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ORIGINAL ARTICLE

Dose-dependent effect of ghrelin on gastric emptying in rats and the related mechanism of action

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Abstract The aim of this study was to investigate the dose-dependent effect of ghrelin on gastric emptying in rats and the related mechanism of action. Sixty Wistar rats were randomized into control and test groups, which respectively received intraperitoneal injection of normal saline and ghrelin at different doses (0.5 nmol/kg, 1.0 nmol/kg, 1.5 nmol/kg, 2.0 nmol/kg, and 2.5 nmol/kg). After 45 minutes, all rats were gavaged with semisolid paste. The gastric emptying rate was determined 30 minutes later, and the plasma cholecystokinin level was tested by radioimmunoassay. The mean gastric emptying rate in the test groups was significantly higher than in the control group (38.24 ± 7.15% and 27.18 ± 2.37%, respectively, $p < 0.05$). Medium and high doses of ghrelin (1.0 nmol/kg, 1.5 nmol/kg, 2.0 nmol/kg, and 2.5 nmol/kg), but not low dose (0.5 nmol/kg), accelerated the gastric emptying. In addition, the plasma cholecystokinin level in the test groups was significantly higher than in the control group ($p < 0.01$). The gastric emptying rate was positively correlated with the plasma cholecystokinin level ($p < 0.01$). Intraperitoneal injection of ghrelin at medium and high doses significantly accelerated gastric emptying in rats.

Introduction

Ghrelin, a newly discovered 28 amino acid endogenous peptide, is an endogenous ligand of growth hormone secretagogue receptor (GHSR), which induces extensive biological effects after binding to GHSR. Ghrelin promotes secretion of growth hormone, enhances appetite, lowers fat utilization, increases body weight, maintains a positive energy balance, and regulates energy metabolism [1].

Conflicts of interest: All authors declare no conflicts of interests.

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Ghrelin is produced mainly by gastric endocrine cells and enters the blood circulation. Gastric electrophysiological stimulation can markedly promote gastric endocrine cells to produce ghrelin [2]. Research has shown that ghrelin can accelerate gastric emptying and small intestinal propulsion in *Suncus murinus* and mice [3,4]. High doses of ghrelin can significantly enhance contraction of gastrointestinal smooth muscle [5], and intraventricular or intravenous injection of ghrelin can induce postprandial rapid gastric and duodenal contractions in control rats [6]. However, there are few data on the role of ghrelin in the regulation of gastric emptying. We aimed to observe the effects of intraperitoneally injected ghrelin at different doses on gastric emptying in rats to illuminate the regulatory effect of ghrelin on gastric emptying, and to simultaneously detect plasma cholecystokinin level, so as to explore the mechanism of ghrelin in the regulation of gastric emptying.

Methods

Animals and grouping

A total of 60 healthy male Wistar rats weighing 200—250 g were provided by Laboratory Animal Centre of Wenzhou Medical University. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University.

Rats were maintained under a diurnal cycle at 22 ± 3°C with free access to food and water. After 1 week of adaptive raising, the rats were deprived of food for 24 hours and of water for 12 hours, and then randomized into control and test (A—E) groups with 10 rats in each group. The rats in the control and test groups received intraperitoneal injection of normal saline (1 mL) and ghrelin (Sigma, St. Louis, MO, USA) at different doses (A, 0.5 nmol/kg; B, 1.0 nmol/kg; C, 1.5 nmol/kg; D, 2.0 nmol/kg; and E, 2.5 nmol/kg).

Semisolid paste preparation

Five grams of hydroxymethyl cellulose was dissolved in 125 mL distilled water and the following ingredients were added and mixed to make 150 mL semisolid paste (150 g): 8 g milk powder, 4 g sugar, and 4 g starch [7]. The preparation was stored in the fridge for 12 hours and kept at room temperature for 2 hours before use.

Gastric emptying test

The rats in the control group and test groups A—E received intraperitoneal injection of normal saline (1 mL) and 0.5 nmol/kg, 1.0 nmol/kg, 1.5 nmol/kg, 2.0 nmol/kg, and 2.5 nmol/kg octanoyl ghrelin, respectively. Each rat was gavaged with 1 mL semisolid paste after 45 minutes. The tail venous blood was collected 30 minutes later under anesthesia with intraperitoneal injection of pentobarbital (the anesthetic dose of phenobarbital was 8 mg/kg). The abdominal cavity was opened, and the pylorus and cardia were ligated. The stomach was taken out and weighed after being dried with filter paper. It was then cut along the greater curvature, and the gastric content was washed off; the net weight of the stomach was recorded after drying. The difference between the stomach full weight and net weight was defined as the stomach residue weight; the gastric residual rate was the percentage of stomach residue in semisolid paste; the gastric emptying rate was calculated using the following formula:

$$\text{gastric emptying rate} = [1 - (\text{stomach full weight} - \text{net weight})] \times 100\%. \quad (1)$$

At the end of the experiments measuring gastric emptying, animals were euthanized by intraperitoneal injection of excess doses of pentobarbital (the anesthetic dosage of phenobarbital was 40 mg/kg, with the concentration as 2%). After intraperitoneal injection of a large dose of phenobarbital, the rats stopped breathing and their hearts stopped beating, so they were judged as dead.

Detection of cholecystokinin level

The tail venous blood was collected into a tube with 0.3 μL EDTA and 1000 kU aprotinin, followed by cryogenic centrifugation at 3000 g for 10 minutes. The plasma was isolated and the cholecystokinin level was determined using a radioimmunoassay kit (Beijing Haikerui Biotech Center, Beijing, China).

Statistical analysis

All results are presented as mean ± standard error of the mean. The effect of ghrelin on cholecystokinin level in plasma was analyzed by one-way analysis of variance followed by Dunnett's *post hoc* testing. A p value < 0.05 was considered statistically significant. Linear regression was used to study the correlation.

Results

Comparison of gastric emptying

After intraperitoneal injection of ghrelin, the gastric emptying rate in the test groups (38.24 ± 7.15%) was significantly higher than that in the control group (27.18 ± 2.37%; $p = 4.15\times10^{-5}$; Table 1).

Table 1 Comparison of gastric emptying between two groups.

Groups	Cases	Gastric emptying rate (%)
Control	10	27.03 ± 2.91
Test	50	38.24 ± 7.15*

* Compared with control group, $p = 0.0000415$.