Inhibitory effects of intestinal electrical stimulation on food intake, weight loss and gastric emptying in rats

Jieyun Yin,1 Jing Zhang,1 and Jiande DZ Chen1,2

1Veterans Research and Education Foundation, VA Medical Center, Oklahoma City, Oklahoma; and 2Division of Gastroenterology, University of Texas Medical Branch, Galveston, Texas

Yin J, Zhang J, Chen JD. Inhibitory effects of intestinal electrical stimulation on food intake, weight loss and gastric emptying in rats. Am J Physiol Regul Integr Comp Physiol 293: R78–R82, 2007. First published March 15, 2007; doi:10.1152/ajpregu.00318.2006.—The aim was to investigate the effects of intestinal electrical stimulation (IES) on food intake, body weight, and gastric emptying in rats. An experiment on food intake and weight change was performed in 22 rats on a control diet and 10 diet-induced obese (DIO) rats for 4 wk with IES or sham IES. The effect of IES on gastric emptying was performed in another 20 rats in the control group. We found that 1) in control rats, 4-wk IES resulted in a reduction of 18.2% in the total amount of food intake compared with sham-IES (P = 0.02); the rats treated with IES had a weight change of −1 ± 7.8 g (P = 0.03), which was equivalent to a weight loss of 6.2% due to IES when adjusted for normal growing. 2) Acute IES delayed gastric emptying by 20% in the control rats (P < 0.01). 3) In the DIO rats, 1-wk IES with the same parameters as those used in the control rats resulted in a significant reduction in the total amount of food intake (126.6 ± 6.3 g vs. 116.9 ± 3.2 g, P < 0.01). More reduction in food intake was noted, and a significant weight change was also observed when stimulation energy was increased. 4) No adverse events were observed in any of the experiments. In conclusion, IES delays gastric emptying, reduces food intake, and decreases weight gain in control growing rats. These data suggest that it is worthy to explore therapeutic potentials of IES for obesity.

Gastrointestinal motility; obesity; diet-induced obese rats; stimulation parameter; mechanism

OBESITY IS ONE OF THE MOST PREVALENT public health problems in the United States (2). It results from an imbalance between energy expenditure and caloric intake. The current therapeutic strategies for the treatment of obesity are not satisfactory: Behavior modification and pharmacotherapy have been found to be effective only for a short term (1, 2). The surgical treatment induces satisfactory long-term weight loss; its application is, however, very limited because of substantial risks and complications involved (19).

Recently, there is a growing interest in electrical stimulation for the treatment of obesity. Gastric electrical stimulation (GES), as a potential therapy for obesity, has been extensively studied in both animals and humans. Previous open-label studies have shown promising results in food intake and weight loss (4, 5, 7, 15). Similar to the stomach, the small intestine can also be electrically stimulated (3, 18, 11, 20). It has been indicated that intestinal electrical stimulation (IES) was able to completely entrain segmental intestinal myoelectrical activity in dogs, suggesting the feasibility of normalizing intestinal myoelectrical dysrhythmia with IES (11). In animal studies, IES was also found to slow intestinal transit in the intestinal segment proximal to the stimulation site and accelerate transit in the segment distal to the stimulation site (3). These previous findings suggest the capability of IES in altering intestinal motility.

A number of previous studies have explored potential clinical applications of IES in treating intestinal motility disorders, such as short bowel syndrome, Roux stasis syndrome, and dumping syndrome (6, 14, 18). It is unknown whether IES might also be applied to treat obesity. Recently, in our laboratory, a series of studies have been performed in both canines and rodents. IES was found to decrease gastric accommodation to a meal, increase small intestinal transit, and decrease fat absorption (22, 27). Moreover, IES was also found to decrease acute food intake in a canine model (27), suggesting a therapeutic potential for obesity. However, no data are available in the literature regarding the chronic effect of IES on food intake and weight loss in any species.

Therefore, the aim of this study was to investigate chronic effects of IES on food intake and weight loss in rats and its possible mechanism involving gastric emptying.

