Synchronized gastric electrical stimulation improves delayed gastric emptying in nonobese mice with diabetic gastroparesis

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Song G-Q, Chen JD. Synchronized gastric electrical stimulation improves delayed gastric emptying in nonobese mice with diabetic gastroparesis. J Appl Physiol 103: 1560–1564, 2007. First published August 23, 2007; doi:10.1152/japplphysiol.00319.2007.—The aim of this study was to investigate the effect and mechanism of synchronized gastric electrical stimulation (SGES) on gastric emptying in nonobese mice with diabetic gastroparesis (DB-GP). Eight control mice and 48 nonobese diabetic (NOD) mice with two pairs of gastric electrodes were used in this study. The study included seven groups in a randomized order [control (DB), DB-GP, DB + SGES, DB-GP + SGES, DB-GP + Atropine, and DB-GP + SGES + Atropine groups]. In the control, DB, DB-GP, and DB-GP + Atropine groups, gastric emptying was measured in BLAB/cJ mice (control group) or NOD mice with a duration of diabetes of 0–7 days (DB group) or 28–35 days (DB-GP or DB-GP + Atropine group). In the DB + SGES, DB-GP + SGES, and DB-GP + SGES + Atropine groups, the experiment was the same as the corresponding DB, DB-GP, and DB-GP + Atropine groups except that SGES was applied during the experiment. SGES was applied via the proximal pair of electrodes and synchronized with the intrinsic gastric slow waves. The following results were obtained: 1) gastric emptying was delayed in NOD mice with a duration of diabetes of 28–35 days; 2) SGES was able to significantly increase gastric emptying in both diabetic mice and diabetic gastroparetic mice; and 3) the excitatory effect of SGES was completely blocked by atropine. SGES accelerates gastric emptying in NOD mice with diabetic gastroparesis. The effect of SGES on gastric emptying is mediated via the cholinergic pathway. These findings suggest that SGES may have a therapeutic potential for treating patients with diabetic gastroparesis.

Gastroparesis is defined as delayed emptying of a solid meal and is seen in 30–50% of patients with Type 1 or Type 2 diabetes mellitus (5, 9, 15, 21). Upper gastrointestinal symptoms such as early satiety, weight loss, abdominal bloating, abdominal discomfort, nausea, and vomiting occur frequently and impact quality of life seriously in patients with diabetic gastroparesis.

Though gastroparesis affects many diabetic people worldwide, treatment options are very limited. These include medical therapy, surgical therapy, and nutritional support. Surgical procedures, such as gastrectomy and antrectomy, are the last option of treatment since they involve the removal of part or all of the stomach. In patients with severe gastroparesis but normal small intestinal motility, jejunostomy tube feeding may be applied. While this provides nutritional support, it does not cure gastroparesis (27). Consequently, medical therapy is the primary option for gastroparesis. Medications for gastroparesis include metoclopramide, domperidone, cisapride, and erythromycin. While these agents have been used for treating gastroparesis, they have been reported to be of only limited efficacy, and many patients cannot tolerate them due to side effects (3, 16, 20, 34).

Recently, the therapeutic potential of gastric electrical stimulation (GES) for gastroparesis has been explored. A number of studies have been performed to show that long-pulse GES is able to entrain gastric slow waves (13, 14, 17–19, 22, 29–31, 36). A noncontrolled clinical study on nine patients with gastroparesis (mainly diabetic gastroparesis) reported that long-pulse GES significantly improved gastroparetic symptoms and gastric emptying (28). In a multicenter clinical study (1), short-pulse GES was noted to be capable of substantially reducing the frequency of nausea and vomiting. The therapy (called Enterra) received Food and Drug Administration approval for humanitarian use for gastroparesis. However, it does not seem to normalize gastric motility or emptying.

When GES is performed, two kinds of electrical events occur in the stomach: an artificial electrical stimulus and an intrinsic physiological electrical activity. With the current methods of GES, we have noticed that there is not a perfect match between these two electrical events. With short-pulse GES, the frequency is three times higher and the energy much lower than the intrinsic gastric electrical activity. Laboratory data showed that such stimulation had no effects on intrinsic gastric electrical activity (6). With long-pulse GES, the frequency is usually slightly higher than that of the intrinsic electrical activity, and the applied electrical stimulus is not in phase with the intrinsic electrical event (6). That is, the stimuli are applied at random without consideration for the occurrence of the intrinsic electrical activity.

