Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2008 March 7; 14(9): 1419-1424
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Gastric motor effects of ghrelin and growth hormone releasing peptide 6 in diabetic mice with gastroparesis

Wen-Cai Qiu, Zhi-Gang Wang, Wei-Gang Wang, Jun Yan, Qi Zheng

Wen-Cai Qiu, Zhi-Gang Wang, Wei-Gang Wang, Jun Yan, Qi Zheng, Department of General Surgery, The Affiliated Sixth Hospital of Medical School, Shanghai Jiaotong University, Shanghai 200233, China
Author contributions: Qiu WC and Wang ZG contributed equally to this work, Qiu WC and Wang ZG completed the animal experiment, organized the experimental statistical data and wrote the paper; Wang WG and Yan J finished the RT-PCR test; Zheng Q helped write, organize, and correct the paper.
Supported by National Nature Science Foundation of China, No. 30400429
Correspondence to: Qi Zheng, Department of General Surgery, The Affiliated Sixth Hospital of Medical School, Shanghai Jiaotong University, Shanghai 200233, China. sh6_zhengqi@126.com
Telephone: +86-21-64369181 Fax: +86-21-64701361
Received: November 11, 2007 Revised: December 29, 2007

Abstract

AIM: To investigate the potential therapeutic significance of ghrelin and growth hormone releasing peptide 6 (GHRP-6) in diabetic mice with gastric motility disorders.

METHODS: A diabetic mouse model was established by intraperitoneal (*ip*) injection of alloxan. Diabetic mice were injected *ip* with ghrelin or GHRP-6 (20-200 μg/kg), and the effects on gastric emptying were measured after intragastric application of phenol red. The effect of atropine, N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) or D-Lys^3-GHRP-6 (a growth hormone secretagogue receptor (GHS-R) antagonist) on the gastroprokinetic effect of ghrelin or GHRP-6 (100 μg/kg) was also investigated. The effects of ghrelin or GHRP-6 (0.01-10 μmol/L) on spontaneous or carbachol-induced contractile amplitude were also investigated *in vitro*, in gastric fundic circular strips taken from diabetic mice. The presence of growth hormone secretagogue receptor 1a transcripts in the fundic strips of diabetic mice was detected by reverse transcriptase polymerase chain reaction (RT-PCR).

RESULTS: We established a diabetic mouse model with delayed gastric emptying. Ghrelin and GHRP-6 accelerated gastric emptying in diabetic mice with gastroparesis. In the presence of atropine or L-NAME, which delayed gastric emptying, ghrelin and GHRP-6 (100 μg/kg) failed to accelerate gastric emptying. D-Lys^3-GHRP-6 also delayed gastric emptying induced by the GHS-R agonist. Ghrelin and GHRP-6 increased the carbachol-induced contractile amplitude in gastric fundic strips taken from diabetic mice. RT-PCR confirmed the presence of *GHS-R* mRNA in the strip preparations.

CONCLUSION: Ghrelin and GHRP-6 increase gastric emptying in diabetic mice with gastroparesis, perhaps by activating peripheral cholinergic pathways in the enteric nervous system.

Key words: Gastric emptying; Ghrelin; Growth hormone releasing peptide 6; Growth hormone secretagogue receptor; Diabetic mice

Peer reviewer: Leonard R Johnson, Professor, Department of Physiology, University Tennessee College of Medicine, 894 Union Ave, Memphis, TN 38163, United States

Qiu WC, Wang ZG, Wang WG, Yan J, Zheng Q. Gastric motor effects of ghrelin and growth hormone releasing peptide 6 in diabetic mice with gastroparesis. *World J Gastroenterol* 2008; 14(9): 1419-1424 Available from: URL: http://www.wjgnet.com/1007-9327/14/1419.asp DOI: http://dx.doi.org/10.3748/wjg.14.1419

INTRODUCTION

Delayed gastric emptying occurs in more than 50% of patients with chronic diabetes mellitus (DM) and is always associated with impaired quality of life and diabetic control. While this delay is not always clinically apparent, the range of gastrointestinal symptoms may include nausea, vomiting, regurgitation, fullness, and bloating[1]. Diabetic patients with poor gastric emptying have a number of possible metabolic consequences, including poor glycemic control, increased risk of postprandial hypoglycemia and variable drug absorption. At its worst, gastroparesis can lead to intractable vomiting and an inability to feed, and carries a poor prognosis[2].

