Endocrine cells in the ileum of patients with irritable bowel syndrome

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AIM: To study the ileal endocrine cell types in irritable bowel syndrome (IBS) patients.

METHODS: Ninety-eight patients with IBS (77 females and 21 males; mean age 35 years, range 18-66 years) were included, of which 35 patients had diarrhea (IBS-D), 31 patients had a mixture of both diarrhea and constipation (IBS-M), and 32 patients had constipation (IBS-C) as the predominant symptoms. The controls were 38 subjects (26 females and 12 males; mean age 40 years, range 18-65 years) who had submitted to colonoscopy for the following reasons: gastrointestinal bleeding, where the source of bleeding was identified as hemorrhoids (n = 24) or angiodysplasia (n = 3), and health worries resulting from a relative being diagnosed with colon carcinoma (n = 11). The patients were asked to complete the: Birmingham IBS symptom questionnaire. Ileal biopsy specimens from all subjects were immunostained using the avidin-biotin-complex method for serotonin, peptide YY (PYY), pancreatic polypeptide (PP), enteroglucagon, and somatostatin cells. The cell densities were quantified by computerized image analysis, using Olympus cellSens imaging software.

RESULTS: The gender and age distributions did not differ significantly between the patients and the controls (P = 0.27 and P = 0.18, respectively). The total score of Birmingham IBS symptom questionnaire was 21 ± 0.8, and the three underlying dimensions: pain, diarrhea, and constipation were 7.2 ± 0.4, 6.6 ± 0.4, and 7.2 ± 0.4, respectively. The density of serotonin cells in the ileum was 40.6 ± 3.6 cells/mm² in the controls, and 11.5 ± 1.2, 10.7 ± 5.6, 10.0 ± 1.9, and 13.9 ± 1.4 cells/mm² in the all IBS patients (IBS-total), IBS-D, IBS-M, and IBS-C patients, respectively. The density in the controls differed significantly from those in the IBS-total, IBS-D, IBS-M, and IBS-C groups (P < 0.0001, P = 0.0001, P = 0.0001, and P < 0.0001, respectively). There was a significant inverse correlation between the serotonin cell density and the pain dimension of Birmingham IBS symptom questionnaire (r = -0.6, P = 0.0002). The density of PYY cells was 26.7 ± 1.6 cells/mm² in the controls, and 33.1 ± 1.4, 34.1 ± 2.5, and 41.7 ± 3.1 cells/mm² in the IBS-total, IBS-D, IBS-M, and IBS-C patients, respectively. This density differed significantly between patients with IBS-total and IBS-C and the controls (P = 0.03 and < 0.0001, respectively), but not between controls and, IBS-D, and IBS-M patients (P = 0.8, and P = 0.1, respectively). The density of PYY cells correlated significantly with the degree of constipation as recorded by the Birmingham IBS symptom questionnaire (r = 0.6, P = 0.0002). There were few PP-, enteroglucagon-, and somatostatin-immunoreactive cells in the biopsy material examined, which made it impossible to reliably quantify these cells.
CONCLUSION: The decrease of ileal serotonin cells is associated with the visceral hypersensitivity seen in all IBS subtypes. The increased density of PYY cells in IBS-C might contribute to the constipation experienced by these patients.

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Key words: Computer image analysis; Irritable bowel syndrome; Ileum; Peptide YY; Serotonin

Core tip: The present study investigated for the first time the ileal endocrine cells in patients with irritable bowel syndrome (IBS). It included a relatively large cohort of patients and comprising all the IBS subtypes, namely diarrhea (IBS-D), a mixture of both diarrhea and constipation (IBS-M) and constipation (IBS-C). It showed that the density of serotonin cells is reduced in patients with IBS, regardless of the subtype. On the other hand the density of peptide YY (PYY) cells in the ileum of IBS-D and IBS-M patients did not differ from that of controls, but was significantly elevated in those with IBS-C. It was concluded that the reduction of ileal serotonin cells may be connected to the visceral hypersensitivity seen in all IBS subtypes and that increase in the PYY cell density in IBS-C, would slow the intestinal transit and cause constipation.