In this study, we proposed to use synchronized GES. That is, the electrical stimulus was applied only on the detection (occurrence) of each intrinsic electrical event (or slow waves). Since each gastric slow wave represents the depolarization of gastric smooth muscles, the electrical stimulation performed upon the occurrence of the slow waves was expected to enhance the depolarization process and thus induce or enhance gastric contractions. This was similar to inducing a stronger vibration/oscillation of a subject (such as a bridge) by stimulating it at its intrinsic frequency. Therefore, the aim of this study was to investigate the effect and mechanism of synchronized GES on gastric emptying in nonobese mice with diabetic gastroparesis.

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MATERIALS AND METHODS

Animal preparation. Forty-eight female NOD/LtJ mice aged 8 wk were obtained from the Jackson Laboratory (Bar Harbor, ME). As controls, eight age- and sex-matched mice of the nonobese diabetic (NOD) sister strain (8), BALB/cJ, were used. The animals were maintained and experiments performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and protocols were approved by the Animal Research Committee of the Veterans Research Administration, Oklahoma City.

NOD mice were screened for diabetes weekly. Glocusuria was measured semiquantitatively by means of the Chemstrip uGK urine glucose test (Boehringer Mannheim, Indianapolis, IN), which is based on the glucose oxidase/peroxidase method. Animals with detectable glycosuria were considered diabetic. In cases of ambiguous or low levels of glycosuria, diagnosis of diabetes was further tested by assessing glucose in a drop of whole blood obtained by puncturing a tail vessel. Blood glucose measurements were performed with a commercially available glucometer (MediSense Precision Xtra, Abbot Laboratories, MA). An animal was considered diabetic when blood glucose levels were higher than 250 mg/dl (13.87 mmol/l) and stable. The diabetic animals were treated with an intraperitoneal injection of bovine insulin-Zn in suspension, 40 IU/ml (LenteR Mc, Novo). The animals received 1 IU every second day. In case of hyperglycemia, the insulin dose was increased up to 5 IU; in case of hypoglycemia, the animal was injected intraperitoneally with 1 ml glucose solution (25 mg/ml). Glocusuria was measured before every treatment to evaluate the treatment regime.

Eight control mice, 16 NOD mice with diagnosed diabetes for 0–7 days (divided into two diabetic groups), and thirty-two NOD mice with diabetes for 28–35 days (these rats had gastroparesis and were divided into four groups) were used in this study. The animals were weighed on the day of the experiment. After an overnight fast, laparotomy was performed on each mouse under general anesthesia. Before the surgical procedure, the mouse was given ketamine (60 mg/kg, ip) together with xylazine (5 mg/kg, ip) to maintain a deep level of surgical anesthesia and muscle relaxation (leg punch response to toe pinch with forceps disappears). After a midline abdominal incision, the stomach and proximal duodenum was externalized. Two pairs of 28-gauge cardiac pacing wires were implanted on the serosal side of the greater curvature, with one pair in the middle of the stomach and the other in the middle between the proximal pair and the pylorus. One pair was used for stimulation and the other for recording. The connecting wires were subcutaneously brought out to the back close to the neck, and exited dorsally in the scapula region to prevent chewing. The abdomen was then closed, and a small piece of gauze was placed on the wound to prevent the accumulation of secretory fluids in the abdomen. After the abdominal surgery, each mouse was housed in a cage for at least a week until a complete recovery from the surgery.

Experimental protocol. The animals were divided into seven groups [control mice, diabetes (DB), DB with gastroparesis (DB-GP), DB with synchronized GES (DB + SGES), DB-GP with SGES (DB-GP + SGES), DB-GP with atropine (DB-GP + Atropine), and DB-GP + SGES + Atropine]. The mice in the DB group were confirmed with diabetes for 0–7 days. The mice in DB-GP groups had diabetes for 28–35 days. Gastric slow waves were recorded before feeding phenol red for a period of 30 min in all groups. The mice in the control, DB, or DB-GP groups were gavage fed with 0.2 ml of methylcellulose mixed with 10 mg of phenol red. Thirty minutes after feeding, the mouse was killed, the stomach was removed, and gastric retention of phenol red was analyzed. In the DB + SGES or DB-GP + SGES groups, the experiment was the same as the DB or DB-GP groups except that SGES was applied during the 30-min postprandial period. In the DB-GP + Atropine group, the experiment was the same as the DB or DB-GP groups except for the intraperitoneal administration of atropine (50 μg/mg) immediately before feeding. In the DB-GP + SGES + Atropine group, the experiment was the same as the DB-GP + Atropine group except that SGES was applied during the 30-min postprandial period. SGES was applied via the proximal pair of electrodes and synchronized with the intrinsic gastric slow waves measured from the other pair of electrodes. The stimulus was composed of trains of pulses with a frequency of 40 Hz, pulse width of 2 ms, and amplitude of 4 mA. The selection of these parameters was based on our preliminary study in dogs.

