Myogenic mechanism for peristalsis in the cat esophagus

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Myogenic mechanism for peristalsis in the cat esophagus. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G306–G313, 1999.—A myogenic control system (MCS) is a fundamental determinant of peristalsis in the stomach, small bowel, and colon. In the esophagus, attention has focused on neuronal control, the potential for a MCS receiving less attention. The myogenic properties of the cat esophagus were studied in vitro with and without nerves blocked by 1 μM TTX. Muscle contraction was recorded, while electrical activity was monitored by suction electrodes. Spontaneous, nonperistaltic, electrical, and mechanical activity was seen in the longitudinal muscle and persisted after TTX. Spontaneous circular muscle activity was minimal, and peristalsis was not observed without pharmacological activation. Direct electrical stimulation (ES) in the presence of bethanechol or tetraethylammonium chloride (TEA) produced slow-wave oscillations and spike potentials accompanying smooth muscle contraction that progressed along the esophagus. Increased concentrations of either drug in the presence of TTX produced slow waves and spike discharges, accompanied by peristalsis in 5 of 8 TEA- and 2 of 11 bethanechol-stimulated preparations without ES. Depolarization of the muscle by increasing K+ concentration also produced slow waves but no peristalsis. We conclude that the MCS in the esophagus requires specific activation and is manifest by slow-wave oscillations of the membrane potential, which appear to be necessary, but are not sufficient for myogenic peristalsis. In vivo, additional control mechanisms are likely supplied by nerves.

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tions were studied and they are shown schematically in Fig. 1. The preparations were allowed to equilibrate for 1 h before beginning the experiment.

Preparation A. After the esophagus was dissected free of surrounding fascia, it was inverted onto itself, and the entire mucosa was removed by sharp dissection. After the esophagus was returned to its in situ length, circular side out, it was fixed with pins at both ends to the bottom of the bath (Fig. 1A). Circular muscle contractions were monitored by a manometric tube placed intraluminally with three ports situated 1, 2, and 3 cm proximal to the junction of the gastric and esophageal mucosa. The lumens were perfused (0.3 ml/min per lumen) with Krebs solution by low-compliance pressurized infusion pump (Mui Scientific, Mississauga, ON). Longitudinal muscle contractions were recorded by a single isometric force transducer (model FT.03c; Grass Instruments, Quincy, MA), fastened via a lever to a silk suture tied at the gastroesophageal junction.

Preparation B. The esophagus was dissected free of surrounding fascia and opened lengthwise, and the entire mucosa was removed by sharp dissection (Fig 1B). After the esophagus was returned to its in situ length, circular muscle up, it was secured by pinning half of it along its length to the bottom of the bath (Sylgard, Dow Corning, Midlands, MI). Circular contractions were recorded isometrically by three force transducers (FT.03c, Grass Instruments) and fastened by silk sutures tied to the free edge of the esophagus at a distance 1, 2, and 3 cm proximal to the junction of the esophageal and gastric mucosa. Longitudinal contractions were monitored by intraluminal manometry with recording sites 1–3 cm apart and applied directly to the circular muscle, as close to the manometric recording sites as possible (preparation A) or 0.2–0.3 mm from the point of attachment of the isometric transducers (preparation B). Electrical signals were initially DC amplified (Intronix model 1955-1-DC; Intronix Technologies, Toronto, ON) and filtered with a low cutoff of 100 Hz by subsequently passing the signal through a bioelectric amplifier (8811A; Hewlett Packard, Waltham, MA). Contractions and electrical events were recorded on a direct-writing inkpen polygraph (Gould 2800S).

Materials. Atropine sulfate, TEA, and TTX were obtained from Sigma Chemical (St. Louis, MO). Bethanechol chloride was obtained from Merck Frost (Kirkland, PQ).

Statistics. Values are reported as means ± SE. Statistical comparisons were made with Student's t-test. P < 0.05 was considered significant.

