Basal-Granulated Cells in Human Brunner's Glands

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Summary. Basal-granulated cells (BGC) in the human duodenal bulb were observed by light and electron microscopy, and both the cell types and their population densities in the duodenal crypts and in the Brunner's glands were compared.

The number of the BGC in the Brunner's glands was much smaller than in the crypts. On the basis of their ultrastructural features, nine types of BGC, i.e. an EC cell, N cell, D cell, D1 cell, S cell, I cell, G cell, L cell and P cell were identified in the human duodenal bulb. In the duodenal crypts, as is generally recognized, EC cells were most numerous, making up 40% of the total BGC. N cells and D cells were around 10% of the total, and S cells, I cells and L cells were less than 10%, respectively. By contrast, in Brunner's glands, D cells and small granule-containing cells such as S cells, I cells and D1 cells were predominant, accounting for about 80% of the total BGC. EC cells and N cells were about 10% or less, respectively.

These results indicate that the Brunner's glands are definitely different from the ordinary intestinal mucosa in regard to their BGC population, and are considered to have endocrine functions mainly performed by D1 cells, S cells and I cells.

Since the late 1960s, basal-granulated cells (BGC) in the digestive tract have been extensively studied by electron microscopy. SOLCIA et al. (1978) and GRUBE and FORSSMANN (1979) have identified, from a morphological point of view, 14 or 15 different types of BGC, which include the EC cell, D cell, D1 cell, S cell, I cell, N cell, L cell and so on. In terms of the rate of occurrence of BGC, it has been recognized that EC cells are most numerous throughout the digestive tract from the duodenum to the colon (KOBAYASHI et al., 1970; OSAKA et al., 1973; SASAGAWA et al., 1973; CRISTINA et al., 1978). On the other hand, in Brunner's glands a few types of BGC such as EC cells, D cells, D1 cells, and G cells have been identified by light (FRENSEN and HOLZKI, 1968; RENDA et al., 1976) and electron microscopy (LEESON and LEESON, 1966; 1968; FORSSMANN et al., 1969; KOBAYASHI et al., 1970; CAPELLA et al., 1976) using human and non-human materials. However, these studies do not describe in detail the features of BGC in the Brunner's glands.

The present study was carried out in order to systematically examine the variety and the rate of occurrence of BGC in human Brunner's glands. At the same time, the duodenal crypts were also examined for comparison, as they were considered to be representative mucosa of the gut with respect to BGC. Comparing the results thus

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obtained in the Brunner's glands and duodenal crypts, the characteristics of the Brunner's glands in terms of BGC were demonstrated.

MATERIALS AND METHODS

Duodenal tissues were obtained during surgical operations on six patients (Table 1) suffering from gastric cancer (3 cases; one case was used for silver impregnation), gastric ulcer (1 case) and duodenal ulcer (2 cases). Small blocks of tissue were extirpated from the duodenal bulb which appeared macroscopically normal. For silver impregnation, specimens were fixed in Bouin's fluid, dehydrated through a graded ethanol series and embedded in paraffin. Sections 5 to 7 μm thick were stained either by modified Masson-Hamperl's argentaffin (Singh, 1964), by Grimelius' argyrophil (1968), or by Hellerström-Hellman's method (1960). For electron microscopy, the specimens were fixed immediately in a fixative containing 1.25% glutaraldehyde and 4% paraformaldehyde (Karnovsky, 1965) in 1/15 M Sörensen's phosphate buffer adjusted to pH 7.4 for 2 hrs at 4°C. They were post-fixed in a 1% osmium tetroxide solution adjusted to pH 7.4 by the same buffer for 1 hr, dehydrated through a graded ethanol series, and embedded in Epon 812. Thin sections were cut with a diamond knife on a Porter Blum MT II ultramicrotome. They were double-stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and observed in a JEM 100B electron microscope.

One hundred BGC were randomly selected from both the duodenal crypts and the Brunner's glands in five patients. Accordingly, a total of 1000 BGC (500 BGC for the Brunner's glands and 500 for the duodenal crypts) were examined. The BGC were classified mainly on the basis of the morphological characteristics of their basal granules. In cases where the granules were seemingly of the same dimension, the sizes of 100 granules of each type of BGC were measured—in an attempt to get an accurate size—on electron micrographs taken of the section on which latex particles of 109±2.7 nm in diameter (Dow Chemical Company) had been placed as a reference.

