IMMUNOHISTOCHEMICAL DEMONSTRATION OF PP-, SOMATOSTATIN-, ENTEROGLUCAGON- AND VIP-LIKE IMMUNOREACTIVITIES IN THE COCKROACH MIDGUT

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ABSTRACT

The midgut epithelium of the cockroach, *Periplaneta americana* contains basal-granulated (endocrine) cells with PP-, somatostatin- and enteroglucagon-like immunoreactivities. The three immunoreactivities are located in different endocrine cells, the PP-immunoreactive cells being most numerous.

The midgut is provided with inner circular and outer longitudinal musculature, the latter being heavily innervated. The nerve fibers here are found to contain VIP-, somatostatin- and PP-like immunoreactivities, each occurring in different fibers. VIP-immunoreactive fibers are most numerous, followed by somatostatin-immunoreactive ones.

This study supplements the previous electron microscope observations of the same material (10). Moreover, it provides data for considering the correlation between neurons and paraneurons in animal evolution.

KEY WORDS cockroach midgut / *Periplaneta americana* / immunohistochemistry / basal-granulated cell / PP / somatostatin / enteroglucagon / VIP

Recently the fine structure of basal-granulated cells in the midgut of the cockroach, *Periplaneta americana* was reported by two of the present authors (10). They demonstrated that the basal-granulated cells of the cockroach corresponded well structurally to those established as gut endocrine cells in vertebrates, and that they included at least four cell types, which suggested occurrence of a number of peptide products. The occurrence of granule-containing nerves along the longitudinal muscles of the midgut was also demonstrated.

The present immunohistochemical study will supplement the electron microscope findings by detecting different kinds of bioactive peptides both in the basal-granulated cells and the nerves in the cockroach midgut.

MATERIALS AND METHODS

Midguts from six adult cockroaches of either sex were used. Specimens were fixed in Bouin's fluid, embedded in paraffin and cut at 4 μ m, partly serially at 1.5-1.7 μ m.

The sections were subjected to indirect immunoperoxidase method (9) or peroxidase-antiperoxidase (PAP) method (13). After dewaxing with xylene and rinsing in 0.01 M phosphate buffered saline (PBS), the sections were incubated for 12-24 hr at 4°C with each of the antisera (diluted in PBS to the concentrations indicated).

Antiserum to porcine insulin (1:360) was raised in a guinea pig; the other antisera were raised in rabbits to bovine pancreatic peptide (BPP, 1:6,000), synthetic somatostatin (1:1,500), synthetic glucagon (C-terminal anti-glucagon

serum, OAL-123, 1:2,000), porcine/bovine glucagon (N-terminal anti-glucagon serum, 1:2,000), synthetic porcine vasoactive intestinal peptide (VIP, R502, 1:2,560), synthetic human gastrin (R1301, 1:1,500), synthetic porcine secretin (R801, 1:800), synthetic porcine motilin (R1104, 1:800) and synthetic bovine neurotensin (R3502, 1:800). The site of antigen-antibody reaction was revealed by rabbit anti-guinea pig IgG

conjugated with peroxidase (Miles Laboratories, Israel) at the dilution 1:50 in PBS or porcine anti-rabbit IgG (1:20, Dako, Denmark) followed by PAP complex (1:50, Dako, Denmark).

Controls comprised incubations with normal guinea pig or rabbit serum and antigen-inactivated serum (10–20 μ g of corresponding peptide in 1 ml of diluted antiserum).

Fig. 1 Cross-section of the cockroach midgut. Numerous PP-like immunoreactive cells are found in the epithelium. $\times 200$

Fig. 2 PP-like immunoreactivity seen in a basal-granulated cell (arrow) in the epithelium and nerve fibers in the outer longitudinal musculature. A terminal-like swelling of a nerve fiber (arrowhead) filled with the reaction product is located in the periphery of a muscle bundle. $\times 800$

Fig. 3 An enteroglucagon immunoreactive cell of probable open type, in the midgut epithelium. $\times 1,000$

RESULTS

General Histology

The microscopic structure of the cockroach midgut was introduced elsewhere (10) and will be described only briefly. The midgut is lined by

tall (80–110 μm) columnar epithelium containing conglomerations of regenerative cells, or nidi, disposed at the base of the epithelium.

The epithelium is wrapped by a thick basal lamina and a thin layer of internal circular muscle fibers. The outside of this musculature

Fig. 4 Two somatostatin-like immunoreactive cells in the midgut epithelium. $\times 800$

Figs. 5 and 6 Somatostatin-like (Fig. 5) and VIP-like (Fig. 6) immunoreactive nerve fibers in the longitudinal musculature (LM). Few immunoreactive nerve fibers are found in the inner circular musculature (CM). $\times 800$

is loosely attached by outer longitudinal muscle fibers, which are gathered into thicker and thinner bundles separated at irregular intervals.

It is noteworthy that these muscle bundles contain numerous nerve fibers which are partly thin but partly very thick, often measuring over 3 μ m. In contrast, very few, if any, nerves seem to supply the inner circular musculature. Nerve cell bodies are not found in the midgut. The nerve fibers mentioned above are thought to be derived from the central nervous system, possibly from the ganglia of the ventral cord.

Peptide Immunoreactivities in Basal-Granulated Cells

The cockroach midgut epithelium was found to contain relatively numerous basal-granulated cells with PP-like immunoreactivity (Figs. 1 and 2). Enteroglucagon-immunoreactive cells (reactive to the N-terminal anti-glucagon serum, but not to the C-terminal anti-glucagon serum) and somatostatin-immunoreactive cells were less numerous (Figs. 3 and 4). Observation of serial adjacent sections treated with different antisera confirmed that the three immunoreactivities resided in different cells.

