow waves. Decanoic acid (1 mM) was infused into the lumen of guinea pig duodenum and jejunum. Video imaging was used to monitor intestinal diameter as a function of both longitudinal position and time. Circular muscle electrical activity was recorded by using suction electrodes. Recordings from sites of segmenting contractions showed they are always associated with excitatory junction potentials leading to action potentials. Recordings from sites oral and anal to segmenting contractions revealed inhibitory junction potentials that were time locked to those contractions. Slow waves were never observed underlying segmenting contractions. In paralyzed preparations, intracellular recording revealed that slow-wave frequency was highly consistent at 19.5 (SD 1.4) cycles per minute (c/min) in duodenum and 16.6 (SD 1.1) c/min in jejunum. By contrast, the frequencies of segmenting contractions varied widely (duodenum: 3.6–28.8 c/min, median 10.8 c/min; jejunum: 3.0–27.0 c/min, median 7.8 c/min) and sometimes exceeded slow-wave frequencies for that region. Thus nutrient-induced segmentation contractions in guinea pig small intestine do not depend on slow-wave activity. Rather they result from a neural circuit producing rhythmic localized activity in excitatory motor neurons, while simultaneously activating surrounding inhibitory motor neurons.

The major motor activity of the small intestine after a meal is the most poorly understood. This is the set of stationary, rhythmic contractions of the circular muscle known as segmentation, which was first described by Cannon over a century ago (7, 8). Segmentation aids in the digestion and absorption of nutrients by mixing food with digestive juices and exposing digested material to the absorptive epithelium. Cannon (8) suggested that the generation and timing of this motor pattern were the result of the activity of the enteric nervous system (ENS); however, this remains unproven. More recently, it has been suggested that the rhythmic contractions that are characteristic of segmentation are regulated by slow waves generated by the interstitial cells of Cajal (ICC) with ENS activity serving a permissive role (15, 28). However, definitive evidence supporting either hypothesis is lacking.

Our limited knowledge about mechanisms controlling segmentation has largely been due to lack of a useful model with which to study segmentation in vitro. We have recently used a high resolution mapping method based on video imaging (2, 16, 22) to show that segmentation can be evoked in isolated guinea pig small intestine in vitro by intraluminal infusion of fatty acids (14) or amino acids (13). The activity evoked by the nutrient stimuli consists of a suite of different motility patterns. The stationary contractions (or segmentation contractions) are localized constrictions that appear simultaneously (within a single frame of the video recording) along their entire length and do not propagate along the intestine. Short length propagating contractions are constrictions that propagate slowly either orally or anally for 2–3 cm along the intestine. Whole-length propagating contractions are similar to the propulsive contractions that are evoked by saline distension of an entire segment and typically appear at the oral end of a segment and propagate rapidly along its entire length. In some cases, whole-length contractions propagate orally. This complex suite of nutrient-induced motility patterns is abolished by blocking the activity of the ENS, indicating that they depend on neural activity. Furthermore, the motility pattern is abolished by hexamethonium, which suggests that nicotinic cholinergic receptors are involved in the underlying neural circuitry (14). It was concluded from indirect evidence that the stationary contractions, which are characteristic of segmentation in vivo, are independent of slow-wave activity. However, as yet there is no direct evidence as to the nature of the output of the neural circuits regulating segmentation that are activated by intraluminal nutrients. The present study was designed to address this critical question.

We used extracellular recording techniques correlated with video imaging to examine the electrical activity of the circular muscle associated with the stationary contractions induced by decanoic acid and individual amino acids in vitro. This also allowed a direct test of the earlier conclusion that the properties of the stationary contractions induced by nutrients are independent of slow waves in this preparation. This was essential because a study correlating electrical activity with contractile events induced by prolonged saline distension of the same preparations has concluded that propulsive activity was due to induction of slow wave-like activity in the muscle via release of acetylcholine from excitatory motor neurons (11).

Methods

Tissue preparation for correlated extracellular recordings and motility studies. Guinea pigs of either sex (250–380 g) were killed by being stunned and having their carotid arteries severed. This proce-

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ture was approved by the University of Melbourne Animal Experimentation Ethics Committee. Segments of duodenum or jejunum were prepared as described previously (14) (Fig. 1A).

