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REGULAR ARTICLE

Ashok Raina · Shirlee Meola · William Wergin · Michael Blackburn · Geetha Bali

Antennal ampullary glands of *Helicoverpa zea* (Lepidoptera: Noctuidae)

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Abstract In adult moths, the cephalic aorta terminates in an apical sack from which extends a pair of optic and antennal vessels that lie on either side of the esophagus, at the dorsoanterior surface of the brain. The base of each antennal vessel is dilated to form an ampulla that contains an oval mass of tissue, the antennal ampullary gland (AAG). An ultrastructural study revealed that the AAG of the corn earworm moth, *Helicoverpa zea* (Lepidoptera, Noctuidae), is composed of a single type of 40–50 parenchymal cells that produce secretory granules. The secretory material is released into the lymph channel of the ampullary vessel, suggesting that the AAG is an endocrine gland. Unlike the prothoracic gland and the corpus allatum, the AAG does not receive direct neural innervation; however, portions of the aortal muscle, associated with the ampullary wall, contain neurosecretory terminals and some of their products may also affect the AAG. No morphological differences were found between the AAG of males and females, with the exception that the glands in males were slightly larger. The function of the AAG remains unknown at this time. Because the AAG is located within the ampulla of the antennal vessel, one could assume that the product(s) of this gland may influence the response of the antennal sensory neurons to external stimuli.

Keywords Endocrine gland · Accessory circulatory organ · Antennal ampulla · Moth, *Helicoverpa zea* (Insecta)

A. Raina (✉)
USDA, ARS, Formosan Subterranean Termite Research Unit,
1100 Robert E. Lee Blvd., New Orleans, LA, 70179, USA
e-mail: araina@srrc.ars.usda.gov
Tel.: +1-504-2864290
Fax: +1-504-2864235

S. Meola
USDA, ARS, Southern Plains Agricultural Research Center,
2881 F&B Road, College Station, TX, 77845, USA

W. Wergin
USDA, ARS, Bldg 177B, Nematology Laboratory,
Beltsville, MD, 20705, USA

M. Blackburn
USDA, ARS, Bldg 011A, Insect Biocontrol Laboratory,
Beltsville, MD, 20705, USA

G. Bali
Department of Biotechnology,
Bangalore University, Bangalore, India

Introduction

The flow of hemolymph in insects is achieved for the most part by a single dorsal vessel, and accessory circulatory organs that assist in circulating hemolymph in peripheral organs. Because of the importance of the head appendages in feeding and receiving sensory stimuli, the cephalic circulatory systems of insects, especially those of cockroaches, several hymenopterans and a few moth species, have been extensively studied (Jones 1977; Matus and Pass 1999; Pass 1985, 1988, 2000). The cephalic aorta in most orders of insects contains antennal circulatory organs that facilitate the exchange of hemolymph to the vital sensory organs of these appendages. Many of these circulatory systems contain ampullae, also termed "antennal hearts," which are saccular organs that regulate circulation of hemolymph into the antennae. Lepidoptera have paired antennal ampullae located at the anterior boundary of the dorsal aorta and at the base of the antennal vessel that extends into each antenna (Hessel 1969). The antennal ampullae of Lepidoptera differ structurally from those of most other species of insects in that the former originate from the frontal sac of the aorta while that of the latter species originates from the hypodermis of the cuticle (Pass 1985). In Lepidoptera, each antennal vessel is dilated at its base. This dilation, referred to as an ampulla, contains a spherical mass of tissue first described in *Bombyx mori* by Selvatico in 1887. Schneider and Kaissling (1959), in their comprehensive study of the nervous system and blood vessels in the head and antennae of *B. mori,* refer to the ampulla as filled with a fine fibrous connective tissue-like mass of unknown function. However, they did not rule out a

possible endocrine function for this structure. Hessel (1969), in his study of the dorsal vessel and associated structures in Lepidoptera, reported that the tissue mass was particularly well developed in Noctuidae. He, however, speculated that the spindle-shaped cells in the mass were reminiscent of connective tissue and therefore not endocrine in nature.

In order to determine the morphology and possible function of the tissue found within the ampulla of the antennal vessel of Lepidoptera, we undertook an ultrastructural study of this organ in the corn earworm, *Helicoverpa zea*.

Materials and methods

Experimental animals and tissue

Helicoverpa zea were reared on artificial diet in the laboratory and maintained under a photoperiodic regime of 15 h light and 9 h darkness and corresponding temperatures of 26°C and 22°C respectively. Heads of both male and female *H. zea* were dissected in 0.1 M phosphate-buffered saline (PBS) containing 0.15 M sodium chloride at pH 7.2. The cephalic portion of the aorta was carefully removed and fixed for electron microscopy.

