Abnormal Gastrointestinal Endocrine Cells in Patients with Diabetes Type 1: Relationship to Gastric Emptying and Myoelectrical Activity

M. El-Salhy & B. Sitohy
Section for Gastroenterology and Hepatology, Dept. of Medicine, Institution of Public Health and Clinical Medicine, University Hospital, Umeå, Sweden


Background: Gastrointestinal symptoms in patients with diabetes are believed to be caused by gastrointestinal dysmotility and secretion/absorption disturbances, and the gut endocrine cells play an important part in regulating these two functions. Studies on animal models of human diabetes type 1 revealed abnormality in these cells, but it is unknown whether abnormality also occurs in patients with diabetes. Methods: Eleven patients with long duration of diabetes type 1 and organ complications, as well as gastrointestinal symptoms, were studied. Endocrine cells in different segments of the gastrointestinal tract were detected by immunocytochemistry and quantified by computerized image analysis. Gastric emptying was measured by scintigraphy and gastric myoelectric activity was determined by electrogastrography. Results: An abnormal density of gastrointestinal endocrine cells was found in patients with diabetes. This abnormality occurred in all segments of the upper and lower gastrointestinal tract investigated, and included most of the endocrine cell types. The patients showed delayed gastric emptying, which correlated closely with the acute glucose level, but did not correlate with HbA1c. Gastric emptying also correlated closely with the density of duodenal serotonin and secretin cells. The patients exhibited bradygastrias and tachygastrias. These dysrhythmias, however, did not differ significantly from controls. Conclusions: The endocrine cells are the anatomical units responsible for the production of gut hormones, and the change in their density would reflect a change in the capacity of producing these hormones. The abnormality in density of the gastrointestinal endocrine cells may contribute to the development of gastrointestinal dysmotility and the symptoms encountered in patients with diabetes.

Key words: Computerized image analysis; diabetes type 1; electrogastrography; gastric emptying; gastrointestinal endocrine cells; immunocytochemistry

Magdy El-Salhy, M.D., Ph.D., Section for Gastroenterology and Hepatology, Dept. of Medicine, University Hospital, SE-901 85 Umeå, Sweden (fax. +46 90 14 39 86, e-mail. magdy. el-salhy@ medicin.umu.se)

Gastrointestinal symptoms such as nausea and vomiting, diarrhoea, constipation and abdominal pain are common in patients with diabetes mellitus (1–5). They are believed to be caused by gastrointestinal dysmotility and secretion/absorption disturbances (6). The endocrine cells of the gut secrete peptides and amines that play an important part in regulating both motility and absorption/secretion of the gastrointestinal tract (7–9). It is conceivable that gastrointestinal dysmotility and disturbances in absorption/secretion can be accompanied by an abnormality in these cells. This assumption is supported by the findings in animal models of human diabetes (10–14). To the best of our knowledge, the gastrointestinal endocrine cells in patients with diabetes have not yet been studied.

The aim of the present study was to quantify the gastrointestinal endocrine cells in patients with type 1 diabetes in order to establish whether or not an abnormality occurs. In further investigating the possible role of such abnormality in gastrointestinal dysmotility, the outcome was correlated to gastric emptying and myoelectrical activity.

Methods

Patients

Twelve patients with diabetes type 1 (9 women and 3 men; mean age 45 years, range 28–78) were investigated. Clinical data were obtained from medical records and are summarized in Table I. All patients underwent 75Se-homocholic taurine-conjugated bile acid (SeHCAT) and hydrogen breath tests. Both tests were normal in all patients, thus excluding bile acid malabsorption and small-bowel bacterial overgrowth. As controls for the endocrine content, biopsy specimens of the corpus and antrum of the stomach, bulbus and pars descendens duodeni (as a representative of the small intestine) and the rectum (as a representative of the large intestine) were selected. The duodenum and the rectum were chosen because they
contain all the endocrine cell types in the respective gastrointestinal segment and in large number (15). An overview of the controls used to determine the endocrine cell content is given in Table II. Fifteen healthy volunteers without gastrointestinal complaints (10 women and 5 men; mean age 47 years, range 25–65) served as controls for gastric scintigraphy, and 38 healthy subjects (26 women and 12 men; mean age 37, range 18–65) as controls for electrogastrography. The investigation was approved by the local ethics committee at Umeå University.

**Gastrointestinal endoscopy**

After an overnight fast, a gastroduodenal endoscopy was performed in patients and controls. During the endoscopy procedure, three or four forceps biopsies were obtained from the corpus (major curvature), the antrum, the bulbus duodeni and the pars descendens duodeni (distal to papilla of vateri). In addition, biopsies from the antrum were taken and used in the CLO test for *Helicobacter pylori*. Colonoscopy was performed and three or four biopsies were taken from the dorsal wall of the rectum, about 15 cm from the anus.

