Effect of electrical stimulation of the LES on LES pressure in a canine model

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SEVERE GASTROESOPHAGEAL REFUX DISEASE (GERD) is relatively common and may be associated with serious complications like esophageal strictures, Barrett esophagus, and esophageal cancer. Symptoms recur frequently, even when medical therapy is maintained (15, 16, 32). The need for long-term medical treatment has increased the interest in permanent or semipermanent therapies such as fundoplication. Unfortunately, surgical procedures that change gastroesophageal anatomy and function produce symptoms that diminish quality of life (4, 18, 23). It is widely accepted that the severity of GERD and the incidence of its complications are proportional to the degree of lower esophageal sphincter (LES) weakness (34). There is a need for new treatment modalities that improve LES barrier function and decrease the incidence of complications related to GERD.

Although electrical stimulation (ES) of the esophagus in vivo is unstudied, several types of gastric electrical stimulation have been explored as methods to modify gastric motor function. One obvious target for ES is modulation of the gastric pacemaker that generates SWs. SWs are rhythmic oscillations of membrane potential (~3/min) that arise from interstitial cells of Cajal (26). They control the spatial and temporal distribution of peristaltic gastric contractions. A constant-current, square-wave electrical pulse (30- to 500-ms duration) delivered to the pacemaker region can initiate a slow wave that propagates toward the antrum. Delivering such pulses at a rate slightly faster than that of the intrinsic pacer frequency entrains the pacemaker and drives it at the rate of the electrical stimulus. This is called gastric electrical pacing. Although gastric pacing can drive SW production in the stomach, it does not evoke peristaltic contractions (5). In addition, and by unknown mechanisms, ES with these parameters delivered to the stomach produces a significant increase in LES pressure in dogs (38, 39).

The effect of high-frequency (in the range of pulses/s) ES on gastric motor function has been explored in humans with gastroparesis and dogs (5). Delivering this type of ES to the pacemaker region does not affect slow wave generation or propagation. It improves nausea, vomiting, and symptoms of GERD in patients with gastroparesis but inconsistently affects gastric emptying (1, 2, 21). Symptom improvement may be related in part to gastric relaxation (35, 37). Like gastric pacing, high-frequency ES of the stomach increases LES pressure in humans and dogs (19, 38, 39).

Stimulation of the gastric wall with trains of short-duration, high-amplitude, square-wave electrical pulses causes smooth muscle contraction at the electrode site and for a few centimeters along the circumferential and the longitudinal axes of the stomach. This form of ES is termed neural electrical stimulation (NES) because it activates neural elements intrinsic to the muscularis propria. NES works independently of intrinsic slow wave generation and propagation systems. It produces circular smooth muscle contraction in the stomach and colon (25, 33).

Almost all studies exploring the effect of the direct ES on esophageal and LES smooth muscle function were performed in vitro using stimulation parameters much like NES. EFS (10-s trains of 1-ms, 30-V square wave pulse) makes esophageal smooth muscle strips contract. However, contraction of

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longitudinal and circular muscles differs as a function of stimulus pulse rate. Maximal circular muscle contraction occurs in the range of stimulus frequencies from 5 to 20 Hz and diminishes linearly as stimulus frequency is increased further (22). Longitudinal muscle contraction is 50% maximal at a stimulus frequency of 10 Hz and increases to a maximum at 40 Hz. This disparity in stimulus-response arises because the neuromuscular control of these muscles differs (22). Circular muscle contraction is controlled by nitric oxide (NO)-generating neurons that are activated at lower stimulus frequencies, and longitudinal muscle contraction is mediated by cholinergic neurons that are activated at higher stimulus frequencies.

