**ULTRASTRUCTURE OF TWO TYPES OF ENDOCRINE
CELLS IN THE MIDGUT EPITHELIUM
OF *SPODOPTERA EXIQUA* HÜBNER, 1808
(INSECTA, LEPIDOPTERA, NOCTUIDAE)**

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Abstract. Midgut epithelium of Lepidoptera consists of four types of cells: columnar, goblet, endocrine and regenerative. Among endocrine cells two types have been distinguished: “open” and “closed”. In all five larval instars of *Spodoptera exiqua* (Lepidoptera, Noctuidae) only “open” endocrine cells were observed, which means that their apical membranes contact with the midgut lumen and form microvilli like columnar cells of epithelial character. Among them granular and vesicular cells are distinguishable. The number of vesicular endocrine cells increases distinctly in the 5th larval instar. The structure of endocrine cells in the midgut epithelium of *S. exiqua* and their differentiation from the regenerative cells, studied in transmission electron microscope, are described.

Key words: endocrine cells, midgut epithelium, granular cells, vesicular cells, Lepidoptera

INTRODUCTION

Lepidopteran midgut is composed of four types of cells: columnar, goblet, endocrine and regenerative (ANDRIÉS and TRAMU, 1985; LEHANE and BILLINGSLEY, 1996; PINHEIRO *et al.*, 2003; LEVY *et al.*, 2004). Endocrine cells have been observed between epithelial cells in the midgut epithelium of the majority of

insects, but they have been described mainly for Lepidoptera, Ephemeroptera, Trichoptera and Plecoptera (MONTUENGA *et al.*, 1989; NISHITSUTSUJI-UWO and ENDO, 1981; ROST-ROSZKOWSKA, 2008). They are responsible for the regulation of enzyme synthesis and secretion (MONTUENGA *et al.*, 1989; CHAPMAN, 1998). In some insect species only one type of endocrine cells has been found while in others several different types have been detected (MONTUENGA *et al.*, 1989; NEVES *et al.*, 2002, 2003). They are described as oval, pyramid or bowl-shaped cells (ANDRIÉS and BEAUVILLAIN, 1988; CAVALCANTE and CRUZ-LANDIM, 1999), and originate from the regenerative cells. In recent years the structure of endocrine cells has been described. They differ structurally from the other epithelium cells (ENDO and NISHITSUTSUJI-UWO, 1981; GÜL *et al.*, 2001; LEVY *et al.*, 2004). The presence of somatostatin, pancreatic polypeptide and FMRFamide-related peptides has also been analyzed in the cytoplasm of endocrine cells (ENDO *et al.*, 1982; ANDRIÉS and TRAMU, 1985; ZUDAIRE *et al.*, 1998). Their activity is associated with the presence of a great number of electron dense secretory granules, which are mainly accumulated in their basal cytoplasm (RAES and VERBEKE, 1994; BILLINGSLEY and LEHANE, 1996; LEVY *et al.*, 2004).

The aim of our studies was to analyse the ultrastructure of endocrine cells in relation to their endocrine activity in the midgut epithelium of all larval instars of *Spodoptera exiqua* (Insecta, Lepidoptera), a species of great economic importance.

MATERIAL AND METHODS

The material for the study originated from the laboratory culture of *S. exiqua*. Larvae (1st – 5th) and isolated midgut were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4 (4°C, 2 h) and postfixated with 2% OsO₄ (4°C, 1.5 h). After dehydration in a graded series of alcohols (50%, 70%, 90%, 96%,

Fig. 1. Midgut epithelium in all larval stages of *Spodoptera exiqua* is formed by columnar (c), endocrine (e), goblet (g) and regenerative (arrow) cells. Midgut lumen (l). Light microscope, bar = 80µm.

Fig. 2. 1st larval instar of *S. exiqua*. Endocrine cells (e), regenerative cells (r), columnar cell (c), basal lamina (arrow). TEM, bar = 24µm.

Fig. 3. 3rd larval instar of *S. exiqua*. Regenerative cell (r), endocrine cell (e), goblet cell (g), basal lamina (arrow), nucleus of the regenerative cell (n). TEM, bar = 20µm.