Electron microscopy

Tissue intended for electron-microscopic examination was processed by two different procedures. In the first procedure, the cephalic aorta with the gland was dissected in PBS and fixed for 48 h in a solution of 3.4% glutaraldehyde and 2.3% paraformaldehyde in 0.1 M PBS. The tissues were then rinsed in three changes of cacodylate buffer, postfixed in 2% osmium tetroxide in 0.1 M sodium cacodylate, rinsed in three changes of distilled water and dehydrated in a graded series of ethanol followed by 100% acetone. The tissues were embedded in Spurr's resin (Spurr 1969) and sectioned on an ultramicrotome either at 1 μm and stained with azure B-methylene blue (Hayat 1970) or thin sectioned (70 nm) and stained with 2% aqueous uranyl acetate and Reynolds lead citrate (Reynolds 1963). The thick resin sections were viewed and photographed on an Olympus A-H2 Vanox microscope, while the thin sections were viewed with a Phillips 300 transmission microscope at 60 kV.

In the second procedure, the cephalic aorta were fixed in 3% glutaraldehyde in 0.05 M phosphate buffer, pH 6.8. Following fixation, the samples were washed six times in the phosphate buffer, transferred to phosphate-buffered 2% osmium tetroxide, dehydrated through a series of ethanol solutions and gradually infiltrated and embedded in Spurr's resin. Thin sections (80–90 nm) were cut on a Reichert/AO Ultracut microtome, stained for 30 min in uranyl acetate followed by 5 min in lead citrate and viewed in a Hitachi H-500 transmission electron microscope.

Results

Histology

The terminology used for histological descriptions is that of Eaton (1988) and the ultrastructural terminology is that of Smith (1968). The cephalic portion of the aorta, lying along the dorsal surface of the esophagus, extends through the circumesophageal commissure and terminates in a widened structure called the frontal sac (FS). At the anterior surface of the brain, two pairs of vessels arise from the frontal sac: the optic vessels (OV) and the antennal vessels (AV) (Fig. 1A). The optic vessels extend dorsolaterally over the brain, terminating as inflated sacs

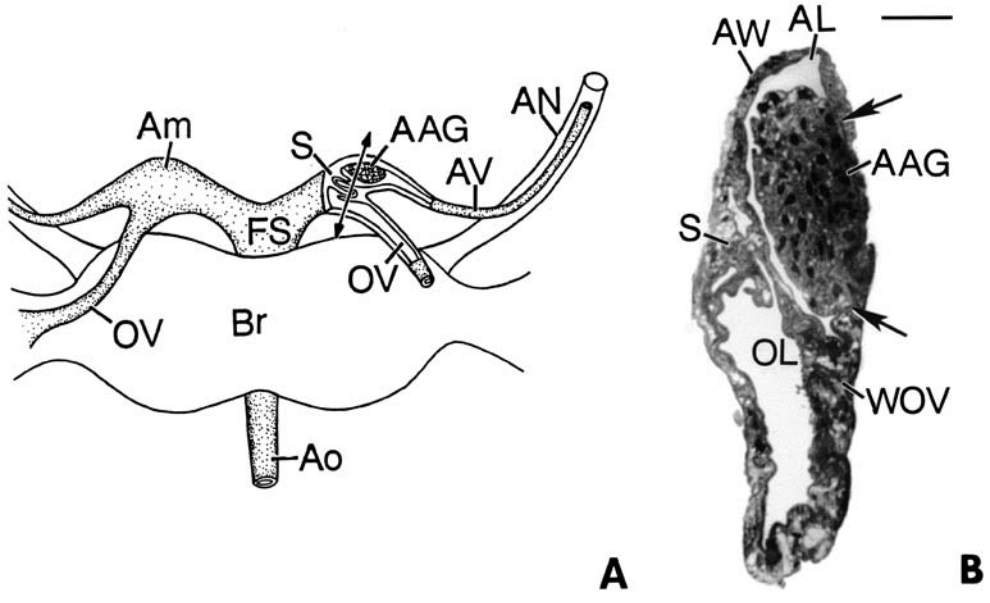

Fig. 1 **A** Schematic dorsal view of the morphology of the circulatory organs in the head of *Helicoverpa zea* (*Am* ampulla, *AN* antennal nerve, *Ao* aorta, *AV* antennal vessel, *Br* brain, *AAG* antennal ampullary gland, *FS* frontal sac, *OV* optic vessel, *S* septum). *Double-headed arrow* indicates the angle of section through the antennal ampulla of **B**. **B** Transverse section through the optic and antennal vessels. The antennal ampullary gland lies in the ampulla of the antennal vessel, attached (*arrow*) to the wall of the ampulla (*AW*). A broad, incomplete septum (*S*) separates the ampullary lumen (*AL*) from the lumen of the optic vessel (*OL*) (*WOV* wall of optic vessel). *Scale bar* 100 μm

