Intramural mechanism of esophageal peristalsis: Roles of cholinergic and noncholinergic nerves

(latency of contraction/latency gradient/smooth muscle/atropine/enteric nerve)

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Communicated by Eugene Braunwald, February 21, 1984

ABSTRACT We examined the role of peripheral cholinergic and noncholinergic mechanisms in esophageal peristalsis. Intramural nerve elements in rings of circular muscle from six different levels of the opossum esophagus were stimulated transmurally so as to cause neurally mediated muscle contractions. Stimulus frequency was varied from 2 to 40 Hz. An increase in stimulus frequency caused an increase in latencies of contractions in rings from distal esophageal sites and a decrease in latencies in rings from proximal sites. This resulted in a marked slowing of the calculated peristaltic speed. Increasing stimulus frequency also caused an increase in duration and amplitude of contractions. These effects were reversed by atropine (0.1 μM), suggesting that higher stimulus frequencies recruited more cholinergic nerves. In the presence of atropine, increasing the stimulus frequency caused an increase in latencies of contraction at all sites, suggesting that increasing stimulation frequency applied to noncholinergic nerves causes an increase in latencies of contraction at all sites. The results of this study indicate that both noncholinergic and cholinergic nerves play a role in the peripheral mechanism of esophageal peristalsis. Cholinergic nerve stimulation reduces the latency and enhances the amplitude and duration of contractions seen with noncholinergic nerve stimulation alone. The influence of cholinergic innervation is most prominent proximally and decreases distally along the smooth muscle portion of the esophagus. This peripherally located gradient of cholinergic innervation plays an important role in determining the speed and amplitude of esophageal peristalsis.

The act of swallowing is associated with a peristaltic wave of esophageal contractions. This peristaltic activity in the skeletal muscle portion of the esophagus is due to sequential activation of lower muscle neurons in the vagal nuclei (1, 2). However, in the smooth muscle portion, this peristaltic activity may be due to peripheral intramural mechanisms (1, 2). This is evidenced by studies showing the following. (*i*) After bilateral vagotomy, balloon distension in the esophagus causes peristalsis in the smooth muscle portion (secondary peristalsis) (2, 3). (*ii*) *In vitro* transmural stimulation of circular muscle strips from different esophageal levels shows that strips from more proximal sites have shorter latencies of contraction than strips from more distal sites (4). (*iii*) Electrical stimulation of the peripheral end of the decentralized vagus nerve causes peristaltic contractions in the smooth muscle portion of the esophagus (5, 6). In addition, the speed and polarity of peristalsis can be modified by altering the parameters of the electrical stimuli applied to the decentralized vagal nerves (6), thereby demonstrating that peripheral mechanisms possess the ability to modulate the speed of esophageal peristalsis.

The nature of the nerves involved in the peripheral regulation of peristalsis is not fully known. It has been suggested by some investigators that peristalsis is due to noncholinergic nonadrenergic inhibitory nerves that produce a period of inhibition followed by rebound contraction (4, 7, 8). Some investigators, however, deny the presence of any cholinergic innervation to circular esophageal smooth muscle, thereby implying that cholinergic nerves play no role in peristalsis (7, 8). Other investigators have described cholinergically mediated peristaltic contractions *in vitro* in response to transmural stimulation (9, 10) and *in vivo* in response to vagal stimulation or swallowing (9, 11–13). These investigators suggest that peristalsis is due to cholinergic nerves. This latter view, however, fails to explain the mechanism by which stimulation of cholinergic excitatory nerves would result in an aborally increasing gradient of latencies of contraction which is responsible for peristalsis.

We examined the roles of intramural cholinergic and noncholinergic nerves in determining the amplitudes, durations, and gradients of latencies of esophageal contractions *in vitro*. Our studies suggest that both cholinergic and noncholinergic nerves are involved in peristalsis. Noncholinergic nerves are responsible for a period of inhibition followed by rebound contraction. The cholinergic nerves modulate the speed, amplitude, and duration of peristaltic contractions associated with noncholinergic inhibitory nerves.

