Upsala J Med Sci 107: 89–99, 2002

Gastric Emptying in Animal Models of Human Diabetes: Correlation to Blood Glucose Level and Gut Neuroendocrine Peptide Content[1]

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ABSTRACT

Gastric emptying was measured in non-obese diabetic (NOD) and in obese diabetic mice. The feces were collected and the water content was determined. The neuroendocrine peptides known to regulate gastrointestinal motility, namely secretin, gastric inhibitory peptide (GIP), motilin, somatostatin, peptide YY (PYY), substance P, vasoactive intestinal polypeptide (VIP) and galanin, were measured in tissue extracts of different segments of the gut by radioimmunoassay. Whereas the gastric emptying of NOD mice was significantly slower than that of controls, that of the obese diabetic mice was unaltered. The gastric emptying of NOD mice, but not that of obese diabetic mice, correlated with the blood glucose level. The feces weight and water content in NOD mice was significantly higher than controls. The feces water content in obese diabetic mice was significantly lower than that of controls. The concentrations of antral somatostatin, VIP and galanin, and duodenal secretin as well as jejunal motilin in NOD mice were higher than those of controls. Duodenal GIP and colonic PYY concentration in NOD mice was lower than controls. Duodenal GIP and VIP, and colonic somatostatin and VIP levels were lower in obese diabetic mice than controls. Secretin and motilin levels correlated with gastric emptying in NOD mice. The high duodenal concentration of secretin might reflect high synthesis and release of this hormone, and may therefore be among the factors that caused slow gastric emptying in the NOD mice. The increase in concentration of motilin observed in NOD mice may be caused by impaired release of this hormone as a result of hyperglycemia. Whereas the high concentrations of antral VIP and galanin and the low level of colonic PYY in diabetic NOD mice may contribute to the development of diarrhea in NOD mice, the decreased levels of duodenal and colonic VIP and colonic somatostatin in obese diabetic mice may account for the constipation encountered in these animals.

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1) Received 29 August 2002
Revised manuscript accepted 7 November 2002

INTRODUCTION

Gastrointestinal symptoms in patients with diabetes mellitus are common (1–5). Nausea and vomiting, abdominal pain, diarrhea, constipation and fecal incontinence are often encountered in these patients (1–5). Gastrointestinal symptoms in patients with diabetes are attributed to disturbed gastrointestinal motility (6,7). Gastrointestinal dysmotility in diabetes is believed to be caused by autonomic neuropathy and/or hyperglycemia (6, 7). The neuroendocrine system of the gut secrets peptides that play an important role in regulating gastrointestinal motility (8–10). It is conceivable, therefore, to assume that a disturbance in this regulatory system may contribute to the pathogenesis of gastrointestinal complications in diabetes. This assumption is supported by the findings in animal models of human diabetes, where several abnormalities in this system have been reported (11–14, 15–23). It is not clear, however, whether these models have gastrointestinal motility disorders and symptoms similar to patients with diabetes.

The aim of the present study was to establish whether there is a slow gastric emptying in animal models of human diabetes type 1 and 2, where abnormality in the gut neuroendocrine system has been reported (16–19, 22, 23). Furthermore, this study aimed to establish whether diarrhea or constipation occurs in these animals. It was also intended to correlate the outcome with a possible abnormality in the neuroendocrine peptide levels.