RESULTS

Spontaneous activity. A total of 20 experiments were performed with preparation A. Sixteen of the 20 preparations (80%) initially showed spontaneous brief bursts of spike potentials (0.5–2 s) superimposed on a small depolarization and occurring at the onset of longitudinal muscle contraction. These phasic events of longitudinal muscle occurred with a frequency of 0.9–7.8 min⁻¹ (Fig. 2A). In 10 preparations the spike burst was followed at each recording site by hyperpolarization lasting 5–10 s and occurring during the longitudinal muscle contraction. These spike potentials were simultaneous at each recording site and were associated with low amplitude (<2 mmHg) increases in intraluminal pressure measured by manometry, coordinated with the longitudinal muscle contraction and the electrical activity described previously. The spontaneous spike bursts and accompanying longitudinal contractions persisted in the presence of 10⁻⁶ M TTX (Fig. 2B).

However, the depolarization-hyperpolarization changes accompanying these longitudinal contractions were blocked, suggesting neuronal involvement in their genesis.

Effects of muscle stretch. Spontaneous peristaltic electrical or mechanical activity was not seen in preparation A, except in a single preparation (Fig. 3), which became distended by the intraluminal perfusion. This preparation exhibited prolonged periods of electrical membrane potential oscillations with superimposed spiking, occurring both spontaneously and after ES. The rate of distal progression of the electrical activity was ~0.7 cm/s. The manometric recording did not show...
peristaltic contractions because of the common cavity created by fluid distension. When the esophagus was vented to allow the egress of fluid, repetitive activity ceased.

To further assess the effects of stretch, four experiments were done using preparation B (Fig. 1B). The esophagus was opened lengthwise so that stretch could be applied to the circular muscle while simultaneously recording contraction of this muscle layer by attached isometric force transducers. Muscle stretch alone (1–4 g) applied systematically to each of three sites and simultaneously at all sites resulted in irregular contractions of longitudinal and circular muscle, accompanied by brief spike bursts but no peristaltic electrical or mechanical activity. However, stimulation with bethanochol produced regular slow-wave-like membrane depolarizations with superimposed spiking, accompanied by contractions of circular muscle (Fig. 4). Depolarizations had duration of 6 ± 1 s and rate of 11.0 ± 1.1 min⁻¹. In two preparations the slow waves and accompanying contractions were peristaltic at 0.17 and 0.27 cm/s.

ES and effects of intrinsic nerves. Representative records of the responses to ES of preparation A are shown in Fig. 5. A short-duration stimulus (0.5 ms), used to selectively activate nerve rather than smooth muscle, applied as a single pulse produced a predominant hyperpolarization response frequently followed by a slight depolarization (Fig. 5A). Similar inhibitory junction potentials have been reported previously for both vagal and field stimulation of cat and opossum esophageal tissue (4, 7, 11, 12, 22, 25). A single long-

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Fig. 2. Preparation A shows spontaneous activity. A: control; spontaneous phasic contractions of longitudinal (Long) muscle are associated with small depolarization-depolarization sequence, occurring simultaneously at all 3 sites (top traces). Occasional low-amplitude, circular muscle is seen (arrows). B: after addition of 10⁻⁶ M TTX, spontaneous longitudinal contractions continue, as do spike bursts (top traces). However, subsequent hyperpolarization sequence is blocked.

Fig. 3. Preparation A after becoming distended by perfusion fluid from manometry tube showed continuous slow-wave-like electrical activity (top traces, sites 1–3) spontaneously (traces at right) and after single 500-ms pulse stimulus (S). Slow waves are propagated distally at 0.67 cm/s. Manometric record (bottom traces, sites 1–3) shows common cavity phenomenon.

