Crural diaphragm inhibition during esophageal distension correlates with contraction of the esophageal longitudinal muscle in cats

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Liu, Jianmin, James L. Puckett, Torahiko Takeda, Hwoon-Yong Jung, and Ravinder K. Mittal. Crural diaphragm inhibition during esophageal distension correlates with contraction of the esophageal longitudinal muscle in cats. Am J Physiol Gastrointest Liver Physiol 288: G927–G932, 2005.—Esophageal distension induces simultaneous relaxation of the lower esophageal sphincter (LES) and crural diaphragm. The mechanism of crural diaphragm relaxation during esophageal distension is not well understood. We studied the motion of crural and costal diaphragm along with the motion of the distal esophagus during esophageal distension-induced relaxation of the LES and crural diaphragm. Wire electrodes were surgically implanted into the crural and costal diaphragm in five cats. In two additional cats, radiopaque markers were also sutured into the outer wall of the distal esophagus to monitor esophageal shortening. Under light anesthesia, animals were placed on an X-ray fluoroscope to monitor the motion of the diaphragm and the distal esophagus by tracking the radiopaque markers. Crural and costal diaphragm electromyograms (EMGs) were recorded along with the esophageal, LES, and gastric pressures. A 2-cm balloon placed 5 cm above the LES was used for esophageal distension. Effects of baclofen, a GABA_β agonist, were also studied. Esophageal distension induced LES relaxation and selective inhibition of the crural diaphragm EMG. The crural diaphragm moved in a caudo-cranial direction with expiration and inspiration, respectively. Esophageal distension-induced inhibition of the crural EMG was associated with sustained cranial motion of the crural diaphragm and esophagus. Baclofen blocked distension-induced LES relaxation and crural diaphragm EMG inhibition along with the cranial motion of the crural diaphragm and the distal esophagus. There is a close temporal correlation between esophageal distension-mediated LES relaxation and crural diaphragm inhibition with the sustained cranial motion of the crural diaphragm. Stretch caused by the longitudinal muscle contraction of the esophagus during distension of the esophagus may be important in causing LES relaxation and crural diaphragm inhibition.

lower esophageal sphincter relaxation

TWO SPHINCTERS, SMOOTH MUSCLE lower esophageal sphincter (LES) and skeletal muscle crural diaphragm, guard the lower end of the esophagus for its antireflux barrier function (5, 20). Under certain physiological conditions, i.e., belching and vomiting, both of these sphincters relax simultaneously to allow gastric contents to reflux into the esophagus (21, 31). Transient LES relaxation (TLESR), a major mechanism of gastroesophageal reflux and belching is accompanied by simultaneous relaxation of the LES and inhibition of the crural diaphragm (18, 19). The mechanism of simultaneous LES and crural diaphragm inhibition is not well understood.

Esophageal distension induces simultaneous relaxation of the LES and crural diaphragm in anesthetized cats (1, 14), but the mechanism is also not clear. Esophageal distension results in excitation of vagal afferents, which in turn may mediate inhibition of the inspiratory neurons responsible for the crural diaphragm contraction. Altschuler et al. (2), however, failed to find such a mechanism. Our previous observations suggest a possible peripheral mechanism of crural diaphragm inhibition (at the level of crural diaphragm) in cats. However, the precise nature of peripheral mechanism of crural diaphragm inhibition is not known (14).

Swallow-induced as well as esophageal distension-mediated relaxations of the LES are associated with distal esophageal longitudinal muscle contraction that results in esophageal shortening (6–8). What is not clear is whether there is a cause and effect relationship between the LES relaxation and esophageal shortening. The effect of esophageal shortening on the motion and inhibition of the crural diaphragm is also not clear. In the present experiments, we studied the motion of the crural diaphragm during esophageal distension-induced LES and crural diaphragm relaxation in cats. Our observations indicate that there is a close temporal correlation between longitudinal muscle contraction of the distal esophagus with the LES relaxation and crural diaphragm inhibition.

MATERIALS AND METHODS

Animal preparation. These studies were performed in seven female adult cats, weighing 3.0–3.7 kg. The study protocol was approved by the Subcommittee on Animal Studies of Veterans Affairs San Diego Healthcare System. Under general anesthesia, a midline laprotomy was performed, and two groups (3 in each group, 2 active and 1 ground) of stainless steel wire electrodes (MYO/WIRE; A & E Medical, Farmingdale, NJ) were implanted into the left diaphragm. One group of electrodes was placed ~2 mm from the hiatus and the other group into the costal diaphragm ~4 mm from the costal-vertebral angle (Fig. 1). Electrodes were positioned parallel to each other and spaced 1 cm apart. The other ends of the wire electrodes were tunneled through the abdominal wall and exited at the back of animal. In addition to the wire-electrodes, we sutured two platinum wires (0.5-mm diameter, 10-mm length) into the outer wall of the distal esophagus as radiopaque markers to study motion of the distal esophagus in the three cats. The abdomen was closed, and animals were allowed to recover for at least 1 wk before the physiological recordings.

