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Ghrelin gastrokinetic action in patients with neurogenic gastroparesis

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ABSTRACT

Ghrelin has been shown to accelerate gastric emptying in animals where its effect appeared mediated through the vagus nerve. We aimed to verify the gastrokinetic capacity of ghrelin in human. Patients with gastroparesis attributed to a neural dysregulation by diabetes ($n = 5$) or surgical vagotomy ($n = 1$) were evaluated. The emptying of a test meal (420 kcal) was determined by the C^{13} octanoic acid breath test. Saline or synthetic ghrelin 1–4 μg/kg were given in 1 min bolus at the end of the meal. *T*-lag and *T*-1/2 were shorter during ghrelin than during saline administration [33 ± 5 min versus 65 ± 14 min ($p < 0.01$) and 119 ± 6 min versus 173 ± 38 min ($p < 0.001$)]. Ghrelin injection therefore accelerated gastric emptying of a meal in humans even in presence of a deficient gastric innervation.

1. Introduction

Ghrelin is synthesized in endocrine cells of the gastric mucosa [6]. The most characteristic actions of this recently discovered peptide include the stimulation of growth hormone (GH) release [6,12,17], of appetite and nutrient ingestion [13,20,22], as well as of digestive motility [1,4,8,19].

When injected in mice [1,3], rats [4,8,19], or dogs [18], ghrelin accelerated the gastric emptying of a meal. The gastrokinetic effect of ghrelin was strong enough to reverse delayed gastric emptying in experimental models of gastric ileus [3,18,19].

The motor action of ghrelin appears to be mediated by neural mechanisms. Indeed, ghrelin receptors were difficult to identify on muscles [2], and the action of ghrelin was blocked by antimuscarinic agents or by vagotomy in animals [8].

The primary aim of this study was to document the gastrokinetic effect of ghrelin in humans. Secondary aims included to verify its action in gastroparesis patients, and to gain new information on its mode of action.

2. Methods

The study was approved by the Research and Ethics Committee of Centre hospitalier de l'Université de Montréal. Approval for the experimental use of ghrelin in humans has been obtained from Health Canada.

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2.1. Study subjects

Subjects with symptomatic gastroparesis were included in this study. Gastroparesis had to be confirmed by a scintigraphic gastric emptying test for solid nutrients performed over the last 2 years. The origin of the gastroparesis had to be attributed to neural dysregulation by conditions such as diabetes mellitus, surgical vagotomy, etc. The subjects had to be free of significant heart, lung or kidney dysfunction as well as of any severe health abnormality related or not to their current condition.

Six patients with symptomatic gastroparesis were recruited into the study. All were female; mean age was 50.5 years (36–60), mean height was 154 cm (143–164 cm); and mean weight was 74 kg (54–100 kg). Five patients were suffering from long standing diabetes treated by insulin; all these patients had neurovascular complications (orthostatic hypotension and proteinuria was present in all of them); 1 patient was suffering from severe gastroparesis following a truncal vagotomy performed 20 years ago for peptic ulcer disease well controlled since then.

2.2. Gastric emptying test

Gastric emptying function was evaluated by the C^{13}-octanoic acid breath test, as described by Ghoos et al. [5], to measure *T*-lag (time to empty the first 10% of gastric content) and *T* (time to empty 50% of the labeled meal from the stomach) according to the calculations proposed by Lee et al. [7].

The test meal consisted of one egg labeled with C^{13}-octanoic acid (100 mg), ham (one slice, 50 g), bread (two slices), butter (10 g), milk (2% fat; lactose-free; 200 ml), orange juice (100 ml), and water (100 ml) for a total caloric content of 420 kcal (protein: 18%, carbohydrates: 45%, lipids: 37%).

2.3. Material

C^{13}-octanoic acid 100 mg (purchased from Cambridge Isotope Laboratory, Inc., MA, USA) was mixed with egg yolk before cooking. Control saline (NaCl 0.9) or synthetic human ghrelin (purchased from Merck Biosciences, Clinalfa Division, Switzerland; 20 μg/ml diluted in NaCl 0.9) was injected i.v. in a 1 min bolus given in the left basilic vein. Breath specimens were collected during the test procedure in glass tubes (16 mm × 100 mm) to be counted later in a mass spectrometer for measurement of expelled $^{13}CO_2$ from the lungs after the absorption of the egg labeled with C^{13}-octanoic acid by the small intestine.

3. Experimental protocol

Fasting patients came to the laboratory at 8 a.m. The test meal was ingested from time −10 to 0 min. Breath samples, vital signs (heart rate and blood pressure) and blood glucose (measured by the patient personal glucose meter machine) were obtained at time −30, 0, +15, +30, +40, +60, +90, +120 and +150 min. Blood glucose had to be less than 12 mmol/l prior to breakfast, and diabetic patients injected their insulin dose according to their usual therapeutic regimen for the proposed caloric content and their current basal glycemia.

To compare the effect of ghrelin to basal control conditions, the test subjects were submitted to a cross-over experimental design with a similar experiment being realized at two different days 1 week apart. On day 1, the patients were randomized to receive a bolus injection of saline or ghrelin 1 μg/kg given in 1 min at the end of the meal. On day 8, they were administered the ghrelin or saline solution not given earlier.

After counting of the breath samples collected from the two experiments described above, and measurement of the gastric emptying rate, subjects showings no acceleration of gastric emptying in response to ghrelin 1 μg/kg in comparison to saline were retested with a higher dose of ghrelin (4 μg/kg) given between days 15 and 35 under similar experimental conditions.

3.1. Data analysis

Paired data were compared by Student's test.

4. Results

4.1. Effect of ghrelin on gastric emptying

With the administration of ghrelin 1 μg/kg, four subjects accelerated their gastric emptying rate. *T*-lag in these patients was decreased by 15, 20, 50, and 55%, respectively, while *T*-1/2 was reduced by 11, 12, 44, and 54%. In two subjects (one diabetic and one vagotomized), ghrelin 1 μg/kg failed to accelerate the gastric emptying rate and the ghrelin dose was increased to 4 μg/kg. In these two patients, the gastric emptying rate was then accelerated (*T*-lag and *T*-1/2 were decreased by 14 and 76% and by 20 and 57%, respectively). Combining the positive results obtained with both doses of ghrelin, the *T*-lag was reduced from 65 ± 14 min during control conditions to 33 ± 5 min with ghrelin ($p = 0.002$); *T*-1/2 declined from 173 ± 38 min in control conditions to 119 ± 16 min with ghrelin ($p = 0.001$). These data are presented in Fig. 1.

4.2. Tolerance to ghrelin administration

Ghrelin injection was well tolerated; none of the patients reported side-effects with the peptide infusion. Heart rate as well as systolic and diastolic blood pressures were unchanged (data not shown). Mean blood glucose concentrations increased from 7.4 mmol before the meal to 10.0 mmol/l after the meal during the basal condition, whereas they rose from 11.6 ($p = 0.08$ in comparison to saline) to 14.8 ($p = 0.01$ in comparison to saline) when ghrelin was given; this suggested that hyperglycemia could occur in response to ghrelin administration. However, if blood glucose concentrations were expressed as percentages of the fasting levels to take into consideration the variations in the pre-prandial conditions (difference in basal blood glucose was close to significance), post-prandial glucose levels increased to 159% with saline versus 175% with ghrelin (p = NS), suggesting that ghrelin was not responsible for exaggerated blood glucose levels after the meal.