Axial stretch: a novel mechanism of the lower esophageal sphincter relaxation

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Dogan I, Bhargava V, Liu J, Mittal RK. Axial stretch: a novel mechanism of the lower esophageal sphincter relaxation. Am J Physiol Gastrointest Liver Physiol 292: G329–G334, 2007. First published October 5, 2006; doi:10.1152/ajpgi.00351.2006.—Swallow and esophageal distension-induced relaxations of the lower esophageal sphincter (LES) are associated with an orad movement of the LES because of a concurrent esophageal longitudinal muscle contraction. We hypothesized that the esophageal longitudinal muscle contraction induces a cranially directed mechanical stretch on the LES and therefore studied the effects of a mechanical stretch on the LES pressure. In adult opossums, a silicon tube was placed via mouth into the esophagus and laparotomy was performed. Two needles with silk sutures were passed, 90° apart, through the esophageal walls and silicon tube, 2 cm above the LES. The tube was withdrawn, and one end of each of the four sutures was anchored to the esophageal wall and the other end exited through the mouth to exert graded cranially directed stretch on the LES by using pulley and weights. A cranially directed stretch caused LES relaxation, and with the cessation of stretch there was recovery of the LES pressure. The degree and duration of LES relaxation increased with the weight and the duration of stretch, respectively. The mean LES relaxation in all animals was 77.7 ± 4.7%. The required weight to induce maximal LES relaxation differed in animals (714 ± 348 g). Nω-nitro-L-arginine, a nitric oxide inhibitor, blocked the axial stretch-induced LES relaxation almost completely (from 78 to 19%). Our data support the presence of an axial stretch-activated inhibitory mechanism in the LES. The role of axial stretch in the LES relaxation induced by swallow and esophageal distension requires further investigation.

Axial stretch: a novel mechanism of the lower esophageal sphincter relaxation

Lower esophageal sphincter (LES) muscle is in a state of constant or tonic contraction, which is due to both myogenic and neurogenic elements of the LES (11, 17). In response to swallow and esophageal distension the LES relaxes, which is mediated through a vagal inhibitory pathway (9, 20). The vagal inhibitory afferent nerves that mediate LES relaxation synapse with the myenteric neuron located in the esophagus and LES. Upon activation of the inhibitory myenteric neuron the latter releases nitric oxide, which is the major inhibitory neurotransmitter that induces LES relaxation (18, 21, 29, 31).

Swallow as well as esophageal distension-induced relaxation of the LES is associated with a cranial or oral movement of the LES. Animal and human studies show that the LES moves in an oral direction, up to 20 mm, with swallow and esophageal distension (4, 6, 8, 19). The oral movement of the LES is due to the longitudinal muscle contraction of the esophagus, which occurs with swallow as well as with esophageal distension (3, 26). In fact, it is well known that the oral or cranial movement of the LES creates an artifact in the LES pressure recording when the latter is monitored with the side hole of a manometry catheter (7). We hypothesized that the longitudinal muscle contraction of the esophagus causes a cranially directed mechanical stretch on the LES that activates an inhibitory pathway to the LES. Therefore, the goal of our study was to determine the effects of cranially directed mechanical stretch on the LES pressure.

Methods

Studies were performed in seven adult opossum of either sex, weighing between 4 and 6 kg. The animal safety committee of the San Diego Veterans Affairs Health Care System approved the protocol for these studies. Animals were initially sedated with an intramuscular injection of ketamine (50 mg/kg) and then anesthetized with inhaled gaseous mixture of 2% isoflurane and oxygen, applied through a facial mask. A venous cannulation was performed to maintain an intravenous access. The heart rate and blood oxygen saturation of the animals were monitored continuously by pulse oximetry during the entire experiment. Following completion of surgery, inhalation anesthetic gas was removed and anesthesia was maintained by an initial intraperitoneal and subsequent intravenous injection of pentobarbital sodium. The animal was given oxygen through a face mask during the entire experiment.

