Acta Biologica Hungarica 55 (1–4), pp. 301–313 (2004)

EMBRYOGENESIS OF THE HISTAMINERGIC SYSTEM IN THE POND SNAIL, *LYMNAEA STAGNALIS* L.: AN IMMUNOCYTOCHEMICAL AND BIOCHEMICAL STUDY*

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(Received: August 31, 2003; accepted: December 1, 2003)

Embryogenesis of the histaminergic system in the pond snail, *Lymnaea stagnalis,* was investigated by means of immunocytochemistry and HPLC assay. From the earliest onset of the of histamine-immunoreactive (HA-IR) elements, the labelled neurons were confined to the pedal, cerebral and buccal ganglia, whereas no IR cells within the pleural, parietal and visceral ganglia were detectable during the embryogenesis. Peripheral projections of the embryonic HA-IR neurons were missing. No transient HA-IR neurons could be found either inside or outside the CNS. The first HA-IR elements appeared at about E55% of embryonic development, at the beginning of metamorphosis, and were represented by three pairs of neurons located in the cerebral ganglia. Following metamorphosis, four pairs of HA-IR neurons were added; two of them occurred in the pedal (E65% stage of development) and two in the buccal (E90% stage of development) ganglia. During embryogenesis, HA-IR fibers were present in the cerebro-pedal connectives and in the cerebral, pedal and buccal commissures, whereas only little arborization could be observed in the neuropil of the ganglia. HPLC measurements revealed a gradual increase of HA content in the embryos during development, corresponding well to the course of the appearance of immunolabeled elements. It is suggested that the developing HAergic system plays a specific role in the process of gangliogenesis and CNS plasticity of embryonic *Lymnaea.*

Keywords: Histamine – gastropods – molluscs – *Lymnaea* – embryogenesis – immunocytochemistry – HPLC

INTRODUCTION

The biogenic imidazole amine, histamine (HA), is an important chemical messenger widely distributed within the nervous system of both vertebrates and invertebrates. The discovery of HA, its synthesizing and degrading enzymes, and specific HA receptors in the nervous tissue of different species suggest that HA is a neurotransmitter or a neuromodulator [19, 28].

In the vertebrate brain, HA has been detected in three cellular compartments of the central nervous system (CNS): in hypothalamic tuberomammillary neurons and their

* Presented at the 10th ISIN Symposium on Invertebrate Neurobiology, July 5–9, 2003, Tihany, Hungary.

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processes [30], perivascular mast cells [29], and isolated microvessels [10]. The mammalian central HAergic system appears to regulate general activities and general metabolism, such as arousal state, anxiety, activation of the sympathetic nervous system, stress related release of hormones from the pituitary gland and of central aminergic neurotransmitters, antinociception, water-retention and supression of eating [4].

Among invertebrates, the localization and possible role of HA was investigated in detail first of all in the insect visual system. In insect photoreceptors, its presence and function as a neurotransmitter was demonstrated and firmly established by immunocytochemistry and electrophysiology, respectively [14, 15, 16, 28]. The occurrence of HA is, however, not restricted to the visual, or other sensory systems [1, 5, 23] of arthropods, but it was also found in interneurons and efferent nerve cells in the central nervous system of insects [18, 27, 31], crustaceans [26], chelicerates [33], and molluscs [13, 37]. Investigations on *Aplysia* have revealed much of the physiological and pharmacological properties of the HAergic neurons [22], and the transmitter role of HA in the feeding system has well been established [7]. In the course of a recent study, we have described the distribution and biochemical properties of HAergic elements in the central and peripheral nervous system of adult *Lymnaea stagnalis* and *Helix pomatia* [17], showing a relative high number of HA-containing neurons in the CNS, and a rich HAergic innervation of the ganglionic neuropils and certain peripheral tissues as well. HPLC assay, uptake-release experiments, and the demonstration of specific membrane effects of HA also suggested a signaling role of this monoamine in the nervous system of pulmonate snails. At the same time, there is no data on the developmental aspects of the HAergic system either in gastropods, or in other groups of invertebrates.

Studies on transmitter expression by developing neurons have recently been performed on a variety of model animals of invertebrate neurobiology. Investigations on gastropods, including freshwater pulmonates, such as *Lymnaea*, *Helisoma* and *Planorbis*, are greatly facilited by the relative simplicity of their nervous system and the identification of physiologically well-characterized neurons and neural networks. In addition, detailed knowledge has been accumulated on the morpho- and organogenesis of these animals, making them even more suitable for studies in developmental neurobiology [24, 25, 32]. The embryogenesis of neurons, which contain serotonin [21, 39], FMRFamide [8, 40], octopamine [12], dopamine/catecholamines [9, 42], and nitrogen monoxide [34, 35, 36], has been described, showing a difference in timing and location of appearance of the different signaling systems during development. The adult nervous system of *Lymnaea* and its signal molecules have also been the focus of numerous biochemical, anatomical, physiological and behavioral studies for a long time, furnishing important information on the cellular organization of different networks underlying behavior [2, 3, 6, 11, 20, 38, 43, 44].

Based on the above-mentioned data, the aim of the present study was to analyze the development of the HAergic system in the embryonic pond snail, *Lymnaea stagnalis*, with special attention to the spatio-temporal pattern of the distribution of HAergic neurons. The study included the determination of the exact time of appear-

ance of HAergic neurons in the CNS using immunocytochemistry, and the measurement of HA concentrations at different embryonic developmental stages applying HPLC technique.

