Gastric Neuroendocrine Cells and Secretory Products

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INTRODUCTION

Gastric neuroendocrine cells constitute about one percent of the volume of the oxyntic mucosa. Ultrastructural studies have identified at least six distinct cell types in the endocrine cell population (Table 1). Enterochromaffin-like (ECL)\textsuperscript{b} cells are the most numerous, constituting about 30 percent of the endocrine cell population, followed by P-cells and D-cells (somatostatin cells) 24 percent and 22 percent respectively (Table 2). The endocrine cells are mainly located in the middle and lower third of the mucosal thickness. The vast majority of these cells are randomly interspersed between the parietal and chief cells lining the oxyntic glands [1-3]. Occasionally endocrine cells can also be identified between mucosal neck cells in the superficial third of the mucosa as well as in association with nerve fibers and Schwann cells in the lamina propria. Even though these various cell types have a random spatial distribution, it is of interest that the ECL-cells are preferentially located adjacent to the chief cells and that their cell density within the mucosa normally shows little regional variation [3, 4].

Available evidence supports the origin of the gastric endocrine cells from the same stem cells, located in the upper neck region (the “renewal” zone) of the gastric gland from which superficial/foviolar, mucous-neck, chief, parietal and pyloric/cardial gland cells take origin [5]. In the pyloric-type mucosa, in humans, gastrin (G-cells) is usually concentrated in the deep mucous-neck and upper mucous-gland zone just below the renewal zone of the glands. The G-cells of the pyloric mucosa have been found to be renewed through replication of other gastrin cells as well as from undifferentiated progenitor cells of the renewal zone and then migrate downwards together with mucous-gland cells [6, 7]. Mature ECL-cells have recently been shown not to divide (N. Wright, personal communication), therefore renewal should take place from the progenitor cells of the upper neck region. Gastrin, argyrophil, argentaffin and mucous cells have been found to be intimately admixed in endocrine-exocrine pyloric adenocarcinomas [8, 9]. Moreover, “amphicrine” muc-o-endocrine cells may occur in gastric microcarcinoidosis and adeno-carcinoma [10].

During human fetal development endocrine cells first appear in the immature, glycogen-rich gastric epithelium (at 9 to 10 weeks) as small proto-endocrine cells with scattered ribosomes, reduced glycogen content, abundant mitochondria, microfilaments and a few small round secretory granules resembling those of P-cells. By the 10th week, fairly differentiated EC-, D- and GLI-cells have been observed by immunocytochemistry and ultrastructural studies. Pyloric G-cells and oxyntic ECL-cells appear later around the 14th week. Besides typical vesicular granules with the coarsely granular content, ECL-cells display dense solid, elongated granules resembling those of EC-cells or small round homogenous granules resembling those of P- or D1-cells [11].

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\textsuperscript{b}Abbreviations: ECL, enterochromaffin like; NSE, neuron specific enolase; HDC, histidine decarboxylase; CgA, chromogranin A.
Table 1. Neuroendocrine cells of the gastric mucosa.

| Cell types                  | Percent of endocrine cell population mean ± SD |
|-----------------------------|-----------------------------------------------|
| (human oxyntic mucosa)      |                                               |
| ECL-cells                   | 30 ± 9                                        |
| P-cells                     | 24 ± 7                                        |
| D (somatostatin) cells      | 22 ± 4                                        |
| D₁ cells                    | 9 ± 8                                         |
| EC-cells                    | 7 ± 5                                         |
| X cells                     | < 1                                           |
| Non-granulated cells        | 8 ± 4                                         |

D'Adda et al. 1989

Table 2. Neuroendocrine cells of the gastric mucosa.

| Cell types                  | Percent of endocrine cell population         |
|-----------------------------|----------------------------------------------|
| (human pyloric gland area)  |                                               |
| G-cells                     | 40-60                                        |
| EC-cells                    | 20-40                                        |
| D-cells                     | 10-20                                        |
| P-cells                     | 1-5                                          |

Socia et al. 1989.

