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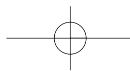

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Gastrointestinal Transit in an Animal Model of Human Diabetes Type 2: Relationship to gut neuroendocrine peptide contents[1]

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ABSTRACT

Gastrointestinal transit (GIT) was determined in obese diabetic mice (ob/ob, Umeå/Bom). Blood glucose level, and insulin concentration in the serum and pancreas extracts as well as neuroendocrine peptide contents were measured in several segments of the gut. GIT was significantly slower in the obese diabetic mice, but was not correlated with the blood glucose level, serum insulin, or pancreatic insulin content. GIT was correlated with duodenal secretin content and colonic vasoactive intestinal peptide (VIP) content, but not with the content of other neuroendocrine peptides in different segments investigated. The antral gastrin content in obese diabetic mice was significantly higher than in controls. The concentration of secretin in obese diabetic mice was higher than in controls. Whereas the contents of peptide YY (PYY) and somatostatin were higher in obese diabetic mice, the contents of substance P and VIP were lower. The increased content of duodenal secretin and decreased content of colonic VIP may be among the factors that cause slow GIT in obese diabetic mice. The changes in the colonic contents of PYY, VIP and somatostatin may cause low intestinal secretion and, together with slow GIT, give rise to constipation, which is a common symptom in diabetes.

INTRODUCTION

Diabetes mellitus is a disease that affects several organ systems, including the gastrointestinal (1). Gastrointestinal manifestation is common, varying between 76% and 25% in patients with diabetes (2–5). Symptoms that occur in diabetes are dysphagia, nausea and vomiting, abdominal pain, diarrhoea, constipation and faecal incontinence (1–5). In addition to morbidity, gastrointestinal symptoms have a major impact on glycaemic control in diabetic patients (6).

Gastrointestinal symptoms in patients with diabetes are believed to be caused by

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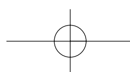


disturbed gastrointestinal motility and, abnormal secretion and absorption. The neuroendocrine system of the gut plays an important role in regulating these two functions (7–9). In animal models of human diabetes, several abnormalities in this regulatory system have been reported (10–21).

The aim of the present study was to establish whether there is a motility disturbance in an animal model of human diabetes type 2 (obese diabetic mouse), where abnormality in the gut neuroendocrine system has been reported (19–21). It was also intended to at establish the correlation of disturbances in the neuroendocrine system and a possible motility dysfunction.

MATERIALS AND METHODS

Animals

Eleven 15-week-old male homozygous (ob/ob, Umeå/Bom-0b) mice and 11 age-matched male lean (+/+) control mice (Bomholtgård Breeding and Research Centrum, Denmark) were used. The mice were kept in our vivarium, 5 to each cage in a room illuminated for 12h/day with artificial light. 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 an Ecur-Test stick (Boehringer Mannheim). All the obese diabetic mice had glucosuria ranging between 2 and 4 (5.6–55 mmol/l). The control lean mice did not show glucosuria. After the gastrointestinal transit had been determined, the animals were killed, and blood samples were taken by heart puncture. Tissue samples of the splenic part of the pancreas, antrum, proximal duodenum and distal colon were excised. The splenic part of the pancreas was removed and divided into two parts. One part was placed in 4% buffered formaldehyde for morphological studies, while the other part was put together with tissue samples from the gastrointestinal tract, frozen in liquid nitrogen and kept at –70°C for radio-immunoassays. The investigation was approved by the local committee of animal ethics at Umeå University.

Description of the animals used

The animals were weighed on the day of the experiment. Blood glucose level was measured with glucometer (Precision Q.I.D., Medisense, Waltham, MA, USA). Serum level of insulin and the pancreatic insulin content were determined by radioimmunoassay. The fixed tissue samples of the pancreas were embedded in paraffin wax and cut at 5 μm. The sections were stained with haemotoxylin-eosin and immunostained for insulin by the avidin-biotin complex (ABC) method (Dako A/S, Glostrup, Denmark) as described in detail previously (22). The antibodies used were rabbit anti-porcine insulin (Euro-Diagnostica, Malmö, Sweden, code no. 2263PINS, dilution 1:200). The area of the islets was measured in 10 randomly chosen fields from each individual in 2-4 sections stained with haemotoxylin-eosin and with a X4 objective. The results from each field were tabulated, computed and statistically analysed automatically. The Quantimet 500MC image processing and ana-

lysis system (Leica, Cambridge, England) linked to Olympus microscope type BX50 was used. The software used in this system was Leica's Windows-based image analysis tool kit "QWIN" (version 1.02) and an interactive programming system, QUIPS (version 1.02). When quantification was performed in this system with X4, each pixel corresponded to 2.12, and each field seen in the monitor represented 1.3 mm^2 area of the tissue. The islet area was determined by using interactive measurements in the manual menu. This was carried out by drawing a line around the islet viewed in the field.