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Data recording. Under light anesthesia with Telazol (2 mg/kg), the animals were placed under an X-ray fluoroscope. Tracking the motion of implanted electrodes and radiopaque marker placed on the esophagus monitored the motion of the diaphragm and esophagus. A reverse-perfused sleeve (11) manometry catheter was placed through the mouth into the esophagus and stomach to record pressures in the stomach, LES, and esophagus at 2 and 8 cm above the LES. Crural and costal diaphragm electromyograms (EMGs) were recorded along with the pressures. A 2-cm-long balloon with a diameter of 2.5 cm (when filled with 10 ml of air) was placed 5 cm above the upper edge of the LES to induce esophageal distension. Cats were positioned in the prone position on the X-ray table, and fluoroscopic images (left oblique view) were videotaped. Images were synchronized with the pressure and EMG recordings using a video time coding device (Thalaner Electronics, Ann Arbor, MI). Recordings were made before and after the administration of baclofen (2.4 mg/kg iv). Esophageal distensions were performed by manually inflating the balloon with 10 ml of air for 20 s using a syringe. A 1-min period was allowed in between esophageal distensions. The animals breathed spontaneously during the entire experiments. The blood $P_{O_2}$, blood pressure, and body temperature were monitored via a physiological monitor.

**Data analysis.** The fluoroscopic images were digitized for measurement of the motion of various markers. The crural diaphragm and a cranial excursion were measured by tracking the motion of the tip of wire electrode relative to a fixed bony landmark (the lower edge of a thoracic vertebra). The motion was measured in x-axis as well as y-axis coordinates. The vertical motion was measured as the absolute difference between the y-axis coordinate of the wires relative to the vertebra. Two types of crural diaphragm motions were observed: 1) phasic motion with inspiration and expiration and 2) sustained cranial motion during esophageal distension. The phasic motion was calculated from the y-axis coordinates of the wire at end inspiration and at end expiration. The sustained cranial motion was calculated from the y-axis coordinates of the wire before and during esophageal distension at end inspiration. The mean of three inspiration-related motions before esophageal distension, three inspiration-related motions during esophageal distension, and a mean value were determined. Similarly, for LES pressure, a mean of three end-expiratory values before esophageal distension and the lowest end-expiratory pressure during esophageal distension were determined. Radiopaque markers implanted on the esophageal wall were used to study the motion of the distal esophagus. These distances were also measured in reference to a fixed bony landmark and as absolute differences between the y-axes coordinates at end inspiration and at end expiration, before and during esophageal distension. The LES pressure was measured at end expiration in millimeters of mercury in reference to the end-expiratory gastric pressure. The crural diaphragm EMG amplitude was measured in arbitrary units. Duration of balloon distension-induced LES relaxation was measured between the points where the pressure dropped to 50% of baseline to the point where it returned to the 50% of the basal LES pressure. Similarly, the duration of crural diaphragm-EMG inhibition was measured between the points when the EMG dropped to 50% of the baseline value to its return to the 50% of the baseline value. The duration of crural diaphragm cranial motion was measured between the points of an increase above the basal end-inspiration position to its return to the basal end-inspiration position. Percent LES relaxation and percent crural diaphragm EMG inhibition during esophageal distensions were calculated.

Data are shown as means ± SE. Paired t-tests were used to determine the statistical significance.
RESULTS

Crural and costal diaphragm moves cranially and caudally with expiration and inspiration, respectively (Fig. 2). The range of phasic motion between inspiration and expiration is 8.89 ± 1.38 mm. The crural diaphragm motion or the diaphragmatic descent is in phase with the increase in the crural diaphragm EMG activity.

During esophageal distension, there is a sustained excursion of the crural diaphragm in the cranial direction, the duration of which is approximately the same as the duration of esophageal distension (18.54 ± 0.49 and 20.25 ± 0.35 s, respectively). There is a close temporal correlation between the cranial motion of the crural diaphragm and the inhibition of the crural diaphragm EMG activity. The inspiration-related motion of the crural diaphragm (phasic motion) is significantly reduced during sustained elevation of the crural diaphragm. The crural diaphragm EMG activity and LES pressure are inhibited during esophageal distension, the durations of which are also similar to duration of esophageal distension (Fig. 3). Similar to crural diaphragm, the costal diaphragm also moves in the craniocaudal direction with expiration and inspiration, respectively. However, the costal diaphragm EMG activity and costal diaphragm motion are not affected by esophageal distension (Fig. 4).