MATERIALS AND METHODS

Animals

Embryos used in this study originated from egg masses laid in our laboratory. Adult specimens of the pond snail, *Lymnaea stagnalis* were collected from the Kis-Balaton Reservoir, kept thereafter in aquaria supplied with aerated running lakewater, and fed on lettuce. Egg masses were also maintained in aquaria. Stages of embryonic development were determined on the basis of specific morphological, morphometric and behavioral features, according to Meshcharyakov [24] and Morrill [25]. Developmental stages were expressed as a percentage of total embryonic development, where 0% (E0) corresponded to the first egg cleveage and 100% (E29) to hatching.

Immunocytochemistry

Immunocytochemical experiments were performed on whole-mount embryos, applying the two-step indirect immunofluorescence technique. Embryos were removed from their egg capsule, the shell and the visceral mass was cut and the body mass was immersed in freshly prepared fixative solution, containing 4% 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDAC, Sigma) diluted in 0.1 M phosphate buffer (PB) for 1.5 hours at room temperature, followed by additional fixation for 4 hours at 4 °C. Finally, the embryos were postfixed with 2% paraformaldehyde (PFA) diluted in 0.1 M PB for 1 hour at room temperature. Prior to fixation, partial proteolytic digestion was performed for 1.5 min at room temperature in 0.25% protease (type XIV, Sigma) in phosphate buffered saline (PBS), in order to ensure the better penetration of immunocytochemical reagents into the tissue. After fixation, preparations were thoroughly washed in several changes of 0.1 M PB. The immunocytochemical procedure consisted of the following steps: (i) 60 min at room temperature in PBS containing 0.25% BSA and 0.25% Triton-X 100 (PBS-TX-BSA); (ii) 24 h incubation at 4 °C with a polyclonal anti-HA antiserum raised in rabbit against HA coupled to succinylated keyhole limpet hemocyanin with EDAC (DiaSorin, Stillwater, U.S.A.), diluted to 1 : 1000 in PBS-TX-BSA (4% Triton-X-100); (iii) incubation with tetramethylrhodamine-isothyocyanate tagged swine-anti-rabbit IgG (TRITC, DAKO) diluted to 1 : 50 in PBS-TX-BSA for 5 h at room temperature. Preparations were mounted in a 3 : 1 mixture of glycerol and PBS, viewed and photographed in a Zeiss Axioplan compound microscope equipped with the appropriate filter sets.

HPLC

Whole embryos (4–15 embryos for each HPLC assay, 4 parallel experiments for all stages) were homogenized in 100 μl 2% perchloric acid. The HA concentrations were measured by HPLC, according to the method of Yamatodani et al. [45]. The perchloric extract was centrifuged and the aliquots of the supernatant were injected by a CMA/200 automatic sample injector (CMA/Microdialysis, Stockholm, Sweden) onto a 60-mm-long TSK-GEL Histaminepak cation exchange column (TOSO-HAAS) and eluted with 0.25 M KH_2PO_4. The column effluent was then mixed on-line with a solution of 0.1% ortho-phthalaldehyde (OPA, Fluka Chemie) and another solution of 2 M NaOH and 0.2 M boric acid, and derivatized in a reaction loop held at 45 °C, and then the reaction was terminated by on-line mixing with a 3 M solution of phosphoric acid and the fluorescent OPA-derivatives were measured in a Merck/Hitachi F-1050 fluorescence spectrophotometer at 450 nm with excitation at 360 nm. The data were collected and analyzed on a Merck/Hitachi D-2500 chromatointegrator (Hitachi). The mobile phase and the three reaction solutions were all pumped with Pharmacia LKB 2150 HPLC pumps (Pharmacia LKB Biotechnology).

RESULTS

Appearance and distribution of the HA-IR neurons in embryos

In *Lymnaea* embryos before stage E50% (end of veliger stage) no immunoreactivity could be detected. HA immunoreactivity was first expressed by neurons within the CNS at E55% stage of embryonic development (onset of metamorphosis), a time point which corresponds to the premetamorphic veliger stage. At this early developmental stage when nerve cells just begin to proliferate within the central ganglia, three intensely stained, bilaterally symmetric HA-IR cell pairs could be found in the anterodorsal quadrant of the embryo, an area corresponding to the cerebral ganglia (Figs 1, 4). The labeled cells were localized close to each other in the right and left cerebral ganglia. The morphology and size of these neurons were similar: small size (diameter 20–25 μm) unipolar cells sent a fine axon processes through the developing ganglia into the cerebro-pedal connectives and the pedal commissure (Fig. 1B–D). Hence the cerebro-pedal loop could already clearly be identified at this time of development of the HAergic system (Fig. 1A, C).

By stage E65% (postmetamorphotic, adult-like form), the first HA-IR neurons represented by one pair of cells could be visualized in the pedal ganglia (Figs 2, 4). The morphology and size of these cells, showing also bilateral symmetry, were similar to those found in the cerebral ganglia. Hence, at this early postmetamorphic stage, altogether 8 cells expressing HA-immunoreactivity were present in the cerebral and pedal ganglia of the developing CNS. The cerebral- and pedal commissures, as well as the cerebro-pedal connectives contained labeled fibers originating from these neurons.

