Gastric electrical stimulation inhibits postprandial antral tone partially via nitric pathway in conscious dogs

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Sun, Ying, and J. D. Z. Chen. Gastric electrical stimulation inhibits postprandial antral tone partially via nitric pathway in conscious dogs. Am J Physiol Regul Integr Comp Physiol 290: R904–R908, 2006. First published November 10, 2005; doi:10.1152/ajpregu.00842.2004.—Gastric electrical stimulation (GES) has recently been explored as a therapeutic option for gastrointestinal motility disorders or obesity. The mechanism behind it is not fully elucidated. The aims of this study were therefore to study the effects of the GES with different parameters on antral tone and to explore the involvement of the nitric pathway. Eight dogs equipped with a gastric cannula and one pair of serosal electrodes in the greater curvature 4 cm above the pylorus were studied on separate days. The study was composed of seven randomized sessions in the fed state [control, GES with different parameters, and GES plus neuronal nitric oxide synthase (nNOS) inhibitor]. Each session included three consecutive 30-min periods (baseline, GES, and recovery). GES was performed with long pulses or pulse trains. The antral volume was measured using an intragastric balloon connected with a barostat device. It was found that 1) postprandial antral tone was reduced with GES with all tested parameter settings, reflecting as a significant and substantial increase in antral volume ranging from 179 to 309%; 2) the inhibitory effect of GES on antral tone was partially blocked (decreased by 39.5%) with an nNOS inhibitor; and 3) mild symptoms were induced with GES and found to be correlated with the GES-induced increase in antral volume. We conclude that retrograde GES with long pulses or pulse trains inhibits antral tone, and this inhibitory effect is partially mediated via the nitric pathway. These results suggest that retrograde GES may have a therapeutic potential for obesity.

GASTRIC ELECTRICAL STIMULATION (GES) has recently been explored as a therapeutic option for gastrointestinal motility disorders and obesity. On the basis of stimulation pulse width, GES can be classified into the following three categories: long pulses (in the order of ms), short pulses (in the order of μs), and pulse trains. GES with long pulses at the physiological frequency of the intrinsic gastric slow wave has been reported to entrain gastric slow waves and normalize gastric dysrhythmia (22, 24, 25). Slow wave entrainment by GES can promote gastric emptying if the stimulation is applied in the primary pacemaker area. GES with short pulses at a frequency a few times of the gastric slow wave is able to improve symptoms of nausea and vomiting without entraining gastric slow waves in both dogs and gastroparetic patients (1, 6). GES with pulse trains was noted to accelerate the movement of gastric solid contents in anesthetized dogs (27), possibly via a neural mechanism. According to the location of stimulation electrodes, GES can be classified into retrograde and forward stimulation. Retrograde stimulation was reported to delay gastric emptying of digestible solids and liquids in healthy dogs (9). Forward stimulation was found to improve dyspeptic symptoms and gastric emptying in patients with gastroparesis (11, 12, 23), including diabetic gastroparesis and gastric stasis after Roux gastrectomy (26). Recently, implantable gastric stimulation with pulse trains was reported to induce early satiety and reduce body weight in patients with morbid obesity (7).

Mechanisms involved in the effects of GES on gastric motility and food intake have not been well studied. Although GES has been reported to reduce the tone of the proximal stomach (19, 20), its effects on the tone of the distal stomach (antral tone) have not been studied. Nitric oxide (NO) is considered as a major inhibitory neurotransmitter of nonadrenergic noncholinergic nerves. NO has been reported to regulate the accommodation reflex of the fundus (37) and the peristaltic reflex of the intestine (14). A recent study suggests that there are much less nitric oxide synthase (NOS)-containing neurons in the antrum compared with the fundus (45). Assuming that GES inhibits antral tone, it is unknown whether NOS is involved.

The aims of this study were therefore to study the effects of GES on antral tone assessed by barostat and the possible involvement of NOS via administration of neuronal NOS (or nNOS) inhibitor in conscious dogs.

MATERIALS AND METHODS

Preparation of Animals

The study was performed in eight healthy female hound dogs (17–21 kg). After an overnight fast, the dogs were anesthetized with intravenous infusion of thiopental sodium (5 mg/kg, Pentothal; Abbott Laboratories, North Chicago, IL) and maintained with inhalation of isoflurane (1–2%; Abbott Laboratories). A midline laparotomy was performed, and one pair of 28-gauge cardiac pacing wires (A & E Medical, Farmingdale, NJ) was implanted on the serosal surface of the distal stomach along the greater curvature 4 cm above the pylorus. Two electrodes in the pair were 1 cm apart. The electrode wires were subcutaneously tunneled through the anterior abdominal wall along the right side of the trunk and were placed outside the skin around the right hypochondrium for attachment to the recorder or stimulator. A cannula was placed on the anterior gastric wall 10 cm above the pylorus. All dogs were given a recovery period of at least 2 wk. The Institutional Animal Care and Use Committee at the Oklahoma City...
Veterans Affairs Medical Center approved the surgical and experimental protocol.