Gastric emptying. The method used to measure gastric emptying in this study was similar to that described previously by Scarpiognato et al. (35) and applied in our previous study (25). Methylcellulose was dispersed in water at 80°C at a final concentration of 1.5% under continuous stirring. The solution was allowed to cool to 37°C, and then phenol red (50 mg/ml), used as a nonabsorbable marker, was added. A volume of 0.2 ml of the phenol red solution was given orally into the stomach through a stainless steel tube that was removed immediately after delivering the solution intragastrically. Thirty minutes after the injection of the meal, the animal was euthanized by means of 1 ml KCl administered intracardially under anesthesia. The stomach was clamped at the pylorus and the gastroesophageal junction and removed. It was then cut open and its contents placed in 100 ml of 0.1N NaOH and settled for 60 min at room temperature. Afterward, 5 ml of supernatant was taken out of the solution and put into a test tube with 0.5 ml of trichloroacetic acid (20% wt/vol), then centrifuged at 3,000 rpm for 30 min. The contents of the centrifuged tube were then poured into another test tube and mixed with 4 ml of 0.5N NaOH. The absorbance of the sample was read at a wavelength of 560 nm with a spectrophotometer. Gastric retention was calculated based on the amount of phenol red recovered from the stomach 30 min after the meal.

Recording and analysis of gastric myoelectric activity. A multichannel recorder (AcqknowledgeIII, EOG 100A, Biopac System, Santa Barbara, CA) was used to record gastric myoelectric activity via all available electrodes not used for stimulation during the entire study. The signals (two channels at baseline, one channel during GES) were displayed on a computer monitor and saved on hard disk by an IBM-compatible 486 PC. The low- and high-cutoff frequencies of the amplifier were 0.05 and 35 Hz, respectively. For the analysis of gastric slow waves, the signals were further lowpass filtered with a cutoff frequency of 1 Hz and downsampled at 2 Hz. Previously validated computerized spectral analyses were performed to derive the percentage of normal gastric slow waves from the recordings. The myoelectric recordings obtained from the most distal pair of electrodes were used for the computation of the following parameter.

The percentage of normal gastric slow waves was defined as the percentage of time during which regular 4–6 cpm slow waves were presented over a specific analyzed period. It was computed by means of the adaptive spectral analysis method. In this method, each recording was divided into blocks of 1 min without overlapping. The power spectrum of each 1-min recording was calculated and examined to see if the peak power was within the range of 4–6 cpm. The 1-min recording was called normal if the peak power was within the 4–6 cpm range. Otherwise it was defined as dysrhythmia. The definition of normal slow wave frequency range (4–6 cpm) was based on a previous study (25).

GES synchronized with gastric intrinsic slow waves. Gastric slow waves were recorded during the baseline period. The phase shift between the proximal channel and middle channel was calculated. During the synchronized GES period, the proximal pair of electrodes was used for stimulation, whereas the distal pair was used for recording slow waves. Each stimulus was delivered at the detection of slow wave peaks from the distal pair with adjustment of phase shift in slow waves between the two pairs. This ensured that each stimulus was actually delivered at the occurrence of the slow wave peak at the stimulation site (Fig. 1).

Statistical analysis. The results are expressed as means ± SE. ANOVA was used to compare the data among three or more groups,
and Student’s t-test was used to assess the difference between each pair of groups. \( P < 0.05 \) was considered statistically significant.

RESULTS

Development of diabetes. Twenty-six NOD mice were excluded from the study due to 1) failure in the development of diabetes (15 mice); 2) death resulting from complications of diabetes (5 mice); or 3) severe gastric dysrhythmia occurring in the DB-GP group (6 mice). Diabetes was induced successfully in the other 40 mice (Table 1). The blood glucose level in these mice was 112 ± 27 mg/dl before the development of diabetes and 353 ± 86 mg/dl 0–7 days after the development of diabetes (\( P < 0.001 \)). A significant weight gain was noted in these diabetic mice. The body weight 0–7 days after the development of the diabetes was 23.7 ± 2.0 g, while that of controls at the same age was 17.6 ± 1.3 g (\( P < 0.01 \)).