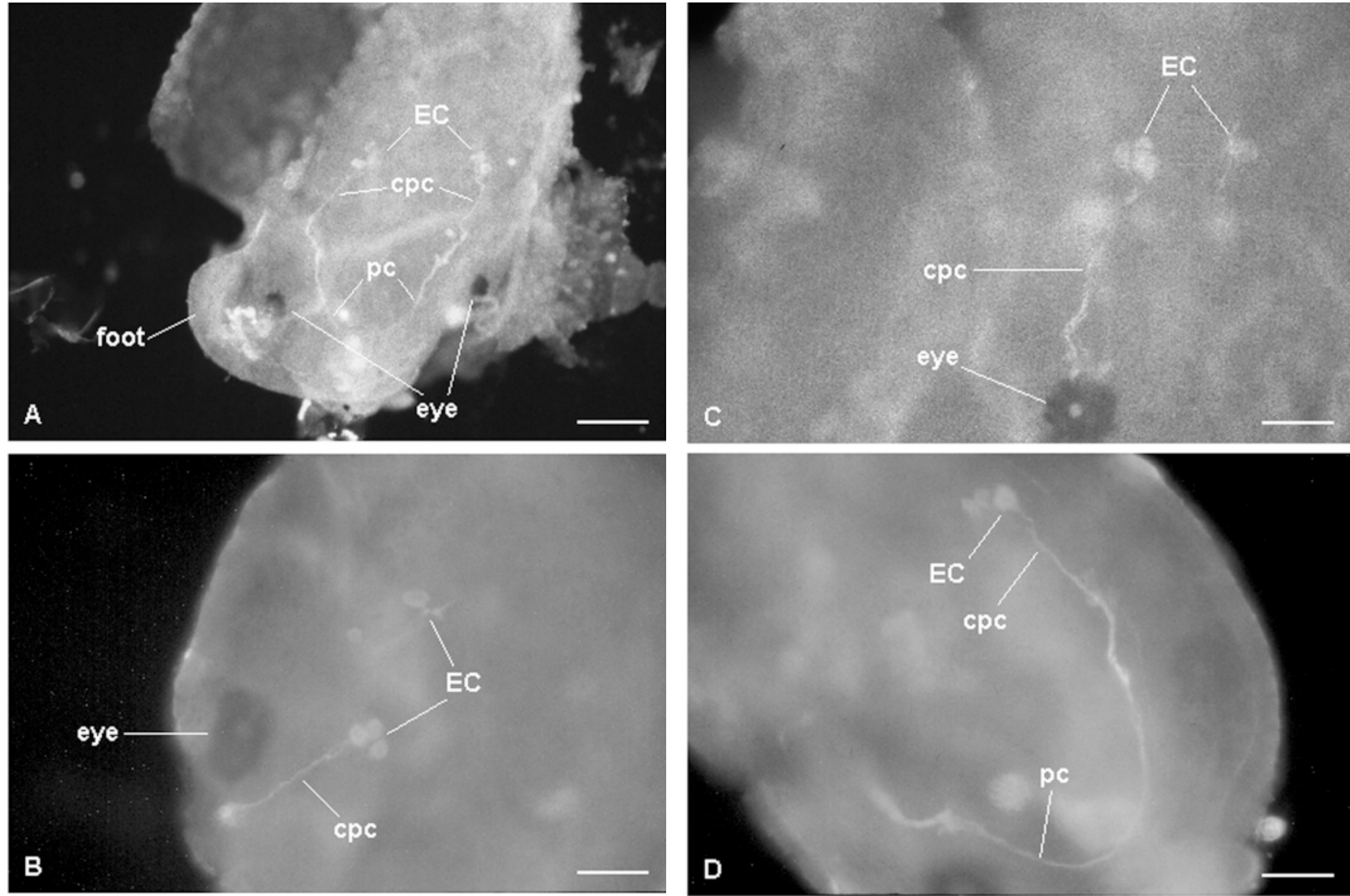

Fig. 1. HA-IR elements in *Lymnaea* embryos at E55% developmental stage. Whole-mount preparations, TRITC-labeling. (A) Low magnification fronto-horizontal view of an embryo, showing HA-IR neurons (EC) in the cerebral ganglia, and also labeled axon processes in the cerebro-pedal connectives (cpc) and pedal commissure (pc). (B, C) – Higher magnification of the HA-IR cells (EC) in the cerebral ganglia, and their processes in the cerebro-pedal connectives (cpc). (D) – HA-IR fibers, originating from EC neurons, in the cerebro-pedal loop formed by the cpc and pc. Ventral view. Scale bars: 40 µm in A, 80 µm in B, C and D

