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Extrinsic ghrelin in the paraventricular nucleus increases small intestinal motility in rats by activating central growth hormone secretagogue and enteric cholinergic receptors

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ABSTRACT

Background/Objectives: Ghrelin is a brain-gut peptide that regulates gastrointestinal (GI) motility. We hypothesized that the excitatory effect of ghrelin on the paraventricular nucleus (PVN) increases GI motility by activating the central growth hormone secretagogue receptor (GHSR) and central neuropeptide Y (NPY) signaling pathways, leading to increased enteric cholinergic activity.
Methods: Thirty-six male Sprague Dawley rats were maintained on duodenal catheterization and PVN cannulation. Small intestinal transit (SIT) was observed and rats were divided as follows: experimental animals received ghrelin injections in the PVN (0.03, 0.08, or 0.24 nM); 1 nM GHSR antagonist D-Lys3-GHRP6 alone; 1 nM D-Lys3-GHRP6 before ghrelin injection in the PVN, respectively. Electrophysiologic parameters of the interdigestive myoelectric complex (IMC) were examined by administration of 0.24 nM ghrelin in the PVN after small intestinal electrode implantation and PVN cannulation. GI cholinergic pathway activation was analyzed after intravenous atropine administration. The involvement of central NPY signaling was evaluated by injecting an anti-NPY immunoglobulin (IgG) in the PVN. Neuronal expression of c-Fos in the brain and GI tract was examined using immunohistochemistry.
Results: Injection of ghrelin in the PVN dose-dependently accelerated SIT, and this excitatory effect was competitively inhibited by a GHSR antagonist. The excitatory effect of ghrelin on IMC activity was diminished by GHSR antagonism and NPY neutralization, as well as by blockade of peripheral muscarinic acetylcholine receptors. Extrinsic ghrelin significantly upregulated c-Fos expression in the PVN and other central nuclei, as well as in the enteric nervous plexuses of the stomach, duodenum, and proximal colon. The ghrelin-induced upregulation of central and enteric c-Fos expression was also dependent on central GHSR activation.
Conclusions: Ghrelin positively regulates GI motility by exciting both central and enteric neurons, including those of the PVN, by activating GHSR and NPY pathways, and peripheral muscarinic acetylcholine receptors.

1. Introduction

The central nervous system (CNS) and enteric nervous system (ENS) play a synergistic role in regulating gastrointestinal (GI) motility through the brain-gut axis [21]. Among the large number of peptides participated in the brain–gut axis, ghrelin is primarily produced by endocrine cells in the oxyntic mucosa of the stomach, and increases GI motility through both central and peripheral mechanisms by acting as an endogenous ligand for the growth hormone secretagogue receptor (GHSR) [4,11,23]. In the GI tract, ghrelin is highly expressed from the stomach to the colon, especially in the gastric fundus; whereas in the CNS, ghrelin is mainly detected in the hypothalamus, as well as occasionally in the thymus, kidney, pancreas, and ovaries of humans and rodents [6,34]. Ghrelin may also regulate acid secretion, feeding behavior, and energy metabolism [18,37].

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In the CNS, GHSR is mainly expressed in hypothalamic nuclei including the paraventricular nucleus (PVN), arcuate nucleus (ARC), and dorsomedial hypothalamic nucleus (DMH) [28,33]. Among these nuclei, the PVN, which expresses high levels of GHSR, acts to regulate feeding behavior, satiety, and GI motility by integrating the efferent and afferent signals in the brain-gut axis, a complex interactive network composed of a large number of neuroendocrine and autonomic pathways [15,27,36]. The proto-oncogene c-Fos, a member of the immediate early gene family, acts as an intranuclear "third messenger" expressed at an extremely low level in neurons under physiologic conditions [4]. Substances like ghrelin can induce rapid and significant upregulation of c-Fos expression, detected by immunohistochemistry, which acts as a signpost for stimulated neuronal activation [29].

The interdigestive myoelectric complex (IMC) is a distinct pattern of GI electromechanical activity observed during fasting. Due to its typical characteristics of periodicity, phase, and propagation, the IMC is thought to function as a housekeeper to sweep interdigestive residue through the GI tract over four distinct phases. Systemic injection of ghrelin has been reported to stimulate IMC activity in experimental animals [22,40].

However, it is unclear whether ghrelin regulates the IMC by exciting the PVN in the brain. It also remains unknown how central ghrelin acts on the ENS through the interactive network of the brain-gut axis. We hypothesized that the excitatory effect of ghrelin is mediated by central GHSR and that administration of ghrelin to the PVN indirectly stimulates an enteric cholinergic pathway that extensively involves both central and enteric neurons.

