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Ghrelin accelerates gastric emptying via early manifestation of antro-pyloric coordination in conscious rats

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Abstract

Ghrelin is known to enhance gastric motility and accelerate gastric emptying of liquid and solid food in rats. As solid gastric emptying is regulated by the coordinated motor pattern between the antrum and pylorus (antro-pyloric coordination), we studied the correlation between solid gastric emptying and antro-pyloric coordination in response to ghrelin. Rats were given 1.5 g of solid food after a 24-h fasting. Immediately after the ingestion, ghrelin (0.4–8.0 μg/kg) or saline was administered by intraperitoneal (IP) injection. Ninety minutes after the feeding, rats were euthanized and gastric content was removed to calculate gastric emptying. To evaluate the antro-pyloric coordination, strain gauge transducers were sutured on the antrum and pylorus. The incidence of postprandial antro-pyloric coordination was compared between ghrelin-and saline-injected rats. In saline-injected rats, gastric emptying was 58.3±3.7% (n=6). Ghrelin (4.0–8.0 μg/kg), accelerated gastric emptying. Maximum effect was obtained by ghrelin (4.0 μg/kg), which significantly accelerated gastric emptying to 77.4±3.7% (n=6, $p<0.05$). The number of antro-pyloric coordination 20–40 min after feeding was significantly increased in ghrelin-injected rats, compared to that of saline-injected rats (n=4, $p<0.05$). It is suggested that enhanced antro-pyloric coordination play an important role in accelerated solid gastric emptying induced by ghrelin.

Keywords: Growth hormone Secretagogue receptor; Postprandial gastric contraction; Vagus nerve

1. Introduction

Ghrelin was discovered as an endogenous ligand for growth hormone secretagogue receptor (GHS-R) [1]. Ghrelin enhances food intake [2,3] and stimulates gastrointestinal (GI) motility [4] and promotes solid gastric emptying in humans [5], rats [6] and mice [7]. Gastric emptying of non-nutrient liquid is also accelerated by ghrelin in rats [6,8,9] and mice [10,11].

However, it is not well established how ghrelin mediates the acceleration of gastric emptying. Two major pathways are suggested; vagal pathway and intrinsic neural pathway.

Ghrelin receptors are synthesized in vagal afferent neurons and transported to the afferent terminals. Blockade of the gastric vagal afferent abolished ghrelin-induced feeding and GH secretion, indicating that the gastric vagal afferent is the major pathway conveying ghrelin's signals for starvation and GH secretion to the brain [12].

Others also showed the presence of GHS-R in the afferent nerve in the nodose ganglion which projects to the stomach [13]. The stimulatory effect of ghrelin on gastric motility is abolished by the pretreatment with atropine or bilateral vagotomy in rats [4]. These suggest that the stimulatory effect of ghrelin on gastric emptying is mediated via vagal pathways.

On the other hand, intravenous administration of ghrelin alters gastric contractions from fed pattern to fasted pattern even in vagotomized rats [14], suggesting that the effect of ghrelin on gastric motor activity is mediated via vagal-independent pathway in vagotomized rats.

In vitro muscle strip study showed that ghrelin enhances electrical field stimulation (EFS)-evoked contractions in rats [6,15,16] and mice [17], suggesting the direct action of ghrelin on the gastric myenteric plexus.

The mechanism of regulating gastric emptying is different between liquids and solids. The emptying of liquids from the

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stomach is thought to be primarily a function of the pressure gradient between the stomach and the duodenum [18]. On the other hand, the antral pump and pyloric opening are importance factor for the emptying of solid foods. Large solid particles are retained in the stomach by the pyloric closure and retropelled and triturated in the antral mill [18,19].

The coordinated motor pattern between the antrum and pylorus is observed in emptying period in humans [19] and dogs [20]. This has also been demonstrated as antro-pyloric coordination in rats [21–23]. It is generally accepted that coordination between the antrum and pylorus is an important factor in the emptying of solid foods [18,19,22,24,25].