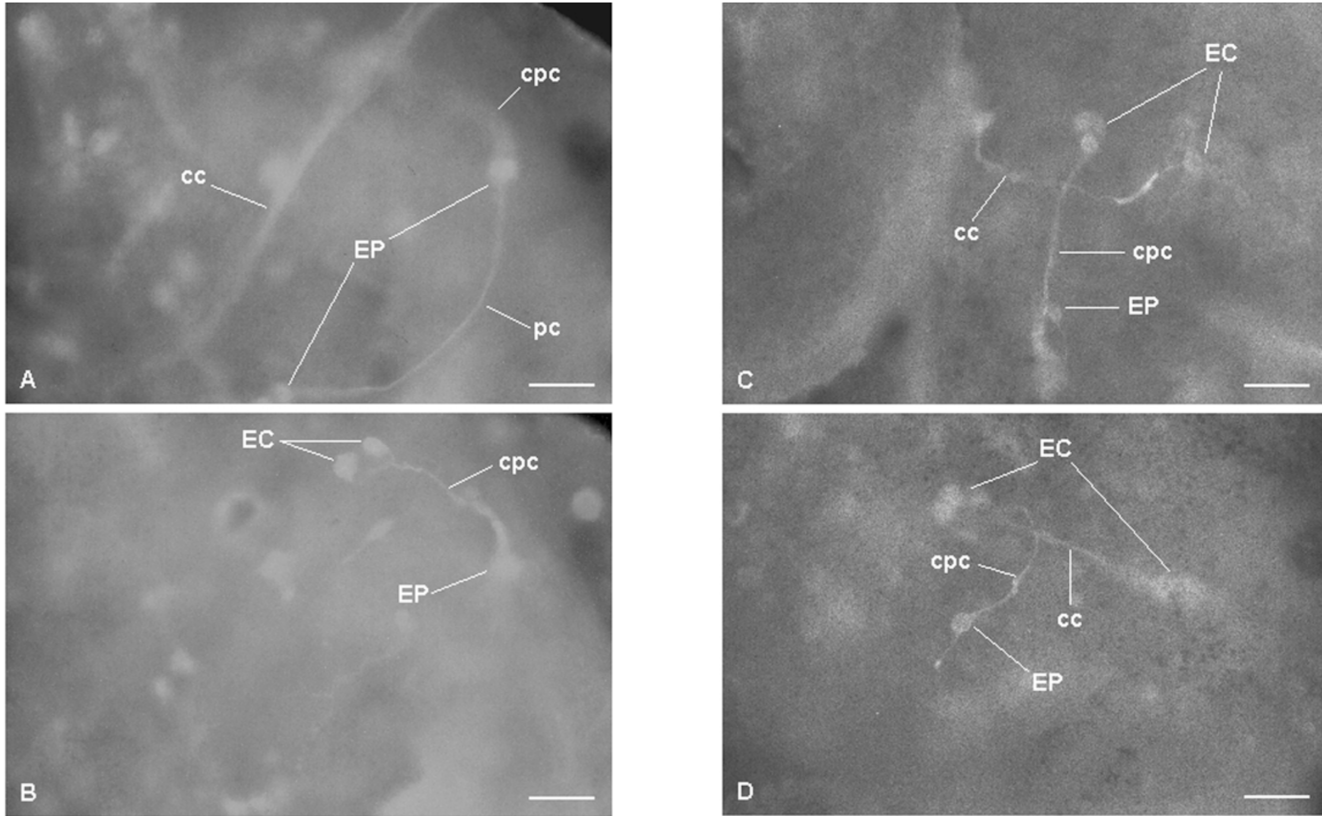

Fig. 2. By E65% developmental stage, the first pair of HA-IR neurons (EP) in the pedal ganglia becomes detectable (A–C). Both the pedal (pc) and cerebral (cc) commissures, and the cerebro-pedal connectives (cpc) contain labeled fibers originating from the cells located in the cerebral (EC) and the pedal (EP) ganglia, respectively (A–D). A – ventral view, B, C and D – dorsal view. Whole-mount preparations, TRITC-labeling. Scale bars: 80 μm