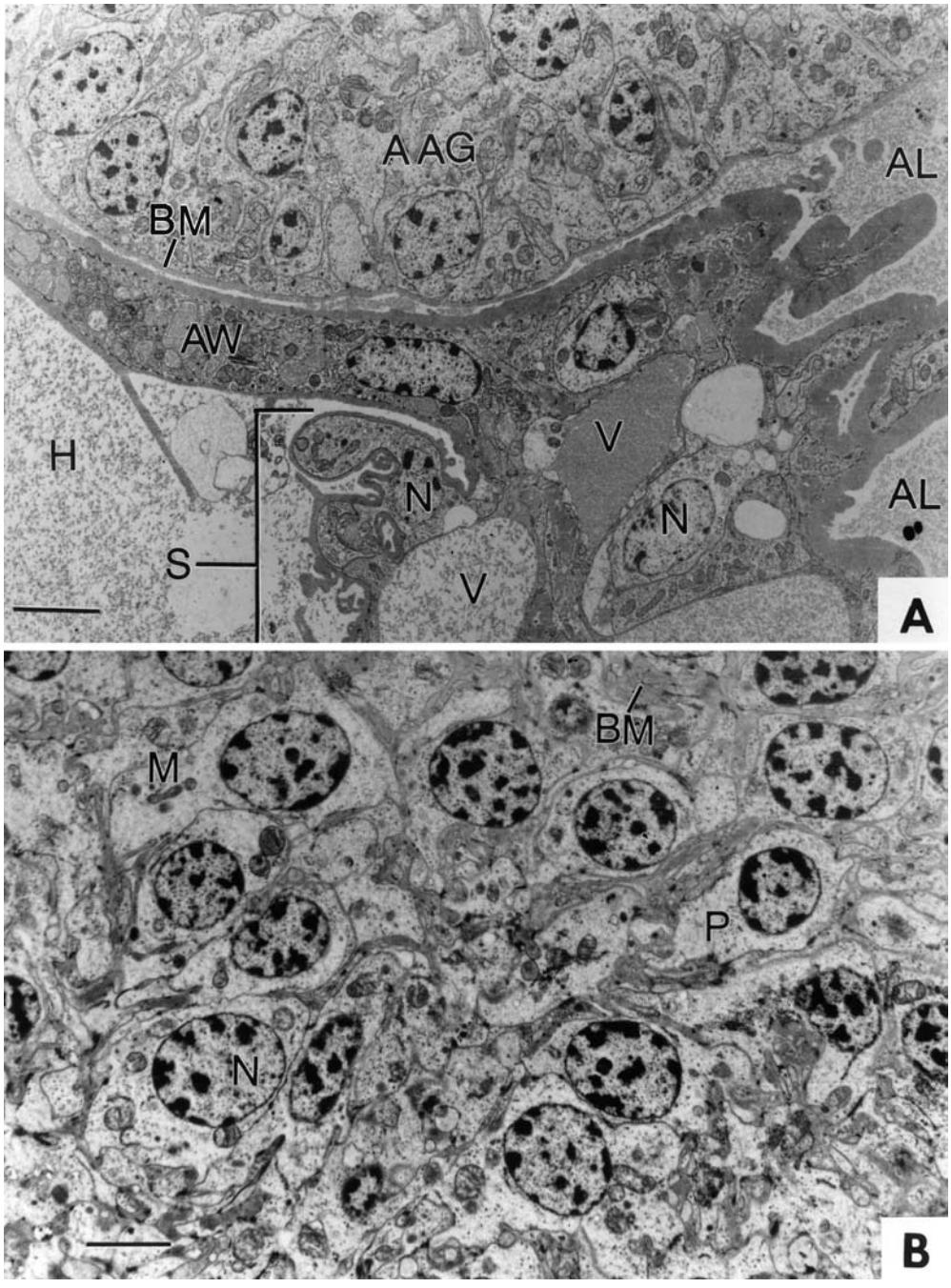

Fig. 2A, B Transmission electron micrographs of the antennal ampullary gland of *H. zea*. **A, B** A section through the antennal vessel reveals that the surface of the AAG is covered only by a basement membrane (*BM*) and that the septum (*S*) separating the antennal and optic vessels is formed by a series of infoldings of the ampullary wall (*AW*), and contains large vacuoles (*V*) filled with flocculent material of different electron density (*H* hemocoel, *N* nucleus of epithelial cell of septum). **B** AAG showing the compact structure of this tissue. The nuclei (*N*) of the parenchymal cells (*P*) of the AAG are round to oval and contain abundant chromatin clusters. Mitochondria (*M*) are scattered throughout the cytoplasm. The parenchymal cells, with their fibrous, moderately dense basement membrane (*BM*) that lines the intercellular channels, are the only cellular components of this tissue. *Scale bars* 10 μm

