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The Endocrine Cells in the Human Pyloric Antrum. An Electron Microscope Study of Biopsy Materials*

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Received July 8, 1970

The pyloric antrum has been long known as the site of gastrin secretion but the cellular source of this hormone had not been considered until recently. Some authors have postulated that this hormone is secreted from a modified type of nerve cell in Meissner's plexus of the pyloric antrum (BAUGH et al., 1958; WOODWARD et al., 1963). In their light and electron microscopic studies, Italian pathologist SOLCIA and his co-workers proposed a special type of basal-granulated cell in the antropyloric mucosa as the source of gastrin. These cells which they called G cells were argyrophil but not argentaffin and consisted of a rounded cell body and microvilli-covered apex extending to the antropyloric lumen (SOLCIA, VASSALLO and SAMPIETRO, 1967; CAPELLA, SOLCIA and VASSALLO, 1969; SOLCIA, VASSALLO and CAPELLA, 1969; VASSALLO, SOLCIA and CAPELLA, 1969). Similar findings were given by Swiss researchers (FORSSMANN and ORCI, 1969). An important difference in opinion between the two research groups consists in that the Italian group identified the G cell with the pancreatic D cell which they regarded also as the source of gastrin, whereas the Swiss group acknowledged both cells as distinctly different types. Pancreatogenic tumors in the Zollinger-Ellison syndrome have been studied by some authors with the light or electron microscope. Here again we find a dispute as to whether the tumor cells correspond to the pancreatic D or to the pyloric G or to both cells (CREUTZFELDT, CREUTZFELDT and PERINGS, 1969; see also the review by FUJITA, 1968). The problem is complicated by the fact that the tumors previously related to the Zollinger-Ellison syndrome are now known to include different types, i.e., at least gastrin producing and secretin producing types (see the review by KRAFT, TOMPKINS and ZOLLINGER, 1970).

Besides the G cell and the enterochromaffin cell which is known to be widely distributed in the gastrointestinal mucosa and to be the source of serotonin, some authors identified one or more additional types of basal-granulated cells in the antropyloric mucosa under the electron microscope (ORCI et al., 1968; CAPELLA, SOLCIA and VASSALLO, 1969; VASSALLO, SOLCIA and CAPELLA, 1969). This finding seems important and deserves further investigation because it implies possible secretion of further unknown hormones from the pyloric antrum.

The light and electron microscopic studies on the G cells and other basal-granulated cells have been performed mainly in laboratory animals and there are only a few papers which include descriptions of the human pyloric antrum (SOLCIA and SAMPIETRO, 1965; SOLCIA, VASSALLO and SAMPIETRO, 1967; SOLCIA, VASSALLO and

*The study was supported by a research grant (No. 48412) from the Ministry of Education of Japan.

CAPELLA, 1969). As knowledge of the human gastrointestinal hormones and their sources is believed to be indispensable for the pathophysiologic interpretations of clinical cases of gastrointestinal disorders, a series of electron microscope studies on the endocrine elements of the human digestive tract is now being carried out in our research group.

Following our recent report on the occurrence of multiple cell types in the duodenal mucosa taken from surgical operations, this paper reports the findings in the biopsy tissue of the pyloric antrum.

Materials and Methods

Patients and their diagnoses are shown in Table 1.

Table 1

Patient	Year	Sex	Endoscopic diagnosis
1	51	♀	Gastric polyp at antrum and atrophic gastritis
2	61	♂	Gastric ulcer at angle and atrophic gastritis
3	58	♂	Gastric ulcer at angle, atrophic gastritis and duodenal ulcer
4	51	♂	atrophic gastritis
5	37	♂	atrophic gastritis
6	48	♂	atrophic gastritis
7	24	♂	atrophic gastritis

After surveying the condition of the gastric mucosa, small pieces, two from each patient and not exceeding $1.0 \times 1.0 \times 2.0$ mm, were obtained from the antral mucosa of the lesser curvature under direct vision using a fiber-gastroscope FGS-BL (Machida Co.). As soon as possible, the biopsy materials were fixed in 2.5% glutaraldehyde buffered to pH 7.0–7.2 with phosphate. Each biopsy block was then cut into four smaller blocks, placed in a fresh quantity of the same fixative for about 2 hrs, and subsequently transferred through several rinses in phosphate buffer containing 10% sucrose. The specimens were then postfixed in a 1.0% OsO_4 for about 4 hrs, dehydrated in a graded series of ethanol, and embedded in Luft's Epon. By light microscopy of thick sections stained in toluidine blue specimens with apparent pathological changes and intense metaplasia were eliminated. Ultrathin sections were cut on a Porter-Blum microtome equipped with a glass knife. The sections were stained with both uranyl acetate and Millonig's lead and examined in a Hitachi HU 125ds electron microscope at 75 kV.