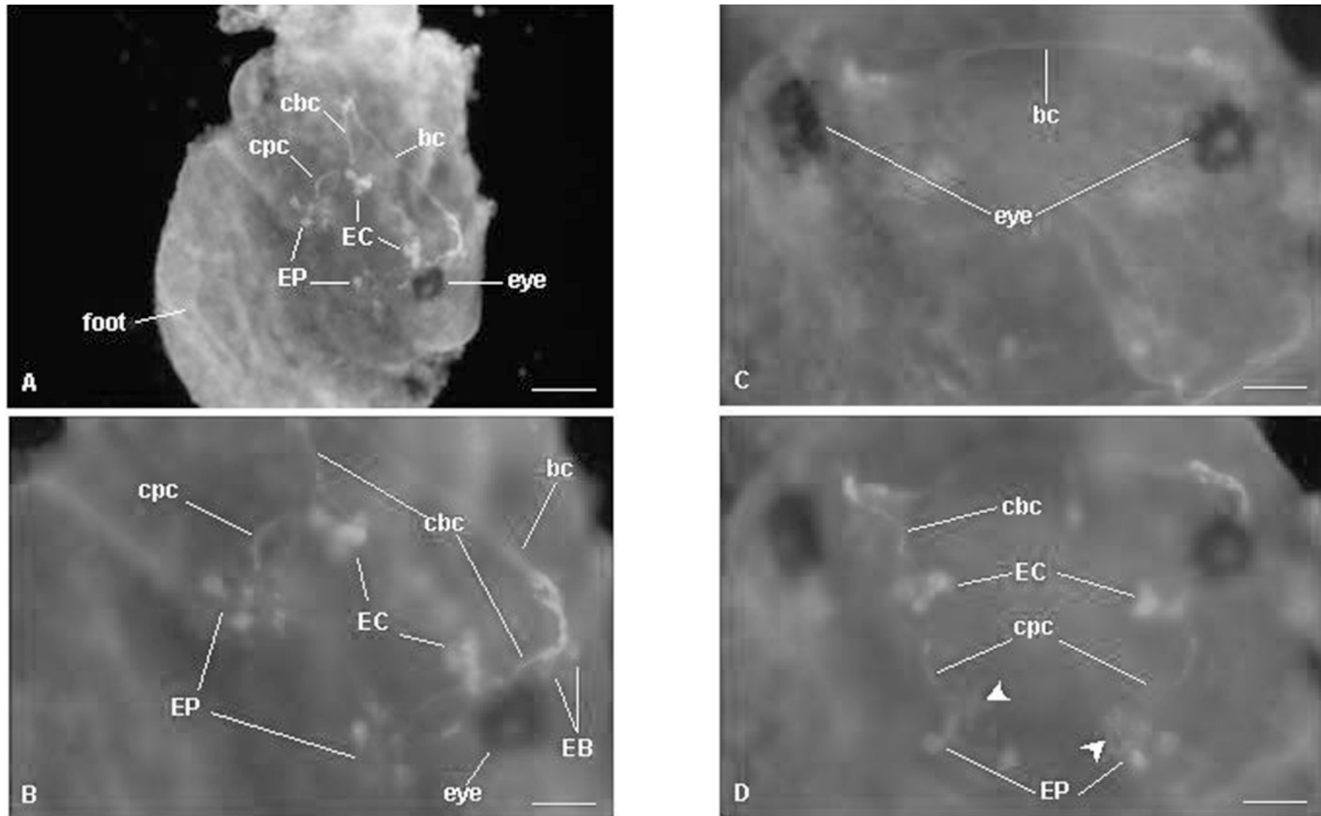

Fig. 3. HA-IR cells at E85–90% developmental stage in the cerebral, pedal and buccal ganglia of *Lymnaea* embryos. (A) Low magnification lateral view of an embryo, showing HA-IR neurons in the cerebral (EC) and pedal ganglia (EP), as well as labeled fibers in the buccal commissure (bc), and the cerebro-buccal (cbc) and cerebro-pedal (CPC) connectives. (B) Higher magnification detail of A. Two faintly labeled cells (EB) can be seen in the buccal ganglion, and one additional cell (EP) appear in each pedal ganglion. (C, D) Dorsal view of the anterior (head) region, showing the exact anatomical location of HA-IR cells and the organization of their projections. Note IR varicose arborization (arrowheads) in the pedal ganglia. Whole-mount preparation, TRITC-labeling. A and B – lateral view, C and D – dorsal view. Scale bars: 40 µm in A, 80 µm in B, C and D

At E90% stage of embryonic development, one day before hatching, an additional pair of neurons exhibited HA-immunoreactivity in the pedal ganglia (Figs 3A, B and D, 4), and two pairs of immunoreactive neurons in the buccal ganglia were added to the already existing small population of HA-IR neurons (Figs 3A, B and D, 4). These cells represented the last HA-IR neurons detected during the embryogenesis of *Lymnaea*. HA-IR processes could also be demonstrated in the buccal commissure (Figs 3A–C, 4).

Hence altogether 14 HAergic neurons could be found before hatching in the developing CNS of the pond snail, *Lymnaea stagnalis*, and these HA-IR neurons were confined to the cerebral, pedal and buccal ganglia (Fig. 4). No IR cell bodies in the pleural, parietal or visceral ganglia could be observed during the embryogenesis, and only little arborization of fibers was present in the neuropil of these ganglia. No peripheral projections and peripheral cells labeled by anti-HA antibody were detectable either, the axon processes of the HA-IR neurons remained within the ganglion ring until hatching.

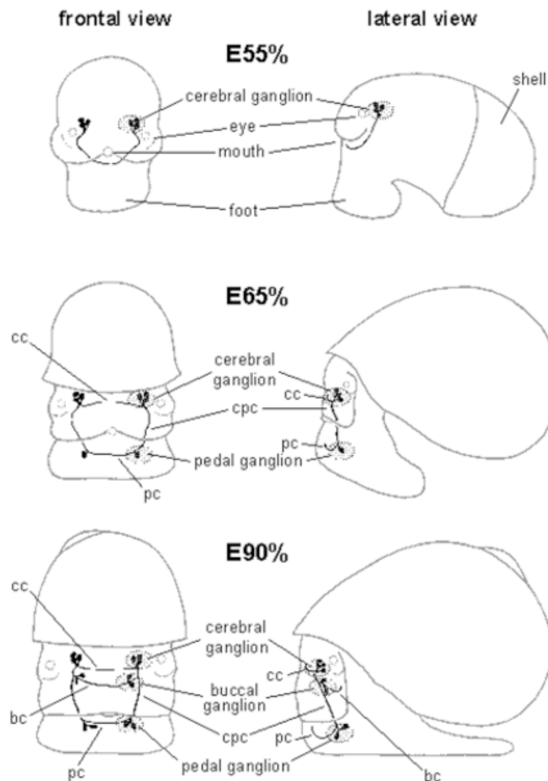

Fig. 4. Schematic drawing of the embryonic development of HA-IR elements in the CNS of *Lymnaea stagnalis*. Frontal and lateral views depicted at E55%, E65% and E90% developmental stages. cc – cerebral commissure, cpc – cerebro-pedal connective, pc – pedal commissure, bc – buccal commissure

