Investigations on Neurosecretion in the Central and Peripheral Nervous System of the Pulmonate Snail *Lymnaea stagnalis* (L.)

Some Preliminary Results*

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In all central ganglia, except for the buccal and pedal, of the pond snail *Lymnaea stagnalis* neurosecretory cell groups have been described using the classic neurosecretory stains paraldehyde fuchsin and chrome haematoxylin (LEVER *et al.*, 1961). In each of the cerebral ganglia a group of phloxinophilic cells in addition to two groups of Gomori-positive cells has been studied in some detail by light microscopy (JOOSSE, 1964; BOER, 1965) as well as by electron microscopy (BOER *et al.*, 1967).

However, the selectivity of the Gomori methods has limitations for studying neurosecretory materials, because other substances like glycogen, lysosomes and connective tissue elements also take up the stains (SIMPSON *et al.*, 1966). Therefore the Alcian blue/Alcian yellow technique, which has been introduced for the identification of neurosecretion—in the preoptic-neurohypophyseal tract in *Rana temporaria*—by PEUTE and VAN DE KAMER (1967), was applied with slight modifications. After oxydation with potassium permanganate the Alcian blue solution was used at pH 0.5 for staining strong acid groups and the Alcian yellow solution at pH 2.5 for identifying weak acid groups. It appeared that in this way the neurosecretory materials in this snail could be easily distinguished from other Gomori-positive tissue elements. Furthermore it was demonstrated that there exist at least three different types of Gomori-positive cells staining light-green, yellow-green and dark-green, respectively. The colours indicate that the ratio of strong acid groups to weak acid groups is different in the neurosecretory substances concerned. In addition to these cells, two Gomori-negative cell types which were not regarded as neurosecretory before, were found. The one stained strongly with Alcian yellow and the lipid stain Sudan black B, while the other could be identified by an affinity for phloxin and a moderate reaction with Sudan black B.

With the electron microscope it was established that each of the types of histochemically different neurosecretory materials consisted of elementary granules of characteristic size and appearance.

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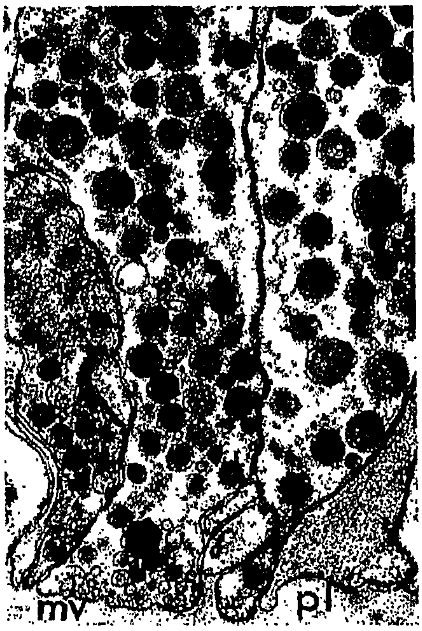

Fig. 1. Axon endings in the periphery of a visceral nerve containing elementary granules of the phloxinophilic type of neurosecretion. The occurrence of transparent granules (axon at the left) and microvesicles (*mv*) indicates release; *pl* perineurial layer. ×20,500

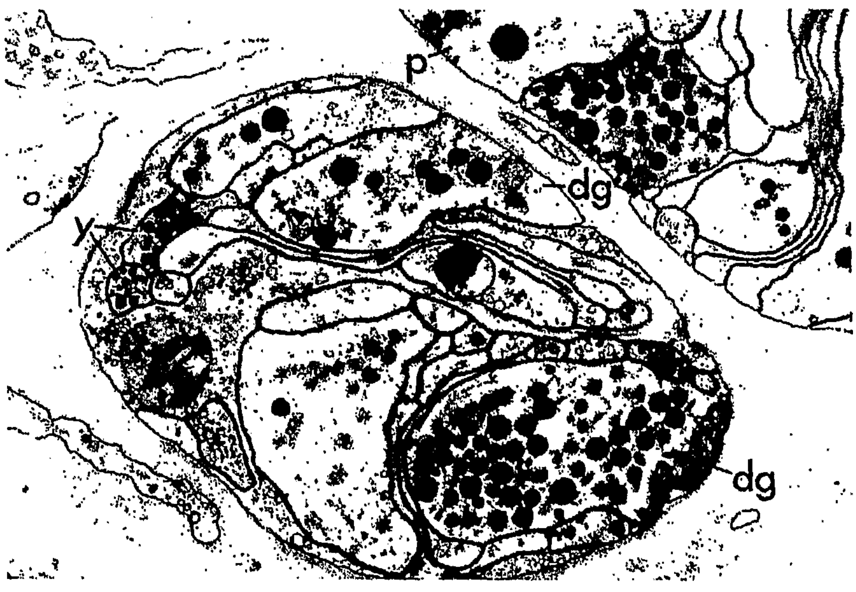

Fig. 2. Small nerves in the connective tissue capsule near the parietal ganglion. The axons contain elementary granules of the "dark-green" (*dg*), "yellow" (*y*), and phloxinophilic (*p*) type of neurosecretion. ×14,000

Apparently the periphery of the lip nerves and the intercerebral commissure serve as neurohaemal areas for the neurosecretory cell groups in the cerebral commissure (JOOSSE, 1964; BOER *et al.*, 1967). Storage of neurosecretory material at the periphery of connectives and nerves appears to be a general phenomenon at least in this snail. Axon endings of the neurosecretory cell groups in the pleural, parietal and visceral ganglia were observed in the peripheral regions of the connectives between these ganglia and especially of the parietal and visceral nerves. Indications of release of neurosecretory material were frequently found (Fig. 1).

Fig. 3. Axons (*ax*) containing the "yellow" type of neurosecretory elementary granules in the connective tissue near the ureter epithelium; *g* glial cell; *u* basal infoldings of ureter cells. ×17,200

Neurohaemal zones in *Lymnaea stagnalis* seem to be even more extensive since it was further observed that tiny nerves containing several types of stainable materials penetrate the perineurial layer around the ganglia and nerves, forming a network near blood vessels, blood spaces and capillaries in the connective tissue capsule surrounding the central nervous system and the proximal parts of the nerves (Fig. 2). In this capsule also release generally occurs.

Moreover accumulations of neurosecretory material were observed in peripheral parts of the nervous system as far as the nerve endings in some effector organs. Axons containing the "yellow" type of neurosecretion were observed in the visceral nerve and in the branches of this nerve which innervate the kidney. These axons are ending non-synaptically in the connective tissue between the folds of the ureter epithelium (Fig. 3). This part of the kidney is assumed to be involved in transport of salts and water (WENDELAAR BONGA and BOER, 1969). Release of the contents of the elementary granules in this area appeared to take place in a neurosecretory way.

In order to investigate whether this material might have a direct hormonal effect upon the ureter, groups of three animals were exposed to solutions of different osmolarity (de-mineralized water, a 0.56% NaCl sol., and tap water) and studied at the electron microscope level. After two hours the percentage of axon profiles showing release phenomena in the group exposed to de-mineralized water when compared to animals in tap water increased from about 20% to approximately 70%. In snails placed in the saline solution there was a slight decrease. Per snail 450 axons were examined. This result suggests that this type of peripherally released neurosecretion exerts an influence on the water balance in this species.

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