**Histopathology and immunocytochemistry**

Tissue specimens were fixed in 4% buffered paraformaldehyde overnight, embedded in paraffin wax, and cut into 5-μm-thick sections. These were stained by haematoxylin-eosin and van Gieson, and immunostained with the avidin-biotin-complex (ABC) method (Dakopatts, Glostrup, Denmark) as described earlier in detail (16). Briefly, the sections were

<table>
<thead>
<tr>
<th>Table I. Clinical data of patients with diabetes investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient no.</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
</tbody>
</table>

* Fast values measured before endoscopy.

<table>
<thead>
<tr>
<th>Table II. An overview of the controls and the site of biopsies used in determining the endocrine cell content</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of subjects</strong></td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>28</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>34</td>
</tr>
</tbody>
</table>

* Women/men; ** mean (range).
immersed in 0.5% hydrogen peroxide in Tris buffer, pH 7.6, for 10 min to inactivate endogenous peroxidase. They were then incubated with 1% bovine serum albumin for 10 min to occupy the non-specific binding sites. The sections were then incubated overnight with primary antiserum/antibodies at room temperature. Incubation with the secondary antibody, biotinylated swine anti-rabbit, or swine anti-mouse (in the case of monoclonal antibodies) IgG diluted 1:200, was carried out at room temperature for 30 min. The sections were then incubated with avidin-biotin-peroxidase complex (diluted 1:200) at room temperature for 30 min. Peroxidase was detected by immersing the sections in 50 mL Tris buffer containing 25 mg 3,3′-diaminobenzidine tetrahydrochloride (DAB) and 10 μl of 30% H₂O₂ followed by light counter staining in Mayer’s haematoxylin. All the gastrointestinal segments were investigated with antisera/antibodies against serotonin and somatostatin. Antrum and duodenum were studied with antisera against cholecystokinin (CCK)/gastrin C-terminus, duodenum with antisera against secretin and gastric inhibitory peptide (GIP) and rectum with antisera against pancreatic polypeptide (PP), peptide YY (PYY) and enteroglucagon. A detailed account of the antisera used is given in Table III. Specificity controls were the same as those described previously (16). Briefly, the sections were incubated with non-immune serum instead of the primary antibodies, or with the primary antibody preincubated with the corresponding or structurally related antigen.

**Computerized image analysis**

This was performed by the Quantimet 500MC image processing and analysis system (Leica, Cambridge, UK) linked to an Olympus microscope, type BX50. The program used in this system is QWIN (Leica’s Windows-based image analysis tool kit, version 1.02). In addition, the system included QUIPS (version 1.02), an interactive programming system. The slides were coded and the performer was not aware of the identity of the sections. Measurements were performed with an ×20 objective. At this magnification, each pixel of the image corresponds to 0.414 μm, and each field in the monitor represented a tissue area of 0.04 mm². Measurements were made in 20 randomly chosen fields in the stomach and rectum, and the vilus and crypt parts of the duodenum for each individual and peptide. These fields were selected from three to four sections, 50 μm apart.

The parameters measured were: the number of immunoreactive cells and the area of epithelial cells. Using QUIPS, an automated standard sequence analysis operation was created, as described in detail earlier (17). Briefly, the number of immunoreactive cells was counted using field measurements. The areas of the epithelial areas were measured using a threshold setting. The data from each field were tabulated, computed and statistically analysed automatically. Section thickness was determined microscopically by reading the screw at the upper and lower section surfaces. The number of endocrine cells/mm³ of the epithelial cells was estimated as described previously (17).

**Scintigraphic measurements of gastric emptying**

Scintigraphic measurement of gastric emptying was performed as described earlier (18). Briefly, after an overnight fast, gastric emptying of solid food was carried out after the subjects had ingested a standard meal consisting of an omelette (311 kcal) and 150 mL soft drink (70 kcal), with total energy of 381 kcal. The omelette was made of 2 eggs (57.5–65 g), 100 mL milk (3% fat), 15 g wheat flour, 5 g margarine and 15 MBq ⁹⁹Tc-labelled macroaggregated albumin (Pulmonate; Amersham International plc, Little Chalfont, UK). The omelette was cooked in a microwave oven at 1000W for 3 min. The omelette comprised 18 g protein, 19 g fat and 17 g carbohydrate. The meal was eaten within 10 min and measurement began immediately after ingestion, with the subjects seated in an upright position with the gamma camera in an anterior position. The region of interest corresponding to the stomach was outlined to determine the gastric counts for each frame. Data were acquired for 120 min, 60 sec per 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 using a program created in Excel (Microsoft, Calif., USA).

