The myogenic component in distention-induced peristalsis in the guinea pig small intestine

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Donnelly, Graeme, Timothy D. Jackson, Krista Ambrous, Jing Ye, Adee Safdar, Laura Farraway, and Jan D. Huizinga. The myogenic component in distention-induced peristalsis in the guinea pig small intestine. Am J Physiol Gastrointest Liver Physiol 280: G491–G500, 2001.—In an in vitro model for distention-induced peristalsis in the guinea pig small intestine, the electrical activity, intraluminal pressure, and outflow of contents were studied simultaneously to search for evidence of myogenic control activity. Intraluminal distention induced periods of nifedipine-sensitive slow wave activity with superimposed action potentials, alternating with periods of quiescence. Slow waves and associated high intraluminal pressure transients propagated aborally, causing outflow of content. In the proximal small intestine, a frequency gradient of distention-induced slow waves was observed, with a frequency of 19 cycles/min in the first 1 cm and 11 cycles/min 10 cm distally. Intracellular recording revealed that the guinea pig small intestinal musculature, in response to carbachol, generated slow waves with superimposed action potentials, both sensitive to nifedipine. These slow waves also exhibited a frequency gradient. In addition, distention and cholinergic stimulation induced high-frequency membrane potential oscillations (~55 cycles/min) that were not associated with distention-induced peristalsis. Continuous distention produced excitation of the musculature, in part neurally mediated, that resulted in periodic occurrence of bursts of distally propagating nifedipine-sensitive slow waves with superimposed action potentials associated with propagating intraluminal pressure waves that caused pulsatile outflow of content at the slow wave frequency.

Gastrointestinal motility; interstitial cells of Cajal; neural control; myogenic control; slow waves; frequency gradient.

Peristalsis is a complex motor pattern involving cooperation between neural and myogenic mechanisms (6, 8, 13, 36). The guinea pig small intestine has been intensively studied as a model for peristalsis, focused on neural control mechanisms (7, 49). Peristaltic propulsion is often seen to be due to a reflex whereby excitatory motor neurons contract a segment of circular muscle while the segment aboral to the contracted segment is simultaneously relaxed by inhibitory neurons (46). This hypothesis requires “programmed neural circuits” to induce these sequential contractions (48). There is little doubt that enteric neurons can perform this reflex of ascending excitation and descending inhibition. However, whether or not such a neuronal reflex solely or primarily causes most motor patterns in vivo is questionable. Tonini et al. (42) suggest that initiation of peristalsis via ascending excitatory reflexes may be rare, since this reflex requires rapid-onset stimuli that may never occur during the normal passage of liquid contents in the small intestine. The ascending excitatory reflex can be dissociated from the initiation of peristalsis in the guinea pig small intestine (42), demonstrating that, although neural components are necessary, additional mechanisms, possibly myogenic, must be involved. Brookes et al. (4) have provided further evidence for an integrative role of smooth muscle cells, indicating that peristalsis does not simply represent the output of neural circuitry.

