Baclofen Blocks Lower Esophageal Sphincter Relaxation and Crural Diaphragm Inhibition by Esophageal and Gastric Distension in Cats

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This study was supported by an NIH grant 1RO1 DK 51604-02

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ABSTRACT

Background: Esophageal distension and transient lower esophageal sphincter (LES) relaxation (TLESR) are accompanied by simultaneous relaxation of the LES and inhibition of crural diaphragm. Recent studies indicate that baclofen decreases the frequency of TLESR, however, its effect on the crural diaphragm is not known.

Aim: To evaluate the effects of baclofen on LES relaxation and crural diaphragm inhibition induced by gastric distension and esophageal distension in cats.

Methods: Five adult cats underwent surgical implantation of wire electrodes into the crural and costal diaphragm for measurement of their EMG activity respectively. One week after the surgery animals were lightly sedated and recordings were performed using a manometry catheter equipped with a 2.5 cm balloon. The effects of baclofen, 10 µmol/kg i.v., on the graded esophageal distension and gastric distension induced LES and crural diaphragm responses were studied.

Results: Distension of the esophagus and stomach induces relaxation of the LES and inhibition of the crural diaphragm simultaneously. Baclofen blocks both the esophageal and the gastric distension induced relaxation of the LES and inhibition of the crural diaphragm. The magnitude of response to baclofen was significantly larger for the crural diaphragm inhibition than for the LES relaxation.

Conclusion: Baclofen, a GABA<sub>B</sub> receptors agonist, blocks reflex inhibitory pathway to the LES and crural diaphragm. The reflex inhibitory pathway to the crural diaphragm is more sensitive to blockade by baclofen than the reflex LES inhibitory pathway.
INTRODUCTION

Transient lower esophageal sphincter relaxation (TLESR) is the major mechanism of gastroesophageal reflux (GER) in normal subjects and patients with reflux disease (8, 23). Recent studies in animals and humans reveal that baclofen, a GABAB agonist, inhibits TLESR (3, 11, 13, 28). All of the studies documenting the blockade of TLESR by baclofen used the LES relaxation criteria to identify TLESR (10). During TLESR, along with LES, there is also inhibition of the other major component of the anti-reflux barrier, i.e., the crural diaphragm (21). Distension of the stomach in dogs induces TLESR, which is also associated with inhibition of the crural diaphragm (17). Simultaneous relaxation of the LES and inhibition of crural diaphragm also occurs during distension of the esophagus in cats (1). The effect of baclofen on the crural diaphragm inhibition during TLESR and esophageal distension is not known. The goals of our study were two folds; 1) to determine the effects of baclofen on the gastric distension mediated LES relaxation and crural diaphragm inhibition, the two important components of the TLESR. 2) To determine the effects of baclofen on the esophageal distension mediated relaxation of the LES and inhibition of crural diaphragm.

MATERIALS AND METHODS

Diaphragm Electrodes Implantation

These studies were conducted in 5 female adult cats, weighing between 3 to 3.7 kg. The protocol for the study was approved by “The Animal Ethics Committee of the University of California, San Diego and San Diego VA Hospital.” Under general anesthesia induced by Xylazine 1.5 mg/kg i.m and maintained by isoflurane 2-3% inhalation, a mid-line laparotomy was performed. Six stainless steel wire electrodes (MYO/WIRE, A & E
Medical Co., Farmingdale, NJ) were implanted into the left diaphragm, three into the crural and other three into the costal diaphragm, in each cat. These electrodes have a 1 cm bare wire, which is embedded into the muscle. The wire electrodes were parallel to each other and spaced 1 cm apart. The other ends of the electrodes were tunneled through the abdominal wall and exited outside the body on the left side of the abdomen. The laparotomy incision was closed and the animals were allowed to recover for at least one week before the actual recording sessions.