We know of no experiments in vivo assessing the effects on LES motor function of different types of ES delivered to the LES. Recently, an acute study of three anesthetized dogs showed that high-frequency (20 Hz) stimulation delivered to the LES causes an increase in its pressure (3). We hypothesized that ES delivered locally to the LES in vivo modulates LES pressure. We chose stimulus parameters similar to those proven to change LES tone in vitro and other types of ES that when applied to stomach increase LES pressure or generate smooth muscle contraction. In addition, we decided to explore the mechanism by which NES affects LES pressure.

MATERIALS AND METHODS

Four healthy, female mongrel dogs (weight 20–25 kg) completed the experiment. All dogs underwent esophagostomy to facilitate esophageal manometry and implantation of a pair of electrodes at the LES for ES of the sphincter tissue. After recovery, the effect of five different types of ES on LES pressure was explored: 1) sham stimulation; 2) low-frequency stimulation (6 cycles/min); 3) high-frequency (50 Hz) stimulation; 4) high-frequency (20 Hz) stimulation; and 5) NES (see Table 1 for details). Each stimulus protocol was performed on a separate day, in a random order with at least 48 h between consecutive studies in each animal. During each session continuous recordings of LES pressure and esophageal motility were obtained by use of a standard esophageal manometry recording system. Changes in LES pressure were analyzed for the different sessions. At the end of the study, esophageal manometry was performed under anesthesia and the effect of NES on LES pressure was tested alone and with the intravenous (IV) administration of an inhibitor of NO synthase [N^2-nitro-L-arginine methyl ester (L-NAME)] and atropine. The protocol was reviewed and accepted by the Institutional Animal Care and Use Committee at Cedars Sinai Medical Center, Los Angeles, CA.

Preparation of the Animals

Esophagostomy. Dogs were fasted for 18 h and received preoperative carprofen (4.0 mg/kg po, Pfizer Animal Health, Exton, PA) on the morning of surgery. They were anesthetized with IV thiopental (20 mg/kg, Abbott Laboratories, Chicago, IL) and inhaled isoflurane (1–2%, Abbott Laboratories). A paramedial esophagostomy was performed by a procedure described previously by McMahon et al. (24). A 15-mm esophageal plastic dilator was positioned through the mouth into the esophageal lumen to facilitate the identification of the esophagus. Then a esophagectomy was created following the long axis of the esophagus. The skin wound was closed and the esophagectomy site was marked with external stitches. Dogs wore soft collars to protect the esophagectomy site. Medications were given for postoperative pain.

Implantation of LES electrodes and esophagostomy. Four weeks after the first surgery, the dogs underwent open laparotomy under general anesthesia. The stomach was identified and one pair of temporary stimulation wires (A&E Medical, Farmingdale, NJ) was implanted in the musculature of the LES with endoscopic guidance such that the long axis of each electrode was positioned parallel to the longitudinal axis of the esophagus. The electrodes were positioned 2 cm apart on the LES, placing them nearly 180° from one another around the circumference of the LES. The vagus nerves were identified and the electrodes were implanted away from them. The wires were secured to the LES wall, tunneled underneath the skin to come out on the back, between the shoulder blades, and were secured to the skin.

During the same operation, the skin over the esophagectomy was cut open under endoscopic guidance and a modified gastrostomy tube (Standard PEG kit, Boston Scientific, Spencer, IN) was introduced to keep the fistula track opened and to serve as a direct access to the esophagus. The esophagostomy tube was kept plugged at all times except during manometry recordings. During this operation, a standard Dent-sleeve manometry catheter was introduced into the esophagus via the esophagectomy, and under endoscopic guidance it was positioned with the sleeve across the gastroesophageal junction. The distance from the esophagostomy opening was noted so that the sleeve could easily be positioned across the LES during experiments. At the end of the procedure, a soft collar was placed over the esophagostomy tube and electrodes, and the dogs wore jackets that blocked access to these devices. Medications were given for postoperative pain.

Esophageal Manometry

Dogs were allowed to recover for 2 wk after the second surgery before stimulation and esophageal manometry were performed. Standard esophageal manometry recordings were obtained from each dog on separate days during the stimulation sessions.

