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doi:10.3748/wjg.14.6299

World J Gastroenterol 2008 November 7; 14(41): 6299-6302
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

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Effects of ghrelin on interdigestive contractions of the rat gastrointestinal tract

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Received: October 15, 2008 Revised: October 30, 2008
Accepted: November 6, 2008
Published online: November 7, 2008

Abstract

Ghrelin causes interdigestive contractions of the stomach in rats. However, it remains unknown whether ghrelin causes interdigestive contractions in the small intestine. Four strain gauge transducers were implanted on the antrum, duodenum, proximal and distal jejunum. After an overnight fast, gastrointestinal (GI) contractions were recorded in freely moving conscious rats. Spontaneous phase Ⅲ-like contractions were observed at every 13-16 min in rat GI tract. The fasted motor patterns were replaced by the fed motor pattern immediately after food intake. Two minutes after finishing the spontaneous phase Ⅲ-like contractions in the antrum, acyl ghrelin (0.8, 2.4 and 8.0 μg/kg per min) was continuously infused for 30 min. Three-five minutes after the starting ghrelin infusion, augmented phase Ⅲ-like contractions were observed at the antrum, duodenum, and jejunum. Ghrelin infusion (0.8, 2.4 and 8.0 μg/kg per min) significantly increased motility index of phase Ⅲ-like contractions at the antrum and jejunum in a dose dependent manner, compared to that of saline injection. Thus, it is likely that exogenously administered ghrelin causes phase Ⅲ-like contraction at the antrum, which migrates to the duodenum and jejunum. The possible role of 5-HT, in addition to ghrelin, in mediating intestinal migrating motor complex (MMC), is discussed.

Key words: Phase Ⅲ-like contractions; Strain gage transducers; Motility index

Taniguchi H, Ariga H, Zheng J, Ludwig K, Takahashi T. Effects of ghrelin on interdigestive contractions of the rat gastrointestinal tract. *World J Gastroenterol* 2008; 14(41): 6299-6302 Available from: URL: http://www.wjgnet.com/1007-9327/14/6299.asp DOI: http://dx.doi.org/10.3748/wjg.14.6299

GHRELIN AND INTERDIGESTIVE GASTRIC MOTILITY

In the interdigestive state, the stomach and small intestine show a remarkable motor pattern, known as the migrating motor complex (MMC)[1]. MMC consists of three phases; phase I (period of motor quiescence), phase Ⅱ (period of irregular low amplitude contractions) and phase Ⅲ (period of regular high amplitude contractions). In humans and dogs, MMC is usually observed every 90-120 min in the interdigestive state. In contrast, in rats, MMC cycle is less than 20 min and not so regular, compared to humans and dogs[2,3]. Exogenously administered motilin does not induce phase Ⅲ-like contractions in rats. Motilin or its receptors are not found in rats[4].

Ghrelin, a 28-amino acid peptide, was discovered as the endogenous ligand for growth hormone secretagogue receptor (GHS-R) from the rat stomach[5]. Because of a structural resemblance to motilin, ghrelin is known as the motilin-related peptide[6,7]. Ghrelin administration causes phase Ⅲ-like contraction at the antrum and duodenum in conscious rats[3,8]. We recently showed that gastric spontaneous phase Ⅲ-like contractions were abolished by ghrelin receptor antagonists[9]. This suggests that endogenous ghrelin regulates spontaneous phase Ⅲ-like contractions of the rat stomach.

However, it still remains unknown whether ghrelin regulates intestinal phase Ⅲ-like contractions in rats. In the current study, we investigated whether exogenously administered ghrelin stimulates phase Ⅲ-like contractions of gastrointestinal (GI) tract in conscious rats.

EFFECTS OF GHRELIN ON THE INTERDIGESTIVE GI CONTRACTIONS

Male Sprague-Dawley rats weighing 280-340 g were kept

in-group cages under conditions of controlled temperature (22-24℃), humidity and light (12 h light cycle starting at 7:00 am) with free access to laboratory chow and water. Protocols describing the use of rats were approved by the Institutional Animal Care and Use Committee of Zablocki VA Medical Center (Milwaukee) and carried out in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals". All efforts were made to minimize animal suffering and to reduce the number of animal in experiments.