Gastrointestinal transit

The gastrointestinal transit in both obese diabetic and lean control mice was performed by a slight modification of the method described by Smites and Lefebre (23). Briefly, after an overnight fast the animals were given perorally during 3 min 0.2 ml of a standard meal. This consists of 10% charcoal in 5% Arabic gum aqueous suspension. After 20 min, the animals were killed and the gastrointestinal tract was dissected out carefully without stretching and the distance travelled by the charcoal and the total length of the small intestine were measured. Gastrointestinal transit was expressed as the percentage of the distance travelled by charcoal of the total small intestine.

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 homogenisation and centrifugation for 20 min at 700X g. The supernatant was neutralised with 1 M NaOH and stored at –70°C until the time for assay. Insulin was determined in pancreatic tissue extracts and serum. Somatostatin, vasoactive intestinal peptide (VIP), substance P, neurotensin, neuropeptide Y (NPY), and galanin were measured in all gastrointestinal tract specimens investigated. Gastrin was measured in the antrum and duodenum, gastric inhibitory peptide (GIP), secretin, cholecystokinin (CCK) in the duodenum, and peptide YY (PYY) in the colon.

Peptide content of the various investigated parts was determined using commercially available RIA kits. Thus, insulin was determined using rodent standard insulin kit from DiaSorin Inc. (Stillwater, MN, USA), gastrin, CCK, somatostatin, substance P, VIP, neurotensin and NPY were measured using RIA kits from Eurodiagnostica (Malmö, Sweden), and secretin, GIP, PYY and galanin with RIA kits from Peninsula Laboratories (Belmont, Calif., USA). The assays were performed according to the protocols supplied by the manufacturers, in duplicates of undiluted extracts and of 1:2 diluted extracts (Table 1). 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-guinea pig (insulin) or goat anti-rabbit IgG (the other peptides) serum and PEG 6000 were added. Following incubation, bound and free labels were separated

Table 1. Summary of the radio-immunoassay procedures in different neuroendocrine peptides.

Petide	Standard sample or control	Antibody	Incubation time and temperature*	Labelled peptide	Incubation time and temperature*	Double antibody solid phase	Incubation time and temperature*	Antibody raised against	Antibody % cross-reactivity
Insulin	200 μL	100 μL	0	100 μL	16-20 h at 4°C	500 μL	15-25 min at room temperature	Porcine insulin*	!00% human, porcine and rat insulin; 30% pro-insulin; and l<0.01% with C-peptide of insulin
Secretin	100 μL	100 μL	16-24 h at 4°C	100 μL	16-24 h at 4°C	200 μL	90 min at room temperature	Porcine secretin	100% with porcine, human and rat insulin. O with chicken secretin, amylin amide, CCk, enkephalin, insulin, NPY, PHI, somatostatin, substance P, and VIP
GIP	100 μL	100 μL	16-24 h at 4°C	100 μL	16-24 h at 4°C	200 μL	90 min at room temperature	Human GIP	100% human and porcine GIP; and 0 amylin amide, bombesin, big gastrin, gastrin-1, GRP, insulin, and substance P
CCK	200 μL	500 μL	2 days at 4°C	500 μL	4 days at 4°C	100 μL	30-60 min at 4°C	Synthetic CCK 26-33, sulphate	100% CCK; and <0.01% gastrin-17
Gastrin	100 μL	200 μL	0	200 μL	60 min at room tepmerature	500 μL	30 min at room temperature	Synthetic human gastrin-17	100% gastrin-17; 83% gastrin-17, sulphated; 61% gastrin-34; 36% CCK-8; and <0.02% gastrin 1-14, GRP, VIP, motilin, glucagon, somatostatin-14 and C-peptide
Somatostatin	100 μL	200 μL	18-24 h at 4°C	200 μL	18-24 h at 4°C	400 μL	130-60 min at 4°C	Synthetic cyclic somatostatin	100% cyclic somatostatin; 100% Tyr[1]-somatostatin, 50% linear somatostatin and 25% Des.ala-gly-somatostatin
PYY	100 μL	100 μL	16-24 h at 4°C	100 μL	6-24 h at 4°C	200 μL	90 min at room temperature	Rat PYY	100% rat and porcine PYY; 9% human PYY; 0.1%NPY; 0.01 PP; 0 enkephalin, galanin, glucagon, secretin, substance P, and VIP
Substance P	100 μL	200 μL	18-24 h at 4°C	200 μL	18-24 h at 4°C	500 μL	30-60 min at 4°C	Synthetic substance P	100% substance P; 103% substance P sulphoxide; 24% substance P 4-11; <0015 substance P 7-11, neurokininA and b, kassinin, eledosin, physalaemin, bombesin, and neuromedin B and C
VIP	200 μL	200 μL	24 h at 4°C	100 μL	16-24 h at 4°C	500 μL	30-60 min at 4°C	Porcine VIP	100% VIP 1-28 (whole sequence); 90% VIP 7-28; 83% VIP 11-28; 71% VIP 18-28; <2.5%VIP 1-6 ; 1-16,1-18,1-22; < 0.01 secretin, GIP, glucagon, PP, substance P, and somatostatin
Neurotensin	200 μL	400 μL	18-24 h at 4°C	400 μL	40-48h at 4°C	500μL	130-60 min at 4°C	Synthetic neurotensin 1-13	100% neurotensin 1-13; < 1% neurotensin 1-12, 1-11, 1-10, 1-8, 1-6, 4-13, 7-13, 8-13, 9-13, and 10-13
NPY	200 μL	200 μL	8-24 h at 4°C	200 μL	8-24 h at 4°C	100 μL	30-60 min at 4°C	NPY	100% NPY, <2% PYY, < 1% PP
Galanin	100 μL	100 μL	16-24h at 4°C	100 μL	16-24 h at 4°C	200 μL	90 min at room temperature	Porcine galanin	100% galanin; 0% amylin amide, insulin, NPY, PHM-27, secretin, substance P and VIP