Present management of diabetic gastroparesis involves empirical use of prokinetic drugs such as domperidone, metoclopramide, cisapride[2,3] and erythromycin[4]. The effects of these drugs, however, are unpredictable. One possible explanation for this lack of sustained response to treatment is that gastroparesis may be originally associated with progressive autonomic neuropathy[5,6].

Ghrelin, a 28-amino acid peptide with an octanoyl moiety at Ser^3, was discovered in 1999 as the endogenous

ligand for the growth hormone secretagogue receptor (GHS-R), now often referred to as the ghrelin receptor[7]. The ghrelin receptor, originally called GHS-R1a, has also been called GRLN receptor since the discovery of ghrelin[8]. This receptor was first characterized, cloned and identified as the receptor for a family of synthetic ligands known as growth hormone secretagogues, which stimulate the release of growth hormone (GH)[9]. Ghrelin and its receptor have been localized to the gastrointestinal tracts of many mammalian species, including the mouse, rat and humans[7,10-13]. In rats[14], mice[15,16] and dogs[17], ghrelin has been found to increase gastric emptying, and the site of action may involve the enteric nervous system. In rats and mice, a gastroprokinetic-like activity of ghrelin may be observed *in vitro* as an increase in neuronally mediated contractions evoked by electrical field stimulation (EFS)[11,14]. Growth hormone-releasing peptide-6 (GHRP-6) is a synthetic peptide that causes release of GH, similar to the effect of ghrelin, but through an as yet unknown mechanism. Diabetic gastroparesis is a disabling condition with no consistently effective treatment; however, the effect of ghrelin and its synthetic peptide GHRP-6 on diabetic mice with gastroparesis has not been reported. Therefore, we investigated the potential therapeutic significance of ghrelin and GHRP-6 in diabetic mice with gastric motility disorders.

MATERIALS AND METHODS

Chemicals

Rat ghrelin, GHRP-6 and D-Lys^3-GHRP-6 were obtained from Tocris Cookson (Bristol, UK). N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) was purchased from Bachem (Bubendorf, Switzerland). Atropine sulfate, phenol red and alloxan were obtained from Sigma (St Louis, Missouri, USA).

Preparation of experimental animals

C57 mice weighing 18-22 g were obtained from the experimental Animal Center of the Shanghai Academia Sinica. All procedures were approved by the Medical Ethics Committee of Shanghai Jiaotong University. Mice were housed in stainless steel cages at a controlled temperature (22 ± 2℃) and 60%-65% relative humidity with a normal 12 h light/dark cycle. Six mice were randomly selected as normal controls, and the rest were fed a high-fat diet. After exposure to the high-fat diet for 3 wk, the mice were fasted overnight with free access to water, and injected intraperitoneally (*ip*) with alloxan (0.2 g/kg body weight) dissolved in sterile normal saline solution. Seventy-two h later, the fasting blood glucose levels of the mice were determined using the glucose oxidase method with a Glucose Analyzer. Mice with a blood glucose level greater than 11.1 mmol/L were defined as diabetic (DM) mice. DM mice continued to feed without control of blood glucose for 4 wk; then, the mice that were defined to be DM mice with gastroparesis, as confirmed by subsequent tests, were used for further investigations.

Studies of gastric emptying in vivo

Mice were allowed free access to water 12 h before the experiment. DM mice were injected with either ghrelin (0, 20, 50, 100, or 200 μg/kg; *ip*) or GHRP-6 (0, 20, 50, 100, or 200 μg/kg; *ip*) in a random order. Modulation of the effects of the growth hormone secretagogue receptor (GHS-R) agonists by pharmacological blockers was tested by *ip* administration of atropine (1 mg/kg), L-NAME (50 mg/kg) or D-Lys^3-GHRP-6 (5 μmol/kg) 15 min before administration of the GHS-R agonist (ghrelin 100 μg/kg or GHRP-6 100 μg/kg). Each drug treatment group consisted of at least six DM mice. An additional group consisting of at least 6 DM mice were injected with saline as normal controls.

