Cholinergic stimulation induces asynchrony between the circular and longitudinal muscle contraction during esophageal peristalsis

Hariprasad Korsapati, Arash Babaei, Valmik Bhargava, and Ravinder K. Mittal
Division of Gastroenterology San Diego Veterans Affairs Health Care System; and University of California, San Diego, California

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Cholinergic stimulation induces asynchrony between the circular and longitudinal muscle contraction during esophageal peristalsis. Am J Physiol Gastrointest Liver Physiol 294: G694–G698, 2008. First published January 10, 2008; doi:10.1152/ajpgi.00458.2007.—In healthy subjects, a close temporal correlation exists between contractions of the circular muscle (CM) and longitudinal muscle (LM) layers of the esophagus. Patients with nutcracker esophagus show disassociation between the peak of contractions of the CM and LM layers and the peak of contraction 1–3 s apart (Jung HY, Puckett JL, Bhalla V, Rojas-Feria M, Bhargava V, Liu J, Mittal RK. Gastroenterology 128: 1179–1186, 2005). The purpose of the present study was to evaluate the effect of acetylcholinesterase inhibitor (edrophonium) and acetylcholine receptor antagonist (atropine) on human esophageal peristalsis in normal subjects. High-frequency intraluminal ultrasound imaging and manometry were performed simultaneously during swallow-induced peristalsis in ten normal subjects. Standardized 5-ml water swallows were recorded 2 cm above the lower esophageal sphincter under three study conditions: control, edrophonium (80 μg/kg iv), and atropine (10 μg/kg iv). A close temporal correlation exists between the peak pressure and peak wall thickness during the control period. The mean time lag between the peak LM and peak CM contraction was 0.03 s. After edrophonium administration, the mean contraction amplitude increased from 101 ± 9 mmHg to 150 ± 20 mmHg (P < 0.05) and mean peak muscle thickness increased from 3.0 ± 0.2 mm to 3.6 ± 0.3 mm (P < 0.01), and duration of both CM and LM contractions were also increased. Furthermore, the mean time difference between the peak CM and LM was increased to 1.1 s, (ranging 0.2 to 3.4 s) (P < 0.0001). We conclude that cholinomimetic agent induces discoordination between the two muscle layers of the esophagus.

MATERIALS AND METHODS

Subjects. Ten normal subjects with a mean age of 34 ± 14 yr (SD) (4 men, 6 women) without history of gastrointestinal disease or abdominal surgery were studied. The Human Investigation Committee of the University of California, San Diego approved the study protocol, and each subject signed an informed consent before participation in the study protocol.

Study protocol. After an overnight fast, recordings were obtained with the subjects in right recumbent position. An eight-lumen manometry catheter (Dent sleeve;Mui Scientific, Mississauga, ON, Canada) with four circumferentially placed side holes, located 2 mm from the distal end, and an intravascular ultrasound (US) catheter was used to record simultaneous pressure and US images (Fig. 1). The remaining four side holes were located in 5-cm increments above the first four radially arranged channels (i.e., at 5, 10, 15, and 20 cm above the first set of holes). The catheter assembly consisted of a 30-MHz, 3.2-F MicroRail US catheter (Cardiovascular Imaging Systems, Sunnyvale, CA), which was placed through the core lumen of the manometry catheter such that the US transducer was located just distal to the tip of the manometry catheter (Fig. 1). This catheter arrangement allowed us to record pressure and US images from the same region of the esophagus (within 2–3 mm). Furthermore, the advantage with this catheter assembly is its ability to capture full 360° tomographic US images of the esophagus. The US transducer was encased in a water-filled polyethylene bag of the same diameter as the manometry catheter. The catheter assembly was introduced through the nose into the stomach after topical anesthesia of the nasal cavity and oropharynx by using 1% lidocaine gel and 1% benzocaine spray.