on the dorsal surface of this organ. The proximal portion of each antennal vessel is enlarged, forming an ampulla approximately 100 μm in diameter from which arises the narrow tubular portion of the antennal vessel. A white, ovoid mass of tissue consisting of closely packed cells, ensheathed by a thick fibrous basement membrane (BM), lies within the lumen of each ampulla (Figs. 1A, B, 2A). We have called this tissue the antennal ampullary gland (AAG). These structures, approximately 70×90 μm in size, are slightly larger in the male *H. zea*. No difference was detected between the sexes in the morphology of the AAG. Thick (1.0 μm) resin sections reveal the AAG to be a mass of 40–50 cells with prominent nuclei and located at the base of the antennal vessel, adjacent to the septum (S) that separates the antennal ampulla from the lumen of the optic vessel (Figs. 1A, B, 2A). The AAG are loosely attached to the wall of the ampulla.

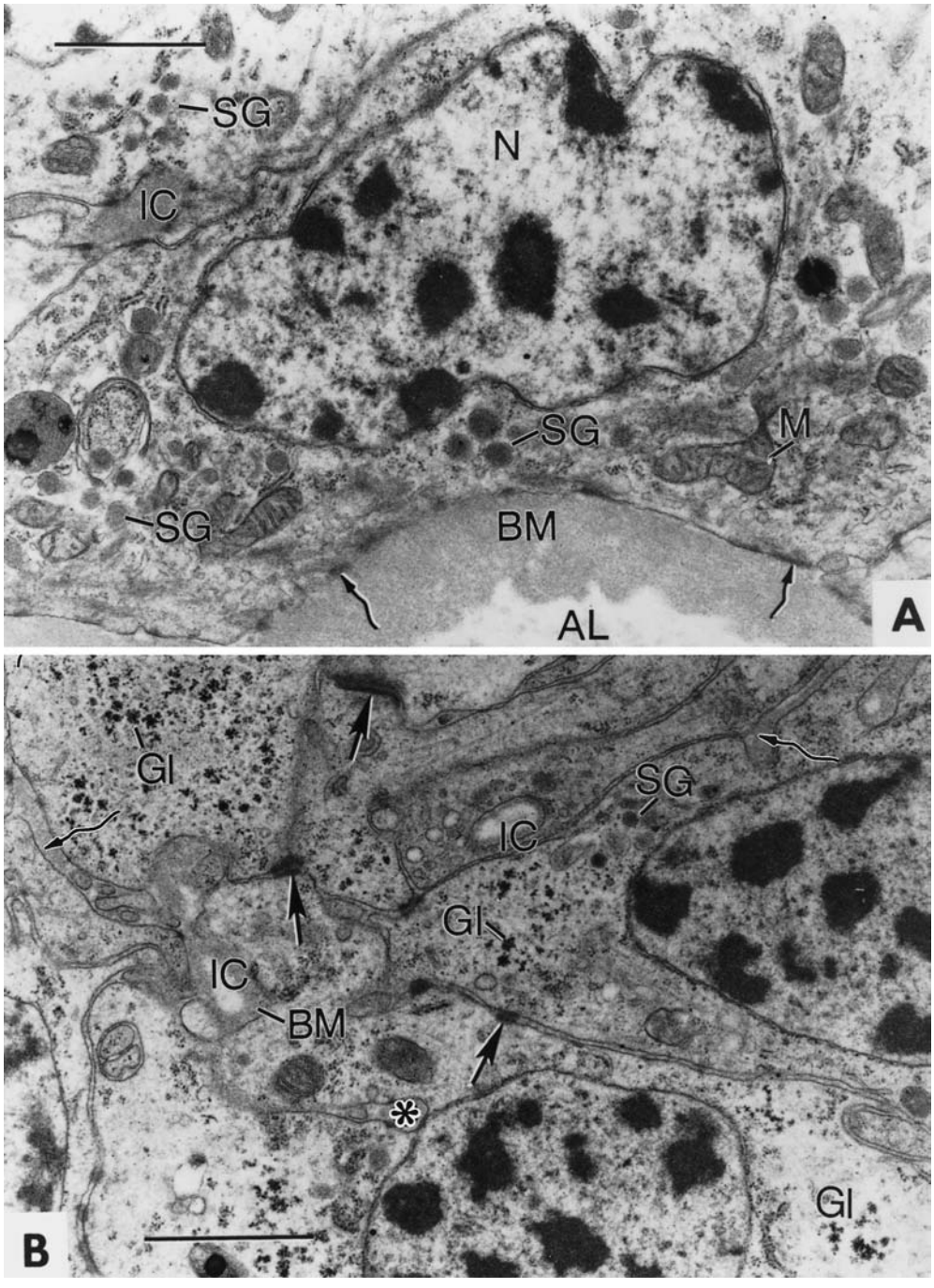

Fig. 3A, B Antennal ampullary gland of *H. zea.* **A** Two parenchymal cells of the AAG-containing clusters of membrane-bound, moderately electron-opaque secretory granules (*SG*) lying among short segments of rough endoplasmic reticulum. This cell has numerous mitochondria. A thick basement membrane, connected to the plasma membrane of the parenchymal cell by hemidesmosomes (*wavy arrows*), ensheathes the gland and covers the surface of the intercellular channels (*IC*). **B** In addition to the secretory granules, some