**Data Recordings**

Animals were fasted overnight before each recording session. On the study day, telazol (Ford Dodge Animal Health, Ford Dodge, Iowa) 10 mg/kg was given intramuscularly to induce anesthesia. An intravenous line was established for maintenance of anesthesia and the injection of baclofen. The anesthesia was maintained with telazol, 5 mg/kg i.v., administered every 20 minutes. Animals were placed on a heating pad for maintenance of body temperature. The animals breathed spontaneously during the entire experiments. A manometric catheter was placed through the mouth into the esophagus and stomach for measurement of the esophageal, LES and stomach pressures. The manometry catheter was equipped with a 6 cm-long reverse perfused sleeve sensor to measure the LES pressure and side holes to measure pressure in the stomach (2 cm below the LES) and esophagus at 2 and 8 cm above the LES. A 2 cm-long balloon with a diameter of 2.5 cm (with 10 ml air) was placed with its center at 4 cm above the LES. Each of the side holes and the sleeve sensor was perfused at a rate of 0.5 ml/min, using a pneumohydraulic infusion system (Arndorfer Inc., Milwaukee, WI). The diaphragm electromyogram (EMG) signals were recorded using an amplifier (J & J electronic). The integrated EMG
output from the amplifier was recorded along with the pressures on a computer using a PC-Polygraph (Medtronic, Minneapolis/St. Paul, MN). Distension of the esophagus was performed by manually inflating the balloon with 4, 6, 8, and 10 ml of air, each for 30 seconds using a hand held syringe. Each balloon volume was tested 3 times with a 1 minute resting period in between the distensions. Baclofen (Sigma, St. Louis, MO), 10 \( \mu \text{mol/kg} \) bolus was injected i.v., and 2 to 3 minutes after the injection of baclofen, esophageal distensions were repeated. The effects of gastric distension on the LES and crural diaphragm were studied on a separate day in each animal, by injecting 60 ml of air into the stomach. The air injections were completed over a period of 5-10 seconds using a hand held syringe. Our preliminary studies revealed that gastric distension with 60 ml air induced long periods of LES relaxation consistently. The air was kept in the stomach for a period of 60 seconds, and then withdrawn using a syringe. The volume of the withdrawn air was measured. Eighty to 90\% of the injected air was recovered from the stomach after each gastric distension. The gastric distensions were repeated three times in each animal. A 2 minutes interval was allowed between gastric distensions. If an esophageal contraction occurred immediately after the air injection, gastric distension was terminated by withdrawing air from the stomach.

**DATA ANALYSIS**

The LES pressure was referenced to the end expiratory gastric pressure prior to the injection of air into the stomach. The basal LES pressure was measured over a 10 seconds period prior to each esophageal and gastric distension. The basal diaphragmatic EMG was measured as the mean inspiratory oscillations over a 10 seconds period, prior to the distension, in reference to end expiratory baseline. The diaphragmatic EMG was
measured in arbitrary units (a.u.). The percent LES relaxation and the percentage crural
diaphragmatic EMG inhibition during esophageal and gastric distension were calculated.
The incidence of LES relaxation and crural diaphragm inhibition were also calculated.
For the incidence calculations, the LES relaxation and crural diaphragm inhibition were
said to occur only if the drop in pressure and EMG activity was greater than 50% of the
baseline values respectively. The duration of LES relaxation and crural diaphragm EMG
inhibition were measured from the 50% values. The esophageal contraction amplitude,
proximal to the balloon (8 cm above the LES) was measured during esophageal
distension. Data were analyzed using paired t-tests and are presented as mean ± SEM.

RESULTS

Effect of Esophageal Distension on the LES and Crural Diaphragm

Esophageal distension by the balloon induced simultaneous relaxation of the LES and
inhibition of the crural diaphragm. The LES relaxation was observed for the tonic LES
pressure, as well as for the inspiration induced LES pressure oscillations. Along with
LES relaxation, esophageal distension also induced inhibition of the inspiratory crural
diaphragm EMG activity. The LES relaxation during esophageal distension ranged from
80-96%. We did not observe a graded response of LES relaxation with the graded
increase in the balloon volume, in the range of balloon volumes tested. The crural
diaphragm inhibition was of the magnitude of 60-78% and was also not related to the
balloon volume. The LES relaxation during esophageal distension lasted 21.8 ± 0.4
seconds and crural diaphragm inhibition lasted 21.0 ± 0.6 seconds. An esophageal
contraction proximal to the site of esophageal distension (Figure 1) was also observed,
the amplitude of which was directly related to the volume of air injected into the balloon.
Figure 1. Effects of esophageal distension on the LES and crural diaphragm. This record shows two periods of esophageal distension. Each distension induced relaxation of the LES and inhibition of the crural diaphragm EMG activity. Note an esophageal contraction proximal to the balloon at the onset of distension and an esophageal contraction distal to the balloon following deflation of the balloon.

