POINT OF VIEW

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Electron microscopic observations on the H-organ of Lepidoptera

HEINER BIRKENBEIL

Sächsische Akademie der Wissenschaften zu Leipzig, AG Prof. Dr. H. Penzlin, Erbertstraße 1, Postfach 100322, 07703 Jena, Germany

Lepidoptera, *Galleria mellonella*, *Manduca sexta*, H-organ, connective tissue, neurohaemal organs, ultrastructure

Abstract. The H-organs of lepidopteran larvae, which have been previously described as neurohaemal organs, were reinvestigated using electron microscopy. The results of this study revealed that there are no signs of neurosecretion in the H-organs of *Galleria mellonella* and *Manduca sexta*. Moreover, the H-organs consist of fibroblasts and are considered to be connective tissue and not neurohaemal organs.

INTRODUCTION

In the course of their search for a thoracic endocrine centre other than the prothoracic glands, Abou-Halawa & Sláma (1986) detected a new tissue structure in lepidopteran larvae and identified it as a neurohaemal organ. They named it H-organ because of its shape and documented its presence in a variety of lepidopteran larvae and pupae. Anatomical and histological studies, including staining techniques conventionally used for neurosecretion, led them to the assumption that they had discovered a neurocrine organ belonging to the metameric system of the perisympathetic organs.

During our efforts to investigate moult regulation in *Galleria mellonella* we started an electron microscopy study of these organs in order to correlate their ultrastructural appearance with physiological events.

MATERIAL AND METHODS

Galleria mellonella L. (Pyralidae) larvae, reared in total darkness at 30°C on a standard diet (Sehnal, 1966), were used in the last (seventh) instar.

Manduca sexta L. (Sphingidae) were raised on an artificial diet under a 18 L : 6 D photoperiod at 26°C and used also in the last (fifth) larval instar. The H-organs were fixed in situ with 4% glutaraldehyde, 2% paraformaldehyde in 0.1 M phosphate buffer, pH 7.3, and excised together with the underlying nerve cord. After washing with buffer, they were postfixed with 1% OsO_4 in phosphate buffer, dehydrated with acetone and embedded in Durcupan ACM. Thin sections were stained with uranyl acetate and lead citrate and examined with a Tesla electron microscope. In addition, total preparations of H-organs of *Galleria* were stained with Weigert's stain for elastic connective tissue (Pearse, 1968).

RESULTS

Galleria mellonella

The anatomical arrangement of the H-organ in *Galleria* larvae was found to be the same as that described by Abou-Halawa & Sláma (1986); the central part is situated dorsal of the connectives, between suboesophageal and prothoracic ganglia, whereas the anterior

and posterior lateral branches extend laterally up to respective ganglia. Cross sections through whole branches in different regions of H-organs did not reveal any nervous constituents (Fig. 1). Instead, a number of profiles of only one cell type are found, and are often arranged in groups that are embedded in well-developed extracellular space containing fibrillar substance. The whole organ is enclosed by a thin basement membrane or tunica propria. In the middle part of the anterior branches, a large trachea is seen and numerous smaller tracheae and tracheolae are distributed throughout the H-organ (Fig. 1). The cells of the H-organ are characterized by an elongated shape. The nuclei are ovoid or irregularly formed. The perinuclear cytoplasm and the neighbouring parts of the long and slender cell processes exhibit cisternae of the rough endoplasmic reticulum (RER), a great number of ribosomes and some mitochondria. More distal parts of the cellular processes are characterized by bundles of intracellular filaments or microtubules that extend parallel to the long axis of the cellular processes (Fig. 2). There are no signs of neurosecretory elements in the entire organ investigated.