METHODS

Ten healthy adult male and female opossums (*Didelphis virginiana*) weighing between 1.8 and 2.8 kg were anesthesized using intraperitoneal sodium pentobarbital (35–50 mg/kg). The chest was opened and the entire intra-thoracic and intra-abdominal esophagus was measured and marked *in situ*. The lower esophageal sphincter (LES) was localized by using a continuously perfused catheter assembly as described (14). The entire esophagus along with a cuff of stomach tissue was then excised and transferred to a bath of Krebs solution equilibrated with 95% O_2/5% CO_2. The tubular esophagus was then stretched to its *in situ* length and pinned to a wax block. In 4 of the animals, transverse rings, 0.7 mm wide, were cut along the pinned esophagus at 2-cm intervals beginning 1 cm above the LES. This produced transverse rings from each esophagus at 1, 3, 5, 7, 9, and 11 cm above the LES. In the other 6 animals, rings were cut at 11 cm and 1 cm above the LES. Excess serosa as well as mucosa were removed from each transverse strip by using a dissecting microscope. Each ring was then mounted in a muscle bath that was continuously perfused with Krebs solution equilibrated with 95% O_2/5% CO_2. The muscle bath consisted of a small block of Plexiglas with a central chamber 3 ml in volume containing the muscle ring bathed in Krebs solution. A heated reservoir surrounded this central core so as to maintain the bath temperature at 37°C. The Krebs solution consisted

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Abbreviation: LES, lower esophageal sphincter.
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of glucose, 11.5 mM; bicarbonate, 21.9 mM; phosphate, 1.2 mM; sodium, 138.5 mM; calcium, 2.5 mM; magnesium, 1.2 mM; potassium, 4.6 mM; chloride, 125.0 mM. One end of each ring was attached to a force–displacement transducer (model UC2, Gould, Plantsville, CT) and the other was fixed to the chamber for continuous monitoring of isometric tension. Transducer output was recorded on an ink-writing polygraph (model R711, Beckman). The rings were stretched to 150% of their original length [equivalent to the optimal length (7)] and recordings were begun after a 1-hr equilibration period.

Electrical field stimulation consisted of passing trains of current pulses between two platinum wire electrodes running parallel to the muscle rings within the bath. These electrodes were connected to a stimulator (model S48, Grass).

To study the influence of stimulus frequency on the contractile response at various esophageal levels, stimulus frequency was altered from 2 to 5, 10, 20, and 40 Hz while train duration, pulse duration, and voltage were maintained at 1.0 sec, 1.0 msec, and 80 V, respectively. Each muscle strip was stimulated three separate times using a particular combination of stimulus parameters, resulting in a total of 15 stimuli for each of the six sites. The interval between 2 successive stimuli was maintained at greater than 20 sec since stimulation at shorter intervals resulted in a decrease in the amplitude of contraction. In animals in which muscle rings were cut only 11 and 1 cm above the LES sites, determination of control latencies, amplitudes, and durations of contractions was carried out prior to any drug treatment. Atropine was subsequently added to the bath so as to attain concentrations of 0.2 and 10 μM. Bubbling of 95% O_2/5% CO_2 into the bath was maintained while perfusion with Krebs was discontinued. Absence of perfusion during a 30-min period had no effect on control contractions. At equilibration intervals of 5 and 15 min, the various stimulus parameters were again administered and contractile responses were measured. No differences in contractile responses between atropine bath concentrations of 10 and 0.2 μM were seen. Tetrodotoxin was also added to a bath concentration of 0.1 μM and bethanechol was used at a bath concentration of 1 μM.

A muscle contraction, as recorded by the force–displacement transducer (Gould UC2) on the ink-writing polygraph, was defined as any pen deflection greater than 2 mm above the baseline at a gain of at least 0.2 g/cm. The latency period of a contraction was defined as the time from initiation of the electrical stimulus to the onset of the pen deflection. Latencies of contractions at 11- and 1-cm sites were used to estimate a calculated value for speed of peristalsis expressed as cm per sec. This was derived by dividing the distance between the 11- and 1-cm sites (10 cm) by the difference in latencies of contractions at the two sites.

The data were analyzed using three-way analysis of variance with repeated measures and significant interactions tested by the Newman–Keuls test. The significance of differences in contraction amplitudes, durations, and latencies of contraction were tested by Student's *t* test. Values reported are expressed as mean ± SEM.

