Developmental Expression of the Octopamine Phenotype in Lobsters, Homarus americanus

HENNING SCHNEIDER, PRATISHA BUDHIRAJA, ISMENI WALTER, BARBARA S. BELTZ, ERIN PECKOL, AND EDWARD A. KRAVITZ
Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115 (H.S., P.B., I.W., E.A.K.); Biology Department, Wellesley College, Wellesley, Massachusetts 02181 (B.S.B., E.P.)

ABSTRACT
We have used immunocytochemical methods to examine the sequence of appearance of octopamine-immunoreactive neurons during development, and to try to correlate that appearance with the emergence of behavioral or physiological capabilities. The first octopamine neurons express their transmitter phenotype at approximately 43% of embryonic development. The last cells show immunostaining at the 3rd larval stage. In the wild, therefore, immunoreactivity in cells appears over a 9–12 month period. In contrast, serotonin-immunoreactive neurons stain early in embryonic development and the last serotonin-immunoreactive cells appear at about the same time the first octopamine-immunoreactive neurons show staining. The pattern of appearance of octopamine-immunoreactive cells is cell type-specific. A pair of brain cells and the descending interneurons stain first. Additional brain cell staining is seen throughout embryonic development. The ascending interneurons appear next, and a general anterior-posterior gradient typifies their emergence over a relatively short portion of embryonic life (E 48–62%). The neurosecretory cell staining appears last, is segment-specific, begins at about 62% development, and continues to the 3rd larval stage. The emergence of immunostaining for amine neurotransmitters within groups of identified neurons at precise times in development may specify possible functional units. With at least one group of cells, this possibility seems plausible: the three pairs of claw octopamine neurosecretory cells show immunostaining as a unit.

INDEXING TERMS: amines, invertebrate, nervous system, ontogeny, immunocytochemistry

It is both a pleasure and an honor for us to have been invited to submit this manuscript to the special issue of the Journal of Comparative Neurology dedicated to Sandy Palay. Sandy is a long-term colleague and friend of the members of the Neurobiology Department at Harvard Medical School, whose work we admire a great deal and whose contributions to the field of neuroanatomy are enormous. One of us (E.A.K.) has fond memories of an exciting discussion group formed by neuroanatomists, neurophysiologists and biochemists at Harvard Medical School in the early days before Neuroscience was the enormous field it is today. Particularly vivid are the memories of Sandy’s spectacular electron microscopic profiles of central nervous system synapses and synaptic junctions that illuminated our discussions of the roles of vesicles in transmitter release and helped resolve questions on the nature of transmission in the central nervous system. We dedicate this paper to him.

The phenolamine octopamine is the major amine derived from tyrosine in crustacean and in insect nervous systems (Stevenson and Spörhase-Eichmann, 1995, for review). Octopamine is the invertebrate analog of norepinephrine and it has been implicated in motor control and agonistic behavior in crustaceans. In the American lobster, Homarus americanus, octopamine injections trigger postural extension by activating slow extensor and inhibiting slow flexor

Accepted November 21, 1995.

Ismeni Walter’s present address is Biologische Anstalt Helgoland, Marine Station, 27498 Helgoland, Germany.

Erin Peckol’s present address is Program in Developmental Biology, Department of Anatomy, University of California, San Francisco, San Francisco, CA 94143-0422.

Address reprint requests to Dr. Henning Schneider, Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, Boston, MA 02115. E-mail: schnei@warren.med.harvard.edu

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motor circuits (Livingstone et al., 1980; Harris-Warrick and Kravitz, 1984; Harris-Warrick, 1985; Kravitz, 1988). The octopamine actions are opposite to those triggered by serotonin injections which favor the readout of slow flexor motor programs (Livingstone et al., 1980; Harris-Warrick and Kravitz, 1984). Extended and flexed postures are expressed by subordinate and dominant lobsters during agonistic encounters, thereby possibly linking the amine actions with agonistic behavior (Livingstone et al., 1980; Kravitz, 1988, for review). Modulatory actions of octopamine in crustacean motor systems also have been described in escape reflexes in crayfish, Procambarus clarkii (Glanzman and Krasne, 1983; Krasne and Glanzman, 1986), in the beating of swimmeret appendages in the crayfish, Pacifastacus leniusculus (Mulloney et al., 1987), in the stomatogastric system of the spiny lobster, Panulirus interruptus (Flamm and Harris-Warrick, 1986; Heinsel, 1988), and in optokinetic responses of the crab, Leptograpsus variegatus (Erber and Sandeman, 1989). Pre- and postsynaptic sites are targeted by the amine, with activation of the CAMP and phosphatidyl inositol signaling pathways (Battelle and Kravitz, 1978; Florey and Rathmayer, 1978; Breen and Atwood, 1983; Fischer and Florey, 1983; Flamm et al., 1987). In addition, octopamine increases the sensitivity of sensory organs (Pazstor and Bush, 1987, 1989), enhances the strength and frequency of the heartbeat (Sullivan and Barker, 1975; Battelle and Kravitz, 1978), and facilitates the hemolymph clotting (Battelle and Kravitz, 1978).