MATERIALS AND METHODS

Subjects

Forty-two Sprague-Dawley rats (male, 300–350 g Charles River Laboratory, Wilmington, MA) and 10 diet-induced obese (DIO) rats (male, 450–550 g, Charles River Laboratory, Wilmington, MA) were used in this study. The DIO rats were purchased at an age of 8 wk and were validated by the vendor before being delivered to our laboratory. The DIO rats were fed with high-fat diet (D12266B, Research Diets, New Brunswick, NJ), which was composed of 16.8 kcal% of protein, 51.4 kcal% of carbohydrate and 31.8 kcal% of fat. All rats were housed in the microisolator cage equipped with filter hoods under controlled temperature (20°C) and with a 12:12-h light-dark cycle and had free access to water and solid food (Labdiet, PMI Nutrition International, Brentwood, MO) for the control rats and a high-fat diet for the DIO rats. Both control and DIO rats underwent surgery for placement of stimulation electrodes at the age of 14 wk. The surgical and experimental procedures were approved by the Animal Care and Use Committee of the Veterans Affairs Medical Center, Oklahoma City, OK (for the food intake experiments) and by the Animal Care and Use Committee of University of Texas Medical Branch, Galveston, TX (for the acute gastric emptying study).

Surgical Procedure

After an overnight fast, the rat was operated under anesthesia using ketamine hydrochloride (60 mg/kg ip; Phoenix Pharmaceuticals, St. Joseph, MO) and xylazine (8 mg/kg ip; Phoenix Pharmaceuticals) mixture. Supplement anesthetics were given throughout the surgery as needed. A midline laparotomy was performed, and one pair of bipolar
electrodes (A&E Medical, Farmingdale, NJ) was implanted on the serosal surface of the duodenum about 4 cm distal to the pylorus. The interval between the two electrodes in the pair was 0.5 cm. The connecting wires were subsequently tunneled through the anterior abdominal wall along the right side of the trunk to the central dorsum. The abdominal wall and skin were closed in a simple interrupt pattern. Experiments were initiated after the rats were completely recovered from the surgery, usually 7 days after the operation.

**Experimental Design**

Food intake and weight loss in control rats. The experiment was performed in the 22 control rats. Before the study, the rats were acclimated to experimental conditions for 1 wk; during this time, food was available in a restrainer for 2 h daily between 0900 and 1100. The restrainer was designed to allow the rat to have enough space to take food. The food was placed in the restrainer near the animal’s head. The duration of 1-wk acclimation was determined to ensure that the animals were habituated to eating within 2 h the equivalent of food they would eat normally during an entire day (see Ref. 26). After the acclimation period, the rats were randomly divided into two groups. Eleven rats were used as the control and treated with sham IES for 4 wk, and the other 11 age- and weight-matched rats were treated with IES for 4 wk. The experiment was performed daily from 0900 to 1100. During that period, the rats were brought to the laboratory and placed in the restrainer (to prevent the connecting wires of the electrodes being chewed off). During the daily treatment period with IES or sham IES, the rats were brought to the laboratory and placed in the restrainer with the electrode wires connected to a custom-made portable stimulator, which was turned on for IES and off for sham-IES. Stimulating parameters were set at a frequency of 20 pulses/min, a pulse width of 100 ms (millisecond) and amplitude of 6 mA. This set of parameters was modified from our previous study, which indicated that fat absorption was reduced with IES (22). Unlimited regular solid food was provided during the 2 h with IES or sham-IES. No feeding except water was provided when the rats returned to regular cages. The amount of food consumed by the rat during the experimental period was calculated daily; the weekly cumulative food intake was calculated. Body weight of each rat was measured at the beginning and end of the 4-wk treatment period. For the 11 rats in the IES group, the impedence of the electrodes was measured twice a week to ensure the conductivity of the electrodes.