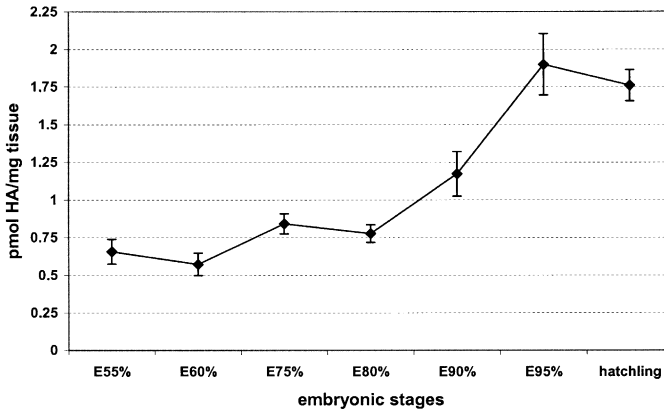

Fig. 5. Changes of HA concentrations at different developmental stages in *Lymnaea* embryos. Note the significant increase of HA content from the late, E80% stage of embryogenesis

Table 1
HA concentrations at different embryonic stages of *Lymnaea stagnalis*

Developmental stages	[HA] pmol/mg tissue
E55% (n = 4 × 15)	0.66 ± 0.08
E65% (n = 4 × 12)	0.57 ± 0.07
E75% (n = 4 × 10)	0.84 ± 0.07
E80% (n = 4 × 10)	0.78 ± 0.06
E90% (n = 4 × 8)	1.17 ± 0.14
E100% (n = 4 × 5)	1.90 ± 0.20
Hatchling (n = 4 × 5)	1.76 ± 0.10

HPLC assays

Concentrations of HA were determined from the E50–55% embryonic stage to hatching (E100%). Data are summarized in Figure 5 and Table 1. At E55% stage of embryogenesis, when the first HA-IR elements appeared, HA could be assayed in a low concentration (0.66 ± 0.08 pmol/mg tissue). The low concentration values did not change significantly during the following three stages (0.57 – 0.84 pmol/mg tissue), but at E90% stage a rapid increase of HA content (1.17 ± 0.14 pmol/mg tissue) could be measured, followed by the highest HA levels assayed at E95% developmental stage (1.9 ± 0.2) and in hatchlings (1.76 ± 0.1).

DISCUSSION

Our immunocytochemical and HPLC data show unequivocally that a small set of HAergic neurons is present in the developing CNS of the pond snail, *Lymnaea stagnalis*. The first HA-IR cells appeared at the beginning of the formation of the cerebral ganglia (E55% developmental stage), followed until hatching by five additional pairs of labeled neurons distributed in the cerebral, pedal and buccal ganglia. The changes of HA content measured by HPLC correlated well with gradual appearance of the HA-IR elements at different stages after metamorphosis.

The first FMRFamide-, dopamine- and serotonin-IR neurons appear in *Lymnaea* very early, during the trochophore-veliger stages (E15-30% of embryogenesis), outside the future CNS, when no central ganglia yet exist. However, these latter cells cease to express their transmitter phenotype by hatching, and therefore are called transient cells [8, 39, 41, 42]. In contrast, we have not found any transient HA-IR neuron either in- or outside the CNS during the embryonic development of *Lymnaea*, and this also was characteristic for the octopaminergic system appearing very late, one day before hatching [12]. The embryogenesis of neurons belonging to different signaling systems in the *Lymnaea* CNS shows two basic types of temporal pattern: i) early (veliger) appearance followed by a gradual increase until hatching in cell number and arborization pattern (serotonin, FMRFamide [21, 39, 40, 41]); ii) late appearance of neuronal populations with chemical specificity (dopamine, octopamine [12, 42]). These findings were also supported by the parallel biochemical detection of the different bioactive substances with HPLC or radioimmunoassay. The embryogenesis of the HAergic system does not seem to fit into any of the two developmental types described above, but represents a third way of the embryogenesis of chemical specifity of neurons in *Lymnaea*. At E55% of development three pairs of HA-IR cells appear in the cerebral ganglia, to which four pairs will be added, hence a very small set of altogether 14 neurons is present in the CNS of hatching. In addition, the localization of the HA-IR neurons is confined to three ganglia, the cerebral, pedal and buccal ones, and the labeled cells display little arborization in the neuropil. This suggests that the embryonic HAergic system plays a specific, anatomically (synaptically) limited, local role in gangliogenesis and events of early neuronal plasticity.

Compared to the number of HA-IR neurons (approximately 130) in the adult *Lymnaea* CNS [17], the total number (14) of embryonic HAergic cells represent only about a tenth of the fully developed, adult system. Similarly to the embryonic distribution, the vast majority of the HA-IR neurons are located in the cerebral, pedal and buccal ganglia of the adult *Lymnaea* [17], but in adults the neuropil of all central ganglia and several peripheral nerves are densely supplied by labeled processes. In adults, a part of the peripheral tissues, such as the lip and foot, are also innervated by HA-IR elements, and the statocysts attached to the pedal ganglia, contain 7 to 8 HA-immunoreactive sensory cells. This shows unequivocally that the development of the HAergic system does not stop at hatching, but numerous HA-IR cells will be added to the already existing cell clusters of the central ganglia. It also involves the appear-

ance of other, novel cell groups in the three ganglia of the developing CNS, and also the peripheral HAergic system evolves during postembryogenesis. All these findings indicate that postembryogenesis (juvenile life) is the decisive period for the maturation of the HAergic system, during which it would acquire not only its final distribution and anatomical shape, but also the functional/regulatory role(s) at both central and peripheral levels would be fixed at this time.