After overnight fasting, the rats were anesthetized with intraperitoneal injection of pentobarbital sodium (45 mg/kg). Through a midline laparotomy, strain gauge transducers were implanted on the serosal surface of the antrum, duodenum and jejunum. Duodenal transducers were implanted at 5 cm distal from the pylorus. Jejunal transducers were implanted at 15 cm (the proximal jejunum: J-1) and 25 cm (the distal jejunum: J-2) distal from the pylorus, respectively. The wires from transducer were exteriorized through abdominal wall, ran under skin toward the back. Intravenous catheter was inserted into right jugular vein, and similarly exteriorized to the back, as previously reported[2,9]. The catheter was filled with heparinized saline (100 U/mL) to prevent coagulation. Wires and a catheter were protected by a protective jacket (Star Medical, Tokyo, Japan). After the surgery, rats were housed individual and were allowed to recover for one week before the experiments.

After the implantation of transducers, rats were given food once daily at 12:00 pm-16:00 pm, as previously reported[2]. Experiments of GI motility recording were started at 9:00 am every day. The wires from the transducer were connected to the recording system (PowerLab model 8SP, ADI instruments, Colorado Springs, CO). GI contractions were measured with free access to water in freely moving conscious rats. Spontaneous phase Ⅲ-like contractions were observed for 2-3 h. Phase Ⅲ-like contractions were defined as clustered potent contractions with amplitude of more than 4 g, as previously reported[10].

Fujino *et al*[3] reported that bolus injection of acyl ghrelin (1 g/rat; iv) induced phase Ⅲ-like contraction in conscious rats. In general, bolus injection of certain peptides abruptly increased its plasma level. In our previous study, acyl ghrelin (0.8 μg/kg per min) was continuously infused for 5 min and potent phase Ⅲ-like contractions were observed in the antrum in rats[9]. In our current study, acyl ghrelin (0.8, 2.4 and 8.0 μg/kg per min) was continuously infused for 30 min. Acyl ghrelin was purchased from Tocris Cookson (Ellisville, MO).

Motility index (MI), area under the curve, was calculated using a computer-assisted system (PowerLab, ADInstruments, Colorado Springs, CO). MI in GI tract was compared thirty minutes before and during the infusion of acyl ghrelin. Saline infused rats served as controls.

Results were shown as mean ± SE. ANOVA followed by student's *t*-test was used to assess the difference among groups. A P value < 0.05 was considered to be statistically significant.

It has been showed that spontaneous phase Ⅲ-like contractions are observed at 12-15 min intervals of the stomach[3,9,10] in conscious rats. In our current study, cyclic changes of contractions were detected in the antrum, duodenum, J-1 and J-2 including a quiescence period (phase Ⅰ-like contractions) followed by a grouping of strong contractions (phase Ⅲ-like contractions). Spontaneous phase Ⅲ-like contractions were observed at every 13-16 min in rat GI tract. The fasted motor patterns were replaced by the fed motor pattern immediately after food intake[11].

Two minutes after finishing the spontaneous phase Ⅲ-like contractions in the antrum, acyl ghrelin (0.8, 2.4 and 8.0 μg/kg per min) was continuously infused for 30 min. Three-five minutes after the starting ghrelin infusion, augmented phase Ⅲ-like contractions were observed at the antrum, duodenum, J-1 and J-2 (Figure 1).

Ghrelin infusion (0.8, 2.4 and 8.0 μg/kg per min) significantly increased MI of phase Ⅲ-like contractions at the antrum and jejunum compared to that of saline injection, in a dose-dependent manner (Figure 2).

It is well established that exogenously administered ghrelin causes phase Ⅲ-like contractions in the interdigestive state at the antrum and duodenum in rats[3]. However, it is not clear whether intestinal phase Ⅲ-like contractions are affected by ghrelin administration.

We evaluated the effects of peripherally infused ghrelin on gastrointestinal phase Ⅲ-like contractions in freely moving conscious rats. We demonstrated that ghrelin infusion induced phase Ⅲ-like contractions in the antrum, duodenum, proximal and distal jejunum in a dose-dependent manner (0.8-80 μg/kg per min). This suggests that gastric phase Ⅲ-like contractions induced by exogenously administered ghrelin migrate distally to the small intestine.