Food intake and weight loss in DIO rats. This was a preliminary study to investigate whether similar results could be obtained in a rodent model of obesity. Ten DIO rats were used in this experiment: five in the IES group and five in the sham IES group. The acclimation period was decided based on the stability of daily food intake; it took 2 wk for DIO rats to get used to the experimental setting: being fed in a restrainer and being fed only for 4 h daily (0900–1300). The experimental protocol after the acclimation period was slightly different from that in the control rats and included 2-wk of sham IES or IES with the same parameters as used in the control rats, 1 wk of sham-IES for both groups (this allowed for a within-subject comparison, which was necessary due to the smaller sample size), and 1 wk of sham IES or IES with increased stimulation energy (the stimulation pulse was increased from 100 to 300 ms). The animals were fed using the high-fat diet. Food intake was measured daily after each experimental session. The body weight of the animal was measured weekly every Monday before the experimental session. The increase in daily feeding hour from 2 to 4 h was to make the study more physiological and to make the amount of food intake more consistent. The increase in stimulation parameters was prompted by the results obtained from the first 2 wk of IES.

Adverse events. Behavior changes, including frequent movement and jawing, as well as feces conditions, such as diarrhea and constipation, were closely monitored and noted during the experiment in the 4-wk treatment of IES or sham-IES for the assessment of adverse events resulting from IES. The behavior changes were assessed by a colleague who blinded to the research protocol on the basis of their severity during the daily experimental period (severity: 0, never; 1, mild; 2, moderate; 3 severe). The total score was calculated. The condition of the feces was observed during and after the experiment.

Gastric emptying. Twenty of the 42 control rats were used for the gastric emptying test, 10 with sham IES as a control and the other 10 with IES. Each rat was fasted for 12 h before the gastric emptying test. Methylcellulose was dispersed in water at 80°C at a final concentration of 1.5% under continuous stirring. The solution was allowed to cool down to 37°C, and then phenol red (0.5 mg/ml), which was used as a nonabsorbable marker, was added. A volume of 1.5 ml of the phenol red solution was given orally into the stomach through a 16-gauge stainless-steel feeding needle, which was removed immediately after delivery of the solution. In the IES group, IES with the same parameters as in the food intake experiment in the control rats, was initiated immediately after the feeding and lasted for 30 min. Thirty minutes after the ingestion of the meal, the animal was rapidly euthanized by decapitation under anesthesia, and the stomach was clamped at the pylorus and the gastroesophageal junction, and removed. The stomach was placed in 100 ml of 0.1 N NaOH and cut into small pieces and then homogenized for 30 s; the suspension was allowed to settle 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 TCA (20% wt/vol), and centrifuged at 3000 rpm for 30 min. The contents of the centrifuged tube were then transferred into another test tube and added with 4 ml of 0.5 N NaOH. The absorbance of the sample was read at a wavelength of 560 nm with a spectrophotometer. Gastric retention was calculated on the basis of the amount of phenol red recovered from the stomach 30-min after the meal.

**Statistical Analysis**

All data are presented as means ± SE. Unpaired t-test was applied to investigate the difference in food intake, weight change, and gastric emptying between the sham IES group and the IES group in the control/obese rats. In the DIO rats, longitudinal paired t-test was also applied to investigate the difference in food intake and weight change between the week with IES and the week with sham IES. A P value of < 0.05 was considered statistically significant.

**RESULTS**

Effects of IES on Food intake in Control Rats

During the 1-wk acclimation period, there was no significant difference in the amount of daily food intake between the two groups (14.4 ± 0.6 g vs. 14.6 ± 0.5 g, P = 0.6), indicating that the two groups had similar food intake before the experiment. IES resulted in a significant reduction of food intake compared with the sham IES rats. During the 4-wk study period, the total food intake was 297.8 ± 14.3 g in the sham IES rats; a significant reduction of 18.2% was found in the rats with IES (243.5 ± 15.4 g, P = 0.02 vs. the control rats) (Fig. 1). As shown in Fig. 2, a significant reduction in food intake was noted during the 1st, 2nd, and 4th wk between the sham IES rats and the IES rats. In the 1st wk, the weekly food intake was 73.7 ± 5.2 g in the control rats and 55.3 ± 4.9 g in the rats with IES (P = 0.02). Similarly, IES significantly decreased weekly food intake during the 2nd and 4th wk compared with the controls (2nd wk: 74.0 ± 4.2 g vs. 60.3 ± 3.8 g; 4th wk: 73.6 ± 2.7 g vs. 62.8 ± 3.5 g, P = 0.03). The difference in food intake during the 3rd wk between the two groups was marginal (P = 0.07). These data indicated that the rats were not adapted to IES during the 4-wk treatment period.
Effect of IES on Body Weight in Control Rats

