The Direct Release of Neurosecretory Material from the Cell in the Pars Intercerebralis of *Monema flavescens* (Lepidoptera : Heterogeneidae)

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This paper deals with the direct release of neurosecretory material from neurosecretory cells in the pars intercerebralis of *Monema flavescens* WALKER. In the perineurium of the pars intercerebralis, a few dendritic-like cytoplasmic processes, originating from the neurosecretory B cells, were found. In the diapause stage, no neurosecretory granules were found in the cytoplasmic processes. However, as diapause started to terminate, neurosecretory granules were transferred from the cell body to the cytoplasmic processes. As a result, many neurosecretory granules were found in the cytoplasmic processes. These findings suggested the transport of neurosecretory granules by way of cytoplasmic processes. On the bases of these observations, a hypothetical releasing theory of neurosecretory granules was proposed

INTRODUCTION

In general, neurosecretory material synthesized in the cell body is transported only to the endings from which release occurs. With respect to the transport of neurosecretory materials, two main pathways have been distinguished. Axonal transport is a well-known pathway among the two. This is based on the following evidence: (1) Stainable material along axons of neurosecretory cells is readily demonstrated cytologically and can be interpreted as the pathway of secretory material (ARVY, et al., 1953; THOMSEN, M., 1954); (2) There is an accumulation of secretory material at the proximal end of a transected axon of neurosecretory cells, accompanied by a depletion of secretory material at the distal end of the cutting (SCHARRER, 1952). Further, a ligature made on a nerve containing neurosecretory axons results in accumulation of secretory material on the side near the relevant cell bodies (THOMSEN, E., 1954; HARKER, 1960); (3) *In vitro* observation of neurosecretory material passing along axons has been reported (CARLISLE, 1953; HILD, 1954). Release from the perikaryon itself, as well as release along the axon, is conceivable. The second pathway is direct release from the cell body however, it does not represent the most common form. This type is assumed by virtue of the fact that vacuoles and vesicles are found in the peripheral region of the cytoplasm with the disappearance of neurosecretory material (WIGGLESWORTH, 1940; REHM, 1950; WIKLIAMS, 1952; ICHIKAWA, 1955; KOBAYASHI, 1957). NISHITSUTSUJI-UWO (1961) reported that the release of neurosecretory material from the cell body may take place directly into canaliculi. Further, in crustacea, MATSUMOTO (1958) reported that after transection of neurosecretory neurons, accumulation of secretory material does not occur. This result strongly suggested that neurosecretory cells are able to release their secretory material

from the cell body.

In a previous paper (TAKEDA, 1972b), it was demonstrated that in the prepupa of *Monema flavescens* the neurosecretory B cells in the pars intercerebralis become active regarding the release of their secretory granules from the cytoplasm in the period of diapause break. Further, a possibility was postulated that neurosecretory granules are released directly from the pars intercerebralis (TAKEDA, 1970 a, b). This finding lead the author to investigate a pathway other than axonal transport in the perineurium of the pars intercerebralis. Therefore, the present paper deals with a problem of direct release of neurosecretory materials from the cell body.

MATERIALS AND METHODS

Materials used in this study were prepupae of the slug moth, *Monema flavescens* WALKER. They were collected in the field and were kept under natural conditions. The prepupae of this species enter diapause in late September and terminate diapause at the end of January. They were fixed at intervals from September to the following March. Some of the prepupae were also incubated at 20°C to estimate the degree of diapause intensity (TAKEDA, 1970, 1976 c).

For light microscopic observations, the brains were fixed for 24 hours with Bouin. After fixation, they were dehydrated with butyl alcohol, embedded in paraffin wax and sectioned at 3 μ m. The serial sections were stained mainly with Clark's paraldehyde fuchsin. For electron microscopic observations, the brains were rapidly extirpated under a dissecting microscope, immediately prefixed with cold 2.5% glutaraldehyde in phosphate buffer (pH 7.4) for 90 minutes and postfixed with cold 2% osmium tetroxide in Veronal buffer (pH 7.4) for 3 hours. They were passed through cold 50%, 70% and 90% ethyl alcohol for 5 minutes, respectively, and then immersed in absolute ethyl alcohol for 45 minutes at room temperature. After dehydration, they were placed in absolute acetone for one hour and were gradually infiltrated in Epon 812 (LUFT, 1961). The Epon blocks were trimmed and sectioned by means of an ultramicrotome with glass knives. For staining, lead citrate was used (REYNOLDS, 1963). The micrographs were taken with a JEM-T63 electron microscope.