We have previously shown that GHS-R antagonists significantly inhibited spontaneous phase Ⅲ-like contractions in conscious rats[9], suggesting that endogenously released ghrelin regulates spontaneous phase Ⅲ-like contractions. We also showed the correlation between the plasma ghrelin levels and occurrence of gastric phase Ⅰ- and Ⅲ-like contractions of the antrum[9]. However, it is not clear whether intestinal phase Ⅲ-like contractions are regulated by endogenously released ghrelin. Previous report showed that ghrelin stimulates motility in the rat small intestine and that the stimulatory effect of ghrelin is mediated *via* cholinergic neurons of the myenteric plexus[12].

Our recent study showed that GHS-R antagonists inhibited phase Ⅲ-like contractions at the antrum, but not the duodenum and the jejunum[11]. Previous studies also showed that GHS-R antagonists did not affect phase Ⅲ-like contractions in the duodenum[8].

It is likely that exogenously administered ghrelin (a pharmacological dose of ghrelin) causes phase-Ⅲ like contraction at the antrum, which migrates to the jejunum. In contrast, endogenously released ghrelin causes spontaneous phase Ⅲ-like contractions at the antrum, which do not migrate to the small intestine.

It has been demonstrated that 5-HT is involved in mediating interdigestive contractions of the small intes-

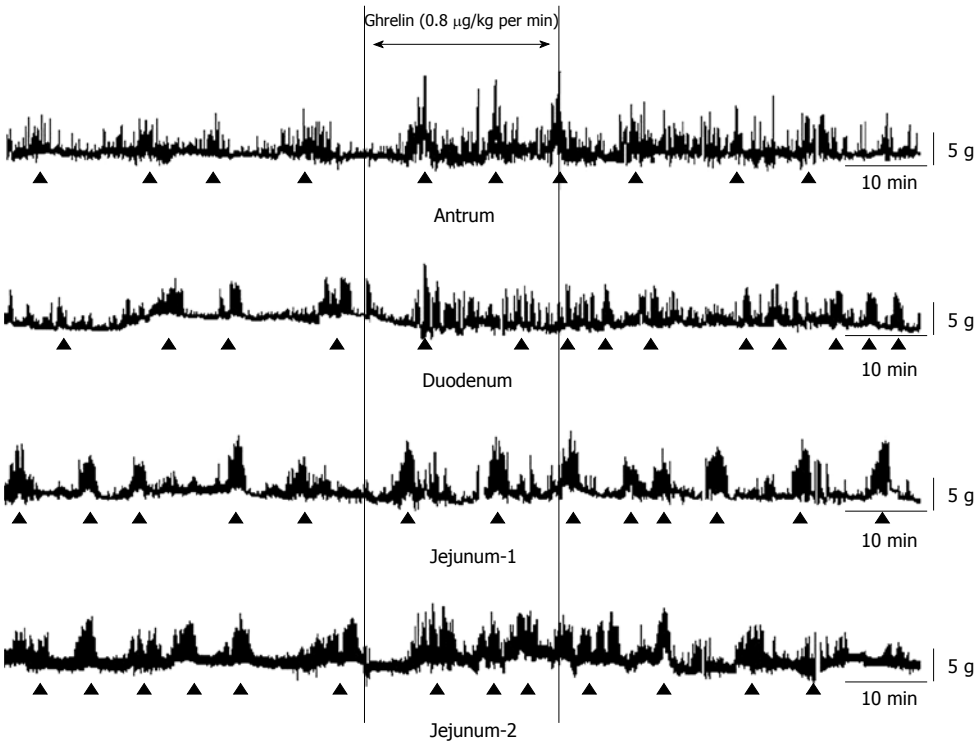

Figure 1 Effect of ghrelin infusion (0.8 μg/kg per min) on spontaneous phase Ⅲ-like contractions in conscious rats. Two minutes after finishing the spontaneous phase Ⅲ-like contractions in the antrum, acyl ghrelin (0.8 μg/kg per min) was continuously infused for 30 min. Three-five minutes after starting the ghrelin infusion, phase Ⅲ-like contractions were observed in response to ghrelin infusion at the antrum, duodenum, jejunum-1 and jejunum-2 (▲ indicates phase Ⅲ-like contractions).