**Table III. Detailed account of the antibodies/antisera used**

<table>
<thead>
<tr>
<th>Antisera raised against</th>
<th>Working dilution</th>
<th>Code number</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin*</td>
<td>1:800</td>
<td>M 758</td>
<td>Dakopatts, Glostrup, Denmark</td>
</tr>
<tr>
<td>Porcine secretin</td>
<td>1:500</td>
<td>R787502 B 33-1</td>
<td>Euro-Diagnostica, Malmö, Sweden</td>
</tr>
<tr>
<td>Porcine gastric inhibitory polypeptide (GIP)</td>
<td>1:1600</td>
<td>R786403</td>
<td>Euro-Diagnostica</td>
</tr>
<tr>
<td>Synthetic gastrin-34</td>
<td>1:3000</td>
<td>R-783511</td>
<td>Dakopatts</td>
</tr>
<tr>
<td>Synthetic human somatostatin</td>
<td>1:4000</td>
<td>A566</td>
<td>Dakopatts</td>
</tr>
<tr>
<td>Synthetic polypeptide YY (PYY)</td>
<td>1:2000</td>
<td>B52-1</td>
<td>Eurodiagnostica</td>
</tr>
<tr>
<td>Synthetic human pancreatic polypeptide (PP)</td>
<td>1:1000</td>
<td>A619</td>
<td>Dakopatts</td>
</tr>
<tr>
<td>Pancreatic glucagon</td>
<td>1:2500</td>
<td>B-31</td>
<td>Eurodiagnostica</td>
</tr>
</tbody>
</table>

All the antisera raised in rabbits except *, which are monoclonal antibodies.

1 = specific for C-terminus of CCK/gastrin; 2 = specific for glucagon N-terminus and cross-reacts with both pancreatic glucagon and enteroglucagon.
**Electrogastrography (EGG)**

EEG was performed with a Digitrapper (Synetics Medical, OS version 2.0, hardware 03.0) for signal trapping, and the results were analysed with Synetics EGG software version 6.3. After skin preparation, the electrodes were applied as follows: one active electrode in the ventral mid-line, between the xiphoid process and umbilicus and the other active electrode on the left side of the abdomen, 5 cm distally angulated at 45°. The reference electrode was placed on the right side of the abdomen, 15 cm from the first electrode. Registration was done in 30 min after an overnight fast and after a standard meal. The meal consisted of 500 g fruit yoghurt, one slice of crpped bread and 200 mL orange juice. This meal contained 10.6 g protein, 6.5 g fat and 110.6 carbohydrates, with a total energy of 342 kcal.

**Statistical analysis**

Comparisons between patients and 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**

**Gastrointestinal endoscopy**

The stomach and duodenum, as well as the rectum, of both the patients and the controls were macroscopically normal. One of the patients with diabetes (no. 1) and four of the controls exhibited a positive CLO test, indicating infection with *H. pylori*.

**Histopathology and immunocytochemistry**

Histopathological examination of the stomach and duodenal biopsies from patients and controls revealed normal histology, thus excluding coeliac disease as a cause of diarrhoea. The rectal biopsies were normal except for one patient (no. 1) and three controls whose mucosa displayed a slight proctitis.

In the corpus, serotonin- and somatostatin-immunoreactive (IR) cells were found in both patients with diabetes and controls. In the antrum, in addition to serotonin- and somatostatin-IR, CCK/gastrin C-terminus-IR cells were detected. In the stomach the endocrine cells were localized mainly in the glandular tissue. The endocrine cells were round- to basket-shaped.

In the duodenum, serotonin-, secretin-, GIP-, CCK/gastrin C-terminus- and somatostatin-IR cells were observed in both patients and controls. Serotonin-IR cells were found both in villi and crypts. CCK/gastrin- and secretin-immunoreactive cells were confined almost exclusively to the villus; somatostatin- and GIP-immunoreactive cells were mainly localized within the crypts. The shape of these cells varied from flask-shaped to basket-shaped, with a basal process running parallel to the basement membrane.

In the rectum, serotonin-, peptide YY (PYY)-, pancreatic polypeptide (PP)-, and somatostatin-IR cells were found in both patients and controls. On the other hand, enteroglucagon-IR cells were detected in controls, but not in patients with diabetes. The endocrine cells occurred mostly in the middle part of the crypts of Lieberkühn. Their shape varied from basket-shaped to flask-shaped (Fig. 1).

