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MORPHOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON THE NEUROSECRETORY CELLS OF DEVELOPING AND ADULT PARAMPHISTOMUM CERVI (DIGENEA: PARAMPHISTOMATIDAE)

B. C. GUPTA, V. R. PARSHAD and S. S. GURAYA

Department of Zoology, College of Basic Sciences and Humanities, Punjab Agricultural University, Ludhiana

Abstract. Only one type of neurosecretory cells is present in the developing as well as in the adult *Paramphistomum cervi*. In four week-old worms, the neurosecretory cells contain only bromphenol blue-positive material, periodic acid-Schiff-positive material appears in eight-week old worms. In the adult worm, the neurosecretory material is glycoprotein with few phospholipid granules. In maturing worms the amount of neurosecretory material gradually increases in the axon, revealing its role in the maturation of gonads.

The presence of neurosecretory cells in adult trematodes was described by many workers (Ude 1962, Grasso 1967a, b, Matskasi 1970, Harris and Chang 1972, Mehrotra and Bhatia 1979, Shymasundari and Hanumatha Rao 1980), but little is known about their development and nature of secretion during the juvenile stages of the worms. At the same time, the literature regarding their role in the reproduction is inadequate, except for Ramakrishna et al. (1980) and Kalyankar and Kansal (1981), who reported neuroendocrine control of the maturation in *Fischoederius cobbaldi* and *Proalarioides tropidonotois vidyarthi*, respectively. The present paper describes in detail the histochemical changes in the neurosecretory cells during various developmental stages and in adult worms. Their role in the maturation of gonads is discussed.

MATERIAL AND METHODS

The mode of collection of *Paramphistomum cervi* of known age, as well as various histochemical techniques employed, were the same as described in Gupta et al. (1983), except for paraldehyde-fuchsin staining (Thompson and Hunt 1966).

RESULTS

The brain of developing and adult worms has two types of cells, i.e. sensory cells and the neurosecretory cells. The sensory cells are of two types, i.e. of type I, which are small and unipolar, while those of the type II are larger and bipolar in appearance. They have a high nucleus: cytoplasm ratio and contain a round, darkly stained nucleus with compact chromatin material. These sensory cells are frequently present in the oral sucker, posterior sucker and below the body wall. The neurosecretory cells differ from the secretory cells in the large body and prominet axon, dense cytoplasm, very prominent nucleolus and presence of paraldehyde-fuchsin-positive material in their perikaryons and axons. The neurosecretory cells are present both in the centre and in the periphery of the brain.

In 4-week-old worm, two neurosecretory cells are present in the brain. They contain