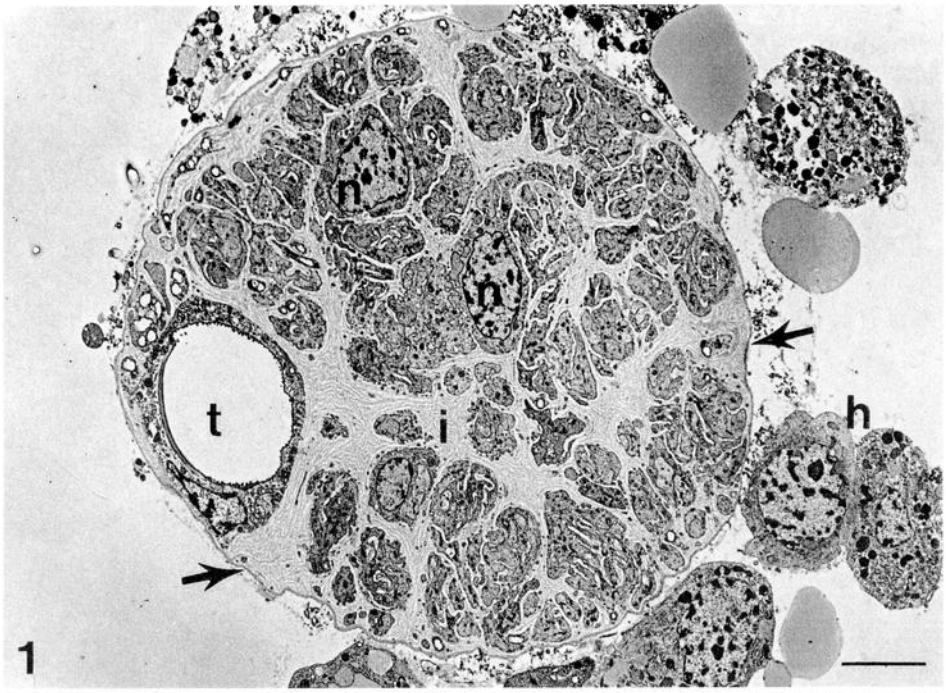
Manduca sexta

In *Manduca* larvae, the anatomical appearance of the H-organ is similar to *Galleria*. The anterior branches pass the immediate vicinity of the prothoracic glands. Cross and longitudinal sections revealed that they also contain only one type of elongated, thin cells embedded in a fibrillar extracellular matrix and surrounded by a tunica propria (Fig. 3). In contrast to the H-organs of *Galleria*, the nuclei are also of elongated shape and occupy nearly the whole cellular diameter. Cytoplasmic areas containing ribosomes and dilated cisternae of the RER (Figs 4, 5) indicate protein synthesis and are characteristic of fibroblasts (François, 1978); these areas surround other parts with tightly packed microtubules. In longitudinal sections, the fibrillar nature of the extracellular matrix is conspicuous (Fig. 5). In entire cross sections of anterior arms of H-organs, only two small profiles containing neurosecretory granules were found; these may have originated in peripheral neurosecretory cells. Otherwise, a motor nerve crossing the central part of the H-organ, without any signs of neurosecretion, is the only portion of the nervous system detected in the H-organs.

DISCUSSION

In a series of previously published papers (Abou-Halawa & Sláma, 1986; Bhargava & Sláma, 1987; Sláma, 1988), the H-organ found in larvae of *Galleria mellonella* and other Lepidoptera was described as a newly discovered neurohaemal organ. The methods used were vital or supravital staining with methylene blue, histological staining with azocarmine, and histofluorescence studies with acridine orange and the Falck-Hillarp method. These studies lead to the assumption that the H-organ could be a neurohaemal organ containing glial and neurosecretory cells and this organ could be a member of the metameric neurohaemal organs of the perisympathetic nervous system. Axons of the anterior branches were assumed to innervate the prothoracic glands.