RESULTS

The brain of *M. flavescens* was surrounded by a thick perineurium about 30–40 μ m (Fig. 1A, P). There were two types of neurosecretory cells in the pars intercerebralis, the A cells and the B cells. Each neurosecretory cell consists of a cell body and a long axon. It is the B cells that are linked with the diapause break. Neurosecretory granules of the B cells stained pink with Gomori's, deep red with Mallory's, and deep purple with Clark's staining, respectively (Fig. 1). The A cells were not so acidophilic as the B cells; further, the A cells were usually larger than the B cells. So, the B cells were easily distinguished from the A cells (TAKEDA, 1972 b).

In the perineurium of the pars intercerebralis, there were a few dendritic-like cytoplasmic processes which originated from the neurosecretory B cells. They spread branches toward the outside of the perineurium. In the perineurium, no cytoplasmic processes came from the neurosecretory A cells. In the cytoplasmic processes, cell organellas, especially mitochondria (Fig. 2 and Fig. 4 A) and glycogen particles,

Fig. 1. Higher magnification of the neurosecretory B cell with elongated cytoplasmic processes (CP) directed to the perineurium (P). G, neurosecretory granule; Gl, glycogen particle; N, nucleus. $\times 16,000$. Inserted Fig. A shows the neurosecretory B cell (B) and the perineurium (P) in the half area of the pars intercerebralis.

were found as in the cell body. The cytoplasmic processes became narrow with their penetration to the outer perineurium (Figs 2 and 4). However, their breadth varied near the cell body as found in Fig. 1.

As reported previously (TAKEDA, 1972 b), the cytoplasm of the B cells was filled with many neurosecretory granules which were synthesized in Golgi apparatus from the beginning of diapause in late September. These granules disappeared from the cell body with the progression of diapause break, leaving vesicles and vacuoles in the cytoplasm.

In the stage of diapause (Fig. 4 A) and post diapause, there was no neurosecretory granules in the cytoplasmic processes. But as diapause started to terminate, many neurosecretory granules appeared in the cytoplasmic processes (Figs 2 and 3). They were naked granules, not coated with membrane; they lost their coated membrane in the cytoplasm at the beginning of migration (TAKEDA, 1972 b). In this stage, there were few neurosecretory granules in the cell body of the B cells. Therefore, it is certain that neurosecretory granules found in the cytoplasmic processes originated from the cell body.

In the same stage, granular hemocytes gathered in the pars intercerebralis. These hemocytes did not contain neurosecretory granules detectable by the histochemical

Fig. 2. Migrating neurosecretory granules (G) across the perineurium (P). Well-branched cytoplasmic processes (CP) become narrow. M, mitochondria; g, glycogen particle. $\times 20,000$.

test. The period of gathering granular hemocytes coincided with the disappearance of secretory granules from the cell body of the B cells. Then, these hemocytes became positive for aldehyde fuchsin stain. This seems to indicate the giving and receiving of neurosecretory granules between the B cells and granular hemocytes. Detailed experiments were reported previously (TAKEDA, 1971 a, 1976 b).

The cytoplasmic processes, therefore, seem to mediate between these cells, the neurosecretory B cells and granular hemocytes. It can be assumed that neurosecretory granules migrate across the perineurium in the pars intercerebralis by way of the cytoplasmic processes. However, any figures which show contact between the cytoplasmic processes and granular hemocytes were not obtained, probably because of fixational difficulty.

On the outside of the perineurium branches became narrower (Fig. 4 b and c). Therefore, granules were sometimes barely able to pass them. Granules were rarely found out of the cytoplasmic processes (Fig. 3).

As found in other neurosecretory cells, axonal transport was also observed in the B cells in other stages, except the stage of diapause break. The difference of the

Fig. 3. A part of the cytoplasmic process found in the perineurium (P). Some neurosecretory granules (G) are found out of the perineurium. $\times 20,000$.

neurosecretory pathway seems, perhaps, to be based on the difference of the nature and action of neurosecretory substances.