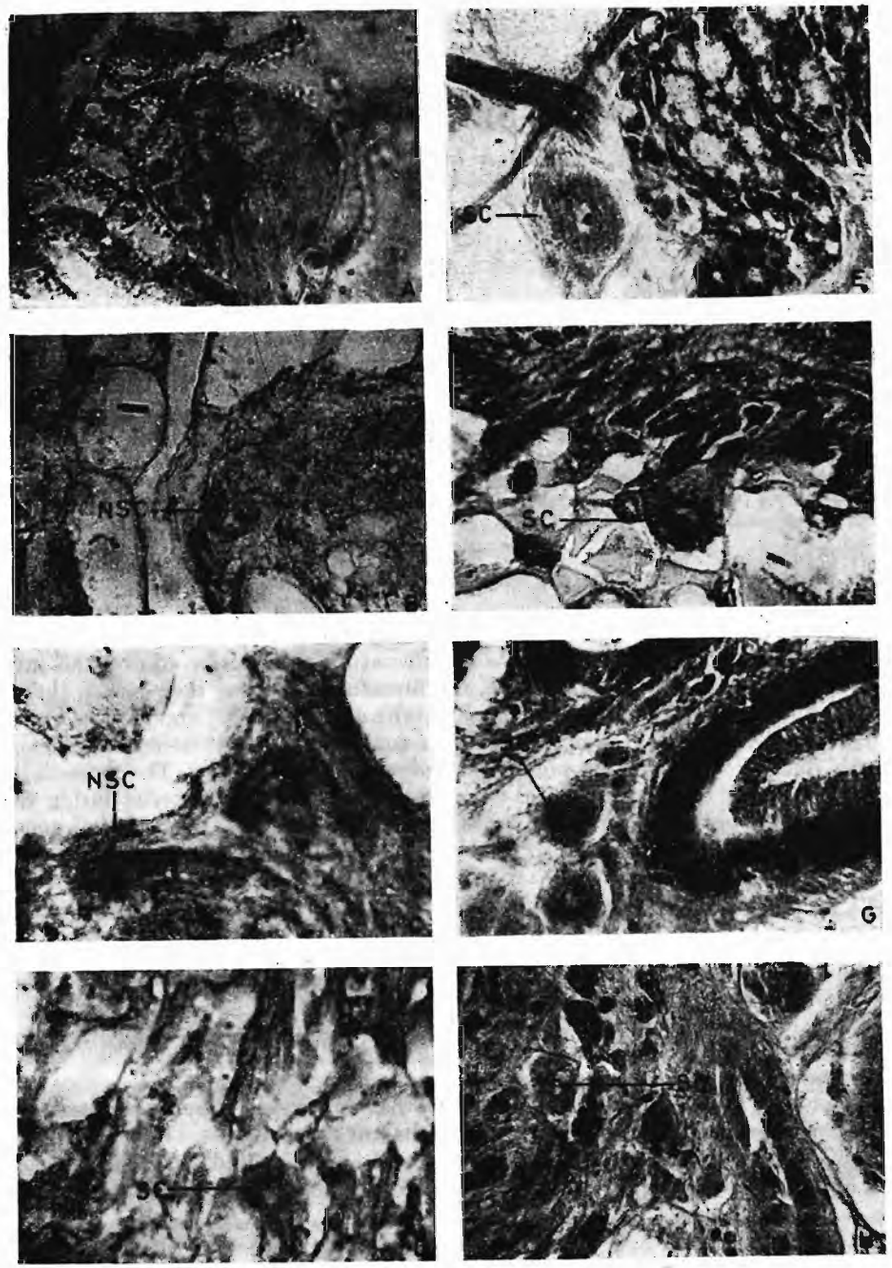

Fig. 1. Presence of neurosecretory cells (NSC) and secretory cells (SC) in various organs of *Paramphistomom cervi*. A — Brain of eight week-old worm showing the presence of NSC. Mercuric bromphenol blue (HgBB) × 270. B — NSC in the brain of ten weeks-old worm. HgBB × 270. C — NSC in the brain of adult worm. Note the presence of secretory material in the body and axon. Paraldehyde Fuchsin × 270. D — SC present in the posterior sucker. Polychrome × 270. E — SC near the vitelline follicle. HgBB × 270. F — SC below the body wall. HgBB × 270. G — near the intestinal caeca. Toluidine blue × 270. H — SC in the wall of metraterm. Polychrome × 270.

a round nucleus and show a high cytoplasm: nucleus ratio. Their cytoplasm contains a small amount of bromphenol blue (HgBB)-positive material. In 6-week-old worms, the number of neurosecretory cells in the brain increases to three. They stain positively with HgBB but do not contain any periodic acid Schiff (PAS)—and Sudan black B (SBB)—positive material. In 8-weeks-old worms, the size and number of neurosecretory cells further increase to 5 (Fig. 1A). Few PAS-positive granules start to appear in them. In 10-week-old worms, the neurosecretory cells are quite large in size and contain a prominent round nucleus. Their number in the brain increases to 8 (Fig. 1B). Their cytoplasm does not show any reactin with SBB. In 16-week-old worms, as many as 10 neurosecretory cells, typical of adults, are present in the brain. They have a round body and a long tapering axon. The amount of HgBB material increases and SBB-positive material appears in the neurosecretory cells. Till 10 weeks, the neurosecretory material, which was seen mainly in the perikaryon of the neurosecretory cells, is present both in perikaryon, as well as in the axon during the 16th week.

The neurosecretory cells in the brain of adult worms, are quite large. They measure 16—24 μm in the outline and have a single, large and round nucleus measuring 6—10 μm in diameter (Fig. 1C). The nucleus contains a single prominent nucleolus which may be present in the centre or displaced towards the periphery. The chromatin material is granular and is scattered throughout the nucleoplasm. The number of cells increases to about 14. They contain paraldehyde-fuchsin-positive material both in the perikaryon and in the axon, with more accumulation in the latter.

The cytoplasm of the neurosecretory cells in the adult worms stains strongly with HgBB. The protein contains both free and bound—NH_2 groups, as revealed by their positive reactions with ninhydrin-Schiff and chloramine T-Schiff tests. However, the protein does not contain any SS-group and tyrosine, as they do not stain with performic acid Schiff and Millon reaction, respectively. The cytoplasm of the neurosecretory cells stains pink with methyl green-pyronin technique, showing their basophilic nature.

The neurosecretory cells stain positively with PAS technique showing the presence of carbohydrates, but do not give any reaction with Alcian blue-PAS and Alcian blue, pH 2.5, revealing the absence of acid mucopolysaccharides. However, they stain positively with SBB after treating them with 25 % acetic acid indicating the presence of bound lipids in them. Few phospholipid granules are also present as revealed by their weak affinity with acid-hamatin test. Since the neurosecretory material stains both for proteins and carbohydrates, it appears to be glycoprotein in nature, with few phospholipid granules.