Prior to each session the dogs were fasting overnight, and on the morning of the procedure they were fed 312 g of canned food (Pedigree, MasterFood USA, Vernon, CA). The procedure was done 1 h after the meal and under mild sedation with oral acepromazine (0.5 mg/kg, Fort Dodge Animal Health, Forth Dodge, IA). Esophageal manometry was performed by using a multilumen perfused catheter assembly that incorporated a sleeve sensor for monitoring LES pressure (Mii Scientific, Mississauga, ON, Canada) and a low-compliance water-perfusion pump (Arndorfer Medical Specialities, Greendale, WI). The esophageal manometry catheter was placed through the esophagostomy into the esophagus and was positioned with the sleeve straddling the LES and the other four side openings of the catheter located in the

Table 1. Types of stimulation

<table>
<thead>
<tr>
<th>ES Type</th>
<th>Pulse Frequency</th>
<th>Train Duration, s</th>
<th>Pulse Duration, ms</th>
<th>Pulse Amplitude</th>
<th>Train Frequency, trains/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-frequency</td>
<td>6 cycles/min</td>
<td>375</td>
<td>5 mA</td>
<td>Continuously</td>
<td></td>
</tr>
<tr>
<td>High-frequency</td>
<td>50 Hz</td>
<td>10</td>
<td>1</td>
<td>5 mA</td>
<td>1</td>
</tr>
<tr>
<td>High-frequency</td>
<td>20 Hz</td>
<td>10</td>
<td>1</td>
<td>5 mA</td>
<td>1</td>
</tr>
<tr>
<td>NES*</td>
<td>50 Hz</td>
<td>6</td>
<td>20</td>
<td>10 V*</td>
<td>1</td>
</tr>
<tr>
<td>Sham-stimulation</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

This table depicts the parameters used in each type of electrical stimulation (ES) applied to the lower esophageal sphincter (LES). Each type of stimulation was applied at random, on separate days and for 20 min. NES, neural electrical stimulation. *Peak-to-peak.
The curve (AUC) were determined for 20 min for each of the following.

Euthasol (Delmarva, Midlothian, VA) was administered as an IV bolus and 2 min later NES was delivered for 10 min.

Aldrich, St. Louis, MO) was administered IV over a few minutes to reach a total dose of 50 mg/kg. Twenty to 30 min after the medication administration period (*P < 0.05 compared with baseline).

For the second experiment LES pressure was measured for the entire ES duration in each period. Changes in the mean LES pressure are presented as raw data and as a percentage change from the preceding baseline. ANOVA for repeated measures was used to assess differences between the three periods (NES alone, NES + t-NAME and NES + t-NAME + atropine). The duration of the contractions and relaxations was also measured during each period.

Data are presented as means ± SD and P < 0.05 was considered statistically significant.

**RESULTS**

**Effect of LES Electrical Stimulation on LES Pressure**

Esophageal manometry was well tolerated by all animals. Baseline LES pressure was 36.3 ± 4.3 mmHg. A sustained and statistically significant increase in LES pressure was observed only during low-frequency stimulation: prestimulation 32.1 ± 12.8 mmHg vs. stimulation 42.4 ± 18.0 mmHg vs. poststimulation 50.2 ± 23.6 mmHg, P < 0.05. The AUC also significantly increased during and after stimulation: prestimulation 39,320.3 ± 15,722 vs. stimulation 51,294 ± 21,826 vs. poststimulation 59,823 ± 28,198 mmHg*s, P = 0.01 (Fig. 1, Table 2).

LES relaxation during saliva swallows was unaffected either during or following ES of the LES. Manometry recordings were performed for 1 h and transient relaxations of the LES were seen infrequently during those periods. There were no significant changes in LES pressure with the other types of ES (Table 2).