MATERIAL AND METHODS

Animals

As an animal model of human type 1 and type 2 diabetes, female non-obese diabetic (NOD) mice aged 13-weeks and 15-week-old male homozygous (ob/ob, Umeå/Bom-0b) (Bomholtgård Breeding and Research Centrum, Denmark) were used. As controls, age- and sex-matched mice of a NOD mice sister strain, BLAB/cJ mice and lean homozygous (+/+) mice (Bomholtgård Breeding and Research Centrum) mice were used (24). The mice were kept in a vivarium, five to a cage, in a room illuminated by a 12h/12h light-dark cycle. They were fed a standard pellet diet (R 34, Lactamin, Vadstena, Stockholm, Sweden) and given water *ad libitum*. The urine of each mouse was tested daily with Ecur-test strips (Boehringer Mannheim). The animal was considered diabetic when the level of glucosuria was higher than 50 mmol/l and remained at this level. The NOD mice were treated by i.p Injection of bovine insulin-Zn in suspension, 40 I.u./ml (Lente^{R} Mc, Novo). The animals received 1 i.u. every second day. If an animal lost weight or appeared to be unwell, the blood glucose level was measured in a blood sample taken from the tail. In the case of hyperglycemia, the insulin dose was increased up to 5 i.u. or was injected with 1 ml glucose solution (25 mg/ml) i.p. In the case of hypoglycemia. Glucosuria is measured before every treatment to evaluate the treatment regime. NOD mice were not treated with insulin in the day of experiment. The obese diabet-

ic mice did not receive any treatment. Ten NOD mice with 28–35 days diabetes and 10 obese diabetic mice with 20 days diabetes, as well as 10 BLAB/cJ and 10 lean mice, were used in this study.

After gastric emptying had been determined and the animals were killed, blood samples were taken by heart puncture, and blood glucose levels were measured with glucometers (Precision Q.I.D., Medisense, Waltham, MA, USA), using Sensor Electrode Plus (Medisense). Tissue samples from the antrum, proximal duodenum, jejunum and distal colon were excised. The antrum was chosen to represent the stomach, the duodenum and jejunum to represent the small intestine and the colon to represent the large intestine. The tissue samples were frozen in liquid nitrogen and kept at –70°C for radioimmunoassays. The local committee on animal ethics at Umeå University approved the investigation.

Gastric emptying

Gastric emptying was performed by a slight modification of the method described by Smits and Lefebvre (25). Briefly, after an overnight fast the animals were orally administered a standard meal for 2 min. This consisted of 0.2 ml of a solution of 50 mg phenol red in 100 ml 1.5% methylcellulose, which was constantly stirred and held at 37°C. The meal was administered by gavages and the animals were killed after 20 min. After laparatomy, the stomach was quickly ligated at the lower esophageal sphincter and pyloric region and removed. The stomach was opened, its contents were poured into a test tube and the stomach was washed thoroughly with 4 ml distilled water. At the end of the experiment, 2 ml 1M NaOH was added to each tube to develop the maximum intensity of color. The solutions were assayed with a spectrometer at 560 nm. Gastric emptying percentage was calculated according to the following formula, where amount of phenol red recovered after 0 min is the amount administered by gavage:

100X (1-amount of phenol red recovered after 20 min/amount of phenol red recovered after 0 min)

Assessment of diarrhea or constipation

The animals were kept in metabolic cages two days before they were killed. They were left to adapt for one day and then the feces were collected the second day. The feces were weighed and dried in an oven at 100°C for 3 h and weighed again. The water content was calculated using the following formula:

100 X (1 – feces weight after drying/feces weight before drying)

Radioimmunoassays (RIA)

The tissue specimens were allowed to thaw and then weighed. The peptides were extracted by boiling the tissue in 3 ml 0.5 M acetic acid, followed by homogenization and centrifugation for 20 min at 700 xg. The supernatant was neutralized with 1 M NaOH and stored at –70°C until the time for assay. Somatostatin, substance P, vasoactive intestinal peptide (VIP) and galanin were determined in all gastrointesti-

nal tract specimens investigated except the jejunum. Secretin and gastric inhibitory peptide (GIP) were measured in the duodenum; motilin in the jejunum; and peptide YY (PYY) in the colon.