Fig. 4. Example of continuous slow-wave-like membrane potential oscillations, superimposed spike bursts (top traces, sites 1–3) and circular muscle contractions (bottom traces, sites 1–3) recorded in preparation B after stimulation with 10⁻⁶ M bethanochol. Slow waves with spike bursts and accompanying contractions are propagated distally at 0.27 cm/s.
duration pulse (500 ms) not only stimulated intrinsic nerves, but also directly activated muscle as evidenced by the twitch contraction of the longitudinal muscle. The electrical responses to a long-duration stimulus were qualitatively similar to the 0.5-ms pulse but of greater magnitude (Fig. 5, A and B). In some preparations, the after-depolarization with superimposed spiking was accompanied by a circular muscle contraction, consistent with a typical off contraction (Fig. 5B) (30). Neither the short nor long-duration stimulus when applied as a single pulse produced a peristaltic response. The hyperpolarization and depolarization responses to both short- and long-duration single pulses were abolished by 10^-6 M TTX (Fig. 5, C and D), as were off contractions. The long-duration pulse still produced a twitch contraction of the longitudinal muscle (Fig. 5, D and E) and depolarization accompanied by circular muscle contraction with adequate stimulation (500 ms at 100 V as shown in Fig. 5E).

Cholinergic stimulation. With nerves blocked by 10^-6 M TTX, stimulation by bethanechol (10^-7 to 10^-6 M) enhanced the circular muscle contraction to direct myogenic activation by broad-pulse ES. In 5 of 7 preparations, a brief electrical depolarization with superimposed spiking and an associated second component to the muscle contraction were observed (Table 1 and Fig. 6). Both the spike potentials and the rapid pressure rise were peristaltic distally when the stimulus was applied at the proximal site and reversed with stimulation at the distal site (Fig. 6). Higher concentrations of bethanechol (10^-4 to 10^-5 M) often produced continuous slow-wave-like electrical oscillations and superimposed spiking accompanied by phasic circular muscle contraction (Fig. 7A). These slow waves were similar to those observed with distension of the esophagus (Fig. 3) and with bethanechol stimulation of preparation B (Fig. 4). Slow waves had a duration of 5.7 ± 0.3 s and a rate of 8.4 ± 1.1 min^-1 at maximum bethanechol concentration (n = 7). In some preparations the amplitude of the slow waves, the presence of spiking, and the amplitude of the accompanying contractions tended to wax and wane with a period of 1.3 ± 0.1 min (n = 4), as shown in Fig. 7A. However, in contrast to preparation B, this continuous slow-wave activity and the repetitive contractions were not peristaltic.

High K^+. Because cholinergic excitation of smooth muscles occurs in part by membrane depolarization, we examined the effects of increasing bath K^+ concentration in this preparation. In the presence of 10^-6 M TTX, increasing bath K^+ (10−40 mM) enhanced the amplitude of the circular muscle contraction to broad-pulse ES (not shown), similar to the effect seen with bethanechol (Fig. 6). High K^+ also induced low-amplitude, repetitive circular muscle contractions (4.5−9 min^-1) associated with slow-wave-like electrical oscillations with spiking (Fig. 7D). Neither the spontaneous repetitive contractions nor ES contractions were peristaltic. The high K^+-induced slow waves were transient, and the effect of cholinergic blockade could not be assessed.

TEA. TEA has certain similarities in its action to cholinergic activation in cat esophageal muscle (28), and others have shown that TEA can facilitate myogenic peristalsis in the presence of neural blockade in the opossum esophagus (18, 27). Hence, the effects of TEA were examined in the present model. As observed in the presence of bethanechol (Fig. 6) and high K^+,

Table 1. Myogenic peristalsis facilitated by bethanechol or TEA in the presence of 10^-6 M TTX