Image acquisition and spatiotemporal maps. A Logitech Quickcam pro camera was positioned 6–7 cm vertically above the organ bath to record video images of a 4–5 cm length of intestine. Images were captured at a frame rate of 30 per s with a resolution of 640 × 480 using Logitech Quickcam software (Version 5.0) and acquired directly to computer in “avi” format. The videos were processed offline with edge-detection software developed in-house using the MatLab system (V6.1) to produce spatiotemporal maps. The maps were analyzed to measure the frequencies of isolated stationary contractions occurring in the same intestinal region. These methods have been described in detail previously (14).

Extracellular recording. The extracellular suction electrodes consisted of thin walled capillary glass (outer diameter 1.0 mm, inner diameter 0.78 mm) containing 250 μm Ag-AgCl wire. The top of the glass electrode was connected to flexible Silastic tubing (Dow Corn ing no. 508-004; OD 1.65 mm, ID 0.76 mm), which was then connected via a three-way stopcock to a 5-ml syringe used to apply suction and to a 60-cm vertical column of liquid to maintain the suction once the electrode was attached. The silver wire was pushed through the wall of the tubing and threaded down the lumen to the tip of the glass electrode that contained physiological saline as the conducting solution. The other end of the wire emerging from the tubing was coiled to provide flexibility and soldered to a gold pin to allow connection to the headstage. Electrical activity was recorded using a World Precision Instruments KS-700G amplifier (×1 amplification) and a Biopac Systems DA 100 amplifier (×200 amplification) and was stored on a personal computer using AcqKnowledge 3.7.2 software.

Protocol for extracellular recordings. After the tissue was set up, 10 ml of physiological saline was flushed through the lumen and the preparation left to equilibrate for 1 h. During the equilibration period, the tissue was held at an intraluminal pressure of 2 cmH2O and was mostly quiescent. After the equilibration period, either single or multiple extracellular electrodes were attached to the serosal surface of the intestinal segment and electrical recordings were made for 30 min (Fig. 1A). Following this, the inflow pressure was increased in 1-cm steps at intervals of 30 s, and the electrical activity associated with propulsive contractions was recorded. After three measurements, physiological saline containing decanoic acid (1 mM), L-phenylalanine (30 or 50 mM), or L-tryptophan (30 mM) was flushed into the lumen. The tissue was again equilibrated at 2 cmH2O, and electrical recordings were made with decanoic acid or an amino acid present in the lumen. Extracellular electrodes were attached to regions where stationary contractions consistently occurred at one site and also next to segmenting regions. Following recordings under control conditions, or with decanoic acid in the lumen, antagonists were added to the bath for 30–40 min. The antagonists were washed from the bath and recordings were made to check reversibility of the drugs.

Preparation and protocol for combined intracellular and extracellular recordings. Intracellular recordings were made from duodenum, jejunum, and ileum to identify the basic properties of slow waves in each region. Segments of intestine (5 cm in length) were opened along the mesenteric border and pinned flat to the floor of an organ bath with the mucosal surface uppermost. The bath was perfused at a constant rate of 6 ml/min with physiological saline (composition in mM: 118 NaCl, 4.6 KCl, 1 NaH2PO4, 25 NaHCO3, 1.2 MgSO4, 11 d-glucose, 2.5 CaCl2, bubbled with 95% O2 and 5% CO2) maintained at 37°C.

The L-type calcium channel blocker nicardipine (2.5 μM) was added to the perfusate to reduce contraction of the muscle layers and thus increase stability of the impalements. Distension stimuli to naturally excite reflex pathways were applied via rubber balloons in the base of the bath. Distending from the serosal side allows pathways excited by distension to be activated separately from those excited by mechanical deformation of the mucosa (32). The anal end of the segment was folded over to allow impalement of circular muscle cells via the