RESULTS

Effect of Stimulus Frequency on Latencies of Contractions at Different Levels of the Esophagus. The effect of increasing the stimulus frequency from 2 to 40 Hz on the latencies of contractions at six different esophageal sites is shown in Fig. 1*A*. At the more proximal 11-, 9-, and 7-cm sites, stimulation using 40 Hz resulted in a shorter latency of contraction than stimulation using 2 Hz. The decrease in latency of contraction as the stimulus frequency was increased from 2 to 40 Hz was greatest at the 11-cm site and smallest at the 7-cm site. On the other hand, at the 5-, 3-, and 1-cm sites, increases in
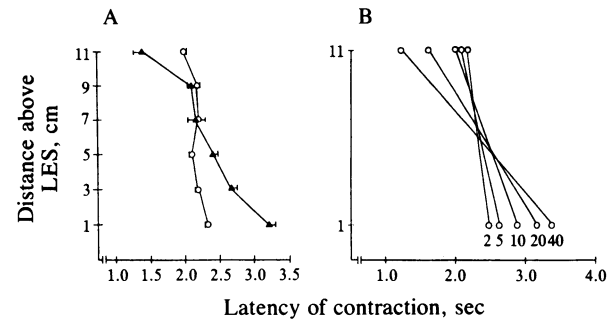

FIG. 1. (*A*) Effect of stimulus frequency on latencies of contraction at different sites along the esophagus. Increasing the stimulus frequency from 2 (o) to 40 (▲) Hz resulted in a decrease in latencies of contraction at the more proximal sites (11, 9, and 7 cm above the LES) and an increase in latencies at the more distal sites (5, 3, and 1 cm above the LES). (*B*) Effect of increasing stimulus frequency (2, 5, 10, 20, and 40 Hz) on latencies of contractions at the 11- and 1-cm sites. At the 1-cm site, there was a gradual increase in latency of contraction with increases in stimulus frequency. However, at the 11-cm site, increasing stimulus frequency resulted in a decrease in latencies of contraction. Standard errors for each data point fall within the plotted circles.

stimulus frequency produced an increase in the latencies of contraction. Increasing the stimulus frequency produced the greatest increase at the 1-cm site and a less pronounced increase at the 3- and 5-cm sites.

The effect of stimulus frequency on latencies of contractions at the 11- and 1-cm esophageal sites is shown in Fig. 1*B*. Data are presented from these two sites because they show the most pronounced effects. Increasing the stimulus frequency from 2 to 40 Hz at the most proximal (11-cm) site resulted in a decrease in the latency of contraction from 2.13 ± 0.06 sec to 1.21 ± 0.07 sec ($P < 0.05$, $n = 12$). However, at the most distal (1-cm) site, increasing the frequency from 2 to 40 Hz resulted in an increase in latency from 2.48 ± 0.07 sec to 3.39 ± 0.10 sec ($P < 0.05$, $n = 12$). Because of these opposing effects of stimulus frequency on the latency of contraction at the proximal and distal sites, the difference in latencies of contractions between these two sites was smallest at a stimulus frequency of 2 Hz and progressively increased as the frequency was increased to 40 Hz. This accounted for the decrease in speed of peristalsis with increasing stimulus frequency shown in Table 1.

Effect of Atropine on Latencies of Contractions at the 11- and 1-cm Sites. The influence of atropine (0.2 μM) on latencies of contractions at the 11- and 1-cm sites is shown in Fig. 2. At the 11-cm site, atropine treatment resulted in an increase in latencies of contraction at stimulus frequencies of 10 Hz or more ($P < 0.05$, $n = 9$). At the 1-cm site, atropine treatment also resulted in an increase in latencies of contrac-

Table 1. Effect of atropine on differences in latencies of contraction and calculated speed of peristalsis between 11- and 1-cm sites at different stimulus frequencies ($n = 9$)

Stimulus frequen-cy, Hz	Control		Atropine treatment	
	Difference in latencies, sec	Calculated speed, cm/sec	Difference in latencies, sec	Calculated speed, cm/sec
2	0.25 ± 0.10	40.0	0.36 ± 0.09	30.5
5	0.59 ± 0.14	16.9	0.58 ± 0.08	17.2
10	0.84 ± 0.10	11.9	0.85 ± 0.05	11.7
20	1.55 ± 0.17*	6.4	0.91 ± 0.06*	10.9
40	2.25 ± 0.06*†	4.4	1.13 ± 0.08*†	9.1

*Significantly different from control ($P < 0.05$).
†Statistically different with increasing frequency ($P < 0.05$).

