Excitatory effects of synchronized intestinal electrical stimulation on small intestinal motility in dogs

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Submitted 20 February 2007; accepted in final form 2 October 2007

Yin J, Chen JD. Excitatory effects of synchronized intestinal electrical stimulation on small intestinal motility in dogs. Am J Physiol Gastrointest Liver Physiol 293: G1190–G1195, 2007. First published October 4, 2007; doi:10.1152/ajpgi.00092.2007.—The aim of this study was to investigate effects of synchronized intestinal electrical stimulation (SIES) on small intestinal motility in dogs. Seventeen dogs were equipped with a duodenal cannula for the measurement of small bowel motility using manometry; an additional cannula was equipped in six of the dogs with 1.5 m distal to the first one for the measurement of small intestinal transit. Two pairs of bipolar electrodes were implanted on the small intestinal serosa with one for the measurement of small intestinal transit. We found that SIES induced small intestinal contractions during phase I of the migrating motor complex (MMC) (contractile index or CI: 5.2 ± 0.6 vs. 10.3 ± 0.7, P = 0.003); 2) in the fed state, SIES significantly improved glucagon-induced small intestinal postprandial hypomotility (CI: 3.4 ± 0.5 vs. 6.0 ± 0.3, P = 0.03); 3) SIES significantly accelerated small intestinal transit delayed by glucagon (70.4 ± 3.1 vs. 44.5 ± 3.1 min, P < 0.01); 4) there was a negative correlation between the CI and transit time (r = −0.427, P = 0.048); and 5) the excitatory effect of SIES was blocked by atropine. SIES may have a therapeutic potential for treating patients with small intestinal disorders.

SMALL INTESTINAL MOTOR DISORDERS may result in delayed or accelerated small intestinal transit. Delayed transit is common in patients with diabetes, pseudo-obstruction, etc. (5, 6, 25, 29, 34). In diabetic patients, impaired gastrointestinal motility was observed not only in the stomach, but also in the small intestine (6). Bursts of nonpropagated contractile waves and abnormal postprandial duodenal chyme transport were reported in diabetics (6, 25).

Conventionally, gastrointestinal electrical stimulation can be divided into two types according to the stimulation frequency. In one method, stimulation is performed at a physiological frequency. A number of studies have reported that stimulation at the physiological frequency entrained or normalized gastrointestinal slow waves (2, 7, 19, 21). In the other method, stimulation is performed at a pathophysiological frequency, i.e., a frequency higher than the normal physiological frequency of the gut. In animal models, it has been reported that stimulation at the pathophysiological frequency induced gastric tachygastria in dogs (30), reduced food intake in obese Zucker rats, and inhibited small intestinal motility in dogs (18, 38); therefore this method of stimulation may have a potential for treating obesity. Recently, a new method called synchronized gastric electrical stimulation (SGES) was proposed. In this method, stimuli are synchronized with the intrinsic slow waves. Zhu et al. (41) reported that SGES enhanced antral motility and accelerated liquid gastric emptying. Based on the findings of SGES, we hypothesized that synchronized stimulation might also be applicable to the small intestine, and synchronized intestinal electrical stimulation (SIES) might enhance small intestinal motility and accelerate intestinal transit.

Therefore, the aim of this study was to investigate the effects of SIES on small intestinal contractions in both fasting and fed states, small intestinal transit delayed by glucagon, and the correlation between the small intestinal transit time and small intestinal contractions in dogs. We also aimed to investigate the underlying mechanism of SIES.