* vortex and mix before incubation

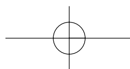


Table 2. Characteristics of the animals used (all values expressed as mean ± SE).

	Body weight (g)	Blood glucose (mmol/l)	Serum insulin (pg/ml)	Pancreatic insulin (ng/g)	Islet area (μm^2)
Lean controls	34±0.9	6.6±0.6	33±14.4	2 184±302	8 490±2 535
Obese diabetic	53±1.3***	17.4±1.6***	70±21.5*	6 670±2 132**	35 127±6 879***

*=P<0.5; **=P<0.01; ***=P<0.001.

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 azaid.

Statistical analysis

Comparisons between diabetic obese mice and lean controls were performed with the Wilcoxon non-parametric test, and correlation with Spearman non-parametric test. P-values below 0.05 were deemed significant.

RESULTS

Description of the animals

The characteristics of the animals used are summarised in Table 2. The pancreatic islets in the obese diabetic mice were much larger than those of the lean controls.

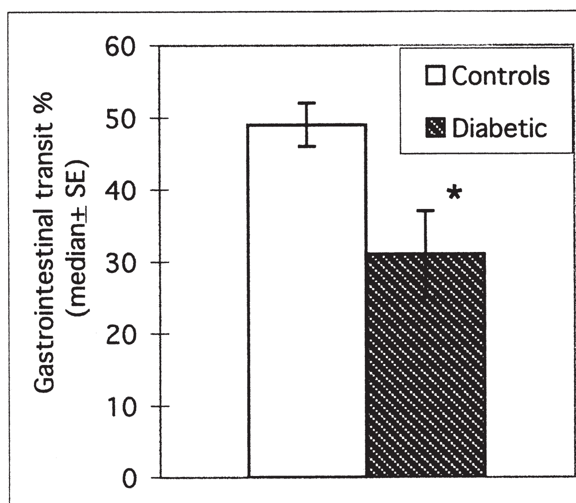
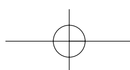


Fig. 1. Gastrointestinal transit in obese diabetic mice and lean controls. *P<0.05.