INTRODUCTION

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal functional disorder that is characterized by abdominal discomfort or pain, altered bowel habits, and bloating/abdominal distension[1,2]. IBS reportedly has a prevalence of 5%-20% and an incidence of about 200 per 100000 of the adult population[2-9]. IBS reduces the quality of life considerably in IBS patients and is an economic burden to society for various reasons, including overconsumption of healthcare resources and increased sick leave[10]. However, IBS is not known to be associated with the development of serious disease or with increased mortality[11]

The diagnosis of IBS is based on the assessment of symptoms and detailed, accurate, and clinically useful definitions of the syndrome that have been elaborated by the working parties responsible for producing the latest Rome III Criteria[13,14]. In addition to these criteria, warning symptoms (so-called red flags) such as age > 50 years, short history of symptoms, nocturnal symptoms, weight loss, rectal bleeding, anemia, and the presence of markers for inflammation or infections should be excluded. IBS patients are sub-grouped on the basis of differences in the predominant bowel symptoms into diarrhea (IBS-D), constipation (IBS-C), both diarrhea and constipation (IBS-M), and un-subtyped IBS in patients with insufficient abnormality of stool consistency to meet criteria for IBS-C, -D, or -M[13,14]. Because of the overlap of symptomology with celiac disease and microscopic colitis, some gastroenterologists (including the present authors) believe that these disorders should be excluded in addition to applying the Rome Criteria[13].

Abnormalities in the endocrine cells in the stomach, duodenum, colon, and rectum have been reported in patients with IBS[16-29], but the ileal endocrine cells have not been investigated previously. The endocrine cell types differ markedly between the distal and proximal small intestine, probably due to the quite different functions performed by these two parts of the intestine. The proximal small intestine contains serotonin, secretin, cholceystokinin (CCK), gastric inhibitory polypeptide (GIP), and somatostatin cells, while the ileum has the same endocrine cell types as in the large intestine, namely serotonin, peptide YY (PYY), pancreatic polypeptide (PP), enteroglucagon, and somatostatin cells[30].

A recent study observed endocrine cell depletion in the ileum of patients with sporadic IBS[31]. This depletion was detected by chromogranin A, which is a common marker for endocrine cells[32-34]. The aim of the present study was to clarify the affected endocrine cell types by examining various ileal endocrine cells in the same cohort of IBS patients investigated using chromogranin A.

MATERIALS AND METHODS

Patients and controls

Ninety-eight patients (77 females and 21 males; mean age 35 years, range 18-66 years) with IBS according to Rome III Criteria were included in the study[13,14]. The IBS subtypes were distributed as follows: 35 patients with IBS-D, 31 patients with IBS-M, and 32 patients with IBS-C. Symptoms had been present in all of the patients for many years, and the onset of their IBS symptoms could not be associated with any events, in particular gastrointestinal or other infections. All patients underwent a complete physical examination and were investigated using the following blood tests: full blood count, electrolytes, inflammatory markers, liver tests, and thyroid function tests. They also underwent further gastroscopy with duodenal biopsies, which were used to exclude celiac disease. All of the patients had been tested previously (i.e., before they were referred to us) for lactose intolerance and the presence of intestinal infectious agents including parasites in the stool; the results of all of these tests were negative.

For comparison, the control group comprised 38 subjects (26 females and 12 males; mean age 40 years, range 18-65 years) who had submitted to colonoscopy for the following reasons: gastrointestinal bleeding,
where the source of bleeding was identified as hemorrhoids (n = 24) or angiodysplasia (n = 3), and health worries resulting from a relative being diagnosed with colon carcinoma (n = 11).

The patients were asked to complete the: Birmingham IBS symptom questionnaire. The Birmingham IBS symptom score questionnaire is a disease specific score to measure the symptoms of patients with IBS. It has been developed to be suitable for self-completion and has been found to be acceptable to patients. Its dimensions have good reliability, external validity and sensitivity[35]. The questionnaire comprises 11 questions based on the frequency of IBS related symptoms. Each question has a standard response scale with symptoms all being measured on a 5-point Likert scale ranging from 0 ("none of the time") to 5 ("all of the time"). There are three underlying dimensions: pain (3 items), diarrhea (5 items) and constipation (3 items)[35].

The study was performed in accordance with the Declaration of Helsinki and was approved by the Regional Committee for Medical and Health Research Ethics West, Bergen, Norway. All subjects gave oral and written consent to participate.