MATERIAL AND METHODS

Animal Model and Surgical Procedures

The study was performed in 17 healthy female hound dogs (20–24 kg). After an overnight fast, the dog was anesthetized with initial intravenous infusion of thiopental sodium (5 mg/kg; Abbott Laboratories, North Chicago, IL) and maintained on IsoFlo (isoflurane 1.5%, inhalation anesthesia, Abbott) in oxygen-nitrous oxide (1:1) carrier gases delivered from a ventilator following endotracheal intubation. The dog was monitored with the assessment of tongue color, pulse rate, and breath rate. Two pairs of 28-gauge cardiac pacing wires (A & E Medical, Farmingdale, NJ) were implanted along the serosal surface of the small intestine with an interval of 5 cm; the proximal one was 35 cm distal to the pylorus. The two electrodes in each pair were about 0.5 cm apart. The electrodes were penetrated into the subserosal layer and were affixed to the small intestinal serosa by nonabsorbable sutures. The wires were tunneled through the anterior abdominal wall subcutaneously along the right side of the trunk and were placed outside the skin around the right hypochondrium for attachment to the recorder or the stimulator. A cannula was placed in the duodenum, 20 cm beyond the pylorus, for the assessment of small intestinal contractions. In six dogs, an additional intestinal cannula was implanted 150 cm distal to the first cannula for the assessment of small intestinal transit. The dogs were transferred to the recovery cage after receiving medications for postoperative pain control. The study was initiated after the dogs were completely recovered from the surgery, usually 2 wk after the surgery. The study was approved by the Animal Care and Use Committee of the University of the Texas Medical Branch at Galveston, Texas.

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Experimental Protocol

Experiment 1: effects of SIES on fasting small intestinal contractions. This experiment was performed in six dogs with a single cannula in the duodenum. Each dog was studied in two sessions on 2 separate days in a randomized order; one served as a control, and the other was for SIES. The study was performed in the fasting state. In the control session, intestinal contractions were recorded without any interventions for a period of time that included a complete period of phase III of the migrating motor complex (MMC) and a 40-min period (mostly phase I of MMC) immediately after the completion of phase III. The protocol of the SIES session was the same except that SIES was performed during the 40-min recording period after the completion of phase III.

Experiment 2: effects of SIES on postprandial small intestinal contractions. This experiment was performed in the same six dogs as in experiment 1. It consisted of two sessions on 2 separate days in a randomized order. After an overnight fast, each dog was fed with 375 g (413 kcal) solid meal (Pedigree; Masterfoods, Vernon, CA) to induce postprandial small intestinal contractions; a bolus of glucagon (2.87 × 10⁻² μmol/kg) was injected intravenously 20 min after the feeding, and the recording was made for another 20 min. The 20-min recording after injection was chosen based on the short half-life of glucagon, ranging from 8–18 min. The protocol of the SIES session was the same except that SIES was performed during the entire 40-min postprandial period. Glucagon was chosen to establish an acute model of intestinal hypomotility because it was shown in the literature, as well as in our laboratory, to have an inhibitory effect on intestinal motility and induce hyperglycemia (28, 36, 37).

Experiment 3: effects of SIES on small intestinal transit. This experiment was performed in another six dogs implanted with dual cannulas. The method of small intestinal transit was modified from a previous study (31). Each dog was studied three times on 3 separate days in a randomized order: control, glucagon injection, and glucagon plus SIES. After an overnight fast, each dog was fed with 237 ml liquid meal (Boost; Novartis Medical Health, Minneapolis, MN) to induce postprandial motility patterns, and, for the assessment of small intestinal transit, small intestinal contractions were recorded simultaneous. In the glucagon session, glucagon (2.87 × 10⁻² μmol/kg) was bolus injected immediately after the feeding. The protocol of the SIES session was the same as the glucagon session except that SIES was performed during the entire postprandial period. To study the difference between conventional IES (not synchronized) with slow waves and SIES, conventional IES (non-SIES) was also performed in four of the six dogs using the same protocol.

Experiment 4: effects of non-SIES on fasting small intestinal contractions and involvement of cholinergic pathway. An additional five dogs were used in this experiment. The experiment design was the same as experiment 1 except that non-SIES instead of SIES was performed in the stimulation session. In an additional session, atropine (2.88 × 10⁻² μmol/kg) was used to test the cholinergic pathway of SIES. It consisted of a 20-min baseline recording, a 20-min recording after injection of atropine, and a 30-min recording with SIES. The dose of atropine was chosen based on our previous experiment and shown to be high enough to block the cholinergic pathway without inducing slow wave dysrhythmia (41).