A map of octopamine-immunoreactive neurons describing the location of cell bodies and projection paths has been constructed for the American lobster, Homarus americanus (Schneider et al., 1993). Approximately 86 octopamine-immunopositive cells, mostly with long projections, are formed throughout the entire central nervous system of postlarval (4th stage) lobsters, the earliest stage that resembles adult animals. Using anatomical criteria, 52 of these cells were divided into 4 groups: (1) neurosecretory anterior and posterior crotch cells, (2) descending interneurons, (3) thoracic ascending interneurons, and (4) supersophagal (brain) ganglion neurons. Many of these cells persist in juvenile and adult lobsters (Schneider et al., 1993). The serotonin neuron system also has been mapped (Beltz and Kravitz, 1983), and shown to be fully formed by the midembryonic stage (Beltz and Kravitz, 1987). For a subgroup of the serotonin cells, the neurosecretory neurons, a functional role has been defined. These cells act as gain setters in posterior motor circuits (Ma et al., 1992). No information is available about the formation or function of the octopaminergic system. Here we present an analysis of the appearance of the octopamine phenotype during ontogeny.

Lobster development proceeds through embryonic, prelarval, larval and postlarval stages (Herrick, 1895; Charmantier, 1991, for review). The first half of embryonic development is characterized by dramatic changes in the external and internal morphology of embryos, but development slows during the second half (Helly and Beltz, 1991). The only discernible behavior during embryonic life is the tailflip reflex, which first appears in midembryonic life (Cole and Lang, 1977; Helly et al., 1993). During the first few weeks after hatching, animals molt four times, and during this larval period lead a planktonic, top-dwelling existence. Larval animals bear little resemblance to adult lobsters: they keep afloat in a flexed posture by rapidly moving their large thoracic exopodites. With the metamorphic molt to the 4th stage, the postlarval lobsters resemble adult animals (for review see Charmantier et al., 1991). Now they swim rapidly with their bodies extended, propelled by beating, fully formed swimmerets (Davis and Davis, 1973; Ennis, 1986). Later in the 4th stage, lobsters reduce their swimming activity, settle to the substrate and begin to dig burrows for shelter. In adult lobsters, a similar posture is triggered by octopamine injection (Livingstone et al., 1980).

Early in decapod development, substantial changes in locomotory systems take place, which can be associated with particular larval and postlarval stages. For example, the thoracic exopodites degenerate over the 3rd to 4th developmental stages (Govind et al., 1988) while the swimmeret system gradually matures over the pelagic period (Davis and Davis, 1973; Kirk and Govind, 1992). This restructuring of locomotory systems is accompanied by changes in postural systems (Kirk and Govind, 1983). At the neuronal level exopodite motoneurons degenerate (Govind et al., 1988), abdominal extension and flexion motor neurons grow and mature (Kirk and Govind, 1985), and transmitter phenotypes change (Beltz et al., 1990). Useful clues about the function of octopamine neurons might be gathered by comparing the timing of appearance of the transmitter phenotype with changes taking place in development. Other investigators have used a similar developmental approach to gather important information about the emergence of learning behavior in Aplysia (Nolen and Carew, 1994), ec dysis behavior in moths (Truman, 1992; Riddiford et al., 1994), and mating behavior in nematode worms (Loer and Kenyon, 1993).

