Distribution of catecholamine-containing, serotonin-like and neuropeptide FMRFamide-like immunoreactive neurons and processes in the nervous system of the actinotroch larva of Phoronis muelleri (Phoroniida)

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Summary. Glyoxylic-acid-induced fluorescence of catecholamines and antibodies against serotonin and FMRFamide were used to study the distribution of putative neurotransmitters in the actinotroch larva of Phoronis muelleri Selys-Longchamps, 1903. Catecholamines occur in the neuropile of the apical ganglion, in the longitudinal median epistome nerves, in the epistome marginal nerves, and in the nerve along the bases of the tentacles. The tentacles have latero-frontal and latero-abfrontal bundles of processes that form two minor nerves along the lateral ciliary band of the tentacles, and a medio-frontal bundle of processes. Monopolar cells are located on the ventro-lateral part of the mesosome. Processes are located along the posterior ciliary band and as a reticulum in the epidermis. Serotonin-like immunoreactive cells and processes are located in the apical ganglion, in the longitudinal median epistome nerves, and as a dorsal and ventral pair of bundles along the tentacle bases. Processes from the latter extend into the tentacles as the medioabfrontal processes. The latero-abfrontal processes form a minor nerve along the ciliary band. The dorsal bundles forms the major nerve ring along the tentacles and processes extend from it to the metasome. Processes are located along the posterior ciliary band. FMRFamide-like immunoreactive cells and processes are found in the apical ganglion, in the longitudinal median epistome nerves and as a pair of lateral epistome processes projecting towards the ring of tentacles. In the tentacles, a pair of latero-frontal processes are found; these form a minor nerve along the ciliary band. A band of cells can be seen along the tentacle ring.

Key words: Cilia – Catecholamine-containing neurons – FMRF-like immunoreactivity – Serotonin-containing cells – Larval tentacles – Neurotransmitters – Phoronis muelleri (Phoroniida)

During the last 10 years, immunocytological methods have been applied to invertebrates, especially for studying the nervous system (for review, see Thorndyke and Golds-worthy 1988). The aims of the research presented here are to detect the putative neurotransmitters, catecholamines (CA), serotonin (5-hydroxytryptamine, 5-HT) and the biogenic tetra-peptide phe-met-arg-phe-NH₂ (FMRFamide), by glyoxylic-acid-(GA-) induced fluorescence, and by antibodies against serotonin and FMRFamide; to determine the distribution of these transmitters; and to compare the distribution with the location of the nerve processes, as found by an ultrastructural investigation (Hay-Schmidt 1989).

The planktotrophic actinotroch larva was first described by J. Müller (1846); it represents the larval stage of the phoronids as established by Kowalevsky (1867; see Leuckart 1867). The body of the actinotroch larva can be divided into three regions (Fig. 1): an anterior epistome (prosome, oral hood), containing the protocoel, which usually forms a hood over the mouth and which is provided with a marginal ciliary band; a median mesosome with tentacles provided with a lateral ciliary band used in feeding, and containing the mesocoel, the intestine, stomach, and a pair of protonephridia; a posterior metasome, which contains the metacoel, the intestine and the metasomal pouch (at metamorphosis, this will become the adult "body"), and which is provided with a posterior locomotory ciliary band around the terminal anus.

The phoronids are normally regarded as protostomatus coelomates, and the actinotroch larva as a modified trochophora larva (Hyman 1959; Lacalli, in press.). Recent studies by Nielsen (1985, 1987) on ciliary bands show that both adult and larval phoronids have an upstream collecting system with monociliary cells used in feeding. An identical type of feeding system is found in adult and some larval brachiopods, adult pterobranchs, larvae of echinoderms (bipinnaria larvae of Astroidea, auricularia larvae of Holothuroidea, pluteus larvae of Echinoidae and Ophiuroidea), and in the tornaria larvae of enteropneusts. The phoronids and brachiopods (two of the three lophophorate phyla; the third is the bryozoans) are therefore placed within the "deuterostomatous" coelomates (Nielsen 1985, 1987). Thus, a careful study of the actinotroch larva (and the planktotrophic brachiopod larvae) might lead to the conclusion either that these larvae de facto are trochophores, or that they occupy a central position within respect to the evolution of the deuterostomatous coelomates from some presumably protostomatous coelomate.

GA-induced fluorescence of catecholamines has been studied in different invertebrate groups: Nematoda (Sharpe...
and Atkinson 1980), Insecta (Budnik and White 1988) and Echinodermata (Bisgrove and Burke 1987). Serotonin-like immunoreactivity has been shown in: Platyhelminthes (Reuter 1987; Reuter et al. 1988), Insecta (Klemm et al. 1986; Valles and White 1988) and Echinodermata (Bisgrove and Burke 1987). FMRFamide-like immunoreactivity has been extensively studied in the Coelenterata (Grimmelikhuijzen et al. 1988) and Mollusca (Cottrell et al. 1988); but for reviews, see Greenberg et al. (1985, 1986). Greenberg and Price (1988).

Materials and methods

Actinotroch larvae (86 specimens) of Phoronis muellerae Selys-Longchamps, 1903 with 10–28 tentacles were taken from plankton samples at Kristinebergs Marine Biological Station (Sweden) on 4th–11th October 1987.