Effects of Baclofen on LES Pressure and Crural Diaphragm EMG During Baseline and Esophageal Distension

The basal LES pressure was not significantly affected by baclofen. The animal’s breathing or the inspiratory pattern was significantly altered by baclofen. All animals showed an increase in the amplitude of inspiratory crural and costal EMG activity. The mean inspiratory crural diaphragm EMG increased from 91.3 ± 4.8 au to 119.0 ± 7.5 au.
in five cats, \( (p < 0.002) \) (Figure 2, Figure 3). Some of the animals developed cyclic but brief periods of apnea in inspiration after baclofen administration.

**Figure 2.** Effect of baclofen on the LES pressure and crural diaphragm EMG. The inspiratory EMG activity increased following baclofen administration. Also note, the periods of apnea in inspiration. Esophageal distension, following baclofen administration, induced partial LES relaxation and no inhibition of the crural diaphragm EMG.
Figure 3. Effect of baclofen on the LES pressure and crural diaphragm EMG activity. The basal LES pressure was not affected by baclofen. On the other hand, baclofen caused an increase in the inspiratory crural diaphragm EMG activity.

The percent LES relaxation and crural diaphragm inhibition were significantly reduced by baclofen. The incidence of LES relaxation (> 50% drop in LES pressure) was 100% for all the distension volumes in the control period and was not significantly altered by baclofen (78%, 100%, 82% and 81% with 4, 6, 8 and 10 ml distensions, respectively). Pooled data revealed that the magnitude of LES relaxation was significantly reduced by baclofen at all volumes of esophageal distensions (Figure 4).

Figure 4. Pooled data of the effects of baclofen on the LES relaxation and crural diaphragm inhibition. The LES relaxation and crural diaphragm EMG inhibition were both reduced by baclofen. However, the magnitude of reduction of crural diaphragm
inhibition by baclofen was significantly greater than its effect on the reduction of LES relaxation.

Baclofen also reduced the incidence and magnitude of crural diaphragm EMG inhibition during esophageal distension (77 ± 1% vs. 13 ± 2% during esophageal distension with 10 ml, p < 0.001). Following baclofen administration, it was more difficult to elicit crural diaphragm inhibition than it was to elicit LES relaxation. In other words, the blockade of crural diaphragm inhibition was more sensitive to baclofen than the blockade of LES relaxation. The duration of LES relaxation was significantly reduced by baclofen (21 ± 0.6 vs. 16.5 ± 0.6 seconds, p < 0.001). The amplitude of esophageal contraction, proximal to the distension site was reduced by baclofen (from 38 ± 4 mm Hg to 22 ± 8 mm Hg during 10 cc distension). However, the difference in the contraction amplitude before and after baclofen was not statistically significant (p = 0.10).

**Effect of Gastric Distension on the LES and Crural Diaphragm**

Gastric distension with 60 ml air induced a prolonged period of LES relaxation (mean 48 seconds) and an increase in the esophageal pressure (a common cavity phenomenon). The latter was defined as an increase in intra-esophageal pressure of >2 mm Hg. The LES relaxation occurred within 30 seconds of the completion of air injection into the stomach. The LES relaxation was accompanied by inhibition of the crural, but not the costal diaphragm EMG activity, and was usually terminated by a peristaltic esophageal contraction (Figure 5). The LES relaxation and crural diaphragm inhibition was observed during 91% of the gastric distensions. The percent LES relaxation and crural diaphragm
inhibition during all gastric distensions in five cats was 76 ± 1% and 77 ± 1%, respectively (Figure 8). The duration of LES relaxation and crural diaphragm inhibition were 48 ± 3 seconds and 41 ± 3 seconds, respectively.

