

Immunohistochemical identification and localisation of gastrin and somatostatin in endocrine cells of human pyloric gastric mucosa

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The detailed description of the distribution of endocrine cells G and D producing important hormones that regulate activation of other cells in the human stomach may be a valuable source of information for opinions about mucosa changes in different diseases of the alimentary tract. The density and distribution of immunoreactive G and D cells in the pylorus of humans (donors of organs) were evaluated. The pylorus samples were collected after other organs were harvested for transplantation. The number of G cells in the pyloric mucosa of healthy people was higher than the number of D cells. G and D cells were distributed between columnar cells of epithelium mucosa. Multiform endocrine cells generally occurred: gastrin in the middle third of the mucosa and somatostatin cells in the basal half of the pyloric mucosa. The investigation of the pyloric part of the healthy human stomach showed a characteristic distribution of cells that reacted with antisera against gastrin and somatostatin. (Folia Morphol 2012; 71, 1: 39–44)

Key words: G-cells, D-cells, immunohistochemistry, human pylorus

INTRODUCTION

Gastrointestinal hormones are secreted by endocrine cells, which are distributed throughout the mucosa of the gastrointestinal tract. Endocrine cells play an important regulatory role in the gastrointestinal tract.

Gastrin, somatostatin, and other gastrointestinal hormones regulate the functions of the gastrointestinal tract such as secretion of intestinal and fundic glands, and nutrient and drug absorption [17, 20].

The secretion of hydrochloric acid is an important function of the human stomach. Gastric acid aids protein digestion, facilitates the absorption of iron, calcium, and vitamin B₁₂, and prevents bacterial overgrowth [7, 17]. In humans, acid is continuously secreted by parietal cells of gastric mucosa and is precisely regulated by a variety of neurocrine, paracrine, and endocrine signals in order to achieve the correct amount of acid secretion required by the specific situ-

ation [12, 19]. Too much acid can lead to peptic ulcer disease, gastroesophageal reflux disease, and stress-related erosion/ulcer disease. Too little acid can interfere with the absorption of certain nutrients, predispose to enteric infection, and interfere with the absorption of some medications [17]

Important regulators of gastric acid secretion are gastrin G cells and somatostatin D cells. Gastrin, the main stimulant of acid secretion during meal ingestion, is produced in G cells of the gastric antrum and duodenal mucosa, but the major source of serum gastrin is antral G cells [19, 24]. The pyloric gland area, the hallmark of which is the gastrin or G cell, comprises 20% of the antrum [5]. The pyloric gland also contains D cells, which produce somatostatin. Somatostatin is the main inhibitor of basal gastric acid secretion. The regulation of gastric acid secretion is achieved by the interplay between two major gas-

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tric endocrine cells: the gastrin G cell and the somatostatin D cell. Each of these agents acts directly on the parietal cell as well as indirectly by modulating the secretion of neuroendocrine cells [19].

Gastrin indirectly stimulates acid secretion through induction of histamine release from enterochromaffin-like (ECL) cells. Histamine subsequently stimulates gastric acid secretion through H₂ receptors on parietal cells [3, 18, 29]. Increased acid levels then stimulate putative chemoreceptors on pyloric D cells to secrete somatostatin and block further release of gastrin and gastric acid. Thus gastric acid secretion is regulated by a negative feedback mechanism involving somatostatin [6, 18].

The alterations in the relative numbers of gastrin G cells and somatostatin D cells may play a key role in gastroduodenal disease.

Due to the difficulties in obtaining human samples our current level of knowledge concerning the DNES cells is founded on the results of research conducted on animals.

The limited data available regarding the description of the endocrine cells of the human gastrointestinal tract is based on a restricted number of samples collected during surgical procedures or biopsies. In the present paper, the authors investigated the distribution, morphology, and number of G cells and D cells in the healthy human pyloric gastric mucosa, in order to provide basic materials for studying gastrointestinal endocrinology, gastroenterology, and prevention of disease in the digestive system, and its treatment.

MATERIAL AND METHODS

Nineteen donors of organs with normal gastric mucosa were used in the study. There were 10 men with mean age 43.9 years, range from 21 to 65 years, with mean body weight 83.25 kg and 9 women with mean age 46.3 years; range from 19 to 58 years with mean body weight 66.33 kg.

Each patient was treated in the Intensive Care Unit due to brain damage caused by brain injury or by cerebral haemorrhage, and only in a few cases was the brain damage secondary in origin due to primary cardiac arrest. Patients were artificially ventilated and received circulatory support and parenteral and enteral nutrition. No one received steroids.

Each patient presenting clinical symptoms of brain death was considered as an organ donor. The irreversible brain damage was confirmed by special clinical examination and with angiography (no blood flow within the brain arteries).