The establishment of the HAergic system in the embryonic nervous system of *Lymnaea* is obviously an early and stereotypic ontogenetic event. The small number and easy identification of the embryonic HA-IR neurons as well as their clear spatio-temporal pattern of emergence shown in this study provide a solid background for future work to determine the possible role of HA in neurogenesis and behavioral ontogeny of *Lymnaea*, as well as the exact time of the anatomical and functional onset of the HAergic system in the course of postembryogenesis, in free-living juveniles.

ACKNOWLEDGEMENTS

The skillful technical assistance of Ms. Zsuzsanna N. Fekete, Ms. Éva Karácsonyi and Mr. Boldizsár Balázs is much appreciated. This work was supported by grants form the Hungarian Scientific Research Fund (OTKA), Nos. 29559 and 34106, to K. E.

REFERENCES

1. Battelle, B. A., Calman, B. G., Andrews, A. W., Grieco, F. D., Mleziva, M. B., Callaway, J. C., Stuart, A. E. (1991) Histamine: a putative afferent neurotransmitter in *Limulus* eyes. *J. Comp. Neurol. 305*, 527–542.
2. Benjamin, P. R., Burke, J. F. (1994) Alternative mRNA splicing of the FMRFamide gene and its role in neuropeptidergic signalling in a defined neural network. *BioEssays 16*, 335–342.
3. Benjamin, P. R., Staras, K., Kemenes, G. (2000) A systems approach to the cellular analysis of associative learning in the pond snail *Lymnaea*. *Learning and Memory 7*, 124–131
4. Brown, R. E., Stevens, D. R., Haas, H. L. (2001) The physiology of brain histamine. *Prog. Neurobiol. 63*, 637–672.
5. Buchner, E., Buchner, S. M. G., Hofbauer, A., Pak, W. L., Pollack, I. (1993) Histamine is a major mechanosensory neurotransmitter candidate in *Drosophila melanogaster*. *Cell Tissue Res. 273*, 119–125.
6. Chase, R. (2002) *Behavior and its neural control in gastropod molluscs.* Oxford Univ. Press, New York.
7. Chiel, H. J., Weiss, K. R., Kupfermann, I. (1990) Multiple roles of a histaminergic afferent neuron in the feeding behavior of *Aplysia*. *Trends Neurosci. 13*, 223–227
8. Croll, R. P., Voronezhskaya, E. E. (1996) Early elements in gastropod neurogenesis. *Dev. Biol. 173*, 344–347.
9. Croll, R. P., Voronezhskaya, E. E., Hiripi, L., Elekes, K. (1999) Development of catecholaminergic neurons in the pond snail, *Lymnaea stagnalis*: II. Postembryonic development of central and peripheral cells. *J. Comp. Neurol. 404*, 297–309.
10. Edvinsson, L., MacKenzie, E. T., McCullogh, J. (1993). Histamine. In: Edvinsson, L., MacKenzie, E. T., McCulloch, J. (eds), *Cerebral blood flow and metabolism*. Raven Press, New York, pp. 313–324.

11. Elekes, K., Kemenes, G., Hiripi, L., Geffard, M., Benjamin, P. R. (1991) Dopamine-immunoreactive neurones in the central nervous system of the pond snail *Lymnaea stagnalis*. *J. Comp. Neurol. 307*, 214–224.
12. Elekes, K., Voronezhskaya, E. E., Hiripi, L., Eckert, M., Rapus, J. (1996) Octopamine in the developing nervous system of the pond snail, *Lymnaea stagnalis* L. *Acta Biol. Hung*. *47*, 73–87.
13. Elste, A., Koester, J., Shapiro, E., Panula, P., Schwartz, J. H. (1990) Identification of histaminergic cells in *Aplysia*. *J. Neurophysiol*. Vol. 64, No. 3. 736–744.
14. Hardie, R. C. (1987) Is histamine a neurotransmitter in insect photoreceptors? *J. Comp. Physiol. 161A*, 201–213.
15. Hardie, R. C. (1988) Effects of antagonists on putative histamine receptors in the first visual neuropil of the housefly (*Musca domestica*). *J. Exp. Biol*. *138*, 221–241.
16. Hardie, R. C. (1989) A histamine-activated chloride channel involved in neurotransmission at a photoreceptor synapse. *Nature 339*, 704–706.
17. Hegedűs, E., Kaslin, J., Hiripi, L., Kiss, T., Panula, P., Elekes, K. (2004) Histaminergic neurons in the central and peripheral nervous system of gastropods (*Helix, Lymnaea*): an immunocytochemical, biochemical and electrophysiological approach. *J. Comp. Neurol.* (in press).
18. Homberg, U., Hildebrand, J. G. (1991) Histamine-immunoreactive neurons in the midbrain and suboesophageal ganglion of the sphinx moth *Manduca sexta*. *J. Comp. Neurol. 307*, 647–657.
19. Hough, L. B. (1988) Cellular localisation and possible functions for brain histamine: recent progress. *Prog. Neurobiol 30*, 469–505.
20. Kemenes, G., Elekes, K., Hiripi, L., Benjamin, P. R. (1989) A comparison of four techniques for mapping the distribution of serotonin and serotonin-containing neurons in fixed and living ganglia of the snail, *Lymnaea*. *J. Neurocytology 18*, 193–208.
21. Marois, R., Croll, R. P. (1992) Development of serotonin-like immunoreactivity in the embryonic nervous system of the snail *Lymnaea stagnalis*. *J. Comp. Neurol*. *322*, 255–265.
22. McCaman, R. E., Weinreich, D. (1985) Histaminergic synaptic transmission in the cerebral ganglion of *Aplysia*. *J. Neurophysiol*. *53*, 1016–1037.
23. McClintock, T. S., Ache, B. W. (1989) Histamine directly gates a cloride channel in lobster olfactory receptor neurons. *Proc. Natl. Acad. Sci. USA 86*, 8137–8141.
24. Meshcheryakov, V. N. (1990) The common pond snail *Lymnaea stagnalis* L. In: Detlaff, D. A., Vassetzky, S. G. (eds) *Animal species for developmental studies*. Plenum Press, New York, London, pp. 69–132.
25. Morrill, J. B. (1982) Development of the pulmonate gastropod, *Lymnaea*. In: Harrison, F. W., Cowden, R. R. (eds) *Developmental biology of freshwater invertebrates*. Alan P. Liss, New York, pp. 399–483.
26. Mulloney, B., Hall, W. M. (1991) Neurons with histamine-like immunoreactivity in the segmental and stomatogastric nervous system of the crayfish *Pacifastacus leniusculus* and the lobster *Homarus americanus*. *Cell Tissue Res*. *266*, 197–297.
27. Nässel, D. R., Pirvola, U., Panula, P. (1990) Histamine-like immunoreactive neurons innervating putative neurohemal areas and central neuropil in the thoracic-abdominal ganglia of the flies *Drosophila* and *Calliphora*. *J. Comp. Neurol. 297*, 525–536.
28. Nässel, D. R. (1999) Histamine in the brain of insects: a review. *Micr. Res. Tech*. *44*, 121–136.
29. Panula, P., Karlstedt, T., Sallmen, T., Peitsaro, N., Kaslin, J., Michelsen, K. A., Anichtchik, O., Kukko-Lukjanov, T., Lintunen, M. (2000) The histaminergic system in the brain: structural characteristics and changes in hibernation. *J. Chem. Neuroanat. 18*, 65–74.
30. Panula, P., Yang, H. Y., Costa, E. (1984) Histamine-containing neurons in the rat hypothalamus. *Proc. Natl. Acad. Sci. USA 81*, 2572–2576.
31. Pirvola, U., Tuomisto, L., Yamatodani, A., Panula, P. (1988) Distribution of histamine in the cockroach brain and visual system: an immunocytochemical and biochemical study. *J. Comp. Neurol*. *276*, 514–526.
32. Raven, C. P. (1966) *Morphogenesis: The analysis of molluscan development.* Pergamon Press, Oxford.

