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HORMONAL CONTROL OF FEMALE REPRODUCTION AND MOLTING IN DECAPOD CRUSTACEANS

T. SUBRAMONIAM

Department of Zoology, University of Madras, Guindy Campus, Chennai – 600 025, India.

SUMMARY

Endocrine regulation of reproduction and molting in Crustacea was first demonstrated by the experimental evidence that eyestalk removal led to accelerated ovarian maturation and the onset of precocious molting. Subsequent immunocyto chemical studies as well as HPLC separation of neuropeptides followed by suitable bioassay established the occurrence of two neuropeptides namely gonad inhibiting (GIH) and molt-inhibiting (MIH) neurohormones in the X-organ/sinus gland complex of stalk-eyed malacostracan crustaceans. While the molt promoting hormones have been decidedly shown to be the ecdysteroids, as in other arthropods, many hormonal factors of diverse chemical nature have been proposed for controlling female reproduction. Neurosecretory hormones from brain/ thoracic ganglia, methyl farnesoate, a structural homologue to insect Juvenile hormone III (JH III) and a variety of steroidal hormones are the principal candidates for control of female reproduction, especially vitellogenesis. As molting continue to occur in the adult crustaceans, hormonal coordination of both molting and reproduction becomes vital to accomplish fast body growth and increased fecundity in these aquaculture-targeted arthropods. Steroidal control of such a coordination is stressed in this review.

Key words : Crustaceans; Molting; Neuropeptides

INTRODUCTION

Crustaceans are primarily aquatic in distribution, with a few species venturing into terrestrial habitats. Such a diversity in their distribution is reflected in a fascinating range of reproductive strategies to ensure the continuance of the species. A defining feature in the female reproductive biology of the common crustacean is its ability to produce a large number of yolky eggs, which are invariably brooded in the pleopodal region pending the release of the zoea larvae. A notable exception to this rule is the penaeoid shrimps, in which the eggs are spawned directly into the seawater. Here, the eggs are less yolky and the embryonic development is quite simple with the release of naupliar larvae.

Another feature of interest is that most crustaceans continue to molt throughout the reproductive adulthood, in contrast to the pterygote insects, in which molting ceases following adult emergence. This condition necessitates the completion of reproductive activities during the rather extended intermolt phase when metabolic demands for the new cuticle formation will not exist. Thus, in the majority of large- bodied decapod crustaceans molting and female reproduction are temporally set apart in order to judiciously channelise the organic storage material for both new cuticle formation and vitellogenesis in a biphasic manner. Conversely, in shrimps, prawns, isopods and amphipods, there is a closeness or overlapping in the molting and reproductive processes without showing any real antagonism between them. Such an inextricable linkage between molting and reproduction is accomplished by a delicate multihormonal interaction, existing uniquely in crustaceans. For example, while molting in Crustacea is controlled solely by ecdysteroids as in insects, several hormonal factors have been proposed in the control of reproductive processes (1). In all oviparous vertebrates,

vitellogenin synthesis is transcriptionally controlled by the steroid hormone estradiol (2). Among invertebrates, only in cyclorraphan dipteran insects, ecdysteroid control of vitellogenin synthesis has been demonstrated at transcriptional level (3). Furthermore, molting and reproduction in crustaceans are ultimately controlled by eyestalk inhibitory neuropeptides that act upon the endocrine glands producing hormones that stimulate these two major physiological processes. This review is a critical appraisal of the interactive role played by various hormonal factors in coordinating molting and reproduction in decapod crustaceans.

CRUSTACEAN HORMONES

Neuropeptides

The principal hormones produced by crustaceans can be included into the following three chemical categories, *viz,* neuropeptides, terpenoids and steroids. By far, the neuropeptides are the best-studied hormones in respect of their structural elucidation and physiological functions. Unlike insects, both molting and reproduction are negatively controlled by a group of neuropeptides belonging to a family of crustacean hyperglycemic hormones (CHH). They are molt inhibiting hormone (MIH) and the gonad-inhibiting hormone (GIH). These neuropeptides are produced by the neurosecretory perikarya in the medulla terminalis (X- organ) and axonally transported into the neurohaemal organ (sinus gland) where they are stored until release.