**NES Effect on LES Pressure: Mechanisms of Action**

NES induced an initial LES contraction followed within a few seconds by LES relaxation and then a slow resumption of resting pressure over a 1-min period (Fig. 2). This response was seen every time NES was delivered to the LES. To study the nature of the response, IV infusions of an NO synthase inhibitor, t-NAME, and later of atropine were given to the dogs. The changes in LES pressure induced by NES at baseline and after the administration of t-NAME and atropine are displayed in Fig. 2 and Table 3.

Resting LES pressure was increased by t-NAME. It also increased the amplitude of the initial LES pressure rise in response to NES; however, this was not statistically significant. Finally, t-NAME markedly attenuated or abolished LES relaxation following its initial contraction. Subsequent IV administration of atropine diminished resting LES pressure to near baseline levels and abolished the initial rise in LES pressure induced by NES (Fig. 2, Table 3).

**Table 2. Effect of LES electrical stimulation on LESP**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>Stimulation</th>
<th>Poststimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-frequency</td>
<td>32 (12.9)</td>
<td>42.4 (18)</td>
<td>50.2 (23.8)*</td>
</tr>
<tr>
<td>High-frequency, 50 Hz</td>
<td>39.4 (13.2)</td>
<td>43.9 (14.1)</td>
<td>41.7 (14.9)</td>
</tr>
<tr>
<td>High-frequency, 20 Hz</td>
<td>32.7 (10.9)</td>
<td>36.6 (10.8)</td>
<td>38.7 (9.5)</td>
</tr>
<tr>
<td>NES</td>
<td>41.9 (18.1)</td>
<td>40.6 (19.4)</td>
<td>40 (19)</td>
</tr>
<tr>
<td>Sham stimulation</td>
<td>36.3 (6)</td>
<td>35.4 (9.8)</td>
<td>41.4 (13.6)</td>
</tr>
</tbody>
</table>

Values are shown as means (SD). Different types of LES electrical stimulation were applied during 20 min, in random order and on separate days. LESP, LES pressure. *P < 0.05 compared with baseline.
is nearly completely abolished by inhibition of NO synthase (12). After blockade of NO synthase, a prominent cholinergic contraction occurs during EFS (13). Several studies suggest that the response of LES muscle to EFS depends somewhat on the characteristics of the stimulus (13, 14, 20, 36). For example, human LES muscle strips that generate a biphasic response to EFS contract and relax maximally at stimulus frequencies around 3 Hz. Muscle strips that only relax with EFS respond maximally at frequencies around 5–10 Hz (13, 14). Studies like these consistently demonstrate that the resting and active neuromuscular properties of the LES are complex and depend on the interplay of myogenic with excitatory and inhibitory neural mechanisms. They raise the possibility that applying ES to the LES in vivo may alter LES function and that the type of response may depend on characteristics of the ES and the location of its delivery.

Previous studies in vivo demonstrated that both low- and high-frequency ES applied either to the midstomach or fundus increases LES pressure (38, 39). However, until now nobody studied how direct electrical stimulation of the LES may affect sphincter pressure in a chronic animal model. This study was designed to evaluate the effects of four types of ES on LES function. We found that only low-frequency, long-pulse stimulation caused a significant and sustained increase in LES pressure. Moreover, the elevated LES pressure was sustained for more than 20 min after the stimulation was stopped. This finding is promising since it suggests that continuous ES of the LES may not be needed to maintain LES closure and protect against gastroesophageal reflux. Long-term treatment studies will need to be done in animals and humans to determine whether this approach can be used to treat gastroesophageal reflux.