Increased interest in the myogenic component of motor control has come from recent evidence that interstitial cells of Cajal (ICC) generate gastrointestinal pacemaker activity (23, 40). This has led to the hypothesis that, in the small intestine, the major function of the ICC associated with Auerbach’s plexus is the initiation of slow wave activity (16, 37, 45). Also, in the human, one of the major components of intestinal motor control is the presence of electrical slow waves (6), and abnormal ICC networks are now being implicated in human intestinal motor abnormalities (19, 21). The guinea pig small intestine has two networks of ICC that, at both the light microscopic and electron microscopy levels, are very similar to their equivalents in the mouse, dog, and human (20, 26, 27, 41, 51). However, the presence of slow waves in the guinea pig small intestine has been in question for many years. Kuriyama et al. (29, 30) showed that the guinea pig longitudinal muscle generates action potentials, either irregularly, in discrete bursts, or in a continuous manner (29, 30). The occurrence of a “slow” component was not common but increased on pharmacological stimulation. Slow components were always associated with action potentials and were abolished in the presence of...
TTX. These early studies clearly demonstrated that in the guinea pig slow waves are not omnipresent as in the small intestine of other species. With extracellular field stimulation, single slow depolarizations could be evoked and, usually, action potentials developed on their crests. These were generated through activation of cholinergic nerves since they were blocked by atropine (18). Bolton (1) noted that in the presence of acetylcholine or carbachol the slow component became more pronounced and could carry a burst of action potentials (1, 2). These slow waves could obtain a relatively constant frequency between 12 and 30 cycles/min. Recently, it has become apparent that muscarinic stimulation can evoke oscillatory activity in single, isolated smooth muscle cells (25). Such oscillatory activity is blocked by ryanodine and heparin, suggesting involvement of intracellular calcium stores (24, 25). Although these individual studies point to one or more types of electrical oscillatory activity occurring in the guinea pig small intestine, it is not clear how the activity from single cells relates to activity recorded from tissue or how activity generated in tissue relates to patterns of motor activity in intact segments of the intestine. The nature of these slow components remains unclear and has not been studied in relation to distention-induced neural activity.

The objective of the present study was to explore the evidence for a role of myogenic activity in the control of peristalsis. This was accomplished through a study combining electrical, mechanical, and flow measurements in the guinea pig intestine using a recently developed model of distention-induced peristalsis. For supporting evidence, intracellular recordings were obtained of spontaneous and induced electrical activities. These data were presented in abstract form at the International Motility Society meeting in Brugge, Belgium in 1999 (14).

METHODS

Male guinea pigs (Charles River Laboratories) weighing 250–300 g were killed by inhalation of CO2 gas in an enclosed container. The small intestine was exposed by a midline abdominal incision, and a 10-cm tubular segment of intestine was removed of spontaneous and induced electrical activities. The small intestine was exposed by a midline abdominal incision in 1999 (14).

RESULTS

Distention-Induced Activity

When a 6-cm segment from the proximal small intestine of the guinea pig was cut out and immediately placed in an organ bath, the musculature was found not to generate any type of spontaneous electrical ac-
activity, in contrast to intestines of other species. However, when 2–3 cmH\textsubscript{2}O intraluminal pressure was applied, slow waves with superimposed action potentials developed. In this example, the burst duration was 1.4 ± 0.1 min and the interburst quiescent periods lasted 0.9 ± 0.4 min. Slow waves occurred synchronized at the three recording sites (1.0 cm between each site; top three traces). A slow wave with superimposed action potentials was associated with a transient increase in intraluminal pressure, here recorded underneath the second electrode (bottom trace). The amplitude of the intraluminal pressure was related to the number and amplitude of the action potentials generated. Slow waves propagated at 1.2 cm/s. The transient increases in intraluminal pressure were associated with visible ring indentations in the intestinal segment due to circular muscle contraction, and these indentations were seen to propagate aborally at the same velocity. Each propagating event was associated with outflow of fluid (a). The outflow was pulsatile at exactly the slow wave frequency.

Fig. 1. Distention-induced peristalsis: relationship between slow waves, action potentials, intraluminal pressure, and outflow. On distention, evoked by input of H\textsubscript{2}O in a 6-cm segment of proximal small intestine to an intraluminal pressure of 3.5 cmH\textsubscript{2}O, bursts of slow waves with superimposed action potentials developed. In this example, the burst duration was 1.4 ± 0.1 min and the interburst quiescent periods lasted 0.9 ± 0.4 min. Slow waves occurred synchronized at the three recording sites (1.0 cm between each site; top three traces). A slow wave with superimposed action potentials was associated with a transient increase in intraluminal pressure, here recorded underneath the second electrode (bottom trace). The amplitude of the intraluminal pressure was related to the number and amplitude of the action potentials generated. Slow waves propagated at 1.2 cm/s. The transient increases in intraluminal pressure were associated with visible ring indentations in the intestinal segment due to circular muscle contraction, and these indentations were seen to propagate aborally at the same velocity. Each propagating event was associated with outflow of fluid (a). The outflow was pulsatile at exactly the slow wave frequency.