of the parenchymal cells also contain glycogen granules (*Gl*). Segments of the intercellular channels (*IC*) are lined with the basement membrane (*wavy arrows*). These channels lie between adjacent cells, with desmosomes (*arrows*) linking the cell membranes. The intercellular channels can extend to the surface of the outer nuclear membrane (*asterisk*). *Scale bars* 1 μm

Ultrastructure

Antennal ampullary gland

The cluster of cells in AAG have well-defined cell membranes and round to ovoid nuclei that contain numerous clusters of chromatin scattered throughout the karyoplasm (Figs. 2B, 4A). Occasionally, a nucleus with a more irregular shape is also found (Fig. 3A). The gland appears to consist of only one type of cell, which is relatively small, approximately 2.4–2.8 μm in diameter. Long, sinuous processes of adjacent AAG cells interdigitate, giving this gland a compact appearance (Fig. 4A). Electron-dense, membrane-bound granules, 120–200 nm in diameter, are present in the cytoplasm of these cells, along with numerous mitochondria, short segments of rough endoplasmic reticulum, free ribosomes, Golgi complexes and glycogen granules (Fig. 3A, B). The electron-dense granules accumulate near the periphery of the AAG cells (Fig. 3A). Intercellular channels (IC, Figs. 3A, wavy arrows, B, 4A), originating from the surface, extend throughout the gland, possibly resulting in access to nutrients carried in the hemolymph, as well as removal of waste products and secretory material released