Peptide content of the different investigated parts was measured using commercially available RIA kits. Thus, somatostatin, motilin, substance P and VIP were measured using RIA kits from Eurodiagnostica (Malmö, Sweden); and secretin, GIP, PYY and galanin with RIA kits from Peninsula Laboratories (Belmont, CA, USA). The assays were performed according to the protocols supplied by the manufacturers, in duplicates of undiluted extracts and of 1:2. Briefly, standards and samples were incubated with respective antibodies and then incubated with the appropriate [^{125}I]-tracer. The antibody-bound [^{125}I]-tracer was separated from the unbound fraction using the double antibody-polyethylene glycol (PEG) precipitation technique. Thus, goat anti-rabbit IgG serum and PEG 6000 were added. Following incubation, bound and free labels were separated by centrifugation for 15 min at 1700 xg at 4°C. The supernatant was removed by aspiration and the precipitate was counted in an automatic gamma counter. The assay buffer was 0.05 M phosphate containing 0.25% human serum albumin and 0.02% methiate or 0.05% sodium azid.

Statistical analysis

Comparisons between diabetic mice and controls were made with the Wilcoxon non-parametric test and correlation with Spearman's non-parametric test. P-values below 0.05 were considered significant.

RESULTS

The blood glucose level of NOD mice treated with insulin was 7.1+1.4 mmol/l (mean+SE), and for controls was 5.1+0.04. There was no statistical difference between NOD mice and controls regarding the blood glucose level (p=0.8). The blood glucose level of obese diabetic mice was11.5+0.8 mmol/l (mean+SE). The corresponding figure for lean controls was7.4+0.3. There was a significant statistical difference between obese diabetic mice and controls regarding the blood glucose level ($p<0.0001$).

Gastric emptying

The results of gastric emptying measurements are given in Fig.1.The gastric emptying of NOD mice was significantly slower that that of controls (p=0.008). There was no significant difference between obese diabetic mice and controls regarding gastric emptying (p=0.3). The gastric emptying in NOD mice correlated with the blood glucose level (p=0.001, r= – 0.9). It did not, correlate with however, gastric emptying in obese diabetic mice (p=0.3).

Assessment of diarrhea and constipation

Feces weight and water content are presented in Fig. 2. The feces weight and water

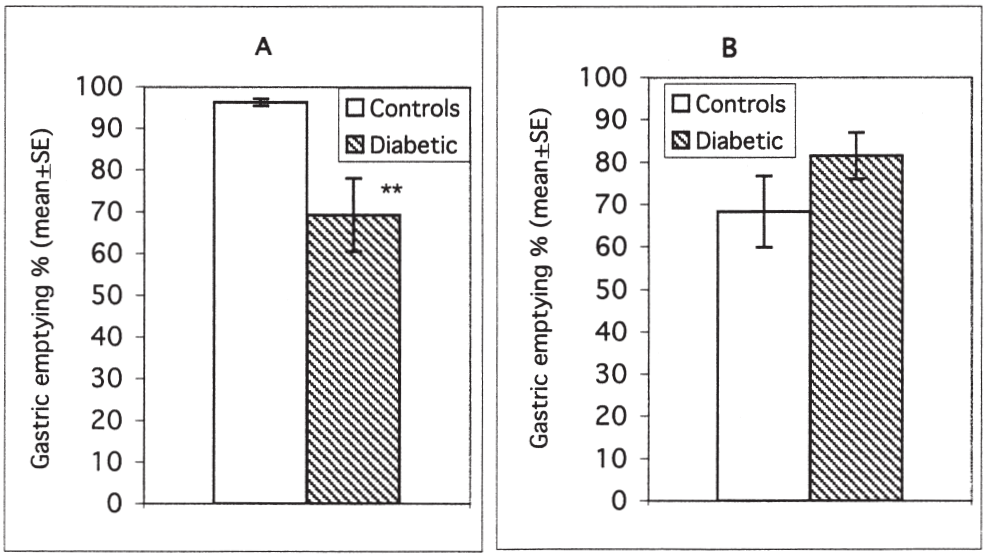

Fig. 1. Gastric emptying in non-obese diabetic (A) and in obese diabetic mice (B). **P<0.01.

