GUT ENDOCRINE CELLS IN INSECTS: THE ULTRA-STRUCTURE OF THE GUT ENDOCRINE CELLS OF THE LEPIDOPTEROUS SPECIES

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ABSTRACT

Endocrine cells in the epithelia of the larval, pupal, and adult midguts of seven species of Lepidoptera were examined under an electron microscope. Gut endocrine cells, or basal-granulated cells, are dispersed throughout the midgut of all species in all developmental stages. They are pyramidal or bowl-shaped and their fine structures are essentially the same among the species, but somewhat different between larval and adult stages. Some of them seem to be open in type, standing on the basal lamina and extending toward the epithelial surface a cytoplasmic process covered by a tuft of microvilli.

Endocrine granules are usually round with a high electron density and homogeneous texture and encircled by a distinct membrane derived from the Golgi complex. The majority of them are small-sized (~100 nm) electron dense granules. Medium-sized (150-250 nm) and large-sized (300-500 nm) ones are occasionally found.

Since the gut endocrine cells observed in the lepidopterous species correspond to only a small portion of the entire cell population, an attempt to classify these basal-granulated cells into types seems difficult at present.

KEY WORDS basal-granulated cell / gut endocrine cell / midgut / insect / *Bombyx mori* / *Pieris rapae* / *Papilio xuthus* / *Samia cynthia ricini* / *Lymantria dispar* / *Galleria mellonella* / *Ephestia cautella*

In a previous paper (13), we described the ultrastructure of endocrine cells in the midgut and enteric caeca of a hemimetabolous insect, the cockroach. Gut endocrine cells developed in the regenerative cell mass, the nidus, and were singly intercalated among the columnar cells. Because of the presence of basally concentrated granules, these cells were given the name of basal-granulated cells and apparently corresponded to the endocrine element

of the gut in mammals. They were temporarily classified by reference to the granules into four cell types: the Type I-a cell was reminiscent of the M cell, the Type I-b cell of the L cell, the Type I-c cell of the D-like cell, and the Type II cell of the EC cell of the human alimentary mucosa (cf. 11, 12).

The present paper concerns the gut endocrine cells of holometabolous insects, the Lepidoptera. During the past several years, we have been studying the histopathology of the larval midgut tissues affected by a *Bacillus* toxin in seven lepidopterous species (cf. 3) and have found basal-granulated cells dispersed in the midgut epithelia both in experimental and control series of all species. Later, we examined the pupal and adult midguts as well, and recognized that they were present in all develop-

Abbreviations in Figure Labels: BG, basalgranulated cell; BI, basal infoldings of the cell membrane; BL, basal lamina; C, columnar cell; ER, endoplasmic reticulum, FG, foregut; G, goblet cell; GO, Golgi complex; HG, hind gut; M, mitochondria; MG, midgut; MT, microtubules; MV, microvilli; N, nucleus; R, regenerative cell; T, tracheole

mental stages. The gut endocrine cells in lepidopterous species, however, represented only a very small portion of the entire cell population and were somewhat different from those observed in the midgut of the cockroach. This paper describes the fine structure of basal-granulated cells in the midguts (mainly the larval midgut) of seven lepidopterous species.

MATERIALS AND METHODS

Insects

For the common cabbageworm, *Pieris rapae*, pupae and adults were collected in the field. Eggs were laid on the cabbage leaves and from the second instar onward larvae were reared on a semisynthetic diet. The fifth-instar larvae and adults were employed. Observations have been done mainly with this species. For the gipsy moth, *Lymantria dispar*, egg masses were collected during winter in the field and stocked at 4°C in constant darkness. In spring, the eggs were subjected to a long-day photoperiod at 25°C. Larvae were reared with oak leaves (*Quercus* sp.) and the fourth instars were used. For the swallowtail, *Papilio xuthus*, eggs and young larvae were collected in the field and reared on leaves of Bengal quince, *Poncirus trifoliata*, and 1-day-old fifth-instar larvae were

examined. For the almond moth, *Ephestia cautella*, eggs were transferred into rice-bran autoclaved at 120°C for 20 min. The fourth-instar larvae were used. Eri-silkworms, *Samia cynthia ricini*, have been reared in our laboratory for years either with an artificial diet or with fresh leaves of the castor-oil plant, *Ricinus communis*. We used 1-day-old fifth-instar larvae and young pupae reared on fresh leaves. For the wax moth, *Galleria mellonella*, stock cultures were reared on honeycombs and the sixth-instar larvae were used. For the silkworm, *Bombyx mori*, a racial hybrid, Shinrei-Shuho, was reared on fresh mulberry leaves and the third-instar larvae, young and old pupae, and young adults were observed.