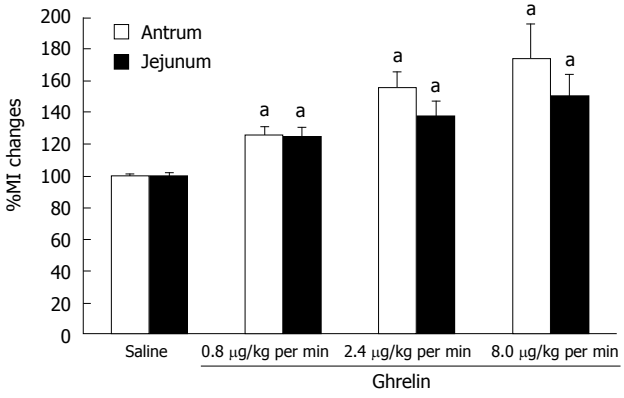

Figure 2 Effect of ghrelin on % changes of MI of phase Ⅲ-like contractions of GI tract ($^aP < 0.05$ *vs* saline, $n = 5$). Ghrelin infusion (0.8, 2.4 and 8.0 μg/kg) dose-dependently increased MI of phase Ⅲ-like contractions of the antrum and jejunum ($^aP < 0.05$ *vs* saline, $n = 5$).

tine in rats[13]. Subcutaneous or intravenous administration of 5-HT can induce intestinal migrating myoelectrical activity in rats[14,15]. Intestinal migrating myoelectrical activity was reduced by a 5-HT_3 antagonist, but not by a 5-HT_4 antagonist in conscious rats[15]. However, others showed that intestinal migrating myoelectrical activity was reduced by a 5-HT_4 antagonist, as well as a 5-HT_3 antagonist[16].

Our recent study showed that phase Ⅲ-like contractions at the jejunum, not the antrum and duodenum, were significantly attenuated by 5-HT_4 antagonists. In contrast, 5-HT_3 antagonists did not affect phase Ⅲ-like contractions in all of upper GI tract[11]. These suggest that spontaneous phase Ⅲ-like contractions at the jejunum is mediated *via* 5-HT_4 receptors, but not 5-HT_3 receptors.

5-HT_3 receptors[17] and 5-HT_4 receptors[18] are located on the cholinergic neurons of the myenteric plexus as well as sensory neurons of the intestinal mucosa[19]. Nerve endings of sensory neurons may well be the targets for the 5-HT released from enterochromaffin (EC) cells[20]. It is generally accepted that 5-HT stimulates intrinsic nerve fibers *via* 5-HT_4 receptors[21], while 5-HT stimulates extrinsic nerve fibers *via* 5-HT_3 receptors[19,22] in rats.

5-HT_3 receptors are located on the nerve terminal of vagal afferent of the duodenal mucosa in rats[23]. 5-HT released by mucosal stimuli initiates peristalsis by activating 5-HT_4 receptors on sensory CGRP neurons of the rat colon *in vitro*[24]. These suggest that 5-HT_4 receptors play a major role in mediating an intrinsic neural reflex. It is conceivable that luminally released 5-HT from duodenal EC cells initially stimulates duodenal phase Ⅲ-like contractions *via* 5-HT_4 receptors located on intrinsic primary afferent neurons (IPAN).

It has been shown that ghrelin receptors are synthesized in vagal afferent neurons and transported to the afferent terminal. This is the major pathway conveying ghrelin signals for starvation and growth hormone secretion to the brain[25]. Blockade of the gastric vagal neuron by vagotomy or perivagal application of capsaicin abolished ghrelin-induced feeding, GH secretion, and activation of NPY-producing and GHRH-producing neurons[25]. Ghrelin-induced acid secretion is also abolished by bilateral vagotomy[26].

Our recent study showed that spontaneous phase Ⅲ-like contractions were completely disappeared in vagotomized rats[11]. These results suggest that ghrelin-induced spontaneous phase-Ⅲ like is mediated *via* vagal pathways.

Spontaneous phase Ⅲ-like contractions are mainly regulated by ghrelin in the antrum, while spontaneous phase Ⅲ-like contractions are regulated by 5-HT in the jejunum. Released ghrelin from the gastric mucosa initi-

ates gastric phase Ⅲ-like contractions *via* vagal dependent pathways. Released 5-HT from intestinal EC cells induces intestinal phase Ⅲ-like contractions *via* IPAN in rats.

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S- Editor Xiao LL **E- Editor** Ma WH