Neuroanatomy of *Cornudescoides kulkarnii* n. sp., a gill parasite of *Mystus vittatus* in Meerut (UP), India

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Abstract Chemical named 5-bromo indoxyl acetate has been used to describe the nervous system of a viviparous monogenean *Cornudescoides* Kulkarni (1969), a gill parasite of *Mystus vittatus*. Central nervous system consists of paired cerebral ganglia from which anterior and posterior neuronal pathways arise. These neuronal pathways are interlinked by cross connectives and commissures. Paired dorsal, ventral and lateral nerve cords emanate from the cerebral ganglia, connected at intervals by transverse connectives. Huge arrangement of dorsal, ventral and lateral nerve cords and their innervations have been examined. Peripheral nervous system (PNS) includes innervations of the alimentary tract, reproductive organs and attachment organs (anterior adhesive areas and haptor). Both the CNS and PNS are bilaterally symmetrical, and better developed ventrally than laterally and dorsally.

Keywords *Cornudiscoides kulkarnii* · *Mystus vittatus* · Monogeneans · Neuroanatomy

Introduction

Phylum Platyhelminthes is a highly diversified and versatile phylum, a fact that is reflected in the structure of nervous system, both in its gross morphology and in the cellular types and their secretory inclusions. Absence of coelom and a proper circulatory system in flatworms means that any long-distance control of processes, such as growth and development, is likely to be accomplished by a

neurosecretory or peptidergic component of the nervous system. In this way, the flatworm nervous system may function not only as a nervous system *per se* but also as an endocrine system by releasing modulatory substances into the intercellular space close to target cells or organs, in a synaptical or non-synaptical way. Development of nervous system in early bilaterian flatworms would appear to have been dominated by two themes: (1) an anterior concentration of sensory and nervous tissue to form a ganglionic mass or primitive brain; and (2) the consolidation of peripheral neurons into a number of large longitudinal nerve cords. It is assumed, that concentration of associative neurons in the anterior end of the early active flatworm arose with the advent of bilateral symmetry and from selective pressures generated by the expanding need to coordinate the input from frontal sensory receptors and from the activity of the two sides of the body. Thus, development of bilateral symmetry, rather than cephalization, most likely necessitated the evolution of brain, thereby preventing the two sides of ancestral flatworm from engaging in contradictory activities.

Aims in studying the nervous system of flatworms are generally twofold. One is the scientific interest, since in view of the group's unique evolutionary position, information is likely to be forthcoming about early development of the nervous system in general; the other aim concerns the parasitological-medical aspect which ultimately seeks a means of controlling or, indeed, eradicating the parasites from their hosts. In targeting the nervous system, the hope lies in elucidating novel chemotherapeutic agents that act specifically on some neuronal signal substance or receptor of the worm, or along the chain of its synthesis or degradation, with minimal side effects on the host. Parasitic flatworms need to: (1) secure attachment on or in the host, and (2) produce sufficiently large numbers of progeny to

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ensure genetic continuity. The nervous system has developed around these problems.

During the present study 30 specimens were used for study of morphology and 10 worms were used for the study of nervous system in these parasites. The authors used indoxyl acetate as substrate to demonstrate the nervous system of *C. kulkarnii*. Central nervous system (CNS) and peripheral nervous system (PNS) were found to be highly reactive and stained extensively in a dark blue colour.

Materials and methods

Fishes for the present investigation were collected from freshwater bodies of Meerut region. In all 45 specimens were studied. Thirty specimens were studied for morphological studies and 15 specimens were studied for neuroanatomy. Worms collected in live condition were washed thoroughly with cold distilled water. Study of nervous system was made with the help of histochemical localization of esterases. It is one of the very common neuropharmacological elements, commonly found in the nervous system of all animals including monogeneans as suggested by Halton and Jennings (1964). Washed worms were incubated for 24 h at 38°C in the following medium-

- i) Tris buffer 0.2 M at pH 8.3 : 2.0 ml
- ii) Oxidant : 1.0 ml
- iii) 1 M Calcium chloride : 0.1 ml
- iv) 2 M Sodium chloride : 5.0 ml
- v) Distilled water : 2.0 ml

Oxidant: Potassium ferrocyanide: 210 mg, Potassium ferricyanide: 155 mg, Distilled water: 100 ml

1.3 mg 5-bromo indoxyl acetate was dissolved in 1.0 ml 100% alcohol and substrate solution was added to it. Washed worms were incubated in this medium for 24 h at 38°C. As a result of which, the nervous system was stained blue. Permanent mounts were made after dehydrating through ascending grades of alcohol, clearing in Xylene and mounting in Canada balsam. All the prepared slides were observed under the microscope within a week. The stain fades away after 8–10 days. Camera lucida drawings were drawn. Microphotographs were taken using Motic microscope and image analyzing system.