Gastric slow waves. The percentage of normal 4–6 gastric slow waves was 71 ± 5% in control, 68 ± 6% in DB, and 65 ± 8% in DB-GP. There were no significant differences among groups. SGES had no effects on gastric slow waves.

Complications of diabetes in gastric emptying. Gastric emptying was significantly delayed in NOD mice with a duration of diabetes of 28–35 days. The gastric emptying at 30 min in the mice with a duration of diabetes of 28–35 days (DB-GP group) was 59.0 ± 6.3%, which was significantly lower than that in the control mice (78.7 ± 5.9%, \( P < 0.01 \)) or the mice with diabetes of 0–7 days (DB group, 77.6 ± 10.2%, \( P < 0.01 \)). However, no significant difference was noted between 0- and 7-day diabetic mice and control (\( P > 0.05 \); Fig. 2).

Involvement of cholinergic pathway with SGES. The excitatory effect of SGES was completely blocked by atropine. Atropine showed a little but not significant inhibition in gastric emptying. When SGES was performed in the presence of atropine (DB-GP + SGES + Atropine group), gastric emptying was the same as that in the DB-GP (57.3 ± 10.1% vs. 59.0 ± 6.3%, \( P > 0.05 \)) or DB-GP + Atropine groups (55.1 ± 12.4% vs. 59.0 ± 6.3%, \( P > 0.05 \); see Fig. 3).

DISCUSSION

In this present study, we have found that 1) gastric emptying was delayed in NOD mice with a duration of diabetes of 28–35 days; 2) synchronized gastric electrical stimulation was able to normalize gastric emptying in both diabetic mice and diabetic gastroparetic mice; and 3) the excitatory effect of SGES was completely blocked by atropine.

Recent epidemiology studies have indicated that the complication of gastroparesis has been found in 50% of patients with Type 1 and 30% of patients with Type 2 diabetes (5, 9, 15, 21). A survey of Type 1 diabetic patients has shown that

Table 1. Blood glucose level and weight in the control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Diabetes (n=40)</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>15.8±0.5</td>
<td>16.5±1.6</td>
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<tr>
<td>8 wk</td>
<td></td>
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<tr>
<td>0–7 days after DB in DB group</td>
<td>16.6±1.3</td>
<td>23.7±2.0*†</td>
</tr>
<tr>
<td>Blood glucose, mg/dl†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 wk</td>
<td>81±14</td>
<td>112±27</td>
</tr>
<tr>
<td>0–7 days after DB in DB group</td>
<td>101±33</td>
<td>353±86*†</td>
</tr>
</tbody>
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Values are means ± SE; \( n \), no. of rats. DB, diabetes. *\( P < 0.01 \) vs. 8 wk. †\( P < 0.01 \) vs. 0–7 days after DB in DB group.

Effects of synchronized gastric electrical stimulation on gastric emptying. SGES was able to significantly increase gastric emptying in both diabetic mice and diabetic gastroparetic mice. Compared with the DB or DB-GP groups, gastric emptying was significantly enhanced in diabetic mice with SGES (DB + SGES group, 85.2 ± 4.9%, \( P = 0.017 \) vs. DB group) and in gastroparetic mice with SGES (DB-GP + SGES, 72.8 ± 11.6%, \( P = 0.016 \) vs. DB-GP; see Fig. 2). The improved gastric emptying in gastroparetic mice with SGES was similar to that in the control group (72.8 ± 11.6% vs. 78.7 ± 5.9%, \( P = 0.18 \)).

Fig. 1. Synchronized gastric electrical stimulation (SGES). Top: natural slow waves. Bottom: stimulus delivered at the appearance of each slow wave peak.

Fig. 2. Effects of SGES on gastric emptying. Gastric emptying was slower in the diabetic gastroparesis group (DB-GP; diabetic mice of 28–35 days) than that in the control or diabetic (DB; diabetic mice of 0–7 days) groups. SGES accelerated gastric emptying in DB and DB-GP groups. *\( P < 0.01 \) vs. Control or DB; **\( P < 0.01 \) vs. corresponding group without SGES.

