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Low density of ghrelin cells in the oxyntic mucosa correlated to slow gastric emptying in patients with type 1 diabetes

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Abstract. Ghrelin is a peptide hormone that has been isolated from the stomach and localized to endocrine cells in the oxyntic mucosa. Ghrelin acts synergistically with GH-releasing hormone and increases appetite and feeding. It also accelerates gastric and small intestinal motility in rodents. Patients with diabetes suffer from slow gastric emptying, giving rise to nausea and vomiting. The present study was undertaken to establish the possible role of ghrelin in slow gastric emptying observed in patients with longstanding type 1 diabetes, and to correlate the results with the metabolic status of these patients. Eleven patients with type 1 diabetes along with 10 and 15 healthy volunteers as controls underwent gastrointestinal endoscopy/biopsy or gastric scintigraphy. Gastric emptying in patients and controls was measured by scintigraphy. Sections from biopsies of the oxyntic mucosa and duodenum were immunostained for ghrelin with the avidin-biotin complex method. The density of the cells was quantified with computerized image analysis. Both the lag phase and half-emptying time (T_{50}) were higher in patients with diabetes than in healthy volunteers. The T_{50} was correlated with the blood glucose level. The density of ghrelin-immunoreactive cells in the oxyntic mucosa of patients with diabetes was significantly reduced compared to the healthy controls. Ghrelin cell density was correlated with both the lag phase and T_{50}, as well as with blood glucose level. The present finding of reduced density of ghrelin cells in patients with type 1 diabetes, which was well correlated with gastric emptying, indicates the possible role of ghrelin in the pathophysiology of gastroparesis observed in diabetes.

Introduction

Ghrelin is a peptide hormone that has been isolated from the stomach (1). It originates mostly from endocrine cells in the oxyntic mucosa of the stomach, though small amounts have also been found in the small intestine and arcuate nucleus of the hypothalamus (1,2). Ghrelin has several functions, the best known of which is its growth hormone (GH)-releasing effect in the pituitary, where it acts synergistically with GH-releasing hormone (1,3). Ghrelin also increases appetite and feeding (4,5), and stimulates the orexigenic pathways through neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (6,7). Additionally, ghrelin has been found to accelerate gastric and small intestinal motility in rodents (8,9).

Patients with diabetes suffer from slow gastric emptying, which gives rise to nausea and vomiting and worsens their metabolic control (10-12). As ghrelin appears to play an important role in regulating gastric motility, the present study was undertaken to establish the possible role of ghrelin in slow gastric emptying observed in diabetes patients. To this end, the density of ghrelin cells was estimated and correlated to gastric emptying and the metabolic status of patients with longstanding type 1 diabetes.

Materials and methods

Patients and healthy subjects. Eleven patients with type 1 diabetes (9 women and 2 men) with a mean age of 45 years (range 28-78 years) participated in the study. Clinical data are summarized in Table I. All patients underwent hydrogen breath and ^{75}Se-homocholic taurine-conjugated bile acid (SeHCAT) tests. Both tests were normal in all patients, excluding small-bowel bacterial overgrowth and bile acid malabsorption.

Ten healthy volunteers without gastrointestinal complaints (8 women and 2 men; mean age 32 years, range 22-50 years) served as controls for gastrointestinal endoscopy and biopsy. Another 15 healthy volunteers without any gastrointestinal complaints (10 women and 5 men; mean age 47 years, range 25-65 years) served as controls for gastric scintigraphy. The investigation was approved by the local committee on ethics.

Scintigraphic measurements of gastric emptying. Scintigraphic measurement of gastric emptying in diabetic patients and controls was performed as previously described (13). Briefly, after overnight fasting, gastric emptying of solid food was carried out after the subjects had ingested a standard meal consisting of an omelette (311 kcal) and a 150-ml soft drink (70 kcal). The omelette contained 15 MBq ^{99}-Tc-labelled

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Table I. Clinical data of the patients.

Patient no.	Gender	Age (years)	Diabetes duration (years)	Organ complications	Gastrointestinal (GI) symptoms	GI symptom duration (years)	Blood Glucose[a] (mmol/l)	HbA_{1c} [a] (3.5-5.3)
1	F	78	48	Retinopathy; polyneuropathy	Nausea and vomiting; diarrhoea	7	9.5	8.6
2	F	31	25	Retinopathy; polyneuropathy; nephropathy	Nausea and vomiting; constipation	8	10.0	10.5
3	F	63	41	Polyneuropathy	Nausea and vomiting; constipation	20	9.0	6.7
4	F	46	19	Retinopathy; polyneuropathy	Nausea and vomiting; diarrhoea	7	9.5	8.6
5	F	53	25	Retinopathy; polyneuropathy;	Constipation	2	18.0	8.9
6	F	33	14	None	Nausea and vomiting; diarrhoea	4	11.0	8.7
7	F	33	27	Nephropathy	Nausea and vomiting; diarrhoea	4	8.0	12.8
8	F	30	16	Retinopathy; nephropathy	Nausea and vomiting; diarrhoea	10	13.0	10.7
9	F	49	39	Retinopathy; polyneuropathy	Nausea and vomiting; constipation/diarrhoea	13	9.0	6.5
10	M	26	15	Retinopathy; polyneuropathy	Diarrhoea	1	16.0	9.6
11	M	50	20	Polyneuropathy	Diarrhoea	2	6.0	6.6