DISCUSSION

In insects, migration of neurosecretory granules to the storage organs, i.e. corpus cardiacum, by way of axonal pathway has been clearly demonstrated, especially in *Leucophaea maderae* (SCHARRER, 1952; MARKS, et al., 1972; MARKS, et al., 1973), *Calliphora*, *Aphis* and *Synagis* (THOMSEN, M., 1952), *Bombyx mori* (ARVY, et al., 1953), *Calliphora erythrocephala* (THOMSEN, E., 1954), *Periplaneta americana* (HARKER, 1960), *Schistocerca septemfasciata* (STRONG, 1965), *Dysdercus koenigii* (DOGRA, 1967), *Anacridium aegyptium* (GIRARDIE and GRANIER, 1973; GIRARDIE et al., 1974), *Leptinotarsa decemlineata* (SCHOONEVELD, 1974), and *Manduca sexta* (NIJHOUT, 1975). In *M. flavescens*, axonal transport was also usually found in each neurosecretory cell (TAKEDA, unpublished data).

In *B. mori*, KOBAYASHI (1957) reported that neurosecretory material of colloidal nature is transported to the corpus cardiacum and, further, to the corpus allatum, by way of axonal pathway. On the contrary, neurosecretory material of foam-like nature is released directly from the cell body to the tissue gap of the brain and finally to the circulatory system. Characteristically, vacuoles are found only in the posterior portion of the cytoplasm which does not extend to the axon. In *Papilio xuthus*, ICHIKAWA (1955) also found many vacuoles in the peripheral region of active neurosecretory

Fig. 4. Elongated cytoplasmic processes. A: Starting area of the cytoplasmic process in the stage of pre-diapause. Neurosecretory granules are absent. Glycogen particles and mitochondria are seen. B: A part of cytoplasmic processes, somewhat near the cell body. Near the trachea (Tr), neurosecretory granules are seen. C: A part of the top area of cytoplasmic processes. Cytoplasmic processes become more narrow. Migrating neurosecretory granules are seen. G, neurosecretory granule; M, mitochondria; gl, glycogen particle; P, the perineurium. $\times 6,500$.

cells. Although he used only light microscopy, he found that granules which contain neurosecretory substance of colloidal nature connect tightly to the cytoplasmic membrane and secretory materials are directly released from the cell body to the intracellular spaces of the brain, leaving vacuoles in the peripheral region. In other insects, similar results were also obtained in *Rhodnius prolixus* (WIGGLESWORTH, 1940), *Ephestia kühniella* (REHM, 1950), *Hyalophora cecropia* (WILLIAMS, 1952), *Schistocerca gregaria* (HIGHNAM, 1961) and *M. flavescens* (TAKEDA, 1972 b). In *Nephtys californiensis* (Annelida), CLARK (1959) reported that many vacuoles appear all over the peripheral region of the active neurosecretory cells, suggesting direct release of secretory material from the cell body.

On the other hand, other forms have been found in several insects. In *Iphita limbata* (WIGGLESWORTH, 1970) and *D. koenigii* (DOGRA, 1967) neurosecretory axons

from the pars intercerebralis end in the wall of the aorta, where their secretory materials were released into the hemolymph. In the suboesophageal ganglion of *Carausius morosus*, there are two ventral neurosecretory cells which give off axons that ramify in the dorsal region of the ganglion. Here, neurosecretory material gathers and is released into the hemolymph (WIGGLESWORTH, 1970). In the case of diuretic hormone in *R. prolixus*, axons from neurosecretory cells located in the fused thoracic and abdominal ganglion enter the nerves and fan outward as they run to all parts of the abdomen. Along the course of these nerves, neurosecretory axons give off branches which end in bulbous enlargements immediately below the nerve sheath (MADDRELL, 1966). The opening for release of neurosecretory material may be located in definite neurohaemal organs or at individual nerve endings. In *C. morosus* and *Phormia terrae-novae*, which have multipolar neurones situated abundantly in the peripheral region of the ventral part, release of neurosecretory material occurs from the dendrite (FINLAYSON and OSBORN, 1968). In the fly, *Sarcophaga bullata* and other flies, including *Phormia regina* and *Drosophila melanogaster*, circumstantial evidence was present for direct transport of secretory materials from neurosecretory cells to the target organ by way of continuous connective tissue membrane. Neurosecretory material passes to the periphery of secretory cells and accumulates in channels within the basement membrane. The channels converge and pass along connective tissue strands, or may pass directly, into channels within the sheath of the target organ. Secretion-filled channels have further been found within the basement membrane (WHITTEN, 1964). In *Philosamia cynthia ricini*, NISHITSUTSUJI-Uwo (1961) reported that the neurosecretory cells in the pars intercerebralis are enmeshed with neuroglial cell processes, among which there exist canaliculi communicating with a blood sinus. She speculated that neurosecretory material is released directly from the cell body into the blood system. However, she did not find secretory materials within canaliculi. In the thoracic ganglion of *Eriocheir japonica* (Crustacea), MATSUMOTO (1954) suggested that neurosecretory material migrates to capillary vessels surrounding the cell body.