Immediately after the injection of the drug, 5 mg/kg body weight phenol red test meal (0.5 g/L in 0.9% NaCl with 1.5% methylcellulose) was administered intragastrically with an orogastric canula. The mice were sacrificed 20 min later. The stomach was clamped with a string above the lower esophageal sphincter and a string beneath the pylorus to prevent leakage of phenol red. The stomach was cut just beneath the strings and was frozen at -70℃ until measurement of gastric emptying. Gastric emptying was determined spectrophotometrically using a previously described method[18,19]. The stomach of each mouse was cut just above the lower esophageal sphincter and the pyloric sphincter. Phenol red remained largely in the lumen of the stomach, although some was trapped in the mucus layer of the stomach, and a very small amount of phenol red was reabsorbed in the mucosa after 20 min. The stomach and its contents were submerged in 5 mL of 0.1 mol/L NaOH. The stomach was minced, and these samples contained the total amount of phenol red present in the stomach. The samples were further diluted in 10 mL 0.1 mol/L NaOH and left at room temperature for 1 h. Five mL of the supernatant was then centrifuged at 800 × *g* for 20 min. The absorbance was read at a wavelength of 546 nm with a spectrophotometer (Shanghai Yixian Company, China), and the phenol red content present in the stomach was calculated. The percentage of gastric emptying of the mice was calculated as (infusion-remained/infusion) × 100%.

Contractility measurements in vitro

DM mice were sacrificed by cervical dislocation, and the stomach was removed and rinsed with ice cold saline. Circular muscle strips, freed from mucosa (length 8-10 mm, width 0.2 mm) were cut from the fundus and suspended vertically in an organ bath filled with Krebs solution (120.9 mmol/L NaCl, 2.0 mmol/L NaH_2PO_4, 15.5 mmol/L $NaHCO_3$, 5.9 mmol/L KCl, 1.25 mmol/L $CaCl_2$, 1.2 mmol/L $MgCl_2$, and 11.5 mmol/L glucose) warmed at 37℃ and gassed with 95% O_2/5% CO_2. One end of the strip was fixed to a hook on the bottom of the chamber while the other end was connected by a thread to an external isometric force transducer (BK Company, USA) at the top. After 1 h of equilibration at optimal stretch (0.75 g), the reproducibility of the contractile response to carbachol (0.1 μmol/L) was assessed. Mechanical responses in the smooth muscle strips were measured using an isometric force transducer and stored on a computer for analysis using the SMUP-E biological signal processing system (Chengdu Equipment Factory, China). To investigate the modification

of neuro-effector transmission by GHS-R agonists, the response was studied in the presence and absence of carbachol (0.1 μmol/L), which, when used, was added to the tissue bath 0.5 min before application of the GHS-R agonists. The effect of GHS-R agonists on spontaneous or carbachol (0.1 μmol/L)-induced contractile activity in DM mouse fundic muscle strips was studied by measuring the mean contractile amplitude of the muscle strips.

Measurement of the growth hormone secretagogue receptor by RT-PCR

Total RNA was prepared from DM mouse fundic muscle strips using Trizol reagent (Invitrogen, Carlsbad, CA). Single-stranded cDNA was synthesized using an oligo (dT) anchor primer and Superscript™ Ⅱ RNase H⁻ reverse transcriptase (Gibco BRL, NY, USA). The obtained cDNA served as a template for polymerase chain reaction, consisting of 35 cycles of amplification (95℃ for 10 min, 94℃ for 50 s, 60℃ for 30 s, 72℃ for 30 s) with a final elongation of 10 min at 72℃ using 0.5 U of Taq DNA polymerase (Promega, Sweden) and 0.5 μmol/L primers (forward: 5'-CGACCTGCTCT GCAAACTC-3' and reverse: 5'-CACGCCCACCAGCACGAAGA-3'). PCR using intron-spanning mouse β-actin primers (forward: 5'-CCTGTATGCCTCTGGTCGTA-3' and reverse: 5'-CCATCTCCTGCTCGAAGTCT-3'), demonstrated that cDNA was present and devoid of genomic DNA contamination. The expected sizes of GHS-R and β-actin fragments were 217 bp and 260 bp, respectively. All primers were selected from conserved regions identified by the alignment of published sequences for GHS-R mRNA in Genbank. PCR products were separated by electrophoresis on 1.4% agarose gels and photos of the separated products were taken.

