

CHAPTER-I

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I INTRODUCTION

In recent years, the studies in Molluscan physiology have given great importance to neuroendocrine control over reproduction. It is hoped that such studies may help to control molluscan crop pests or a means for their population control (Godan, 1983). Among the various molluscan classes, neuroendocrinology of class gastropoda has been well studied for many reasons - i.e. (1) They are serious pests to horticulture and agriculture (Hunter, 1969; Runham and Hunter, 1970). (2) They also serve as a good indicators for soil and water pollution (Godan, 1983). (3) Gastropod neurons are giant cells so they are best for neurophysiological studies. (4) They are economically important. (5) They are also important in medical and veterinary practice, as they serve as intermediate hosts for some parasites of human and domestic animals.

1 A BRIEF REVIEW OF NEUROENDOCRINOLOGICAL STUDIES IN

GASTROPODS :

A) NEUROSECRETORY SYSTEM IN GASTROPODS :

The neuro-endocrinological studies in gastropod molluscs have concentrated their attention to find out neurosecretory cells in the different neurosecretory organs. Scharrer (1935) first observed the large secretory cells in the central nervous system in Opisthobranchs, Aplysia and Pleurobranchia.

Scharrer and Scharrer (1937) pointed out that the cells in the central nervous system have neurosecretory function in all molluscan groups. Gabe (1954) first confirmed the presence of neurosecretory cells in cerebral ganglia of a pulmonate gastropoda. Letter on he (Gabe, 1966), also gave a complete account of neurosecretory cell types in Mollusca with the help of light microscopy. Lever (1957) gave a comparative account on neurosecretion in various pulmonate molluscs. However, the credit of present knowledge of neurosecretion in stylommatophoran pulmonates goes to van Mol (1967) and that of basommatophorans goes to Joosse (1964) and Wendelaar Bonga (1970, 1972). Then the information about neurosecretion in pulmonates was consolidated and published by Boer and Joosse (1975) and in entire phylum Mollusca by Geraerts (1976 a,b).

B) LOCALIZATION OF NEUROSECRETORY CELLS :

There is controversy about the localization of neurosecretory cells in gastropods. According to some, they are located in cerebral ganglia (Gabe, 1954; Krause, 1960; Laryea, 1969; Nagbhushanum and Kulkarni, 1971a,b; van Minnen and Reichart, 1980; Joosse et al., 1982), in dorsal bodies located above the cerebral ganglia (Lever, 1957; 1958a, b; Bern, 1962, Gabe, 1966, Simpson et al., 1966a,b; Geraerts, 1976a,b; Highnam and Hill, 1977), in optic glands (Lane, 1964a, b; Gabe, 1966; Simpson et al., 1966 a; Highnam and Hill, 1977) and in other organs like follicular gland (Bern, 1962), Semper's gland,

arterial gland, pleural, pedal and visceral ganglia (Nagbhusanum and Mane, 1973).

C) NEUROHAEMAL ORGANS :

According to Antheunisse (1963) the neurosecretory materials from the neurosecretory cells pass through the glia cells in Dressena. But most of the evidences indirected their passage through the axons to neurohaemal organs. The release sites in some gastropods are widely distributed at the peripheries of nerves, connectives and commissures (Wendelaar Bonga, 1970; 1972) and these release sites are not concentrated in one or few organs within the connective sheaths surrounding the nervous system (Nolte and Kuhlmann, 1964; Wendelaar Bonga, 1970) or in wall of the blood capillaries or auricle (Martoja, 1972).

Here one point should be noted that in most of gastropods, the neurohaemal organs are not distinct, but the tracts of neurosecretory axons extend from perineurium of commissures, connectives and nerves associated with the central nervous system or in the connective tissue surrounding these structures. The neurosecretory material is released in these areas by exocytosis (Wendelaar Bonga, 1970) and scattered neurosecretory perikarya may also be present in the perineurium (Dyer and Cowdon, 1973).

In some gastropods, peripheral neurosecretion is observed (Swindle and Benjamin, 1976). The number of release sites could be a strategy for increasing speed of the release of a hormone (Swindle and Benjamin, 1976). Joosse et al.

