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Gastric/Intestinal Electrical Stimulation Modulates Appetite Regulatory Peptide Hormones in the Stomach and Duodenum in Rats

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Background: Gastric/intestinal electrical stimulation (GIES) has been used to suppress appetite in the treatment of obesity with promising results. However, the mechanisms by which GIES benefits obese patients are not completely understood. This study investigated the acute effects of GIES on gastric and intestinal tissue levels of peptide hormones related to satiety and appetite in rats.

Methods: 32 rats were divided into 4 groups: 1) sham stimulation, 2) gastric electrical stimulation (GES) with pulse trains, 3) GES with long pulse, and 4) duodenal electrical stimulation (DES) with pulse trains. After 2 hours of GIES, the rats were sacrificed immediately, and gastric fundus, duodenum and distal colon were harvested and extracted. Hormone levels of ghrelin, obestatin, cholecystokinin-8 (CCK-8) and peptide YY (PYY) were measured by radioimmunoassay (RIA).

Results: 1) The mean gastric fundus ghrelin level was 1789.04±362.81 pg/mg in the sham stimulation and significantly decreased with GES of pulse trains (597.85±195.33 pg/mg, *P*=0.012), GES of long pulse (754.6±282.6 pg/mg, *P*=0.039) and DES (731.69±110.84 pg/mg, *P*=0.037). 2) The mean duodenal CCK-8 concentration was 413.27±42.14 pg/mg in the sham stimulation and significantly increased by DES (762.6±98.75 pg/mg, *P*=0.013) but not in others. 3) Neither gastric obestatin nor distal colonic PYY was altered by any of GES or DES.

Conclusions: GIES significantly impacts appetite-related peptide hormones in gastric and duodenal tissues. Acute GIES-induced manipulation of gut peptide hormones related to appetite and satiety is a non-pharmacologic, well-tolerated clinical procedure that could substantially contribute to the successful treatment and long-term management of obesity.

Key words: Gastric/intestinal electrical stimulation, RIA, ghrelin, cholecystokinin-8, peptide YY, appetite, obesity

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Introduction

Gastrointestinal (GI) peptide hormones are integral contributors to the numerous peripheral signals that regulate food intake and energy balance. GI peptide hormones such as ghrelin, obestatin, cholecystokinin-8 (CCK-8) and peptide YY (PYY) that are synthesized and released from the GI tract have multi-potential capabilities through a complex interplay from mechano- and chemoreceptor, signaling events, then to ultimately regulate satiety and appetite. They can interact locally with specific receptors on vagal afferent axons passing to the brain.[1-3] These gut peptides can also be released directly into the bloodstream, allowing their delivery to distant sites of action to relay information regarding nutritional status.

Ghrelin increases can induce weight gain and adiposity by stimulating food intake and decreasing fat use or energy expenditure.[4-6] Obestatin is a new ghrelin-associated peptide isolated from the rat stomach. Contrary to the appetite-stimulating effects of ghrelin, it suppresses food intake and decreases

body weight gain.[7] Cholecystokinin-8 (CCK-8) is an important GI satiety signal.[8] Peptide YY (PYY-3-36) is also a gut-derived hormone with reported anorectic properties.[9] These GI peptide hormones that control appetite and satiety have enormous potential as novel therapeutic targets in the treatment of human obesity and its long-term management.[10]

Gastric/intestinal electrical stimulation (GIES) has increased satiety and decreased food intake with resultant weight loss in clinical and animal studies. Gastric pacing in animals caused altered eating behavior and weight loss.[11,12] Recently, implantable gastric stimulation (IGS) has been shown to induce satiety and weight loss in patients with morbid obesity.[13-15] Additionally, duodenal electrical stimulation (DES) results in inhibitory effects on gastric motility/secretion, fat absorption, gastric emptying and food intake, yielding weight loss in animals and humans.[16,17]