A midline laparotomy was performed and the esophagogastric junction was identified. A silicon tube, 3 mm in diameter, was placed via the mouth of the animal into the esophagus. A curved needle with a silk suture was passed through the esophageal wall, silicon tube (placed in the lumen of the esophagus), and opposite wall, 2 cm above the LES. The tube was removed and silk suture of ~50-cm length was left on each side of the esophageal wall. Another needle with silk suture was again passed through the esophageal wall, the silicon tube, and the opposite esophageal wall, in a manner similar to the first one, but 90° apart and in a plane perpendicular to the esophagus. Again, sutures of equal length, ~50 cm in length, were left behind on each side of the esophagus. The silicon tube was withdrawn, and one end of each of the four sutures was anchored to the esophageal wall by use of a plastic cuff as shown in Fig. 1. A small amount of tissue glue (Nexaband, Abbott Laboratories, Chicago, IL) was applied between the plastic cuff and esophageal wall to further anchor the two. The silicon tube was again passed into the esophagus and stomach, and this time a curved needle with fine silk suture was passed through the esophageal wall, tube, and opposite esophageal wall, ~1 cm above the esophagogastric junction. Sutures of equal length (~40 cm) were left on each side. The tube was withdrawn from the mouth, and two silk sutures (exiting through the mouth) were tied to the manometric catheter, just above the sleeve sensor. By pulling the end of sutures outside the esophagus, the catheter was easily advanced into the esophagus and positioned across the esophagogastric junction. The upper end of the sleeve sensor, located above the esophagogastric junction, was anchored to the esophageal wall with another plastic cuff. The proximal ends of the four sutures exited...
through the mouth and were used to exert graded cranial stretch on the LES, by use of pulley and weights as shown in Fig. 1.

Study protocol. Cranial stretch was exerted on the LES using weights, starting with 100 g and up to 1,200 g. Once the optimal weight that produced maximal LES effect was determined, several observations were made with each weight. The effect of maximal weight on esophageal motion was determined by measuring the motion of a marker placed on the suture at the incisor tooth of the animal as shown in the Fig. 1. The effects of increasing the duration of stretch were determined by applying weight that produced maximal effects for the various time durations: 5, 10, 30, and 60 s. The effect of nitric oxide inhibitor [N\(^{\text{G}}\)-nitro-L-arginine (L-NNA), 20 mg/kg, intravenous bolus] on the stretch effect was determined. The effect of distension of the esophagus on the LES pressure was determined by using a 2-cm balloon, placed 5 cm above the LES, both before and after L-NNA administration. Animals were euthanized with an intravenous injection of potassium chloride solution (2 mmol/kg) at the completion of the experiment in accordance with the euthanasia guideline of San Diego Veterans Affairs animal committee (2).

Data analysis. The LES pressure was measured as the end-expiratory pressure above the gastric pressure. Both percent LES relaxation and residual LES pressure during cranial stretch and balloon distension-induced relaxations were determined. Duration of LES relaxation was determined from the time when the LES pressure dropped to 50% of the prestretch value to the time when it returned to 50% of the prestretch value.

Statistical analysis. Differences between the LES pressure during the cranial stretch and balloon distensions were determined by paired t-test. Data are presented as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Relationship between applied weight and cranially motion of the esophagus. The marker on the suture, located at the incisor, moved away from the incisor and toward the applied weight with the application of weight. Increasing the weight increased the distance of the marker movement. With the weight that caused maximal LES relaxation in each animal, the marker movement, ranging from 15 to 17 mm (1.6 ± 0.1 cm), was observed.

Effect of applied weight on the LES pressure. Application of weight caused LES relaxation and with the removal of weight there was recovery of LES pressure (Fig. 2A). The latency of response between the application of weight and onset of LES relaxation was less than 1 s. The minimal weight that induced any LES relaxation ranged from 100 to 200 g. The degree of LES relaxation increased as the applied weight increased up to a certain weight, and further increase in the applied weight did not increase the LES relaxation significantly (Fig. 2B). Similarly, the residual LES pressure decreased with the increasing weights. Complete or 100% LES relaxation with the cranial stretch was not observed in any of the animals tested. The mean LES relaxation induced by stretch in our experiment was 77.7 ± 4.7%. The weight that produced maximal LES relaxation differed in each animal and ranged from 400 to 1,000 g. Esophageal distension with a balloon (10 ml), on the other hand, resulted in significantly (P = 0.008) larger LES relaxation (97 ± 1.3%) than induced by stretch, in all animals tested (Fig. 3).