In this communication, we describe the appearance of octopamine-immunoreactive neurons during embryonic and larval development of the American lobster. A cell-type-specific pattern of appearance of immunostaining supports our previous classifications of octopamine-immunoreactive cells based on projection patterns. All of the descending and ascending interneurons are labeled by抗体, but descending interneurons appear earlier than ascending interneurons. In contrast, the neurosecretory cells continue to appear until the 3rd larval stage. Subgroups of these cells develop their amine phenotype as sets at precise larval stages, suggesting possible functional subdivisions. The development of the octopaminergic system appears to be independent of the changes in the locomotory systems.

MATERIALS AND METHODS

Animals

Embryos and larvae were obtained from a lobster-rearing facility located at the New England Aquarium, Boston, MA. Eggs at different stages of embryonic development were removed from the ventral surface of the abdomen of egg-bearing female lobsters. The percent staging system for lobster embryos (Helly and Beltz, 1991), based upon eye index (Perkins, 1972), was used to determine the age of each embryo. The length and width of the eyes of animals were measured through the transparent eggshell prior to dissection. Larval stages were determined by noting the external morphological features of animals as described in Herrick (1895).
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Dissection and immunocytochemistry

With embryos, eggs were immobilized in ice-cold saline, eggshells cut open and the embryos removed from the yolk by dissection. At early stages of development, embryos were cut open to expose the nerve cord, but cords were not removed from the embryos until after fixation because the tissues were too fragile. With later stage embryos and with larvae, animals were immobilized in ice-cold saline and the nerve cords were dissected free of the organism in a lobster physiological saline solution (462 mM NaCl, 26 mM CaCl2, 16 mM KCl, 8 mM MgCl2 and 10 mM HEPES, pH 7.4, modified from Otsuka et al., 1967) and pinned out on Sylgard-coated petri dishes. Preparations were immersed, as quickly as possible, in a fixative containing 6% glutaraldehyde, 30% saturated picric acid in water, 1% sodium metabisulfite, and 0.5% glacial acetic acid for 1.5 to 2 hours. They then were washed in a buffer solution containing 0.1 M phosphate buffer, pH 7.4, 462 mM NaCl, 16 mM KCl, 1% sodium metabisulfite (PBS-LS) overnight. After a half hour incubation in the same buffer with 0.3% Triton X-100 (PBS-LSX), preparations were incubated in 5% sodium borohydride in PBS-LS. Following a subsequent wash in PBS-LSX, nerve cords from embryos were incubated in a polyclonal octopamine antibody solution at a dilution of 1:1,000 (in PBS-LSX, Eckert et al., 1992; Schneider et al., 1993) for up to 5.5 days. Tissues then were washed for 6 hours in PBS-LSX with frequent changes in buffer, followed by incubation with a Cy3-conjugated affinipure goat anti-rabbit IgG (Jackson Immunologicals, West Grove, PA) at a dilution of 1:50 in 0.1 M phosphate buffer, pH 7.4, 462 mM NaCl, 16 mM KCl, 0.3% Triton X-100 (PBS-LX) for about 16 hours. Preparations were washed in the same buffer without Triton (PBS-L), dehydrated in increasing concentrations of isopropanol, cleared and mounted on glass slides in methyl salicylate.

Immunostaining in larvae was also performed with a monoclonal mouse octopamine antibody (1:5,000; BIOMAR Diagnostics Systems Inc., Marburg, FRG). Dissections, incubation solutions and incubation times were as above, but the fixative was different: 3% formaldehyde, 3% glutaraldehyde, 1% sodium metabisulfite in PBS-L. A Cy3-labeled anti-mouse IgG (Jackson Immunologicals) was applied as the secondary antibody in a 1:50 dilution in PBS-LSX.

The preparations were examined using a confocal microscope (Biorad MRC 600). In general, 50 optical sections, 1.5 to 2 μm apart, were taken from single wholemount preparations. Images of ganglia were reconstructed and photographed from a flat screen monitor. Tentative identification of cell bodies was based on the results presented by Schneider et al., 1993). Negatives were scanned and combined using Adobe Photoshop 2.5.1. or a Macntosh Quadra 840AV.

RESULTS

A total of 99 embryos and 52 larvae were used in these studies. Ten embryos were stained between 12% and 35% of development. Starting at 35% embryonic development, 5–7 preparations were analyzed for every 5% of embryonic development, and 10–14 stainings were performed for each larval stage. The staining intensity of preparations at similar developmental stages differed, but did not influence the total number of cell bodies stained.