Synchronized Intestinal Electrical Stimulation

Two pairs of electrodes were implanted along the serosal surface of the small intestine with an interval of 5 cm. The proximal pair was used for stimulation, and the distal one was used for recording small intestinal slow waves. The stimulus was delivered based on the slow wave peak detected by window discriminator (model no. 121; World Precision Instruments, Sarasota, FL). An adjustable electrical stimulator (model no. A310, World Precision Instruments) was used to connect to the wires from the small intestine. The parameter used for stimulation was with on-time of 0.5 s, pulse frequency of 20 Hz, pulse width of 2 ms, and amplitude of 4 mA. This set of parameters was shown to be effective in improving gastric hypomotility in a previous study (41). The stimulation parameters for non-SIES were the same as SIES except that the stimuli were delivered at a fixed frequency equal to the mean frequency of the intrinsic intestinal slow waves: pulse train on-time of 0.5 s and off-time of 2.5 s (that is, 20 pulse trains/min), and pulse frequency of 20 Hz, width of 2 ms and amplitude of 4 mA.

Measurement of Small Intestinal Transit

Immediately after a liquid meal, a 10-ml solution mixed with phenol red (0.5 mg/ml) and 1.5% methylcellulose was injected through the proximal intestinal cannula for the assessment of intestinal transit. The selection of phenol red as a marker was based on our previous studies (17, 31, 39). The small intestinal content was then collected through the distal cannula every 2 min for the first 10 min and then every 5 min until the disappearance of phenol red. The disappearance of phenol red was determined by visual observation of sample color; after the first observation of light yellow color, five more samples were collected before the termination of the experiment to ensure the completion of phenol red, which was previously validated (31). The absence of phenol red in the last collections was further validated by the spectrophotometric method. The small intestinal transit time was defined as the time between the injection of phenol red and the complete disappearance (absence of phenol red in three consecutive collections) of phenol red collected from the distal cannula.

Detection and Analysis of Small Intestinal Contractions

A manometric catheter (Medtronic; Synectic Medical AB, Stockholm, Sweden) with five side holes (an interval of 5 cm) for pressure measurement was inserted through the duodenal cannula to the small intestine. Small intestinal contractions were recorded from all five sensors. The manometric assembly was continuously perfused with low-compliance water by a pneumatic hydraulic capillary infusion system. All the sensors were located in the jejunum with the most proximal one 15 cm distal to the proximal cannula.

After the study was completed, the small intestinal contraction was analyzed. The pressure waves from the second most distal sensor, which was 25 cm distal to the proximal intestinal cannula, showed the best quality and were chosen to be analyzed. This was to ensure the consistency of analyses. The contractile activity of the small intestine was assessed by using the mean area under the contractile wave curve (mean AUC) per min, a parameter called contractile index (CI). A higher value in CI presented increased contractile activities of the small intestine.

Statistical Analysis

All data are presented as means ± SE. Paired student’s t-test was applied to investigate the effects of SIES on small intestinal contractions and small intestinal transit. One-way ANOVA was used to investigate the difference among sessions of control, glucagon, and SIES plus glucagon. Pearson correlation was used to investigate the correlation between the small intestinal transit time and the CI. P < 0.05 was considered statistically significant.

RESULTS

Effects of SIES on fasting small intestinal contractions. In the fasting state, after the occurrence of phase III, SIES induced small intestinal contractions. The contractile index during minutes 11 to 50 after phase III was 5.2 ± 0.6 in the control session and significantly increased to 10.3 ± 0.7 with SIES (n = 6, P = 0.003, Fig. 1).
Effects of SIES on postprandial glucagon-induced small intestinal hypomotility. In the fed state, glucagon substantially and significantly inhibited small intestinal contractions. The contractile index was $11.3 \pm 0.7$ during the 20-min postprandial baseline recording and reduced to $3.4 \pm 0.5$ during the 20-min postprandial period after the injection of glucagon ($P < 0.001$). SIES significantly improved glucagon-induced small intestinal postprandial hypomotility. The contractile index was significantly increased from $3.4 \pm 0.5$ in the control session to $6.0 \pm 0.3$ in the session of SIES ($n = 6$, $P = 0.03$, Fig. 2).