Fig. 2. Distention-induced slow waves in 2 different experiments (A and B). The extracellular recordings were obtained using AC amplification that gives some variability in the shape of the compound slow waves recorded. The intestinal segments were quiescent without stimulation, and the slow waves with superimposed action potentials were evoked by 2.5 cmH\textsubscript{2}O intraluminal pressure. In each experiment, slow waves (top traces) were correlated 1:1 with transient increases in intraluminal pressure (bottom traces) caused by local ring contractions of circular muscle, resulting in outflow of content.

slow waves and transient intraluminal pressure changes. Occasionally, fast oscillations were also observed at a frequency of 54 ± 3 cycles/min (Fig. 4). These were not associated with increases in intraluminal pressure or outflow.
To investigate the possibility that the aboral propagation of the induced slow waves was due to an underlying frequency gradient, similar to that of classic slow waves (10, 39), the frequency of induced slow waves in the most proximal 3-cm section was compared with a more distal section starting at 11 cm distal to the pylorus. Both segments were studied with an identical intraluminal pressure of 3 cmH$_2$O. The slow wave frequency within the bursts in the proximal segment was 18.6 ± 4.0 cycles/min, and in the more distal segment it was 11.0 ± 0.7 cycles/min. This frequency gradient was observed in each segment studied, with the frequency of slow waves in the more distal segment being significantly lower ($n = 5; P < 0.001$).

To investigate the role of NO in mediating the quiescent periods, L-NAME was added to preparations under conditions of regular periodic activity at intraluminal pressures between 2 and 3 cmH$_2$O. Examining a 20-min period of activity, 3.6 ± 0.2 periods of quiescence occurred for an average duration of 2.7 ± 0.6 min, which is 53 ± 14% of total time ($n = 6$). After addition of L-NAME (4 mM), the slow waves with superimposed action potentials occurred continuously, with quiescent periods only occurring in one preparation for 10% of the time in the 20-min period following L-NAME.

To investigate a cholinergic component in the distention-induced activity, the action of atropine was studied ($n = 15$). The effect of atropine ($2 \times 10^{-6}$ M) was quite variable between preparations and over time. In eight preparations, activity was abolished, although in three of these preparations some activity appeared again after 10–20 min. In seven preparations, regular activity remained after addition of atropine at unchanged frequency but at a 38 ± 12% reduced amplitude (Fig. 5).

The effect of TTX ($5 \times 10^{-7}$M) was also variable ($n = 15$). In most preparations activity disappeared or some activity remained at 15 ± 10% of the original amplitude in a nonbursting manner. In five preparations, activity remained at regular frequency (Fig. 5).

All activity was always abolished by the L-type calcium channel blocker nifedipine ($10^{-6}$M; $n = 10$) as well as by the removal of distention ($n = 10$). In the presence of CPA ($10^{-6}$M; $n = 5$), the slow wave frequency was 10.1 ± 1.1 cycles/min compared with 9.8 ± 1.2 cycles/min before the addition of CPA ($P > 0.05$; no significant difference).

Because of the sensitivity of the induced activity to atropine, the hypothesis that pharmacological stimulation of muscarinic receptors could mimic this activity was tested. First, the segments were subjected to stretch to evoke the regular burst type activity. In those preparations in which the activity was inhibited by TTX ($5 \times 10^{-7}$M), the effect of carbachol ($10^{-7}–10^{-5}$ M) was studied. Carbachol induced a variety of patterns of electrical activity: fast oscillations without superimposed spikes at 69.0 ± 10.5 cycles/min, variable action potential activity, and periodic appearance of slow waves with superimposed action potentials at a slow wave frequency of 24.7 ± 4.8 cycles/min. Carbachol, under these conditions, did not evoke a regular pattern of propagating slow waves.