Neuroendocrine cells of the gastric mucosa are identified by the argyrophil staining technique of Grimelius, which identifies all oxyntic endocrine cells except for the D-cells. The Sevier-Munger argyrophil technique selectively stains the ECL-cells and a small subset of the EC- and D₁-cells [12]. All the endocrine cells of the gut mucosa can be stained by immunohistochemical technique using such markers of neuroendocrine differentiation as chromogranin A, neuron specific enolase (NSE) and synaptophysin. Immunohistochemical techniques using antibodies against secretory products of endocrine cells have now been accepted and replaced many of the old histochemical techniques. However, this technique is applicable only when the secretory products of a particularly cell type is known and present in detectable amounts. One of the secretory products of the ECL-cell, histamine, is displaying such a problem where immunohistochemical visualization of ECL-cells requires special procedures such as freeze-drying, vapor-fixation and cryostat sectioning [12].

ECL-CELLS

In humans, the ECL-cells are normally confined to the oxyntic mucosa of the stomach where they constitute its predominant endocrine cell type [1, 13]. Scattered randomly in the lower and intermediate third of the mucosal gland, these cells are preferentially associated with chief cells. The ECL-cells are essentially identified with chromogranin A immunohistochemical staining together with the Sevier-Munger argyrophil technique. At the ultrastructural level the ECL-cells show typical secretory granules showing electron dense granules and also electron-lucent vesicles [14]. One of the most significant biological characteristics of the ECL-cells is their sensitivity to the trophic influence of gastrin. These cells, therefore frequently undergo hyperplasia in chronically hypergastrinemic states [14].
Table 3. Secretory products from neuroendocrine gastric cells.

| Cells  | Products                                      |
|--------|-----------------------------------------------|
| ECL    | Chromogranin A (splice products)              |
|        | Chromogranin B                               |
|        | Synaptophysin                                 |
|        | Histamin                                      |
|        | Histidine decarboxylase (HDC)                 |
|        | Calbindin (Gastrocalcin)                      |
| G-cells| Gastrin                                       |
| D-cells| Somatostatin                                  |
| Other  | PP                                            |
|        | Serotonin                                     |
|        | Calcitonin                                    |
|        | HCG-α subunits                                |

SECRETORIAL PRODUCTS FROM NEUROENDOCRINE GASTRIC CELLS

The different secretory products from neuroendocrine gastric cells that have been identified today are listed in Table 3. The main secretory products of the ECL-cells are chromogranin A and histamine but also chromogranin B, synaptophysin, histidine decarboxylase (HDC) and serotonin [15, 16] can be identified. It has also been claimed that the ECL-cells can produce calbindin, the 28KD calcium binding protein [17, 18]. Another agent that is involved in calcium homeostasis, called gastrocalcin has been reported by Håkanson et al. [19]; however, it has not yet been biochemically characterized. Finally, the ECL-cells are also able to produce the alpha subunits of human choronic gonadotrophin, particularly in situations with hyperplasia or malignant transformation. The other cell types, G-cells, are producing gastrin and the D-cells, somatostatin. Other known secretorial products from the gastric endocrine cells are pancreatic polypeptide, serotonin, PYY and calcitonin.

CHROMOGRANINS/SECRETOGANINS

These proteins, which are acidic polypeptides, found ubiquitously in the soluble matrix of dense-core secretory granules in neurons and endocrine cells. The so-called “granins” include chromogranin A and B and secretogranin II or chromogranin C. The granins have been proposed to play multiple roles in the secretory process. Intracellularly, granins play a role in targeting peptide hormones and neurotransmitters to granules of the regulated pathway by virtue of their ability to aggregate in the low-pH, high calcium environment of the trans-Golgi network [20-23]. One of the most important secretory products from the ECL-cells is chromogranin A (CgA). Although CgA was isolated already in the end of 1960s, the biological function of this glycoproteins is not yet established. In Table 4, some of the biological functions are listed. Some conserved features of the mature CgA protein are polyglutamic acids, calcium-binding sites and several pairs of basic amino acids (Figure 1). The first two features are important for its intracellular function, and the latter characteristics suggested that peptides could be released from the molecule by precursor processing enzymes. Several biological active peptides are encoded within this chromogranin A molecule (Figure 1) such as vasostatins (CgA1-76, CgA1-113), pancreastatin
Table 4. Chromogranin A.