Observations

General Aspect of Endocrine Cells in the Pyloric Antrum

The cells of basal-granulated type were found in the deeper half of the mucosa. They were either barrel-like or pyramidal cells intercalated between the exocrine cells of pyloric glands.

In every endocrine cell type the basal part of the cell directly faced the basement membrane of the epithelium while the apical, narrowed cytoplasm reached the antral lumen. The apical end of the cell was covered by more or less numerous microvilli. Junctional complexes occurred on the lateral surface of the cell immediately adjacent

Fig. 1. A G-cell intercalated between the excretory cells of pyloric gland. The basal granules consisting of a round sac and a loose and irregular core are characteristic of this cell type. $\times 25,000$

Labels common to all figures: L lumen, j junctional complex, c centriole, g Golgi complex, m mitochondria, r granular endoplasmic reticulum, f cytoplasmic filaments, ly lysosomes, bm basement membrane.

to the lumen. Desmosomes occurred here and there on their boundary against adjacent cells.

The nucleus of the cell was generally oval in shape and shifted more or less conspicuously towards the base of the cell.

At least four different cell types—EC cell, G cell, third type cell and D-like cell, as they will be tentatively called in this paper—could be identified in this study. These three cell types occurred constantly in all the antra examined. We obtained some electron micrographs suggesting the occurrence, though in a small number, of a few other cell types, but our findings on these cells are still insufficient.

Features of Individual Cell Types

1. Enterochromaffin cell (EC cell)

Cells of this type pyramidal in shape were found intercalated in the basal portion of the pyloric gland but less frequently than in the human duodenum.

As is well known (TONER, 1964; CARVALHEIRA, WELSCH and PEARSE, 1968; FORSSMANN et al., 1969; CAPELLA, SOLCIA and VASSALLO, 1969; VASSALLO, SOLCIA and CAPELLA, 1969), the EC cell is identified by its relatively slender pyramidal form and by its electron dense granules of characteristically distorted shapes. As a detailed description of this cell type was given in our previous report on the duodenum (KOBAYASHI, FUJITA and SASAGAWA, 1970), it will be not repeated here.

Occasionally was found a cell with roundly vacuolated granules whose core was similar to the granule of the EC cell.

2. G cell

Rounded cells with characteristically vacuolated specific granules with a vague core were found very frequently in the deeper portion of the pyloric glands. This type of cell corresponded to that described by SOLCIA, VASSALLO and CAPELLA (1969) and by VASSALLO, SOLCIA and CAPELLA (1969) in the pyloric mucosa of man and animals under the name of "G cell."

The granules were numerous and filled the infranuclear and paranuclear regions of the cell. The membrane sac of the granules was smoothly round and measured about 200 m μ in diameter. The dark core of the granule generally appeared cloud-like with vague and irregular contour and was surrounded by a wide clear zone. In some granules the core substance was partly attached to the inner side of the sac, which in this case appeared dark and thick. In some granules of a smaller size the core substance, dark and homogeneous, filled the granule sac more or less completely and the clear halo disappeared. In a few G cells the granules appeared not as such but rather as vacuoles, leaving few core substances within them. Also in such cells small round granules filled with dark homogeneous material remained here and there.

The G cell was relatively rich in mitochondria whose profiles, mostly oval but partly elongated into irregular forms, were scattered through the cytoplasm. Golgi complexes, not very large, were found in the supra or paranuclear region. Specific granules of a dense and small type were gathered around the Golgi apparatus whose vesicles partly contained a dark core. Thus, the elaboration of specific granules in the Golgi elements was likely.

Free ribosomes, mostly forming polysomes, were scattered in moderate amounts.