**Experimental Protocol**

The study was performed in the following eight randomized sessions: control without stimulation, GES with long pulses (2 sessions), GES with train of pulses (3 sessions), GES with NOS inhibitor, and preprandial session. Before each study session, the dog was fasted overnight with free access to water. Each session (except GES preprandial session) consisted of the following three 30-min postprandial periods: baseline, GES (or no GES), and recovery. In the GES with nNOS inhibitor session, specific nNOS inhibitor 7-nitroindazole (7-NI, 25 mg/kg ip) was injected 1 h before the preprandial recording. In the GES preprandial session, two 30-min sessions were included, and GES was applied during the second 30 min. GES was applied during the second 30-min postprandial period.

**Parameters of GES**

Stimulation parameters for two GES sessions using long pulses included: 1) pulse width of 300 ms, amplitude of 10 mA, and a physiological frequency of 5.5 cycles/min (cpm) and 2) the same as the first but with a pathological frequency of 10 cpm. Stimulation parameters for three GES sessions using pulse trains were 1) train on-time of 2 s, off-time of 8 s, and pulses within the trains with a frequency of 20 Hz, width of 5 ms, and amplitude of 6 mA; 2) same as set 1 except with a train off-time of 3s; and 3) same as set 2 except with a pulse amplitude of 10 mA. In the sessions with GES plus NOS inhibitor and preprandial GES, the stimulation parameters were the same as set 2. These parameters were chosen to study the effects of GES with different pulse frequencies (physiological or pathological), different train frequencies, or different stimulation amplitudes on antral tone.

**Placement of Catheter and Measurement of Antral Tone/Volume**

In each study session, the dog was in a standing position and slightly restrained from movement with a sling. The gastric fistula was uncorked, and a double-lumen polyvinyl tube with an adherent noncompliant plastic bag (450 ml capacity), finely folded, was introduced through the gastric cannula toward the direction of the distal stomach (Fig. 1). The catheter was secured on the cannula with tape. The polyvinyl tube was connected to a computer-driven programmable barostat device (G & J Electronics, Willowdale, Ontario, Canada). A constant intragastric pressure was set with the minimal distending pressure plus 2 mmHg. After a 15-min equilibration period, either the preprandial antral volume was recorded for 60 min or the dog was fed with a solid meal of 395 kcal (1% fiber, 8% protein, 3% fat). The postprandial antral volume was continuously recorded for 90 min. The barostat device was operating in such a way that it pumped air in the intra-antral bag when there was a reduction in antral tone, and it withdrew air out from the intra-antral bag when there was an increase in antral tone so that a constant pressure was maintained within the bag. Accordingly, the measured antral volume was inversely proportional to antral tone (35, 36, 40).

**Assessment of Behaviors**

Behaviors of the dogs before and during the stimulation period were recorded in each session. These included salivation, licking tongue, murmuring, movement because of discomfort, and dry or liquid vomiting. The severity of the symptoms was classified into the following four degrees: none = 0, mild = 1, moderate = 2, and severe = 3. Vomiting was noted separately and scored as three. Accordingly, the highest symptom score for each dog would be 15. This method of assessment was validated in a previous study (6).

**Calculation of Stimulation Energy**

Stimulation energy, E, was calculated according to the following formula

$$ E = \text{pulse width} \times \text{no. of pulses/min} \times \text{amplitude}^2 $$

$$ \text{Pulse trains: E/min} = \text{on time (s)} \times \text{pulse frequency (Hz)} \times \text{pulse width} \times \text{trains/min} \times \text{amplitude}^2 $$

**Statistical Analysis**

Results in the text and Figs. 1–5 are expressed as means ± SE. Statistical significance of differences (P < 0.05) between the mean values in different sessions was assessed with one-way ANOVA and a post hoc Tukey’s test. Mann-Whitney Rank Sum Test and Kruskal-Wallis one-way ANOVA on Ranks were used to assess the difference in the behavior score among different sessions. Linear regression was used for analyzing the relationships between the gastric volume and the stimulation energy or the behavior score. The software program used was SigmaStat (Jandel Scientific, San Rafael, CA).

**RESULTS**

**Effects of GES on Antral Tone in Postprandial State**

GES with long pulses. GES with long pulses at 10 cpm significantly decreased postprandial antral tone. The gastric volume during the GES period was 278.8 ± 47.1 ml, which was significantly and substantially higher than 68.1 ± 22.5 ml (increased 309%) in the corresponding period in the control session without GES (P < 0.005, see Fig. 2). GES at 5.5 cpm, however, did not result in a significant change in antral tone (P = 0.06).