GIES has been demonstrated to impact a variety of complex pathophysiologic mechanisms, including inhibition of the intrinsic gastric electrical activity and GI motility,[18-20] direct effects on the central nervous system,[21,22] and hormonal modulation of satiety and/or appetite. Previous studies have demonstrated that Gastric electrical stimulation (GES) alters the plasma levels of selected GI satiety-related peptides and also alters the expression of neuropeptides in neurons of the hypothalamus.[23-27] Long-term treatment with IGS reduces blood levels of CCK and somatostatin, and basal levels of plasma GLP-1 and leptin with no effect on plasma ghrelin levels. Acutely, 2-hour GES decreases plasma levels of insulin and glucose in dogs.[25] Recently, our studies have shown that 2-hour treatment of GES increases the expression of oxytocin and decreases the expression of ghrelin in the hypothalamus in rats.[26]

Nothing is known about the effects of acute electrical stimulation on the peptide levels in gastric and intestinal tissues. The aim of this study, therefore, was to evaluate gut tissue levels of ghrelin, obestatin, CCK-8 and PYY in rats with GES and DES with various stimulation parameters. We hypothesized that direct acute electrical stimulation of gastric and intestinal tissues would initiate a significant and rapid modulation of GI peptide hormones related to satiety and/or appetite in gastric and intestinal tissues of fasted rats.

Materials and Methods

Animals

The study was approved by the Institutional Animal Care and Use Committees of the University of Texas Medical Branch at Galveston and performed at the University of Texas Medical Branch. Adult male Sprague-Dawley® rats (300-350 g, Harlan Sprague-Dawley, Houston, TX) were housed in cages in a temperature-controlled environment with a temperature of 22˚C, 40% humidity and a 12-h light-dark cycle. Rats had free access to regular chow pellets and drinking water. There was 1 week of acclimatization prior to the initiation of these experiments. After an overnight fast, all rats underwent surgery under anesthesia. A midline laparotomy was performed and 1 pair of 28-gauge cardiac pacing wires (A&E Medical, Farmingdale, NJ, USA) were implanted either on the serosal surface of the stomach in the distal antrum of the great curvature 0.5 cm proximal to the pylorus or the duodenum 0.5 cm distal to the pylorus as shown in Figure 1a. The electrodes (stainless steel wire) in each pair were 0.3 cm apart, penetrated subserosa and were affixed to the gastric serosa by non-absorbable sutures. The connecting wires were tunneled subcutaneously through the anterior abdominal wall along the right side of the trunk, and led outside the skin to the back of the neck for attachment to the stimulator. The abdominal

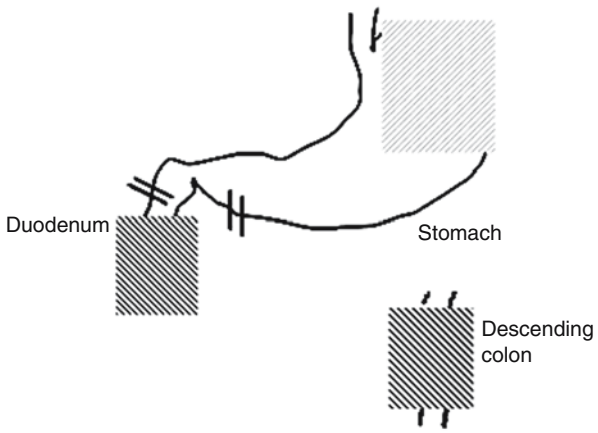

Figure 1a. Experimental model for gastric and duodenal electrical stimulation. The pair of electrodes near the gastric greater curvature and the duodenum were used for gastric electrical stimulation and duodenal electrical stimulation, respectively. Tissues were harvested from the shaded regions as indicated.

wall and skin were closed in a simple interrupted pattern. After surgery, all animals were able to ambulate and had unrestricted access to food and water. GIES was initiated 3 days after surgery and electrode implantation.