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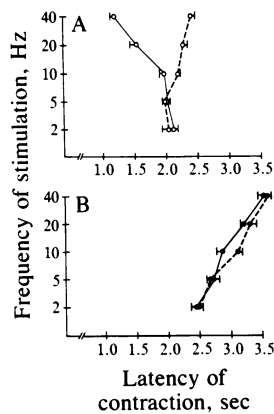*Proc. Natl. Acad. Sci. USA 81 (1984)* 3597

FIG. 2. Effect of stimulus frequency on latencies of contraction at the 11- (*A*) and 1-cm (*B*) sites before (—) and after (- - -) treatment with 0.2 μM atropine. At the 11-cm site, atropine treatment resulted in an increase in latency of contractions at stimulus frequencies of 10 Hz and greater. At the 1-cm site, atropine treatment produced a significant increase in latency of contractions only at a stimulus frequency of 10 Hz.

tion at stimulus frequencies of 10 Hz ($P < 0.05$, $n = 9$). At other frequencies, this difference was not significant.

Influence of Stimulus Frequency and Atropine Treatment on the Calculated Speeds of Peristalsis. The effect of stimulus frequency on the calculated speed of peristalsis between the 11- and 1-cm sites both before and after treatment with atropine is shown in Table 1. The calculated speed of peristalsis was slowest (4.4 cm/sec) at a stimulus frequency of 40 Hz and progressively increased to 40.0 cm/sec as the stimulus frequency was decreased to 2 Hz. After treatment with atropine (0.2 μM), there was a 3-fold increase in the calculated speed of peristalsis as the stimulus frequency was decreased from 40 Hz to 2 Hz. The effect of atropine was more marked with stimulus frequencies of 40 Hz than with 2 Hz. With a 40-Hz stimulus, atropine treatment resulted in a more than doubling of the calculated speed of peristalsis [from 4.4 cm/sec to 9.1 cm/sec ($P < 0.05$, $n = 9$)]. This was due to a marked increase in latency of contraction at the 11-cm site following atropine treatment and lack of effect at the 1-cm site. With 2-, 5-, and 10-Hz stimuli, the speeds of peristalsis changed insignificantly after treatment with atropine ($P > 0.05$, $n = 9$).

Effect of Stimulus Frequency and Atropine Treatment on the Amplitudes of Contractions at the 11- and 1-cm Sites. As shown in Fig. 3*A*, at the 11-cm site, prior to treatment with atropine, the amplitude of contraction increased from 0.15 ± 0.04 g to 0.34 ± 0.08 g as the stimulus frequency was increased from 2 to 40 Hz ($P < 0.05$, $n = 9$). At the 1-cm site, the amplitude of contraction increased from 0.90 ± 0.16 g to 1.15 ± 0.20 g as the stimulus frequency was increased from 2 to 40 Hz ($P < 0.05$, $n = 9$).

At the 11-cm site, atropine treatment produced a significant reduction in the amplitudes of contraction at all stimulus frequencies ($P < 0.05$, $n = 9$). Despite large doses of atropine (10 μM), contractions could not be abolished at any stimulus frequency. At the 1-cm site, treatment with atropine resulted in a decrease in contraction amplitudes, although these decreases were not significant for a particular stimulus frequency ($P > 0.05$, $n = 9$). As shown in Fig. 3, the amplitudes and durations of contraction of muscle strips at the 1-cm site were greater than those at the 11-cm site. Because we did not normalize the strips on the basis of muscle mass, the importance of these differences is not known.

Effect of Stimulus Frequency and Atropine on Durations of Contractions. As shown in Fig. 3*B*, at the 11-cm site, prior to treatment with atropine the duration of contractions increased from 1.59 ± 0.05 sec to 3.29 ± 0.15 sec as the stimulus frequency was increased from 2 to 40 Hz ($P < 0.05$, $n = 9$). After treatment with atropine, there was a decrease in the duration of contractions ($P < 0.05$, $n = 9$). This decrease was most prominent at higher stimulus frequencies. At the 1-cm site, there was an increase in the duration of contraction from 4.05 ± 0.05 sec to 4.38 ± 0.07 sec as the stimulus fre-
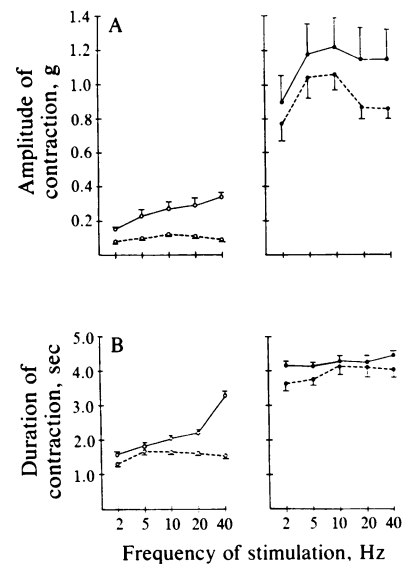