Embryonic development

Embryos as early as E12% up to E100% were prepared for octopamine immunoating but no cell bodies or neurites were detectable until E43%. The pattern of appearance of cell bodies during embryonic development is summarized in Figure 1.

E43%

The first octopamine-immunoreactive cell bodies appear around E43%. These include a single pair of cell bodies in the brain (Fig. 2A), and two pairs of large cell bodies on the ventral surface in the 3rd and 4th neuromere of the subesophageal ganglion (Fig. 3A). The latter are identified by their location as descending interneurons (Schneider et al., 1993). These first three cell pairs show granular staining that is restricted to the cytoplasm surrounding the large nucleus. Neurites are not detectable at this time and the typical punctate neuropil staining of the brain and subesophageal ganglion, seen at later developmental stages, is very weak.

E48%

By E48% the cell bodies of the descending interneurons and brain cells become much more prominently labeled. In addition, one new pair of cell bodies is stained in the brain and five new pairs are observed in the subesophageal ganglion (Fig. 1). Three of the new pairs of cell bodies in the subesophageal ganglion are located on the dorsal surface in the 4th, 5th and 6th neuromeres, the typical location of ascending interneurons (Schneider et al., 1993). On the ventral surface, two new pairs of cell bodies appear in the 5th and the 6th neuromeres, where the posterior neurosecretory crotch cells are located (Fig. 2B). As before, the newly appearing cells are weakly labeled and show granular staining in the cytoplasm. The staining intensity of cell processes in the neuropil has increased dramatically, however, and varicosities are clearly seen in the eyestalks and brain neuropil areas with the exception of the olfactory and accessory lobes. In the subesophageal ganglion, octopamine-immunoreactive axons are observed in a ventral tract that appears to originate from the ascending interneurons (Fig. 3B).

E62%

As embryonic development proceeds, staining appears in additional cell bodies in the brain (Fig. 2B) and the subesophageal ganglion, and the first cell body staining appears in the thoracic ganglia (Fig. 4A). Immunoreactive ascending interneurons are seen in the first and second thoracic ganglia and posterior crotch cells stain in the second thoracic ganglion. Neuripil regions of thoracic ganglia are filled with labeled varicose processes. Dorsally, axons of the descending interneurons are easily visible, but the processes of crotch cells are not. The staining of crotch cells is light, as it is in 4th stage larval lobsters. In the brain, a fourth pair of cell bodies is stained and varicosities are intensely labeled in the neuropil. The axonal and dendritic processes of the cells, however, only stain partially and cannot be traced back to cell bodies. From this point forward in embryonic development relatively few new immunoreactive cell bodies will appear, and the general...
development of octopamine immunostaining appears to slow down, as does the overall development of the embryo (Helluy et al., 1993). Only seven new immunolabeled cell body pairs will appear over the last 40% of embryonic development.

**E69%**

At E69% the first pairs of anterior crotch cells are labeled in the last two segments of the subesophageal ganglion (Fig. 1C). This completes the development of the posterior part of the subesophageal ganglion as far as octopamine immunoreactivity is concerned. No new cell body immunostaining is seen in the brain (Fig. 2C) or the thoracic ganglia (Fig. 4B) during this intervening period.

**E82%**

The last two pairs of neurosecretory cells that appear in embryonic development show up at this time. These are the anterior crotch cells in the second thoracic ganglion and the posterior crotch cells in the third thoracic ganglion (Fig. 4C). The second thoracic ganglion now contains a complete set of immunoreactive cell bodies. Surprisingly, however, the octopamine-immunoreactive neurosecretory cells of the first thoracic ganglion are not yet detectable, and will not show immunolabeling until the 3rd larval stage. In the brain, two more pairs of cell bodies show labeling and an overall increase in neuropil staining is seen (Fig. 2D).