Molt inhibiting hormone (MIH)

As molting alternates with female reproductive cycle in many crustacean species, the interactive role of MIH and GIH in controlling the programming of these two events assumes significance. MIH is known to negatively regulate synthesis of the molting hormone, ecdysteroid, by the Y-organ (4). Several studies have demonstrated the effect of eyestalk ablation resulting in the rapid increase in hemolymph ecdysteroid titer, reminiscent of those seen during premolt (1). MIH peptides are also structurally and functionally interrelated with the CHH molecules in the lobster *Homarus americanus* (5) and the crayfish *Procambarus clarkii* and *P. bouvieri* (6). Eyestalk neuropeptides exhibiting both CHH and MIH activities have also been demonstrated in the penaeid shrimp, *Penaeus japonicus* (7).

Molt inhibiting hormone is regarded as the prime regulator of ecdysteroid synthesis in crustaceans. The inhibition pathway of steroidogenesis in Y-organ proceeds via transadolase, the key enzyme needed in the conversion of cholesterol into ecdysteroids. MIH causes rapid and sustained increase in cyclic GMP causing phosphorylation of transadolase resulting in its inactivation (8). A recent study on the shore crab, *Carcinus maenas,* (9) further elucidated the possible control mechanisms on molting by determining the inhibitory action of MIH and CHH on the target tissue, Y-organ. Their receptor binding studies using displacement (Kd) and saturation experiments (Bmax) revealed little variation among all stages of molt cycle, although a significant number of MIH receptors on the Y-organ membrane preparation was noted. Similarly, mRNA levels of both MIH and CHH in the X-organ and their stored peptide contents in the sinus gland during different molt cycle stages were not variant. These results further suggest that MIH mechanism of action primarily relates to intracellular signalling pathways within the Y-organ.

Gonad inhibiting hormone (GIH)

The existence of a gonad inhibiting hormone (GIH; also called VIH, as it possesses appreciable inhibitory effect on vitellogenesis) was first demonstrated by Panouse (10) when he observed rapid ovarian maturation by ablating the eyestalk of the grass shrimp *Palaemon serratus.* The first isolation and partial characterization of GIH were made in the Crab *Cancer magister* using gel chromatography

techniques. It was found to be a 2 kDa peptide with inhibitory effect on the ovarian growth of the shrimp *Crangon crangon*. Quackenbush and Keeley (11) also purified the GIH from the crude extract of the shrimp *Penaeus setiferus* using sephadex G-25 chromatography and determined the molecular weight as 3,300 Da. This peptide inhibited the ^{14}C-leucine incorporation into the vitellogenin of the cultured ovary of *Ucapugilator*, while the incorporation of the radioactivity in the other proteins remained unaffected. The GIH of *P. setiferus* also showed the same inhibitory effect on the yolk protein synthesis in the ovary and hepatopancreas of another penaeid shrimp *P. vannamei* (12).