Frequency of stimulation, Hz

FIG. 3. (*A*) Effect of atropine on amplitudes of contraction at sites 11 and 1 cm above the LES. At the 11-cm site (*Left*), treatment with atropine resulted in a significant decrease in amplitudes of contraction at all stimulus frequencies. At the 1-cm site (*Right*), atropine treatment produced a decrease in amplitudes of contraction only at stimulus frequencies of 20 Hz and greater. (*B*) Effect of atropine on durations of contraction at sites 11 and 1 cm above the LES. At the 11-cm site (*Left*), treatment with atropine resulted in a decrease in contraction duration at all stimulus frequencies. At the 1-cm site (*Right*), atropine treatment produced a decrease in the duration of contractions at all stimulus frequencies. However, this decrease was significant only at stimulus frequencies of 2, 5, and 40 Hz. —, Control; - - - , atropine treatment.

quency was increased from 2 to 40 Hz ($P < 0.05$, $n = 9$). Atropine treatment produced a decrease in the duration of contractions at all stimulus frequencies. However, this decrease was significant only at stimulus frequencies of 2, 5, and 40 Hz ($P < 0.05$, $n = 9$).

Influence of Bethanechol on Latencies and Amplitudes of Contractions. Bethanechol (1 μM) decreased the latencies and increased the amplitudes of contractions at both the 11- and 1-cm sites. At a stimulus frequency of 10 Hz, bethanechol treatment decreased the latencies of contraction from 1.74 ± 0.05 sec to 1.37 ± 0.08 sec at the 11-cm site ($P < 0.05$, $n = 6$) and from 3.14 ± 0.07 sec to 2.03 ± 0.06 sec at the 1-cm site ($P < 0.05$, $n = 6$). Bethanechol treatment also increased the amplitudes of contraction from 0.43 ± 0.03 g to 0.78 ± 0.07 g at the 11-cm site ($P < 0.05$, $n = 6$) and from 1.12 ± 0.05 g to 1.62 ± 0.06 g at the 1-cm site ($P < 0.05$, $n = 6$). The effects of bethanechol were reversed by atropine.

Influence of Tetrodotoxin on Contractions. Tetrodotoxin (0.1 μM) abolished contractile responses at all frequencies of stimulation examined, indicating that these responses are due to stimulation of intramural nerve elements in the muscle strips.

DISCUSSION

These *in vitro* studies show the following. (*i*) The speed of esophageal peristalsis, as calculated from differences in the latencies of contractions of transverse circular muscle rings from various sites, can be profoundly modified by changing the frequency of transmural stimulation. This ability to modify the calculated peristaltic speed is due to the qualitatively and quantitatively different effects of stimulus frequency on latencies of contractions at different esophageal sites. For example, increasing the stimulus frequency results in a decrease in latencies of contractions in rings from upper esophageal segments and an increase in latencies in rings from lower esophageal segments. (*ii*) After atropine treatment, the

differential effects of stimulus frequency on latencies of contractions disappear and increasing the stimulus frequency results in increases in latencies at all esophageal sites. This response to atropine suggests that the differential effects of stimulus frequency are due to involvement of cholinergic nerves.

Our finding that the latencies of contraction of the proximal segments are shorter than those of the distal segments is similar to that reported by Weisbrodt and Christensen (4). They observed a calculated speed of peristalsis of 7.7 cm/sec using a stimulus frequency of 15 Hz. Subsequent studies have shown that the latencies of contractions can be modified by various treatments (15). Our studies show, however, that a calculated speed of peristalsis varying from 4.4 cm/sec to 40.0 cm/sec can be obtained by varying the frequency of transmural stimulation.