Fig. 1. Schematic diagram of experimental preparations. A: intact segment of intestine mounted horizontally in an organ bath and cannulated to allow intraluminal perfusion of nutrient solutions. A video camera is positioned above the bath and extracellular electrodes are attached to the serosal surface of the intestine to allow correlation of changes in intestinal diameter with muscle electrical activity. B: segment of intestine that has been dissected and pinned flat to the base of an organ bath with the mucosal surface uppermost. The anal end of the intestine was turned over on itself to allow access to the serosal surface. Dual intracellular (IC) and extracellular (EC) recordings of slow waves were made with the 2 electrodes (ICE and ECE) positioned within a few millimeters of each other circumferentially. Rubber balloons in the base of the bath were used to stretch the intestinal wall from the serosal side to evoke inhibitory reflexes that were recorded simultaneously by the intracellular and extracellular electrodes.
serosal surface (32). After equilibration for 1 h, circular muscle cells were impaled by advancing the microelectrode (containing 1 M KCl, resistance 60–100 MΩ) through the longitudinal muscle layer into the underlying circular muscle layer. Circular muscle cells with resting membrane potentials between −40 and −50 mV were allowed to stabilize for 1 min. In preparations of duodenum, indomethacin (1 μM) was added to the bath solution to block endogenous prostaglandin synthesis, and hence release, to increase slow-wave activity (18–20). Time controls were performed over a period of 4 h to examine the behavior of slow waves, and their amplitude and frequencies were determined offline.

Dual intracellular and extracellular recordings were made to compare the recording techniques during slow-wave activity and distension induced inhibitory reflexes. First the extracellular electrode was attached, and then circular muscle cells were impaled 5–8 mm away circumferentially (Fig. 1B). Responses to electrical stimulation of inhibitory motor pathways are indistinguishable between recording sites separated by this circumferential distance at the same longitudinal position (4).

Drugs. Drugs used in these experiments included nicardipine, hyoscine, and indomethacin (all from Sigma Aldrich) and TTX and apamin (both from Alomone Labs). Stock solutions of the drugs were initially made up in distilled water and diluted to working concentrations in physiological saline on the day of the experiment. Decanoic acid (Sigma Aldrich) was first dissolved in absolute ethanol and then diluted with distilled water (1:1 ratio) to form a 100 mM stock solution. L-Phenylalanine and L-tryptophan were dissolved in physiologicht solution. L-Phenylalanine and L-tryptophan were dissolved in physiological saline on the day of the experiment to make a final concentration of each amino acid of 30 or 50 mM.

Statistics. Data in the text are reported as means ± SE or medians (SD). Statistical comparisons were made by paired and unpaired t-tests and one-way ANOVA where appropriate. P values <0.05 were taken as indicating statistical significance.

RESULTS

Traces of simultaneous intracellular and extracellular recordings showed excellent correlation during slow-wave electrical activity (Fig. 2, A and B) and during inhibitory junction potentials (IJPs) evoked by oral distension (Fig. 2, C and D). The longitudinal muscle in the guinea pig small intestine does not exhibit IJPs (17). Thus the fact that the intracellular electrode was recording directly from a circular muscle cell and the dual recordings during inhibitory reflexes had identical time courses indicates that the extracellular electrode was recording from the circular muscle layer. These experiments also showed that extracellular electrodes could detect slow waves in circular muscle down to less than 2 mV in amplitude.

Electrical activity underlying decanoic acid induced segmentation. During the 1-h equilibration period (2 cmH2O intraluminal pressure), the segments of intestine were mostly quiescent. Typical electrical recordings taken from serosal extracellular electrodes showed small spontaneous irregular oscillations in membrane potential (Fig. 3B). Increases in intraluminal pressure past a threshold pressure evoked repeated propulsive contractions (a peristaltic reflex) that were initiated at the oral end of the segment and propagated to the anal end (Fig. 3C). The electrical activity associated with an individual propulsive contraction consisted of an excitatory junction potential (EJP) leading to two or more action potentials (APs) (Fig. 3D). The characteristics of propulsive contractions have previously been described in Gwynne et al. (14) and will not be discussed in detail here since they were not the focus of this study.

When physiological saline was in the lumen, slow waves were only seen in 1 of 12 preparations of jejunum (16.1 c/min, 0.6 mV) and were not observed at all in the duodenum (n = 12).