Fig. 2. Effect of SIES on postprandial small intestinal hypomotility. A: injections of glucagon significantly inhibited postprandial small intestinal contractions ($P < 0.001$). SIES significantly improved postprandial small intestinal hypomotility induced by glucagon ($P = 0.03$). B: postprandial small intestinal recording with glucagon injection. C: postprandial small intestinal recording with glucagon and SIES.
Effect of SIES on glucagon-induced delayed small intestinal transit. No significant difference in the time of the first appearance of phenol red was noted among the control, glucagon, and glucagon plus SIES sessions. The time of the first appearance of phenol red was 3.9 ± 0.9 min in the control session, 5.6 ± 0.7 min in the glucagon session (P = 0.2 vs. control), and 3.7 ± 0.6 min in the glucagon plus SIES session (P = 0.07 vs. glucagon). However, there was a significant difference in the total small intestinal transit time among three sessions (ANOVA, P < 0.001). As shown in Fig. 3, the total transit time was 34.0 ± 3.1 min in the control session, increased to 70.4 ± 3.1 min in the glucagon session (P < 0.001 vs. control), and reduced to 44.5 ± 3.1 min in the glucagon plus SIES session (P < 0.001 vs. glucagon, P = 0.1 vs. control).

Similar to experiment 2 with the solid meal, SIES significantly improved glucagon-induced hypomotility after the liquid meal. Injection of glucagon significantly reduced CI from 14.2 ± 1.6 to 9.4 ± 1.4 (P = 0.002); however, the inhibitory effect was at least partially reversed by SIES (9.4 ± 1.4 vs. 12.8 ± 1.4, P = 0.02). Figure 4 shows small intestinal tracings in different sessions after the liquid meal.

Effect of nonsynchronized intestinal electrical stimulation on small intestinal contractions and motility. Not surprisingly, no significant effects were noted with nonsynchronized IES on small intestinal contractions either in the fasting state or in the fed state. In the fasting state, the CI was 5.4 ± 0.6 in the control session and 5.2 ± 0.5 with non-SIES (n = 5, P = 0.6). In the fed state, the total small intestinal transit time in the control session (without stimulation and without glucagon injection) was 34.0 ± 3.1 min; it was significantly increased to 70.4 ± 3.1 min with the injection of glucagon and remained at 72.4 ± 7.9 min with non-SIES (P = 1.0 vs. glucagon without stimulation, P = 0.05 vs. control, n = 4, Fig. 3). Similar ineffective results were noted with nonsynchronized IES in the time of the first appearance of phenol red (4.4 ± 0.7 min, P = 0.8 vs. glucagon without stimulation, P = 0.7 vs. control) and small intestinal contractions (CI: 8.7 ± 0.8, P = 0.6 vs. glucagon without stimulation, P < 0.05 vs. control).

Correlation between the small intestinal transit time and small intestinal contractions. As shown in Fig. 5, there was a negative correlation between the small intestinal CI and small intestinal transit time (r = −0.427, P = 0.047).

Involvement of cholinergic pathway. There was a significant difference among baseline, atropine injection, and SIES (ANOVA, P = 0.03). Atropine completely blocked the excitatory effect of SIES on small intestinal contractions. The CI was 5.1 ± 0.6 during baseline, 3.1 ± 0.4 after injection of atropine (n = 5, P < 0.01 vs. baseline), and 3.3 ± 0.8 with SIES (P = 0.5 vs. atropine). The failure of SIES in increasing the intestinal CI after atropine suggests the involvement of the cholinergic pathway.

DISCUSSION

In the present study, we found that intestinal electrical stimulation synchronized with intestinal slow waves induced intestinal contractions during phase I of the MMC, improved small intestinal postprandial hypomotility induced by glucagon, and accelerated small intestinal transit delayed by glucagon.
Symptom of diarrhea. Although small intestinal motility disturbed intestinal transit in diabetics with autonomic neuropathy, atroclon. Nonsynchronized IES, however, failed to accelerate small intestinal motility. SIES-induced acceleration in intestinal transit was found to be correlated with enhanced intestinal contractions. The excitatory effect of SIES was mediated via the cholinergic pathway.

Up to 76% of patients with diabetes are reported to suffer from gastrointestinal complications (12, 22). Camilleri and Malagelada (6) have reported that motor abnormalities associated with gastroparesis syndrome are not limited to the stomach; small intestines are also frequently affected in patients with diabetic gastroparesis. Recently, Hackelsberger et al. (13) reported impaired small intestinal motility in diabetic autonomic neuropathy, characterized by disturbances in the generation and aboral migration of phase III and a marked postprandial hypomotility. Delayed small intestinal transit was frequently reported in diabetic patients (15, 16, 32, 33). Using the hydrogen breath test, Scarpello et al. (32) reported delayed intestinal transit in diabetics with autonomic neuropathy, attributed to vagal nerve denervation. Delayed small intestinal transit may result in bacteria overgrowth and lead to the symptom of diarrhea. Although small intestinal motility disorder in diabetics is common, treatment options are, however, very limited (11, 14, 15, 35). Antibiotics such as rifaximin and tetracycline were used for the treatment of bacterial overgrowth resulting from impaired intestinal motility. Cisapride was reported to be effective to improve gastrointestinal hypomotility in diabetics. However, the long-term effects of these treatments were not satisfactory, and Cisapride has been withdrawn from the market because of its cardiac toxicity.