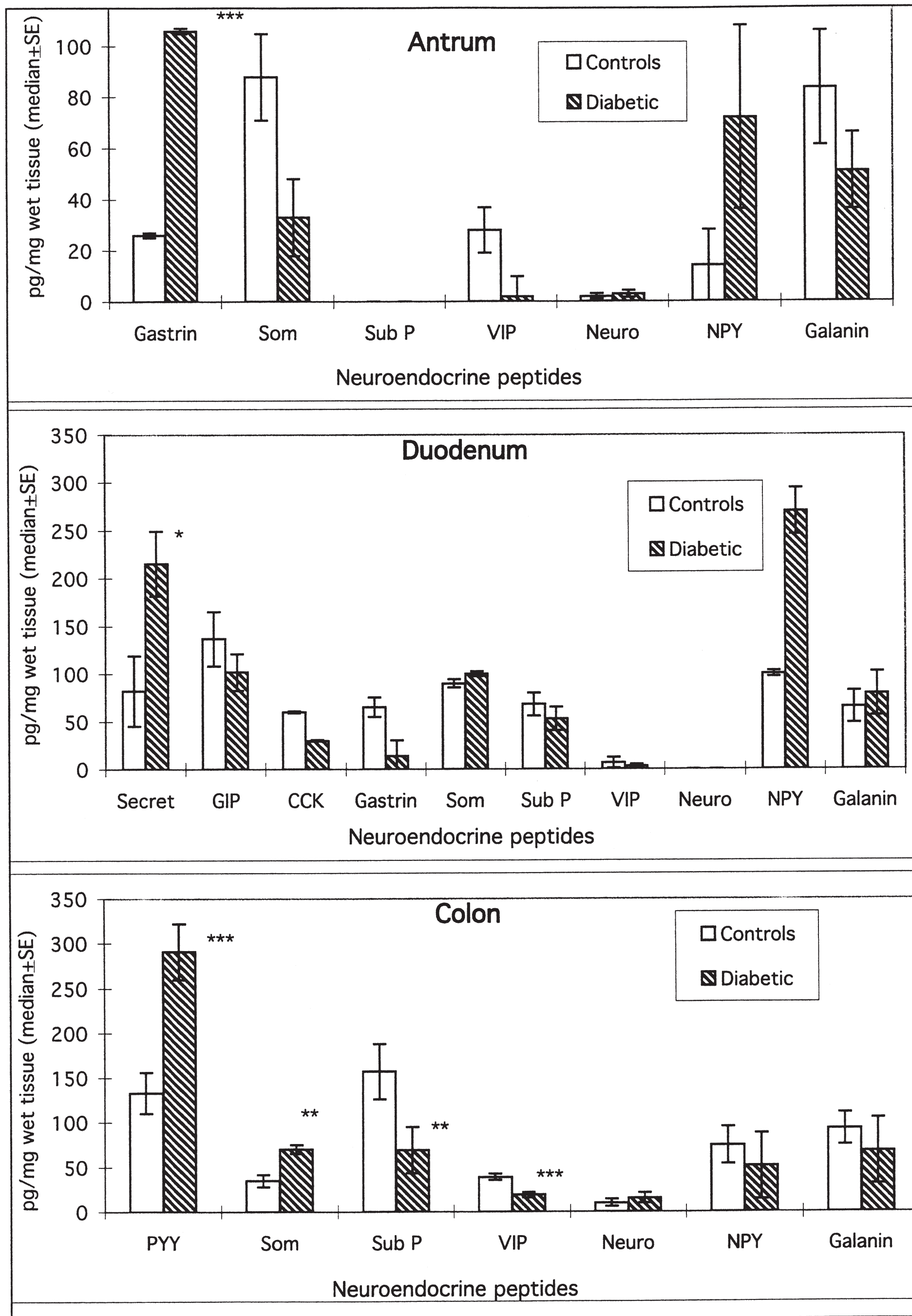


Fig. 2. The concentration of various neuroendocrine peptides in various segments of the gastrointestinal tract investigated. Som=somatostatin; Sub P=substance P; VIP=vasoactive intestinal peptide; Neuro=neurotensin; NPY=neuropeptide Y; Secret=scretin; GIP=gastric inhibitory peptide; CCK=cholecystokinin; PYY= petide YY. *P<0.05; **P<0.01; *''P<0.001.

The pancreatic islets of the diabetic obese mice were predominated by insulin-immunoreactive cells.

Gastrointestinal transit

The gastrointestinal transit in obese diabetic mice and lean controls is illustrated in Fig. 1. The gastrointestinal transit was significantly slower in the obese diabetic mice (P=0.03). The gastrointestinal transit was not correlated to the blood glucose level (P=0.3 r=–0.3), serum insulin level (P=0.2, r=–0.4), or pancreatic insulin content (P=0.4, r=–0.2). Gastrointestinal transit was correlated with duodenal secretin content (P=0.01, r=–0.6) and colonic VIP content (P=0.003, r=0.6). It did not correlate, however, with the content of other neuroendocrine peptides in the different segments investigated.