GES with pulse trains. A significant decrease in gastric tone was noted with GES of pulse trains of all three parameters sets. As shown in Fig. 2, the antral volume was 230.6 ± 65.3 ml (increased 239%) with GES of 8 s off, 263.1 ± 50.8 ml (increased 286%) with GES of 3 s off and 10 mA, and 264.0 ± 35.3 ml (increased 287%) with GES of 3 s off and 6 mA; all of those were significantly and substantially higher than that in the corresponding period in the control session without GES.
The increase in gastric volume with GES was, however, not correlated with the stimulation energy ($r = 0.113$, $P = 0.487$; Fig. 3).

Involvement of NOS with GES-induced Gastric Tone

Pretreatment of 7-NI partially blocked the effect of GES on postprandial antral tone. As shown in Fig. 2 (see columns “3s-off” and “3s-off + 7-NI”), the postprandial antral volume was 66.0 ± 22.1 ml in the control session and increased to 264.0 ± 35.3 ml with GES of 3 s off at the absence of 7-NI ($P < 0.001$ vs. control) and 159.8 ± 22.4 ml with the same GES but in the presence of 7-NI ($P < 0.05$ vs. control and vs. GES without 7-NI). These data indicate that the GES-induced reduction in antral tone was partially blocked (decreased by 39.5%) by 7-NI, suggesting the involvement of nNOS and possibly other mechanisms.

Effects of GES on Antral Tone in the Preprandial State

GES with pulse trains of 3 s off and 6 mA significantly decreased antral tone in the preprandial state. The preprandial antral volume was 89.8 ± 11.1 ml during baseline and 227.9 ± 39.7 ml ($P < 0.05$ vs. control; increased 154%) during GES. The antral volume increase induced by GES in the fasting state was similar with that in the postprandial state with GES at 6 mA ($P = 0.510$).

Effects of GES on Behaviors

Mild illness behaviors were induced with GES of different patterns and parameter sets (Fig. 4, $P < 0.05$, ANOVA). The possible highest score was 15, but the actually observed highest score was 1.5, which was noted during GES of long pulses at a frequency of 10 cpm. With GES of long pulses, an increase in stimulation frequency significantly increased the illness behavior score ($P < 0.05$, 10 vs. 5.5 cpm). However, with GES of pulse trains, no such significant difference was noted among stimulation sessions with different stimulation energy. The illness behavior score in the session with GES plus 7-NI was lower (0.6 ± 0.5) than that in the session with GES without 7-NI (1.4 ± 0.5), but the difference was not statistically significant ($P = 0.24$). A positive correlation was obtained between the total averaged illness behavior score and the antral volume ($r = 0.436$, $P < 0.01$; see Fig. 5), suggesting that the behaviors might be attributed to antral distention or reduction in antral tone.

DISCUSSION

This was, to the best of our knowledge, the first study investigating the effects and mechanisms of GES on antral tone. The main findings were as follows: 1) retrograde GES with long pulses or pulse trains significantly and substantially inhibited antral tone, and this inhibitory effect was partially mediated via the nitrergic pathway and 2) mild symptoms were induced with GES, and the total symptom score was correlated with the GES-induced antral distention or relaxation.

![Fig. 2. Effects of gastric electrical stimulation (GES) with different stimulation parameters on gastric volume. 7-NI, 7-nitroindazole; cpm, cycles/min. Values are means ± SE. Compared with control group, *$P < 0.05$ and **$P < 0.01$. Compared with 3 s off, #$P < 0.05$.](image)

![Fig. 3. Correlation between gastric volume and stimulation energy of GES. Plotted values are means of the gastric volume (ml) and the energy/min in different groups ($r = 0.113$, $P = 0.487$).](image)

![Fig. 4. Effects of GES with different stimulation parameters on behaviors. Values are means ± SE. Compared with GES at normal frequency, *$P < 0.05$.](image)

![Fig. 5. Correlation between the total illness behavior scores and antral volume. Plotted values were total illness behavior score and antral volume ($r = 0.436$, $P < 0.01$).](image)
Similar to the tone of the proximal stomach, the tone of the distal stomach has recently been reported to be of great significance in the physiology of gastric motility and pathophysiology of functional dyspepsia. Antral tone has been thought to play an important role in gastric emptying (17, 33). A reduced antral tone resulted in a delay of gastric emptying (32). The distention of the antrum was reported to cause dyspeptic symptoms in healthy volunteers (19). A wider gastric antrum has been seen in patients with diabetes (38) and functional dyspepsia (15).