Small and flattened cisterns of rough-surfaced endoplasmic reticulum were dispersed throughout. A lamellar accumulation of them, not very large, often occurred in a supranuclear region. Centrioles frequently were encountered at the middle between the nucleus and the cell apex. A few microtubules ran in apparently random

Fig. 2. A G-cell showing basal granules with a clear content. Some dark granules of smaller size are mingled. $\times 15,000$

directions. Lysosomes with a relatively homogeneous material occurred in the supranuclear region.

The rounded cell was either tapered or abruptly constricted towards the apical end, which was covered with microvilli. Pinocytotic invagination and vesicles occurred at the base of the microvilli.

The infranuclear part of the G cell was conspicuously thick but generally only a part of its basal surface faced the basement membrane as a thin cytoplasm of adjacent exocrine cell was inserted beneath the cell base. Some specific granules closely approached the basal plasma membrane of the G cell but we could find no morphological evidence of granule release such as an emiocytotic figure.

Fig. 3 G cell granules in a closer view. A flocculent material and a clear space are surrounded by a membrane sac. A few small granules with dark content are scattered. $\times 37,000$

3. Endocrine cell of a third type

This was a much scarcer but second most frequently found cell type in the human antral mucosa. This designation is only for convenience of description and simply implies "the third type in the antrum." It does not necessarily define its identity with the third type cell of the duodenum previously shown by us (KOBAYASHI, FUJITA and SASAGAWA, 1970).

The cell was a slender pyramid in shape and characterized by specific granules

of a small size ($90\text{ m}\mu$ in diameter) and a high electron density. The membrane sac of the granule was closely attached to the dark and homogeneous granule substance.

In some cells the granule was slightly less electron dense in its core than in its periphery. Occasionally a few large vacuolated granules reminiscent of G cell granules were mingled.

Differing from the above described two types of cell, this third type cell was

Fig. 4. Two endocrine cells of a third type intercalated between the excretory cells of a pyloric gland. The cell in the middle is extended from the epithelial base to the lumen. $\times 10,000$

characterized by a spotted distribution of the granules. This was apparently caused by abundant cytoplasmic filaments, which mainly were gathered around the nucleus and partly extended to divide the cytoplasm into compartments for the granules.

Fig. 5. Basal portion of a third type cell. Notice the unevenly distributed granules of dense and homogeneous substance and abundant occurrence of a filamentous structure. $\times 37,000$

Mitochondria of oval and elongate profiles also tended to be grouped. A few small, flattened cisterns of rough-surfaced endoplasmic reticulum were dispersed. Free ribosomes occurred throughout the cytoplasm in a moderate amount. Golgi complexes of small sizes often were found in the supra or paranuclear cytoplasm. A centriole, if encountered, was at the middle between the nucleus and cell apex. Lysosomes of different sizes and structures occurred both in the supra and infra-nuclear cytoplasm.

The narrowed apical part of the cell opened to the lumen with a radiation of microvilli. The microvilli were covered by a floccular and filamentous surface mucous coat. The apical cytoplasm showed pinocytotic invaginations and vesicles as well as small tubular sacs which often were filled with a dark material.

4. Gastric D-like cell

This cell, generally a slender pyramid in shape, was characterized by basal granules not distinguishable in size and structure from the pancreatic D cells and

from the so-called intestinal D cells observed in our previous study in the human duodenum (KOBAYASHI, FUJITA and SASAGAWA, 1970). The occurrence of this type cells was, however, much less frequent in the pyloric antrum than in the duodenum. The granules were round and relatively large in size ($200\text{m}\mu$ in diameter). The membrane sac of the granule was distinct but closely attached to the granule

Fig 6. A gastric D-like cell showing basal granules of relatively low electron density. $\times 22,000$

substance leaving no clear space. The substance was finely granulated in texture and showed a relatively low electron density.

Fig. 7. Basal granules of a gastric D-like cell in a closer view. $\times 37,000$

Several lysosomes composed of a dark and coarsely granulated substance occurred in the supranuclear portion. Their size seemed to correspond to that of the specific granules. A few lysosomes contained a finely granulated substance not distinguishable from the material of the specific granule.