(1982) have demonstrated the caudodorsal neurosecretory system in snail, Lymnaea stagnalis. According to modern definition of neurosecretory system, the neurosecretory cells, neuronal glands, neurosecretory material and release sites for neurosecretory products are included in the neuroendocrinological study.

D) STAINING METHODS OF NEUROSECRETORY CELLS :

Some investigators have used routine histological stains for demonstration and identification of neurosecretory cells (Scharrer, 1935; Fahrman, 1961; Gabe, 1966). von Mol (1960) described two types of neurosecretory cells in the slug, Arion. and Krause (1960) showed the same number of cell types in Helix pomatia. He designated these cell types as Type I and type II cells. Contrary to this observation, Lever (1957) described five different types of cells in Ferrissia shimekaii. Shinde (1991) noticed seven cell types in Indoplanorbis exustus, whereas Jawalikar (1989) gave a list of about 8-10 cell types of neurosecretory cells in Laevicaulis alte.

On the basis of histochemical reactivities, the neurosecretory cells could be differentiated into three types in Helix aspersa, seven types in Lymnaea stagnalis, ten types in Bulinus truncatus, eleven types in Arion hortensis and thirteen types in Helix pomatia (Wijdenes et al; 1980).

Some specific staining techniques are used to demonstrate neurosecretory cells. In earlier studies, Chrome-Haematoxylin Phloxin (CHP) staining method was used for identification of neurosecretory cells (Gomori,

1941). Then this Gomori's method was modified by Ewen (1962). He suggested Aldehyde Fuchsin (AF) method. Using these techniques, Krause (1960) and Lever (1957) identified various neurosecretory cells in different freshwater pulmonate snails. According to Bern (1962) neurosecretory properties are fixed with CHP and AF positive inclusions in the cytoplasm. Latter on a new specific staining technique of Alcian Blue : Alcian yellow (AB:AY) has been developed. By employing this new technique neurosecretory cells have been detected in L. stagnalis (Wendelaar Bonga, 1970), in B. truncatus (Boer et al., 1977) in D. reticulatis, A. horstensis and H. aspersa (Wijdenes et al., 1980). Nagabhushanum and Kulkarni (1971) used Mallory's Triple (MT).

E) NATURE OF NEUROSECRETORY GRANULES :

The presence of neurosecretory substances is based on staining property of cytoplasmic inclusions but about their validity has controversy due to the presence of pigments and food reserves in molluscs (Bern, 1962). The distinct neuron inclusions suggest the secretory activity in gastropods which attracted many investigators (Scharrer and Scharrer, 1954; Ladislav, 1966). Chou (1957) noticed cytoplasmic inclusions in neurons of Helix aspersa and Lymnaea stagnalis. The neurosecretory inclusions contained cystein rich proteinaceous granules in many pulmonate gastropods (Boer and Joosse, 1975). Bern (1962)

pointed out that the neurosecretory substances were the sources of vascular hormones in gastropods.

The structures of the neurosecretory cells and neurosecretory granules have been studied by few investigators by employing histological methods in Ariophanta lingulata and Vaginulus Sp. (Nagabhushanum and Swarnamayee, 1963, 1964). They found neuron and gland cell characters in neurosecretory cells. Boer and Joosse (1975) have given a histological criteria for identification of neurosecretory cells in molluscan animals. The ultrastructural details in these cells have been revealed only in limited number of gastropod species by Fahrman (1961) Wendelaar Bonga (1970) and van Minnen et al (1977),

Unfortunately, due to lack of classic research methodology, the study of neurosecretion presents unexpected difficulties, since some not related structures such as gliomes or waste pigments also stain with the available methods for demonstration of neurosecretory substances. The consequence is that true neurosecretory cells may remain unidentified, while common nerve cells mimic neurosecretory aspects and the chemical nature of neurosecretion still remains unknown.