Specificity controls showed that the immunostaining was abolished completely after the pre-incubation with the corresponding peptide. Preincubation of the antisera with the structurally related peptides had no effect on the immunostaining. Replacing the antisera with normal rabbit serum or Tris buffer gave no staining.

**Computerized image analysis**

In both the corpus and the antrum, the number of serotonin-IR cells was significantly higher in patients with diabetes than in controls. There was no statistical difference between
patients and controls regarding CCK/gastrin C-terminus- and somatostatin-IR cells (Fig. 2).

The density of serotonin- and secretin-IR cells was higher in both bulbus duodeni and pars descendens duodeni in patients with diabetes than in controls. Whereas the number of CCK/gastrin C-terminus-IR cells increased in bulbus duodeni, it decreased in pars descendens duodeni in the patients compared to the controls. The density of gastric inhibitory peptide (GIP) was higher in pars descendens duodeni of the patients, but not in bulbus duodeni. The number of somatostatin-IR cells in both bulbus duodeni and pars descendens did not differ from that of controls (Figs. 3 and 4).

In the rectum, the density of serotonin- and PYY-IR cells was higher in patients with diabetes than in controls. The number of somatostatin-IR cells was lower than in controls and enteroglucagon-IR cells were lacking completely in the patients. There was no difference between patients and controls regarding PP-IR cells (Fig. 5).

**Scintigraphic measurements of gastric emptying**

In patients with diabetes, the lag phase and $T_{50}$ were $52.8 \pm 9.9$ min and $198.7 \pm 48$ min (mean ± s), respectively. The corresponding figures for controls were $31.7 \pm 0.9$ and $91.4 \pm 1.6$ (Fig. 6). The difference between patients and controls was statistically significant regarding the lag phase and $T_{50}$ ($P = 0.001$ and $<0.001$, respectively). $T_{50}$ correlated with the present blood glucose level ($P = 0.007; r = 0.8$), but not with the lag phase ($P = 0.4; r = 0.3$). Neither the lag phase nor $T_{50}$ correlated with HbA1c ($P = 0.5; r = 0.2$ and $0.6; r = 0.2$, respectively). $T_{50}$ correlated with the density of serotonin-IR cells in bulbus duodeni ($P = 0.03; r = 0.8$), secretin-IR cells in both bulbus duodeni ($P = 0.03; r = 0.8$) and pars descendens duodeni ($P = 0.02; r = 0.8$). Neither the lag phase nor the $T_{50}$ correlated with the density of any of the other endocrine cell types in the various gastrointestinal segments investigated.
Electrogastrography

There was no statistically significant difference between patients and controls in either fasting or the postprandial state regarding period dominant frequency, dominant frequency in normal area, bradygastria, tachygastria or the ratio between period dominant power postprandial and in fasting (Table IV).

Discussion

The changes in the gastrointestinal endocrine cells observed here in patients with diabetes were much more extensive than those seen in an animal model of human diabetes type 1 (non-obese diabetic mouse) (10, 11, 14). Moreover, the type of endocrine cells affected differed from those in the animal model (10, 11, 14). This difference may be due either to the shorter duration of the diabetic state in these animals as compared with the patients examined in the present study, or to this model being not completely representative of human diabetes type 1. Some of the changes in the gastrointestinal cells could be primary, as changes in these cells have been observed in the pre-diabetic state in an animal model of human diabetes type 1 (10, 11, 14).

Table IV. The results of electrogastrography in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>PDF1</th>
<th>PDF2</th>
<th>PDFN1</th>
<th>PDFN2</th>
<th>Brad1</th>
<th>Brad2</th>
<th>Tach1</th>
<th>Tach2</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>3.00</td>
<td>3.00</td>
<td>92.0</td>
<td>95.0</td>
<td>3.4</td>
<td>3.7</td>
<td>4.2</td>
<td>4.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Patients</td>
<td>2.9</td>
<td>3.09</td>
<td>85.6</td>
<td>90.3</td>
<td>3.5</td>
<td>0.7</td>
<td>6.2</td>
<td>6.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

PDF = period dominant frequency; PDFN = % of period frequency in normal frequency area; Brad = % bradygastria; Tach = % tachygastria; ratio* = ratio between period dominant power postprandial and period dominant power in fasting state (volts); 1 = fasting state; 2 = postprandial.
Some of the abnormalities may be secondary to the diabetic state, with the metabolic rearrangement it causes, as other changes in the gut endocrine cells than those observed in the pre-diabetic state have been reported in an animal model of human diabetes type 1 (10, 11, 14). The other changes could be secondary to autonomic neuropathy, as changes in the gastrointestinal endocrine cells have been reported after vagotomy (19–21). Regardless of the cause of the abnormality in the density of gastrointestinal cells in patients with diabetes, this abnormality may result in a dysfunction of the gastrointestinal motility and secretion/absorption.