A Golgi area, not very conspicuous, occurred in the supranuclear portion. Small, probably young type of specific granules could be found in and around this area.

Mitochondria of oval and elongate shapes occurred all over but tended to be accumulated in the portion apical to the Golgi area.

Small and flattened cisterns of rough-surfaced endoplasmic reticulum were dispersed in the cytoplasm. Free ribosomes mostly forming polysomes were moderate in amount.

The apical end of the cell was slightly concave and provided with microvilli, not very numerous. They contained filamentous support and their surface was covered by a mucous surface coat. Tiny vesicles occurred in the apical cytoplasm suggesting pinocytotic activity in the cell surface.

Discussion

Cell types and their possible hormones

In 1965, SOLCIA and his group initiated publication of their studies on the possible cellular sources of gastrointestinal hormones (SOLCIA and SAMPIETRO, 1965). In this

light microscopic study they found toluidine blue-metachromatic and argyrophil cells concentrated in the pyloric mucosa of man and animals and proposed them as the source of gastrin under the name of G cells. On the basis of their stainability and of the electron microscopic findings of the cells (SOLCIA, VASSALLO and SAMPIETRO, 1967), they identified G cells with pancreatic D cells. Though they later acknowledged that the G cell granules differed in fine structure from D cell granules in the cat (VASSALLO, SOLCIA and CAPELLA, 1969), their theory on the identity of both cells was not revised by this finding.

FORSSMANN and his co-workers confirmed in the rat and cat that the G type cells occurred only in the part of the regions of the stomach where gastrin was known to be produced (FORSSMANN et al., 1969; FORSSMANN and ORCI, 1969). Their view is clearly divergent from that of the Italian group in that they regard the G cell as a cell type distinctly different from the pancreatic D cell.

The present paper first evidences a co-occurrence of G and D-like cells in the same region of the gastrointestinal tract. With FORSSMANN and ORCI (1969) we remark that the G cells correspond in their fine structure to at least a certain type of Zollinger-Ellison tumor cells (CREUTZFELDT, CREUTZFELDT and PERINGS, 1969). Taking into account the characteristic concentration of G cells in the deeper portion of the antropyloric mucosa where gastrin is also known to be concentrated (BROOMÉ, FYRÖ and OLBE, 1968) and their distributional and structural coincidence to the gastrin-containing cells shown in an immunofluorescence study by McGUIGAN (1968), we are convinced that the G cells indicated in the present study are the source of gastrin. As for the pancreatic D cell, on the other hand, we believe that its hormone should be investigated by abandoning the theory of its identity with the G cell.

The characteristic concentration of D-like cells in the duodenum (KOBAYASHI, FUJITA and SASAGAWA, 1970) and their sporadic occurrence in the pyloric antrum corresponds to the distribution of the hormone secretin. Although secretin has not been successfully extracted from the pancreas whose islets possess pancreatic D cells (FUJITA, unpublished), it is worthy of attention that certain types of Zollinger-Ellison tumors (non-beta pancreatogenic tumors) have been known to contain secretin instead of gastrin (KRAFT, TOMPKINS and ZOLLINGER, 1970).

The "X cells in human pyloric gland" shown in Figure 9 in the paper by SOLCIA, VASSALLO and CAPELLA (1969) may possibly correspond to our D-like cell.

Though the significance of the cells tentatively designated third type cells in this study is unknown, it is suggested that they may produce a third hormone. On the basis of the spotted distribution of specific granules of small size and round shape as well as of the abundant occurrence of cytoplasmic filaments, this cell may possibly be identical with the third type cell of the duodenum described in the previous paper (KOBAYASHI, FUJITA and SASAGAWA, 1970). Although in that paper it was suggested that the third type cell of the duodenum might possibly correspond to pancreatic A cells, a quantitative comparison of the size of cell granules later revealed that the pancreatic A cell is probably a different kind of cell. It seems worthy to notice that our third type cell resembles the "G cell" of the human pyloric mucosa shown in Figure 6 of the paper by SOLCIA, VASSALLO and CAPELLA (1969).

In our study on the fundic mucosa of the human stomach (KOBAYASHI, SASAGAWA and FUJITA, to be published), no G cells but considerably numerous third type cells

were recognized. The third type cells thus seem invalid as the source of gastrin.