Within 10–15 min of the intraluminal application of decanoic acid, the basal electrical activity in the circular muscle increased in amplitude from control levels. This increased activity was present despite the fact that contractions were not yet observed. Most notably, the amplitude and number of downward deflections or IJPs increased compared with the basal activity (Fig. 4, A and B). Addition of TTX (1 μM; Fig. 4C) or apamin (300 nM; Fig. 4D) to the bath blocked this increase in inhibitory activity (n = 4 in each case). After 10–15 min of increased baseline electrical activity, a complex mixing motor pattern appeared; this consisted of stationary contractions, short-length propagating contractions, and whole-length propagating contractions as described previously (13, 14). A similar latency for the onset of inhibitory activity prior to segmentation contractions was observed with intraluminal infusion of L-phenylalanine (n = 3, jejunum) or L-tryptophan (n = 1, jejunum). However, because of the lower number of preparations using amino acids, the following results refer to activity induced by decanoic acid.

When an electrode was attached to a region where stationary contractions were occurring, an upward deflection or EJP leading to a single AP was recorded in association with each contraction...
The EJP/AP complex was completely abolished by the addition of hyoscine (1 μM) to the bath (Fig. 5C). Slow waves were never seen associated with the EJP-AP complexes underlying stationary contractions. When a recording electrode was either oral or anal to a site of stationary contractions (typically within 1–1.5 cm of the contracting region), large-amplitude IJPs were recorded time locked with the adjacent contraction (duodenum n = 7, jejunum n = 6) (Figs. 6 and 7). In some cases, pairs of extracellular recording electrodes were placed on either side of a contracting region and IJPs were recorded simultaneously both oral and anal to a segmentation contraction (3 preparations of duodenum and 2 preparations of jejunum, Fig. 7). These IJPs were abolished by apamin (300 nM, Fig. 6E). The pattern of inhibition surrounding stationary contractions was also observed in preparations where segmentation was induced by intraluminal application of the amino acids 1-phenylalanine (n = 3) or 1-tryptophan (n = 1) (not illustrated). Slow waves were never observed associated with EJP/AP complexes underlying stationary contractions induced by the amino acids.

A related phenomenon was observed with short-length anally propagating contractions. When such a slowly propagating contraction passed through an orally placed electrode but stopped short of a more anally placed electrode, an EJP/AP complex was recorded at the oral electrode and an IJP was recorded at the anal electrode. This was observed in four of eight preparations in the duodenum with decanoic acid in the lumen and in two of three preparations in the jejunum (Fig. 8). Orally propagating contractions were much rarer, and we were unable to determine whether similar IJPs were seen oral to these contractions.

When apamin was added to the bath after segmentation activity had started, the pattern of contractility was altered such that the proportion of whole length propagating contractions compared with stationary contractions was increased (duodenum n = 4, jejunum n = 4) (Fig. 9). In four preparations (2 duodenum, 2 jejunum), it was possible to compare the propagation speeds of anally propagating whole-length contractions evoked by saline distension, with decanoic acid in the lumen and in the presence of apamin. No significant differences were seen (distension 28.4 ± 3.1 mm/s, decanoic acid 28.4 ± 3.2 mm/s, decanoic acid plus apamin 30.5 ± 2.7 mm/s, n = 4, P > 0.1). Many of the whole length propagating contractions induced by apamin in the jejunum appeared first at the anal end.
Recorded slow-wave frequencies were also remarkably consistent to the bath blocked these downward deflections, indicating that they were markedly increased. Addition of TTX (1 \( \mu \)M) significantly decreased the number of contractions with decanoic acid alone was too small to allow statistical comparisons. However, the incidence of orally propagating whole-length contractions with decanoic acid alone was too small to allow statistical comparisons.