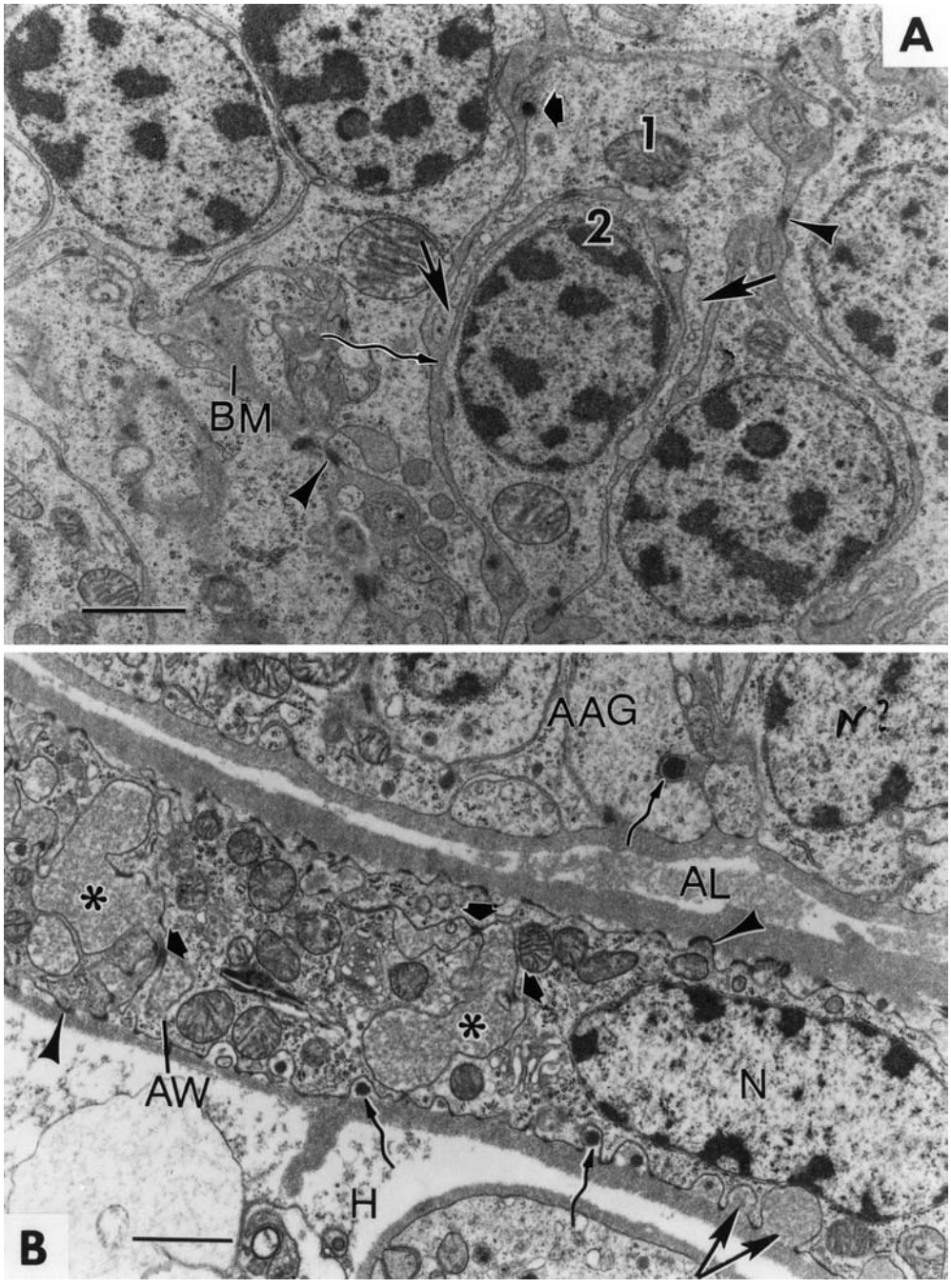

Fig. 4A, B Antennal ampullary gland of *H. zea.* **A** Electron micrograph showing the long cellular process of the AAG cells (*arrows*) and the sinuous channels between adjacent cells (*wavy arrow*). *Arrows* point to processes of cell *1* extending around the cell body of cell *2*. An electron-dense sphere appears to be in the process of being released from the surface of cell 1 (*short arrow*) into an intercellular channel. At intervals, the apposing plasma membranes of the parenchymal cells are linked by desmosomes (*arrowheads*) that occlude the intercellular channels. Basement membrane filling intercellular channel. **B** The ampullary wall of the antennal vessel consists of unicellular layers of epithelial cells separated by intercellular channels filled with a flocculent material (*asterisks*). These channels extend from the outer surface of the ampullary wall (*arrows*), contain electron-dense bodies (*wavy arrows*) and, like the IC of the AAG, are compartmentalized by desmosomes (*broad arrows*) (*N* nucleus of epithelial cell of ampullary wall). *Scale bars* 1 μm

from these cells. These channels are lined with a basement membrane (Figs. 3B, 4A). The cell membranes of adjacent gland cells are connected at intervals by desmosomes (arrows, Figs. 3B, 4A). In addition to the secretory granules, glycogen granules are also found in the AAG cells (Fig. 3B). The cytoplasm of the parenchymal cells at the periphery of the gland has relatively smooth margins and is tightly bound to the thick basement membrane ensheathing the gland, by hemidesmosomes (wavy arrows, Fig. 3A). No tracheae or nerves were found associated with the AAG.

Ampullary wall

The ampullary wall (AW) serves as a sheath surrounding the AAG and thus influences the type of material entering or exiting the ampullary lumen (AL) (Figs. 2A, 4B). The AAG does not float freely in the lumen; instead, one side of the gland is attached to the ampullary wall by filaments of the apposing basement membrane of the AAG and the AW, forming strands that interconnect the two structures with the remaining surface of the AAG projecting into the antennal lumen (Fig. 1A, B). The AW is formed as an extension of the frontal sac (FS) and is contiguous with