A decrease in the calculated speed of peristalsis with increasing frequency of stimulation could be due either to a proportionally greater increase in the latency of contraction at the distal site as compared with the proximal site or to a proportionally greater decrease in latency at the proximal than the distal site. Neither of these possibilities, however, was the basis for the decrease in peristaltic speed seen in our studies. Instead, increasing the frequency of stimulation decreased the latencies at proximal sites and increased them at distal sites. The effect of stimulus frequency on latency of contraction qualitatively changed somewhere in the middle of the smooth muscle portion of the esophagus. This paradoxical effect of increasing stimulus frequencies was reversed by atropine.

These observations can be explained on the basis of the following hypothesis (Fig. 4). Esophageal circular smooth muscle receives both noncholinergic and cholinergic innervation. Both these pathways are involved in peristalsis. Moreover, proceeding distally along the esophagus, there is a gradient of decreasing cholinergic influence and increasing noncholinergic influence.

Stimulation of noncholinergic innervation alone is capable of producing a contraction after a latency period. These responses are obtained in the presence of high (10 μM) doses of atropine and are therefore clearly noncholinergic. The noncholinergic nerves are stimulated at lower frequencies of stimulation. The maximal amplitude of contraction due to

their stimulation is reached at a stimulus frequency of 10 Hz. The latency of contraction resulting from stimulation of noncholinergic nerves increases with increasing frequency of stimulation. This is evidenced by the fact that in the presence of high (10 μM) doses of atropine, an increase in frequency of stimulation results in an increase in latency of contraction at all esophageal sites. However, this increase is more marked in distal than proximal sites and, as a consequence, increasing the stimulus frequency applied to noncholinergic nerves from 2 to 40 Hz causes a 3-fold decrease in the calculated speed of peristalsis (from 30.5 to 9.1 cm/sec). Although the influence of noncholinergic nerves is clear and prominent in the distal segments of the esophagus, their presence in the proximal portions is evidenced by the persistence of contractions despite the presence of atropine at 10 μM. The gradient of increasing noncholinergic innervation distally along the esophagus is further evidenced by the fact that stimulation of noncholinergic nerves (stimulation in the presence of high doses of atropine) causes near maximal contraction in distal rings but only submaximal contraction in proximal rings. The stimulation of the noncholinergic nerves may cause initial inhibition followed by rebound depolarization and contraction (1, 8, 16, 17). The distally increasing gradient of latency of contraction along the esophagus may be due to an increasing amount of neurotransmitter released as a result of greater noncholinergic innervation. The neurotransmitter released by the noncholinergic nerves is not known, although ATP and vasoactive intestinal polypeptide have been suggested (18, 19).

Cholinergic nerves act to reduce the latency and increase the duration and amplitude of contractions seen with stimulation of noncholinergic nerves. The cholinergic nerves are optimally stimulated at higher frequencies of stimulation (20). Cholinergic nerve influence is greatest in the proximal esophageal segments and progressively decreases distally along the esophagus as depicted in Fig. 4. Therefore, in the proximal segments, increasing the frequency of stimulation from 2 to 40 Hz activates a progressively larger number of cholinergic nerves. This increase in stimulus frequency results in a reduction in the latency and an increase in the amplitude and duration of contractions. These effects are clearly cholinergic as atropine at a concentration of 0.2 μM reverses them. Moreover, the muscarinic agonist bethanechol reduces the latencies of contractions and augments the amplitude of contractions. The influence of cholinergic agents on noncholinergically mediated esophageal contractions has been reported previously (15, 21).

Previous studies involving transmural stimulation of esophageal smooth muscle strips have shown that ganglionic blockers have no effect on either the latencies or amplitudes of responses, suggesting that the responses seen with transmural stimulation are due to activation of postganglionic nerve axons (15, 20). Therefore, the influence of atropine appears to be at muscarinic receptors located on the smooth muscle rather than on the intramural ganglia.