F) ROLE OF NEUROSECRETION IN GASTROPODS :

Evidence is being accumulated in recent years to show the role of neurosecretory substances in the control of reproduction in gastropods, which has been reviewed by Fretter and Graham (1964). According to them, such a role is clear only in very few instances. Pelluet and Lane (1961) observed

relation between neurosecretion and cell differentiation in ovotestis of two slugs, A. ater, A. subfuscus and in Milax species. According to them along with reproductive function the neurosecretory products also maintain the normal body activities including animal growth, metabolism and help for harmonious adaptation with the environment. Joosse (1964) and Guyard (1967) noticed the role of neurosecretion in the control of egg laying in L. stagnalis and Helix sp., respectively. According to some investigators the role of control in reproductive activity resides in the cerebral ganglia. Hence the neurosecretory cells of the cerebral ganglia show a maximum activity at the time of gametogenesis (van Mol, 1967; Joosse, 1964; Smith, 1967). As against this, other group of investigators reported that the variations in the neurosecretory cells as a function of the genital state are more important in the visceral ganglion than in the cerebral ganglion in Gundlachia and A. ater, Smith, 1967). Except these examples, the studies involving the role of neurosecretion in the reproductive activity of gastropod molluscs are scarce. Therefore, the study of neuroendocrinology in relation to reproduction in gastropod animals forms an open field for future research. Though some ideas concerning the gametogenesis and spawning and their neuroendocrine control have been suggested.

2 PROBLEMS RELATED TO NEUROSECRETION IN GASTROPODS :

In the exostive review on the physiological aspects of neuroendocrine hormones in molluscs, Martoja (1972) has pointed out that although the

information on neurosecretion and its role in the reproduction has been clearly established at least in some gastropods during the breeding season but its role during the post-breeding season which is usually followed by aestivation phase has seldom been clarified. Hence, it was decided to study the nature and capacity of neurosecretory cells of the gastropod species which go into aestivation, in relation to their breeding-aestivation cycle.

The existing literature indicates that the number of neurosecretory cell types differs in various gastropod species. This fact needs further elaborative investigation which might aid in establishing some sort of relationship between the phylogenetic status of the gastropods and the presence of neurosecretory cell types in the different neurosecretory organs.

There is some indication about the variations in the metabolite contents in the operculate snails and slugs without the shells in gastropod molluscs. Such studies might be interesting in case of neurosecretory activity also. These studies could be further extended to find out the relationship between the presence of shell and absence of shell in the gastropods and the nature and capacity of neurosecretion in the different neurosecretory organs during breeding and aestivation periods.

3 *DETAILS OF THE PLAN OF PROPOSED WORK :*

Some of these problems on the neuroendocrine control in the physiology of reproduction in gastropod species are being undertaken for detailed analysis in this laboratory. The present dissertation concerns with the histological and

histochemical studies on the secretion of neurosecretory cells in the cerebral ganglia and in the optic tentacles and their correlation with the gametogenesis in the ovotestis during breeding season and during aestivation of locally available two pulmonate gastropod species belonging to same order stylommatophora but one without shell i.e. Semperula maculata (Semper) and other with shell i.e. Cryptozona semirugata (Beek).

In the present investigation, it was proposed to carry out detailed-

- i) Analysis of the neurosecretory substances elaborated in the cerebral ganglia and optic tentacles by employing recent and well established histological and histochemical neuroendocrinological methods.
- ii) Analysis of the alterations in the neurosecretion during breeding and aestivation periods in two pulmonate gastropod species during their breeding-aestivation cycle.
- iii) Analysis of the variations in the number and in the secretory products in the neurosecretory cells in the above referred two pulmonate species in relation to presence or absence of protecting shells.

4 OUTLINE OF PRESENTATION OF DISSERTATION :

For the convenience of presentation and ease of understanding, the next part of the dissertation is divided into four chapters. The Second chapter describes in detail the histological and histochemical staining methods employed in the present study. Chapter third describes in detail the anatomy and

histological localization of the neurosecretory cells in the cerebral ganglia and optic tentacles of S. maculata and C. semirugata, the observations on the chemical nature of these cells, variations in the number and concentration of their chemical constituents in the breeding as well as in the aestivation periods. In Chapter fourth the observations of the dissertation have been critically discussed in the light of existing information. The Last (fifth) Chapter gives the general summary and certain ideas have been arrived at and the conclusions are put forward. The last chapter is followed by the Bibliography.