**E98%**

The last pair of octopamine-immunoreactive neurons to stain in the embryo is located in the brain. In marked contrast to what had been seen at earlier times, however, neuropil staining in the brain appears to be reduced just before hatching. Varicosities and axons are barely visible, and the smaller cell bodies show a reduction in their staining intensity (Fig. 2E). Two large pairs of cell bodies remain prominently labeled. A similar “bleaching” of neuropil staining is seen in the subesophageal and thoracic ganglia (Figs. 3E and 4D). Even the neurosecretory cell bodies in the subesophageal and thoracic ganglia appear less intensely labeled when compared to E82%. That this is not just some type of tissue labeling artifact is suggested by the finding that the descending interneurons are clearly visible and appear unchanged in their staining intensity (Fig. 2E).
Fig. 2. Octopamine immunoreactivity in the embryonic brain. A: The first immunolabeled cells are seen at E43% (arrows). B: Three pairs of cell bodies are detectable by E62%. Neuropil staining is prominent. C: By E69%, the staining intensity of the cell bodies has increased and much denser neuropil staining is seen as well. D: By E82%, very dense staining is seen in the neuropil. E: Just before hatching (E98%), the neuropil staining is significantly reduced, but the cell body staining appears unchanged. F: By the 3rd larval stages (L3), the cell bodies and neuropil processes again are strongly labeled. Scale bars = 50 μm.

Larval development of immunostaining

In the first three larval stages, expression of the octopamine phenotype continues in the ascending interneurons, and in the neurons of the neurosecretory system (Fig. 5). In contrast to the generally anterior to posterior acquisition of octopamine immunoreactivity during embryonic development, however, immunolabeling now proceeds mostly in the opposite direction.

1st larval stage (L1)

Lobsters, Homarus americanus, hatch from eggs as a prelarva that rapidly molts to the 1st larval stage. In the 1st
Fig. 3. Octopamine immunoreactivity in the embryonic subesophageal ganglion.

A: By E43%, octopamine-immunoreactive cells appear on the ventral surface of the 3rd and 4th neuromeres of the subesophageal ganglion. Cell bodies in this location typically are members of the descending interneuron group (din).

B: By E48%, the staining intensity of the din group has increased, while the newly appearing posterior crotch cells (p) are weakly labeled.

C: By E69%, the neuropil staining is very prominent, and the anterior (a) and posterior (p) crotch cells stain darkly. D,E: Towards the end of embryonic development, the staining intensity of the neuropil decreases. At E98%, the anterior (a) and posterior (p) crotch cells show a weak, granular labeling, but the staining intensity of the din group appears unchanged. Scale bars = 50 μm.

larval stage, immunolabeling of the pairs of cell bodies of the ascending interneurons of the 3rd, 4th, and 5th thoracic ganglia is seen (Fig. 6A–C). With these additions, the set of ascending interneurons is complete. In addition, staining is observed in the anterior and posterior crotch cells of the neurosecretory system in the 5th thoracic ganglion (Fig. 6C).
Fig. 4. Octopamine immunoreactivity in the embryonic thoracic region of the nerve cord. By the end of embryonic life, only five of the 15 cell pairs of octopamine immunostaining cells ordinarily found in these ganglia are detectable. A: At E62%, the posterior crotch cells (p, arrow) in T2 and the ascending interneurons in T1 and T2 (not shown) are labeled. B: The staining pattern is unchanged at E69% (p, arrows). C: At E82%, the anterior crotch cells (a) in T2 and the posterior crotch cells (p) in T3 are detectable for the first time. D: By the end of embryonic development, the complete set of octopamine-immunoreactive cells is found only in the T2 ganglion: the posterior crotch cells (p) are shown here in a ventral plane of focus. The inset shows the dorsal region of the ganglion with the anterior crotch cells (a) and the ascending interneurons (i). Scale bars = 50 μm.

The general level of staining in neuropil regions remains weak.

2nd (L2) and 3rd larval stages (L3)

The development of the octopamine-immunoreactive components of the neurosecretory system is completed in these two larval stages, with the sequential filling of the gaps in the 4th, 3rd, and 1st thoracic ganglia. In the 2nd larval stage, stained anterior and posterior crotch cells appear in the 4th thoracic ganglion (Fig. 7B,C), and labeled anterior crotch cells are seen in the 3rd thoracic ganglion (Fig. 7A). The staining intensity within neuropil regions remains low. This, however, changes dramatically with molting to the 3rd larval stage. At this point, the staining intensity within the entire nervous system is higher than that seen in any other larval stages. In addition, with the appearance of immunolabeling in the anterior and posterior crotch cells of the 1st thoracic ganglion (Fig. 8), staining is seen in all components of the sets of ascending and descending interneurons and in the neurosecretory cells.