Colonoscopy, histopathology, and immunohistochemistry

Colonoscopy was performed on both the patients and the controls, segmental biopsy specimens were taken from the colon and rectum, and four biopsy samples were taken from the ileum of each subject. The biopsy samples were fixed overnight in 4% buffered paraformaldehyde, embedded in paraffin, and cut into 5-m sections. The sections were stained with hematoxylin-eosin, and immunostained by the avidin-biotin complex (ABC) method using the Vectastain ABC kit (Vector Laboratories). The sections were hydrated and immersed in 0.01% hydrogen peroxide in PBS buffer (pH 7.4) for 10 min to inhibit endogenous peroxidase. After washing in buffer, the sections were treated with 1% bovine serum albumin for 30 min to block the nonspecific binding sites, and then incubated with the primary antiserum/antibody at room temperature for 2 h. The sections were then washed in PBS buffer and incubated with biotinylated swine anti-mouse (in the case of monoclonal antibodies) or anti-rabbit IgG (in the case of polyclonal antibodies) diluted 1:200 for 30 min at room temperature. After washing the slides in PBS buffer, the sections were incubated for 30 min with avidin-biotin-peroxidase complex diluted 1:100, and then immersed in 3,3'-diaminobenzidine (DAB) peroxidase substrate (Vector Laboratories), followed by counterstaining in hematoxylin[10]. The following primary antisera/antibodies were used: monoclonal mouse anti-serotonin (Dako, code no. 5HT-209), polyclonal anti-porcine peptide PYY (Alpha-Dagnostica, code PYY 11A), polyclonal rabbit anti-synthetic-human PYY (Diagnostic Biosystems, code No. 114), polyclonal rabbit anti-porcine glicentin/glucagon (Acris Antibodies, code BP508), and polyclonal rabbit anti-synthetic-human somatostatin (Dako, code no. A560); these antibodies were used at dilutions of 1:1500, 1:1000, 1:800, 1:400, and 1:200, respectively.

Computerized image analysis

Quantification of the endocrine cells was done as described previously[36,37]. Measurements were performed using Olympus cellSens imaging software (version 1.7) on a computer linked to an Olympus microscope type BX 43 with an Olympus camera (DP 26). The number of immunoreactive cells and the area of the epithelial cells were measured. The numbers of endocrine cells in each field were counted manually by pointing and clicking the computer mouse, and the areas of the epithelium containing these cells were drawn manually using the computer mouse. A × 40 objective was used, for which each frame (field) on the monitor represented a tissue area of 0.14 mm². Each individual and peptide hormone was measured in ten randomly chosen fields. Immunostained sections from the IBS patients and controls were coded and mixed, and measurements were made by the same person (El-Salhy M) who was blind to the identity of the sections. The data from the fields were tabulated, and the cell density of the epithelium (in cells/mm²) was computed and statistically analyzed.

Statistical analysis

The gender difference between patients and controls was tested by Fisher’s exact test, and the age difference was tested by the Mann-Whitney nonparametric test. Differences between controls, all IBS patients (IBS-total), IBS-D, IBS-M, and IBS-C patients were tested by the Kruskal-Wallis nonparametric test with Dunn’s post-test. Correlation was done by Spearman nonparametric test. The data are presented as mean ± SE values, and differences with P < 0.05 were considered to be statistically significant.

RESULTS

Gender and age characteristics of patients and controls

The gender and age distributions did not differ significantly between the patients and the controls (P = 0.27 and P = 0.18, respectively). The total score of Birmingham IBS symptom questionnaire was 21 ± 0.8, and the three underlying dimensions: pain, diarrhea, and constipation were 7.2 ± 0.4, 6.6 ± 0.4, and 7.2 ± 0.4, respectively.

Endoscopy, histopathology, and immunohistochemistry

The colon and rectum of both the patients and the control subjects were macroscopically normal. The ileum was macroscopically normal in all except in one control subject and three patients, in whom lymphoid hyperplasia was observed; this condition is a common finding in young individuals and has no pathological relevance. The results of histopathological examinations of the ileum, colon, and rectum were normal in both the patients and the controls.
Serotonin-, PYY-, PP-, enteroglucagon-, and somatostatin-immunoreactive cells were found in the ileum of all of the subjects (i.e., patients and controls), mostly in the crypts. These cells were basket- or flask-shaped.