Observations

Neuroanatomy (Figs. 1, 2, 3, 4, 5)

Central nervous system of *C. kulkarnii* consists of paired cerebral ganglia (Cg) from which anterior and posterior neuronal pathways arise and interlinked by cross connectives and commissures. Peripheral nervous system includes

innervations of the alimentary tract, reproductive organs, attachment organs (anterior adhesive areas and haptor) and sub-tegmental muscles. Both central nervous system and peripheral nervous system are bilaterally symmetrical and better developed ventrally than laterally and dorsally. They display strong staining for cholinesterase activity. No staining was observed in the absence of cholinesterase substrate.

Cholinergic enzyme histochemical staining

The CNS and PNS were found to be highly reactive for ChE, and stained extensively in a dark blue and purple colour. The CNS consists of a thick, curved mass of paired Cg located ventrally just anterior to the pharynx. Several

Fig. 1 Diagram showing the nervous system of *Cornudiscoides kulkarnii* as revealed by cholinergic enzyme histochemical staining Avc (Anterior ventral connective); Cg (Cerebral ganglia); Ap (Anterior projection); Cn (Cerebral ganglia); Pn (Pharyngeal nerves); Pc (pharyngeal connective) Vnc (Ventral nerve cord); Vc (Ventral transverse connective); Phg (Prehaptor ganglion); Lnc (Lateral nerve cord); Vlc (Ventral lateral connective); Dnc (Dorsal nerve cord); Dc (Dorsal transverse connective); Dvc (Dorso ventral connective); Dlc (Dorso lateral connective); V (Ventral neurons); L (Lateral neurons); Ohn (Outer haptor nerve); Ihn (Inner haptor nerve); Ihc (Inner haptor connective); Ahn (Antero lateral hook nerves); Phn (Posterior hook nerves)

Fig. 2 Anterior head region showing cerebral ganglia (Cg) with two anterior projections (Ap) and anterior ventral commissure (Avc). Innervations of pharynx (10× 100×s)

Fig. 4 Testis region showing ventral nerve cord and neurons (10× 100×)

Fig. 3 Middle portion of the body showing Ventral nerve cord and ventral connective near ovary and vaginal opening (male copulatory complex) (10× 100×)

Fig. 5 Haptoral region showing haptoral nerves (10× 100×)

thick projections (Ap) extend from the anterior median region of the cerebral ganglia, imparting to this organ a butterfly-like appearance. Each projection gives rise to cerebral nerves (Cn), which extend anteriorly to enter the head lobes where they innervate the anterior adhesive areas. A pair of pharyngeal nerves (Pn), was detected inside the pharynx, with two pharyngeal connectives (Pc1–Pc2).

Two thick ventral nerve cords (Vnc) arise one from each ventral region of the cerebral ganglia, and run posteriorly where each joins a prehaptoral ganglion (Phg1). Another pair of prehaptoral ganglia (Phg2) is located one on each side of the body a short distance posterior to the first prehaptoral ganglia (Phg1) in the region anterior to the haptor. One thin branch arises from ventral nerve cord from one

side at the point posterior to cirrus and from other side at the point anterior to testis and reconnects to the ventral nerve cord at a short distance. Six ventral transverse connectives (Vc1–Vc6) were also detected, four before Phg1 and two between the two prehaptoral ganglia Phg1 and Phg2.

Two considerably thin lateral nerve cords (Lnc) arise from the posterolateral region of the cerebral ganglia and run posteriorly, one adjacent to each lateral margin of the body where they join the pre-haptoral ganglia Phg1. At regular intervals, Vnc communicate with Lnc by means of four pairs of ventro-lateral connectives (Vlc1–Vlc4). Two thin dorsal nerve cords (Dnc) arise from the posteromedian region of cerebral ganglia and connect posteriorly to the prehaptoral ganglia Phg1. Three pairs of dorsal transverse connectives (Dc1–Dc3) are present between Dnc. Dorsal nerve is connected to the lateral nerve at the point anterior

to cirrus, whilst they are linked to Vnc via three dorso-ventral connectives (Dvc1–Dvc3).

Staining for cholinergic elements revealed the presence of four pairs of large neurons distributed bilaterally down the main body of the worm. These cells are located on the ventral side of the body (V1–V4). First pair of ventral cell bodies (V1) is positioned at the level of cirrus, the second pair (V2) at the level between cirrus and testis, the third pair (V3) in the region of testis and fourth pair (V4) in the region of ovary.