GES has been under intensive investigation for its therapeutic potentials for gastroparesis (1, 6, 22, 24, 25) and obesity (7, 10, 13). Although there have been a number of studies investigating the effects of GES on various components of gastric motility, nothing has been reported on the effect of GES on antral tone. Forward GES or GES at the proximal stomach at the physiological frequency is known to entrain gastric slow waves (24), normalize gastric dysrhythmias (6, 16, 29, 43), improve nausea and vomiting in patients with gastroparesis (2), induce gastric contractions in dogs (9), and accelerate gastric emptying in a canine model (32) and patients with gastroparesis (2). Recently, GES was reported to reduce proximal gastric tone (41). Its effect on proximal tone was related to the intensity of stimulation (34) and was mediated by the nitricergic pathway. The effect and mechanisms of GES on antral tone had not been studied previously.

Antral tone was found for the first time to be substantially inhibited with GES of either long pulses or pulse trains in this study. The inhibitory effect of retrograde GES on the antral tone seems similar to the proximal gastric tone (34). However, we didn’t find a relationship between the achieved antral volume and the stimulation energy in this study. This result was not consistent with our finding in the proximal stomach (34). The discrepancy might be attributed to differences in anatomy, motility activity, and nerve innervation between the proximal and the distal stomach. 1) The proximal stomach serves as a short-term storage reservoir and shows low-frequency, sustained contractions that are responsible for generating a basal pressure within the stomach. However, the distal stomach develops strong peristaltic waves of contraction that increase in amplitude as they propagate toward the pylorus. 2) Innervation of vagal afferent and efferent fibers is different between the proximal and the distal stomach. A study recorded from single gastric afferent fibers found that afferent fibers arise from receptors in the antrum showed modulation in phase with spontaneously occurring antral contractions, whereas afferent fibers from the corpus and fundus discharged at irregular rates with no correlation with the intragastric pressure rises associated with the antral contractions (3). Recording from single efferent fibers in the urethane-anesthesia- tized ferret showed excited firing responded to distension of the gastric corpus while a reduction in firing responded to antral distension (4). 3) We believe that the distal antrum is not as distendable as the proximal stomach. It might have reached the maximal distendable volume with relatively low stimulation energy, and thus no linear correlation was observed.

Concurrent to the antral relaxation, mild symptoms were noted during GES. The correlation between the symptom score and the antral volume observed in this study suggests that the GES-induced antral relaxation may cause symptoms. This is consistent with previous findings of reduced antral tone in patients with dyspeptic symptoms. On the other hand, one should note that the symptoms induced with GES in this study were mild (the highest score was 10% of the possible maximum score), although the reduction in antral tone was substantial. These results are interesting and promising for the application of GES in treating obesity: it might be possible to delay or slow the digestive process without inducing noticeable dyspeptic symptoms. This concept was supported by a recent preliminary clinical study in which GES at the distal stomach with parameters similar to the present study resulted in a delayed emptying of solids and a reduced food intake without any noticeable symptoms in humans (44).

The mechanism of action involved with the GES-induced antral relaxation was also investigated in this study. Previous studies have shown that gastric tone of the proximal stomach in animals and humans is maintained by a vagally mediated cholinergic input (21). Recently, gastric fundic tone was found dependent on continuous nitricergic input in the fasting state (7, 35). One study recently performed in our laboratory (42) showed that the inhibitory effect of GES on proximal gastric tone in dogs was partially abolished by the NOS blocker N\textsuperscript{G}~\text{nitro}-l-arginine. The involvement of NOS in the regulation of antral tone caused by GES has not been reported previously. Immunohistochemical studies have shown a reduced expression of NOS in the antrum compared with the fundus in guinea pigs (39) and in rats (45). The NOS inhibitor N\textsuperscript{G}~\text{monomethyl}-l-arginine did not significantly alter antral motility in humans (18) but seemed to exert a tonic inhibitory action on the porcine antral motility (31). NOS can be divided into Ca\textsuperscript{2+}-dependent nNOS and endothelial NOS and Ca\textsuperscript{2+}-independent inducible NOS. Studies on NOS enzyme activities have revealed that only Ca\textsuperscript{2+}-dependent forms exist in the rat stomach (28, 30) and >90% of NO is attributable to nNOS (28). nNOS plays a key role in the autonomic control of gastrointestinal motility. Therefore, we chose a specific NOS inhibitor (7-Ni) in this study. The results showed a partial prevention of the GES-induced antral relaxation with 7-NI, suggesting the involvement of nNOS. The present data also suggest the involvement of other mechanisms, since the nitricergic pathway accounted for only 40% of the effect. Retrograde GES at the tachygastrial frequency is known to induce gastric dysrhythmia and inhibit antral motility (5). Induction of gastric dysrhythmia with retrograde GES was reported to inhibit postprandial antral motility; conceivably, it should also reduce antral tone (46).

In conclusion, GES at the distal stomach with sufficient output inhibits antral tone in the fed state, and this inhibitory effect is at least in part mediated by nNOS. The GES-induced inhibitory effect on antral tone suggests a therapeutic potential of retrograde GES for obesity.

GRANTS

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REFERENCES