Statistical analysis

Data are expressed as mean ± SE. One-way ANOVA was used for statistical analyses of multiple comparisons, and a *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

Contractility in vivo

Compared with the gastric emptying rate of the normal mice (28.10% ± 1.28%), the gastric emptying rate of the DM mice was significantly reduced (22.90% ± 1.42%, $P < 0.05$. In DM mice, ghrelin accelerated gastric emptying of the semi-liquid meal at doses of 50, 100 and 200 μg/kg; the emptying rate was significantly accelerated from 22.90% ± 1.42% to 27.80% ± 0.97%, 34.50% ± 1.20% and 32.90% ± 1.10% at doses of 50, 100 and 200 μg/kg, respectively ($P < 0.05$, compared to injection of saline) (Figure 1). Similarly, GHRP-6 increased gastric emptying dose-dependently with significant effects at 50, 100 and 200 μg/kg ($P < 0.05$) (Figure 1).

The effect of ghrelin or GHRP-6 on DM mouse gastric emptying was characterized pharmacologically. Ghrelin (100 μg/kg) or GHRP-6 (100 μg/kg) was unable to reverse the inhibition of gastric emptying due to pretreatment

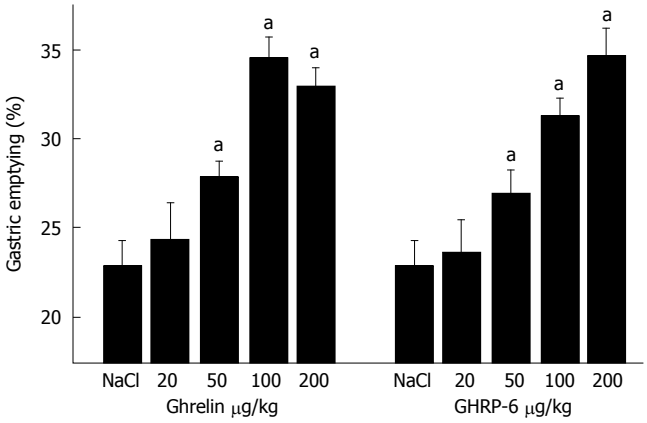

Figure 1 Effect of increasing doses of ghrelin (0-200 μg/kg, *ip*) or GHRP-6 on gastric emptying in DM mice. Bars and error bars represent the mean ± SE of at least six animals. $^aP < 0.05$ *vs* normal saline (NaCl).

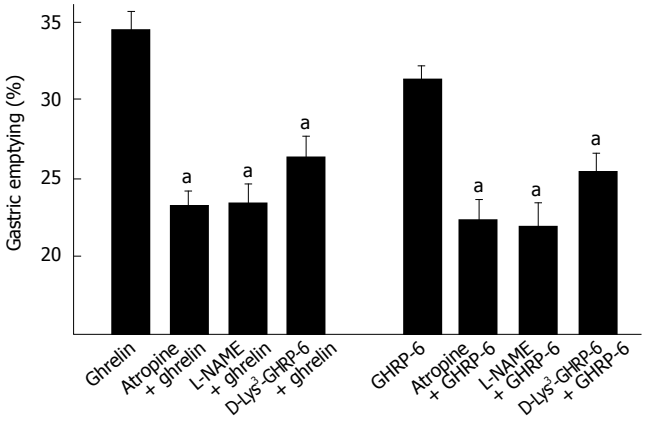

Figure 2 Effects of atropine, L-NAME and D-Lys^3-GHRP-6 on the gastroprokinetics of ghrelin or GHRP-6 in the DM mice. Mice were pretreated with atropine (1 mg/kg), L-NAME (50 mg/kg) or D-Lys^3-GHRP-6 (5 μmol/kg) before administration of ghrelin (100 μg/kg) or GHRP-6 (100 μg/kg), and the effects were compared with those of treatment with ghrelin or GHRP-6 (100 μg/kg) alone. Bar graph and error bars represent the means ± SE of at least six animals. $^aP < 0.05$ *vs* treatment with ghrelin or GHRP-6 (100 μg/kg) alone.

with atropine (1 mg/kg) or L-NAME (50 mg/kg) ($P < 0.05$). Pretreatment of DM mice with D-Lys^3-GHRP-6 (5 μmol/kg) also delayed the accelerated gastric emptying induced by ghrelin or GHRP-6 ($P < 0.05$) (Figure 2).