A number of prior *in vitro* studies have failed to identify any cholinergic innervation to esophageal circular smooth muscle (7, 15). Therefore, it was suggested that the latency gradient of contractions seen *in vitro*, and hence the intramural mechanisms involved in peristalsis, was due solely to the participation of noncholinergic nerves (4). In their studies showing a gradient of latencies of contraction along the esophagus, Weisbrodt and Christensen (4) did not specifically exclude any participation of cholinergic nerves because they did not use atropine in their experiments. The present study clearly shows that the latency gradient along the esophagus is due to involvement of both cholinergic and noncholinergic nerves. It has been reported that vasoactive intestinal polypeptide and acetylcholine may be present in the same nerve endings (22). Therefore, it is a possibility that
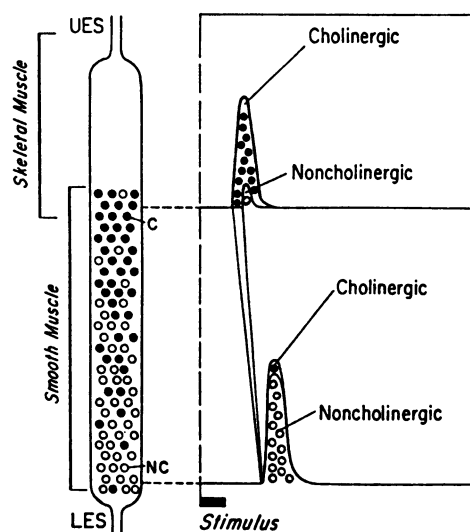

FIG. 4. Schematic drawing illustrating gradients of cholinergic (C) and noncholinergic (NC) nerve influence along the smooth muscle portion of the esophagus. Note that proximally, cholinergic influence is most prominent and that it progressively decreases distally along the esophagus. Noncholinergic influence is most prominent distally and it progressively decreases proximally.

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the observed gradients of cholinergic and noncholinergic innervation may be due to a gradient in the proportion of cholinergic or noncholinergic neurotransmitter in the same nerve terminal. It is interesting that gradients in membrane potentials, potassium concentrations, and densities of neurons along the esophagus have also been reported (23–25).

The conclusions drawn from this *in vitro* study also explain prior *in vivo* observations in which esophageal peristalsis was induced by either vagal stimulation or swallowing. Studies in the opossum involving vagal stimulation of 1-sec duration have shown that atropine increases the latencies of contraction in the proximal portions of the esophagus and has little or no effect on the latencies in the most distal portion (11). Similar to our *in vitro* study, this resulted in an increase in the speed of peristalsis following administration of atropine (11). Moreover, in swallow-induced peristalsis in the opossum, atropine caused a substantial delay in the appearance of the wave pattern in the proximal portion of the smooth muscle segment. Furthermore, atropine markedly reduced the amplitude and duration of contractions in this segment of the opossum esophagus (26). Studies involving the effect of atropine on primary peristalsis in man have also shown that atropine increases the speed of peristalsis by increasing the latencies of contractions, particularly in the proximal smooth muscle part of the esophagus (13). Atropine also reduced the duration and amplitude of contractions in this segment of the human esophagus (13, 27). A similar disconnection of the primary peristaltic wave at the level of the transitional zone between the skeletal and smooth muscle segments by atropine has also been reported in the monkey and cat (28).

Long train stimulation produces contractions shortly after initiation and termination of the stimulus. These responses have been labeled as "on" and "off" contractions, respectively (10, 29). The on contractions are cholinergic, whereas the off contractions are noncholinergic. With short train stimulation, as used in this study, it is impossible to distinguish between on and off contractions. However, the demonstration of atropine-sensitive and atropine-resistant components in our study suggests that the contractions seen using short train stimuli are a mixture of on and off contractions.

In this *in vitro* study, activation of cholinergic nerve endings required stimulation with high stimulus frequencies. This does not imply that *in vivo* during swallowing the cholinergic neurons are stimulated with such high frequency stimulation. Intensity of activation of the cholinergic neurons under physiological circumstances is unknown. These studies only suggest that activation of cholinergic neurons in addition to noncholinergic neurons seems to mimic the physiological behavior of peristalsis. It should also be noted that the speed of peristalsis *in vivo* is slower than the slowest speed observed in these *in vitro* studies.

This study also helps explain why, both *in vitro* and *in vivo*, neither cholinergic contractions nor noncholinergic contractions alone can explain all the facts currently known regarding physiological peristalsis (1, 2, 9, 11–13, 27, 28). The demonstration of peripheral cholinergic and noncholinergic involvement in peristalsis, along with the characterization of their gradients of influence along the esophagus, helps explain many of the previously described facts concerning the peripheral regulation of peristalsis.

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