The complete characterization of GIH was, however, made possible by the sequencing of this peptide molecule using conventional microsequencing (13) and by cDNA cloning (14) in the lobster *Homarus americanus*. GIH of this lobster consists of a 78 residue peptide (Mr. 9135), with an amidated C-terminus and a free N-terminus. Interestingly, this molecule showed considerable sequence similarity (53%) to *Carcinus maenas* MIH (15). This feature, along with the absence of CPRP in the preprocessed precursors of both molecules segregate GIH and MIH into a separate subgroup within the CHH family. Such a subgroup formation is justifiable, physiologically speaking, because MIH and GIH inhibit mutually exclusive events in energy partitioning, namely somatic and gonadal growth. Interestingly, GIH of *H. americanus* exists as two isoforms of identical sequence and mass (13). However, the gonad-inhibiting effect was found only in one isoform, when tested with an *in vivo* heterologous assay developed in the grass shrimp *Palaemonetes varians* (16). The other isoform did not exhibit such biological activity. Following this, Aguilar *et al.* (17) isolated a peptide from the eyestalk of the Mexican crayfish *P. bouveri*, which inhibited the growth of oocytes from *P. vannamei*, *in vitro*. This neuropeptide contains 72-74 amino acid residues and has a molecular weight of 8388 Da and a blocked N-terminus. Structurally, this GIH of the Mexican crayfish is more related to its CHHs than to lobster GIH. Again, its GIH activity in the endogenous vitellogenin synthesis by *P. setiferus* ovaries occurred only at a high concentration of 10 sinus gland equivalents. Surprisingly, in all the above studies, the bioassay to test the activity of GIH was made in heterologous system.

In a recent study, we have used a homologous bioassay system to test the GIH activity in the penaeid shrimp, *Penaeus monodon* (18). RP HPLC separation of X-organ/sinus gland of *P. monodon* yielded two GIH positive fractions. A sandwich enzyme-linked immunosorbent assay was used for the quantification of vitellogenin in the hemolymph of eyestalk ablated *P. monodon*. The two GIH fractions were found to reduce the hemolymph vitellogenin concentration in a time-dependent manner and hence could be identified as vitellogenesis inhibiting hormones I and II. Unlike the other crustacean species, shrimp eyestalk has been shown to possess multiple gonad inhibitory neuropeptides. For example, in the kuruma shrimp, *Penaeus japonicus*, Khayal *et al.* (19) isolated a group of seven peptides, all of them inhibiting the incorporation of radiolabeled methionine into the *in vitro* incubated ovarian fragments of *P. semisulcatus*, indicating vitellogenesis inhibition activity. More interestingly, six of these seven peptides had hyperglycemic activity, and three had molt-inhibiting activity. Cross functional physiological activities of CHH family peptides has already been observed in many crustacean species (20); but the occurrence of many CHH peptides with MIH and GIH activities in the shrimps is not only unique but also indicative of a primitive condition in the origin of CHH family peptide. It is of interest that the eyestalk neuropeptides of *P. monodon* showed several immunopositive fractions for CHH activity (21).

Gonad stimulatory hormone (GSH)

The first evidence for a gonad stimulatory principle in the central nervous system of Crustacea was obtained by Otsu (22), who noticed precocious ovarian development in the crab *Potamon dehaani* after implantation of thoracic ganglia. Injection of the brain extracts from the lobster *Homarus americanus* resulted in the induced ovarian maturation of the shrimp *P. vannamei*, suggesting that the stimulatory factors are not species-specific among shrimp and lobster (23). Interestingly, a gonadal

stimulatory neuropeptide has been reported in the sinus gland extract of the Mexican crayfish *Procambarus bouverri* (24). The putative gonad stimulatory factor from the brain/ thoracic ganglia has not been fully chemically characterized, although it appears to be a 1 kDa peptide inactivated by trypsin (25).