Table 1. Materials

| Case | Age | Sex | Clinical diagnosis | Surgical operation | Used for |
|------|-----|-----|--------------------|--------------------|---------|
| 1    | 44  | male| gastric ulcer      | subtotal gastrectomy | EM      |
| 2    | 79  | male| gastric cancer     | ditto              | EM      |
| 3    | 36  | male| duodenal ulcer     | ditto              | EM      |
| 4    | 49  | male| gastric cancer     | ditto              | EM      |
| 5    | 40  | male| duodenal ulcer     | ditto              | EM      |
| 6    | 65  | male| gastric cancer     | ditto              | SI      |

EM: Electron microscopy  SI: Silver impregnation
RESULTS

Light microscopic observations
BGC could be distinguished in thick Epon sections stained with toluidine blue due to its having a clearer cytoplasm than the adjacent epithelial cells. In the duodenal crypts, numerous BGC were found mainly in their deeper half. Most of them were pyramidal in shape. The granules of some BGC were visible with toluidine blue. Most of the BGC in the duodenal crypts had argentaffin as well as argyrophil characteristics as demonstrated by MASSON-HAMPERL’s or GRIMELIUS’ method, indicating that they were EC cells. The cells stained by HELLERSTRÖM-HELLMAN’s method, which were considered to be D cells, were less prominent in the duodenal crypts.

On the other hand, far fewer BGC were found in the Brunner’s glands than in the duodenal crypts. BGC mostly occurred in a group in the Brunner’s glands. The group of BGC consisted of argyrophil cells as demonstrated either by HELLERSTRÖM-HELLMAN’s or GRIMELIUS’ method (Fig. 1a, b). Only a few argentaffin cells could be found in the Brunner’s glands from the level of the mucosal muscle layer to the internal circular muscle layer (Fig. 1c). The granules of BGC in the Brunner’s glands were hardly visible with toluidine blue, probably due to their smaller dimensions or poor stainability. No BGC were found in the duct of the glands. These light microscopic findings indicated that EC cells were very few, while non-EC cells were predominant in the Brunner’s glands.

Fig. 1. a-c. Human Brunner’s glands as stained by three different silver impregnation methods to demonstrate the argentaffin and argyrophil cells. a. HELLERSTRÖM-HELLMAN’s method. Argyrophil cells (arrows) are found in groups. These cells are considered to be D cells. × 400 b. GRIMELIUS’ method. Argyrophil cells (arrows) are found grouping in some secretory portions of the glands. × 400 c. MASSON-HAMPERL’s method. There is only one argentaffin cell (arrow), suggesting that EC cells are very few in Brunner’s glands. × 400
Fig. 2. Electron micrograph showing a part of the Brunner's glands in the vicinity of the muscularis mucosae. This low-power electron micrograph shows groups of various types of BGC in the Brunner's glands. Demonstrated in this micrograph are: S, I, D, N, EC and G cells. Cells labeled with asterisks could not be classified conclusively. L lumen of the gland, m mucous secretory granules of exocrine cells. ×2,400
Electron microscopic observations

The BGC were classified on electron micrographs according to the nomenclatures proposed at the Lausanne Conference in 1977 (Socia et al., 1978) and to the review written by Grube and Forssmann (1979). In the present study nine different types of BGC could be identified in duodenal crypts and Brunner's glands: EC, G, D, N, L, S, D₁ and P cells (Fig. 2–9). Three types of BGC, i.e. EC, G and D cells were clearly distinguished due to the characteristic features of their granules. L and N cells could be distinguished by their containing large, spherical granules with high electron opacity. Small granule-containing cells such as I, S, D₁ and P cells were difficult to definitively classify from mere granule appearance. Accordingly, the range of the granule sizes was measured referring to the latex particles of 109±2.7 nm in diameter. The size of N cell granules was similarly measured in order to be compared with that of the I cell granules. The following are the morphological characteristics of each BGC dealt with in the present study.

1) EC cells, well-known BGC having characteristically large polymorphous granules with an extremely high electron opacity (Fig. 2).

2) G cells, characterized by large spherical granules with varying electron opacities, approximately 350 nm in diameter (Fig. 2).

3) D cells having large spherical and low electron opaque granules of about 400 nm in diameter (Fig. 3, 4). They have no halo beneath the limiting membrane.

4) L cells containing granules similar in size to, but with much more electron opacity than D cell granules. Some granules were irregular in outline like EC cell granules.

Fig. 3. Electron micrograph showing another part of the Brunner's glands at the level of the muscularis mucosae. This micrograph shows a group of BGC in which D cells are predominant. Cells identified in this micrograph are: D, S, D, and P cells. The asterisk indicates the cell that could not be definitively classified. L lumen of the gland, m mucous secretory granules of exocrine cells. ×2,400
Fig. 4. Electron micrograph showing a basal part of a D cell. The granules are about 400 nm in diameter and have a low electron opacity. $C$ connective tissue, $Nu$ nucleus. $\times 15,000$