Contractility in vitro

Fundic strips from the DM mice showed spontaneous contractile activity after 1 h of equilibration. Ghelin (0.01-10 μmol/L) or GHRP-6 (0.01-10 μmol/L) did not significantly change spontaneous contractile responses in the strips (Table 1). However, in the presence of carbachol (0.1 μmol/L), ghrelin increased the carbachol-induced contractile amplitude at 0.1, 1 and 10 μmol/L. GHRP-6 also increased the carbachol-induced contractile amplitude at 0.1, 1 and 10 μmol/L (Table 2).

Expression of the ghrelin receptor in mouse fundic strips

The presence of *GHS-R* mRNA in the mouse fundic smooth muscle strips was verified by RT-PCR with

Table 1 Effects of GHS-R agonists on the spontaneous contractile amplitudes of DM mouse fundic strips

Group	Spontaneous contractile amplitude of DM mouse fundic strips (mg)				
	0	0.01 μmol/L	0.1 μmol/L	1 μmol/L	10 μmol/L
Ghrelin	20.4 ± 1.15	21.3 ± 0.98	19.8 ± 1.16	22.1 ± 1.58	21.5 ± 1.36
GHRP-6	20.4 ± 1.15	20.8 ± 1.12	21.3 ± 1.74	20.6 ± 1.27	21.3 ± 1.35

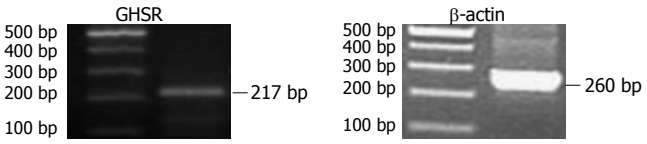

Figure 3 Expression of GHS-R mRNA in gastric fundic strips from DM mice. The band at 217 bp corresponds to the amplified GHS-R cDNA product with the expected length. The band at 260 bp corresponds to the amplified β-actin cDNA product with the expected length.

Table 2 Effect of GHS-R agonists on the carbachol (0.1 μmol/L)-induced contractile amplitudes of DM mouse fundic strips

Group	Spontaneous contractile amplitude of DM mouse fundic strips (mg)				
	0	0.01 μmol/L	0.1 μmolL	1 μmol/L	10 μmol/L
Ghrelin + carbachol (0.1 μmol/L)	60.4 ± 1.21	61.3 ± 1.52	65.7 ± 1.16[a]	70.0 ± 1.58[a]	78.0 ± 1.56[a]
GHRP-6 + carbachol (0.1 μmol/L)	60.4 ± 1.21	62.3 ± 2.14	65.4 ± 1.24[a]	72.0 ± 1.42[a]	82.0 ± 1.75[a]

[a]$P < 0.05$, *vs* carbachol (0.1 μmol/L)-induced contraction amplitude of the DM mouse fundic strips.

gene-specific primers. Analysis of the PCR products by electrophoresis revealed a band with the expected length of 217 bp (Figure 3).

DISCUSSION

We have demonstrated, for the first time, that ghrelin and the synthetic peptide GHRP-6 improve gastric emptying in diabetic mice with gastroparesis. This effect may be mediated through potentiation of peripheral cholinergic pathways in the enteric nervous system.