To test the hypothesis that stretch alone can evoke electrical activity in the musculature, the segments...
were first subjected to distention to evoke the regular burst type activity. After inhibition of activity by both TTX (5 × 10⁻⁷ M) and atropine (10⁻⁶ M), increasing stretch to 3 cmH₂O evoked slow waves with superimposed spikes that were synchronized over the three electrodes at a frequency of 12.1 ± 2.1 cycles/min (n = 5).

To investigate whether the slow waves were a unique effect of muscarinic stimulation or stretch, effects of another excitatory stimulus were studied. Barium chloride excites the musculature through blockade of potassium conductance, and stimulation of calcium conductances has also been used to simulate distention in vitro (5). In the presence of TTX and atropine, barium chloride evoked slow waves with superimposed action potentials, similar to those evoked by distention, which were synchronized over the three recording electrodes. In tissues with distention-induced slow waves at a frequency of 17.5 ± 2 cycles/min, the frequency of slow waves after addition of barium chloride was 16 ± 3 cycles/min (n = 3).

**Intracellular Electrical Activity**

Recording of intracellular electrical activity in isolated circular muscle from the guinea pig small intestine did not reveal regular slow wave activity as seen in intestinal tissues from other species (17, 39). Whereas electrical quiescence was most prominent, two types of electrical activity were encountered without pharmacological stimulation: action potentials with or without some type of underlying slow component and fast membrane potential oscillations (Fig. 6). When sufficient stretch was applied to the tissues, bursts of action potentials occurred spontaneously. Action potentials consisted of a relatively slowly developing prepotential followed by a fast spike. The prepotential had a rate of rise of 12.0 ± 2.1 mV/s, a duration of 1.1 ± 0.3 s, and an amplitude of 4.6 ± 1.0 mV (n = 12). The fast spikes had a rate of rise of 315 ± 33 mV/s, a duration of 0.08 ±
0.02 s, and an amplitude of 17.3 ± 1.3 mV. The frequency of the action potentials was 126 ± 13 cycles/min within the burst, and the amplitude was 20 ± 1.2 mV. Bursts typically lasted ~25 s, with a mean interburst quiescent period of ~50 s. The resting membrane potential was −55.7 ± 2.5 mV. In addition to bursts of action potentials, membrane potential oscillations occurred with a frequency and amplitude of 55 ± 2 cycles/min and 4.2 ± 0.6 mV, respectively. When action potential activity appeared, these membrane potential oscillations disappeared (Fig. 6A).

In response to carbachol (5 × 10⁻⁷ M), smooth muscle cells depolarized from −59.3 ± 1.0 mV to −44.0 ± 2.3 mV (n = 19; P = 0.003). In addition, action potentials developed at a frequency of 158.4 ± 11.9 cycles/min and an amplitude of 19.6 ± 0.5 mV (n = 6) (Figs. 6–8). Within 5–15 min, slow waves in membrane potential developed, resulting in action potentials occurring only on their crest at 244 ± 14 cycles/min (Figs. 6 and 7). Hence, continuous action potential generation changed into distinct slow wave-action potential complexes alternating with periods of electrical quiescence. The slow waves occurred at a frequency of 34.3 ± 7.4 cycles/min (range 18–60, but 80% between 20 and 40 cycles/min), a duration of 1.2 ± 0.2 s, and an amplitude of 13.4 ± 3.3 mV (n = 16) in proximal tissue (between 1 and 3 cm distal to the pylorus). In tissue taken between 11 and 15 cm distal to the pylorus, the slow wave occurred at a significantly lower frequency of 10.3 ± 1.6 cycles/min (range 3.6–15) (P < 0.05), a duration of 1.8 ± 0.2 s, and an amplitude of 15.6 ± 1.9 mV (n = 6). Nifedipine (10⁻⁶ M) abolished the slow wave-action potential complexes with a decrease in frequency and amplitude until loss of electrical activity (Fig. 8A). CPA reduced the slow wave amplitude to 6.2 ± 0.3 mV and also affected the frequency, lowering it from 34.3 ± 7.4 to 24.9 ± 1.9 cycles/min (Fig. 8B).