Gastrointestinal electrical stimulation has been proposed as a potential therapy for gastrointestinal motility disorders (1, 8, 23, 27). A number of studies have demonstrated that gastric electrical stimulation (GES) with short pulses (or high-frequency low-energy stimulation) is capable of improving nausea and vomiting in patients with gastroparesis (1, 20, 23), whereas GES with long pulses is able to pace gastric slow waves, normalize gastric dysrhythmia, and accelerate gastric emptying in patients with gastroparesis or canine models of gastroparesis (8, 24). IES was initially proposed to delay intestinal transit to treat short bowel and dumping syndrome in canine model (3, 10, 26) and enhance intestinal absorption (4, 9); in these methods, IES was performed via electrodes placed in the distal small intestine. Recently, IES delivered via the electrodes placed in the proximal intestine (called forward IES) was shown to accelerate intestinal transit slowed by ileal brake (7). However, none of the previous papers have shown an excitatory effect of GES or IES on gastric or intestinal contractions. Numerous experiments in our laboratory have failed to induce gastric and intestinal contractions using the conventional nonsynchronized electrical stimulation (31, 40).

In the present study, we proposed a novel method of SIES for the treatment of small intestinal hypomotility. The “synchronized” means that the electrical stimulus was applied only on the detection (occurrence) of the peak of each intrinsic electrical event (or slow wave). Three experiments were designed to test the hypothesis: 1) effects of SIES on intestinal contractions in the fasting state in healthy dogs; 2) effects of SIES on glucagon-induced intestinal hypomotility after a solid meal; and 3) effects of SIES on glucagon-induced intestinal hypomotility after a liquid meal. The results of these experiments have consistently shown the excitatory effect of the SIES.

Conceivably, the excitatory effect of SIES on intestinal contractions is expected to accelerate intestinal transit. To prove this, an experiment was designed using the dual-cannula canine model. The transit was delayed by glucagon injection with a 100% increase in the total transit time, and SIES resulted in 63% improvement in the transit. As expected, this improvement in the transit was found to be significantly correlated with the improvement in intestinal contractions. In contrast, the conventional method of nonsynchronized IES with the same stimulation parameters did not yield any improvement in intestinal transit.

SIES is a novel method, and no mechanistic studies have been available in the literature. To understand the mechanism underlying the excitatory effects of SIES on small intestinal hypomotility, atropine, a muscarinic receptor antagonist, was used to investigate the possible involvement of the cholinergic pathway. We found that atropine inhibited small intestinal contractions, and SIES failed to increase small intestinal contractions with the presence of atropine. These findings seem to suggest that the prokinetic effects of SIES on small intestinal motility might be mediated at least, in part, via the cholinergic pathway. Further studies are needed to investigate the exact mechanisms of the SIES. If the prokinetic effect of SIES is truly mediated by the cholinergic nerves, its potency may be dampened when applied in diabetic patients with neuropathy. Clinical studies are needed to investigate the therapeutic potential of SIES in diabetic and nondiabetic patients with intestinal hypomotility. A special method of GES called Enterra Therapy, in which short pulses are used, has found applications in treating nausea and vomiting in patients with gastroparesis. A mechanistic canine study revealed the vagally mediated antiemetic effect of stimulation (8), whereas a multicenter clinical trial showed significant improvement in nausea and vomiting in diabetic patients with gastroparesis (1).

In conclusion, IES synchronized with intestinal slow waves or SIES induces intestinal contractions and accelerates small intestinal transits delayed by glucagon. This novel method may...
have a therapeutic potential for patients with small intestinal disorders.

GRANTS

This work was partially supported by a grant from the American Diabetes Association and a National Institutes of Health Grant (DK-075155-01-A1).

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