**Properties of slow waves in the guinea pig small intestine.** In dissected sheet preparations of small intestine, simultaneous intracellular and extracellular recordings of slow waves were made in the presence of nicardipine (Fig. 2). In the duodenum, jejunum, and ileum, periods of rhythmic slow-wave activity started to appear 90 min after the tissue was dissected and continuous slow-wave activity was usually present by 120 min. Once the slow-wave pattern was established within a preparation, their frequencies remained essentially constant throughout the remainder of the recording period varying by no more than 5.9% in the duodenum (range 2.2%–5.9%, mean 4.1%, \( n = 8 \)) and 6.4% in the jejunum (range 0.8%–6.4%, mean 3.6%, \( n = 12 \)). Recorded slow-wave frequencies were also remarkably consistent between preparations from the same region with medians and standard deviations of slow-wave frequencies in cycles per min (c/min) in duodenum of 19.5 (SD 1.4) c/min (range 17.1–23.0, \( n = 8 \)), in jejunum of 16.6 (SD 1.1) c/min (range 14.4–18.5, \( n = 12 \)), and in ileum of 15.7 (SD 1.1) c/min (range 13.8–17.3, \( n = 12 \)). Slow-wave frequencies were significantly lower in the jejunum (\( P < 0.001 \)) and ileum (\( P < 0.001 \)) than the duodenum but did not differ significantly between the jejunum and ileum. The amplitudes of slow waves were greatest in the jejunum and ileum, but slow waves were not observed in the duodenum unless indomethacin was present, and even then their amplitudes were still significantly smaller than seen in the other regions (duodenum 3.5 ± 0.1 mV, \( n = 8 \); jejunum 4.8 ± 0.1 mV, \( n = 12 \), \( P < 0.001 \); ileum 4.5 ± 0.2 mV, \( n = 12 \), \( P < 0.01 \)). Slow waves were rarely observed in whole segment preparations when decanoic acid was present in the lumen (1 of 12 preparations of duodenum; frequency 20.3 c/min, amplitude 0.4 mV, 2 of 12 preparations of jejunum; frequency 15.9 ± 0.3 c/min, amplitude 0.5 ± 0.02 mV) and were never seen when \( L \)-phenylalanine or \( L \)-tryptophan were present in the lumen. When they were seen, it was during periods of quiescence and their frequencies did not differ from those seen in intact preparations with saline in the lumen or in opened preparations in the presence of nicardipine.

Analysis of spatiotemporal maps produced from video recordings of segmentation induced by decanoic acid showed that when stationary contractions occurred repeatedly in the same location, they exhibited a wide range of frequencies. The median frequency in the duodenum was 10.8 (SD 4.3) c/min (range: 3.6–28.8 c/min, \( n = 30 \), 51 regions) and in the jejunum was 7.8 (SD 5.8) c/min (range: 3.0–27.0 c/min, \( n = 16 \), 62 regions). Thus frequencies of segmenting contractions induced by decanoic acid at a single location were much more variable than the tightly regulated slow-wave activity recorded in the same regions. In addition, the frequencies of some of these segmenting contractions were higher than the maximum slow-wave frequencies in these regions. Furthermore, when the segmentation frequencies were lower than the slow-wave frequencies, the intervals between the stationary contractions were not integer multiples of the intervals between slow waves in the same region. Thus the segmentation contractions were not coupled to slow waves in these preparations.

**DISCUSSION**

This study demonstrates that nutrient-induced segmentation in the guinea pig small intestine is the result of coordinated activity generated within the ENS and is independent of the intrinsic myogenic pacemaker pattern, the intestinal slow waves. Each stationary constriction is associated with a large EJP mediated by muscarinic receptors that leads to a single action potential at the site of the constriction and time-locked IJPs outside the constricted region. These apamin-sensitive IJPs were clearly important, because blocking them converted many stationary constrictions into the propagating constrictions characteristic of peristalsis.

**Recording electrical activity in the circular muscle.** The conclusions of this study critically depend on the reliability of the extracellular recording method for determining the electrical activity of the circular muscle. In particular, previous
studies have found it difficult to record slow waves in the guinea pig small intestine and when they have been recorded they have always been of small amplitude (18, 23). Thus it was important to determine whether the suction electrode method, which allows correlation of electrical with contractile activity in the muscle, had sufficient resolution to detect the slow waves. To address this issue, simultaneous recordings were made with an intracellular and an extracellular electrode from electrotonically coupled sites in opened segments from each small intestinal region. Previous studies indicate that the amplitudes of responses to neural activity would be expected to be identical at the two recording sites (4). We found that the extracellular electrodes were capable of resolving signals that were less than 2 mV in amplitude when recorded with an intracellular electrode. Furthermore, the extracellular electrodes recorded reflexly evoked IJPs with identical time courses to those recorded by intracellular electrodes. This indicates that the suction electrodes were predominantly recording activity in the circular muscle despite being placed on the serosa, because IJPs are not recorded in the longitudinal muscle in response to activation of descending reflexes (17).

A second key question relating to the suction electrodes is whether they modify ongoing motility patterns by themselves. This would interfere with any correlations between nutrient-induced motility patterns and the electrical activity in the muscle. However, it can be seen from Fig. 9 in particular that the suction electrodes have no impact on the motility either by initiating motility patterns at the site of recording or by interrupting propagating patterns that pass this site.