In addition to the neurosecretory cells, some large secretory cells are also present in the anterior and posterior suckers (Fig. 1D), below the body wall (Fig. 1F) in the parenchyma, close to the testes, ovary, Mehli's gland, vitelline gland (Fig. 1E), intestinal caeca (Fig. 1G), and in the wall of metraterm (Fig. 1H). These cells have a large body and long tapering duct. Their body contains a large hypertrophied nucleus containing a prominent nucleolus. Their cytoplasm contains granular material.

DISCUSSION

The present study shows the presence of only one type of neurosecretory cells in *P. cervi*, supporting the observations of Bhatnagar et al. (1980), Sharma and Sharma (1981), both on *Ceylonocotyle scoliocoelium*, and Kalyankar and Kansal (1981) on *Proalarioides tropidonotois*. However, our findings differ from that of Mehrotra and Bhatia (1979) on *Gastrothylax* sp. and Shyamasundari and Hanumantha Rao (1980) on *Paragonimus compactus* and *Euparadistomum* sp., who have reported 2 and 3 types of neurosecretory cells, respectively. Ramakrishna et al. (1980) have

reported only one type of neurosecretory cells in the juvenile stages of *Fischoederius cobbaldi* but during the later stages they observed two types of neurosecretory cells. The presence of sensory cells was reported by Hyman (1951) and Bullock and Horridge (1965). They have reported the presence of bipolar sensory neurons with branched or little branched processes in the sucker and in the body wall. Sharma and Sharma (1981) stated that the large size of the neurosecretory cells of *C. scoliocoelium* differentiates them from the neurons present in the vicinity of nervous and non-nervous tissue.

The presence of protein rich in free and bound NH_2 groups supports the observations of Kalyankar and Kankal (1981) on *P. tropidonotois*, but differs from those of Shyamasundari and Hanumantha Rao (1980) in the absence of sulphydryl-rich groups. However, the presence of carbohydrate moiety and sudanophilic material supports the observations of Shyamasundari and Hanumantha Rao (1980), but differs from those of Kalyankar and Kansal (1981), who have reported the absence of lipids in the neurosecretory cells. The glycoprotein nature of neurosecretory cells demonstrated during the present study, is supported by the observations of Sharma and Sharma (1981) on *C. scoliocoelium*.

However, the significance of the distribution of secretory cells in the developing and adult worms either in association with various reproductive organs or in their vicinity is not known. At present it is difficult to assign any specific function to these secretory cells.

As the worms develop, the number and the size of the neurosecretory cells also increase in *P. cervi*. Grasso and Qualia (1970) demonstrated the role of neurosecretion in the maturation of gametes in Tricladia. Kalyankar and Kansal (1981) and Ramakrishna et al. (1980) gave similar views. They reported that the intensity of neurosecretory material decreases as the worms become mature, but no such depletion was observed during the present studies. Instead, the neurosecretory cells in the adult stain with a slightly more intensity and their number increases. During various developmental stages only a small amount of neurosecretory material was seen in the perikaryon and with the maturation of gonads the neurosecretory material appeared both in the perikaryon and in the axon. This suggests that the neurosecretory cells may play a role in the maturation of gonads. However, Sharma and Sharma (1981) could not find any significant variation in the size and amount of neurosecretory material in juvenile and adult worms of *C. scoliocoelium*.

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МОРФОЛОГИЧЕСКИЕ И ГИСТОХИМИЧЕСКИЕ НАБЛЮДЕНИЯ НЕЙРОСЕКРЕТОРНЫХ КЛЕТОК В РАЗВИВАЮЩИХСЯ И РАЗВИТЫХ ЕКЗЕМПЛЯРАХ *PARAMPHISTOMUM CERVI* (DIGENEA: PARAMPHISTOMATIDAE)

Б. Ц. Гупта, В. Р. Паршад и С. С. Гурая

Резюме. В развивающихся и развитых экземплярах *Paramphistomum cervi* встречается только один тип нейросекреторных клеток. У червей в возрасте 4 недель встречается только материал положительный к бромфеноловому синему. Черви в возрасте 8 недель содержат материал положительный в реакции Шифф-иодная кислота. У развитых червей нейросекреторный материал состоит из гликопротеина и нескольких фосфолипидных гранул. У созревающих червей количество нейросекреторного материала в аксоне постепенно повышается, что свидетельствует о его роли при созревании гонад.

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B. C. G., Department of Zoology, College and Basic Sciences & Humanities, Punjab Agricultural University, Ludhiana-141 004, India