Experimental Design

Rats were divided into 4 groups (8 rats per group): A: Sham stimulation, implanted gastric electrodes and contacted to the stimulator but no electrical stimulation applied; B: GES with pulse trains; C: GES with long pulses; and D: DES with pulse trains. Three days after surgery, the rats were fasted at 1600 hours and electric stimulation was initiated at 0800 hours the next day for 2 hours. After stimulation, the rats were sacrificed immediately. Gastric fundus, duodenum and distal colon tissues were harvested and extracted for ghrelin, obestatin, CCK-8 and PYY3-36 radioimmunoassay (RIA) analysis, respectively. Figure 1a demonstrates the shaded regions of the stomach and intestines that were harvested for this study.

Gastric Electrical Stimulation

GES and DES were applied via the electrodes using an adjustable electrical stimulator (Model A310, World Precision Instruments, Sarasota, FL, USA) at the distal antrum of the greater curvature and the duodenum respectively. The stimulation parameters evaluated in this study were: 1) IGS-3 ms: modified IGS parameters used for obese patients: a pulse frequency of 40 Hz, a width of 3 ms and amplitude 6 mA, and train on-time of 2 s and off-time of 3 s, as shown in Figure 1b. A pulse width of 0.3 ms is commonly used in clinical trials. Recent studies in our laboratory demonstrated that a pulse width of 3 ms was more effective in altering both gastric motility and central neuronal activities.[27] 2) Repetitive long pulses: frequency of 9 pulses/min, a width of 300 ms and amplitude of 6 mA as shown in Figure 1b. GIES with these parameters was shown to reduce food intake and induce weight loss in Obese Zucker rats.[12]

Preparation of Tissues

Tissues from the gastric fundus, duodenum and colon were quickly removed after the rats were sacrificed. They were diced and boiled for 15 min in a 5-fold of solutions that were adjusted to a final concentration of 1 M AcOH and 20 mM HCL to deactivate intrinsic proteases. After cooling, the tissues were homogenized with a Polytron mixer and placed in a centrifuge at 13,000 rpm at 4˚C for 20 min (per recommended RIA protocol for tissue preparation). Tissue sample supernatants were aspirated and protein levels were measured. Four milligrams of tissue samples were loaded on an equilibrated SEP-Pak C18 cartridge (Phoenix Pharmaceuticals, Inc). Equilibration was performed by washing once with 1 ml of buffer B (60% acetonitril in 1% TFA) and three times with 3 ml of buffer A (per manufacturer's protocol). The peptides were then eluted from the columns with 3 ml of buffer B and collected in a 15-ml polystyrol tube, evaporated to dryness and subjected to ghrelin, obestatin, CCK-8 and PYY3-36 specific RIA.[28]