DISCUSSION

Antibody specificity

In this study, we used immunocytochemical methods to examine the appearance of the octopamine phenotype in individual nerve cells and their processes during embryonic and larval development in lobsters (Homarus americanus). The use of antibody alone as a reliable monitor of such expression depends on the specificity of the polyclonal antibody being used and on the level of amine detectable using this reagent. In an earlier study we examined the question of specificity in two ways: (i) by using free octopamine and serotonin as stain blocking reagents; and (ii) by
Fig. 5. The patterns of appearance of octopamine-immunoreactive cells during larval development. As in Figure 1, new cells are shown as filled circles, while old cells are shown as open circles. At the 1st larval stage (L1) five new pairs of cells appear in the posterior part of the thoracic nerve cord. The most dramatic change is in the 5th thoracic ganglion where the complete segmental set of octopamine-immunoreactive cells appears at once. At L2, the anterior crotch cells in T4 and the posterior crotch cells in T3 and T4 appear. At L3, the thoracic set of octopamine-immunoreactive cells is completed with the appearance of the anterior and posterior crotch cells in T1. No further change in staining pattern is observed after the metamorphosis to the 4th larval stage.

direct biochemical measurements of octopamine levels in single cells (Schneider et al., 1993). The results showed that the polyclonal antibody worked reliably and with high specificity. In addition, the patterns of immunolabeled cells observed in different preparations were reproducible. Hence, in using the same polyclonal octopamine antibody for the developmental studies reported here, we did not carry out further tests of specificity. A new monoclonal octopamine antibody (BIOMAR, see Materials and Methods) became available at the end of our experimental series and was used in immunostaining studies with some of the larval preparations. The results were not significantly changed with the use of this antibody. The monoclonal antibody offered a higher sensitivity and lower background, both of which resulted in a more detailed view of immunostaining in neuropil regions. Another change from the previous experimental protocol is that immunofluorescent detection agents and confocal microscopy were used to examine stained tissues in these studies, instead of the avidin-biotin complex and conventional light microscopy. These changes were made to avoid occasional high background signals with the avidin-biotin complex that impeded the detection of weakly labeled structures. Through the use of a Cy3-labeled secondary antibody and the optical sectioning of wholemount preparations, the intensity of the fluorescent emission remained high, even in glutaraldehyde-fixed whole-
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Fig. 6. Octopamine immunoreactivity in the 1st larval stage. In T3 (A) and T4 (B) the cell bodies of the ascending interneurons (i) are seen for the first time on the dorsal surface of the ganglion. C: The complete set of anterior crotch cells (a), posterior crotch cells (p) and ascending interneurons (i) are seen in T5. Scale bars = 50 μm.

mount preparations, which ordinarily show strong glutaraldehyde-induced background staining. Moreover, little fading of the immunofluorescent signal takes place during scanning and it is relatively easy to explore the three-dimensional distribution of octopamine-immunoreactive cell bodies and their processes.

**Pattern of development of octopamine-immunoreactive neurons**

Each octopamine-immunoreactive neuron type has a distinctive developmental pattern during embryonic life. Brain cells and descending interneurons appear first, followed by ascending interneurons and neurosecretory cells. A general anterior-posterior direction of amine expression governs the appearance of the first groups of cells, but the neurosecretory cells are labeled in a segmental pattern that relates to specific developmental stages. The first members of the latter group to stain are in the subesophageal ganglion at about E50% (the posterior crotch cells). These are followed by posterior crotch cells in the 2nd thoracic (E65%), anterior crotch cells in the subesophageal ganglion (E62%), the anterior and posterior crotch cells in the 5th thoracic ganglion (L1), the anterior and posterior crotch cells of the 4th thoracic ganglion and the anterior crotch cells of the 3rd ganglion (L2), and last, the anterior and posterior crotch cells of the 1st thoracic ganglion (L3). Such

Fig. 7. Octopamine immunoreactivity in the 2nd larval stage. A: In T3 the anterior crotch cells (a) are labeled for the first time. Note the difference in staining intensity between the anterior and posterior crotch cell bodies (p). The latter cells appeared first in the embryo (i, ascending interneurons). B: On the dorsal surface of T4, the anterior crotch cells (a) are seen close to the ascending interneurons (i). C: The posterior crotch cells (p) on the ventral side of T4 show the granular staining typical of newly appearing cell bodies. Scale bars = 50 μm.
weeks later (Beltz and Kravitz, 1987; Beltz et al., 1990), the nervous system within the first few days of embryonic development appears to be the major site of synthesis of octopamine and serotonin, leading to the formation of an organism with many morphological and behavioral features of a young lobster (Herrick, 1895; Charmantier et al., 1991). The total span of development is more closely related with other physiological events occurring during embryonic and larval development.

