Baclofen blocks LES relaxation and crural diaphragm inhibition by esophageal and gastric distension in cats

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Liu, Jianmin, Nonko Pehlivanov, and Ravinder K. Mittal. Baclofen blocks LES relaxation and crural diaphragm inhibition by esophageal and gastric distension in cats. Am J Physiol Gastrointest Liver Physiol 283: G1276–G1281, 2002. First published May 1, 2002; 10.1152/ajpgi.00080.2002.—Esophageal distension and transient lower esophageal sphincter (TLESR) relaxation 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. We evaluated the effects of baclofen on LES relaxation and crural diaphragm inhibition induced by gastric distension and esophageal distension in cats. 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 iv) on the graded esophageal distension and gastric distension-induced LES and crural diaphragm responses were studied. 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. Baclofen, a GABAB receptor agonist, blocks the 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.

Materials and Methods

Diaphragm electrodes implantation. These studies were conducted in five female adult cats, weighing between 3 and 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 Veterans Affairs Hospital. Under general anesthesia induced by xylazine (1.5 mg/kg im) and maintained by isoflurane (2–3% inhalation), a midline laparotomy was performed. Six stainless steel wire electrodes (model MYO/WIRE, A & E Medical, Farmingdale, NJ) were implanted into the left diaphragm (3 into the crural and 3 into the costal diaphragm) in each cat. These electrodes have a 1-cm bare wire 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 animals were allowed to recover for at least 1 wk before the actual recording sessions.

Data recordings. Animals were fasted overnight before each recording session. On the study day, telazol (10 mg/kg Fort Dodge Animal Health, Fort Dodge, Iowa) was given intramuscularly to induce anesthesia. An intravenous line was established for maintenance of anesthesia and the injection of baclofen. Anesthesia was maintained with telazol (5 mg/kg iv) administered every 20 min. Animals were placed on a heating pad for maintenance of body temperature. Animals breathed spontaneously during the experiments.

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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 of 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, Milwaukee, WI). The diaphragm electromyogram (EMG) signals were recorded using an amplifier (J & J Electronics). The integrated EMG output from the amplifier was recorded along with the pressures on a computer using a PC-Polygraph (Medtronic, Minneapolis, MN). Distension of the esophagus was performed by manually inflating the balloon with 4, 6, 8, and 10 ml of air, each for 30 s using a hand-held syringe. Each balloon volume was tested three times with a 1-min resting period between the distensions. Baclofen (Sigma, St. Louis, MO) bolus (10 μmol/kg) was injected intravenously, and 3–5 min after the injection of baclofen, esophageal distensions were repeated. 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 s using a hand-held syringe. Our preliminary studies revealed that gastric distension with 60 ml of air consistently induced long periods of LES relaxation. The air was kept in the stomach for a period of 60 s and then withdrawn by using a syringe. The volume of withdrawn air was measured. Eighty to ninety percent of the injected air was recovered from the stomach after each gastric distension. Gastric distensions were repeated three times in each animal. A 2-min 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. LES pressure was referenced to the end-expiratory gastric pressure before the injection of air into the stomach. Basal LES pressure was measured over a 10-s period before each esophageal and gastric distension. The basal diaphragmatic EMG was measured as the mean inspiratory oscillations over a 10-s period before the distension in reference to the end-expiratory baseline. The diaphragmatic EMG was measured in arbitrary units (au). Percent LES relaxation and the percentage of crural diaphragmatic EMG inhibition during esophageal and gastric distension were calculated. Incidence of LES relaxation and crural diaphragm inhibition were also calculated. For incidence calculations, LES relaxation and crural diaphragm inhibition were said to occur only if the drop in pressure and EMG activity was >50% of the baseline values, respectively. Duration of LES relaxation and crural diaphragm EMG inhibition were measured from the 50% values. Esophageal contraction amplitude, proximal to the balloon (8 cm above the LES) was measured during esophageal distension. Data were analyzed using paired Student’s t-tests and are presented as means ± SE.

RESULTS

Effect of esophageal distension on LES and crural diaphragm. Esophageal distension by the balloon induced simultaneous relaxation of the LES and inhibition of the crural diaphragm. LES relaxation was observed for tonic LES pressure as well as for inspiration-induced LES pressure oscillations. Along with LES relaxation, esophageal distension also induced inhibition of the inspiratory crural diaphragm EMG activity. LES relaxation during esophageal distension ranged from 80 to 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. Crural diaphragm inhibition was of the magnitude of 60–78% and was also not related to balloon volume. LES relaxation during esophageal distension lasted 21.8 ± 4.4 s, and crural diaphragm inhibition lasted 21.0 ± 6.4 s. An esophageal contraction proximal to the site of esophageal distension (Fig. 1) was also observed, the amplitude of which was directly related to the volume of air injected into the balloon.