**Neural mechanisms responsible for segmentation.** This study indicates that segmentation in the guinea pig small intestine is due to activity in an enteric neural circuit that produces coordinated firing of excitatory motor neurons innervating the sites of the rhythmic constrictions. This leads to substantial depolarizations of the circular muscle via muscarinic receptors. Each depolarization typically evokes only a single smooth muscle action potential, which contrasts with the depolarizations associated with the propulsive contractions evoked by distension, each of which triggers two or more action potentials. This is consistent with earlier observations indicating that segmentation contractions are less powerful than propulsive contractions (14). On either side of the local-
ized activity of excitatory motor neurons, there is a region in
which firing of inhibitory motor neurons is enhanced, mani-
ifesting as large IJPs adjacent to the constricted region and time
locked to the constrictions. Because both the excitatory and the
inhibitory motor neurons are located within the myenteric
plexus (6), major elements of the neural circuit mediating
segmentation are located within this plexus.

The surrounding IJPs are clearly significant, because block-
ing them with apamin converts predominantly stationary con-
strictions to orally or anally propagating whole-length contrac-
tions. Apamin blocks small-conductance calcium-activated po-
tassium channels activated by ATP, probably via P2Y1
receptors (10, 12) and hence abolishes IJPs but has no effect on
the relaxations mediated by nitric oxide released by the same
inhibitory motor neurons (5). This difference is important in
the regulation of motility, because blockade of nitric oxide
synthesis reduces receptive relaxation of the guinea pig ileum
and lowers the threshold for initiation of propulsive contrac-
tions by saline distension (30, 31). By contrast, apamin has no
effect on receptive relaxation or peristalsis threshold in this
preparation, although it does cause an increase in the magni-
tude of the propulsive contractions (30, 31). This suggests that
the increase in the number of whole-length propagating con-
tractions was not due to a change in the threshold pressure
required to evoke such contractions. Rather it appears that
the propagation of activity within the enteric circuitry is enhanced
in the absence of inhibitory input to the muscle, at least under
conditions where the excitatory drive to the muscle is relatively
weak. IJPs on the anal side of the short slowly propagating
contractions also seen during segmentation may be responsible
for both their inability to propagate over the whole length of
the segment and for their slow propagation speed. Spencer
et al. (25) showed that anally propagating contractions evoked
by either local distension or mechanical stimulation of the
mucosa were markedly enhanced in both strength and speed by
apamin (25). Taken together these observations suggest that
the major role of the apamin sensitive IJPs is to slow or suppress propagation of motor activity, and hence of intestinal contents, rather than to facilitate it as is often assumed.

Although the neural circuit controlling segmentation produces episodes of local excitation and an inhibitory surround, this is superimposed on a background of small, high-frequency IJPs. Thus, in both the regions along the segment between stationary contractions and the quiescent periods between episodic bursts of contractions, contractile activity is depressed. This tonic inhibitory drive may be partially responsible for the absence of recognizable slow waves in the vast majority of preparations (24).

The tonic inhibitory drive to the circular muscle does not appear immediately after exposure to either decanoic acid or the amino acids. Rather it takes 10–15 min for this activity to appear and another 10–15 min for the full segmentation pattern to manifest itself. This slow onset suggests a slow buildup of activity within the enteric neural circuitry, which may be a clue to the mechanism responsible for the overall neural pattern. The oscillations in inhibitory activity postulated to explain the

![Inhibitory junction potentials were recorded simultaneously both oral and anal to isolated segmentation contractions. A: single frame from a video recording of segmentation activity in a jejunal segment (Jej) with L-phenylalanine (50 mM) in the lumen. D: similar image of a duodenal segment (Duo) with decanoic acid in the lumen. Each image shows a stationary contraction (white arrows) occurring between 2 electrodes placed orally and anally. B and C: single IJPs recorded simultaneously at the oral and anal electrodes, time locked with the contraction in the jejunum. E and F: the IJPs recorded simultaneously oral and anal to the segmentation contraction in the duodenum.](http://ajpgi.physiology.org/)
Periodic stationary contractions are characteristic of the recurrent feedback circuits formed by the intrinsic sensory neurons of the myenteric plexus (27). These neurons are chemosensitive and communicate with each other via slow excitatory postsynaptic potentials (3). Oscillations are seen when the characteristic afterhyperpolarizing potentials of these neurons are completely suppressed, which occurs during, and after, very-low-frequency stimulation of their synaptic inputs (1, 9). Thus the time course of the onset of segmentation activity may reflect low-frequency, asynchronous activation of many chemosensitive neurons supplying the mucosa, all feeding into a widely distributed recurrent feedback circuit. What leads to the