General biological properties:

I. Regulation of secretory granule function:
   a. Binding of calcium
   b. Osmotic regulation
II. Participation in the secretory process
III. Precursor of biologically active peptides
IV. Antibacterial effects

(CgA_{248-301}) and parastatin (CgA_{347-419}). Putative receptors for CgA and its fragments are believed to be G-protein-coupled receptors, which are pertussis toxin sensitive [24].

Biological effects of unprocessed CgA include stimulation of brain micro-glial cells and enhanced NO-synthetase activity and antibacterial effects [25]. Pancreastatin is known to inhibit insulin release [26] and ^14^C- amino-purine uptake (histamine stimulated) in parietal cells [27] and has also been described to abrogate the growth of normal pancreas and pancreatic cancer [28]. Parastatin has been reported to inhibit PTH secretion [24, 29, 30].

One of the major sources for circulating CgA is the ECL cells in the oxyntic mucosa (nmol concentration) [31]. It has also been demonstrated that CgA is secreted from most neuro-endocrine tumors and act as an important tumor marker [32, 33]. However, in patients with gastrin-producing tumors, which are stimulating the ECL cells of the oxyntic mucosa, the main source of chromogranin A is not the gastrinoma but the ECL-cells [31]. In a recent study from Borch and co-workers [34], plasma chromogranin A correlated to endocrine cell density in the fundic mucosa and also to serum gastrin concentrations in patients with chronic atrophic gastritis type A. Furthermore, chromogranin A is increased in all patients with gastric carcinoids irrespectively of type, but the type III (non A-CAG) gastric carcinoid presents significantly higher levels of chromogranin A than type I and type II gastric carcinoids, both of them related to gastrin hypersecretion (to be published).

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**Figure 1. Biochemical structure of the chromogranin A molecule.** Arrows denote dibasic cleavage sites. A1(A9) indicate polyclonal antibodies directed against different portions of the molecule. CST, chromostatin; PANCST, pancreastatin; PARAST, parastatin.
It has been speculated over the years whether chromogranin A is circulating as an intact molecule or if it is spliced at dibasic cleavage sites. We have recently generated nine specific antibodies to various regions of the chromogranin A molecule (Figure 1), and we could demonstrate that a significant splicing of the molecule is occurring. Most frequently was a fragment elevated detected by the antibody A3, which is directed to the C-terminal part of vasostatin 1-113. This fragment is also increasing during tumor progression and decreasing during successful treatment (to be published). We have previously reported that chromogranin A is an independent marker of prognosis for midgut carcinoid patients in a multivariate analysis [35]. Therefore, the observation of increasing circulating levels of the vasostatin 1-113 in patients with progressive disease could indicate a growth promoting activity by this splice product. The vasostatins (CgA1-76, CgA1-113) have suppressive effect on vasoconstrictor responses in human blood vessels [36], and a binding protein has been identified in membranes of culture calf aorta cells. Furthermore, the vasostatin has also been reported to mediate autocrine inhibition of parathyroid hormone release and a binding protein of similar molecular size to that in the calf aorta cells has been characterized in isolated bovine parathyroid cells [24]. Recently, Marchisio and co-workers have shown that vasostatin 1-76 is stimulating cell adhesion through interaction with the integrins [37].

SUMMARY

The ECL cell is the most common cell type in the oxyntic mucosa of the stomach. It is producing a number of peptides and amines where histamine and chromogranin A seems to be the most important and abundant products. Recent data indicate a direct correlation between ECL-cell mass and circulating chromogranin A levels. Chromogranin A and its splice products might serve as growth promoting agents in ECL-cell hyperplasia or gastric carcinoids.

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