2. Materials and methods

2.1. Laboratory animals

The study protocol was approved by the Animal Research Committee at Xi'an Jiaotong University in accordance with the National Institute of Health Guidelines for Laboratory Animal Care and Use. Specific-pathogen-free, male Sprague Dawley rats (mean weight, 230 ± 10 g) were obtained from the Center of Laboratory Animal Medicine at Xi'an Jiaotong University and individually housed in rodent cages with free access to standard rodent chow and tap water at a constant temperature of $22 \pm 2\,^{\circ}C$ on a 12-/12-h light/dark cycle.

2.2. Duodenal catheterization

Thirty-six rats were deprived of food but allowed to drink *ad libitum* 18 h before general anesthesia with an intraperitoneal injection of chloral hydrate (400 mg/kg). A sterile polyethylene catheter (0.98 and 0.58 mm outer and inner diameters, respectively; Tuoren, Medical Instrument Co., Ltd. Xinxiang, Hena, China) was intraluminally advanced to 1 cm distal to the pyloric sphincter, exteriorized through a subcutaneous tunnel, and securely fixed on the neck using a 4–0 silk purse-string suture. The external end of the catheter was sealed using a blunt-ended pin. After duodenal catheterization, all rats were acclimated for 7 days before further experiments, and the catheter was flushed with normal saline every day.

2.3. PVN cannulation

A 22-gauge stainless steel cannula (HuaYu, Medical Instrument Co., Ltd. Xi'an, Shaan, China) was implanted into the PVN as previously reported using the following coordinates [25]: 0.3 mm lateral to the midline, 1.5 mm posterior to the bregma, and 7.1 mm below the skull surface. The cannula was anchored to the skull with dental cement and stainless steel screws and sealed with a rubber stopper. All animals were acclimated for another 7 days before further experimentation.

2.4. PVN injection

Ghrelin (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) was dissolved with normal saline at concentrations of 0.03, 0.08, or 0.24 nM (effective for stimulation of colonic propulsion after microinjection into the PVN [36]). A Hamilton microsyringe (Sigma–Aldrich Corporation, St. Louis, MO, USA) was inserted and advanced 0.5 mm beyond the tip of the PVN cannula to deliver active agents or saline (0.5 μl) and maintained for 2 min before withdrawal. The rats were starved for 24 h and acclimated for 30 min in the operating room. The rats were divided into 6 groups ($n = 6$/group) and injected with the following: 0.03 nM ghrelin; 0.08 nM ghrelin; 0.24 nM ghrelin; 1 nM GHSR antagonist D-Lys3-GHRP6 (Anaspec Inc., Fremont, FL, USA); 1 nM D-Lys3-GHRP6 15 min before 0.24 nM ghrelin injection; or normal saline (control).

2.5. Small intestinal transit measurement

For small intestinal transit (SIT) measurement, animals under general anesthesia received 0.1-mL non-absorbable Evans blue (50 mg/mL in methylcellulose solution, Sigma–Aldrich) through the duodenal catheter immediately after injection of relevant drugs as described above and followed by 0.1 mL saline flush. All animals were sacrificed by cervical dislocation 20 min after duodenal administration. The entire small intestine was removed and longitudinally examined for the total length and dye migration distance in a tension-free setting. SIT was determined using the following formula: SIT index (%) = (dye migration distance/total length of the small intestine) × 100%.

2.6. IMC electrophysiologic testing

Under general anesthesia, two pairs of Teflon-coated silver codes (0.5 mm outer diameter) were implanted 2.5 ± 0.5 mm apart in the muscular layer of the small intestine for 56 additional rats and located 5 cm (the duodenum) and 15 cm (the jejunum) distal to the pylorus, respectively. The electrode wires were brought out through a subcutaneous tunnel and fixed on the neck. After electrode implantation, all animals were acclimated for 7 days before PVN cannulation as described above. Before the IMC electrophysiologic testing, all animals were starved for 18 h and acclimated for 30 min in the operating room. After basal IMC activity recording for 1 h, PVN injections were administered as follows: no injection (sham control, $n = 8$), 0.24 nM ghrelin ($n = 6$), and saline (control, $n = 6$). For the ghrelin antagonism experiment ($n = 6$/group), 2 doses of 0.5 μL medication and/or saline were given at a 15 min interval as follows: saline plus saline, saline plus 0.24 nM ghrelin, anti-neuropeptide Y (NPY) IgG (Anaspec, USA, 0.5 μL) plus saline, anti-NPY IgG plus 0.24 nM ghrelin, 1 nM D-Lys3-GHRP6 plus saline, or 1 nM D-Lys3-GHRP6 plus 0.24 nM ghrelin. Silver electrodes were connected to a multi-lead physiological recording system (RM-6280C; Chengdu Instrument Plant, Chengdu, Sichuan, China) to measure small intestinal myoelectric activities. The electrophysiologic testing parameters were as follows: frequency = 1 kHz, time interval = 0.2 s, low-frequency cutoff = 0.8 Hz, and high-frequency cutoff = 100 Hz. Contractions were recorded as electric potential change (μV). The IMC electrophysiologic activity was continuously monitored for another 1 h before the experiment.