Basal LES pressure is reduced from 32.6 ± 5.5 to 6.0 ± 2.3 mmHg (P < 0.05), 87% relaxation during esophageal distension. The crural diaphragm EMG is reduced by 60% during distension (P < 0.05). The craniocaudal excursion of the crural diaphragm between peak of inspiration and expiration is 8.9 ± 1.3 mm. During esophageal distension, there is a sustained cranial excursion of 10.4 ± 1.5 mm for the crural diaphragm (sustained cranial motion). The inspiration/expiration-related excursion during esophageal distension is reduced to 5.4 ± 1.0 mm (a 40% reduction, P < 0.05). The esophageal marker shows a craniocaudal excursion of 3.9 ± 0.2 mm with inspiration and expiration. During esophageal distension there is a sustained cranial motion of the distal esophagus of 8.1 ± 0.3 mm. The craniocaudal excursion between expiration and inspiration of the esophageal marker is reduced to 2.0 ± 0.2 mm during esophageal distension. The cranial excursion of the esophageal marker during esophageal distension suggested shortening of the esophagus related to the contraction of the longitudinal muscle.

Effect of baclofen on the LES pressure, crural diaphragm EMG, and crural diaphragm motion. Cranio-caudal excursion of the crural diaphragm is increased by the injection of baclofen from 8.9 ± 1.3 to 11.0 ± 2.4 mm, but the difference is not statistically significant (P = 0.4) (Figs. 5 and 6). Baclofen reduces the esophageal distension-mediated LES relaxation from 90 to 38% (P < 0.0016) and crural diaphragm inhibition from 50 to 20% (P = 0.004). Baclofen also reduced the sustained cranial motion of the crural diaphragm during esophageal distension from 10.4 ± 1.5 to 2.5 ± 2.5 mm (P < 0.01). The sustained cranial motion of the distal esophageal marker during esophageal distension is also reduced from 8.1 ± 0.3 to 3.6 ± 0.7 mm (Fig. 7).

Fig. 3. Duration of esophageal distension-induced lower esophageal sphincter (LES) relaxation, crural diaphragm (CD) inhibition, and sustained elevation of the crural diaphragm. A 20-s esophageal distension caused LES relaxation, CD-EMG inhibition, and crural diaphragm cranial excursion, which lasted almost the same duration as the esophageal distension.

Fig. 4. Effect of esophageal distension on LES pressure, crural and costal diaphragm EMG, and crural and costal diaphragm motion. Esophageal distension induced a sustained elevation of the crural diaphragm. Note the concurrent crural EMG inhibition and LES relaxation at the time of crural diaphragm elevation. CRD, crural diaphragm; CSD, costal diaphragm; au, arbitrary units.

Fig. 5. Effect of baclofen on the motion of the diaphragm. The phasic motion of the crural diaphragm remains unchanged after the injection of baclofen. The cranial motion of the crural diaphragm is remarkably reduced. The LES relaxation seen in the control period is markedly reduced by baclofen.
DISCUSSION

Our data show the following: 1) esophageal distension induces relaxation of the LES and selective inhibition of the crural diaphragm; 2) crural diaphragm inhibition is temporally related with a sustained cranial excursion of the crural diaphragm and a sustained shortening of the distal esophagus; 3) baclofen inhibits esophageal distension-mediated LES relaxation, crural diaphragm inhibition, and the sustained elevation of the crural diaphragm; and 4) distal esophageal shortening or longitudinal muscle contraction of the esophagus induced by esophageal distension is also inhibited by baclofen.

Our finding of the cranial movement of the distal esophagus during esophageal distension is consistent with the observation made by several other investigators (6 – 8). A swallow-induced peristalsis in the esophagus also causes oral excursion of the distal esophagus (9, 26). Both esophageal distension and swallow-induced peristalsis are associated with relaxation of the LES. TLESR, another type of LES relaxation, is also associated with a strong contraction of the longitudinal muscle of the distal esophagus (28, 29). Therefore, it appears that there is a close temporal correlation between longitudinal muscle contraction in the distal esophagus and LES relaxation. Other evidence of the relationship between longitudinal muscle contraction and LES relaxation comes from studies in patients with achalasia of the esophagus, a condition in which LES relaxation is impaired. There is absence or marked reduction in the oral excursion of the LES in achalasia patients (3, 16). The temporal correlation between longitudinal esophageal muscle contraction and LES relaxation after fundopilation is also interesting. These studies show that the fundoplication diminishes the oral excursion of the distal esophagus and the LES relaxation during swallow as well as TLESR (10, 27). Our observation that baclofen blocks LES relaxation and oral excursion of the distal esophagus further proves a strong temporal correlation between the distal esophageal shortening and LES relaxation.