Serotonin and octopamine show different patterns of expression in development

The most striking feature in the developmental pattern of appearance of octopamine immunostaining is its extended duration throughout embryonic and larval life (Fig. 9). The first octopamine immunostaining cell bodies appear at about 43% of embryonic life. The last octopamine-staining cells appear during the 3rd larval stage, just before the metamorphic molt to the postlarval 4th stage, which leads to the formation of an organism with many morphological and behavioral features of a young lobster (Herrick, 1895; Charpentier et al., 1991). The total span of developmental time over which this occurs can be up to 9 months in the wild. Serotonin, by contrast, appears in the central nervous system within the first few days of embryonic development and the entire system is fully formed several weeks later (Beltz and Kravitz, 1987; Beltz et al., 1990, 1992). The first serotonin-immunopositive fibers appear in the median protocerebrum of the brain around E10%, at the time of the first muscular contractions in the embryo (Beltz et al., 1992). The early appearance has been suggested to relate to possible neurotrophic functions in lobster CNS development (Beltz et al., 1992; Benton et al., 1994), which serotonin also has been suggested to serve in both vertebrate and invertebrate developing systems (Haydon et al., 1987; Lauder, 1987). The serotonin neurosecretory system is fully formed by E50%, when many of the peripheral targets of these neurons have not yet formed. The corresponding octopamine neurosecretory system begins to emerge in late embryonic life and is not complete until the 3rd larval stage. It is not clear why these amine systems differ so widely in their patterns of expression. Perhaps serotonin does serve key nervous system developmental roles not subsumed by octopamine. If so, then the timing of the appearance of octopamine function might be more closely related with other physiological events occurring during embryonic and larval development.

The onset of amine synthesis and appearance of specific behavioral patterns

Our results suggest that the first appearance of octopamine and serotonin cell bodies cannot be linked in a stage-specific way to the emergence of patterns of locomotor or agonistic behavior, even though both amines appear to participate in the regulation of these behaviors. The octopamine and the serotonin systems are fully formed before the motor patterns governing these behaviors are expressed. The serotonin system is complete by 50% of the embryonic development and the octopamine system by the 3rd larval stage, but the behavioral patterns that amines modulate emerge at the 4th and later stages (Herrick, 1895; Lantak et al., 1977; Atema and Cobb, 1980; Fig. 9). It remains possible that the distal synaptic complexes through which these substances influence their peripheral targets may not mature until several developmental stages after the appearance of amines in cell bodies. In addition, a reorganization of the processes of the octopamine-immunoreactive neurons themselves, as has been seen in Manduca sexta (Pflüger et al., 1993), might also take place in the lobsters and could account for later influences on connectivity. Since individual octopamine-immunoreactive cells have been identified that first appear to synthesize this amine during larval life, however, this system may offer easy access to unique amine-containing neurons for examination of their potential regulatory roles in the emergence of behavioral patterns.
In summary, using immunocytochemical criteria, we have shown that differences exist in the maturation of octopaminergic and serotoninergic systems in lobsters, and that the first appearance of octopamine immunolabeling in single neurons cannot be linked to the first appearance of postural and agonistic behavioral patterns in postlarval animals.

ACKNOWLEDGMENTS

We are grateful to Dr. Simone Helluy for kindly introducing us to staging and dissecting techniques of lobster embryos, Drs. Manfred Eckert and Jürgen Rapus for providing the polyclonal octopamine antibody, Dr. Hans Agricola for a sample of the monoclonal octopamine antibody, Dr. Peter Braunig for his suggestions, and Joe Gagliardi for his help with photomicrographs. The work was supported by a NIH grant (NS 25915) to E.A.K. and B.S.B., a DFG grant (Schn 36811.1) to H.S. and a fellowship from the Karl Benz and Gottfried Daimler Stiftung to I.W.

LITERATURE CITED