Fig. 4. 2nd larval instar of *S. exiqua*. Columnar cell (c), “open” endocrine cell (e), microvilli (mv), granular material (probably glycogen) in the cytoplasm of endocrine cell (arrow). TEM, bar = 37µm.

Fig. 5. 4th larval instar of *S. exiqua*. Granular endocrine cell. Asterisks indicate accumulation of granular material (glycogen). TEM, bar = 7µm.

and 100% for 15 minutes each) and acetone (15 min), the material was embedded in Epon 812. Semithin sections, after cutting with the Leica Ultracut UCT25 ultramicrotome, were stained with 1% methylene blue in 0.5% borax and examined in an OLYMPUS BX60 light microscope. Ultrathin sections were cut with the Leica Ultracut UCT25 ultramicrotome and stained with uranyl acetate and lead citrate. They were examined with a Hitachi H500 transmission electron microscope at 75kV.

RESULTS

Midgut epithelium of *S. exiqua* is formed by columnar, goblet, endocrine and regenerative cells (Fig. 1). The endocrine cells are singly distributed among columnar cells and they localize in the neighborhood of regenerative cells (Fig. 2). At the beginning of each larval instar (1st – 5th) it is difficult to distinguish the columnar cells of endocrine character. Regenerative cells are able to proliferate and differentiate into columnar, endocrine and goblet cells (Fig. 3). It is easy to detect the differentiation of goblet or columnar cells, but columnar cells achieve an endocrine character just after reaching the midgut lumen. The regenerative cell first elongates towards the midgut lumen. After microvilli formation and reaching the midgut lumen, it differentiates into columnar or endocrine type. Thus the endocrine cells of the midgut epithelium in *S. exiqua* are of the “open” type, which means that their apical membranes, which form microvilli, contact with the midgut lumen (Fig. 4).

The basal membranes of endocrine cells do not form visible folds. In their cytoplasm numerous Golgi complexes, cisterns of SER and RER and lamellar structures occur (Fig. 5). In the 1st to 5th larval instars two types of “open” endocrine cells were observed: granular and vesicular. The granular endocrine cells have accumulations of electron dense granules which are probably glycogen granules. They are initially accumulated in the basal cytoplasm (Fig. 6). Afterwards, the granules occur also in the perinuclear and apical cytoplasm (Figs. 5, 7). The vesicular endocrine cells are rich in small and large electron lucent vacuoles with visible electron dense core inside. They are present in the entire cytoplasm (Figs. 8, 9). In the 5th larval instar we observed that the number of vesicular endocrine cells increased in comparison with larval instars 1st – 4th.

DISCUSSION

Two major kinds of endocrine cells have been distinguished in the insect midgut epithelium: “open” and “closed” endocrine cells. The apical membrane of the “open” endocrine cells, forming numerous microvilli, contacts the midgut lumen (REINHARDT, 1976; GLÄTTLI *et al.*, 1987), whereas the endocrine cells of the “closed” type do not reach the midgut lumen and their apical membranes do not form microvilli (ANDRIÉS, 1976; BILLINGSLEY and DOWNE, 1986; SERRÃO and CRUZ-

Fig. 6. 4th larval instar of *S. exiqua*. Part of the midgut epithelium. Endocrine cell (e), goblet cell (g), columnar cell (c), nucleus of the columnar cell (n), granular material (asterisk). TEM, bar = 23 μ m.

Fig. 7. 4th larval instar of *S. exiqua*. Apical region of the endocrine cell. Microvilli (mv). Rough endoplasmic reticulum (RER), Golgi complexes (d), granular material (asterisk). TEM, bar = 12 μ m.

Fig. 8. Vesicular endocrine cell in the midgut epithelium of the 2nd instar larva of *S. exiqua*. Vesicles (v), lamellar structures (arrow), nucleus of endocrine cell (n). TEM, bar = 19 μ m.

Fig. 9. Part of the midgut epithelium of the 5th instar larva of *S. exiqua*. Vesicular endocrine cells (e), goblet cells (g), vesicles (v), nucleus of endocrine cell (n). TEM, bar = 23 μ m.