Radioimmunoasssays

The results of determining of the various neuroendocrine peptides in various segments of the gastrointestinal tract are presented in Fig. 2. In antrum, the gastrin content in obese diabetic mice was significantly higher than that of lean controls (P<0.0001). In duodenum, the concentration of secretin in obese diabetic mice was higher than that of controls (P=0.03). In colon, whereas the contents of PYY and somatostatin were higher in obese diabetic mice (P=0.0002 and 0.002, respectively), the contents of substance P and VIP were lower than in controls (P= 0.009 and 0.0005, respectively).

DISCUSSION

The present study showed a slow gastrointestinal transit in the obese diabetic mice, similar to the reported gastrointestinal hypomotility in patients with diabetes (24). This slow gastrointestinal transit was not correlated with the blood glucose level, serum insulin level or the pancreatic insulin content. Acute hyperglycaemia and hyperinsulinaemia (9,13,26). have been found to impair gastrointestinal motility and have been suggested to be a major factor in the development of motility disturbances in diabetic patients (27). It is noteworthy, however, that the effect of chronic hyperglycaemia and hyperinsulinaemia on the gastrointestinal motility, as in this animal model, is not yet elucidated. However, it has been reported that slow gastric emptying in patients with type I diabetes is not correlated to actual blood glucose and glycaemic control (28). Gastrointestinal transit is strongly correlated to the duodenal secretin content and to the colonic VIP level. The high level of duodenal secretin and low level of colonic VIP may therefore be among the factors that caused slow gastrointestinal transit in the obese diabetic mice.

Dietary amines play an important role in regulating gastrin release in rats (29). Starvation in rats selectively reduced antral gastrin content (29,30). Antral gastrin mRNA is inhibited during fasting and is stimulated by ingestion of proteins or ami-

no acids (31). The increase in the antral gastrin content in diabetic obese mice observed in the present study may therefore have been caused by the hyperphagia exhibited by these mice. Secretin in physiological doses inhibits pentagastrin-stimulated acid secretion in rats, dogs and humans (32). The increased duodenal secretin content in the diabetic obese mice shown in this study could be a secondary response to increased gastrin synthesis and release and/or to increased demand on pancreatic secretion of water and bicarbonate. Although the increase in duodenal secretin could be secondary, it could affect the gastrointestinal motility as secretin is known to delay gastric emptying and ant to inhibit contractile activity of small and large intestine (32).

The intestinal peristaltic reflex includes wave of descending inhibition. There is an increasing body of evidence that VIP mediates this component of the peristaltic reflex (33). It is not surprising therefore to find in this study that VIP correlates strongly with gastrointestinal transit. It is rather difficult to establish as weather the present observation of decrease in colonic VIP in obese diabetic mice is primary or secondary to other factors presented by the diabetic state. Although substance P acts as an excitatory neurotransmitter in motor neurones, exciting the smooth muscles of longitudinal and circular muscle and muscularis mucosae (34), it was not correlated with the gastrointestinal transit in the present investigation. One can not exclude, however, that the low content of colonic substance P observed here in obese diabetic mice might affect another part of gastrointestinal motility.

PYY inhibits prostaglandin E_2 (PGE_2)-induced secretion of fluid and electrolytes from jejunum (35). VIP is a strong stimulant of secretion in both small and large intestine (33). Somatostatin inhibits secretion of fluid and bicarbonate, and absorption of calcium, carbohydrates, amino-acids and triglycerides (36). The high colonic content of PYY and somatostatin and the low content of VIP in diabetic obese mice may indicate reduced intestinal secretion in these animals.

Substance P is known to enhance proliferation of T-cells and the macrophage IL-1 production (34). Furthermore, it reduces B-cell Ig production and increases monocyte chemotaxis and cytokine release. It also increases neutrophil isozyme release and phagocytosis (34). VIP also affects the immunocytes, thus, regulating mast cell secretion (37, 38), inhibiting T-cell proliferation and affecting its migration, increasing the cytotoxicity of natural killer (NK) cells. Finally it increases IgM and reduces IgA production of B-cells (33). It is possible that the reduced content of colonic substance P and VIP is related to the state of immune deficiency associated with diabetes.

The present study showed that the obese diabetic mouse, an animal model of human type-2 diabetes, had a slow gastrointestinal transit. This slow transit is strongly correlated to the changes in two neuroendocrine peptides, namely secretin and VIP. It also showed changes in the content of several neuroendocrine peptides that can result in reduced intestinal secretion. These observations may explain the constipation which is a symptom occurring in up to 60% of patients with diabetes (1) and especially in patients with type-2 diabetes (5).

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