<table>
<thead>
<tr>
<th>Type of Response</th>
<th>Type of Response</th>
<th>Rate of peristalsis, cm/s</th>
<th>Rate of peristalsis, cm/s</th>
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</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>Bethanechol</td>
<td>2/11*</td>
<td>5/8†</td>
</tr>
<tr>
<td>Proximal to distal</td>
<td>TEA</td>
<td>5/8†</td>
<td>0.87 ± 0.07</td>
</tr>
<tr>
<td>Distal to proximal</td>
<td></td>
<td>0/11</td>
<td>4/8</td>
</tr>
<tr>
<td>Electrically stimulated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal to distal</td>
<td>5/7</td>
<td>0.80 ± 0.10</td>
<td>5/8†</td>
</tr>
<tr>
<td>Distal to proximal</td>
<td>2/2</td>
<td>0.80 ± 0.28</td>
<td>3/4</td>
</tr>
<tr>
<td>No peristals</td>
<td>4/11</td>
<td>3/8†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *Peristalsis was observed in 2 of 4 esophagi using preparation B. †In 2 preparations most spontaneous peristaltic contractions were proximal to distal, whereas in 3 it was the opposite. ‡In 1 preparation, maximum dose of tetraethylammonium chloride (TEA) tested (5 mM) may not have been adequate.
circular muscle contraction to broad-pulse ES was markedly enhanced by TEA (not shown). In addition, slow-wave-like oscillations of the membrane potential were observed (Figs. 7C and 8). These occurred at a frequency of $5.4 \pm 0.9$ min$^{-1}$ and had an average duration of $7.5 \pm 0.7$ s with 20 mM TEA. The associated spike potentials were usually seen on the upstroke phase of the slow wave and coincided with the onset of circular muscle contraction. These spontaneous electrical oscillations gave rise to circular muscle contractions that were peristaltic either proximally or distally (Table 1 and Fig. 8). Broad-pulse ES produced a slow wave accompanied by a contraction, both of which were peristaltic distally or proximally, depending on the site of stimulation (Fig. 8). The rate of propagation of these events was the same both proximally and distally and was not significantly different from the rate of peristalsis of the spontaneous activity seen in the presence of bethanechol (Table 1). Unlike bethanechol, peristaltic contraction could be initiated by broad-pulse ES in the presence of TEA-induced repetitive spontaneous peristaltic contractions. At TEA concentrations $>30$ mM, oscillations became rapid and appeared to initiate at several sites along the esophagus, obscuring peristaltic activity. Neither spontaneous nor electrically stimulated peristalsis nor TEA-induced slow waves were blocked by 10$^{-6}$ M atropine (not shown).
cholinergic stimulation, muscle stretch, or blockade of esophagus. We used 10 reversed and has never been demonstrated in the though some nerves are TTX-insensitive (3), this is completely blocks all nerves in the esophagus. Al-
mechanism is based primarily on the premise that TTX
stated to produce repetitive electrical slow-wave-type
activity. When adequately stimulated and with nerves blocked by TTX, a single or repetitive electrical oscillation with superimposed spiking and associated contraction can proceed along the cat esophagus in peristaltic fashion. Therefore, in the smooth muscle esophagus of the cat as in the opossum (18, 27), there exists the capability for peristalsis through operation of a myogenic mecha-
nism. The present study demonstrates that this myo-
genic peristaltic contraction is associated with electric-
control activity, similar to the myogenic slow waves
that have been recorded from muscles of the stomach,
small bowel, and colon. The esophageal slow wave
could be produced by depolarization with high K+
cholinergic stimulation, muscle stretch, or blockade of
K+ channels by TEA.

The validity of our demonstration of a myogenic
mechanism is based primarily on the premise that TTX
completely blocks all nerves in the esophagus. Al-
though some nerves are TTX-insensitive (3), this is
exceptional and has never been demonstrated in the
esophagus. We used 10−6 M TTX, a concentration
several times greater than usually needed to ade-
quately block nerves. Furthermore, in each experi-
ment, we confirmed that the nerve-mediated responses
to ES with a short-duration pulse (0.5 ms) were blocked
and could not be overcome with larger pulses (50–500
ms) required for direct muscle activation. We cannot
rule out the possibility that a 500-ms pulse might
stimulate the release of neurotransmitter directly from
nerve varicosities. Even if this were so, the peristaltic
responses we observed would nonetheless not involve
axonal signaling nor represent a physiological contribu-
tion of the intrinsic nerve pathways.