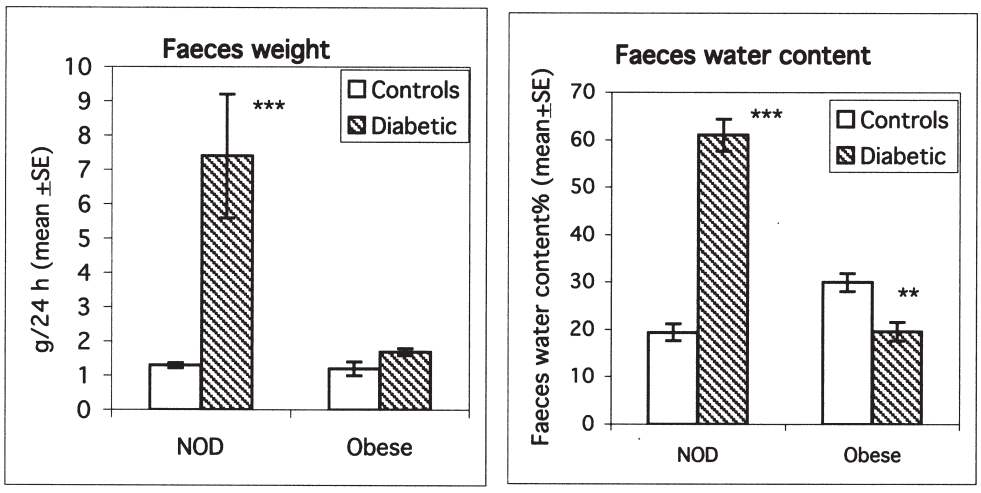

Fig. 2. Feces weight and feces water content in non-obese diabetic and obese diabetic mice. **P<0.01; ***P<0.001.