Our observations are similar to previous studies demonstrating that ES of the gastric body or fundus can modify LES pressure. We previously showed that stimulating the midstomach with low-frequency, long-pulse ES significantly increases LES pressure (38). The study was conducted in acute, sedated animals, but the results were very similar to what we describe here. Another group assessed the effect of ES applied to the gastric fundus on LES pressure in conscious dogs (39). They found that high-frequency, short-pulse ES increased basal LES pressure 22% during stimulation and 33% in the poststimulus period. Low-frequency, long-pulse ES did not affect LES pressure. In our study, stimulating the LES with low-frequency ES increased baseline LES pressure by 33% during stimulation and 56% during the poststimulation period. Contrary to a previous study showing that high-frequency ES (14 and 40 Hz) of the stomach increases LES pressure (38, 39), we observed that 20- and 50-Hz pulse trains applied directly to the LES do not modify LES pressure. We know of one other study in which ES at a frequency of 20 Hz was applied directly to the

**DISCUSSION**

The experiments presented here were undertaken to test the hypothesis that ES applied directly to the LES, in vivo, modulates sphincter function in a chronic animal model. Stimulation parameters that increase LES pressure, if found, may be a useful way to treat gastroesophageal reflux.

In the early 1970s, the neuromuscular properties of the LES were first elucidated by using electrical field stimulation (EFS) to activate neurons intrinsic to transverse muscle strips isolated from the opossum LES. These studies demonstrated that LES muscle generates tone at rest and activation of its intrinsic innervation causes LES relaxation (6, 7). Resting LES tone was felt to be myogenic, i.e., a property of LES muscle, since tetrodotoxin, a neurotoxin that blocks Na⁺ channels, did not abolish it (8, 31). Subsequent studies demonstrated that resting LES tone is modulated by the activity of its intrinsic innervation: cholinergic blockade tends to decrease basal LES tone and inhibition of NO synthase tends to increase it (8, 31). The neurotransmitter primarily responsible for LES relaxation remained an enigma until the early 1990s when a number of lines of evidence proved it to be NO (11, 27–29). LES responses to EFS of its intrinsic innervation vary somewhat depending on what portion of the LES muscle is stimulated and among species (9, 13, 20). EFS of muscle from lower part of the human LES produces relaxation, whereas stimulating the more proximal LES produces a biphasic response consisting of relaxation during EFS followed by contraction (13). Relaxation induced by EFS can be augmented by cholinergic blockade and

**Table 3.** Effect of NES on LESP when applied alone and after the administration of l-NAME and atropine

<table>
<thead>
<tr>
<th></th>
<th>LESP Baseline, mmHg</th>
<th>Initial Peak, % change</th>
<th>Peak Duration, s</th>
<th>Relaxation, % Change</th>
<th>Relaxation Duration, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>NES</td>
<td>20.7 (3.7)</td>
<td>+31 (22.7)</td>
<td>1.7 (0.5)</td>
<td>-68.8 (28.6)</td>
<td>8.1 (0.8)</td>
</tr>
<tr>
<td>NES + l-NAME</td>
<td>35.7 (5.1)†</td>
<td>+76 (48.6)</td>
<td>6.7 (3.9)</td>
<td>-28.2 (34.1)†</td>
<td>4.4 (3.8)</td>
</tr>
<tr>
<td>NES + l-NAME + Atropine</td>
<td>24.1 (6.8)</td>
<td>+27.3 (39.9)</td>
<td>3.9 (0.7)</td>
<td>-20.4 (13.5)†</td>
<td>4.3 (3.8)</td>
</tr>
</tbody>
</table>

The data show the changes in LESP caused by NES and how those changes are modified by a nitric oxide inhibitor [N°-nitro-l-arginine methyl ester (l-NAME)] and an anticholinergic drug (atropine). Data presented as mean (SD). *P < 0.001 compared with NES alone. †P < 0.05 compared with NES alone.
LES in three anesthetized dogs. In that study, LES pressure rose significantly with pulse amplitudes >8 mA (3). We did not find the same effect in our study, perhaps because we used a lower pulse amplitude (5 mA vs. >8 mA) or a longer pulse duration (1 ms vs. 200 μs) that we chose on the basis of a previous in vitro study (22).