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A station pull-through technique was used to determine the location of the LES. Data were acquired at 2 cm above the LES. Five standardized 5-ml water swallows were recorded during the control period, 1 min after intravenous administration of the edrophonium (80 μg/kg), and 5 min after atropine (10 μg/kg). Atropine was injected 30 min after edrophonium. All swallows were induced 30 s apart, and subjects refrained from swallowing in between. Pressures were recorded on a computer interfaced to Polygraph ID or Polygram 98 (Medtronic Synectics, Shoreview, MN). US images were recorded on a S-VHS tape recorder by using HP Sonos 100 platform (Hewlett-Packard, Watertown, MA). Pressure and US recordings were synchronized with a digital timer (Thalner Electronics, Ann Arbor, MI) that encodes analog time on the video images and an event marker on the polygraph with a 10-ms resolution.

**Measurements and data analysis.** US images were digitized with a frame grabber (Pinnacle Express; Pinnacle, Mountain View, CA) interfaced to a personal computer with Adobe Premiere 6.0 (Adobe Systems, Mountain View, CA). US images and manometry data were analyzed for five swallows to determine the baseline and peak pressures during contraction, as well as the baseline and peak thickness of the muscularis propria during contraction. Two-dimensional US images (B-mode) were converted to M-mode images and synchronized with the manometry data with proprietary programs. From these plots, the duration of contraction, amplitude, and time difference between the peak pressure (CM) and peak muscle thickness (LM) were calculated with Sigma Scan Pro (Jandel Scientific, San Rafael, CA). The time lag between the peak LM and peak CM contractions was determined for each contraction, and it was a positive number when LM contraction precedes CM contraction. On the other hand, when the CM contraction peaks first, the time lag was a negative value.

**Muscle thickness and pressure calculations.** Esophageal muscle cross-sectional area (CSA) measured from the B-mode images is an accurate marker of LM contraction (4, 10). However, measurements of muscle CSA on a frame-by-frame basis over prolonged periods are extremely time consuming and tedious (10). Furthermore, at times, due to partial dropout in the US images, the CSA quantitation is not possible. Several studies show an excellent correlation between the muscle CSA and muscle thickness as an indicator of LM activity, especially during the part of esophageal contraction when there are no changes in the esophageal lumen, as is the case during the entire period of manometrically recorded contraction or the pressure wave (7, 10). For these reasons, we measured muscle thickness rather than muscle CSA as the marker of LM contraction.

Resting muscle thickness was calculated during a 10-s period where there was no esophageal contraction activity. An increase in the muscle thickness was considered significant if it exceeded more than 20% of the baseline value. Respiratory variations in muscle thickness are, in general, about 10% of the baseline thickness. For each swallow-induced contraction, the maximum muscle thickness was measured.

Baseline pressure was defined as the end expiratory pressure before the onset of contraction. The onset of manometric contraction was defined at the point of the rapid upstroke on the pressure wave (rate of pressure rise >40 mmHg/s). This point usually follows the bolus pressure wave, which is the initial slow and small increase in pressure, usually 10 mmHg above the baseline esophageal pressure. The end of pressure wave was defined as the point at which it returned to the baseline esophageal pressure value.

After identifying each event on manometry, simultaneous US image data were acquired and converted to 32-equispaced M-mode...
images (every 11.25°), passing through the center of the US catheter. M-mode images, corresponding to image plane passing orthogonally through the esophageal wall muscle that did not show significant echo dropout over the study period, were selected for image analysis. For this M-mode image two edges were manually drawn by using Sigma Scan Pro, 1) the inner edge of the CM and 2) the outer edge of the LM. The distance between these two borders was computed in mm as the muscle thickness. Muscle-thickness data were time aligned with the manometric data by using the time encoder.

Statistical analysis. Data are shown as means ± SE. All statistical comparisons were done by first calculating the mean value for the parameter (of all swallows) in a given subject for each of the interventions and then comparing, except when indicated as comparison of number of swallows, as seen in Fig. 7. Student’s t-test was used for parametric data comparison. For the calculation of which muscle layer is contracting first: for each subject, the percentage of contractions with CM first and with LM first was calculated for each condition, control and after the administration of edrophonium and atropine. These paired percentages were compared across conditions by using nonparametric Wilcoxon matched-pairs signed-rank test.