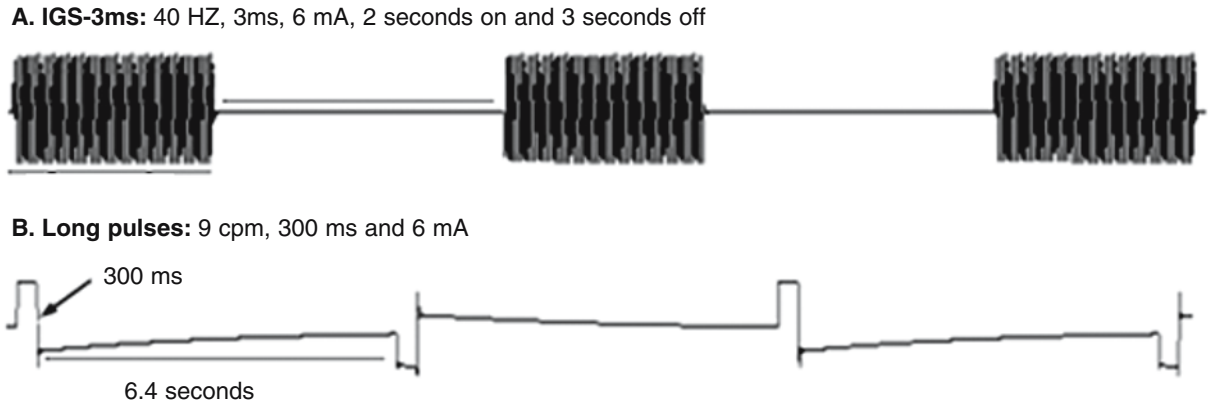

Figure 1b. Stimulation parameters applied included: A) pulse trains (IGS-3ms): 40 Hz, 3ms, 6 mA, 2 seconds on and 3 seconds off; B) long pulses: 9 cpm, 300 ms and 6 mA.

Hormone Determinations (RIA)

Ghrelin, obestatin, CCK-8 and PYY tissue concentrations were determined by a double-antibody radioimmunoassay (RIA) procedure.[29] First, we performed RIA on serial dilutions of one normal tissue sample to determine the optimal titration concentrations to place our samples into the linear range of the assay. There was a good correlation between the tissue protein levels and the peptide hormone levels, as shown in Figure 2. Based on our pilot studies, variable concentrations of extracted protein preparations were needed from the gastric, duodenal and distal colonic regions for ghrelin, CCK-8 and PYY 3-36 quantization assays (25 ug, 50 ug and 100 ug, respectively, as shown in Figure 2). Gastric obestatin could be detected only using 1 mg of tissue samples. Analyses were performed according to the manufacturer's recommendations (Phoenix, Pharmaceuticals Inc, Belmont, CA, USA). The tracer was radio-iodinated (^{125}I) rat peptide and the minimum detectable amount for ghrelin was 5.4 pg/tube with 5% inter- and 13% intra-assay coefficients of variation (CV). For obestatin, 10 pg/tube was detectable with 5-7% inter- and 10-12% intra-assay CV; CCK-8: 5.9 pg/tube was detectable with 5-7% inter- and 10-12% intra-assay CV; and PYY: 1 pg/tube was detectable with the intra-assay variation <8.42% and inter-assay variation <14.52%. Rabbit anti-rat peptide serum (100 ul) was added for the detection of each hormone. After incubation for 16-24 hours at 4˚C, ^{125}I–peptide (100 ul) was added to each assay tube and incubated for another 16-24 hours at 4˚C. Then, 100 ul goat anti-rabbit IgG serum and 100 ul normal rabbit serum were added to each assay tube. The precipitates were separated by centrifugation (3,000 rpm, 20 min, 4˚C) and the radioactivities were counted using an auto-Gamma counting system (PACKARD Instrument Co, Meriden, CT). Each sample was assayed and a standard curve was obtained from the measurements in duplicate.

Statistical Analysis

Statistical analysis was performed using SPSS 11.5 software. All data are represented as mean±SE. One-way analysis of variance (ANOVA) and unpaired Student's *t*-test were used to test for statistical significance compared to sham stimulation. A *P* value of <0.05 was considered significant.