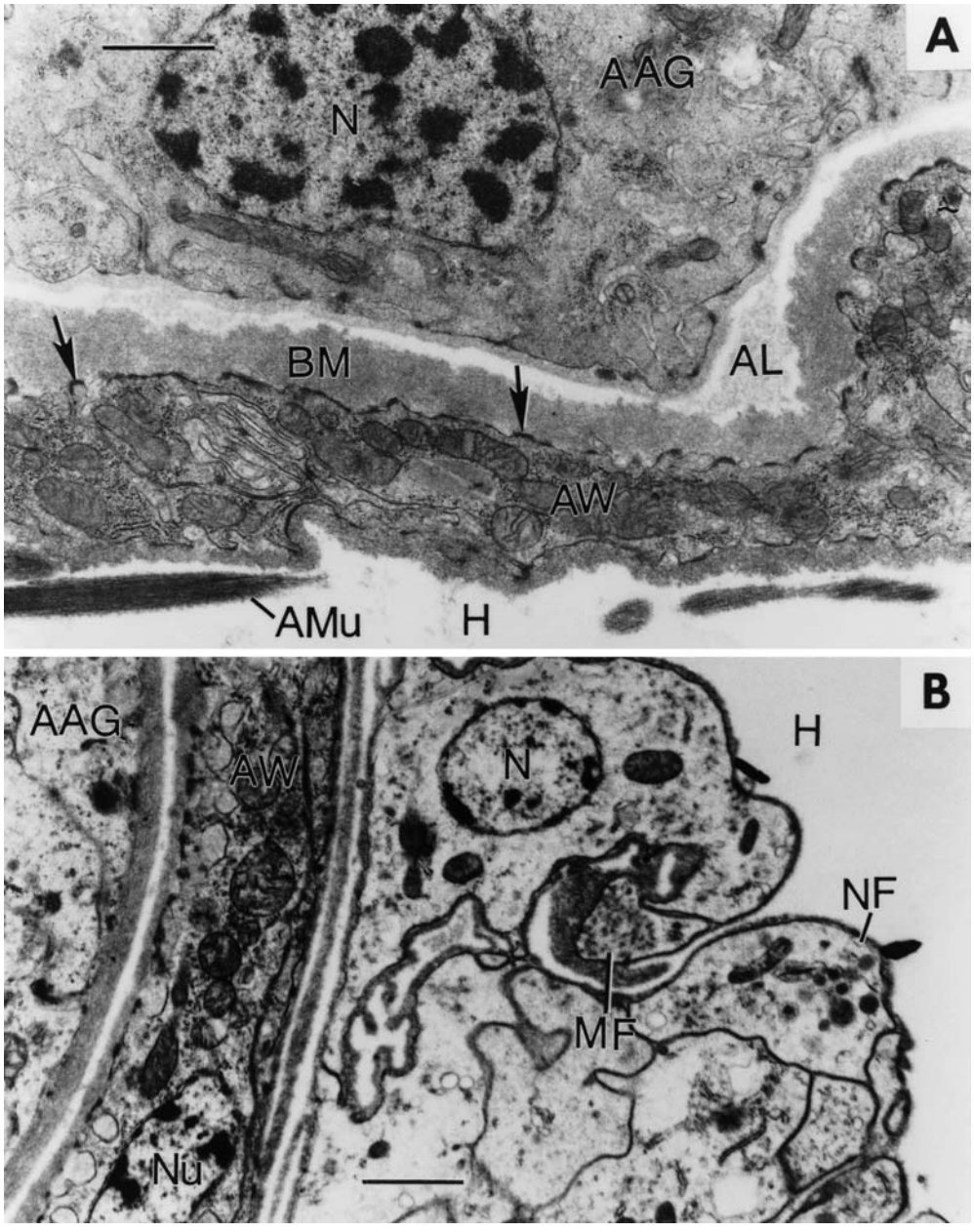

Fig. 5A, B Antennal ampullary gland of *H. zea.* **A** Filaments of aortal muscle (*AMu*) extend over the surface of the outer ampullary wall. *Arrows* indicate hemidesmosomes connecting the plasma membranes of the epithelial cells to the inner basement membrane. **B** Neurosecretory fibers (*NF*) terminate on the surface of the aortal muscle (*MF* muscle fiber, *N* nucleus of aortal muscle fiber, *Nu* nucleus of epithelial cell of ampullary wall). *Scale bars* 1 μm

the wall of the optic vessel (WOV) (Fig. 1A, B). The antennal and optic vessels are separated at the point of divergence from the frontal sac by a septum (S) (Figs. 1A, B, 2A). Folds of the ampullary wall project into the lumen of the ampulla from the septum. Extensive invaginations of the hemocoel and lumen of the ampulla occur as vacuoles within the folds of the septum. These invaginations become enclosed by cytoplasm of the septal epithelial cells and by the thick basement membrane overlying these cells (Fig. 2A). The contents of the vacuoles are a flocculent material that differs in electron density (Fig. 2A) depending upon the source of the vacuole (hemocoel or luminal) and possibly by condensation of the fluid within the vacuole due to uptake of the fluid component of the vacuoles by the septal epithelial cells. Less prominent versions of these vacuoles form channels containing flocculent material, throughout the length of the ampullary wall (Fig. 4B). Hemidesmosomes connect the ampullary wall to the thick basement membrane, covering both the inner and outer surfaces of the ampulla (Figs. 4B, 5A). Intermittent segments of aortal muscle are closely apposed to the outer surface of the ampullary wall (Fig. 5A, B). Neurosecretory fibers (NF), which form an extensive arborization over the aortal muscle, create neurosecretory motor junctions on these muscle fibers, especially near the nucleus of a myofibril (Fig. 5B). The neurosecretory fibers are derived from a single nerve, whose origin could not be ascertained. No tracheae are found within the wall of the antennal vessel, although they are found adjacent to the

antennal vessel and tracheoles terminate in the adjacent aortal muscle.