We found that the increase in LES pressure was sustained for several minutes after low-frequency ES was stopped. We did not try to elucidate the mechanisms for this prolonged effect. In a previous series of studies intended to elucidate the mechanism by which gastric ES affects LES pressure, gastric ES using low- and high-frequency parameters produced a prolonged increase in LES pressure. This increase in LES pressure was not associated with a rise in hormones or peptides that affect the LES, such as motilin, gastrin, and neurotensin (38). Other potential mechanisms by which gastric ES may increase LES pressure were not addressed. Another group found that the rise in LES pressure produced by high-frequency gastric ES is prevented by atropine, suggesting that gastric ES activates cholinergic neural pathways (39). Since a proportion of both preganglionic vagal neurons and myenteric motor neurons supplying the LES are cholinergic, it is difficult to determine which of these neurons are affected by ES. It is unlikely that gastric ES activates preganglionic vagal fibers, because the increase in serum pancreatic polypeptide observed with vagal stimulation is not seen (38).

Unlike what is seen with EFS of isolated LES muscle, only NES produced LES relaxation in vivo. The second part of our study was designed to elucidate the mechanisms by which NES affects the LES. Stimulation of the LES using NES parameters evoked an initial contraction followed by a prolonged relaxation. This response is not exactly the same as described with EFS of isolated LES muscle, where the relaxation occurs before the contraction (12, 13, 27). Inhibition of NO synthesis modified the LES response to NES in two ways: first, it abolished or dramatically attenuated the relaxation, and second, it increased the amplitude of the initial contraction. There is ample evidence that NO mediates LES relaxation produced by swallowing, vagal stimulation, esophageal distention, or EFS of isolated LES muscle (30, 36). In each of these cases, LES relaxation is mediated by NO that is for the most part synthesized in myenteric motor neurons. There is, however, also evidence that NO can be made in smooth muscle of the LES (10, 32). Our observation that NGES-induced LES relaxation is NO dependent is entirely consistent with what is known of LES motor physiology. We are unable to tell whether NES is causing LES relaxation by stimulating preganglionic vagal neurons, myenteric neurons, or smooth muscle cells. In addition, changes in NES parameters, for example, stimulus duration and/or peak-to-peak amplitude, might produce different results.

The NES-induced contraction is cholinergic, as are NES-induced contractions of the stomach and colon (25, 33). Its augmentation by inhibition of NOS indicates that the final LES motor response to NES results from the integration of excitatory, cholinergic and inhibitory, nitrergic influences. Our observation that NES causes a contraction prior to LES relaxation is somewhat different from what is seen during normal swallowing and with EFS of LES muscle strips. Swallowing produces LES relaxation that may or may not be followed by contraction. These differences indicate that the pattern of neural activation produced by NES with the parameters utilized in our study does not mimic that during normal swallowing. EFS of isolated LES muscle also generates relaxation that may or may not be followed by contraction. Inhibition of NOS attenuates the relaxation and allows the expression of a cholinergic contraction during the stimulus, which is followed by an attenuated relaxation. This pattern of response is comparable to what we see in vivo with NES stimulation of the LES. Again, this suggests that the pattern of neural activation by NES in vivo differs from that produced by EFS of isolated muscle. The small rise in LES pressure caused by inhibition of NOS suggest that NO may play a role in LES resting tone, as has been suggested by other studies (13).

In summary, direct electrical stimulation of the LES in vivo with low-frequency pulses significantly increases LES pressure in awake dogs. This effect holds future promise as a treatment for GERD if confirmed in further studies. NES of the LES with the specific parameters utilized in the present study causes a transient LES contraction that is cholinergically mediated and a prolonged relaxation that is NO dependent.

DISCLOSURES

E. E. Soffer and J. Conklin have patent pending.

REFERENCES

G394  ELECTRICAL STIMULATION ON LES IN VIVO IN DOGS