After brain death was diagnosed and the individual's death was recognized by the Committee, organ samples of about 1 cm² were taken from each body (from the same part of the pylorus) after the other organs (kidneys, liver, heart) were harvested for transplantation.

Ethical issues

The study protocol was approved by the Ethics Committee of the Medical University of Białystok (R-I-002/345/2007), and written informed consent was obtained from each participant or from members of the patient's family.

The tissues were immediately fixed in Bouin's solution and routinely embedded in paraffin. The stomach sections were cut perpendicular to the mucosal surface and included the entire depth of the mucosa. Specimens were stained with haematoxylin and eosin (H+E) for general histological examination, and processed for immunohistochemistry for the detection of gastrin and somatostatin.

Light microscopic immunohistochemistry

Procedure. The paraffin blocks were cut into 4- μ m sections (3 sections from each subject) and attached to positively charged glass slides.

Immunohistochemistry was performed using an EnVision Plus-HRP Detection Kit (Dako; Glostrup, Denmark) [4]. Immunostaining was performed using the following protocol. Paraffin-embedded sections were deparaffinised and hydrated in alcohols. For antigen retrieval, the sections were subjected to pretreatment in a pressure chamber heating for 1 min at 21 psi (one pound force per square inch [1 psi] equates to 6.895 kPa, conversion factor provided by United Kingdom National Physical Laboratory) at 125°C using Target Retrieval Solution Citrate pH 6.0 S 2369 (Dako; Glostrup, Denmark). After being cooled to room temperature, sections were incubated with Peroxidase Blocking Reagent S 2001 (Dako; Glostrup, Denmark) for 10 minutes to block endogenous peroxidase activity.

The sections were incubated overnight at 4°C in a humidified chamber with the diluted polyclonal rabbit: anti-gastrin (1:800, A 0568 Dako; Glostrup, Denmark), and anti-somatostatin human antiserum (1:20 000; H-031-30 Phoenix Pharmaceutical Inc.), followed by incubation with secondary antibody (conjugated to horseradish peroxidase-labelled polymer). Bound antibodies were visualized by 1-min incubation with liquid 3,3'-diaminobenzidine substrate chromogen. The sections were finally counterstained in Vector QS haematoxylin, mounted, and

evaluated under light microscope. Appropriate washing with Wash Buffer S 3006 (Dako; Glostrup, Denmark) was performed between each step.

The specificity tests performed for the gastrin and somatostatin antibody included: negative control, where the antibodies were replaced by normal rabbit serum (Vector Laboratories; Burlingame, CA) at the respective dilution, and positive control was done for specific tissue recommended by producer.

Quantitative analysis

Immunopositive cells were counted in 5 visual fields in one section, each field being 0.785 mm², at a magnification of 200× (20× the lens and 10× the eyepiece). Three specimens in each of the 19 subjects were analysed.

The analysis of the preparations and their photographic documentation were performed with an Olympus B×50 light microscope with a video circuit and a Pentium 120 PC computer with NIS Elements AR 3.10 NIKON software for microscope image analysis.

The cell count was expressed as the mean number of G and D cells per visual field.

Statistical analysis

All the presented data were statistically analysed by means of the software package Statistica Version 7.0. Descriptive statistics (mean, SD) were calculated for age, weight, and gastrin-positive and somatostatin-positive cell numbers. Results were expressed as mean ± SD.

RESULTS

Light microscopic immunohistochemistry

Immunoreactive endocrine cells for gastrin and somatostatin were identified in the pylorus of the healthy human in this study.

There was no immunoreactivity when the primary antibodies were omitted from the staining procedure.

Pyloric endocrine cells were characterised on the basis of cytoplasmic staining. All the immunoreactive (IR) cells showed a dark brown colour, and in the cell body and cytoplasmic processes, the secretory granules were seen. The shape of IR endocrine cells was distinctive and variform, and quite different from the epithelial cells. The nuclei were located in the middle part of cell body, and showed a spherical shape.

Gastrin and somatostatin cells were distributed between columnar cells of gastric epithelium.