Methyl farnesoate

Mandibular organ (MO), first described by Le Roux (26), is now confirmed to be an endocrine gland, secreting a sesquiterpenoid compound, methyl farnesoate (MF). Its functional role in the control of vitellogenesis was first indicated by Hinsch (27) from her observation that the active MO implants stimulated ovarian growth in the immature female spider crab, *Libinia emarginata*. Subsequent measurement of MF level in the hemolymph and MO demonstrated an increased synthesis and secretion of MF during vitellogenesis in this crab, suggesting a role in crustacean reproduction (28). MF is the unepoxidated form of the insect juvenile hormone (JH III), and hence appears to have a gonadotropic role, like JH in the insects (29). MF secretion by MO during vitellogenesis has also been described in other crustacean species such as the penaeid shrimps, *P. duorarum* and *P. vannamei* (30, 31). Laufer *et al.* (32) reported stimulation of ovarian maturation in the pre-reproductive females of crayfish *Procambarus clarkii* by treatment with extraneous MF. Injection of MF in the vitellogenic female crayfish also accelerated the process significantly. In a recent study, Rodriguze *et al.* (33) showed the positive effect of MF on oocyte growth when injected alone or in combination with 17β-estradiol. In addition, a higher level in the incorporation of labeled leucine was also induced by MF in the isolated pieces of ovary. Obviously, MF has a positive influence on the vitellogenic activity within the ovary of this crayfish. It is of interest to note here that the crayfish ovary has been repeatedly shown to engage in auto synthesis of yolk proteins, unlike many other decapod crustaceans (34). However, in the American lobster, *Homarus americanus,* Waddy *et al.* (35) did not find any correlation of MF changes with vitellogenesis. In *Macrobrachium rosenbergii*, injection of MF into eyestalk ablated juvenile males and females also did not stimulate vitellogenin production (36). However, other studies have demonstrated positive effects of juvenoids (JH III) on ovarian development in the freshwater crab, *Paratelphusa hydrodromous* (37) and MF in the shrimp, *P. vannamei.* It has been suggested that MF may activate protein kinase C in the ovaries and thereby stimulating vitellogenesis (38).

Despite its conflicting role in the control of vitellogenesis among different decapods, MO's activity seems to be under the control of the eyestalk neuropeptides, as evidenced by several eyestalk ablation studies. The involvement of cGMP as a second messenger in the inhibitory activity of the sinus gland factor on the MF synthesis was demonstrated by Tsukimura *et al.* (1993). Complete characterization of the eyestalk neuropeptide inhibiting MF synthesis by MO (MOIH) was first made by Waineoright *et al.* (39), who purified two MOIH from the HPLC-separation of sinus gland in the crab, *Cancer pagurus*. Both the peptides have 78 amino acid residues and a molecular weight of 9235 Da with free N- and C-termini. Sequence alignment studies with other CHH family peptides revealed 50-60% similarity with MIH of *C. pagurus*. However, MOIH had only limited and variable activity in the MIH assay. Later on, Liu and Laufer (40) have reported three MOIHs from the eyestalk of the spider crab, *Libinia emarginata*, one of which is structurally a CHH.

Steroidal control of reproduction

Ecdysteroids

Ecdysteroids, the principal hormonal factors in the inducement of molting in arthropods, also play a definitive role in the transcriptional activation of vitellogenin gene in certain insects such as the dipteran flies (41). A similar role for ecdysteroids in crustacean vitellogenesis is inconclusive.

However, several reports have appeared in the literature to implicate ecdysteroids in the female reproductive activities. Arvy *et al.* (42) found evidence that there is a rise in the hemolymph ecdysteroids coincident with the initial stages of gametogenesis such as oogonial and spermatogonial mitoses in the shore crab, *Carcinus maenas*. In amphipods and isopods, hemolymph vitellogenin level parallels with ecdysteroid titer during vitellogenic cycle, suggesting a role in the vitellogenin synthesis (43, 44). Similarly, in the freshwater prawn, *Macrobrachium nipponense*, Okumura *et al.* (45) found a close correlation between the hemolymph ecdysteroid titer and the corresponding ovarian maturation stages during a reproductive molt cycle. Interestingly, in all the above crustaceans, female reproductive cycle alternates with the molt cycle. Therefore, the independent role of ecdysteroids in stimulating vitellogenesis cannot be ascertained in the above forms, as the hemolymph ecdysteroids titer also changes concurrently for the molting cycle. A recent study on the radioimmunoassay of 20-hydroxyecdysone (20E) in the hemolymph and ovary of the mole crab *Emerita asiatica* in different stages of molting and female reproductive cycle has revealed its controlling effect on both molting and reproduction (46). These authors found a gradual rise in hemolymph ecdysteroids during intermolt stage, followed by a steep rise in the premolt stage corresponding, to vitellogenic activities in the ovary (intermolt) and molting (premolt), respectively. When the intermolt crabs were injected with 20E, protein levels in the hepatopancreas, ovary and hemolymph increased significantly indicating its metabolic role in yolk protein synthesis. In crustaceans, there is evidence that yolk proteins are synthesized both in the ovary and hepatopancreas (47). Hence, it is likely that both these organs could be the targets for ecdysteroid action to induce/ maintain vitellogenin synthesis. On the contrary, in the shore crab, *Carcinus maenas*, there is total non-involvement of ecdysteroids in vitellogenesis, as the Y-organ removal did not stop this (48). This study did not, however, preclude the possibility that ecdysteroids could be synthesized in the ovary itself, as in adult insects (49).