Figs 1–2. H-organ of *Galleria mellonella*. 1 – cross section of an anterior branch demonstrating only profiles of presumed fibroblasts embedded in an extended extracellular matrix (i) and surrounded by a tunica propria (arrows). h – hemocytes; n – nuclei; t – trachea. Scale bar = 5 μm. 2 – longitudinal section of cellular processes exhibiting numerous ribosomes and RER (arrows) as well as intracellular microtubules (mt). m – mitochondria. Scale bar = 1 μm.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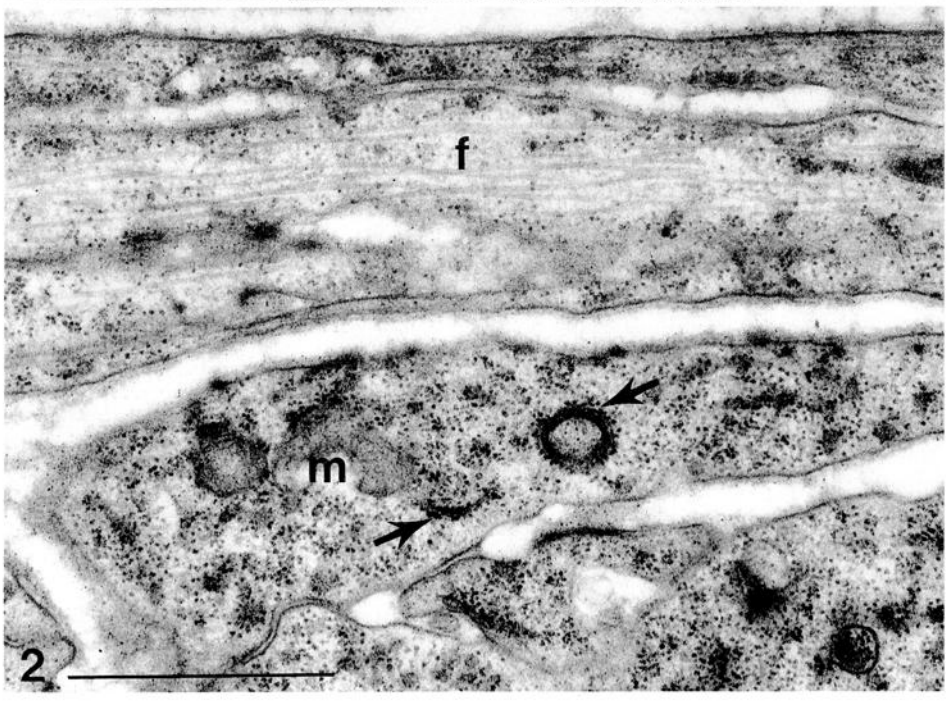
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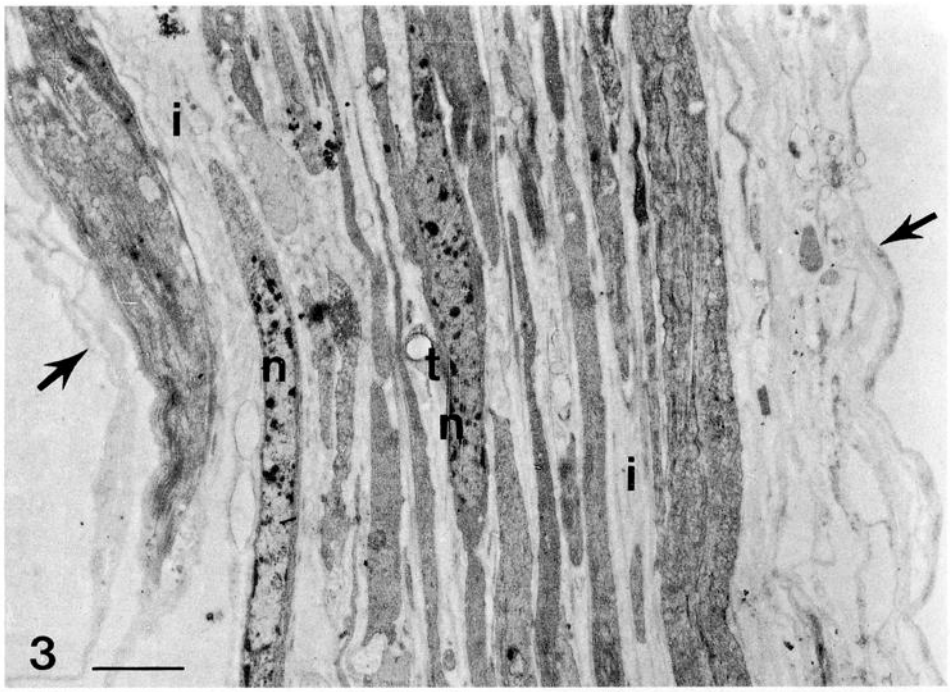
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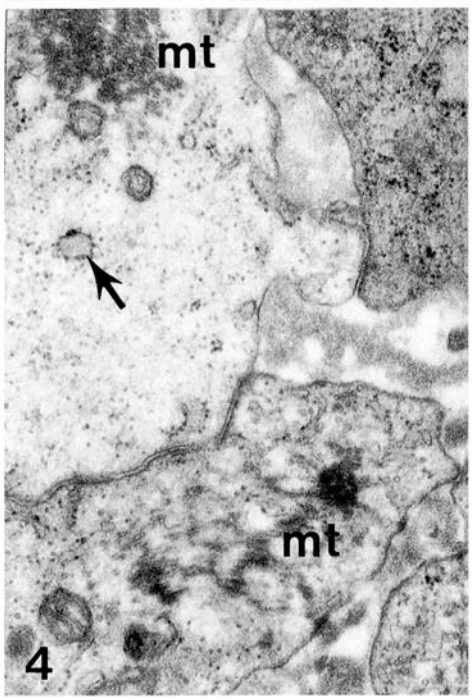
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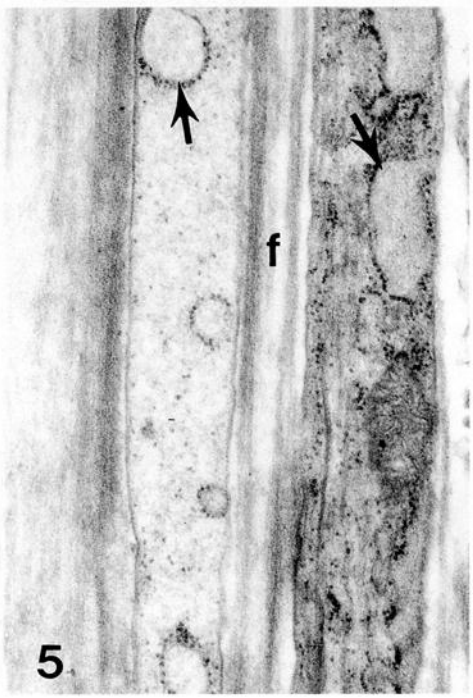
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Our electron microscopy investigations revealed that there are neither axons passing through the anterior branches of the H-organ (Fig. 1) nor any signs of neurosecretory elements, except for two small nerve fibre profiles that contain granules and were found in the H-organ of *Manduca*. Furthermore, the whole H-organ consists of only one cell type that resembles the fibroblasts of the mesenteric connective tissue described in *Periplaneta* (François, 1978) or those of the elastic connective tissue shown in the antennal heart of *Melolontha* (Pass, 1980). These cells contain numerous intracellular fibres that are oriented parallel to the long axis of the long, thin cells, and the ultrastructural constituents of protein synthesis, e.g. dilated