Carbachol also evoked regular membrane potential oscillations at a much higher frequency without action potentials (n = 8; Fig. 6B and Fig. 9). The frequency was 55 ± 2 cycles/min, and the amplitude was 4.4 ± 1.5 mV. The frequency of these oscillations was similar to that of the corresponding oscillations observed as spontaneous activity. The carbachol-induced fast oscillations were abolished by nifedipine (10⁻⁶ M). In response to CPA (10⁻⁶ M), the oscillation frequency reduced progressively until loss of electrical activity occurred (Fig. 9). After washout of CPA, electrical activity did not recover to previous levels. This regular oscillatory activity was disrupted in time by the development of action potentials in most cases.

Development of slow waves was not unique to stimulation of muscarinic receptors. Barium chloride (0.5 mM) evoked bursts of action potentials as well as continuous action potential activity (Fig. 10). Bursts of
action potentials occurred at variable intervals similar to spontaneous bursts, with the mean duration between bursts being 56.9 ± 8.0 s and the bursts lasting for 23.0 ± 3.3 s (n = 6). The frequency of continuous action potentials was variable at 186 ± 44 cycles/min, whereas frequency of action potentials within a burst was more stable at 87 ± 15 cycles/min. In the presence of barium chloride, slow waves developed, leading to the action potentials occurring as bursts superimposed on the slow waves (Fig. 10). The frequency of the slow waves was 8 cycles/min, with a rate of rise of 4.5 ± 0.6 mV/cm, an amplitude of 12.7 ± 1.1 mV, and a duration of 4.7 ± 0.6 s (n = 3).

Stretch alone could also evoke slow wave activity in the presence of TTX (5 × 10^{-7} M) and atropine (10^{-6} M). Stretch levels well above those normally applied, and possibly outside of the physiological range, were needed. Nevertheless, action potential activity developed with underlying slow wave activity at 59.1 ± 2.5 cycles/min at the most proximal site and 16.2 ± 2.1 cycles/min 15 cm distal to the pylorus (n = 7).

DISCUSSION

Continuous distention of the proximal small intestine evoked periods of rhythmic, aborally propagating slow wave activity. Each propagating slow wave was associated 1:1 with an aborally propagating contraction. This led to outflow of intestinal content that was pulsatile, occurring at the slow wave frequency. This distention-induced activity was in part mediated by distention-evoked activity of enteric nerves. Cholinergic nerves played an important role in the excitation of the musculature, and nitrergic nerves played a crucial role in the induction of the periods of quiescence, thereby determining, at least in part, the frequency of the periods of excitation. The slow waves were almost always associated with superimposed action potentials. Interestingly, when sections of proximal and more distal intestine were studied separately, a much higher slow wave frequency occurred in the duodenum compared with more distal sites. Hence, a unique characteristic of the slow waves generated by the guinea pig small intestine compared with small intestines of other species is their dependence on a stimulus, e.g., distention. This study demonstrates the interaction between myogenic and neural components of motility control in the guinea pig small intestine.

Slow waves evoked in a segment of intestine by distention were abolished by nifedipine. Similarly, slow waves evoked by muscarinic stimulation, barium chloride, or stretch in tissue studied with intracellular electrodes were also nifedipine sensitive. These induced slow waves exhibited an intrinsic frequency gradient. Hence distention can induce slow wave activity that propagates aborally, probably because of the intrinsic frequency gradient. This provides an explanation for the consistent aboral propagation of the slow waves. During excitation, the proximally-induced slow waves will lead and pace more distal sections, with resulting aboral propagation (10). The propagation velocity was between 0.8 and 2.5 cm/s, in the same range as propagation associated with classic slow waves.