The question of ecdysteroid-control of vitellogenin could be resolved only by molecular studies pertaining to their receptor activities. In insects, the action of ecdysteroids is similar to vertebrate steroid hormones: the transcriptional control being mediated via two nuclear receptor superfamily members, ecdysone receptors (EcR) and its heterodimeric partner, ultraspiracle (USP). Such dimerization of nuclear receptors is vital to induce transcription of vitellogenin gene in the cyclorraphan dipteran flies such as *Aedes aegypti* (50). In crustaceans, EcR has been identified in blastimal tissues of regenerating limbs of *Uca pugilator*, but it dimerizes with retinoid X receptor, RXR (51). Recently, Durica *et al.* (52) found co-expression of these two receptors (UpEcR and UpRXR) in the ovary of *U. pugilator* during ovarian cycle, suggesting that the ovary is a potential target tissue for ecdysteroid hormonal control. In the epidermis and eyestalk tissues, other ecdysteroid- responsive genes such as HHR3 has also been identified in the lobster, *Homarus americanus* (53). However, the occurrence of ecdysteroid response element in the promotor region of the vitellogenin gene is yet to be identified in the crustaceans. Ecdysteroids in the protostome arthropods could be considered as the functional homologue of vertebrate estradiol in the control of vitellogenin synthesis. They are also chemically detected in parasitic and free-living nematodes. But their physiological role is not determined. Three steroid hormone receptors, belonging to retinoid receptor (RXR), thyroid hormone superfamily of genes, have been identified in *Caenorhabditis elegans* (54). Interestingly, another ecdysone response gene, E78A has also been reported in this nematode. Apparently, there is widespread occurrence of steroid receptors, including those of ecdysone, in the invertebrate phyla suggesting diversified physiological roles.

Ovarian accumulation of ecdysteroids

Despite uncertainties prevailing over the direct role of ecdysteroids in crustacean vitellogenesis, ovary of several crustacean species accumulate significant quantities of these hormones during maturation (49). They are speculated to be either sequestered from hemolymph or synthesized in

the ovary or the developing embryos. In *E. asiatica*, there is evidence that they are sequestered along with yolk protein precursors and accumulated during the entire intermolt period (1). However, the free ecdysteroid level in the ovary is drastically declined during previtellogenic stage. This decline in the ovarian ecdysteroids is inversely related to rising hemolymph ecdysteroids suggesting a release from the ovary (46, 55). Nevertheless, significant quantities of free and conjugated ecdysteroids are retained in the spawned eggs. In crustacean embryos, all the three major forms of free ecdysteroids, *viz,* ecdysone, 20E and ponasterone A (PoA) are also found as conjugates. Chemically, these conjugates can be esters with phosphoric acid or long chain fatty acids as in insects (56). Among several ecdysteroid species reported in crustaceans, ponasterone A is significant, as it is involved in the regulation of embryonic cuticle formation in the shore crab *Carcinus maenas* (57). Electron microscopic investigations on the embryogenesis of the European lobster, *Homarus gammarus* have also adduced further evidence that the embryonic envelop secretion is controlled by the ecdysteroids accumulated within the eggs (58). The ecdysteroids have a definitive role in the brood development of egg carrying decapods is clearly evidenced in a recent study on *Emerita*. In this crab, Gunamalai *et al* (46) reported that hatching of the embryos, attached to the pleopods of the ovigerous females occur under a high titer of hemolymph ecdysteroids. 20E injection at C_3 stage crabs indicated a significant reduction in time duration of pleopodal embryonic development leading to hatching of zoea larvae (Fig.1). Evidently, the augmented hemolymph ecdysteroid titer helps in the synchronization of embryo hatching and the premolt changes, as occurring under the normal premolt conditions. Taken together, the above data indicate the importance of ecdysteroids in accomplishing molt-related reproductive processes such as brood development and larval hatching in those decapods that incubate the eggs, carried in the pleopod, facilitating normal ecdysis without affecting brood development.