**Figure 5.** Effects of gastric distension on the LES pressure and crural diaphragm EMG activity: Sixty ml air was injected slowly into the stomach with the help of a hand held syringe. Note relaxation of LES and inhibition of crural diaphragm EMG activity. Costal diaphragm activity increased during periods of LES relaxation. Also, note an increase in esophageal pressure or a common cavity during LES relaxation. The LES relaxation was terminated by a peristaltic esophageal contraction.

**Effect of Baclofen on the LES Relaxation and Crural Diaphragm Inhibition Induced by Gastric Distension**
Gastric distension induced LES relaxation and crural diaphragm inhibition was blocked by baclofen (Figure 6).

**Figure 6.** Effect of baclofen on gastric distension induced LES relaxation and crural diaphragm inhibition: Note the absence of LES relaxation and crural diaphragm inhibition in response to distension.

Baclofen reduced the incidence of LES relaxation response from 91% to 28% (p < 0.01) (Figure 7). Similarly, the incidence of crural diaphragm inhibition, in response to gastric distension, was reduced by baclofen (91% vs. 11%). The duration of LES relaxation and crural diaphragm inhibition were significantly reduced by baclofen as well (48.5 ± 2.8 sec vs. 19 ± 0.9 sec for LES and 41.1 ± 3.1 sec vs. 3 ± 0 sec for crural diaphragm; p < 0.05). Pooled data show that the magnitude of LES relaxation during gastric distension
in the control and post-baclofen periods were 76 ± 0.8% and 30 ± 1.3% respectively. The magnitude of crural diaphragm inhibition was also significantly reduced by baclofen (77 ± 1% vs. 17 ± 1%), (p<0.01, Figure 8).

**Figure 7.** Incidence of LES relaxation and crural diaphragm inhibition before and after baclofen, in response to gastric distension with 60 ml of air. The relaxation/inhibition was said to occur if the reduction in LES pressure and inhibition in crural diaphragm EMG activity was greater than 50% of the baseline activity. The incidence of both, LES relaxation and crural diaphragm inhibition was significantly reduced by baclofen.  

**Figure 8.** Pooled data on the effects of baclofen on the gastric distension induced LES relaxation and crural diaphragm inhibition. Note that the LES relaxation and crural diaphragm inhibition were significantly reduced by baclofen.
DISCUSSION

Our data indicates that distension of the esophagus and stomach elicits simultaneous relaxation of the LES and inhibition of crural diaphragm in the lightly anesthetized cats. Baclofen, a GABA<sub>B</sub> agonist, antagonizes LES relaxation and crural diaphragm inhibition induced by esophageal and gastric distensions. Our findings (in cats) of the effects of baclofen on LES relaxation and crural diaphragm inhibition are in agreement with the inhibitory effect of baclofen on the TLESR observed in other animal species and humans (3,11, 13, 27). Most importantly, we find that in spite of the differences in the pathways that may mediate LES and crural diaphragm inhibition during esophageal and gastric distensions, baclofen can target both components of the anti-reflux barrier function, i.e., LES and crural diaphragm, which are crucial in preventing reflux of gastric contents into the esophagus (20).
Transient LES relaxation was first reported in humans using a sensor that allowed continuous monitoring of the LES pressure over extended periods of time, i.e. sleeve device (8). Even though the majority of studies on TLESR have been conducted in humans, the phenomenon has been recorded in dogs (6, 11, 18), cats and ferrets (3). The LES relaxation is the major criteria for identification of TLESR in the human as well as animal studies (10). It is now clear, however, that along with the LES, several other regions i.e., the esophagus (27), stomach and crural diaphragm, are inhibited during TLESR. Crural diaphragm inhibition during TLESR has been reported in humans (21), and dogs (17, 24). It seems that the inhibition of the crural diaphragm is essential for the occurrence of gastroesophageal reflux during TLESR because in the absence of LES tone gastroesophageal reflux does not occur unless there is inhibition of the crural diaphragm (12, 20, 22).