The slow waves in membrane potential observed in the guinea pig small intestine that were associated with propagating contractions were named slow waves. This is consistent with nomenclature used by Bolton (1) in reference to spontaneous and cholinergically induced activity in the guinea pig ileum and by Kuriyama et al. (29) in a study showing in vitro activity in the longitudinal muscle of the guinea pig jejunum.
These slow waves are in several ways distinct from classic slow waves occurring in the small intestine of other species (39). First, they are not present in the absence of stimulation; second, they are sensitive to nifedipine and hyperpolarizing agents; third, they can occur periodically; and finally, they occur in a wide range of frequencies. However, the present study shows that they are functionally very similar to the classic slow waves with respect to their role in slow wave-directed peristalsis (9), in particular the presence of an intrinsic frequency gradient. Induced slow waves are also found in other organs, notably the human colon (12, 15) and the esophagus (34). Hence the exploration of the guinea pig ileum may have importance for our understanding of other tissues that rely on stimulus-induced slow waves (35).

Continuous distention of the intestine initiated a pattern of neural excitation and inhibition. The entire segment under study was alternately excited through excitatory nerves and inhibited through nitric oxide-synthesizing nerves. The excitation, however, is not a simple excitation by cholinergic nerves. First, the effect of atropine was variable, with near normal activity possible in its presence, and second, the regular propagating slow wave activity could not be easily mimicked by the addition of carbachol. On the basis of the current data, distention-induced excitation is probably mediated by a combination of cholinergic and other excitatory nerves as well as direct effects of stretch on the musculature. Another candidate for mediating excitation is substance P, since activation of NK₁ receptors can stimulate peristaltic contractions in the guinea pig (38). The “clock” determining the rhythm of the bursting activity, i.e., a few minutes of activity alternating with a few minutes of quiescence, likely resides in the enteric nervous system with an apparent large role of nitrergic nerves. This is similar to patterns of activity observed in other gut organs such as the mouse colon (31) and the cat ileum (47). Should this neural excitation be referred to as a reflex? Costa and co-workers (42, 46) have studied reflex activity on distention in the guinea pig ileum. After gradual distention, one wave of peristalsis is observed, whereupon the experiment is terminated. Had the experiment not been terminated, rhythmic patterns of activity would likely have developed that were similar to those observed in the present study. They concluded that although ascending excitation develops on distention of a segment of intestine, the neural activity involved in generating peristalsis is more than just a reflex. Indeed, distention evokes a positive feedback mechanism, resulting in the recruitment of a large number of excitatory motor neurons to initiate peristalsis (28, 42). Repetitive firing of Dogiel type II sensory neurons will excite other AH neurons, which will then lead to massive synchronous activation of motor neurons. The present study shows that this will lead to muscular excitation of an entire segment in which the evoked slow waves cause the propagating nature of the contractions.

Is the initiation of the slow waves in the guinea pig small intestine participating in slow wave-driven peristalsis “simply” due to (neuronal) stimulation of smooth muscle or do ICC play a role? Kohda et al. (24) showed that single smooth muscle cells can generate membrane potential oscillations in response to muscarinic stimulation, mediated by inositol 1,4,5-trisphosphate-induced calcium release. This activity was very sensitive to blockade of calcium pumps of the sarcoplasmic reticulum by CPA, although the sensitivity to L-type calcium channel blockers was not studied. In single cells at room temperature, this activity reached a frequency of 8 cycles/min. In tissue at 37°C, Bolton recorded such activity at ~50 cycles/min. This activity is similar to the membrane potential oscillations we observed at ~55 cycles/min throughout the proximal and middle small intestine. CPA abolished this activity. Since both the carbachol-induced slow waves with superimposed action potentials and the distention-induced slow waves are relatively insensitive to CPA with respect to frequency, we propose that the fast oscillatory activity is not related to slow wave-induced peristalsis. This is confirmed by our observation that the fast oscillatory activity did not induce intraluminal pressure changes (Fig. 4). The fast oscillatory activity is likely related to spontaneous or carbachol-induced cyclic calcium release from smooth muscle sarcoplasmic reticulum. There is also no evidence at the moment that the smooth muscle-derived oscillations display a frequency gradient comparing different parts of the intestine.