Discussion

Both structurally and functionally, the antennal ampulla of Lepidoptera differs from that of other insects in which this organ has been studied (Pass 1998; Wassenthal 1998). Like other insects, the antennal heart of Lepidoptera consists of an ampulla and a tubular vessel (Pass 1998), but the antennal ampulla of Lepidoptera differs from that of other insects in that it contains a mass of tissue which appears to have a secretory function, and thus we termed this tissue the antennal ampullary gland (AAG). In addition, the antennal ampulla of Lepidoptera also differs from that of most insects, in that it originates directly from the frontal sac of the dorsal aorta (Wassenthal 1998) rather than from the hypodermal wall of the integument (Pass 1985). The antennal ampulla of Lepidoptera contains a septum which, according to Pass (2000), appears to be present only in true pulsatile organs where it may regulate blood flow. Therefore, since a septum is present, fulfilling the function of directing blood flow, the possibility that the cluster of cells in the antennal ampulla of Lepidoptera also acts as a mechanism for flow regulation as suggested by Schneider and Kaissling (1959) would be redundant. An ultrastructural examination revealed that the AAG consists of a single type of cell that is much smaller than those in other endocrine glands of Lepidoptera. For example, each cell of the prothoracic gland of *Diatraea grandiosella* is 35–60 μm (Yin and Chippendale 1975) and each cell of the corpus allatum of *Hyalophora cecropia* is 55 μm (Waku and Gilbert 1964) or roughly half the size of the entire AAG in Lepidoptera. The ultrastructure of the parenchymal cells of the AAG revealed the presence of abundant rough endoplasmic reticulum and Golgi complexes from which electron-dense, membrane-bound spherical vesicles originate. The secretory granules are within the size range (100–500 nm) reported for endocrine secretory granules (Lehane 1998). The electron-dense granules produced by the AAG cells accumulate at both the periphery of the gland and where intercellular channels extend into the cortex. The granules appear to diffuse through the cell membrane in a merocrine type of release. The overall appearance of the AAG is similar to that described by Johnson et al. (1985) for the corpora allata of the cockroach, *Diploptera punctata*. As in the latter, the AAG contain parenchymal cells that are irregularly shaped with long, cytoplasmic processes that interdigitate with those of neighboring cells, and where their plasma membranes are in close proximity they are joined by desmosomes. In both the AAG and the corpus allatum of Lepidoptera, these cellular projections are interconnected by desmosomes (Meola and Meola 1983) which provide tissue stability and serve as selective permeability barriers (Alberts et al. 1983). In addition, hemidesmosomes occur on the surface of both CA and AAG cells where their free surface is in contact with the supporting tissue whether connective tissue or basement membrane. The parenchymal cells of the AAG differ from the CA cells in that they do not contain abundant smooth endoplasmic reticulum as is found in the parenchymal cells of the corpus allatum of Lepidoptera (Waku and Gilbert 1964); instead they have an abundance of short segments of rough endoplasmic reticulum as reported in the parenchymal cells of prothoracic glands of moths (Yin and Chippendale 1975), indicating that these cells may be producing a protein/peptide product rather than a lipid-based product. In addition, the AAG cells also contain glycogen granules as do the endocrine cells of the prothoracic glands of insects. However, unlike the cells of the prothoracic gland, those of the AAG do not have extensive invaginations of their plasma membranes. Alternately, the AAG has a system of intercellular channels that provide the cells of the gland with access to the hemolymph.

Like the AAG, the ampullary wall also contains an extensive system of extracellular channels. These channels are filled with a flocculent material and extend the width of the cells, and thus may act as a conduit for material in the hemocoel and ampullary lumen. The system of channels containing flocculent material in the ampullary lumen is similar in appearance to those in the cytoplasm of fibroblasts in the connective tissue of the ejaculatory duct of the adult locust, *Locusta migratoria* (Ashhurst 1985). Although the ampullary wall is both tracheated and receives direct neural innervation, as do the corpus allatum and prothoracic glands of insects (Waku and Gilbert 1964; Yin and Chippendale 1975; Meola and Meola 1983), the AAG itself receives neither, indicating that exchange of respiration and hormonal regulation are hemolymph borne via the extensive system of intracellular channels within this gland. Because dissected material was used for the ultrastructural analysis in this study, it was not possible to determine the origin of the nerve that innervates the ampullar region. However, Eaton (1988) reported that in Lepidoptera the corpora cardiaca give rise to a pair of dorsal corpora cardiaca nerves (NCCD). A fiber from each NCCD joins a large arterial nerve (ArN) that originates from the dorsal surface of the antennal lobe. The ArN appears to innervate both the optic and antennal vessels. Pass et al. (1988), who used cobalt backfill, reported that in the cockroach *P. americana* an antennal heart nerve extending from the retrocerebral complex originated from soma located in the anteriormost region of the subesophageal ganglion.

The function of the AAG cells may be analogous to the antennal neurosecretory cells of the mosquitoes (Meola et al. 2000; Meola and Sittertz-Bhatkar 2002) in modulating the sensory input of environmental stimuli. Meola et al. speculated that in mosquitoes these cells in turn were affected by feedback mechanism of products including hormones of other cells. Further studies are needed to determine the chemistry of the product or products of the AAG and the physiological role of this organ in Lepidoptera.

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