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Intestinal electric stimulation modulates neuronal activity in the nucleus of the solitary tract in rats

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Abstract

Intestinal electric stimulation (IES) has been shown to produce an inhibitory effect on gastric motility and secretion. The possible central mechanism of this entero-gastric inhibitory effect induced by IES is unknown. The objective of this study was to evaluate the effects of various IES on the activity of neurons in nucleus tractus solitarii (NTS). We examined the extracellular neuronal activity in NTS of the medulla in pentobarbital anesthetized, paralyzed, ventilated male adult rats. The aortic depressor, superior laryngeal, and carotid sinus nerves were crushed or sectioned bilaterally to avoid neuronal responses in NTS to cardiovascular baroreceptors. After NTS neurons with gastric input were identified, responses of single neurons in NTS to IES were determined. IES with different parameters was performed via a pair of platinum electrodes sutured onto the serosal surface of the duodenum 2 cm below the pylorus. IES with different parameters activated 39–72% of the solitary tract nucleus neurons responsive to gastric distension. Moreover, we demonstrated that IES activated the neuronal activity in NTS, which was stimulation energy dependent. The modulatory effect of IES on the central neurons receiving vagal inputs may contribute to the neural mechanisms of IES therapy for the treatment of patients with obesity and gastrointestinal motility disorders.

Keywords: Intestinal electric stimulation; Entero-gastric inhibitory reflex; Nucleus tractus solitarii; Gastrointestinal motility disorders; Obesity

Mechanical or chemical stimuli of the small intestine may result in an inhibition of gastric motility and secretion. For example, distension of the small intestine-induced gastric relaxation in dogs [24,31] and in human [4]. Perfusion of an acid [30], nutrient [2], or sodium oleate solution [7] into the small intestine suppressed proximal gastric motility [1,27], decreased gastric tone and reduced gastric acid secretion [30]. These entero-gastric inhibitory reflexes have been elucidated to be cholinergic vagal nerve related [3,7,25]. Studies have shown that the infusion of dextrose [34], glucose [20] or a liquid diet [21,39] through a duodenal cannula induced Fos expression in the nucleus of the solitary tract (NTS), providing indirect evidence that neural activation arising from small intestinal stimuli is integrated at the level of the NTS. However, the effect of intestinal electric stimulation (IES) on the activity of vagal central neurons has not been examined.

Intestinal electric stimulation has been shown to have various effects on gastrointestinal functions. IES is capable of entraining small intestinal slow waves [16], accelerating intestinal transit slowed by ileal brake [8] and altering small intestinal absorption of water, glucose, sodium [13] and/or fat [32], preventing vomiting and motion sickness-like symptoms [18]. Moreover, IES has been demonstrated to inhibit gastric contractions and slow gastric emptying [5,17]. Recently, IES has been proposed for treating obesity [18,32]. However, the central mechanisms underlying the effects of IES on gastrointestinal functions are poorly understood. One study [23] showed that both passive gastric distension and enhanced antral contractions induced by a nonexcitatory gastric electrical stimulation (GES) activated distension-sensitive vagal afferents signaling to the central nervous system. Our recent study also showed that GES mod-

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ulated the activity of neurons with gastric input in the NTS [26]. Yet, it has never been examined whether the activity of NTS neurons is related to the IES energy.

Therefore, the aims of this study were: (1) to assess the effect of various IES on the activity of the NTS neurons that innervate the stomach; (2) to determine the relationship between IES parameters and neuronal activity in the NTS.

Animal preparation: Data were obtained from nine male Sprague–Dawley rats (372.7 ± 12.8 g, Charles River Laboratories, Greensboro, NC, USA). Experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Under anesthesia with sodium pentobarbital (60 mg/kg, i.p.), the catheters were inserted into the right carotid artery of the rats to monitor blood pressure and into the left jugular vein to inject fluids and drugs. A continuous intravenous infusion of pentobarbital (5–10 mg/kg/h) was given throughout the experiment. A plastic tube was inserted into the trachea for artificial ventilation using a volume-control pump (55–60 strokes/min, 3.0–5.0 ml stroke volume). The aortic depressor, superior laryngeal, and carotid sinus nerves were crushed or sectioned bilaterally to avoid neuronal responses in NTS to cardiovascular baroreceptors [19,37]. Pancuronium bromide (0.4 mg/kg, i.v.) and supplemental doses (0.2 mg/kg, i.p.) were given to paralyze the rats as needed during the experiment. A thermostatically controlled heating pad and overhead infrared lamps were used to maintain rectal temperature between 36 and 38 °C.