All insects were reared at 25°C, except for *Galleria* (30°C), under a 16-hr photoperiod.

Electron Microscopy

Electron microscopic observations were restricted to the anterior-middle region of the midgut. Regardless of whether the species was nocturnal or diurnal, the midguts were excised at mid-day and fixed with 2.5% buffered glutaraldehyde in 0.1M phosphate buffer, pH 7.4, for 60 min at 4°C. The tissues were then washed several times with the same buffer, and post-fixed with 1% buffered OsO₄ for

Fig. 1 Schematic representation of metamorphosis of the alimentary tract in a lepidopterous insect. This drawing is based on the case of the common cabbageworm. The striated area shows the midgut. Changes in midgut epithelium during metamorphosis are shown below.

Fig. 2 A pyramidal-shaped endocrine cell of the larval midgut in the gipsy moth. The cell stands on the basal lamina and extends toward the gut lumen. Many endocrine granules are seen in the infra- as well as supranuclear region. Mitochondria are abundant in the infranuclear region, and their sizes are smaller than those of columnar cells. $\times 17,000$

Fig. 3 A bowl-shaped endocrine cell of the larval midgut in the almond moth. It contains small-sized (~ 100 nm), electron dense granules. This type of granules is found in over 70% of the total endocrine cells observed. Undulations of the basal cell membrane may partly correspond to exocytotic profiles. $\times 27,000$

60 min at 4°C . The tissues were dehydrated in an ethanol series, embedded in Epon 812, and sectioned with an LKB-8800 microtome. After double-electron staining with uranyl acetate and lead hydroxide, the sections were examined with a JEM-100 CX electron microscope.

RESULTS

The digestive tract of lepidopterous larvae is divided into three principal sections: foregut, midgut, and hindgut. The midgut occupies almost the entire body cavity in the larval stage (Fig. 1). It is cylindrical, repeatedly constricted, and comprised of an outer muscular layer, a basal lamina, and a monolayer of epithelial cells consisting mainly of tall columnar and goblet cells ($70\text{--}80\ \mu\text{m}$ in height). The structure of the columnar cells is similar to that seen in the adult cockroach midgut.

The goblet cells are found only in larval midguts of lepidopterous species. They have a flat nucleus lying generally in the basal cytoplasm and contain a large cavity with an apical opening into the lumen of the midgut. Numerous thin cytoplasmic projections extend into the cavity. Each projection contains a mitochondrion (1, 3). Unlike the goblet cells of mammals, the goblet cells of Lepidoptera are believed to be the principal agent of active potassium transport from the hemolymph to the lumen of the midgut (1). They are found as frequently as columnar cells in the middle region of the midgut (3).

The regenerative cells are small and singly dispersed in the epithelium (3) except for those of *Galleria* which lie as a mass like those in the cockroach midgut (13). New columnar and goblet cells (possibly endocrine cells as well) develop from the regenerative cells during the larval-larval ecdysis. In the stage of the

Fig. 4 Basal part of an endocrine cell in the larval midgut of the common cabbage worm. The basal cell membrane is irregularly infolded. The endocrine granules are small-sized (~ 100 nm) and encircled by a clear membrane. $\times 60,000$

Fig. 5 Well-developed Golgi complex in the supranuclear region of the endocrine cell in the larval midgut of the common cabbage worm. Different stages in granule development can be seen. $\times 66,000$

Fig. 6 Medium-sized (150–250 nm) endocrine granules found in the common cabbageworm larva. This type of granules is found in ~20% of the total endocrine cells observed. $\times 50,000$

Fig. 7 Large-sized (300–500 nm) endocrine granules of the basal-granulated cell in the Eri-silkworm larva. This type of granules is only occasionally found. $\times 50,000$

early pharate pupa, the regenerative cells begin to proliferate and differentiate into only tall columnar cells (80 μm in height) replacing the old epithelial cells during larval-pupal ecdysis (15). Imaginal epithelial cells are short and cuboidal (20 μm in height) and gradually replace the old pupal cells during pupal-adult ecdysis (10).

The *endocrine cells* or *basal-granulated cells* are dispersed in the epithelia of the larval, pupal, and adult midguts. They are generally pyramidal (Fig. 2) or bowl-shaped (Fig. 3) extending toward the lumen side. We surmised that many of them could be 'open' in type (Fig. 9), although so far we have obtained only a few typical examples.

The nucleus is usually a distorted sphere in shape and lies in the basal cytoplasm (Figs. 2, 8 and 9c). It contains relatively little chromatin scattered in its center, whereas the chromatin of cockroach gut endocrine cells is often found at the periphery of the nuclear membrane.