RESULTS

A total of 150 contractions were analyzed in ten subjects, 50 during the control period, 50 after the administration of edrophonium, and 50 after atropine (5 swallows from each subject during each of the three conditions). US images showed that each swallow resulted in a bolus-induced distention of the esophagus followed by the collapse of the esophageal lumen and increase in the thickness of both CM and LM layers. Figure 2 shows the pressure tracing from one of the subjects during control period, after edrophonium, and after atropine infusions at 2, 7, 12, 17, and 22 cm above the LES.

After edrophonium, the mean contraction amplitude increased from a control value of 101 ± 9 mmHg to 150 ± 20 mmHg (P < 0.05), and it decreased to 40 ± 6 mmHg after atropine infusion (P < 0.0001). With edrophonium, the duration of the pressure wave increased from 3.7 to 4.9 s (P < 0.05) and decreased to 2.7 s (P < 0.001) following atropine infusion (Fig. 3).

Muscle thickness (LM contraction). Baseline muscle thickness (CM and LM combined) increased from a control value of 1.47 ± 0.16 mm to 1.65 ± 0.19 mm after edrophonium (P < 0.05), and it decreased to 1.33 ± 0.1 mm after atropine infusion. However, the difference between control and atropine period was not statistically significant (Fig. 4). Peak muscle thickness during contraction in the control period increased from 3.0 ± 0.2 mm to 3.6 ± 0.3 mm after edrophonium (P < 0.01) and decreased to 2.3 ± 0.18 mm following atropine infusion (P < 0.005) (Fig. 4). Duration of the LM contraction increased from 4.6 ± 0.4 s during control to 6.4 ± 0.8 s after edrophonium (P < 0.05), and it decreased to 3.7 ± 0.2 s after atropine (P < 0.05) (Fig. 5).

Dissociation between the peak LM and CM contractions. A close temporal correlation exists between the peak pressure and peak muscle thickness during the control period. The mean time lag between the peak LM and peak CM contraction was 0.03 s (range 0.08 to 0.18 s) (Fig. 6) with LM contraction peaking before the CM contraction. After edrophonium, the mean time difference between the peak LM and CM increased to 1.1 s (range 0.2 to 3.4 s) (P < 0.0001). After atropine infusion, even though mean time lag between the two peaks is more than during the control period (0.2 s), the increase was not statistically significant compared with control.

Which muscle layer contracts first? Based on our previous studies, as well as the present study, the time lag between the peaks of the LM and CM contraction in the control period is less than 0.2 s. Henceforth, we chose 0.2 s as a cutoff value and considered the CM and LM contractions to be simultaneous if the time lag was less than 0.2 s. Of the 50 contractions analyzed in the control period, 42 (84%) were simultaneous; in two contractions, CM peaked first, and, in the remaining six, LM contraction peaked first (Fig. 7). After edrophonium administration, in 48/50 contractions, the LM peaks earlier (P < 0.005) than the CM, and, in the remainder, they are simultaneous (4%). After administration of atropine, CM peaked first in 12 contractions (P < 0.05), LM peaked first in 18 contractions (P = NS) (Fig. 7), and, in the remaining 20, the two layers contracted simultaneously.

DISCUSSION

Our results show the following: both the CM and the LM layers of esophagus contract in a precisely coordinated fashion; during peristalsis in normal subjects, acetylcholinesterase in-
The inhibitor (edrophonium) and acetylcholine receptor antagonist (atropine) increase and decrease the contraction pressures, as well as the baseline and peak muscle thickness, respectively, and edrophonium, which increases the available acetylcholine at the neuromuscular junction, induces discoordination between the contractions of the two muscle layers of the esophagus.