**Computerized image analysis**

**Serotonin cell density**: The density of serotonin cells in the ileum was 40.6 ± 3.6 cells/mm² in the controls, and 11.5 ± 1.2, 10.7 ± 5.6, 10.0 ± 1.9, and 13.0 ± 1.4 cells/mm² in the IBS-total, IBS-D, IBS-M, and IBS-C patients, respectively. The serotonin cell density differed significantly between the controls and the IBS-total and IBS subgroups (P < 0.0001). Posttests showed that the density in the controls differed significantly from those in the IBS-total, IBS-D, IBS-M, and IBS-C groups (P < 0.0001, P = 0.0001, P = 0.0001, and P < 0.0001, respectively) (Figures 1 and 2). There was a significant inverse (negative) correlation between the serotonin cell density and the pain dimension of Birmingham IBS symptom questionnaire (r = -0.6, P = 0.0002). There was no significant correlation between the total score of Birmingham IBS symptom questionnaire, the diarrhea or constipation dimension (r = -0.05, P = 0.8; r = -0.4, P = 0.8; and r = -0.2, P = 0.2 respectively). The density of PYY cells was 26.7 ± 1.6 cells/mm² in the controls, and 33.1 ± 1.4, 27.5 ± 1.4, 34.1 ± 2.5, and 41.7 ± 3.1 cells/mm² in the IBS-total, IBS-D, IBS-M, and IBS-C patients, respectively. The PYY cell density differed significantly between the controls and the IBS-total and IBS subgroups (P < 0.0001). This density differed significantly between patients with IBS-total and IBS-C and the controls (P = 0.03 and < 0.0001, respectively), but not between controls and, IBS-D, and IBS-M patients (P = 0.8, and P = 0.1, respectively) (Figures 3 and 4). The density of PYY cells correlated significantly with the degree of constipation as recorded by the Birmingham IBS symptom questionnaire (r = 0.6, P = 0.0002). There was not significant correlation between the cell density of PYY and the total Birmingham IBS symptom questionnaire, the pain-, or diarrhea dimension (r = 0.2, P = 0.2; r = 0.2, P = 0.06; and r = 0.1, P = 0.5 respectively).

**PP, enteroglucagon, and somatostatin cell densities**: There were few PP-, enteroglucagon-, and somatostatin-immunoreactive cells in the biopsy material examined, which made it impossible to reliably quantify these cells.

**DISCUSSION**

The present study showed that the density of serotonin cells in the ileum is reduced in patients with sporadic (nonspecific) IBS, regardless of the subtype. This finding is similar to that reported in the colon of IBS patients[10]. On the other hand the density of PYY cells in the ileum of IBS-D and IBS-M patients did not differ from that of controls, but was significantly elevated in those with IBS-C. The observations made here on PYY cell density differ from those reported in the colon, where the density of PYY cells was reduced in IBS patients.

Abnormalities in the endocrine cells in the stomach, duodenum, colon, and rectum have been reported in patients with IBS[16-29]. The density of ghrelin cells in the stomach is lower in IBS-C and higher in IBS-D than in healthy controls[10]. In the duodenum, the cell densities of GIP and somatostatin are decreased in both IBS-D and IBS-C[18]. The densities of duodenal secretin and CCK cells are decreased in IBS-D but unchanged in IBS-C[18]. The duodenal serotonin cells are not affected in both IBS-D and IBS-C[18]. Postinfectious IBS was found to be associated with increased numbers of duodenal CCK cells but decreased numbers of serotonin cells[17]. Colonic serotonin and PYY cell densities have been found...
to be low in both IBS-D and IBS-C[19]. In the rectum of patients with sporadic (nonspecific) IBS, the densities of PYY and enteroglucagon cells were significantly lower and that of somatostatin cells was significantly higher in both IBS-D and IBS-C than in the controls, whereas the serotonin cell density in these patients did not differ from that in healthy controls[21,38]. Rectal serotonin and PYY cell densities in postinfectious IBS have been reported to be elevated[23,25,27,39,40].

Serotonin cells are the predominant endocrine cell type in the ileum, which could account for the general reduction of ileal endocrine cells reported elsewhere[31]. Each intestinal crypt contains four to six pluripotent stem cells that differentiate through a series of cellular precursors (progenitors) into all epithelial cell types including enterocytes, goblet cells, Paneth cells, and endocrine cells[41-57]. It is possible that the reduction in ileal serotonin cells in IBS patients is due to abnormal cell differentiation from stem cell. This assumption gets support from the findings that depletions of endocrine cell in rejected ileum transplants are associated with marked depression in the expression of neurogenin-3 (NEUROG3) which is a early progenitor for endocrine cells and NeuroD, which is a transcription factor expressed by cells derived from NEUROG3[58]. Furthermore, a mutant NEUROG3 has been described in patients with congenital malabsorption diarrhea and lack of intestinal endocrine cells[59].