Although basal infolding of the cell membrane are almost absent in adult cockroaches, some are found in the Lepidoptera. The basal infoldings (Figs. 3 and 4, BI) of the endocrine cells are irregular and shorter than those of the columnar cells.

The gut endocrine cells have several well-developed Golgi complexes in the supra-, para-, and infranuclear regions, where we found sequential stages of granule development (Fig. 5). Immature granules develop inside the Golgi vesicle and are wrapped with a limiting membrane as described in a previous paper (13). Numerous small vesicles are present near the developing granules. The endoplasmic reticulum is of relatively poor quantity in the endocrine cells and found only as the granular type. The mitochondria are ovoid or elongated and abundant in the infranuclear region (Figs. 2 and 4). They are smaller than those of the columnar cells. In slender cells, bundled microtubules sometimes run toward the apical portion in parallel with the long axis of the cell

Fig. 8 A basal-granulated cell of the adult midgut of the silkworm. Generally, endocrine granules are fewer in the adult stage than in the larval one. Besides the electron-dense granules, many vesicles with coarser texture can be seen (inset). $\times 12,500$ (inset, $\times 50,000$)

(Fig. 9, MT). Extremely elongated mitochondria (Fig. 9, M) may be mingled in parallel with them.

The *endocrine granules* are somewhat different from those observed in gut endocrine cells of the adult cockroach. They are rather similar in shape and size to the neuroendocrine granules observed in the axons extending to the basal lamina of the cockroach midgut (13). The granules of the lepidopterous gut endocrine cells are generally much smaller than those of the cockroaches. Although they are usually abundantly found in the infranuclear cytoplasm, they are often rich in whole cytoplasm (Fig. 2). Most of them are round and clearly membrane-bound. Irregular type granules have only occasionally been found in the species observed.

The most common feature of the granules is a round shape with a high electron density and homogeneous texture. The granules show a clear space between the dense substance and the membrane sac. This space is not clear in the granules situated near the basal lamina. Granules are either small (Fig. 4),

~ 100 nm (in over 70% of the total endocrine cells observed), or medium sized (Fig. 6), 150–250 nm, and some are slightly distorted in shape. Large-sized granules (300–500 nm) with high electron density are slightly distorted in shape and only occasionally observed (Fig. 7). The homogeneous dense substance is encircled by a distinctive membrane. A clear halo is often found beneath the membrane.

Fig. 9 Open-type basal-granulated cell found in the swallowtail larva. Figs. a, b, and c show the apical, middle, and basal part of the same cell, respectively. This cell is $80\text{ }\mu\text{m}$ tall, standing on the basal lamina and reaching the gut lumen with an apical cell process, which is covered by a tuft of microvilli (Fig. 9a). Numerous microtubules run in parallel with the long axis of the cell (a, b). Small-sized granules are seen around the nucleus which is slightly distorted in shape. Since this figure was taken from the midgut 10 min after the administration of $1\text{ }\mu\text{g}$ of *Bacillus thuringiensis* δ -endotoxin *per os*, early pathological changes have already appeared in the neighbouring columnar and goblet cells. $\times 10,000$

Fig. 9

Among these dense granules, a smaller number of other types of granules, round in shape and small with a coarser texture are sometimes found: small dense granules (~ 100 nm) incompletely filled with secretory substance; small round granules (100–200 nm) of considerably electron-lucent substance and looser texture, sometimes extremely scanty; and small round granules (~ 100 nm) packed with many tiny vesicular granules. These subsidiary granules essentially correspond in size and shape to the major electron-dense compact granules either of ~ 100 or 150–250 nm in size. These granule types were found with higher frequency in the adult (Fig. 8) than in the larval midgut.

We found open-type endocrine cells in *Papilio*, *Ephesia*, *Pieris*, and *cockroach* (unpublished data). Cells are of a pyramidal or a bar-like shape as tall as 70–100 μm , standing on the basal lamina with a cytoplasmic expanse and extending toward the epithelial surface. The apical cytoplasm is covered by a tuft of microvilli (2 μm in height). Numerous microtubules run in parallel with the long axis of the cell as if to support the slender cytoplasm. Fig. 9 shows an open-type basal-granulated cell (a: apical, b: middle, and c: basal part of the cell) of *Papilio*. Since this figure was taken from the midgut 10 min after the oral administration of 1 μg of *Bacillus thuringiensis* δ -endotoxin, early pathological changes have already appeared in some columnar and goblet cells: the columnar cells are slightly swollen and collapse of their microvilli has begun, showing an irregular swelling and distortion (Fig. 9a, C); a goblet cell shows relatively high electron density in the cytoplasm (Fig. 9c, G). However, so far, there have been no pathological changes observed in the ultrastructure of endocrine cells and we found similar open-type endocrine cells in the intact midguts. Therefore, Fig. 9 is assumed to be a typical example of the open-type gut endocrine cell in insects.