Haptor is extensively innervated by two outer (Ohn) and one inner (Ihn), relatively thick haptoral nerves. The outer of these arise one from each posterior prehaptoral ganglia Phg2 while the inner haptoral nerves are derived from the ventral connective (Vc6). Inner haptoral nerves are connected with each other by means of inner haptoral connective (Ihc). The outer and inner haptoral nerves run ventrally in a posterior direction before branching into a plexus of numerous fine nerves in the anterior region of haptor. There are two dorso-lateral haptoral nerves (Dhn) arising one from each posterior prehaptoral ganglion (Phg2), and these run posteriorly and eventually branch into posterior hook nerves (Phn) and one pair of antero-lateral hook nerves (Ahn).

Discussion

CNS of *C. kulkarnii* comprises mainly a mass of cerebral ganglia and three pairs of ventral, lateral and dorsal longitudinal nerve cords, connected by transverse commissures. It is better developed ventrally than dorsally and laterally. In these respects, the CNS of *Cornudiscoides* resembles in basic structure to that of all previously studied flatworms, including monogeneans, digeneans and cestodes.

In the present study, intense reaction of Cholinergic components have been observed in the cerebral ganglia which lie immediately anterior to the pharynx, together with longitudinal nerve cords and nerve fibers which innervate head lobes, pharynx and haptor. A striking feature of all monogeneans studied hitherto has been the finding of a ring commissure around the mouth. In the present study, a comparable commissure has been detected. Rohde (1968) suggested oral commissure is a character which distinguishes the nervous system of monogeneans from that of digeneans.

It is important to note that some reproductive organs of *Cornudiscoides* notably the ootype and cirrus are innervated by cholinergic nerves. It indicates that cholinergic nerve innervating the anterior region of the gonopore help coordinate events involved in the process of laying eggs. Cable et al. (1996) suggested that the neuropeptide secretions may

influence movements of ova and vitelline cells, release of spermatozoa and mehlis gland secretion, peristaltic contraction of the ootype which shape the egg, and regulate the release of egg from the ootype into the uterus. The present study also agrees with the previous findings.

Neuromusculature of haptor of *Cornudiscoides* is richly innervated with cholinergic elements, suggesting that major role is motor function for the haptoral nerves.

CNS of *C. kulkarnii* as revealed by cholinergic staining conforms to the basic orthogonal pattern described for other monogeneans. Typically, the PNS innervates the alimentary system, reproductive organs, attachment organs and subtegumental muscles. These neural pathways and their organization have previously been described in 13 other monogeneans: *Diplozoon paradoxum* (Halton and Jennings 1964), *Polystoma integerrimum* (Rahemo and Gorgees 1987), *Gyrodactylus salaris* (Reuter 1987), *Pseudodactylogyrus anguillae* (Buchmann and Møllergaard 1988; Reda and Arafa 2002), *Eudiplozoon nipponicum* (Lyukschina and Shishov 1988; Zurawski et al. 2001), *Diclidophora merlangi* (Maule et al. 1990, 1990), *Pseudodactylogyrus bini* (Reda and Arafa 2002), *Entobdella soleae* (Marks et al. 1994), *Discocotyle sagittata* (Cable et al. 1996), *Protopolystoma xenopodis* (Mc Kay et al. 1991), *Macrogyrodactylus clarrii* (El-Naggar et al. 2004), *Macrogyrodactylus congolensis* (El-Naggar et al. 2007), *Chauhanellus indicus* (Rastogi et al. 2007) and *Silurodescoides vistulensis* (Rastogi et al. 2008).

The authors feel that nerves supplying the head lobes of monogeneans play an important role in temporary attachment of anterior adhesive sacs (head organs) during locomotion and feeding. These nerves are also responsible for coordination of muscular movements involved in the release of histolytic enzymes from the gland cells (cephalic glands) of the anterior adhesive apparatus. Extensive innervation of the head lobes, particularly the adhesive sac nerves, resembles that of *M. clarii* (El-Naggar et al. 2004, 2001; Arafa et al. 2003) and *M. congolensis* (El-Naggar et al. 2007). Contact perception (and possibly contact chemoreception) by the pro-haptor is likely to be important during temporary leech-like locomotion.