The novel finding of our study is that similar to LES there is also a strong temporal correlation between relaxation of the crural diaphragm and a cranial excursion of the crural diaphragm. What causes cranial excursion of the crural diaphragm during esophageal distension? We suspect that it is related to the contraction of the longitudinal muscle of the distal esophagus because the esophagus is anchored to the crural diaphragm by the phrenoesophageal ligament. Therefore, a contraction of the distal esophageal longitudinal muscle contraction will be expected to cause a cranial motion of the crural diaphragm (Fig. 8). Martin et al. (15) described a dorsal movement of the crural diaphragm during gastric distension-mediated TLESR, an event associated with inhibition of the crural diaphragm. However, they did not make a distinction between sustained and phasic cranial motion of the crural diaphragm. It is interesting that in their experiment, unlike ours, no cranial motion of the crural diaphragm occurred during TLESR. The difference in the two experiments may be related to the fact that we studied esophageal distension-mediated crural diaphragm inhibition. On the other hand, Martin et al. used gastric distension...
to induce crural diaphragm inhibition. Simultaneous blockade of the cranial excursion and inhibition of the crural diaphragm by baclofen proves that there is a close temporal correlation between the two events.

Baclofen, a GABA<sub>B</sub> agonist, reduces frequency of TLESR (4, 12) by acting at multiple sites in the vagal nerve pathway (17, 24, 25). It reduces distension-related discharges in the afferent as well as efferent fibers in the vagus nerve (24). GABA<sub>B</sub> receptors have also been found in the reticular formation of brain stem (dorsal vagal complex) as well as in the myenteric synapse of the LES (17). In a previous study (18), we reported that baclofen, in addition to blocking esophageal distension-mediated LES relaxation, also inhibits relaxation of the crural diaphragm. In the present investigation, we found that baclofen inhibits oral excursion of the crural diaphragm and distal esophagus. Blockade of esophageal shortening by baclofen implies that it inhibits distension-mediated contraction of the longitudinal muscle of the distal esophagus, a finding that has not been reported earlier.

The mechanism of crural diaphragm inhibition during esophageal distension and gastric distension is not known. One possible hypothesis is that the vagal afferents stimulated by esophageal distension inhibits inspiratory neurons that induce crural diaphragm contraction. In support of this hypothesis is the observation that bilateral cervical vagotomy blocks esophageal distension-mediated inhibition of the crural diaphragm (1). Studies by Altschuler et al. (2), however, failed to find inhibition of the medullary inspiratory neurons during esophageal distension and proposed that the crural diaphragm inhibition might occur at the level of the spinal cord or the phrenic nerve nucleus. Oyer et al. (22, 23) recorded EMG activity of the crural diaphragm and neurogram of the phrenic nerve branch supplying the crural diaphragm. Interestingly, there was greater inhibition of the crural diaphragm EMG activity than the phrenic nerve activity during esophageal distension. Their explanation for the observation was that the phrenic nerve branch they recorded could have contained nerve fibers that were not exclusively destined for that part of the crural diaphragm, which is completely inhibited. In an earlier study, we stimulated the crural diaphragm contraction directly by electrical stimulation of the muscles (14) and then performed esophageal distension. The latter induced crural diaphragm relaxation, which suggested to us that there is a peripheral mechanism of crural diaphragm inhibition. The sustained elevation of the crural diaphragm observed in our present study is related to the stretch caused by the longitudinal muscle contraction on the crural diaphragm through the phrenoesophageal ligament. It is possible that the stretch caused by longitudinal muscle contraction on the crural diaphragm is the peripheral mechanism of crural diaphragm inhibition. In support of the stretch hypothesis of the crural diaphragm inhibition is our finding that baclofen, which blocks distal esophageal shortening, also prevents crural diaphragm inhibition. Further studies are required to prove the cause and effect relationship between distal esophageal longitudinal muscle contraction with the LES and the crural diaphragm relaxation.

**GRANTS**

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**REFERENCES**