Effects of increase in the duration of applied weight on the duration of LES relaxation. The duration of LES relaxation was fairly close to the duration of the applied weight, and it increased as the duration of applied weight increased (Fig. 4A). Figure 4B shows a plot of the duration of stretch with the duration of LES relaxation along with the line of identity.

Effect of nitric oxide inhibitor on the stretch-induced LES relaxation. The effects of L-NNA administration on the balloon distension and stretch-induced LES relaxation are shown in Fig. 5. Balloon distension-induced LES relaxation was almost completely abolished (97 ± 1.3 vs. 16.5 ± 3.1%) by L-NNA. Similarly, there was near complete inhibition of stretch-induced LES relaxation (77.7 ± 4.7% vs. 19.4 ± 4.2%) by L-NNA. The data with the stretch-induced LES relaxation by weights of 800 g and 1,000 g and the effects of L-NNA are shown in Fig. 6.

DISCUSSION

The observation that LES moves in the oral or cranial direction with a swallow has been known for long time. Edwards and coworkers (1) noticed on fluoroscopy that surgically placed radiopaque clips at the esophagogastric junction move in the oral direction with swallow. Simultaneous radiographic and manometric studies by Clark et al. (4) and Dodds et al. (6) described the cranial or oral movement of the LES in detail and emphasized its significance in relationship to the LES pressure recording. The emphasis of their findings was that the cranial movement of the LES during a swallow results

Fig. 1. Schematics diagram of experimental design. The upper end of sleeve sensor is located above the esophagogastric junction and anchored to the esophageal wall with a plastic cuff. Another cuff is used to anchor 4 silk sutures to exert cranial stretch. The distances between lower esophageal sphincter (LES), cuffs, and diaphragm are approximate values. Distention balloon located 4 cm above the proximal end of the sleeve was positioned above the diaphragm.
in a relative movement between the side hole of the manometry catheter and the LES, thereby resulting in a motion artifact in the LES pressure recording that may overestimate LES relaxation. As a result of their observations, Goyal and Rattan (10) started to use a catheter pinning technique in which manometry catheter and LES were pinned together surgically. For continuous recording of LES pressure in humans, Dent (5) devised a sleeve sensor to overcome the problem of relative movements between pressure sensor and LES. Cranial movement of the LES during swallow and esophageal distension occurs because concurrent with LES relaxation there is also a longitudinal muscle contraction of the esophagus.

A concurrent longitudinal muscle contraction with LES relaxation may mean that the two events happen to occur together without a cause-and-effect relationship between the two, or alternatively the two events may be related to each other in a causal fashion. We hypothesized and tested the hypothesis that a cranially directed stretch on the LES may be the cause of LES relaxation. The ideal experimental design to test our hypothesis would have been to induce isolated longitudinal muscle contraction, using either pharmacological agent or nerve stimulation, and then study its effect on the LES pressure. However, such a method of inducing isolated longitudinal muscle contraction is not known and it appears that during all the known stimuli that cause longitudinal muscle contraction (e.g., swallow, esophageal distension, and vagal stimulation) there is also LES relaxation. We designed a unique experiment in which longitudinal muscle contraction was simulated by a cranially directed mechanical stretch on the LES and studied its effect on the LES pressure. Our data, for the first time, show a dose-dependent effect of cranial stretch on the LES relaxation. We observed that weight of 400 to 1,000 g induced a major LES relaxation (70–80%). One may argue whether our experimental design truly simulates the effects of longitudinal muscle contraction on the LES. We cannot answer the question whether the amount of cranial stretch we used is physiological and is similar to what happens during a swallow and esophageal distension, the two physiological stimuli that cause longitudinal muscle contraction and LES relaxation. Several observations, however, suggest that our findings could have physiological relevance. The weights that caused maximal LES relaxation resulted in a cranial movement of 15–17 mm of the anchor point (close to LES) in our experiments, which is similar to what happens during swallow in humans (8, 19). A similar range of motion is also seen during esophageal distension-induced LES relaxation in the cat (16). Most importantly, the cranial stretch-induced LES relaxation was blocked by the nitric oxide inhibitor L-NNA in our experiments. The latter implies that the LES relaxation in our experiment is not a mechanical artifact; rather, it is mediated by an inhibitory neurotransmitter. Nitric oxide is the major inhibitory neurotransmitter in the gastrointestinal tract and in

