Serotonin in the morphogenesis of ascidian nervous system

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Abstract — The monoamine serotonin (5-HT) exerts key neuromodulatory activities in all animal phyla and it is supposed to play a morphogenetic role during the development. Nevertheless this aspect of serotonin activity is still poorly understood. By immunofluorescence and confocal laser scanning microscopy we studied the serotonergic nervous system of ascidian larvae belonging to the three orders of Aplousobranchia, Phlebobranchia and Stolidobranchia. A basic homology of the serotonergic nervous system was observed in all the species studied as most serotonergic components of nervous system are localized in the brain. However a lot of differences are shown from the fine cellular localization. Our results revealed that divergent developmental strategies exist during terminal differentiation, probably following different adaptation constraint.

Key words: Aplousobranchia, Ciona intestinalis, nervous system development, neurotransmitters, Phlebobranchia, Stolidobranchia, serotonin

INTRODUCTION

Ascidians are the most basal branch of the chordate phylum, which includes the vertebrates, and belong to the subphylum Urochordata, which diverged from the last common ancestor of all chordates at least 520 million years ago (Chen et al. 2003).

As chordate, they show a body plan (at least in the tadpole larval stage) and embryonic development very similar to those of vertebrates (Passamanek and Di Gregorio 2005). Asciidiacea are divided into three order Aplousobranchia, comprising mainly colonial species and considered the basal taxon; Phlebobranchia, comprising mainly solitary species; Stolidobranchia, comprising both colonial and solitary species and considered the most derived order (Burighel and Cloney 1997).

The ascidian larva is tadpole shaped and consists of a trunk and a tail with locomotory function. Larvae of colonial species bears in the trunk adult organs in an advanced level of differentiation, while in larvae of solitary species adult organs develops during metamorphosis from endoderm and mesenchyme of the trunk. The larva of the most studied solitary ascidian, Ciona intestinalis, contains about 2600 cells and only six tissue types: ectoderm, nervous system, notochord, muscle, endoderm and mesenchyme. The central nervous system (CNS) is formed of about 330 cells, of which about 100 are neurons (Nicol and Meinertzhagen 1991), and consists of four main regions along the anteroposterior axis: the rostral sensory vesicle, containing the sense organs, the neck and the visceral ganglion which form the “brain” in the trunk; the caudal nerve cord which houses the naked motor fibers coming from the visceral ganglion that synapse with the muscle cells of the tail. (Bone 1992; Cole and Meinertzhagen 2004). An increasing number of data on gene expression pattern along the anteroposterior axis suggests that the CNS of the ascidian larve is homologous to the vertebrate nervous system and could be considered a miniature model of the vertebrate one, with simple gene equipment (Meinertzhagen et al. 2004; Lemaire 2006).

The peripheral nervous system (PNS) of ascidian larvae consists of epidermal sensory neurons which extend long cilia into the tunic, as revealed by electron microscopy. They have been proposed to constitute mechanosensory organs and were found at more or less regular intervals in the epidermis of the tail and trunk (Torrence and Cloney 1982). At least two sensory neurons are found in each of the three adhesive papillae, at the
anterior tip of the larvae. The emerging axons make connections with the sensory vesicle forming the papillar nerves (Sotgia et al. 1998; Gianuzzi et al. 1999). About ten neurons are precisely positioned along the papillar nerves and at least other two groups have been observed in dorsal and posterior position to the sensory vesicle, along the connection with the visceral ganglion (Takamura 1998; Lemaire et al. 2002; Candiani et al. 2005) (Fig. 1).

The monoamine serotonin (5-HT) exerts key neuromodulatory activities in all animal phyla and it is supposed to play a morphogenetic role during the development in various experimental systems, from embryonic development of the nervous system, to metamorphosis and neuronal maturation (Buznikov et al. 2001). In vertebrate embryos, serotonin (5-HT) binds to cytoskeletal elements of the neuroepithelial cells and may regulate changes in cell shape and morphogenetic cell movements in neural tube closure (Lauder 1993) and in migration of neural crest cells (Nebigil et al. 2000).

In ascidians, the presence of 5-HT was first suggested by Erspamer (1946). It was firstly recognized in chromaffin cells of the digestive tracts (Lauder 1993) and in some cells of the endostyle of adults of Ciona, Ascidia, Asciidiella, Phallusia, and Styela (Georges 1985; Fritsch et al. 1982). By immunofluorescence and confocal microscopy serotonin was then localized in brain vesicle, in some primary neurons of the adhesive papillae and in primary neurons scattered in the tail epidermis of the larvae of Phallusia mammillata (Pennati et al. 2001).

In the present study, we comparatively examined the presence of serotonin in the CNS of larvae of various ascidian species, belonging to three orders.
Leica TCS-NT (Leica Microsystems, Heidelberg, Germany), equipped with laser argon/krypton, 75 mW multiline. Control experiments were performed pre-adsorbing anti serotonin antibody with 10µM serotonin (Sigma, Italy); in these cases no positive signal was detected.