The patients included in this study showed delayed gastric emptying, a finding which agrees with previous observations in patients with diabetes (6, 28). The present finding that T50 correlates closely with the acute glucose level of the patient is in agreement with earlier studies where hyperglycaemia has been found to impair gastric emptying and gastric motility (6, 22–25). It has been suggested that hyperglycaemia is a major factor in the development of gastroparesis in diabetic patients (6). On the other hand, in the present study, gastric emptying did not correlate with HbA1c, which agrees with previous observations (26). It seems that gastric emptying is affected only by the present blood glucose level and not by chronic hyperglycaemia. The patients exhibited bradygastrias and tachygastrias, as reported earlier (6, 27). However, these dysrhythmias did not differ significantly from controls, which is in agreement with previously reported observations (28).

Serotonin has been found to stimulate gastric antrum, the small intestinal and colonic motility, as well as accelerating gastric emptying, and both small and large intestinal transits (29–34). The density of serotonin-IR cells was higher in patients with diabetes than in controls in all gastrointestinal segments. This increase may be one of the factors that cause diarrhea, a symptom most of the patients examined here suffered from. The number of small intestinal serotonin-IR cells correlated closely with gastric emptying. The increase in small intestinal serotonin-IR cells could be a secondary response to the delayed gastric emptying in these patients.

Secretin delays gastric emptying and inhibits contractile activity of small intestine and colon (35, 36). The density of secretin-IR cells was significantly higher in patients with diabetes compared with controls and was closely correlated with gastric emptying. It is reasonable, therefore, to conclude that the increased density of secretin cells may be one of the factors causing delayed gastric emptying in these patients. GIP plays a major part as an ‘incretin’ in the mechanism by which gut factors contribute to the enhancement of insulin secretion after a meal (37). The increased intensity of small intestinal GIP-IR cells in patients with diabetes may be caused by the absence of insulin release in response to hyperglycaemia in these patients.

Gastrin has an excitatory effect on the smooth muscles, exerted by a direct effect on the corpus and antrum and by an indirect effect mediated by the release of acetylcholine (7). Despite this stimulatory motor activity of the corpus and antrum, gastrin delays gastric emptying of both solids and liquids (38). CCK retards gastric emptying in most species studied by inducing relaxation of the proximal part of the stomach and constricting the pyloric sphincter (39). Furthermore, CCK stimulates slow motor activity and decreases intestinal transit time (39). The antisera used here reacts with CCK/gastrin C-terminus and, accordingly, cross-reacts with both CCK and gastrin cells. One may speculate that the decrease in density of CCK/gastrin-IR cells in bulbus duodeni may be one of the factors that delays gastric emptying. On the other hand, the decrease in density of CCK/gastrin-IR cells in pars descendens duodeni may be compensatory to the increase in bulbus duodeni. Ileal brake is the phenomenon observed in humans, in which perfusion of the ileum and colon with fats slows intestinal transit and delays gastric emptying (40). PYY is believed to be one of the mediators of ileal brake (40). The increased number of PYY-IR cells in patients with diabetes may be a secondary response to accelerated intestinal transit in these patients. This increase would, as a side effect, contribute further to delaying gastric emptying. Somatostatin stimulates the early phase of gastric emptying and inhibits the late phase of gastric emptying and gastric migrating motor complex (41). The decrease in colonic somatostatin-IR in patients with diabetes observed here may therefore contribute to the motility disorders in these patients. The significance of the absence of enteroglucagon-IR cells observed here in the colon of patients with diabetes is difficult to interpret, as the biological action of this peptide is not yet clear (43). It is noteworthy, however, that vincristine-induced constipation in rats is accompanied by disturbances in the colonic enteroglucagon-IR cells (43).

The endocrine cells are the anatomical units responsible for the production of gut hormones, and consequently the change in their density would reflect a change in the capacity of producing gut hormones. The present study revealed abnormal density of gastrointestinal endocrine cells in patients with diabetes. This abnormality was extensive and included most of the endocrine cell types in both the upper and lower gastrointestinal tract. This abnormality may contribute to the development of gastrointestinal dysmotility and symptoms encountered in patients with diabetes. One should keep in mind, however, that endocrine cells of the gut integrate and interact with each other and with the enteric and autonomic nervous system. One should consider the present findings as an imbalance in the endocrine cell part of a fairly complicated regulatory mechanism of the gut.

Acknowledgements

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

References