Effects of baclofen on LES pressure and crural diaphragm EMG during baseline and esophageal distension. 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. Mean inspiratory crural diaphragm EMG increased from 91.3 ± 4.8 to 119.0 ± 7.5 au in five cats (P < 0.002) (Figs. 2 and 3). Some of the animals developed cyclic but brief periods of apnea in inspiration after baclofen administration. 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 (Fig. 4). 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). After baclofen administration, it was more difficult to elicit crural dia-
phragm 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. Duration of LES relaxation was significantly reduced by baclofen (21 ± 0.6 vs. 16.5 ± 0.6 s, \( P < 0.001 \)). The amplitude of esophageal contraction proximal to the distension site was reduced by baclofen (from 38 ± 4 to 22 ± 8 mmHg during 10-ml 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 of air induced a prolonged period of LES relaxation (mean 48 s) and an increase in the esophageal pressure (a common cavity phenomenon). The latter was defined as an increase in intraesophageal pressure of >2 mmHg. LES relaxation occurred within 30 s of the completion of air injection into the stomach. 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 (Fig. 5). LES relaxation and crural diaphragm inhibition were observed during 91% of gastric distensions. The percent LES relaxation and crural diaphragm inhibition during all gastric distensions in five cats were 76 ± 1 and 77 ± 1%, respectively (see Fig. 8). Durations of LES relaxation and crural diaphragm inhibition were 48 ± 3 and 41 ± 3 s, respectively.

**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 (Fig. 6). Baclofen reduced the incidence of LES relaxation response from 91 to 28% (\( P < 0.01 \)) (Fig. 7). Similarly, the incidence of crural diaphragm inhibition, in response to gastric distension, was reduced by baclofen (91 vs. 11%). Duration of LES relaxation and crural diaphragm inhibition were significantly reduced by baclofen as well (48.5 ± 2.8 vs. 19 ± 0.9 s for LES and 41.1 ± 3.1 vs. 3 ± 0 s for crural diaphragm; \( P < 0.05 \)). Pooled data show that the magnitudes 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%) (\( P < 0.01 \), Fig. 8).

**DISCUSSION**

Our data indicate that distension of the esophagus and stomach elicits simultaneous relaxation of the LES and inhibition of crural diaphragm in the lightly anesthetized cat. Baclofen, a GABAB agonist, antagonizes LES relaxation and crural diaphragm inhibition induced by esophageal and gastric distensions. Our (3, 11, 13, 27) 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. Most importantly, we find that, despite 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 antireflux barrier function, i.e., LES and crural diaphragm, which are crucial in preventing reflux of gastric contents into the esophagus (20).

TLESR was first reported (8) in humans using a sensor that allowed continuous monitoring of the LES pressure over extended periods of time, i.e., sleeve device. Although 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 criterion 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 human studies (13), the frequency of spontaneous TLESR is reduced by >50% with a single oral dose of baclofen (40 mg). 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 literature, it is not clear whether 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 LES relaxation and crural diaphragm inhibition induced by esophageal distension (1, 6) is also blocked by baclofen.

Our data show 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 (18, 20) indicate that TLESR is a neural reflex, which is mediated via vagal afferent, brain stem, and vagal efferent pathways. It appears that gastric distension stimulates mechanoreceptors

Fig. 4. Pooled data of the effects of baclofen on the LES relaxation (A) and crural diaphragm inhibition (B). 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.

Fig. 5. Effects of gastric distension on the LES pressure and crural diaphragm EMG activity. Air (60 ml) 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. LES relaxation was terminated by a peristaltic esophageal contraction.

Fig. 6. Effect of baclofen on gastric distension-induced LES relaxation and crural diaphragm inhibition. Note absence of LES relaxation and crural diaphragm inhibition in response to distension.
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 solitarii (NTS) of the vagal complex. The NTS in turn stimulates motor neurons of the dorsomotor 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 the 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 mechanism). However, Altschuler et al. (2) failed to find inhibition of inspiratory medullary motor neurons and suggested that the cervical spinal motor neuron may be the site of inhibition. We recently reported 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 mechanoreceptor 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

Fig. 7. Incidence of LES relaxation (A) and crural diaphragm inhibition (B) before and after baclofen, in response to gastric distension with 60 ml of air. Relaxation/inhibition was said to occur if the reduction in LES pressure and inhibition in crural diaphragm EMG activity was \( >50\% \) of the baseline activity. The incidence of both LES relaxation and crural diaphragm inhibition was significantly reduced by baclofen.

Fig. 8. Pooled data on effects of baclofen on gastric distension-induced LES relaxation (A) and crural diaphragm inhibition (B). Note that the LES relaxation and crural diaphragm inhibition were significantly reduced by baclofen. \( *P < 0.01 \).
be targeted by baclofen. Besides baclofen, general anesthe-
sia (7, 14) and a blocker of nitric oxide synthase (nitro-
arginine methyl ester) (5, 15) have also been shown to inhibit gastric distension, as well as esoph-
geal distension-induced relaxation of the LES and in-
hibition of the crural diaphragm.

In summary, our study shows that gastric distension in lightly anesthetized cats elicits simultaneous relaxa-
tion of the LES and inhibition of crural diaphragm, a response resembling TLESR. Our observation that ba-
clofen can block LES relaxation and crural diaphragm inhibition induced by esophageal and gastric disten-
sion 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 pathways.

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