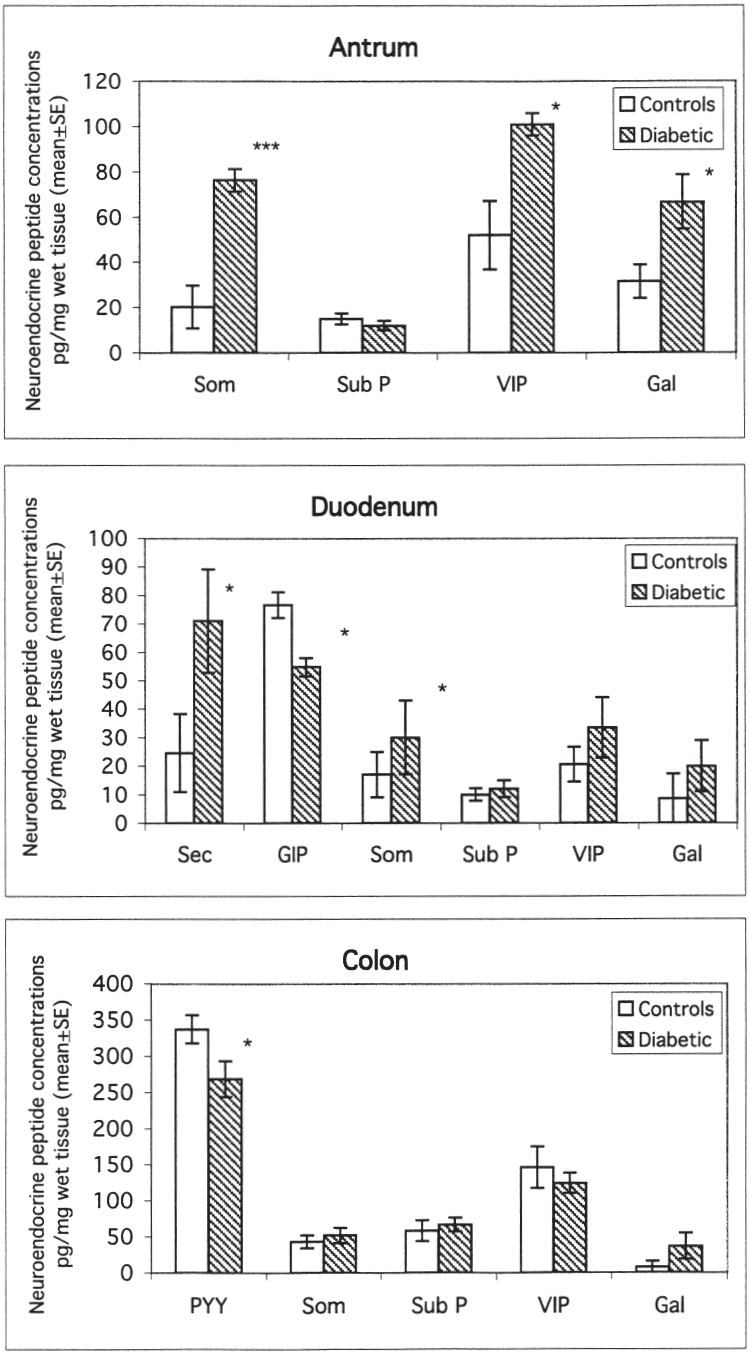

Fig. 3. The concentrations of various neuroendocrine peptides in different segments of the gut of non-obese diabetic mice. Som=somatostatin; Sub P=substance P; VIP=vasoactive intestinal polypeptide; Gal=galanin; Sec=secretin; GIP=gastric inhibitory polypeptide; PYY= peptide YY; Enterogl=enteroglucagon; *P<0.05; **P<0.01.