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References

- Arafa SZ, El-Naggar MM, Kearns GC (2003) Scanning electron microscope observations on the monogenean skin parasite *Macrogyrodactylus congolensis* (Prudhoe, 1957) Yamaguti, 1963. *Acta Parasitol* 48:272–279
- Buchmann K, Møllergaard S (1988) Histochemical demonstration of the inhibitory effect of Nuvan and Neguvon on cholinesterase

- activity in *Pseudodactylogyrus anguillae* (Monogenea). Acta Vet Scand 29:51–55
- Cable J, Marks NJ, Halton DW, Shaw C, Johnston CF, Tinsley RC, Gannicott AM (1996) Cholinergic, serotonergic and peptidergic components of the nervous system of *Discocotyle sagittata* (Monogenea:Polyopisthocotylea). Int J Parasitol 26:1357–1367
- El-Naggar MM, Arafa SZ, El-Abbassy SA, Kearn GC (2001) Chaetotaxy of monogeneans *Macrogtyrodactylus clarii* and *M. congolensis* from the gills and skin of the catfish *Clarias gariepinus* in Egypt, with a note on argentophilic elements in the nervous system. Folia Parasitol 48:201–208
- El-Naggar MM, Arafa SZ, El-Abbassy SA, Stewart MT, Halton DW (2004) Neuromusculature of *Macrogtyrodactylus clarii*, a monogenean gill parasite of the Nile catfish *Clarias gariepinus* in Egypt. Parasitol Res 94:163–175
- El-Naggar MM, Arafa SZ, El-Abbassy SA, Stewart MT, Halton DW (2007) Neuromusculature of *Macrogtyrodactylus congolensis*, a monogenean skin parasite of the Nile catfish *Clarias gariepinus*. Parasitol Res 100:265–279
- Halton DW, Jennings JB (1964) Demonstration of the nervous system of the monogenetic trematode *Diplozoon paradoxum* Nordmann by the indoxyl acetate method for esterases. Nature 202:510–511
- Ljukshina LM, Shishov BH (1988) Biogenic amines in the nervous system of *Eudiplozoon nipponicum* (Monogenea) (in Russian). In: Sakharov DA (ed) Simple nervous systems. Nauka, Moscow, pp 173–176
- Marks NJ, Halton DW, Kearn GC, Shaw C, Johnston CF (1994) 5-Hydroxytryptamine-immunoreactivity in the monogenean parasite *Entobdella soleae*. Int J Parasitol 24:1011–1018
- Maule AG, Halton DW, Johnston CF, Shaw C, Fairweather I (1990a) The serotonergic, cholinergic and peptidergic components of the nervous system in the monogenean parasite *Diclidophora merlangi*. Parasitology 100:255–274
- Maule AG, Halton DW, Johnston CF, Shaw C, Fairweather I (1990b) A cytochemical study of the serotonergic, cholinergic and peptidergic components of the reproductive system of the monogenean parasite *Diclidophora merlangi*. Parasitol Res 76:409–419
- Mc Kay DM, Halton DW, Maule AG, Johnston CF, Shaw C, Fairweather I (1991) Putative neurotransmitters in two monogeneans. Helminthologia 28:75–81
- Rahemo ZIF, Gorgees NS (1987) Studies on the nervous system of *Polystoma integerrimum* as revealed by acetylthiocholine activity. Parasitol Res 73:234–239
- Rastogi R, Mishra D, Rastogi R, Sharma V, Singh HS (2008) On a new species of genus *Silurodescoidea* (Achmerow, 1964) Gussev, 1973 with redescription, copulation biology and neuroanatomy of *S. vistulensis* (new combination) from Meerut (UP), India. Asian J Exp Sci 22:335–348
- Rastogi P, Mishra D, Singh HS (2007) Neuroanatomy of gill parasite *Chauhanellus indicus* (Monogenea: Ancyrocephalinae) from *Mystus seenghala* (family: Bagiridae) in Meerut (UP), India. J Parasit Dis 31:49–53
- Reda ES, Arafa SZ (2002) Cholinergic components of the nervous system of the monogenean gill parasites, *Pseudodactylogyrus bini* and *P. anguillae* from the eel *Anguilla anguilla* in Nile delta waters. Egypt J Zool 38:41–54
- Reuter M (1987) Immunocytochemical demonstration of serotonin and neuropeptides in the nervous system of *Gyrodactylus salaris* (Monogenea). Acta Zool 68:187–193
- Rohde K (1968) Das nervensystem der gattung *Polystomoides* Ward, 1977 (Monogenea). Z Morphol 62:58–76
- Zurawski TH, Mousley A, Mair GR, Brennan GP, Maule AG, Gelnar M, Halton DW (2001) Immunomicroscopical observations on the nervous system of adult *Eudiplozoon nipponicum* (Monogenea: Diplozoidae). Int J Parasitol 31:779–783