Fig. 3. Involvement of cholinergic pathway with SGES. The excitatory effect of SGES was completely blocked by atropine. *\( P < 0.01 \) vs. DB-GP or DB-GP + Atropine.
30–60% had upper gastrointestinal symptoms, particularly postprandial nausea, vomiting, and abdominal discomfort (10, 37). When ingested food lingers too long in the stomach, it may cause problems like bacterial overgrowth from fermentation of the food. Also, the food can harden into solid masses called bezoars that may cause nausea, vomiting, and obstruction in the stomach (4, 38).

The NOD mouse, a model for human Type 1 diabetes, was first developed by Makino and colleagues in Japan (26). Similar to diabetic patients with delayed gastric emptying, a slow gastric emptying was noted in NOD mice with a duration of diabetes of 28–35 days in this study. This is consistent with a previous study (8). In addition, we assessed gastric emptying in NOD mice with a duration of diabetes of 0–7 days and found that a slow gastric emptying did not occur. Accordingly, pathological disorders of diabetes might be more severe in NOD mice with a duration of diabetes of 28–35 days. Based on the finding of this study, the NOD mice with a duration of diabetes of 28–35 days may be a good model of diabetic gastroparesis. In addition, it is reported that diabetic gastroparesis is a disorder that predominantly affects women (11). This was the reason that female NOD mice were selected in our study. While the exact cause of slow gastric emptying in diabetes is still not clear, peripheral and autonomic neuropathy, myopathy, and hyperglycemia have been reported to be possible underlying mechanisms (7).

To date, medical treatment for diabetic gastroparesis is very limited in the USA. A medical device (called Enterra Therapy) that utilizes GES has been approved for the treatment of severe nausea and vomiting in patients with gastroparesis (2, 23, 24). However, it has no consistent effects on gastric motility or emptying. In this present study, a novel method of GES was used: SGES was delivered in synchronization with the physiological gastric myoelectrical activities instead of delivered at a fixed frequency without synchronization. In this proposed method, the configuration and waveforms of electrical stimulation were determined based on a number of pathophysiological factors of gastroparesis, including impaired fundic relaxation, gastric dysrhythmia, gastric antral hypomotility, and delayed gastric emptying. Some preliminary studies in our lab showed that SGES was able to enhance antral and small intestinal contractions in both fasting and fed states in dogs. In this study, we found that GES synchronized with gastric slow waves was capable of increasing gastric emptying in both diabetic mice and diabetic gastroparetic mice. The acceleration of gastric emptying with SGES suggests its therapeutic potential for treating diabetic gastroparesis.

The cholinergic pathway of the gut plays an important role in the regulation of gastrointestinal motility. Therefore, we tested the involvement of the cholinergic pathway in the acceleration of gastric emptying with SGES. Atropine, a competitive antagonist of ACh and other muscarinic agonists, can effectively inhibit the effects of vagal impulses, thus decreasing gastric tone and motility. Our results showed that the effect of synchronized GES on gastric emptying was abolished by atropine, suggesting the involvement of the cholinergic pathway. Previous studies also showed the similar results and indicated that vagal afferent and/or efferent pathways are involved in the regulation of GES on gastric motility (6, 12, 32, 33). In addition to the vagus nerve, enteric nervous and sympathetic nerves also play important roles in the control of gastric motility. Although vagotomy was not performed in this study and the exact effect of vagotomy on the SGES was unknown, we anticipate that SGES would still be effective if vagotomy were performed. This is because the vagus nerve might have been damaged to some extent already in the DB-GP group and yet the improvement in gastric emptying was still noted with SGES.

The findings of the study have demonstrated the therapeutic potential of this innovative SGES method for diabetic gastroparesis. Although this study was focused on the stomach, a similar methodology may also be applied to small intestine and colon for the treatment of motor disorders of the other parts of the gut. The implementation of this therapy is similar to conventional GES, except that it requires the implantation of 2 pairs of electrodes (one for the detection of gastric slow waves and the other for stimulation). The placement of stimulation electrodes can be achieved by a laparoscopic procedure or even an endoscopic procedure. The stimulator can be placed subcutaneously. The safety of this procedure has already been proven in a number of studies (1, 6).

In conclusion, synchronized gastric electrical stimulation accelerates gastric emptying in NOD mice with diabetic gastroparesis. The effect of SGES on gastric emptying is mediated via the cholinergic pathway. These findings suggest that SGES may have a therapeutic potential for treating patients with gastroparesis.

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REFERENCES