The immunoreactive basal-granulated cells were singly intercalated among the columnar and regenerative cells of the epithelium (Fig. 1). The immunoreactive substance was concentrated in the infranuclear cytoplasm which lay on the basal lamina. Among the high columnar cells of the epithelium, the thin, faintly stained supranuclear cytoplasm of the basal-granulated cells was extended upwards but could not be traced so far as to determine whether it reached the gut lumen (Figs. 1 and 3). Some immunoreactive cells were located among the regenerative cells in the peripheral part of nidi. As the cells in this position were crescent-shaped, it was unlikely that their apical process could reach the lumen (Figs. 1 and 2).

Immunoreactivity for VIP, insulin, gastrin, secretin, motilin or neurotensin could not be

demonstrated in any cells of endocrine type.

Peptide Immunoreactivities in Nerve Fibers

Immunoreactivities for three different peptides were demonstrated in different nerve fibers in the outer longitudinal muscle bundles. Numerous fibers were found to contain VIP-like immunoreactivity (Fig. 6). Somatostatin-immunoreactive fibers were less numerous (Fig. 5), while there were still fewer PP-immunoreactive fibers (Fig. 2).

Some nerve fibers were conspicuously thick and filled with the intensely immunoreactive substance (Figs. 2 and 6). These terminal-like axons were closely juxtaposed with or telescoping into muscle fibers, forming probable neuromuscular junctions. The terminal-like axons tended to be located in the periphery of a muscle bundle, and thus their outside surfaces were mostly exposed to the body cavity which, in the insect having the open circulation system, contains hemolymph (Figs. 2, 5 and 6).

Immunoreactivity for glucagon, insulin, gastrin, secretin, motilin or neurotensin could not be demonstrated in nerve fibers. No neuron somata were present in the midgut of the cockroach.

Table 1 sums up the distribution of peptide-immunoreactivities in the basal-granulated or endocrine cells and nerve fibers.

DISCUSSION

The present immunohistochemical study demonstrates three peptide-like immunoreactivities in endocrine cells and three in nerve fibers in the cockroach midgut. This finding is compatible with the electron microscope observation (10) that different kinds of endocrine and neurosecretory granules were contained in the basal-granulated cells and nerve fibers, respectively. Occurrence of multiple peptidic secretions in endocrine cells and neurons is exactly the same phenomenon as seen in verte-

Table 1 *Distribution of Peptide Immunoreactivities in the Cockroach Midgut*

	PP	Enteroglucagon (GLI)	Somatostatin	VIP
Endocrine cell	+++	+	+	-
Nerve fiber	+	-	++	+++

brates.

The fine-structural features of the cockroach basal-granulated cells (10) strongly suggest that they are paraneurons as the gut endocrine cells in vertebrates. This view is supported by the present result that they share with nerves two typical neuronal peptides, PP and somatostatin.

By analogy to the gut endocrine cells in vertebrates, it seems reasonable to postulate that the basal-granulated cells may release their secretions in response to chemical information in the gut lumen or mechanical stimulation in the epithelium. The electron microscope observation (10) indicates that some cells are open to the gut lumen. The present microscopic observation demonstrates that many of the cells extend an apical process. It is presumed that some, if not all, of the cells of this shape may be open to the lumen, although their direct contact with the lumen could not be evidenced in the present study because of the conspicuous height of the epithelium and because of the unstainability of the apical cell process.

Topographic anatomy in the cockroach midgut may support the speculation that the inner circular muscle may be controlled mainly by paracrine effects of epithelial endocrine cells, the outer longitudinal muscle by nerves. Immunoreactivity laden nerve fibers were found to be in contact with and telescoping into muscle fibers in the present study. This completely corresponds to the electron microscope finding (10) on the terminal-like swellings of nerve fibers containing neurosecretory granules and forming a synapse-like connection with muscle fibers.

It is noticed that the nerve fibers and their terminal-like portions are much more numerous than expected for the control of muscle fibers. Moreover, a large part of the nerve fiber surface faces the body cavity. Like other insects, the cockroach has an open circulatory system, and the nerves in question are, together with muscle fibers, so to speak floating in the hemolymph. It seems thus possible to postulate that a part of the neurosecretions, including the peptides demonstrated in this study, may be released into the hemolymph, in addition to controlling the function of muscle fibers.

It is striking that insect neurons and paraneurons possess peptides with as closely related molecular structures as to be stainable with the antisera produced by the use of mammalian peptides. PP and somatostatin, whose immunoreactivities were demonstrated both in endocrine and nervous elements in the cock-

roach gut, are known as common neuronal and paraneuronal peptides in vertebrates. It is noteworthy that enteroglucagon (GLI) is restricted to the endocrine cells, while VIP to the nervous elements, just as they are in the digestive organs in some species of vertebrates (8).

Recent immunohistochemical studies have indicated that vertebrates' bioactive peptides may be contained in neurons and paraneurons of insects (4, 5, 11, 12, 16), molluscs (1, 2, 6) and animals representing more primitive forms of arthropodal evolution like the earthworm which has been demonstrated to contain PP- and VIP-like immunoreactivities in its neurons (14). Even the coelenterate, hydra, which represents one of the most primitive multicellular animals has been claimed to have the substance P-like (3, 15) and gastrin/CCK-like (7) immunoreactivities in its neurons. If one accepts the view that coelenterates are located at the stem of the two evolutionary routes towards vertebrates and towards arthropoda, the above data suggest that certain bioactive peptides developed in a very primitive stage of animal evolution and came to be shared by both vertebral and arthropodal lines in evolution.

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