Fig. 8. IJPs were recorded ahead of an anally propagating contraction that did not reach the anally placed electrode. A: STM of mixing activity induced by decanoic acid in a jejunal segment. The white box surrounds a short anally propagating contraction that is initiated at the oral electrode but stops before reaching the anal electrode. B: still image of this contraction (white arrow) taken from the video recording. C and D: extracellular recordings from the 2 electrodes at the time of the contraction. An EJP-AP complex (arrow) is seen at the oral electrode and an IJP (arrow) is recorded simultaneously at the anal electrode.
differential appearance of excitation and inhibition at different sites has not yet been identified, but future modeling may indicate relevant mechanisms.

Segmentation is independent of slow-wave activity. The results of this study provide direct support for the inference drawn from our previous study (14) that slow waves do not have a role in either generation or timing of segmentation contractions in guinea pig duodenum or jejunum. The primary evidence for this is simply that the stationary constrictions characterized as segmentation were not associated with slow waves. Rather these depolarizations were abolished by hyoscine, indicating that they were excitatory junction potentials mediated by acetylcholine acting at muscarinic receptors. It is important to note here that although there is good evidence that cholinergic neuromuscular transmission in the gut occurs via intramuscular ICC acting as mediators (29), the pacemaking interstitial ICC associated with the myenteric plexus are not normally innervated (21). Indeed, slow waves were not seen at contraction sites during the quiescent periods between episodes of contractile activity and were rare in the relaxed regions between stationary constrictions. This contrasts with the conclusions of Donnelly et al. (11), who reported that prolonged saline distension of the upper small intestine evokes slow-wave activity sufficient to produce bursts of action potentials.

Both the stimuli used here and the distensions used by Donnelly et al. (11) were distributed along the intestine and maintained for substantial periods of time. The motor activity that they produced presumably involved the same final motor neurons. However, the onset of the activity was substantially different with distension activating the propulsive motility pattern much earlier than the chemical stimuli-evoked segmentation. A more rapid onset may reflect a more vigorous activation of the enteric neural circuitry and hence a different behavior of the recurrent circuits responsible for the segmentation activity. In particular, the modeling studies of these circuits indicate that if the afterhyperpolarizing potentials in these neurons are suppressed, but not abolished, the neurons tend to fire in a continuous, rather than oscillatory, fashion (26). This in turn would lead to tonic activation and continuous exposure of the smooth muscle and interstitial cells of Cajal to acetylcholine as postulated by Donnelly et al. (11).

Whatever the explanation for the differences between our conclusions and those of Donnelly et al. (11), analysis of the frequencies of the stationary constrictions in the spatiotemporal maps provides further evidence that slow waves are not involved. Stationary contractions varied markedly in frequency at different sites within and between preparations, whereas the slow waves recorded intracellularly from opened preparations were very regular. This might be due to variable excitability of the muscle resulting in only some slow waves generating the action potentials required for circular muscle constriction. However, the highest frequency contractions were up to 30% more frequent than slow waves recorded in either the duodenum or jejunum, which is impossible if the rhythmicity is due to slow waves. Furthermore, the intervals between segmentation constrictions were not integer multiples of those between the slow waves in the same segments, a necessary condition if slow waves set the period of the rhythmic segmentation contractions.

A final comment. Although slow waves clearly play little or no role in the timing of segmentation contractions in the guinea pig small intestine, this does not mean that they are not important in other species where their amplitudes are much larger. The guinea pig appears to be a special case in which the neurally generated activity producing localized constrictions with an inhibitory surround can be clearly identified. In other species, the output of this neural circuit would be superimposed on the slow-wave activity so that the final motility...
pattern in the fed state would be a result of both. Identifying this final pattern and the contributions of each component will be an important challenge for the future.

GRANTS

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