Ghrelin, a recently discovered peptide hormone, is primarily produced by endocrine cells in the oxyntic mucosa of the stomach in rats and humans[7,12]. Ghrelin has also been found in the small intestine, testis, pituitary gland, ovary, liver, pancreas, kidney, placenta and hypothalamus, in both humans and rodents[7,11]. Ghrelin is a natural ligand for GHS-R, and its receptor is found all over the body, including in the bowel, pancreas, stomach, heart, lungs and brain[7,12,20]. In addition to its effect on growth hormone secretion by activating GHS-R in the pituitary gland, ghrelin enhances appetite, increases food intake[21,22], mediates energy balance, regulates glucose metabolism and insulin release[23], stimulates gastric acid secretion[24] and promotes anxiety[25]. It is well known that many gastrointestinal peptides participate in the regulation of gastrointestinal functions. Ghrelin is one of these candidate gastrointestinal peptides, because it is predominantly present in gastric endocrine cells and is secreted into the bloodstream. In fact, the potential of ghrelin and its synthetic peptide GHRP-6 as a prokinetic agent has been shown previously in *in vitro* and *in vivo* studies. Previous studies on the effect of ghrelin on gastric motility have demonstrated the involvement of vagal and central ghrelin receptors. Thus, the effect of ghrelin on gastric emptying is blocked by atropine and vagotomy in rats and mice[18,20]. Peripheral ghrelin may stimulate fasted small intestinal motor activity through receptors on vagal afferents, which activate neuropeptide Y-containing neurons in the brain, as suggested by experiments in rats[26]. In addition, expression of the ghrelin receptor in the rat nodose ganglion has been confirmed using RT-PCR[27]. In addition to the known vagal pathways, ghrelin and GHRP-6 accelerate gastric emptying and small intestinal transit by activating cholinergic excitatory pathways in the enteric neuron system[14,15,17]. Moreover, ghrelin has been shown to increase gastric emptying in patients with gastroparesis, and it has been proposed that ghrelin or its analogues may represent a new class of prokinetic agents for the treatment of gastroparesis[28,29]. In our study, we investigated the effects of ghrelin and GHRP-6 on gastric motility in diabetic mice with gastroparesis. Our findings indicate the potential of ghrelin as a therapeutic approach for gastrointestinal motility disorders.

In our study, the gastric emptying rate in the DM mice was significantly reduced relative to the normal mice. Ghrelin and GHRP-6 accelerated gastric emptying of the diabetic mice with gastroparesis. In the presence of atropine or L-NAME, which delayed gastric emptying, ghrelin and GHRP-6 (100 μg/kg) failed to accelerate gastric emptying. D-Lys^3-GHRP-6 also delayed gastric emptying induced by GHS-R agonists. Gastric emptying is a complex process involving excitatory and inhibitory nerves, which may contribute to both acceleration and retardation of the emptying process. L-NAME, which blocks inhibitory nitrergic nerves, delayed gastric emptying, probably by interfering with gastric accommodation and pyloric relaxation. Therefore, the effect of ghrelin may involve both excitatory and inhibitory pathways, as suggested by the inability of ghrelin to overcome the delay induced by L-NAME and atropine. Ghrelin has been shown to induce release of nitric oxide in the rat stomach, and in our study, a nitrergic pathway could be involved in the acceleration of gastric emptying because the prokinetic effect *in vivo* was lost in the presence of L-NAME. The GHS-R antagonist D-Lys^3-GHRP-6, also blocked the effect of the GHS-R agonists, and this result indicates that the effect of GHS-R agonists on gastric motility

occurs through GHS-R and likely does not involve cross interactions with other receptors. Ghrelin and GHRP-6 increased the carbachol-induced contractile amplitudes in fundic strips taken from DM mice, and this finding also indicates that GHS-R agonists accelerate gastric emptying of semi-liquid through the activation of GHS-R receptors, possibly located on local cholinergic enteric nerves. Moreover, the presence of *GHS-R* mRNA in the strip preparations was confirmed by RT-PCR.

It remains controversial whether ghrelin can exert a protective effect on gastric mucosa, although previous studies have suggested ghrelin might induce gastric mucosal lesion in rats by increasing acid secretion. It is unlikely the improvement in gastric emptying in DM mice induced by ghrelin or GHRP-6 could be explained by a protective effect of ghrelin and GHRP-6 on gastric mucosa. Acid may inhibit gastric emptying, but the effect of ghrelin on acid secretion remains a controversial issue itself[24,30,31].