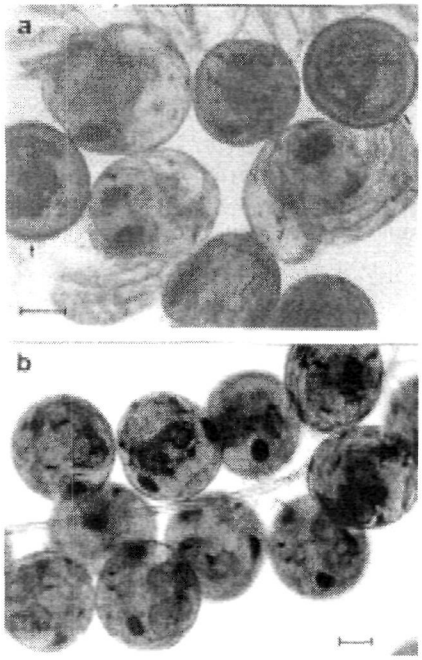

Fig. 1. Precocious hatching of pleopodal embryos in 50 μg/ crab injected at C_3 stage (a), control animals (b) remained at stage VII of embryonic development. Arrow indicates the undeveloped embryos in fig. 1a. The embryos of the crabs under observation were plucked and wet-mounted on glass slides in clean filtered seawater and photographed on Labex, Labovision (India) microscope at a magnification of 4X. Scale bars represent (a) 820 μm; (b) 460 μm (from Gunamalai *et al.*, 2003).

Vertebrate steroids

In recent years, the occurrence of vertebrate steroids such as estradiol and progesterone together with their metabolic products have been identified in the ovary and hepatopancreas of several decapod crustacean species (49). These steroid hormones also exhibit characteristic fluctuations during gonadal maturation suggesting a role in the control of reproduction in several crustaceans. In

the freshwater prawn, *Macrobrachium rosenbergii*, estradiol induced the biosynthesis of 17β-hydroxysteroid dehydrogenase, a key enzyme involved in steroid metabolism. Furthermore, exogenous injection of 17β-hydroxyprogesterone resulted in almost 9.6-fold increase of vitellogenin in the vitellogenic female *P. japonicus* (59). Vitellogenin synthesis by ovarian pieces under *in vitro* conditions, when incubated with 17β- estradiol was also reported by Yano and Itakura (60). In a more recent study, Warrier *et al.* (61) estimated the level of both estrogen and progesterone by RIA technique during different vitellogenic stages of the mud crab, *Scylla serrata.* The level of steroids, especially the estradiol was very high in the hepatopancreas in vitellogenic stage I, when peak vitellogenin synthesis has been observed in this tissue (62). In the oviparous vertebrates, estradiol is specifically involved in the transcriptional activation of vitellogenin gene in the liver by binding to its nuclear receptor (63). In spite of several reports suggesting a similar role for the estradiol controlling vitellogenesis in crustaceans, no direct evidence to this effect has been obtained in crustaceans. Ovary in this crab also accumulated significant quantities of these two hormones suggesting a role in embryogenesis. Interstingly, these hormones are bound to yolk proteins and their precursor, vitellogenin, indicating that these hormones may be transported to ovary along with vitellogenin from an extraovarian synthetic site such as hepatopancreas. Crustacean vitellogenin is a lipid-carrying lipoprotein showing structural similarity with mammalian low-density lipoproteins (47). Hence, it is possible that the cholesterol-derived sex hormones such as estrogen and progesterone could also bind to crab vitellogenin at its lipid binding sites. Since lipovitellins are proteolytic products of vitellogenin, lipovitellin-bound steroids could be derived from vitellogenin during its transport from the hemolymph into the oocyte by receptor-mediated endocytosis.

