Symposium on "Gastrointestinal Hormones"

(1) Source Cells and Secretion Mechanism of Gastro-Intestinal Hormones

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The basal-granulated cells dispersed in the mucosal epithelium of the digestive tract represent the sources of gastro-intestinal hormones. Evidence has accumulated for that the basal-granulated cells comprise, besides the long known enterochromaffine (EC) cells, several different types which correspond to different hormones.

This article will first introduce the types, structures and presumed functions of the basal-granulated cells which we have found in the human digestive tract and later will mention our experiments and hypotheses on the possible role of the cells in the occurrence of diarrhea such as in cholera.

1. Types and Distribution on the Basal-Granulated Cells

Since 1969 we examined, mainly by electron microscopy, the types and distribution of the basal-granulated cells in the mucosal tissues biopsied by Dr. Tsutomu Sasagawa (Niigata City Hospital) from different parts of the digestive tract in many male and female subjects. The results indicate the occurrence of at least 10 types of the basal-granulated cells which will be briefly introduced.

(1) EC cell: This is the predominant cell type in most parts of the digestive tract and is characterized by basal granules of very high electron density and uniquely tortuous profiles which are known to contain serotonin.

(2) EC-like cell: This cell which is numerous found in the gastric corpus contains small vesiculated granules with a dark, irregular core. Small and numerous mitochondria are also characteristic of this cell.

(3) G cell: This type is concentrated in the pyloric antrum and represents the source of gastrin. We recently found a few G cells in the duodenal bulb which probably correspond to the "extragastric gastrin". After ordinary glutaraldehyde fixation the G cell granules are mostly vacuolated but the duodenal G cells mainly contained small and solid granules.

(4) D cell: This cell type, which represents the third endocrine element in the pancreas, occurs widely in the gut, especially in the descending portion of the duo-

denum. It contains large, round granules (300-400 m μ in diameter) of relatively low electron density.

(5) D₁ cell: Solcia and his associates first proposed to differentiate D₁ cell from the D cell by its smaller granules. In the human duodenum we recently came to discern a cell type possessing small but conspicuously irregular-sized granules (200-350 m μ) of relatively low electron density. Bundles of cytoplasmic filaments separated the granules into groups. Although it is not clear enough as yet, as to whether this type fully corresponds to the D₁ type of Solcia's group, it may be clever at present to apply the name of D₁ to the cell in question and to avoid a new designation which may cause an unnecessary confusion.

(6) L cell: This cell is so called by its large granules (250-400 m μ) of round shape and high electron density. It is distributed widely in the stomach and intestine, being most numerous in the first portion of the duodenum.

(7) M cell: This cell was so named by us because of its medium sized granules (150-250 m μ) and its uniquely developed crown of microvilli. The granules are uniformly round and less dark than those of the L cell. It is numerous in the first portion of the duodenum.

(8) S cell: This cell is characterized by small granules (100-150 m μ) of round shape and low electron density and by abundant cytoplasmic filaments. It is frequent in the stomach and duodenum.

(9) A-like cell: Resembling the A cell in the pancreas, it contains large granules of a double structure consisting of a dark round core and a paler cortex. This cell is encountered in different parts of the small intestine.

(10) T cell: A small number of this cell, characterized by long (2 μ), tentacle-like microvilli and small round granules (150 m μ), is found in the duodenum.

As for the hormones produced by the ten cell types, only it is generally accepted that the EC cell produces serotonin and the G cell gastrin. Although the D cell once was regarded by many to be the source of gastrin, our observation of this cell after luminal acidification in man and dog (*vide infra*) has indicated that it may produce secretin or one of other antiacidic agents. Recent studies by the research group of Polak, Bussolati and Pearse using immunofluorescence method suggested that the L cell might produce enteroglucagon, S cell secretin and D₁ cell enterogastrone or GIP. These proposed cell-hormone relationships are not convincing enough because the identification of a fluorescing cell under a light microscope with a certain type in electron microscopy is often very difficult and unreliable.

2. General Structure and Function of the Basal-Granulated Cell

The basal-granulated cells, though divided into different types, have structural and functional characteristics common to them, which will be now discussed.