LANDIM, 1996). Among those two types of endocrine cells several different kinds have been described (ANDRIÉS and BEAUVILLAIN, 1988; RAES and VERBEKE, 1994; CAVALCANTE and CRUZ-LANDIM, 1999; GÜL *et al.*, 2001; NEVES *et al.*, 2003). In *S. exiqua* only the “open” type of endocrine cells was observed. They have microvilli, which contact the midgut lumen. In some insect species among the „open” endocrine cells of the posterior midgut two kinds of cells have been distinguished (NEVES *et al.*, 2002, 2003). The cytoplasm of one type contains great numbers of electron dense granules, which are called granular cells, while the other kind has numerous vacuoles with electron lucent contents (RAES and VERBEKE, 1994; GÜL *et al.*, 2001). The endocrine cells of some insect species have been observed to contain electron dense homogenous granules and structures with distinct electron dense core (LEVY *et al.*, 2004). In *Schistocerca gregaria* 10 types of endocrine cells have been described: 7 types present in both the anterior and posterior midgut, and 3 types in the neighborhood of Malpighian tubules openings (MONTUENGA *et al.*, 1989). The character of material accumulated in the cytoplasm of endocrine cells and the number of endocrine cell types are probably associated with the peptides which are produced in their cytoplasm (NEVES *et al.*, 2002, 2003). In *S. exiqua* two kinds of open cells were observed: granular and vesicular. However, in the 5th larval instar the number of vesicular endocrine cells increases, which might be connected with the additional peptides produced and secreted just before pupal stage.

Lepidopteran endocrine cells have electron lucent cytoplasm with numerous Golgi complexes and vacuoles. Their basal membranes do not form any folds. In some insect species glycogen granules have been detected (ENDO and NISHITSUTSUJI-UWO, 1981; NISHITSUTSUJI-UWO and ENDO, 1981; MONTUENGA *et al.*, 1989; BILLINGSLEY, 1990; LEVY *et al.*, 2004; NEVES *et al.*, 2003). Secretory vacuoles and granules are mainly accumulated in the basal cytoplasm (RAES and VERBEKE, 1994; BILLINGSLEY and LEHANE, 1996; CRISTOFOLETTI *et al.*, 2001; LEVY *et al.*, 2004). The structure of endocrine cells in the studied species is similar to that described for many insect species, granular structures are observed in the entire cytoplasm.

The endocrine cells in the insect midgut epithelium are thought to be functionally similar to neurosecretory cells of the vertebrate alimentary tract (BILLINGSLEY and LEHANE, 1996; LEVY *et al.*, 2004). They synthesize polypeptide hormones (ENDO *et al.*, 1982; ANDRIÉS and TRAMU, 1985; ZUDAIRE *et al.*, 1998), which are responsible for proper and specific enzyme secretion and control proliferation and differentiation of the regenerative cells. The 5th larval instar of *S. exiqua* is the last one before pupal stage, and the organism must be prepared for many changes associated with pupation: the secretory functions of the endocrine cells are probably intensified and new hormones synthesized.

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ULTRASTRUKTURA DWÓCH TYPÓW KOMÓREK ENDOKRYNOWYCH
W NABŁONKU JELITA ŚRODKOWEGO *SPODOPTERA EXIQUA* HÜBNER,
1808 (INSECTA, LEPIDOPTERA, NOCTUIDAE)

STRESZCZENIE

Nabłonek jelita środkowego owadów należących do Lepidoptera tworzony jest przez cztery typy komórek: trawienne, kubkowe (kielichowate), endokrynowe oraz regeneracyjne. Wśród komórek endokrynowych rozróżniamy dwa ich rodzaje: otwarte oraz zamknięte.

We wszystkich pięciu stadiach larwalnych *Spodoptera exiqua* (Lepidoptera, Noctuidae) były obserwowane jedynie komórki endokrynowe typu otwartego, których błony apikalne kontaktując się ze światłem jelita tworzą mikrokosmki, podobnie jak komórki trawienne. Wśród nich można wyróżnić zarówno komórki ziarniste zawierające liczne elektronowo gęste granule, a także komórki pęcherzykowate z licznymi elektronowo jasnymi wakuolami. Liczba komórek endokrynowych o charakterze pęcherzykowatym zdecydowanie wzrasta w ostatnim piątym stadium larwalnym badanego gatunku. Struktura komórek endokrynowych, a także proces ich różnicowania z komórek regeneracyjnych został opisany z wykorzystaniem transmisyjnej mikroskopii elektronowej.

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