Neurosecretory Cells in the Abdominal Ganglia of Larvae of *Lucilia caesar* (Diptera)

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Summary

Ten neurosecretory cells can be found in each of the first 5 abdominal ganglia of the larva of *L. caesar* L. These are arranged as two lateral pairs and a single ventral cell in each half of a ganglion. Half the lateral cells go through a cycle of secretory activity before pupation in developing larvae. In diapause larvae while half the lateral cells are in a resting phase the remainder are in various stages of secretory activity. The destination of the product is not known. Chemically this product is, unlike that of brain neurosecretory cells, PAS-positive. It may contain a glyco- or phospholipid or lipoprotein component.

Introduction

Accounts of neurosecretory cells in the ventral ganglia of insects are very rare in comparison with the numerous descriptions which exist of such cells in insect brains. They were first noted in the ventral ganglia of Lepidoptera by Day (1940), and were described in a cockroach by Scharrer (1941). Their occurrence has since been observed in the sub-oesophageal ganglia of Ephemeroptera (Arvy and Gabe, 1952a, b), of Odonata (Arvy and Gabe, 1952c), of *Tenebrio* (Arvy and Gabe, 1953), of *Bombyx mori* (Bounhiol, Arvy and Gabe, 1953), and of *Iphita* (Hemiptera) by Nayar (1953). Lhoste (1953) indicated that the ganglia of the ventral nervous system of *Forficula* contained neurosecretory cells but he did not describe them. Wigglesworth (1955), discussing humoral stimuli concerned in the breakdown of the thoracic gland of *Rhodnius*, suggested that 'perhaps the neurosecretory cells which are so conspicuous in the thoracic and abdominal ganglia of *Rhodnius* and other insects may be concerned'.

Neurones are identified in this study as neuro-secretory cells by their morphological similarity to certain brain-cells of this kind, by their staining characteristics, and by the evidence in some of the axonal transport of secretory granules.

Material and Methods

Serial sections were prepared from material fixed in Bouin, embedded in ester wax, and cut at 6 μ. Sections of the ganglia of larvae in diapause were stained with chrome-haematoxylin-phloxin (Gomori, 1941) and paraldehyde-fuchsin (Halmi, 1952), in both cases after oxidation with potassium permanganate. The histochemical tests employed were alcian blue with neutral red or haemalum-eosin counterstaining (Steedman, 1950); periodic acid / Schiff
(PAS) with and without pretreatment of sections with diastase (McManus, 1946); and toluidin blue for metachromasia (Pearse, 1953). McManus's Sudan black B test for lipids (Pearse, 1953) was applied to material fixed in a formaldehyde-calcium solution. Ganglia of non-diapause prepupae, killed at 5 stages between the cessation of feeding and puparium formation, were sectioned and stained with paraldehyde-fuchsin and the PAS test was applied to these also.

**RESULTS**

Neurosecretory cells could not be identified in the sub-oesophageal or thoracic ganglia of *L. caesar* larvae. A few phloxinophil cells were seen in the sub-oesophageal ganglia of diapause larvae. These might have been in a resting state, like Group 4 cells in the brain (Fraser, 1957), but there was no evidence of a secretory function in the non-diapause prepupal ganglia.

Neurosecretory cells occur in abdominal ganglia 1 to 5. These are pyriform, unipolar neurones measuring 20 to 25 μ long by 10 to 14 μ wide and have a central nucleus about 8 μ in diameter. These cells are symmetrically arranged in each ganglion (fig. 1). Four lie on each side at the ventrolateral angle of the ganglion (cells 1–4) and one on each side in a ventral position (cell 5). The four lateral cells can be separated into an upper posterior (cells 1 and 2) and a lower anterior pair (cells 3 and 4). Activity of the corresponding cells on each side is normally, though not invariably, in phase. The lateral cells in which granules of product are found are classified as Type A and those lacking such a product as Type B. Cells 1 and 2 are usually Type A and cells 3 and 4 Type
B, but any pair may be of the first type and, rarely, 3 of the 4 lateral cells are of one type.

**Neurosecretory cells in abdominal ganglia of diapause larvae**

In sections stained with paraldehyde-fuchsin, secretory granules are found in Type A cells and in cell 5. In cell 5 the granules are rather small (up to 0.5 \( \mu \)) separate, and distinct, and the general facies resembles that of a brain-cell belonging to Group 6 (Fraser, 1957). In some Type A cells there is a high concentration of minute granules (about 0.25 \( \mu \)) while in others the granules are large (up to 0.75 \( \mu \)) and very distinct, but the total quantity of product appears to be less in the latter. The association of granules with vacuoles, notable in most groups of brain neurosecretory cells, is also found in Type A cells. The cytoplasm of Type B cells is uncoloured by paraldehyde fuchsin and there are no positively stained inclusions. There are several large vacuoles in each cell.