The coordination between CM and LM contractions of the esophagus during peristalsis has been the subject of several studies. Sugarbaker et al. (23, 24), with the use of strain gauges, recorded the CM and LM contractions in the opossum esophagus and arrived at the conclusion that the contraction of LM begins before and lasts longer than the CM during peristalsis, but the peaks of contraction of the two muscle layers occur at the same time. Ponderoux et al. (13), with the use of mucosal clips implanted along the length of the esophagus, and Nicosia et al. (7, 10), with the use of US images to study the longitudinal muscle contraction, came to the same conclusion as Sugarbaker. Concurrent manometry and real-time US imaging is a relatively noninvasive method to measure CM and LM contraction of the esophagus in humans (8, 10). Esophageal manometry measures contraction pressures, a direct result of the CM activity. Dynamic changes in the esophageal muscle thickness and muscle CSA, on the other hand, measured by US images, mainly measure LM activity (law of mass conservation) (10). In a previous study (8), we found that manometry actually misses a part of the CM contraction, both at the beginning and at the end of the pressure wave, which we believe is the reason for the apparent earlier onset and longer duration of the LM contraction observed in various studies. In fact, our study shows that the onset, peak, and end of contraction of the two muscle layers are perfectly synchronized. Our present study findings are: 1) esophageal CM and LM contract synchronously during peristalsis in normal subjects, 2) edrophonium and atropine increase and decrease the contraction pressures and baseline and peak muscle thickness, respectively, and 3) edrophonium induces discoordination of contraction between the two muscle layers. Studies by Smith et al. (19–22) show that the colonic CM and LM also contract in a synchronized fashion during peristalsis.

Muscle CSA, rather than the muscle thickness measurement from US images, is an ideal marker to determine the onset, peak, and end of LM contraction (10). However, we used muscle thickness as a surrogate to measure LM contraction in this study for three reasons: 1) measurement of muscle thickness is somewhat easier compared with the muscle CSA and is as good a surrogate marker of the peak of LM contraction, 2) we were only interested in determining the discoordination of the peak of contraction of the two muscle layers because our previous study showed that, in patients with nutcracker esophagus, the discoordination of the two muscle layers occurs at the peak of contraction and not at the onset of contraction (4), and 3) too often the US images have dropout during the recordings, which limits the amount of data that can be analyzed by using a more rigorous approach of measuring muscle CSA compared with the thickness measurements. The major goal of our study was to determine whether
cholinergic hyper- or hypoactivity could induce discoordination at the peak of contraction of the two muscle layers.

Our present understanding of the pathogenesis of spastic esophageal motor disorders is that there is an imbalance between the excitatory and inhibitory innervation of the esophagus in these conditions (1, 9). Patients with achalasia of the esophagus show lack of nitric oxide-containing neurons in the myenteric plexus of the esophagus and LES (3, 6). Mice deficient in nitric oxide show absence of LES relaxation (17), a hallmark of the achalasia of the esophagus. It is also possible that the other spastic motor disorders, i.e., nutcracker esophagus and diffuse esophageal spasm, are related to an excessive excitatory (cholinergic activity) or diminished inhibitory activity of the myenteric neurons (2, 5). An increase in the contraction amplitude, increase in velocity of contraction and simultaneous contractions of the esophagus, are classical manometric findings in patients with spastic motor disorders and may be induced by cholinergic receptor agonist. It is possible that the reason for discoordination of the two muscle layers that we observed and reported earlier in patients with nutcracker esophagus may also be related to the excessive excitatory cholinergic activity at the level of the esophagus. Our findings that edrophonium induces discoordination of the two muscle layers support our hypothesis. We also observed that atropine had an influence on the coordination between the two layers; nevertheless, the effects were less pronounced.

Our study documents asynchrony induced by a cholinomimetic agent but does not address the precise mechanism by which it does so. The possible mechanisms could be at the level of muscle, nerves, or interstitial cells of Cajal, the three possible players in the smooth muscle contractions. It may be that the two muscle layers have differences in the sensitivity to cholinergic activity and that the LM is more sensitive than the CM. We propose that excessive cholinergic activity can explain several physiological findings observed on manometric studies in patients with spastic motor disorders of the esophagus, i.e., an increase in the baseline LES pressure, an increase in the amplitude and duration of the pressure waves, an increase in the velocity of peristalsis, and simultaneous pressure waves. The findings of the present study prove that, in addition to the above, excessive cholinergic activity can also cause an asynchrony of contraction of the two esophageal muscle layers. Future studies should explore whether cholinergic antagonist can reduce discoordination between the CM and LM contraction seen in patients with nutcracker esophagus.

GRANTS
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REFERENCES