33. Schmid, A., Duncker, M. (1993) On the function of histamine in the central nervous system of Arthropods. *Acta Biol. Hung*. *44*, 67–75.
34. Serfőző, Z., Elekes, K., Varga, V. (1998) NADPH-diaphorase activity in the nervous system of the embryonic and juvenile pond snail, *Lymnaea stagnalis*. *Cell Tissue Res*. *292*, 579–586.
35. Serfőző, Z., Elekes, K. (2002) Nitric oxide level regulates the embryonic development of the pond snail *Lymnaea stagnalis*: pharmacological, behavioral, and ultrastructural studies. *Cell Tissue Res*. *310*, 119–130.
36. Serfőző, Z., Veréb, Z., Rőszer, T., Kemenes, Gy., Elekes, K. (2002) Development of the nitric oxide/cGMP system in the embryonic and juvenile pond snail, *Lymnaea stagnalis* L. A comparative *in situ* hybridization, histochemical and immunohistochemical study. *J. Neurocytology 31*, 131–147.
37. Soinila, S., Mpitsos, G. J., Panula, P. (1990) Comparative study of histamine immunoreactivity in nervous systems of *Aplysia* and *Pleurobranchaea*. *J. Comp. Neurol. 298*, 83–96.
38. Vehovszky, Á., Elliott, C. H., Voronezhskaya, E. E., Hiripi, L., Elekes, K. (1998) Octopamine: a new feeding modulator in *Lymnaea*. *Phil. Trans. Roy. Soc. B Lond. 353*, 1631–1643.
39. Voronezhskaya, E. E., Elekes, K. (1993) Distribution of serotonin-like immunoreactive neurons in the embryonic nervous system of lymnaeid and planorbid snails. *Neurobiology 1*, 371–383.
40. Voronezhskaya, E. E., Elekes, K. (1996) Transient and sustained expression of FMRFamide-like immunoreactivity in the developing nervous system of *Lymnaea stagnalis* (Mollusca, Pulmonata). *Cell. Mol. Neurobiol*. *16*, 661–676.
41. Voronezhskaya, E. E., Elekes, K. (2003) Expression of peptides encoded by the FMRFamide gene in the developing nervous system of *Lymnaea stagnalis*. *Cell Tissue res.* (in press).
42. Voronezhskaya, E. E., Hiripi, L., Elekes, K., Croll, R. P. (1999) Development of catecholaminergic neurons in the pond snail, *Lymnaea stagnalis*: I. Embryonic development of dopamine-containing neurons and dopamine-dependent behaviors. *J. Comp. Neurol. 404*, 285–296.
43. Willows, A. O. D. (1985) *The Mollusca: Neurobiology and Behaviour.* Part 1, Vol. 8, Academic Press, Orlando.
44. Willows, A. O. D. (1986) *The Mollusca: Neurobiology and Behaviour.* Part 2, Vol. 9, Academic Press, Orlando.
45. Yamatodani, A., Fukuda, H., Wada, H. (1985) High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. *J. Chromatography 344*, 115–123.