The rats were purchased with body weight ranging from 300 g to 350 g. From the purchase to the end of the 1-wk acclimation, the sham IES group gained 52.4 ± 5.0 g, and this was not different from the IES group (55.3 ± 5.5 g, $P > 0.05$), demonstrating that the two groups had the similar growth rate before the experiment. IES resulted in a significant reduction of normal body weight increase (Fig. 3). After the 4-wk sham stimulation, the control rats gained 24.6 ± 6.4 g, attributed to the fact that the rats were young and were in the growing stage. However, the rats treated with IES had a weight change of 1.0 ± 7.9 g. The difference in the weight change was statistically significant between the two groups ($P = 0.03$). Relative to the control rats (weight of 415.3 ± 21.5 g at the end of the 4 wk), the treated rats showed an equivalent reduction of normal weight gain of 6.2% during the 4 wk.

Effect of IES on Gastric Emptying in Control Rats

IES delayed gastric emptying. The percentage of gastric emptying at 30 min was 79.0 ± 2.0% in the controls and significantly reduced to 63.8 ± 2.0% in the group with IES ($P < 0.01$).

Effect of IES on Food Intake in DIO Rats

Initially, 5 rats were included in the IES group; however, one of them chewed off the connecting electrode wires during the experiment period and therefore was excluded. The remaining four finished the entire study. Compared with the week of sham IES, IES with the same stimulation parameters, as used in the control rats, significantly reduced weekly food intake in the DIO rats, but the efficacy seems less than that in the control rats. The amount of food intake was 128.5 ± 3.0 g during the 1st wk (IES-on), 116.9 ± 3.2 g during the 2nd wk (IES-on), 126.6 ± 6.3 g during the 3rd wk (IES-off), and 110.4 ± 3.4 g during the 4th wk (IES-on with increased pulse width) (Fig. 4A). Compared with the week of sham IES, IES with the control diet during the 2nd wk resulted in a reduction of 7.7% ($P = 0.03$) in food intake, whereas IES with the increased pulse width led to a decrease of 12.8% ($P = 0.01$) in food intake.

Compared to the sham IES group (sham IES for the entire 4 wk), the IES group showed no change in weekly food intake during the 1st and 2nd wk with a pulse width of 100 ms ($P > 0.3$); however, by increasing the pulse width to 300 ms, IES significantly reduced weekly food intake by 12.3% (125.9 ± 3.7 g vs. 110.4 ± 3.4 g, $P = 0.02$).

Effect of IES on Weight Change in DIO Rats

All rats gained weight during the 4-wk experimental period, suggesting that they were still in the growing stage. In the IES
In the present study, we have found that IES reduced food intake and delayed gastric emptying and stimulated DIO rats. IES was also effective in the DIO rats but might require a higher stimulation of energy. No noticeable behavior changes were observed during the entire experiment.

IES has been reported for the treatment of gastrointestinal disorders. In an early study, Kelly and Code (8) showed that stimulation of the canine duodenum led to oral spread of duodenal contractions, slowing in duodenal transit and delaying in gastric emptying. Likewise, Sarr et al. (20) reported a slow transit of chyme in the jejunal segment with electrical stimulation, resulting in enhanced absorption of water, nutrients, and electrolytes. Conversely, Chen and Lin (3) reported that IES accelerated intestinal transit slowed by fat-induced ileal brake. These data have suggested that IES with appropriate parameters is able to alter gastric and intestinal motility.