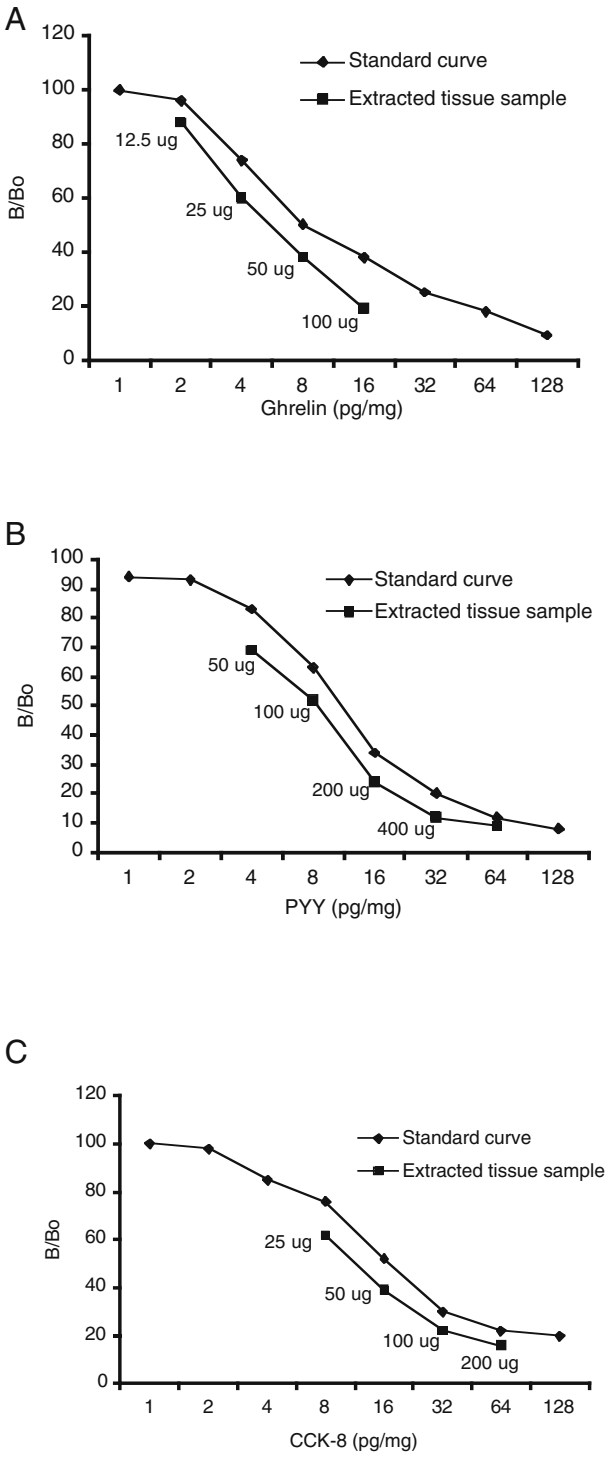

Figure 2. Competition of ^{125}I-peptides binding to peptide antibodies by gastric fundus tissue extracts, duodenum tissue extracts and distal colon tissue extracts. ^{125}I-peptides were incubated with peptide antibodies with different dilutions of tissue extracts and the peptides standard. Pg, pictograms; B, bound; Bo, total bound. A: Ghrelin, B: PYY, C: CCK-8.

Results

Electrical Stimulation Decreased Gastric Ghrelin Levels

Figure 3 demonstrates that the gastric fundus ghrelin level was significantly decreased with both GES and DES. Gastric fundus ghrelin level was significantly decreased in the rats with GES with pulse trains compared to sham stimulation (597.9±195.3 pg/mg vs 1789.0±362.8 pg/mg, *P*=0.012). Gastric fundus ghrelin level was also significantly decreased in the rats with GES of long pulses and with DES (754.6±282.6 pg/mg and 731.7±110.8 pg/mg respectively, *P*=0.039 and 0.037, respectively, compared to sham stimulation).

Gastric Electrical Stimulation does Not Affect Gastric Obestatin Levels

Gastric obestatin levels were not altered by any of the electrical stimulation methods. The obestatin concentration in the stomach was 18.4±1.8 pg/mg in the sham stimulation rats, 17.7±2.3 pg/mg in the rats with GES of pulse trains (P=0.78, vs sham stimulation), 15.0±1.0 pg/mg in the rats with GES of long pulses (P=0.16, vs sham stimulation) and 31.4±7.3 pg/mg in the DES session (P=0.12, vs sham stimulation).