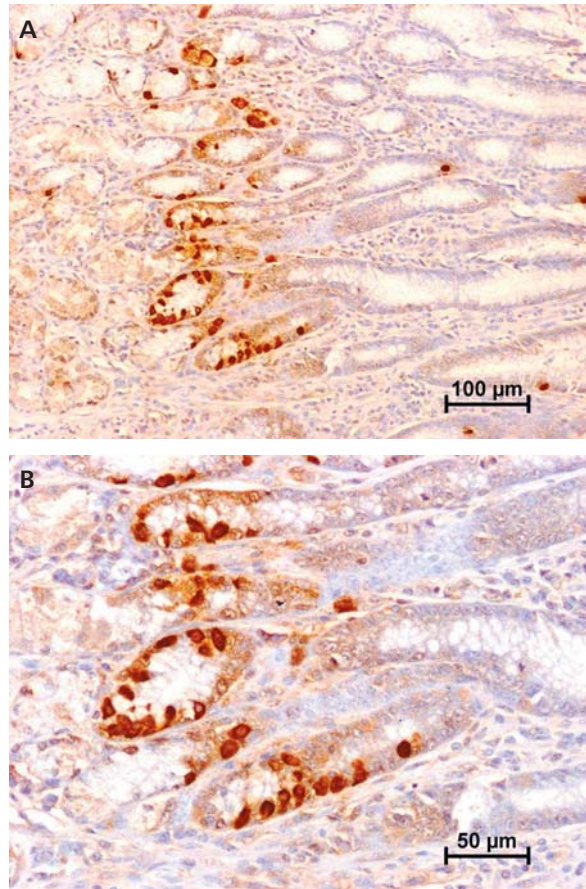


Figure 1A, B. Photomicrographs illustrating the distribution of cells immunoreactive for gastrin in the human stomach (glands of the pyloric mucosa).

The gastrin antiserum visualised a large number of endocrine cells in the pyloric mucosa (mean value 83.6 cells per visual field). Generally, they occurred in the middle third of the mucosa with few in the basal or upper part. Some of the cells were rounded; others were elongated or irregular in shape. The most of the gastrin positive cells appeared as closed type, without a lumen contact, but some of the cells seemed to reach the lumen. Staining intensity of gastrin was strong, but in some gastrin positive cells it was moderate (Fig. 1).

The D cells were identified by means of an antiserum to somatostatin. The somatostatin-immunoreactive cells usually had long slender processes and were less numerous (mean value 31.8 cells per visual field) than gastrin-immunoreactive cells; they were exclusively dispersed in the basal half of the mucosa although scattered cells occurred higher up in the mucosa. Somatostatin-positive cells distributed between gastrointestinal columnar epithelium mainly displayed strong staining of somatosta-

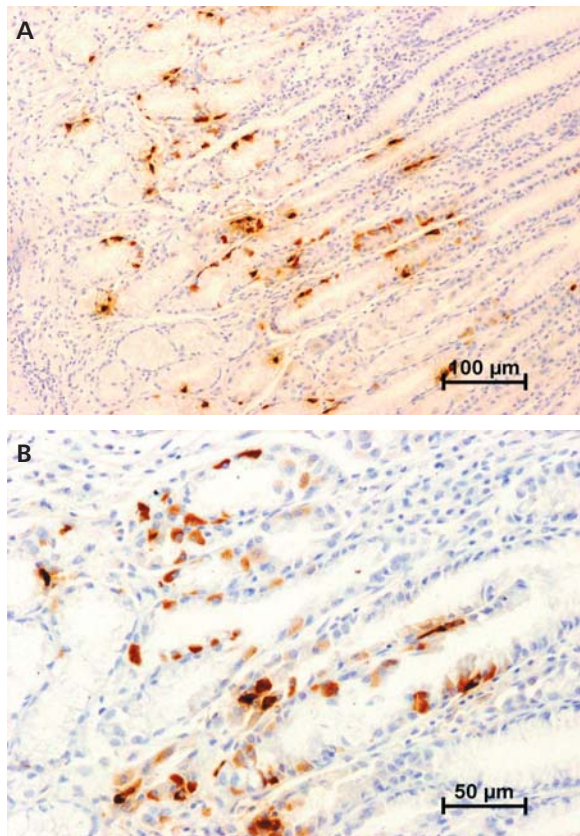


Figure 2A, B. Somatostatin-immunoreactive cells in the human stomach (glands of the pyloric mucosa).

tin. The shape of the cells was irregular, pyramidal, round, or elongated. The apical cytoplasmic processes extended to the stomach lumen, and basal processes extended to the basement membrane or to the neighbouring epithelial cells (Fig. 2).

DISCUSSION

It is well known that regulation of the motility, secretion, and absorption of the alimentary tract is coordinated by neural and hormonal controls [9, 18]. Gastrin is the first peptide to be described and has been localized to specific endocrine-type cells called G cells [22]. Some hormones and neurotransmitters stimulate the release of gastrin, while others, such as somatostatin, inhibit release [27]. Since gastrin was identified, many studies about it and other endocrine-like type cells have been published. Most studies were physiological studies using animals [22, 24, 25]. However, a few focused on the expression of endocrine cells in human gastric mucosa [12, 23, 24, 28].

In the present study, we demonstrated the occurrence and distribution patterns of gastrin- and somatostatin-immunoreactive cells in human pyloric.