The antro-pyloric coordination is abolished by atropine or truncal vagotomy in rats [22]. Treatment by vagal blockade or hexamethonium significantly reduced postprandial antral contractions and pyloric relaxations in dogs [20]. These suggest that the antro-pyloric coordination is mediated via vagal nerve.

In the current study, we studied whether ghrelin affects postprandial antro-pyloric coordination in conscious rats.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 240–290 g were housed in-group cages under conditions of controlled temperature (22–24 °C) and illumination (12-h light cycle starting at 7:00 am) for at least seven days before experiments and maintained on laboratory chow and water. Before the experiment, rats were fasted for 24 h but given free access to water. Protocols describing the use of rats were approved by the Institutional Animal Care and Use Committee of Duke University Medical Center and in accordance with the National Institute of Health “Guide for the Care and Use of Laboratory Animals".

2.2. Substances

Rat octanoylated-ghrelin (Tocris Cookson Inc., MO) were kept in powder form at −70 °C. Ghrelin was dissolved in 0.9% saline immediately before use. Ghrelin or vehicle (saline) was injected intraperitoneally (0.5 ml/rat).

2.3. Measurement of solid gastric emptying

After a 24-h fasting, rats were given 1.5 g of solid meal, as previously described [22]. Immediately after the solid meal ingestion, ghrelin (0.4–8.0 μg/kg; 0.5 ml) or saline (0.5 ml) was applied by intraperitoneal (IP) injection. Ninety minutes after feeding, rats were euthanized by the IP injection of pentobarbital sodium (200 mg/kg). The stomach was surgically isolated and removed. The gastric content was recovered from the stomach, dried, and weighed. Solid gastric emptying was calculated according to the following formula, as previously described [22].

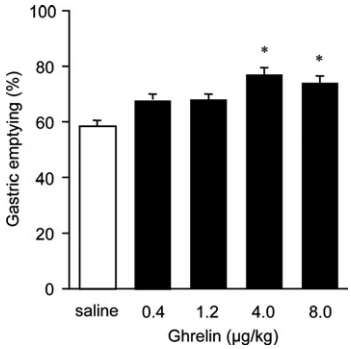

Fig. 1. Effects of ghrelin on solid gastric emptying. IP-administration of ghrelin (0.4–8.0 μg/kg) significantly accelerated gastric emptying in a dose dependent manner. Maximum effect was observed at 4.0 μg/kg of ghrelin, which accelerated gastric emptying at 76.7±3.7% (n=6). Ghrelin (8.0 μg/kg) did not further accelerate solid gastric emptying (*P<0.05 compared to saline-injected group).

Gastric emptying (%)=[1−(dried weight of food recovered from stomach/weight of food intake)]×100.

2.4. Surgery

After an overnight fast, rats were anesthetized with pentobarbital sodium (45 mg/kg, IP, Nembutal; Abbott Laboratories, North Chicago, IL). The stomach was exposed and strain gauge transducers were implanted on the serosal surface of the gastric antrum and pylorus through a midline laparotomy, as previously described [21–23]. The wires from transducers were exteriorized and ran under skin toward the back. Wires were protected by a protective jacket (Star Medical, Tokyo, Japan). After the surgery, rats were housed individually access to a standard diet and tap water. Rats were allowed to recover for one week before the motility recording study.

2.5. Motility recording

After a 24-h fasting, contractions of the antrum and pylorus were recorded in conscious, freely moving rats. The wires from the transducer were connected to a recording system (Power-Lab model 8SP, ADI instruments, Colorado Springs, CO) and fasted gastric contractions were monitored for 2 h. Then, the rats received preweighed 1.5 g of solid meal. Immediately after the solid meal ingestion, ghrelin (4.0 μg/kg; 0.5 ml) or saline (0.5 ml) was applied by intraperitoneal (IP) injection. Postprandial contractions were monitored for 2 h.

2.6. Evaluation of antro-pyloric coordination

As previously described [21–23], antro-pyloric coordination was defined as a single contraction at the antrum that propagated aborally in the pylorus within 10 s and was followed by quiescence period (20 s or more). Individual contractions were defined as >2 g change of >1 s duration.