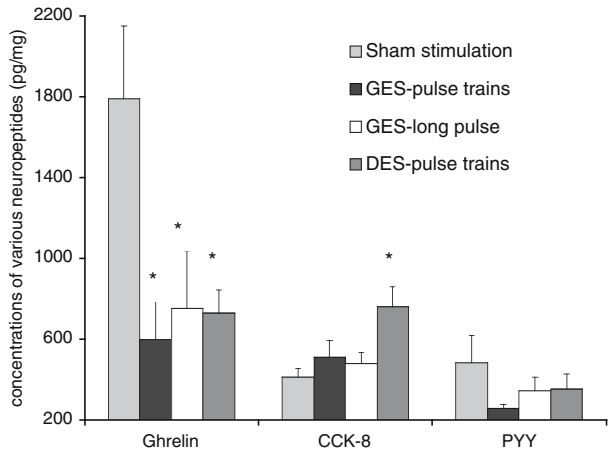

Figure 3. Concentrations of ghrelin in the gastric fundus tissue, CCK-8 in duodenum tissue and PYY in distal colon tissue after gastrointestinal electrical stimulation with different parameters and different locations. Values are mean ± SE. **P*<0.05.

Duodenal Electrical Stimulation Increases Duodenal CCK-8 Levels

The duodenal CCK-8 concentration was significantly increased by DES but not GES. The CCK-8 level was significantly increased with DES compared to sham stimulation (762.6±98.8 pg/mg vs 413.3±42.1 pg/mg, respectively, *P*=0.013) as shown in Figure 3. The duodenal CCK-8 levels were not significantly elevated with GES with pulse trains or GES of long pulses (510.4± 86.0 pg/mg and 479.4±57.6 pg/mg respectively, *P*=0.36 and 0.4 respectively, compared to sham stimulation).

Effects of Electrical Stimulation on Distal Colon PYY Levels

Distal colon PYY levels were not altered by GES or DES. The PYY concentration in the distal colon was 482.16±137.48 pg/mg in the sham stimulation rats and was not significantly altered using GES with pulse trains, GES of long pulses, or DES (259.3±17.5 pg/mg, 346.6±65.9 pg/mg and 353.8±74.9 pg/mg respectively; *P*=0.15, 0.35 and 0.39 respectively, compared to sham stimulation) as shown in Figure 3.

Discussion

Satiety signals are, for the most part, regulated by peptides synthesized and released from specialized enteroendocrine cells in the GI tract. GI neuroendocrine communications between the periphery and the brain regulate energy balance and ingestive behaviors by acting as modulating GI satiety signals. Electrical stimulation has been hypothesized to impact GI satiety signals as changes in eating behaviors in animals and induction of meal-related satiety in obese patients has been reported. In the present study, we found that (1) acute GES and DES with selected stimulation parameters significantly modulated gastric peptide hormones related to satiety and appetite in the GI tissues; GES (pulse trains and long pulse) and DES significantly decreased the ghrelin concentrations in the gastric fundus; (2) DES, but not GES, significantly increased the CCK-8 concentrations in the duodenum; and (3) neither

GES nor DES altered the concentration of gastric obestatin or distal colon PYY.

GIES, reported to change eating behaviors in animals and induce meal-related satiety in obese patients, may operate through direct modulation of the peptide hormones that regulate GI satiety signals. These interactions are largely mediated by the gut-brain peptides such as ghrelin, obestatin, peptide YY 3–36 (PYY_{3-36}) and CCK-8 through negative and positive feedback loops that maintain energy homeostasis.[2,10] Previous studies have identified CCK-8, PYY 3-36 and obestatin, anorexigenic peptides and ghrelin, an orexigenic peptide, as promising therapeutic targets in the battle against obesity.[30]

We have shown in this study that gastric fundus ghrelin levels were significantly decreased by both GES and DES with all the studied parameters. A reduction of ghrelin and its subsequently reduced food intake would induce a state of negative energy balance, which may well contribute to the weight loss resulting from GI electrical stimulation.[31] This finding suggests that the inhibitory effect of electrical stimulation on food intake may be partly due to its influence on inhibiting ghrelin synthesis in the stomach.