DISCUSSION

In insects, there have been only a few reports on cells corresponding to the basal-granulated or endocrine cells distributed in the midgut epithelia. These cells were described in the silkworm (9), mosquitoes (7), fly (2), *Petrobius* (4), flea (14), and cockroach (13). Except for our recent report on gut endocrine cells of the cockroach (13), most of these works contain only a brief description of cells with electron-lucent cytoplasm and many secretory

granules in the infranuclear region of the cytoplasm.

In the cockroach, a hemimetabolous insect, various kinds of gut endocrine cells were observed in the midgut and enteric caeca with high frequency: the endocrine cells could be classified by the fine structure of granules into at least four types (13). Moreover, our immunohistochemical study indicated that PP (pancreatic polypeptide)-, glucagon- and somatostatin-like immunoreactivities are located in different endocrine cells of the cockroach midgut (8). In the holometabolous Lepidoptera, although the basal-granulated cells were also observed in the epithelia of the larval, pupal, and adult midguts, they constituted only a small portion of the entire cell population. Since the midgut of lepidopterous species has a villous structure in the larval stage and occupies a major part of the entire gut (cf. Fig. 1), the number of gut endocrine cells may not be small in total in this stage. Since the alimentary tract plays a minor or no role as a digestive organ in the pupal and adult stages, the endocrine cells seem to decrease in total number after the pupation following reduction of the midgut (cf. Fig. 1).

At present, any attempt to classify the gut endocrine cells in lepidopterous insects into types would be difficult, requiring systematic and laborious observation. As far as the present observations are concerned, the structures of the gut endocrine cells are essentially the same among the species of Lepidoptera. There seem to be some differences between larval and pupal-adult midgut endocrine cells, but we have not enough data after pupation because of difficulty in obtaining fine sections for many reasons (for instance, columnar cells contain a large number of calcium and/or magnesium granules after the pupation).

Since the insect gut endocrine cells are as tall as 70–100 μm and not stained by the routine methods employed for mammalian gut endocrine cells, in ultrathin sections it is a rare incidence to be able to obtain such an intact figure in the direction of the cell axis as demonstrated in Fig. 9. Although in the previous paper we reported that the gut endocrine cells in the cockroach are mostly 'closed' in type, recently we have fortunately obtained the intact figures for the open-type endocrine cells in several insect species including the cockroach. Since most of the basal-granulated cells in insects are pyramidal forms standing on the basal lamina and extending the slender apical process

to the gut lumen, many, if not all of them, could be classified as the 'open' type. Some of them, however, may be the 'closed' type endocrine cells.

According to Fujita and Kobayashi (5), in humans most of the gut endocrine cells are open in type and receptosecretory in nature, and secretion release from their base is controlled by the reception of luminal stimuli at the microvillous apex. Closed-type endocrine cells which are supposed to respond to the stretch and strain of the gut wall, are found only in the gastric corpus. It is striking that even in insects, most of the gut endocrine cells seem to be the 'open' type receptosecretory cells. This fact strongly suggests that the gut endocrine cells are phylogenetically old and ubiquitously present in the animal kingdom.

Interestingly, the endocrine granules of lepidopterous gut endocrine cells are very similar in fine structure and size to the neuroendocrine granules of the Lepidoptera and the cockroach. This fact indicates that in insects as well as mammals neurons and endocrine cells have a common property at the ultrastructural level. Fujita (cf. 5, 6) proposed that peptide-producing endocrine cells and neuroepithelial or sensory cells are continuous in nature to neurons and called them 'paraneurons'. Neurons and paraneurons which all are receptosecretory in nature and contain secretory granules of similar structure, comprise a continuous spectrum and it is impossible to draw a line to discriminate between them. It is surprising that the structure and presumed function of the insect gut endocrine cells and neurons seem to be essentially the same as those of mammals. In fact, vertebrate bioactive peptides are contained in the nerve fibers (neurons) and basal-granulated endocrine cells (paraneurons) of the cockroach midgut. As reported previously (8), PP-, glucagon- and somatostatin-like immunoreactivities are located in the endocrine cells and VIP (vasoactive intestinal polypeptide)-, somatostatin- and PP-like immunoreactivities in nerve fibers.

Our findings in insect gut endocrine cells would support the concept of the 'paraneuron' and should contribute to the phylogenetical considerations on the gut endocrine cells.

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