(1) Production and release of basal granules

The basal-granulated cells possess cytological features which suggest the pro-

duction of protein/polypeptide hormones: The rough-surfaced endoplasmic reticulum (rER) and Golgi complexes are well developed and there are gradations between small granules occurring in the Golgi vesicles and the mature secretory granules. Our radio-autographic study indicates that ^3H -leucine taken up as a material amino acid into the basal-granulated cell first appears in the rER, transported to the Golgi complex and then moves into the secretory granules.

That the mature granules of the basal-granulated cell are released from the cell base by emiocytotic mechanism, i.e., through an Ω -shaped opening of the granule sac, was first evidenced in our electron microscope study. By experimentally lowering the luminal pH value of the dog pyloric antrum with 0.1 N HCl, we succeeded to demonstrate emiocytosis in the D cells. Later we could show granule release in the G cells by elevating the luminal pH values with 3% NaHCO_3 , and that in the EC cells by giving hypertonic (50%) glucose.

These results first elucidated the morphological basis of the hormone release in the basal-granulated cells and, further, evidenced that every type of the basal-granulated cells has its adequate stimulus.

(2) Double production of polypeptide and amine

Recent advance in fluorescence histochemistry has evidenced that the basal-granulated cells contain, in their granules, more or less biogenic amines. The Falck-Hillarp method demonstrates the occurrence of large amounts of serotonin in the EC cells and smaller amounts of catecholamines in some other types. Murine EC-like cells are shown by *o*-phthalaldehyde method to contain histamine.

The ability of the basal-granulated cells uptaking the precursors of amines has recently been confirmed by our radio-autographic studies using tritium-labeled L-DOPA.

It is thus probable that every type of the basal-granulated cells is capable of doubly producing a polypeptide and (a) biogenic amine(s). The EC cell whose aminic hormone, serotonin, has exclusively attracted attention, is now believed to produce also a polypeptide hormone, whose identification will much serve the progress in gastro-enterology.

The aminic and polypeptide secretions are contained in the basal granules, presumably being bound with the complex protein forming the granule matrix. In granule release, these three elements are liberated simultaneously. Thereby, the amine possibly helps the diffusion and escape into circulation of the other elements. It is also supposed that the aminic and polypeptide secretions may exert their functions cumulatively.

(3) Basal-granulated cell as a receptor

A series of our electron microscope studies have elucidated that every basal-granulated cell extends a cytoplasmic process to the mucosal surface and that its end is covered with a tuft of microvilli. The cell is thus open to the lumen. In man only the cells in the gastric corpus are closed in type, i.e., do not reach the mucosal surface.

Fig. 1. Human endocrine cells which show emiocytotic granule release 5 min after acidification of the duodenal bulb (or pyloric antrum). At least these 5 cell types seem to be the sources of the anti-acidic hormones.

We have hypothesized that the open type cell perceives chemical or physico-chemical stimuli in the gastro-intestinal content and regulates, in accordance with that information, its own secretory activity. The occurrence of the marked microvilli and a single cilium, reminiscent of the apical structure of sensory epithelial cells, has supported the idea. This hypothesis of the receptosecretory nature of the basal-granulated cell is difficult to prove directly, but the prompt negative feedback in the gastro-intestinal hormone release through the chemical changes in the luminal content has been satisfactorily explained only by this hypothesis.

The basal-granulated cell can be thus called "taste cell in the gut", though we cannot feel tastes in the gut as the cell does not have nerve connection with the central nervous system as the lingual gustatory cell does.

The closed type cells in the stomach are presumed to be sensitive to some physical (mechanical and thermal) stimuli.

3. Granule Release in Human Basal-Granulated Cells

In some cases of fibroscope biopsy performed by Dr. T. Sasagawa (*vide supra*), either acidic (0.5% HCl) or alkaline solution (3% NaHCO₃) was infused into the portion examined, 5 minutes before biopsy. By applying this method to the pyloric antrum, D and S cells were found to release their granules after the luminal acidification, whereas the G cells showed this reaction after alkalinization.

In the duodenal bulb, it was recently revealed that after acidification of this portion, D, M and L cells released their basal granules by emiocytosis. It is not surprising that more than one cell type reacted to acidification, as, besides secretin,

Fig. 2. A diagram showing the action of cholera toxin causing intestinal hypersecretion (see text). At the bottom right an EC cell releasing its granules is illustrated.

enterogastone and cholecystokinin are known to be released by acid in the duodenum. It seems likely that also enteroglucagon, similar in structure to secretin, may be released under this condition. The above result implies that the D, M, L and S cells are the sources of the antiacidic hormones or the polypeptides of the secretin group. Some postulations from the fluorescence antibody studies on the possible hormones of these cell types were mentioned in the first chapter.