Serotonin activates the submucosal sensory branch of the enteric nervous system that conveys sensation from the gut to the central nervous system[60,61]. Serotonin modulates is known to modulate visceral sensitivity of the gastrointestinal tract[62,63]. It is therefore conceivable to conclude that the reduction of ileal serotonin cells may be connected to the visceral hypersensitivity seen in all IBS subtypes. It is difficult to establish whether the reduction in these ileal serotonin cells is primary or secondary to the visceral hypersensitivity. However, it is tempting to speculate that this abnormality is secondary to the visceral hypersensitivity and represents an adaptation mechanism for reducing the sensation conveyed from the gut to the central nervous system by serotonin. This assumption gets support from the present finding that serotonin cell density is correlated inversely to the pain score obtained by the Birmingham IBS symptom questionnaire. Speaking against this assumption are the findings that tryptophan hydroxylase (TPH)-1, which is the limiting enzyme for the synthesis in serotonin cells, and serotonin transporter (SERT) mRNA levels have been reported to be lower in the rectum and sigmoid colon of IBS patients than control subjects[64]. There was no difference, however, in the levels of TPH-1 and SERT between IBS patients with rectal hypersensitivity and those without[64].

PYY stimulates the absorption of water and electrolytes, and is a major regulator of the “ileal brake”[65-70]. Furthermore, PYY inhibits prostaglandin E2 and vaso-active intestinal polypeptide (VIP), which stimulate intest-
tinal fluid secretion\cite{71-73}. Administration of PYY inhibits diarrhea in experimental mouse models by reducing intestinal fluid secretion and slowing colonic transit\cite{74}. It is thus possible that the increase in the PYY cell density in IBS-C patients, and consequently the increase in PYY, would slow the intestinal transit by strengthening the ileal brake, increasing the absorption of water, and decreasing the secretion of the intestinal fluid in the distal small intestine via inhibiting VIP and prostaglandin E2. These effects would in turn cause constipation. In support of this conclusion is the observation that the density PYY cell correlated to the constipation score calculated from the Birmingham IBS symptom questionnaire.

The above summary indicates that most of the reported abnormalities in gastrointestinal endocrine cells are similar in all IBS subtypes\cite{10,20,28-31}. However, in IBS-C the density of ghrelin cells in the oxyntic mucosa of the stomach was significantly lower than in healthy controls\cite{9}. In addition to its role in regulating appetite and energy metabolism, ghrelin accelerates gastric and small- and large-intestinal motility\cite{75-88}. It can be speculated that the low density of ghrelin cells previously reported and the high density of PYY cells observed in the present study explain why constipation predominates in the IBS-C subtype. On the other hand, in IBS-D patients the density of ghrelin cells in the stomach was significantly higher and the densities of duodenal secretive and CCK were lower than healthy volunteers\cite{10,18}. Secretin inhibits gastric emptying and intestinal motility, and stimulates pancreatic bicarbonate and fluid secretions\cite{10,18,90}. The secretion of pancreatic bicarbonate increases the pH of gut contents, which are highly acidic after leaving the stomach, and this is essential for lipid digestion as pancreatic lipase is irreversibly inactivated below pH 4.0\cite{91}. CCK relaxes the proximal stomach in order to increase its reservoir capacity, inhibits gastric emptying and stimulates gall bladder contractions and pancreatic exocrine secretions of digestive enzymes from pancreatic exocrine glands\cite{91,94}. The low densities of secretin and CCK cells in IBS-diarrhea patients could cause a rapid gastric emptying and acceleration of intestinal motility and ultimately diarrhea in these patients.

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**COMMENTS**

**Background**

Irritable bowel syndrome (IBS) is a common chronic gastrointestinal disorder that is characterized by intermittent abdominal discomfort or pain, altered bowel habits, and bloating/abdominal distension. IBS reduces the quality of life considerably, but it is not known to be associated with the development of serious disease or with an increased mortality rates. Abnormal endocrine cells have been reported in the stomach, duodenum, colon, and rectum in patients with IBS, but the ileal endocrine cells have not been investigated previously.

**Research frontiers**

This study showed for the first time the ileal endocrine cells in patients with IBS are abnormal. It included a large cohort of patients and comprising all the IBS subtypes. It showed that the density of serotonin cells is reduced in patients with IBS, regardless of the subtype. Moreover, it revealed that the density of peptide Y (PYY) cells in the ileum of patients with IBS-D and IBS- did not differ from that of controls, but was significantly elevated in IBS-C. It was concluded that the reduction of ileal serotonin cells may play a role in visceral hypersensi-


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