Glyoxylic acid treatment (26 specimens)

A solution (SPG) of 10.2 g sucrose, 4.8 g KH2PO4, 1.5 g glyoxylic acid crystals in 0.15 l of distilled water adjusted to pH 7.4 by 1 N NaOH (de la Torre and Surgeon 1976) was used to incubate the specimens. Living larvae were either put directly into SPG, or were anaesthetised, in a beaker, in isotonic MgCl2 for 5–10 min before SPG treatment. The larvae were transferred to a slide and dried in a cold air stream for approximately 20 min. They were then heated for 5 min in a 80° C oven, and mounted in paraffin oil under a coverslip and warmed on a hot plate at 80° C for 90 s. If the larvae are kept cool and dark, the fluorescence can be observed for months.

Immunocytochemistry

Specimens (n = 60) were fixed in 4% paraformaldehyde buffered with 0.1 M Millonig’s phosphate buffer and 0.28 M NaCl for 12 h at 4° C, rinsed 4 times in 0.2 M Millonig’s phosphate buffer for 1 h, and washed in phosphate-buffered saline (PBS) with 0.1% Triton X-100, 0.1% sodium azide (PTA) for 10 min. (Specimens can be stored for at least 6 months without a marked loss of antigenicity in PTA at 4° C). Heat inactivated (58° C for 40 min) goat serum (GS) was used to prepare a 3% GS-PTA solution for preincubating the specimens to block non-specific sites. Specimens were preincubated for 12 h at 4° C. The primary antibody (rabbit anti-serotonin (Ra-a-5HT) or rabbit anti-FMRFamide (Ra-a-FMRFamide); Immunonuclear coporation INC) were diluted 1/500 and 1/1000, respectively, 3% GS-PTA, and the specimens were incubated in the relevant antibody for 24 h at 4° C. The specimens were then washed 4 times in PTA for 12 h at 4° C. The secondary antibody was either goat anti-rabbit immunoglobulin (Go-a-RalG G) conjugated with rhodamine isothiocyanate (RTIC) (Sigma), or biotinylated goat-anti-rabbit immunoglobulin (Zymed). Go-a-RalG G-RTIC was diluted 1/100 in 3% GS-PTA and specimens were incubated for 12 h at 4° C. They were washed in PBS four times for 12 h, and then mounted in 1:9 TRIS (pH 7.0) and glycerol solution in 4% n-propyl gallate to reduce photobleaching. Streptavidin-peroxidase and aminoethyl carbazole were added as chromogen (Zymed) to the secondary antibody.

Specimens were viewed with either Leitz Dialux or Aristoplan photomicroscopes, each equipped with a 50 W mercury bulb and epifluorescence objectives. Leitz Ploemopak filters G (excitation: Band pass 350–460 nm; suppression: Long pass 515 nm) and N 2 (excitation: Band pass 530–560 nm; suppression: Long pass 580 nm) were used for GA and RITC, respectively.

Controls

Staining was not seen if the primary antibodies were substituted with 5% non-immune goat serum in PTA. 5-HT immunoreactivity could be removed by absorption of the primary antiserum with 5-HT conjugated to bovine serum albumin (Inestar), or by adding 1 mg 5-HT-hydrochloride (Sigma) per 1 ml 1/500 Ra-a-5-HT, 24 h prior to incubation. FMRFamide immunoreactivity was removed by absorption with 2.5 mM FMRFamide (Diagnostica).

Positive controls. The Ra-a-5-HT antibody was shown to stain perikarya located in the raphae nuclei in rat and mink, whereas no dopaminergic perikarya in the substantia nigra or ventral tegmental area of the mesencephalon of the same animals were stained.

Results

The nervous system of the actinotroch larva has been described light-microscopically by Masterman (1898), Ikeda (1901), Brooks and Cowles (1905), Cori (1939), and Zimmer (1964). Electron-microscopical investigations have been performed by Hay-Schmidt (1989, the results are summarized in Fig. 1), and Lacalli (in press). The larval nervous system consists of an apical ganglion (ag) on the dorsal side of the epistome. The ganglion contains most of the nerve cells. Three median nerves (lnp) project from the anterior part of the ganglion towards the epistome margin. A pair of lateral nerves projects caudally forming the major nerve ring (ma nr), and on reaching the ring of tentacles on the mesosome, they continue laterally and ventrally along the bases of the tentacles. Along the epistome margin, two to three nerves (aen, pen) are found, which terminate when reaching the mesosome. In each tentacle, there are three nerves (tnn) (located on the frontal side). The lateral tentacle nerves of two neighbouring tentacles are continuous, and they form a minor nerve ring (mi nr) along the ring of tentacles. Processes from both the lateral and median tentacle nerves extend along the oral ciliary field (of) (ventral mesosome epidermis) towards the mouth. The density of processes is high in the oesophagus, but nerve processes were not observed to project below the mouth valve into the stomach. In general, the stomach and intestine are devoid of nerve processes. There is a nerve ring (pnr) along the posterior ciliary band. A nerve net of small bundles of processes is located all over the epidermis; it is especially well developed in the oral field.

The function of the different parts of the nervous system in the actinotroch larva has never been investigated; based on the location of nerves, however, the following suggestions can be made: The apical ganglion receives input and sends output through the median epistome nerves and the major nerve ring. The marginal epistome nerves innervate the marginal ciliary band and the marginal muscles, which depress the epistome. The epistome levator muscles (which consist of several minor fibres extending from the epistome margin posteriorly towards the mesosome) are probably innervated by small bundles of processes found in associa-