Fig. 5. A basal part of an N cell. This cell is characterized by round, electron opaque granules measuring 300 nm in diameter. $Nu$ nucleus. $\times 15,000$
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5) N cells, which were characterized by round granules with a high electron opacity, measuring about 300 nm in diameter (Fig. 2, 5, 9d, 10d). 6) I cells containing granules, which appeared similar to, but were somewhat larger in size, and denser in electron opacity than S cell granules, measuring about 260 nm in diameter (Fig. 2, 6, 9c, 10c). 7) S cells, characterized by small spherical granules with a high electron opacity about 200 nm in diameter (Fig. 2, 3, 7, 9b, 10b). 8) D1 cells containing small spherical granules with a high or moderate electron opacity, the diameter of which was approximately 140 nm (Fig. 2, 3, 8, 9a, 10a). Abundant filaments were often found in the peri- or infra-nuclear cytoplasm. 9) P cells, having granules similar in electron opacity to, but somewhat smaller than D1 cell granules, measuring 100 nm in diameter (Fig. 3). The dimensions of N and I cell granules, I and S cell granules, S and D1 cell granules, and D1 and P cell granules were too close to be clearly distinguished from each other by mere observation. However, these granule dimensions were significantly different from each other, as described above (p<0.01), allowing for an adequate differentiation of these confusing BGC.

On the basis of the classification of BGC as done above, the rate of occurrence of BGC in duodenal crypts and Brunner's glands was compared in each case to examine the differences in endocrine functions between these two regions (Fig. 11).

Duodenal crypts

EC cells were most numerous, making up about 40% of all the BGC in the duodenal crypts. Thirteen percent were N cells, 12% D cells, 8% I cells, 7% L cells, 6% S cells, 2% D1 cells, while G cells were less than 1%. The remaining 11% included cells which could not be satisfactorily classified based on presently available morphological criteria.

Fig. 6. A basal part of an I cell. The granules of this cell are somewhat larger than those of S cells, measuring 260 nm or more in diameter. Nu nucleus. ×15,000
Fig. 7. A basal part of an S cell is magnified to show the detail of its granules. The granules are about 200 nm in diameter. Nu nucleus, m mucous secretory granules ×15,000

Fig. 8. A basal part of a D, cell. This cell contains small granules of about 140 nm in diameter. Filaments (f) are abundant in the perinuclear area of the cell. Nu nucleus. ×15,000
Fig. 9. a-d. Highly magnified electron micrographs for the comparison of the granule sizes of D₁ (a), S (b), I (c) and N cells (d). Latex particles (arrows) of 109±2.7nm in diameter were placed on thin sections as references slightly for measuring the granule size. D₁ cell granules are larger, and S cell granules are about twice as large as the latex particles. I cell granules are smaller than N cell granules; the former are about twice or three times as large as the latex particles, while the latter are more than three times as large as the particles. ×30,000
Thus, in the duodenal crypts, large granule-containing cells, such as EC and N cells were characteristically numerous accounting for about 55% of the total BGC in the crypts (Table 2). The finding that EC cells are most numerous in the duodenal crypts is compatible with the fact revealed by the silver impregnation method that argentaffin cells are numerous in duodenal crypts.

Brunner’s glands

D cells were most numerous, accounting for about 30% of all the BGC in the Brunner’s glands. Twenty percent were S cells, 16% D₁ cells, 13% I cells, 11% EC cells, 3% G cells, 2% N cells and 1% P cells. Unclassified cells made up about 5%. It should be

![Fig. 10. a-d. Four histograms showing the distributions of granule sizes of D₁ (a), S (b), I (c) and N cells (d). One hundred granules were measured for each kind of BGC. Some extremely deviating values of granule size were eliminated. The mean values of granule size are 141 nm for D₁ cells, 197 nm for S cells, 258 nm for I cells and 297 nm for N cells.](image)
noted that the Brunner's glands had a large number of D cells and small granule-containing cells such as S cells, I cells and D₁ cells, making up about 80% of the total BGC. Unlike the duodenal crypts, EC cells and N cells were very small in number, making up less than 20%. These electron microscopic findings were also parallel to those obtained by the silver impregnation method that argyrophil cells stained by HELLERSTRÖM-HELLMAN's method were prominent in the Brunner's glands (Fig. 1a, Table 2).

It was revealed that EC, N and D cells were most numerous in the duodenal crypts, occurring at a constant rate for the five cases (χ² < 0.05). In the Brunner's glands, though the density of BGC was less than in the duodenal crypts, D cells and small granule-containing cells such as I, S and D₁ cells were predominant; they also occurred at an almost constant rate for the five cases (χ² < 0.05). The rate of occurrence of these six different types of BGC significantly differed between the duodenal crypts and the Brunner's glands (Table 2).