In the human studies, the frequency of spontaneous TLESR is reduced by more than 50% with a single oral dose of baclofen 40 mg (13). Baclofen has also been reported to inhibit TLESR in dogs (11) and ferrets (3). In all of the studies that documented the inhibition of TLESR by baclofen, the LES relaxation criterion was used to identify TLESR. From the studies in the literature, it is not clear if baclofen has any influence on the crural diaphragm inhibition that accompanies LES relaxation during TLESR. Our study, for the first time, shows that baclofen blocks both components of TLESR, i.e., LES relaxation and crural diaphragm inhibition. Furthermore, we found that the LES relaxation and crural diaphragm inhibition induced by esophageal distension (1, 6) is also blocked by baclofen.
Our data shows quantitative differences in the baclofen induced blockade of the LES relaxation and crural diaphragm inhibition caused by esophageal distension. We found that the incidence as well as the magnitude of crural diaphragm inhibition was much more affected by baclofen than the incidence and magnitude of LES relaxation, a finding that may have clinical relevance. GABA$_B$ agonists, because of their inhibitory effect on the TLESR frequency, may have a role in the treatment of reflux disease. However, the blockade of esophageal distension induced LES relaxation by baclofen is undesirable and can impede esophageal transit thus resulting in symptoms of dysphagia. Our finding that the blockade of esophageal distension induced LES relaxation is less sensitive to baclofen is actually desirable because such an effect is likely to protect against dysphagia.

Several studies indicate that TLESR is a neural reflex, which is mediated via vagal afferent, brain stem and vagal efferent pathways (18, 20). It appears that gastric distension stimulates mechanoreceptors located in the fundus and the lesser curvature of the stomach (9). The afferent signals from these mechanoreceptors traverse via vagal afferent pathways and relay in the nucleus tractus solitareous (NTS) of the vagal complex. The NTS in turn stimulates motor neurons of the dorso motor nucleus of the vagus nerve, (DMV), which in turn sends efferent signals via the vagus nerve to the LES. The mechanism by which gastric distension elicits crural diaphragm inhibition is not known. The esophageal distension mediated relaxation of the LES and inhibition of crural diaphragm is also mediated via esophageal mechanoreceptors, vagal afferents and NTS. How NTS mediates crural diaphragm inhibition is also not known. One possibility is that signals from NTS cause inhibition of crural diaphragm motor neurons (central
However, Altschuler et al failed to find inhibition of inspiratory medullary motor neurons and suggested that the cervical spinal motor neuron may be the site of inhibition (2). We recently reported an evidence for a peripheral mechanism of esophageal distension mediated crural diaphragm inhibition (distal to the spinal cord) (16). The precise nature of this peripheral mechanism of inhibition however, is not clear.

How does baclofen block LES relaxation and crural diaphragm inhibition? Baclofen acts at several sites in the reflex pathway of TLESR. It inhibits the gastric distension mediated mechanoreceptors activity and vagal afferent discharge (25). GABA_B receptors are present in the DMV complex and may have a central site of action, thus influencing the efferent discharges to the LES (19, 26). GABA_B may also reduce the peripheral, preganglionic discharges that mediate LES relaxation (4). Our study does not address the mechanism and site of action of baclofen. However, it is interesting that there are similarities in the two pathways that bring about LES relaxation and crural diaphragm inhibition during esophageal distension and gastric distension that can be targeted by baclofen. Beside baclofen, general anesthesia (7, 14) and blocker of nitric oxide synthase (LNAME) (5, 15) have also been shown to inhibit gastric distension, as well as esophageal distension induced relaxation of the LES and inhibition of the crural diaphragm.

In summary, our study shows that gastric distension in the lightly anesthetized cats elicits simultaneous relaxation of LES and inhibition of crural diaphragm, a response resembling TLESR. Our observation that baclofen can block LES relaxation and crural diaphragm inhibition induced by esophageal and gastric distension suggests the existence of a commonality in the two distinct pathways, one that mediates LES relaxation and the
other crural diaphragm inhibition during TLESR. Further studies are needed to understand the nature of this commonality in the LES and crural diaphragm inhibitory pathway.
REFERENCES