[a]Fast values measured before endoscopy.

macroaggregated albumin (Pulmonate; Amersham Int., Little Chalfont, UK). The meal was eaten within 10 min and measurements were taken immediately after ingestion, with the subject seated in an upright position and the Gamma camera in an anterior position. The region of interest corresponding to the stomach was outlined to determine the gastric count for each frame. Data were acquired for 220 min, 60 sec/frame, at 5-min intervals for 30 min, and thereafter at 10-min intervals. After correction for background scatter and isotope decay, the lag phase and half-emptying time (T_{50}) were calculated.

Gastrointestinal endoscopy. After at least 6 h of fasting, a gastroduodenal endoscopy was performed in the patients and controls. During the endoscopy procedure, two or three biopsies were obtained from the corpus (major curvature) and pars decendens duodeni (distal to papilla of Vateri). In addition, biopsies were taken from the antrum and used in the camplyobacter-like organism (CLO) test for *Helicobacter pylori*.

Histopathology and immunohistochemistry. Biopsies were fixed in 4% buffered paraformaldehyde overnight, embedded in paraffin and cut into 5 μm-thick sections. The sections were stained by H&E and immunostained with the avidin-biotin complex (ABC) method (Dako Cyotmation, Glostrup, Denmark) as previously described in detail (14). The primary antiserum used was anti-ghrelin (polyclonal, code no. 00182, dilution 1:1600, Phoneix Pharmaceuticals, Belmont, CA, USA). The sections were counterstained slightly with haematoxylin. Negative controls included replacing the primary antiserum with 1% bovine albumin and pre-incubating the diluted antiserum with excessive ghrelin (50 μg/ml, NeoMPS, Strasbourg, France) at 4˚C overnight.

Computerized image analysis. Computerized image analysis was performed using the Leica Quantimet 600 MC image processing and analysis system (Leica, Cambridge, UK) linked to an Olympus microscope (type BX50). The system used the QWin program (version 2.6), Leica's Windows-based image analysis tool kit, and included QUIPS (version 2.6), an interactive programming system. Using x20 objectives, each pixel of the computer monitor corresponded to 0.173 μm, and the frame (field) represented an area of 5436 μm^2. The number of ghrelin-immunoreactive cells and the area of the epithelial cells were measured. Using QUIPS, an automated standard sequence analysis operation was created as previously described in detail (15). Briefly, the number of immunoreactive cells was counted using the field measurements. The areas of the epithelial cells were measured using a threshold setting. The data from each field were tabulated and the number of cells/mm^3 was computed and statistically analysed automatically. Measurements were taken in 10 randomly selected fields in the stomach and duodenum of each individual using an x20 objective.

Statistical analysis. Comparison between patients and controls was performed with the Wilcoxon non-parametric test, and correlation with the Spearman non-parametric test. P-values of <0.05 were considered significant.

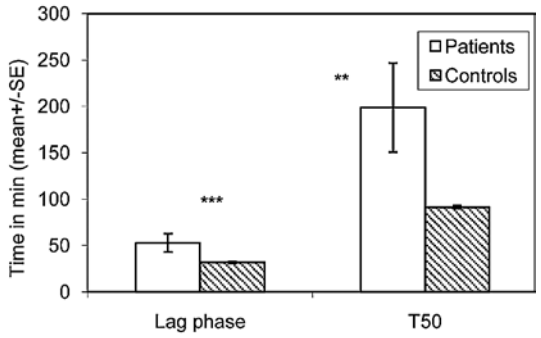

Figure 1. Gastric emptying in patients with diabetes and in controls.

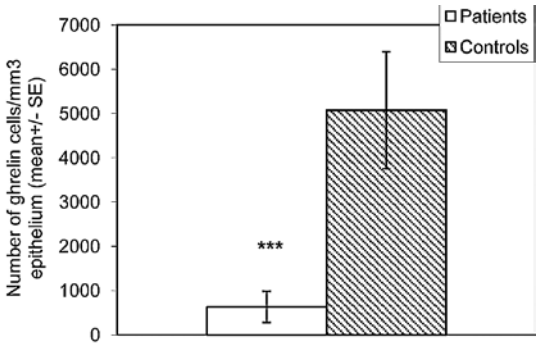

Figure 2. Density of gherlin-immunoreactive cells in the oxyntic mucosa of the stomach in patients with diabetes and in controls.