After chrome-haematoxylin-phloxin staining the cytoplasm of Type B cells proves to be strongly and uniformly phloxinophil. In this they resemble brain Group 4 cells in diapause. The Type A cells may contain very distinct blue-black granules; the rest of the cell, apart from the nucleus, is completely unstained. Alternatively, the granules may be minute and less easily discernible against a grey-purple ground cytoplasm.

The cells with large distinct granules detected by both staining procedures undoubtedly correspond. The ventral cell 5 is not differentiated by the second stain; this is also true of brain Group 6 cells.

The secretory granules of Type A cells do not have a selective affinity for alcian blue, but they are PAS-positive. Pretreatment with diastase proves that the carbohydrate component is not glycogen. Sudanophil granules are usually found in two of the lateral neurones. These are certainly Type A cells. Sudanophil material occurs in variable amounts in other neurones but does not correspond in form, concentration, or distribution to the granules found in Type A. With toluuidin blue the Type A cells stain orthochromatically but the Type B cells exhibit metachromasia (beta): they stain a uniform violet colour. The cytoplasm of the B cells contains an appreciable concentration of a substance not found in any other neurones in the ganglia. This substance cannot be a mucoprotein or mucopolysaccharide since it is not PAS-positive. The results obtained with Type A cells are consistent (Pearse, 1953) with the presence in the granular product of a glyco- or phospholipid or lipoprotein component.

**Neurosecretory cells in the abdominal ganglia of non-diapause larvae**

The first signs of neurosecretory activity appear about 8 h after the cessation of feeding, in the first abdominal ganglion. With paraldehyde fuchsin staining a varying density of small granules can be seen in Type A cells; but Type B cells, identifiable by position and structure, are not selectively coloured. Within 3 days (at 25\(^\circ\) C) the full complement of 20 Type A cells can
be counted. These contain large separate granules, 0.5 to 0.75 μ in size. In the final stage of prepupal life, as the white pupa forms, the amount of product in the cells is diminished and the number of cells of this type has decreased to an average of 15. Type A cells have been found in these ganglia in positions 1 and 2, 1 and 3, and 3 and 4, the other two laterals being in each case Type B. Only 3 or 4 cells that contain positive granules have been found, per individual, in the cell 5 position.

The granules in Type A cells are again stained blue with chrome-haematoxylin while the cytoplasm of Type B cells is, as in diapause ganglia, uniformly phloxinophil. The histochemical tests indicate that the product is again sudanophil and strongly PAS-positive.

**Conclusions**

The axons of these cells take a circuitous route when they enter the medulla and it has not proved possible so far to trace an axon into its nerve. It is improbable that the destination of the granules, seen in axons in numerous sections, is a neurohaemal organ. The lateral and dorsal abdominal nerves of these ganglia all appear to terminate in the muscles of the body-wall and it seems possible, in the light of Finlayson's (1956) work on the abdominal muscles of Lepidoptera, that the secretion of these cells plays some part in the degeneration and regeneration of these muscles during metamorphosis.

The two variants of Type A cells noted in diapause larvae might be functionally different cells, but as the non-diapause material shows, they probably represent different phases in the secretory cycle of similar cells. The Type B cell may represent either a resting phase in the cycle of a Type A cell or a functionally different kind of cell, which is inactive in both types of prepupae but due to function later in the individual's life, or active but producing a non-granular or colloid phloxinophil secretion. If the author's interpretation of the state of Group 4 brain cells during diapause (Fraser, 1957) is accepted, then it can be concluded that the Type B cell is in the resting state. The fact that any one of the 4 lateral cells, each of which has a fixed and definite location in the ganglion, may belong to either category indicates that A and B do in fact represent two aspects of a single type of cell.

Those cells which are active in the ganglia of non-diapause prepupae go through a cycle of activity which appears to be synchronous with the prepupation phase of activity in brain neurosecretory cells. The cells which are active during diapause produce a secretion which is chemically similar to that found in developing prepupae. They appear to be capable of monotonous activity but this is not sustained for the duration of diapause by all cells. Hence during diapause any one of the 4 lateral cells may be found in the resting state or may correspond in appearance with the waxing or waning phases of activity of cells in non-diapause prepupae.

Gabe's (1954) general statement that the neurosecretory product of insects has a carbohydrate component other than glycogen does not hold in the case of brain cells but is true for those in the abdominal ganglia, which are the only
neurosecretory cells found in the *L. caesar* larva whose secretion is PAS-positive.

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