Ghrelin is the only GI hormone known to increase food intake; it affects all aspects of the energy homeostasis system in a concerted manner to promote weight gain.[32] Ghrelin plays a role in determining food intake from meal to meal, and therefore it would be desirable to have an acute or rapid impact on its tissue levels. Expression and secretion of ghrelin are increased by fasting and are reduced by feeding. Previous studies have shown that acute ghrelin blockade in adult animals decreases spontaneous food intake leading to weight loss in the longer studies.[31,33] Total gastrectomy in mice, which decreased the total ghrelin level by 80%, caused reductions in body weight, fat mass and lean mass.[34] Gastric bypass surgery, the most effective treatment for morbid obesity, typically suppresses or at least constrains ghrelin levels.[35] It is possible that an emerging anti-obesity treatment, intermittent stimulation parameters by implantable GES or DES devices, will lead to persistently diminished ghrelin levels; this may in turn contribute to appetite reduction and durable success of weight management.

A significant increase in duodenal CCK-8 concentration was observed with DES, but not GES. CCK-8 was the first gut hormone found to inhibit food intake. CCK-8 is produced by mucosal endocrine cells in the duodenum and released postprandially. Its effects on feeding appear to depend upon local actions near the site of release in stimulating vagal afferent fibers rather than on distant sites relying on humoral transport.[36] In this study, DES directly increased duodenal CCK-8 concentrations where CCK-8 is produced, released and actions targeted. GI signals that influence the brain to stop an ongoing meal are collectively called satiety signals. CCK-8 is truly a short-term, meal-reducing satiety signal. Following its release, CCK-8 elicits multiple effects on the GI system; it inhibits ingestion and eventually brings a meal to termination.[8] As a neuromodulator and/or neurotransmitter in both the central nervous system and the periphery, this brain-gut peptide plays an important role in the regulation of GI responses to nutrient ingestion and forms a negative feedback loop for the control of feeding behaviors in animals and humans.[37,38] A direct control that inhibits meal size can function by amplifying satiety signals or by some combination of these actions. Factors that increase CCK-8 levels may have a potential in the treatment of obesity.

Obestatin levels in the stomach tissue and PYY levels in the distal colon were not affected by acute GES or DES in this study. Obestatin, a 23-amino acid peptide encoded by the ghrelin gene and isolated from the rat stomach, opposes ghrelin's effects on food intake. It is not considered a meal-related signal because serum levels of obestatin were found to be constant in fasting and refeeding with food or water containing dextrose.[7] It is possible that direct acute electrical stimulation may primarily affect meal-related satiety signals.

PYY is considered the most potent anorexigenic substance; it is mainly synthesized and secreted by the ileum and large intestine.[39] We speculated that GES and DES may activate or inhibit local enteroendocrine cells to synthesize gut peptides. The distal colon may be too remote to demonstrate GES or DES-induced stimulation or diminution of colonic PYY levels.

The treatment of obesity has been resistant to most clinical approaches. Issues with appetite have remained one of the significant barriers to successful weight loss and long-term weight maintenance. GIES may offer a promising alternative to the treatment of obesity.[11,13-15] GIES is far less aggressive

than the currently available surgical banding or diversion procedures. It has an acceptable safety profile without any significant side-effects, and thus may eventually become a potentially preferred alternative to long-term drug therapy or bariatric surgery.

In conclusion, acute electrical stimulation of the stomach or duodenum decreases appetite-stimulating gut hormones and increases appetite-inhibiting gut hormones in gastric and duodenal tissues. GES and DES decrease ghrelin levels in the gastric fundus. DES increases CCK-8 levels in the duodenum. Alterations in the synthesis and secretion of these satiety-related peptides may contribute significantly to the altered eating behaviors associated with acute GES and DES in experimental and clinical studies. Manipulation of hormonally-related satiety by electrical stimulation will provide an emerging and promising treatment of obesity.

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