RESULTS

Immunofluorescence and confocal microscopy experiments carried out in metamorphosing larvae of *C. intestinalis* (Phlebobranchia) revealed that serotonin was localized in the brain, in a position posterior to the sense organs, corresponding to the visceral ganglion. (Fig. 2)

The larva of *D. listerianum* (Aplousobranchia) is characterized by eversible papillae and contains two differentiating zooides. Anti-serotonin fluorescence was very evident in the three everted adhesive papillae. In the brain serotonin was localized in the posterior region. The differentiating zooides showed an adult-like pattern of anti-serotonin fluorescence localized in the oesophagus, endostyle and peripharyngeal band. (Fig. 3)

The larvae of *M. vulgaris* (Stolidobranchia) showed anti-serotonin fluorescence restricted to few cells of the brain, near the otolith. The adhesive papillae did not show fluorescence (Fig. 4).

In *S. plicata* (Stolidobranchia) swimming larvae the otolith is well developed and the ocellus remains vestigial and close to the otolith. Serotonin was detected in some cells of the brain, surrounding this single sense organ which is supposed to be functionally homologous to the two organs of other species (photolith). Anti-serotonin fluorescence was also present in the cells of the adhesive papillae, which are fused in a single organ (Fig. 5).

Fig. 2-5 — Immunofluorescence localization of serotonin. (ac, axial complex; ap, adhesive papillae; oc, ocellus; ot, otolith; ph, photolith; z, zooides). Fig. 2 Metamorphosed larva of *Ciona intestinalis* (Phlebobranchia) shows serotonin immunolocalization in posterior brain corresponding to the visceral ganglion (arrow). Fig 3. Larva of *Diplosoma listerianum* (Aplousobranchia) shows serotonin in everted papillae and in a posterior brain region. Differentiating zooides show an adult-like localization of serotonin in oesophagus, endostyle and in peripharyngeal band. Fig. 4 Larva of *Microcosmus vulgaris* (Stolidobranchia) shows serotonin immunolocalization only near the otolith. Fig 5. Larva of *Styela plicata* (Stolidobranchia) shows the serotonin immunofluorescence in adhesive papillae and in the brain, close to photolith. Scale bars equal 50 µm.
DISCUSSION

Our data demonstrate the presence of a serotonergic nervous system in all the species studied. Most serotonergic components of nervous system are localized in brain. However a lot of differences are shown from the fine cellular localization of the serotonin detected by immunofluorescence and confocal microscopy. In C. intestinalis anti-serotonin immunofluorescence appeared in the visceral ganglion. In M. vulgaris serotonin was localized in the anterior CNS, near the sense organs. In S. plicata serotonin was localized both near the single sense organ, the photolith, and in epidermal primary neurons of the palps. In D. listerianum serotonin was largely localized in the everted adhesive papillae and in a posterior region of the brain vesicle.

The pattern of distribution of serotonin does not seem to follow the phylogeny of the ascidians. In previous experiments carried out in P. mammillata (Phlebobranchia) by immunofluorescence and confocal microscopy, 5-HT was localized in the primary neurons of the adhesive papillae and in primary neurons scattered in the tail epidermis. Moreover 5-HT was localized in a group of cells of the brain vesicle, just dorsally to the photoreceptor organ (PENNATI et al. 2001). This pattern of serotonin distribution is very similar to that of S. plicata, a Stolidobranchia, but completely different from that of C. intestinalis, also belonging to the Phlebobranchia order.

Such differences are not surprising, given the heterogeneity of the nervous system details already known in ascidians. In his comparative study of the serotonergic nervous system among Tunicata, STACH (2005) delineated a schematic drawing of the serotonergic nervous system with an anterior aggregate of serotonergic cell bodies located in the brain, in a close connection with the sensory organs, and a network of fibers projecting posteriorly. As the posterior projections are directed toward the visceral ganglion, where motor neurons were identified (MEINHERTZHAGEN et al. 2004) the serotonergic neurons could function in coordination and modulation of tail locomotion in the second part of the larval life, when serotonin could be necessary also to start the morphogenetic events leading to metamorphosis (ZEGA et al. 2005). As the larvae we studied were all collected some hours after hatching, they could be in the physiological condition typical of a premetamorphic status.

The only exception was the C. intestinalis specimen: we showed in the Fig. 2 a metamorphosed larva, with the tail almost completely retracted, as in all the swimming and premetamorphosing larval stages we were not able to obtain a reliable anti-serotonin immunofluorescence. Nevertheless, TPH, the rate-limiting enzyme in the synthesis of serotonin and the most reliable marker of development of the serotonin-producing neurons (ALENINA et al. 2006), is expressed in the posterior brain of C. intestinalis swimming larvæ (PENNATI et al., submitted).

Our results suggest that in ascidians, despite a substantially convergent body plan, developmental strategies had different pathways during terminal differentiation, following different adaptation constraint.

The serotonergic system in all analyzed species, consisted in an anterior complex of numerous neurons, probably sending projections posteriorly, as described by STACH (2005). Thus, the serotonergic system of the ascidian larvae is arranged in a way resembling the general feature delineated by HAY-SCHNARR (2000) for deuterostome larvae.

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REFERENCES