In conclusion, ghrelin and its synthetic peptide, GHRP-6, increase gastric emptying in diabetic mice with gastroparesis, perhaps by activating peripheral cholinergic pathways in the enteric nervous system. Although further studies are needed to determine the underlying mechanisms, we propose that ghrelin or its analogues may represent a new class of prokinetic agents for the treatment of diabetic gastroparesis. Therefore, ghrelin and ghrelin agonists have the potential to become useful therapeutic agents for the treatment of diabetic gastroparesis. However, long term animal experiments and clinical trials are needed.

COMMENTS

Background

Delayed gastric emptying is common in patients with chronic diabetes and is always associated with impairments in both quality of life and diabetic control. Ghrelin is a potent prokinetic peptide. Our aim was to test the effect of ghrelin and its synthetic peptide, GHRP-6, on delayed gastric emptying in diabetic mice.

Research frontiers

This study represents the first investigation into the effects of ghrelin and GHRP-6 on diabetic mice with gastroparesis, using both *in vivo* and *in vitro* approaches.

Related publications

Ghrelin has been under intensive study for its effects on gastrointestinal motor activity and its roles in motility regulation. In addition to influencing food intake and energy balance, ghrelin also possesses prokinetic characteristics mediated by the activation of cholinergic pathways.

Innovations and breakthroughs

Ghrelin has been shown to accelerate gastric emptying in postoperative and septic ileus animal models. However, it has not been studied in a diabetic animal model.

Applications

According to our effective therapy in animal experiments, ghrelin and ghrelin agonists may have the potential to become useful therapeutic agents for the treatment of diabetic gastroparesis.

Terminology

Gastroparesis: A condition of delayed stomach emptying, often seen as a complication of diabetes mellitus.

Peer review

This paper investigated for the first time *in vivo* and *in vitro* that ghrelin and its synthetic peptide, GHRP-6, improves gastric emptying in diabetic mice with gastroparesis, and this effect may be mediated through peripheral cholinergic pathways in the enteric nervous system. These results are potentially significant for the clinical treatment of diabetic gastroparesis.

REFERENCES

1 **Horowitz M**, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samsom M. Gastric emptying in diabetes: clinical significance and treatment. *Diabet Med* 2002; **19**: 177-194

2 **Horowitz M**, Su YC, Rayner CK, Jones KL. Gastroparesis: prevalence, clinical significance and treatment. *Can J Gastroenterol* 2001; **15**: 805-813

3 **Patterson D**, Abell T, Rothstein R, Koch K, Barnett J. A double-blind multicenter comparison of domperidone and metoclopramide in the treatment of diabetic patients with symptoms of gastroparesis. *Am J Gastroenterol* 1999; **94**: 1230-1234

4 **Tack J**, Janssens J, Vantrappen G, Peeters T, Annese V, Depoortere I, Muls E, Bouillon R. Effect of erythromycin on gastric motility in controls and in diabetic gastroparesis. *Gastroenterology* 1992; **103**: 72-79

5 **Ewing DJ**, Clarke BF. Autonomic neuropathy: its diagnosis and prognosis. *Clin Endocrinol Metab* 1986; **15**: 855-888

6 **Balaji NS**, Crookes PF, Banki F, Hagen JA, Ardill JE, DeMeester TR. A safe and noninvasive test for vagal integrity revisited. *Arch Surg* 2002; **137**: 954-958; discussion 958-959

7 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660

8 **Davenport AP**, Bonner TI, Foord SM, Harmar AJ, Neubig RR, Pin JP, Spedding M, Kojima M, Kangawa K. International Union of Pharmacology. LVI. Ghrelin receptor nomenclature, distribution, and function. *Pharmacol Rev* 2005; **57**: 541-546

9 **Howard AD**, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996; **273**: 974-977

10 **Nishi Y**, Isomoto H, Ueno H, Ohnita K, Wen CY, Takeshima F, Mishima R, Nakazato M, Kohno S. Plasma leptin and ghrelin concentrations in patients with Crohn's disease. *World J Gastroenterol* 2005; **11**: 7314-7317

11 **Dass NB**, Munonyara M, Bassil AK, Hervieu GJ, Osbourne S, Corcoran S, Morgan M, Sanger GJ. Growth hormone secretagogue receptors in rat and human gastrointestinal tract and the effects of ghrelin. *Neuroscience* 2003; **120**: 443-453