**DISCUSSION**

The present study has clearly demonstrated that Brunner's glands are not
the mere continuations of duodenal crypts, but are characteristically different from ordinary gut mucosa with regard to BGC. In human Brunner's glands, up to four BGC such as EC, G, D and D1 cells have been noted by electron microscopy, but without quantitative evaluation (Leeson and Leeson, 1968; Kobayashi et al., 1970; Osaka et al., 1974; Capella et al., 1976). In the present study, it is demonstrated that there are at least eight types of BGC in the Brunner's glands, and that non-EC cells such as D, I, S and D1 cells are predominant. This result suggests that Brunner's glands have, besides exocrine functions, notable endocrine functions different from the duodenal mucosa.

Classification of BGC

There are limitations to the classification of BGC when performed from a merely morphological point of view. However, as noted below, it can be said that the nine types of BGC dealt with in the present study were accurately identifiable on the morphological criteria presently available. EC, D and G cells are easy to identify due to the characteristic structures of their secretory granules. Small granule-containing cells such as I, S, D1 and P cells are considered to be clearly distinguishable from one another by using latex particles to statistically measure their granule dimensions. The granular dimensions of the four BGC thus calculated are in accordance with those described in Lausanne (Solcia et al., 1978) and by Grube and Forssmann (1979). As to N cells and L cells, there has been a discrepancy in their granule size between Grube and Forssmann (1979) and Solcia et al., (1978). In the present study the former's opinion has been adopted, i.e. the L cell granules (400 nm) are larger than those (300 nm) of N cells.

It has been immunocytochemically established that EC cells, D cells and G cells secrete serotonin, somatostatin and gastrin, respectively. I cells are obviously identical to cells which secrete cholecystokinin as demonstrated by Polak et al. (1975) and Buchan et al. (1978b). There has been a debate as to the differentiation of S and D1 cells (Osaka et al., 1973; Sasagawa et al., 1973; Osaka et al., 1974; Capella et al., 1976; Solcia et al., 1978; Solcia et al., 1979). The morphological characteristics of S and D1 cells in the present study correspond to those of D1 and S cells, respectively, as proposed by Osaka et al. (1973). Apart from the problem of identification of these two cells, it has been generally supposed that both these two cells are similarly involved in secretin secretion (Polak et al., 1971; Bussolati et al., 1971). N cells, which were occasionally found in the bulbary mucosa in the present study, seem to correspond morphologically to the neurotensin-secreting N cells of Buchan et al. (1978c). However, this fact is not in accordance with the finding by Bloom and Polak (1978) that neurotensin is not secreted in the upper part of the small intestine. The morphological as well as the immunocytochemical identification of N cells remains to be elucidated (Buchan et al., 1978a; Kobayashi et al., 1980).

Endocrine functions of Brunner's glands

It has been widely believed that EC cells are the most numerous BGC throughout the gut from the duodenum to the colon. However, the present study has demonstrated that the most common types of BGC in the Brunner's glands are non-EC cells such as D, S, I and D1 cells. Such a finding has not previously been reported. Recently, Iwanaga has observed that there are many non-EC cells in the Brunner's glands of a 6-month-old human fetus as demonstrated by Grimalius' method as well as by immunocytochemistry (personal communication). From the fact that I, S and D1 cells are predominant, it seems reasonable to consider that cholecystokinin and secretin are secreted from Brunner's glands. Cholecystokinin secreted by I cells is presumed to stimulate...
the bile secretion from the gall bladder, and at the same time to suppress gastric acid secretion (JOHNSON and GROSSMAN, 1970; BROOKS and GROSSMAN, 1970). Secretin, presumably secreted by S cells or D₁ cells, has a stimulating effect on pancreatic juice secretion, and also on the inhibition of gastric juice secretion (WORMSLEY and GROSSMAN, 1964). Furthermore, both cholecystokinin and secretin are considered to have influence on the stimulation of the mucus secretion from the Brunner’s glands (LOVE et al., 1968; STENING and GROSSMAN, 1969). All of these facts indicate that BGC in Brunner’s glands have various effects on the duodenum, including the protection of the duodenal mucosa from the gastric juices. There is a report that the isolated duodenum containing Brunner’s glands does not develop an ulceration, while the jejunum does in response to the gastric juice drainage (FLOREY et al., 1939; GRIFFITH and HARKINS 1956). On the other hand, it has been reported that emiocytosis of some BGC in duodenal crypts can be experimentally induced by application of 0.1 N HCl solution on the duodenal mucosa (OSAKA et al., 1974). This experiment also implies that BGC in Brunner’s glands might perform some hormonal regulations in favor of the protection of the duodenal mucosa.

The fact that there are relatively abundant D cells in Brunner’s glands indicates that somatostatin can not be ignored as a part of the endocrine functions of the Brunner’s glands. By analogy with the morphological and functional aspects of D cells in the pancreas (FUJITA, 1968; RUFENER et al., 1975; RAPTIS et al., 1978), it may be reasonable to presume that D cells and their product, somatostatin in Brunner’s glands might regulate the secretion of the neighboring BGC such as I cells, S cells, and D₁ cells.

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