2. Apical processes of endocrine cells

In the previous study on the human duodenum, we concluded that every cell of endocrine type is provided with an apical process whose end is covered by microvilli and directly faces the alimentary lumen. The present results in the pyloric antrum also coincide in this regard. Not only G cells whose microvilli-covered apex has previously been shown (SOLCIA, VASSALLO and SAMPIETRO, 1967; FORSSMANN and ORCI, 1969) but also EC cells, the third type and D-like cells open into the lumen with microvilli.

It is known that the secretion of gastrin from the pyloric antrum is inhibited by the lowered pH in the lumen and this inhibition is not mediated by a nervous activity (see the review by JORDAN and GARCIA-RINALDI, 1939). We propose a hypothesis that the microvilli-covered cell apex of the G cell may be involved in the perception of information, including the changes in pH, from the lumen. It may then be reasonable to suppose that the cell secretes the hormone gastrin from the cell base in response to the stimuli perceived at the cell apex.

A similar unicellular mechanism of negative feedback seems to account for the structure of the cell apex in other cell types (KOBAYASHI, FUJITA and SASAGAWA, 1970).

3. G cell and its granules

In the pyloric antrum of the cat, FORSSMANN and ORCI (1969) described a secretory cycle in "the gastrin-producing cell" which fully corresponds in fine structure to the G cell in the present study. After fasting for 24 hrs the cells were filled with granules with dense content whereas after refeeding the granules became empty sacs. As far as the more or less clear-looking granules of G cells shown in the present study are concerned, however, we are inclined to regard them not as the result of hormone secretion but as artifacts. The electron micrographs of the G cell granules give us an impression that the content of the G cell granules tend to dissolve during specimen preparation after fixation in glutaraldehyde. Further experiments are needed to settle this problem.

Summary

The mucosa of the pyloric antrum was taken by biopsy from seven patients under a direct inspection with a fiber-gastroscope and studied by electron microscopy. At least four types of basal-granulated cells were found in the deeper layer of the antral mucosa:

1. Enterochromaffin cells were fewer than in the human duodenum. A possible subtype with vacuolated granules was found.

2. G cells with rounded cell body and partly vacuolated basal granules were the most numerous and were considered to be the source of gastrin.

3. Endocrine cells of a third type were characterized by spotted distribution of basal granules of dense and small type and abundant cytoplasmic filaments.

4. Cells tentatively called gastric D-like cells because of their morphologic resemblance to the pancreatic D cells and duodenal D-like cells were found though

less frequently than in the duodenum. A possible secretion of secretin from D type cells was discussed.

5. In all the types of basal-granulated cells, a narrowed apical cytoplasm was extended to the epithelial surface and provided with microvilli projecting into the lumen. A hypothesis of unicellular feedback mechanism in the secretion of gastrin and other antral hormones was proposed on the basis of this fine structure of basal-granulated cells.

ヒトの幽門前庭の内分泌細胞. 生検組織の電子顕微鏡による観察 (内容自抄)

7人の患者から胃ファイバースコープによる直視下に採取した幽門前庭の粘膜を電子顕微鏡で観察した. 前庭粘膜の深部に少なくとも4型の基底顆粒細胞が見いだされた.

1. 腸クロム親性細胞は十二指腸におけるより少ない. その亜型と思われるもので, 空胞化した顆粒をもつ細胞が見いだされた.

2. G細胞は まるい胞体と部分的に空胞化した基底顆粒を特徴とするもので, 4型中もっとも多く出現し, ガストリン分泌細胞と考えられる.

3. 第3型と仮称される細胞には, 暗調の小型円形顆粒が斑紋状に分布し, 細胞質フィラメントが豊富に出現する.

4. “胃のD様細胞”と仮称される細胞は, その微細構造において膵臓のD細胞および十二指腸のD様細胞と区別ができない. その出現頻度は十二指腸よりずっと低い.

5. すべての型の基底顆粒細胞において, 細い細胞質突起が上方へ伸びて上皮表面に達し, 微絨毛をもって内腔へ突出している. ガストリンおよび他の前庭ホルモンの分泌における負のフィードバックの機構を, 細胞のこの微細構造から説明できると考えた.

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