A hypothetical releasing scheme of neurosecretory granules from the B cells in the pars intercerebralis of *M. flavescens* is illustrated in Fig. 5. At first, neurosecretory granules accumulate in the cell body with the progression of diapause (Fig. 5-1). In the stage of diapause break, the B cells become active on releasing neurosecretory granules to the cytoplasmic processes, not to the axon, leaving vacuoles in the cytoplasm. In this stage, granular hemocytes which do not contain aldehyde fuchsin positive substances gather around the pars intercerebralis. Soon after that, neurosecretory granules are transported to granular hemocytes through the cytoplasmic processes. As a result, granular hemocytes are filled with many neurosecretory granules which show the positive reaction for aldehyde fuchsin stain (Fig. 5-2). Then granular hemocytes fall apart from the pars intercerebralis and start to migrate to the target organ (Fig. 5-3), the prothoracic glands (TAKEDA, 1971 a, 1976 b), and activate them to secrete ecdysone (TAKEDA, 1971b, 1972 b, 1976 a).

In *M. flavescens*, it was suggested that, regarding the stage of diapause break, neurosecretory granules in the B cells are transported to granular hemocytes, not from the peripheral region of the cell body or the storage organ by way of axonal pathway, but from the cytoplasmic processes.

Many important problems described below, however, are not answered by the present observations. One is the mode of release of neurosecretory granules to granular

Fig. 5. The general morphology of the half area of the pars intercerebralis and a hypothetical scheme showing the migration of neurosecretory granules by way of cytoplasmic processes. Three large cells are the neurosecretory B cells.

1: Many neurosecretory granules synthesized in Golgi apparatus, accumulate in the cell body of the neurosecretory B cells. 2: Granular hemocytes gather around the pars intercerebralis. Then neurosecretory granules migrate gradually from the cell body to cytoplasmic processes, leaving vacuoles in the cytoplasm, and to granular hemocytes by way of them. 3: Granular hemocytes begin to move from the pars intercerebralis toward the prothoracic glands. As a result, neurosecretory granules entirely disappear from the neurosecretory B cells. P, perineurium; H, granular hemocytes.

hemocytes. For instance, in axonal ending which is the generally accepted point of release of neurosecretory materials, three possible modes of release have been known. One is the theory that neurosecretory granules pass across the membrane as an intact form (ROLICH *et al.*, 1962). This would require that it pass along the intact membrane, or across the membrane after it has been disrupted in some way. A second release procedure is that of exocytosis or reverse pinocytosis: This occurs when the granules and axon membrane fuse, thereby emptying the hormonally-active material into the intercellular space (NORMAN, 1965). The third is the theory that neurosecretory material simply diffuses out of the granules into the axoplasm and then acrosses the cell membrane as individual molecules (HAGADORN, *et al.*, 1963). This would partially account for the presence of small, pale, 'ghost' granules or synaptic-like vesicles, often observed in the vicinity of the supposedly disrupting neurosecretory granules. In the present case, how are neurosecretory granules released and transferred to granular hemocytes? A second problem is the releasing regulatory mechanism of neurosecretory granules. It is well known that neurosecretory cells release their secretory material at axonal endings in response to impulses traveling down the axon (COOKE, 1964; GERSCH, 1972). In addition to this, elevated potassium concentrations lead to hormone release and this is due to a depolarization of neurosecretory axonal endings themselves (MADRELL, 1974). In some insects, calcium ions are also required for

release of neurohormone (GERSCH et al., 1970; MADDRELL, 1974). In *M. flavescens*, by what mechanism is the release of neurosecretory granules controlled? As to the transport of neurosecretory granules of the B cells, two pathways are distinguished; One is the axon and the other is the cytoplasmic process. In *B. mori*, neurosecretory pathways are also known to differ by the nature of secretory substances (KOBAYASHI, 1957). Then, how is the selection of neurosecretory granules to each pathway controlled? These are the problems to be resolved.

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