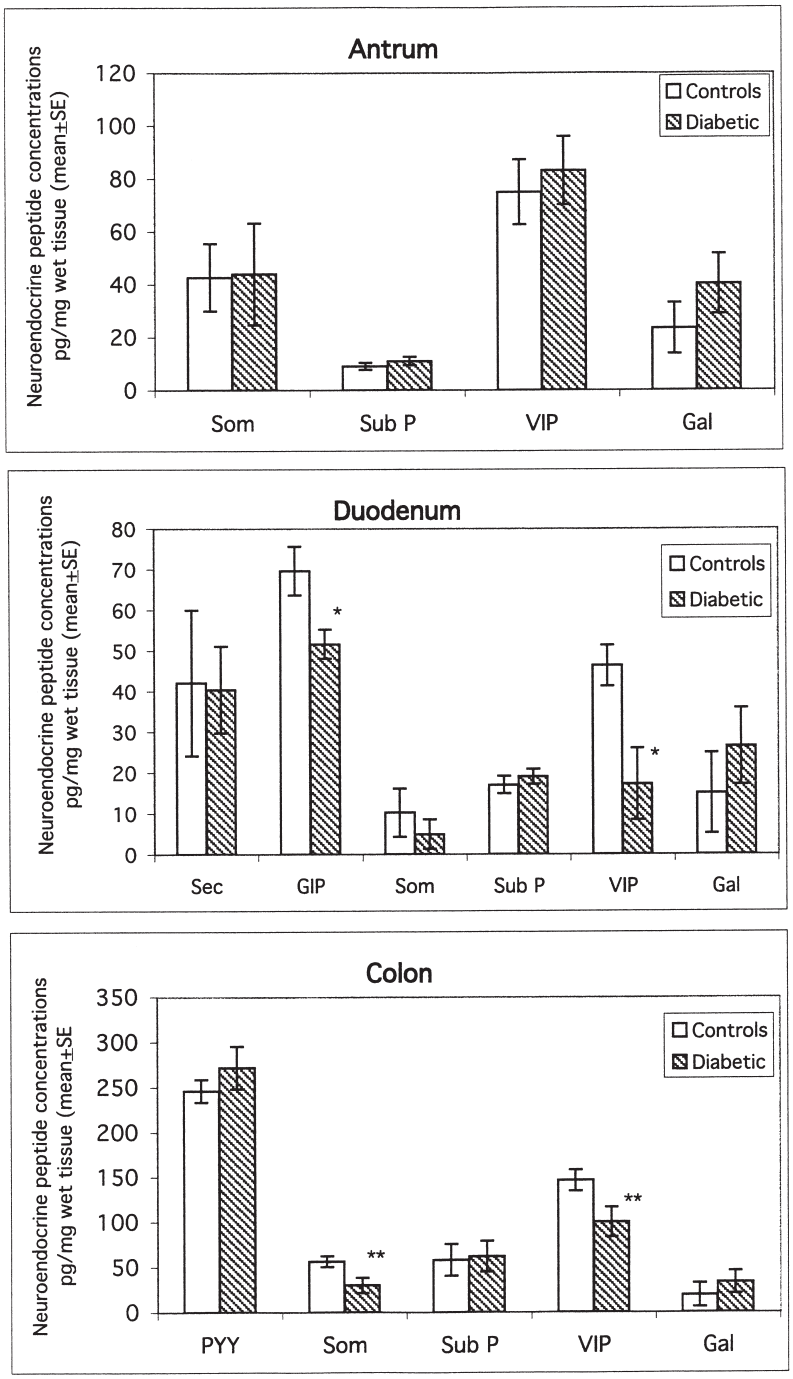

Fig 4. The concentrations of various neuroendocrine peptides in different parts of the gastrointestinal tract of obese diabetic mice. Symbols are the same as in Fig. 3.

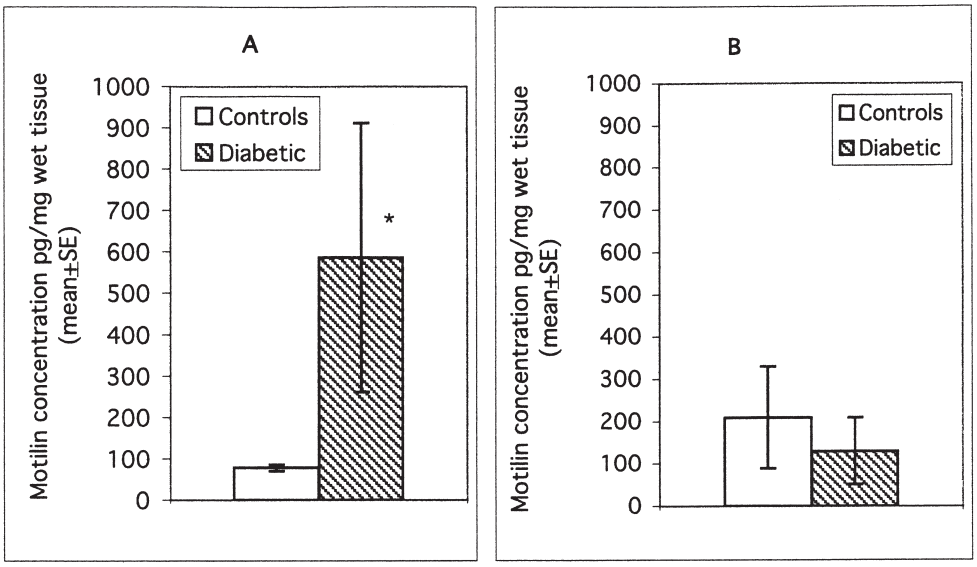

Fig. 5. The level of motilin in tissue extracts of the jejunum of non-obese diabetic (A) and obese diabetic (B) mice. Symbols are the same as in Fig. 3.

content were significantly higher in NOD mice than controls ($p<0.0001$ and $p=0.0004$, respectively). Whereas there was no significant difference between obese diabetic mice and controls regarding the feces weight ($p=0.1$), the feces water content in obese diabetic mice was significantly lower than that of controls ($p=0.008$). The blood glucose level did not correlate with either feces weight or feces water content in NOD or obese diabetic mice.