Sarna et al. (27) and Helm et al. (18) have shown
myogenic peristaltic contraction in the opossum in vivo
and in vitro, respectively, but did not record the associ-
ated electrical events in their studies. However, a
number of investigators have shown, usually in iso-
lated strips, that esophageal circular (4, 6, 21–23, 27)
and longitudinal (5, 23) smooth muscle can be stimu-
lated to produce repetitive electrical slow-wave-type
activity with superimposed spike bursts and associated
repetitive contractile activity. Progression of such com-
bined electrical and mechanical activity along the
esophagus was not assessed. We studied the progres-
sion of this combined activity. In the present study,
electrical recordings were made from the circular muscle
side of the esophagus and therefore reflect primarily
the electrical activity of this layer. Even recordings
from the outer longitudinal muscle layer in the intact
esophagus faithfully pick up the circular muscle activ-
ity with little interference from the longitudinal layer
(29).

We observed spontaneous repetitive activity in the
cat esophagus with nerves intact. However, peristalsis
of this activity or that induced by ES was seen only
when a generalized stimulus of some type was present:
bethanechol (both preparations) or distension (prepara-
tion A). Similarly, after the addition of TTX (prepara-
tion A), we were able to demonstrate electrical or
mechanical peristalsis only in the presence of bethane-
 chol or TEA. In the intact opossum esophagus in vitro,
spontaneous contractile activity was not seen and both
repetitive activity and its peristaltic progression re-
quired stimulation with TEA, bethanechol, or high K+,
with or without TTX nerve blockade (18). Sarna et al.
(27) found that in vivo, myogenic peristaltic responses
in the opossum esophagus were more easily elicited
with multiple electrical stimuli, with local infusion of
TEA, or shortly after the animal was killed by asphyxi-
ation (27). They hypothesized that membrane depolar-
ization facilitated the myogenic response, as did Helm
et al. (18).

Although we occasionally recorded slow-wave-like
activity after the addition of high K+, the activity was
never peristaltic, both electrical activity and contrac-
tions were of low amplitude, and neither were well
maintained. Similarly in the opossum esophagus, K+
stimulation of peristaltic activity in vivo (27) or in vitro
(18) is less effective even with nerves blocked. There-
fore, depolarization of the smooth muscle alone may not
be sufficient for the myogenic oscillatory and peristaltic
mechanism to become established. Mechanisms other
than tissue depolarization are likely to be important in producing conditions suitable for myogenic peristalsis to occur. For example, TEA and bethanechol similarly suppress K$^+$ channel activity (28), an effect that would not be mimicked by depolarization of the tissue by high K$^+$. In contrast to findings in the opossum (18), myogenic peristalsis in the cat esophagus with bethanechol stimulation was observed infrequently. This could be the result of simultaneous stimulation of the entire cat esophagus by bethanechol, resulting in multifocal excitation that obscured peristalsis, reflecting the species difference in the cholinergic sensitivity of peristalsis, with the cat being more sensitive (2, 16).

The present findings in the cat and those reported in the opossum (18, 27) indicate that that there is communication (coupling) within a myogenic mechanism for peristalsis that can operate independent of the extrinsic and intrinsic neural control mechanisms. Our experiments do not determine whether the oscillatory activity and its peristaltic progression are manifestations of a coupled oscillator system (9) or to what extent an active cable-core conductor system is involved (24). Intracellular microelectrode studies using the Abe-Tomita technique have shown high electrical connectivity of the circular muscle layers of the opossum esophagus when measured in the direction of the muscle fibers but poor conduction when measured perpendicular to the circular muscle fibers, that is, in the long axis of the esophagus (8). Morphologically, the smooth muscle cells in the circular layer are arranged in bundles or lamellae that are separated by fibrous tissue septae containing nerves, blood vessels, and other types of cells (15). Therefore, it is not known how communication would occur in the long axis and across the septae that separate the lamellae. Progression of a myogenic contraction would require that the circular muscle electrical oscillation be successively activated by communication through one or more potential mechanisms. These might include 1) circular smooth muscle cells extending through the lamellae and connecting to adjacent muscle bundles, 2) a smooth muscle-ICC network; if the ICC can be considered part of the myogenic system as proposed for elsewhere in the gut, they hold the potential to serve as a muscle-muscle communication pathway (10, 26), and 3) communication with the longitudinal muscle layer such that myogenic activity propagating in the longitudinal layer is transmitted to the circular muscle. Our experiments were not designed to explore or differentiate these possibilities.