Recently, Liu et al. [12] demonstrated gastrin and somatostatin immunostaining in the gastric antral biopsies from patients with dyspeptic complaints. Our findings agree with the distribution patterns reported in these results, but we detected larger number of G and D cells in the pyloric part of the human stomach. These differences might be due to different antisera, methods, and species used in each study. But until now, no report has shown the numbers of endocrine cells in healthy human gastrointestinal tract in such large samples.

The distribution of G and D cells within the human pyloric mucosa and their shape generally agree with the results reported by us in rat stomach [8]. The number of gastrin- and somatostatin-containing cells in the pyloric part of the rat stomach was lower than in the human stomach [8].

The distribution, density, and location of various endocrine cells have some relation to their function. The major endocrine function of gastric pyloric antrum is the secretion of gastrin. Gastrin is produced by G cells in the antral and duodenal mucosa, but the main source of gastrin is the antral G cells. The main inhibitor of antral G-cells is intraluminal acid, acting via release of somatostatin from antral D-cells [16, 26]. These cells are functionally and anatomically closely connected to the G cells.

In this study, we found that the numerous somatostatin-positive cells have long, immunoreactive basal processes.

The current hypothesis suggests that the release of somatostatin from multiple sites along the process allows a single cell to affect several neighbouring cells at the same time.

D-cells have been shown in close contact with neighbouring antral G-cells by long cytoplasmic processes, suggesting the regulatory potency on gastrin-containing cell function [1]. Somatostatin inhibits gastrin secretion and also decreases gastrin mRNA levels by affecting both gastrin gene transcription and mRNA stability [11]. Much data support the hypothesis that somatostatin represents a physiological paracrine regulator of gastrin cell function.

Neuroendocrine cells maintain balance in the epithelium of the gastrointestinal tract, reacting to mechanical events and chemical changes that occur in their direct environment [14]. Normally, gastrin release is suppressed when the luminal antral pH falls below 3. In addition, there is an inhibitory control exerted on gastrin release by cholecystokinin. The inhibition of gastrin release exerted by both gastric acid and cholecystokinin is mediated mainly via the release of somatostatin by D cells within the antral mucosa [12]. An

increase in pH, intake of food, increase of pressure in the stomach, or excitation introduced by the vagus nerve or mucosa nerve plexus could make G cells secrete more gastrin. A decrease in pH stimulated by somatostatin or other gastrointestinal hormones, and excitation of the sympathetic nerve, could inhibit the secretion of gastrin [21, 27, 28].

Gastrin has a unique regional regulatory effect on the secretion of somatostatin in the stomach [21].

Somatostatin widely exists in the gastrointestinal tract, with its highest concentration in the gastric pylorus area. There are two major molecular forms of somatostatin: somatostatin 14 (1.6 K) and somatostatin 28 (3.5 K), both being present in the gastrointestinal tract [25].

In the stomach, somatostatin cells are closely coupled to their target cells (e.g. parietal, ECL, and gastrin cells) either directly via cytoplasmic processes or indirectly via the local circulation [19].

Gastrin G and somatostatin D cells are endocrine cells closely related to the function of the gastrointestinal system. Several studies have now demonstrated both D cell mass and somatostatin secretion were markedly reduced in *Helicobacter pylori* infected patients, whereas the density of G cells and gastrin levels were elevated [12, 13]. These data suggest an intricate balance among immune factors and stomach neuropeptides, including somatostatin and gastrin, in the control of gastrointestinal function.

Alterations in relative numbers of G and D cells and their secretion often affects the normal functions of the digestive tract, even causing clinical symptoms or syndromes.

Circulating native somatostatin has a short half-life. Long-acting analogues like the decapeptide octreotide have been developed and somatostatin analogues have been used to treat intractable diarrhoea, bleeding from oesophageal varices in portal hypertension, dumping syndrome, and gastrointestinal fistulae [10]. Numerous clinical disorders, in addition to neuroendocrine tumours and variceal bleeding, have been treated with long-acting somatostatin analogues, although in many instances there is a lack of prospective, randomized controlled trials [13]. Several lines of evidence have suggested a possible therapeutic benefit from long-acting somatostatin analogues in the treatment of inoperable hepatocellular carcinoma [15]. There has been long-standing interest in the possible therapeutic use of somatostatin or its analogues in the management of obesity and one of its major complications, type 2 diabetes mellitus [2]. The results of these clinical trials should stimulate further studies.

In order to obtain a better understanding of the functional role of gastrin and somatostatin, we investigated their immunocytochemical identification, localisation, and number in the pyloric part of the human stomach. Hopefully, the knowledge, acquired throughout the reported study, may lead to an identification of other, still unknown, roles of gastrin and somatostatin in physiological and pathological conditions.

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