12 **Date Y**, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; **141**: 4255-4261

13 **Gnanapavan S**, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, Bhattacharya S, Carpenter R, Grossman AB, Korbonits M. The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab* 2002; **87**: 2988

14 **Depoortere I**, De Winter B, Thijs T, De Man J, Pelckmans P, Peeters T. Comparison of the gastroprokinetic effects of ghrelin, GHRP-6 and motilin in rats *in vivo* and *in vitro*. *Eur J Pharmacol* 2005; **515**: 160-168

15 **Kitazawa T**, De Smet B, Verbeke K, Depoortere I, Peeters TL. Gastric motor effects of peptide and non-peptide ghrelin agonists in mice *in vivo* and *in vitro*. *Gut* 2005; **54**: 1078-1084

16 **Zhang JV**, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, Hsueh AJ. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 2005; **310**: 996-999

17 **Trudel L**, Bouin M, Tomasetto C, Eberling P, St-Pierre S, Bannon P, L'Heureux MC, Poitras P. Two new peptides to improve post-operative gastric ileus in dog. *Peptides* 2003; **24**: 531-534
18 **De Winter BY**, Bredenoord AJ, De Man JG, Moreels TG, Herman AG, Pelckmans PA. Effect of inhibition of inducible nitric oxide synthase and guanylyl cyclase on endotoxin-induced delay in gastric emptying and intestinal transit in mice. *Shock* 2002; **18**: 125-131
19 **De Winter BY**, De Man JG, Seerden TC, Depoortere I, Herman AG, Peeters TL, Pelckmans PA. Effect of ghrelin and growth hormone-releasing peptide 6 on septic ileus in mice. *Neurogastroenterol Motil* 2004; **16**: 439-446
20 **Xu L**, Depoortere I, Tomasetto C, Zandecki M, Tang M, Timmermans JP, Peeters TL. Evidence for the presence of motilin, ghrelin, and the motilin and ghrelin receptor in neurons of the myenteric plexus. *Regul Pept* 2005; **124**: 119-125
21 **Nakazato M**, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194-198
22 **Asakawa A**, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337-345
23 **Reimer MK**, Pacini G, Ahren B. Dose-dependent inhibition by ghrelin of insulin secretion in the mouse. *Endocrinology* 2003; **144**: 916-921
24 **Masuda Y**, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K. Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res Commun* 2000; **276**: 905-908
25 **Carlini VP**, Monzon ME, Varas MM, Cragnolini AB, Schioth HB, Scimonelli TN, de Barioglio SR. Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem Biophys Res Commun* 2002; **299**: 739-743
26 **Fujino K**, Inui A, Asakawa A, Kihara N, Fujimura M, Fujimiya M. Ghrelin induces fasted motor activity of the gastrointestinal tract in conscious fed rats. *J Physiol* 2003; **550**: 227-240
27 **Sakata I**, Yamazaki M, Inoue K, Hayashi Y, Kangawa K, Sakai T. Growth hormone secretagogue receptor expression in the cells of the stomach-projected afferent nerve in the rat nodose ganglion. *Neurosci Lett* 2003; **342**: 183-186
28 **Murray CD**, Martin NM, Patterson M, Taylor SA, Ghatei MA, Kamm MA, Johnston C, Bloom SR, Emmanuel AV. Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. *Gut* 2005; **54**: 1693-1698
29 **Tack J**, Depoortere I, Bisschops R, Verbeke K, Janssens J, Peeters T. Influence of ghrelin on gastric emptying and meal-related symptoms in idiopathic gastroparesis. *Aliment Pharmacol Ther* 2005; **22**: 847-853
30 **Date Y**, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S. Ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochem Biophys Res Commun* 2001; **280**: 904-907
31 **Dornonville de la Cour C**, Lindstrom E, Norlen P, Hakanson R. Ghrelin stimulates gastric emptying but is without effect on acid secretion and gastric endocrine cells. *Regul Pept* 2004; **120**: 23-32

S- Editor Sun YL **L- Editor** McGowan D **E- Editor** Ma WH