Recording of extracellular action potentials: The rat was placed in a stereotaxic headholder and suspended from thoracic (T_1–T_2) vertebral clamps. A portion of the occipital bone and cerebellum were removed. The dura mater and arachnoid of the exposed medulla were carefully cut and then warm agar (3–4% in saline) was covered to ensure stability for neuronal recording. NTS neurons were searched 0.2–1.0 mm lateral from midline, rostral 1.0 mm and caudal 0.5 mm from obex [22] and 0.5–1.8 mm deep from dorsal surface of medulla. Extracellular action potential discharge was recorded with the carbon-filament glass microelectrodes. The signals from the DC amplifier were sent to an alternating current (ac) filter, and then to the digital oscilloscope, audiomonitor, videotape recorder, and window discriminator (World Precision Instruments). The window discriminator output was led to the Cambridge Electronic Design analog-to-digital converter interfaced with the PC. Spike3 data acquisition software (Cambridge Electronic Design) was used for online and offline analysis.

Visceral stimuli: Gastric distending procedures used have been described previously [12,25]. Briefly, after midline laparotomy, gastric contents were removed through a small incision on the fundus wall. A latex balloon (3–4 cm in length) attached to polyethylene tubing (PE-240) was inserted into the gastric cavity through the incision and fixed on the edge of the incision by a ligature. Intragastric pressure (20 mmHg, 20 s) was applied via a pressure transducer as a searching and examining stimulus. One pair of platinum electrodes (1 cm apart) for delivering IES was sutured onto the serosal surface of the duodenum 2–3 cm below the pylorus. IES with four sets of parameters was applied for 60 s: IES-A (40 Hz, 2s-on and 3s-off, 6 mA, 0.3 ms, typical set of parameters used for treating obesity), IES-B (same as IES-A, except 20 mA, increased stimulation pulse amplitude), IES-C (same as IES-A, except 6 ms, increased stimulation pulse width), and IES-D (12 imps/min, 200 ms, 6 mA, typical set of parameters used to treat gastrointestinal motility disorders). Stimulation energy, *E*, was calculated according to the following formula: *Long pulses*: E/min = pulse width (ms) × number of pulses/min × amplitude^2. *Pulse trains:* E/min = on-time(s) × pulse frequency (Hz) × pulse width × trains/min × amplitude^2.

Histology: At the end of the study, an electrolytic lesion (50 μA DC, 20 s) was made at recording sites to mark the neuronal location. The rat was then killed with an intravenous euthanasia-5 solution or overdose of pentobarbital. The brainstem was removed and placed in a 10% buffered formalin solution. Frozen sections (55–60 μm) of brainstem were examined to find lesion sites (or neuronal recording sites). Locations were drawn on cross sections from the cytoarchitectonic scheme [22].

Data analysis: Spontaneous activity of neurons was determined by counting activity for 10 s and then dividing by 10 to obtain impulses per second (imp/s). Neuronal responses (imp/s) were defined as the difference in neuronal activity between the mean spontaneous activity and the maximal activity during gastric distension and IES. The duration of neuronal responses was measured from the onset of response to the time when neuronal activity recovered to the control level. An increase or decrease in spontaneous neuronal activity (≥20%) was considered an excitatory or inhibitory response to gastric distension and IES. The raw tracings of the neuronal responses to IES were processed by a Spike 3 digital filter to eliminate IES artifacts. Statistical comparisons were made using chi-square (χ^2) analysis and/or ANOVA followed by Tukey's test. Linear regression was utilized for analyzing the relationships between stimulation energy and net neuronal response to IES. Statistical significance was assigned for $P < 0.05$. All data were presented as means ± S.E.

Twenty-three of 98 NTS neurons were responsive to gastric distension (GD, 20 mmHg, 20 s). Of these, 12 and 11 neurons were recorded from the right and left side of the NTS in medulla, respectively. GD increased activity in 19 neurons (83%) and suppressed activity in two neurons. Two neurons had excitatory–inhibitory responses to GD. Characteristics of NTS neuronal response to GD are shown in Table 1.

Of the 23 NTS neurons responsive to gastric distention, IES increased the activity of most NTS neurons with excitatory responses to GD (Table 2). Seventy-two percent of NTS neurons with gastric input responded to IES-B (increased pulse amplitude), which was significantly higher than that of IES-A (40%, $P < 0.05$) and IES-D (39%, $P < 0.05$, Table 2). Sixty-eight percent of NTS neurons with gastric input responded to IES-C (increased pulse width) but this