Of importance is our observation that the slow waves show an intrinsic frequency gradient. Since ICC have been shown to generate the classic slow waves in the small intestine of other species, it is assumed that the frequency gradient of these slow waves is ultimately due to properties of ICC. We speculate that the presence of a frequency gradient in the induced slow waves of the guinea pig small intestine is at least in part due to stimulation of ICC, likely by direct stretch with or without neural involvement. Direct innervation of ICC by cholinergic nerves was recently demonstrated by Ward et al. (43). These induced slow waves propagate in an aboral direction because of the presence of a slow wave frequency gradient. There are several observations consistent with this hypothesis, particularly in comparing the slow waves observed in the guinea pig with those found after cholinergic stimulation in the W mutant mouse that does not have ICC associated with Auerbach’s plexus (16, 45). The slow wave-like activity in the W mutant mouse occurs in a wider range of frequencies and does not have unidirectional propagation (13, 32, 33, 44). In addition, we observed that the generation of propagating slow wave activity could also be achieved without neural activity. Pharmacological stimulation of muscle with barium chloride evokes such activity as well as distention alone in the presence of TTX and atropine. Second, we observed that after distention-induced activity was abolished by atropine and TTX, further distention could evoke propagating slow wave activity, indicating that stretch can evoke
the slow waves through direct action on ICC and/or smooth muscle cells. Hence there are myogenic mechanisms that can evoke propagating slow waves. This leads us to put forward the hypothesis that stimulation of the musculature, including ICC, evokes aborally propagating slow waves due to induction of slow waves at different frequencies, with the highest frequency at the most proximal site.

Whereas single ICC in culture from the mouse small intestine generate spontaneous slow wave activity (23, 40), cultured ICC from the guinea pig do not appear to have intrinsic slow wave activity. Espinosa-Luna et al. (11) demonstrated that small clumps of cells, which included both smooth muscle cells and ICC, never showed slow wave activity. A larger grouping of cells appeared to be required to generate slow wave activity, suggesting that a critical mass of both smooth muscle and ICC may be needed. They did not study whether slow waves could be evoked by cholinergergic stimulation (11). Interestingly, the slow waves that were generated after 8 days in a relatively large clump of cells were nifedipine insensitive, unlike the slow waves that are generated in the guinea pig under more physiological conditions, as reported in this study. The fact that these slow waves in culture could also be insensitive to removal of extracellular calcium makes them different from most slow wave activity studied thus far. Nevertheless, these cultures may be ideal for elucidating the genetic difference(s) between nifedipine-sensitive and -insensitive slow wave activity.

What initiates a peristaltic wave? When distention is gradually applied, electrical activity develops after a certain threshold is reached, and at a critical point, a peristaltic contraction develops (42). Brookes et al. (3) showed that the peristaltic contraction is associated with an electrical oscillation with superimposed spikes and called this electrical oscillation an excitatory junction potential (EJP) (3). The interpretation was that ascending excitation would drive the peristalsis. However, although ascending excitation may accompany peristalsis, it is not the driving force as shown by Costa and co-workers (42). In the experiments by Brookes et al. (3), after the first peristaltic wave was observed the experiment was terminated. Were the experiments continued, the experimental conditions would have been similar to ours. Hence the EJP observed could, in our interpretation, actually be a slow wave accompanying the peristaltic contraction. With continuous distention, periods of neural inhibition alternate with periods of neural excitation. When the phase of neural excitation starts, often some irregular action potential activities develop, and thereafter slow wave activity starts to develop (Fig. 3). Only with the first propagating slow wave does a peristaltic contraction occur. Hence the peristaltic wave is initiated when (neural) excitation of the musculature evokes a propagating slow wave.

In summary, in the guinea pig small intestine, slow wave activity only occurs after stretch/distention or pharmacological stimulation. Distention of a segment of intestine induces rhythmic, propagating slow wave activity that is associated with propulsive contractile activity at the slow wave frequency.

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