The significance of the G cell and gastrin in the pathogenesis of hyper- and hypo-acidity and related disorders has recently been much elucidated. One of our biopsy materials shows that the G cells are markedly increased in the pyloric antrum in a patient who has repeated peptic ulcer in the duodenum. On the other hand, the pathogenic roles of the anti-acidic hormones and their source cells are only poorly understood. The quantitative and qualitative changes of the cells concerned should be examined and evaluated in relations to the levels of the hormones in different gastro-intestinal disorders. The cells in the duodenal bulb, thereby, should be studied as the most important elements in the gastric acid inhibiting mechanism and its disorders.

4. Diarrhea and Gastro-Intestinal Hormones

It is well established that some pancreatogenic tumors producing secretin or enterogastrone (GIP) may provoke severe diarrhea (WDHA syndrome) and that gastro-intestinal hormones such as glucagon, secretin, GIP and gastrin administered in the dog either singly or combined may cause diarrhea.

We revealed that after 50% glucose into the dog duodenum severe degranulation by emiocytosis occurred in the EC cells. This experiment causes diarrhea in the dog and is known to mimic human dumping syndrome. That the formalin-induced fluorescent substance (serotonin) is released from the EC cells in this experimental condition has been demonstrated by Tobe and his associates.

Recently we came to hypothesize that even the diarrhea in cholera may be caused by gastro-intestinal hormones. It is now known that the diarrhea in cholera is due to hypersecreting intestinal mucosa, in which the level of cAMP is markedly elevated. Furthermore, it is known that the cholera enterotoxin evoking diarrhea is very poorly, if any, absorbed into circulation. Thus a working hypothesis can be proposed that the increased cAMP level and the consequent hypersecretion in the intestine are mediated by certain hormones released by the basal-granulated cells stimulated by the toxin.

By a series of experiment introducing crude cholera toxin into the duodenal loop of young rabbits, we found, under the electron microscope, that the EC cells underwent severe degranulation.

Serotonin is thus suspected to cause, as a local hormone, elevated cAMP and hypersecretion in the duodenal crypts and glands. Elevated permeability in the blood vessels in the tissue may accelerate the water release.

It is further postulated that the unknown polypeptide hormone, tentatively called polypeptide X, of the EC cell may also exert a diarrheagenic function.

We propose that both hypertonic glucose which causes severe diarrhea in the dog and cholera toxin which leads to intestinal hypersecretion may be mediated by the secretions from the EC cells. It is further hypothesized that diarrhea provoked by other toxins and allergens may possibly be mediated by the receptosecretory cells in the intestinal mucosa.

Summary

1. Electron microscope studies have revealed that the human gastro-intestinal epithelium contains at least 10 different types of basal-granulated cells as endocrine elements: EC, EC-like, G, D, D₁, L, M, S, T and A-like.

2. The basal granules of the endocrine cells have been demonstrated in man and animals to be released by emiocytosis at the cell base.

3. The basal-granulated cells are believed to be capable of double production of polypeptide and aminic secretions.

4. The basal-granulated cells generally are open to the intestinal lumen with a

tuft of microvilli which is believed to perceive luminal stimuli to regulate the secretory activity of the cell.

5. In human biopsy it was revealed that antral alkalinization caused granule release in the G cells, whereas acidification in the antrum or in the duodenal bulb that in the D, D₁, L, M and S cells. The significance of the duodenal bulb in the homeostatic control of the gastro-intestinal pH values was stressed.

6. Infusion of 50% glucose into the dog duodenum and that of crude cholera toxin into the rabbit duodenum lead to severe degranulation in the EC cells in the mucosal epithelium. The possibility was proposed that intestinal hypersecretion and diarrhea may be caused by serotonin and/or polypeptide hormones released from certain basal-granulated cells stimulated by diarrheagenic agents.

References

Related literature is included in the following papers and not listed here. The most recent articles of ours on the human duodenal bulb and on the experiment using cholera toxin are given below, too.

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