Radioimmunoassays

The results of RIA are given in Figs. 3–5. The concentrations of antral somatostatin, VIP and galanin in NOD mice were higher than those of controls ($p=0.001$, 0.02 and 0.02, respectively). Duodenal secretin level was higher and GIP concentration was lower in NOD mice than controls ($p=0.04$ and 0.01, respectively).

Colonic PYY concentrations in NOD mice were lower than controls ($p=0.04$). Duodenal GIP and VIP, and colonic somatostatin and VIP levels were lower in obese diabetic mice than in controls ($p=0.01$, 0.04, 0.008 and 0.005, respectively). Motilin concentration in the jejunum in NOD mice was significantly higher than that of controls ($p=0.03$). Secretin and motilin levels correlated with gastric emptying in NOD mice ($p=0.008$, $r=–0.9$ and $p=0.02$, $r=–0.6$). There was no correlation between gastric emptying; feces weight or feces water content and the other neuroendocrine peptide concentrations in any of the gastrointestinal segments investigated.

DISCUSSION

The present study showed a slow gastric emptying and diarrhea in an animal model of human diabetes type 1, namely NOD mice. Although constipation occurred in an animal model of human diabetes type 2, the obese diabetic mice, gastric emptying did not differ from that of controls. In both animal models, abnormal levels of the neuroendocrine peptides were found. The type of neuroendocrine peptides and the alteration and localization, however, differed between these two animal models.

The neuroendocrine peptide concentrations in different segments of the gut have been investigated earlier in these animal models (16–20). In these previous studies, both NOD and obese diabetic mice were investigated one week after the onset of diabetes and NOD mice did not receive any treatment. In the present study the animal models studied had a longer duration of diabetes and showed gastrointestinal symptoms similar to diabetic patients. It is not surprising, therefore, that the outcome of RIA analysis in the present study differed from the results of earlier investigations regarding the tissue levels of some neuroendocrine peptides.

Hyperglycemia has been proposed to be one of the major causes of slow gastric emptying seen in patients with diabetes (7). The present finding of a strong correlation between acute blood glucose level and gastric emptying in NOD mice supports this assumption. It is rather puzzling that the gastric emptying of obese diabetic mice was not altered despite high blood glucose concentration. Other mechanisms may be involved in gastric emptying other than hyperglycemia in diabetes type 2.

Secretin and motilin correlated strongly with gastric emptying in NOD mice. Secretin is known to delay (26) and motilin to accelerate gastric emptying and its release is inhibited by hyperglycemia (27). The increase in the small intestinal concentration of motilin observed here in NOD mice may be caused by impaired release. The regulation of the release of motilin is complex and hyperglycemia is only one of the factors that regulate it.

GIP, an incretin hormone, stimulates insulin secretion after meals with elevated plasma glucose (24). In a previous report, the concentration of GIP in duodenal extracts was found to be low in NOD after the onset of diabetes (16). In the present study, the duodenal level of GIP in NOD mice with longer duration of diabetes was also lower than that of controls. Somatostatin inhibits gastrointestinal secretion and motility (29). VIP mediates the intestinal peristaltic reflex and is a strong stimulator of secretion in the small and large intestine (30). Galanin stimulates human small and large intestine contraction (31). On the other hand, PYY is one of the mediators of the ileal brake and inhibits intestinal secretion of fluid and electrolytes (32) The high concentrations of antral VIP and galanin and the low level of colonic PYY in diabetic NOD mice may contribute to the development of diarrhea in these animals. The high level of antral somatostatin in NOD diabetic mice might be a secondary response to the effect created by the previously mentioned peptides. On the other hand, the decreased levels of duodenal and colonic VIP and colonic somatostatin in obese diabetic mice may account for the constipation encountered in these animals.

ACKNOWLEDGEMENTS

This study was supported by grants from the Bengt Ihre's Foundation, Sahlberg's Foundation, Julin's Foundation and the Faculty of Medicine, Umeå University Research funds.

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