![Fig. 2. A: LES relaxation with increasing weights in 1 animal. Horizontal lines above the pressure tracing show the duration of stretch. B: cumulative data in 7 animals. Mean and SE of LES relaxation (%) (dashed line) and LES residual pressure after relaxation (solid line) with increasing weights (n = 7 animals).](image)

Fig. 3. Comparison of LES relaxation induced by 1,000-g axial stretch and balloon distension: Note that the balloon distension-induced LES relaxation was greater than the stretch-induced LES relaxation (P = 0.008, n = 6 animals).
the opossum LES (25). Myenteric neuron, gastrointestinal smooth muscles (13, 27) and interstitial cells of Cajal (30) are all-important sources of nitric release and may be involved in the inhibitory neurotransmission. We cannot answer the question whether nitric oxide released by cranial stretch in our experiment is released by the myenteric neuron, interstitial cell of Cajal, or LES muscle itself. However, on the basis of the observation that in the case of LES, myenteric neuron is most likely responsible for the release of nitric oxide (15), we believe that the cranial stretch causes activation of myenteric neuron, which in turn releases nitric oxide to induce LES relaxation.

Distension of the esophagus induces circumferential stretch on the esophageal wall as well as causes contractions of the circular and longitudinal muscle of the esophagus. Therefore, one cannot be certain which of the above components activates LES relaxation. We studied the effects of an isolated cranially directed mechanical stretch on the LES pressure, which is the unique aspect of our experiment. The reason why axial stretch-induced LES relaxation was less than the balloon-induced LES relaxation in our experiment is not clear but may have to do with the possibility that the balloon distension-induced longitudinal muscle contraction exerts a uniformly distributed axial stretch around the circumference of LES, in contrast to the four-point axial stretch used in our experimental design. Alternatively, there may be separate neural pathways for the two types of LES relaxations. Our study does not address the question whether the swallow induced or esophageal distension-induced LES relaxation is due to an axial stretch-activated inhibitory motor neuron.

Recent studies indicate that besides swallow and esophageal distension, another type of LES relaxation, transient LES relaxation (TLESR), is also preceded by a strong longitudinal muscle contraction of the esophagus (23, 24, 28). The duration of longitudinal muscle contraction during TLESR is similar to the duration of LES relaxation. Furthermore, following fundo-
plication swallow-induced LES relaxation is impaired and manifests as a higher residual LES pressure compared with before fundoplication (12, 22). Transient LES relaxation following fundoplication is also incomplete (12, 22). The current thinking is that the fundoplication-induced impairment of LES relaxation is the result of a mechanical effect “bulking up” of stomach wrap on the LES. We propose that the mechanism by which fundoplication affects LES relaxation may be related to its anchoring effect of the LES that reduces stretch on the LES. Accordingly, Kahrilas et al. (14) observed that the swallow-induced cranial movement of the LES is diminished following fundoplication.

In summary, our data for the first time provide evidence for an axial stretch-activated inhibitory pathway in the LES. Whether such a pathway is involved in the swallow- and distension-induced LES relaxation or TLESR requires further investigation. The significance of our finding is that, if the axial stretch-induced inhibitory neuron does have physiological relevance in LES relaxation, one may be able to design strategies that especially targetcranially directed stretch on the LES in the treatment of gastroesophageal reflux disease.

GRANTS

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Fig. 6. Cumulative data on the effect of L-NNA on stretch-induced percent LES relaxation (A) and residual LES pressure (B). Data are shown as means and SE, before (solid bars) and after L-NNA (open bars). Effects of stretch induced by 800 g are shown on the left and induced by 1,000 g are shown on the right.