In vivo with the nerves intact, swallow-induced peristalsis and the activity following intraluminal balloon distension or vagal stimulation are characterized in the circular muscle esophagus by a preceding hyperpolarization, followed by a depolarization with superimposed spiking and the contraction (12, 22, 25). With nerves intact, we observed similar electrical events in association with the single peristaltic contraction produced by the ES. These events are usually considered the local muscle responses to neural signals directing sequential inhibition and excitation along the esophagus, and consequently neural control has been considered sufficient for regulation of peristalsis. However, the concept fails to consider muscle properties and their potential contribution and involvement. In our experiments, when peristaltic progression of activity occurred in the presence of nerve blockade, only an electrical depolarization or slow wave was seen and the preceding hyperpolarization was absent. Therefore, the slow wave appeared as an actively generated event independent of rebound depolarization, and the wave progressed with a delay along the esophagus without the influence of inhibitory innervation or timed neural excitation. That is, when adequately excited, there is within the muscle itself the capacity to generate a peristaltic electrical slow wave and associated contraction, the characteristics of a MCS.

The relationship between a MCS, including the inducible slow-wave activity we have demonstrated, and normal peristalsis is not known. The esophagus is normally quiescent in vivo except for the single swallow-induced or secondary peristaltic contraction. Sarna et al. (27) proposed that the MCS in the esophagus is consistent with a mechanism involving a chain of bidirectionally coupled oscillators, and in the context of a coupled oscillator system its behavior could be considered a “one-shot” oscillation. It is of interest that the velocity of spontaneous and electrically stimulated myogenic peristaltic activity is similar to that of primary and secondary peristalsis in the opossum (18, 27), although slightly slower in the cat (0.8–1.0 vs. 1.5–2.0 cm/s, respectively). Therefore the myogenic system is intrinsically set to operate in a time frame similar to normal swallow-induced activity, a characteristic feature of linked control systems serving a common function (14). Of particular importance is the integration of the MCS with the neural control involved in normal swallow-induced peristalsis. We speculate that this could include 1) the myogenic system playing no significant role, the peristaltic contraction being controlled entirely by the timing and balance of the excitatory and inhibitory innervation; 2) the MCS serving as the primary mediator of the peristaltic contraction, with nerves regulating the coupling, the excitability, and the oscillatory characteristics, thus modulating the direction, velocity, and amplitude of the contraction, and ensuring the occurrence of a single contraction; and 3) regional differences in muscle properties along the length of the esophagus permitting different muscle responses (e.g., timing, duration of muscle contraction) to the innervation.

Nonetheless, the MCS may have particular relevance to esophageal motor disorders. In this context, simultaneous contractions, reversed peristalsis, and repetitive contractions may be best characterized and understood as alterations of the myogenic system, whether directly or indirectly, by physiological events or disease processes. Neural derangement would be seen as impacting on operation of a MCS. For example, most spastic motor disorders such as achalasia and diffuse esophageal spasm are associated with repetitive contractions. One view of these disorders sees the MCS as overtly expressing its oscillatory self when normal neural
control mechanisms (especially inhibition) fail and the muscle is subject to an excitatory-inhibitory imbalance (1, 13). This imbalance could also explain simultaneous contractions. Furthermore, the potential is present to utilize the MCS in those conditions where central or peripheral neural mechanisms are damaged or ineffective. Therapy such as electrical pacing of the muscle and pharmacological tools could be more specifically directed.

In conclusion, the smooth muscle portion of the cat esophagus is capable of peristalsis by a purely myogenic mechanism in vitro when specifically activated. Under these conditions, a myogenic electrical slow wave is observed. If this mechanism also functions in vivo, it likely requires neuronal input to activate and maintain conditions required for its expression. As such, the myogenic mechanism working in concert with neuronal mechanisms would represent an additional level of motor control in the esophagus. The extent to which this MCS normally contributes to esophageal peristalsis remains for further study.

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