cisternae of RER, which are usually found in fibroblasts, and free ribosomes. Moreover, the cells of the H-organ are embedded in an extended extracellular matrix, also consisting of fibrillar substance, whereas neurohaemal organs are generally characterized by an abundance of tightly packed neurosecretory fibres filled with electron dense granules (Gersch & Richter, 1981). Positive staining of total preparations of H-organs from *Galleria* with Weigert's stain for elastic connective tissue (not shown) augmented these findings. Staining methods used by Abou-Halawa & Sláma (1986) are not specific for neurosecretion. Therefore, it is possible that the proteinaceous material synthesized by fibroblasts may be responsible for the staining reactions mentioned (Abou-Halawa & Sláma, 1986). Elastic fibres are commonly stained by dyes used in neurosecretion techniques (Gersch & Richter, 1981).

In conclusion, it appears that H-organs consist of connective tissue and are not neurohaemal organs.

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Figs 3–5. H-organ of *Manduca sexta*. 3 – low power electron micrograph of a longitudinal section through an anterior arm of a *Manduca* H-organ. Elongated fibroblasts within an extracellular matrix bordered by a tunica propria (arrows) are seen. Scale bar = 2 μm. 4 – cross section showing fibroblasts with ribosomes and dilated cisternae of the RER (arrows) and bundles of microtubule profiles (mt). Scale bar = 1 μm. 5 – longitudinal section of two cellular processes accompanied by extracellular fibrils (f). Arrows show dilated cisternae of RER. Scale bar = 1 μm.