Recently, GES has been reported to reduce food intake and body weight in both animals and humans (4, 5, 7). Similar to GES, in this study, we have found that IES resulted in a significant reduction in food intake (about 18.2%) and weight (6.2%) after a 4-wk period of treatment in the control rats. A previous study reported a significant decrease in food intake with acute IES in dogs (27). The current study was the first to report the chronic effect of IES on food intake and weight loss. Although the treatment lasted only for 4 wk; we believe that in small animals such as rats, it was long enough to elucidate the chronic effects of IES. The reduction of food intake during the 4-wk treatment period was persistent, and no adaptation to IES occurred. Consistent with the reduced food intake, there was a significant weight loss with IES in the control rats.

To verify the applicability of IES in an DIO model, a preliminary experiment of IES in DIO rats was also included in the current study. It is known that the rat model of DIO follows a polygenic mode in inheritance as in much of human obese cases (10, 21). In the current study, we have found that IES with the same parameters as used in the control rats was not effective in reducing food intake until the 2nd wk. Compared with the findings in the control rats, it seemed less effective and therefore, during the 4th wk of the study, IES was performed using an increased pulse width and resulted in a significant decrease in food intake compared with both the sham IES and the diet-controlled rats. These preliminary findings seem to suggest that the DIO rats are more resistant to IES, and a higher stimulation energy may be required when IES is applied in DIO rats. These results were in agreement with a recent study in which the effects of GES on neuronal responses in the ventromedial nucleus, a known satiety center in hypothalamus, were investigated. It was reported that the DIO rats were more resistant to gastric distention as well as GES. To elicit a same level of excitation with GES in the DIO rats, a higher stimulation energy was required (28).

The mechanisms underlying the inhibitory effect of IES on food intake and body weight may include a number of factors. In this study, gastric emptying was found to be delayed with IES. Delayed gastric emptying is expected to increase the time interval between two consecutive meals, whereas the role of gastric emptying in obesity has been reported to be controversial. Both delayed or rapid gastric emptying has been noted in patients with obesity (24, 25, 17). In addition to delayed gastric emptying reported in this study, gastric tone was also reported to be inhibited with IES in a previous canine study (27), and the stimulation-induced gastric relaxation was found to be associated with reduced food intake (16). In another study, a
reduction in fat absorption was noted with IES in rats (22), and the reduction in fat absorption was reported to be associated with accelerated intestinal transit with IES. Besides its peripheral effects on gastric and intestinal motility, IES is also believed to involve the central nervous system. In one previous study, IES was reported to activate the neurons in the nucleus tractus solitarii, suggesting a vagal afferent pathway (23). In a more recent study, IES was found to activate the neurons in the ventromedial nucleus, which is related to satiety (29).

While no noticeable changes in animal behaviors were noted in this study, a possibility that IES might induce malaise and thereby reduced food intake could not be completely ruled out. However, similar acute IES studies have previously been performed in dogs, as well as in humans; no symptoms or discomfort was reported by human volunteers and no noticeable adverse events, such as retching or vomiting, were observed in dogs (3, 12, 13, 16).

Clinical feasibility of IES for treating obesity has recently been explored in a number of clinical studies. In one study, IES was performed using intraluminal ring electrodes (placed under endoscopy) in healthy volunteers (13); similar delay in gastric emptying and a reduction in water intake were noted. An accelerative effect of IES on intestinal transit was also reported with accelerated intestinal transit with IES. Besides its peripheral effects on gastric and intestinal motility, IES is also believed to involve the central nervous system. In one previous study, IES was reported to activate the neurons in the nucleus tractus solitarii, suggesting a vagal afferent pathway (23). In a more recent study, IES was found to activate the neurons in the ventromedial nucleus, which is related to satiety (29).

This work was partially supported by a grant from the National Institutes of Health (DK 063735–01).

REFERENCES

Downloaded from http://ajpregu.physiology.org by 10.220.33.2 on July 1, 2017