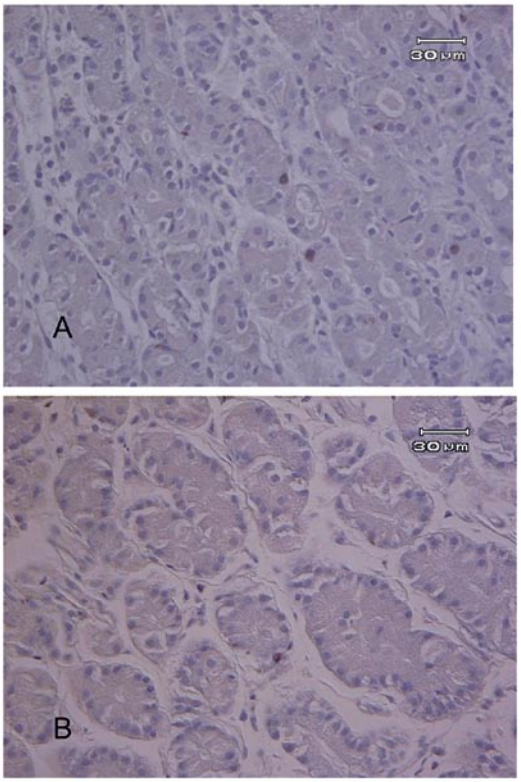

Figure 3. Gherlin-immunoreactive cells in the oxyntic mucosa of (A) a control subject and (B) a patient with diabetes.

Results

Scintigraphic measurements of gastric emptying. The results of gastric emptying are presented in Fig. 1. Both the lag phase and T_{50} were higher in patients with diabetes than in the healthy volunteers (P<0.001 and <0.001, respectively). The T_{50} was correlated with the blood glucose level (P=0.003; r=0.8), whereras the lag phase was not (P=0.4; r=3). Neither the lag phase nor the T_{50} was correlated with HbA_{1c}.

Gastrointestinal endoscopy. The stomach and duodenum of the patients and the healthy volunteers were macroscopically normal. One of the patients and one of the healthy volunteers had a positive CLO test result, indicating infection with *Helicobacter pylori.*

Histopathology and immunohistochemistry. Histopathological examination of the gastric and duodenal biopsies from patients and healthy volunteers revealed normal histology, thus excluding celiac disease. Ghrelin-immuoreactive cells were found in the stomach oxyntic mucosa and among the epithelial cells of the duodenum. In the duodenum, ghrelin cells were few and unevenly distributed, which did not allow for reliable quantification. The cells occurred mostly in the crypts, and were round, flask-shaped or triangular (Fig. 2).

Computerized image analysis. The density of ghrelin-immunoreactive cells in the oxyntic mucosa of patients with diabetes was significantly reduced (P=0.0007) compared to the healthy controls (Fig. 3). Ghrelin cell density in the oxyntic mucosa correlated with lag phase and the T_{50} (P=0.01 and P=0.007; r=-0.7 and r=-0.8, respectively) and with blood glucose level (P=0.003; r=-0.8).

Discussion

The present finding that ghrelin cell density in the oxyntic mucosa was reduced in patients with longstanding type 1 diabetes is in agreement with an earlier observation in rats with streptozotocin-induced diabetes and mouse models of human type 1 diabetes (16,17). The present investigation further demonstrated that gastric emptying is inversely correlated with the density of ghrelin cells in the oxyntic mucosa. As ghrelin has been shown to stimulate gastric motility, it is conceivable that the paucity of ghrelin cells in the oxyntic mucosa is one of the factors causing slow gastric emptying in patients with type 1 diabetes . This assumption is also supported by the close correlation found between gastric emptying and ghrelin cell density. Furthermore, ghrelin injection given as a bolus dose at the end of a meal has been found to accelerate gastric emptying in patients with diabetes and gastroparesis (18,19). Although ghrelin cell density was reduced in patients with diabetes type 1, it was unclear whether these cells compensated for their paucity by increasing ghrelin synthesis and release. Further studies are therefore required, in which the concentrations of total and active ghrelin in tissue extracts and plasma are determined. It is worth noting that plasma levels of active ghrelin in obese patients with type 2 diabetes have been found to be low, and that the ghrelin cell density in the oxyntic mucosa of animal models of human diabetes has been reported to be reduced (17,20).

The present study showed a close correlation between ghrelin cell density in the oxyntic mucosa and glucose level in patients with longstanding type 1 diabetes. As ghrelin is known to increase appetite and feeding, the paucity of ghrelin cells in the patients investigated might be induced by hyperglycaemia over a long period.

Ghrelin shares several structural similarities with motilin and acts on motilin receptors (21-23). The motilin agonist erythromycin has been successfully used in the treatment of gastroparesis in patients with diabetes (11). The present findings suggest that ghrelin might have a therapeutic role in patients with diabetes and gastroparesis.

Acknowledgements

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