A functional role for these vertebrate steroids in ovarian maturation is still enigmatic in crustaceans. Progesterone may have a role in the completion of meiotic maturation of oocytes in the post-vitellogenic period as in vertebrates, but a similar role has been attributed to ecdysteroids in the spider crab, *Acanthonyx* it. Furthermore, the conjugated vertebrate steroids are released into free form in the embryos when the organogenesis is at its peak (61). Therefore, the maternal accumulation of these sex steroids in the eggs could potentiate and facilitate the morphogenetic processes in embryonic development.

CONCLUSION

Recent years have witnessed a real concern for the identification of hormonal factors regulating reproduction in Crustacea, in an attempt to formulate control measures in aquaculture operations. In spite of several attempts taken in this regard, we are still in an uncertain stage of identifying the proximate endocrine gland to stimulate vitellogenesis. By far, eyestalk neuropeptides of decapods have received major attention in the recent past. Crustacean hyperglycemic hormone form the predominant neuropeptide secreted from the X-organ/ sinus gland comprising up to 60% of the total secretory product from the eyestalk. The CHH family neuropeptide, however, includes three important inhibitory hormones to control vital processes such as molting, reproduction and mandibular organ activity. While the MIH and MOIH have direct action on their respective target organs (Y-organ and MO), the target organ for the action of VIH is still elusive. Its proposed antagonistic action on the vitellogenesis stimulating hormones (VSH), originating in the brain/ thoracic ganglia, as hypothesized by earlier workers like Adiyodi and Adiyodi (64) need experimental evidence. An attractive proposition could be that it acts directly on either vitellogenin synthetic site such as hepatopancreas and ovary or at the site of vitellogenin uptake by the ovary. Although correlative evidence is available to this effect, direct evidence indicating the mode of their inhibitory effect is lacking. That it may not act on other two gonad stimulatory hormonal sources is revealed by the fact they have their own inhibitory neuropeptides (MIH and MOIH). Eyestalk neuropeptides including CHH, MIH, MOIH and VIH have all been included into a single CHH family, by virtue of significant structural homology

found among them. It is therfore highly likely that cross-functional activities could also exist among them.

From the existing evidence, there is every reason to believe that the control of reproduction in Crustacea could be multihormonal. Apart from the purported stimulatory neuropeptide from the brain/ thoracic ganglia, methyl farnesoate, ecdysteroids and the vertebrate steroids have been implicated with the control of female reproduction almost in a species-specific manner. Mandibular organ with its contolling neuropeptide, MOIH from the eyestalk is an ideal homologue to the corpora allata and its controlling neuropeptide, allatostatin, of insects in as much as both the glandular secretions have a gonadotrophic function in these arthropods.

Similarly, the steroidal hormones, ecdysteroids, and the vertebrate steroids have also been shown to have a role in the control of reproduction and embryonic development in several crustacean species. The occurrence of two steroid hormones such as ecdysteroids and vertebrate steroids in crustaceans is interesting in the sense that both could induce molting and reproduction as reported for insects and oviparous vertebrates. In a recent unpublished work (Gunamalai and Subramoniam) on *E. asiatica* and *M. rosenbergii*, there is evidence to support such a supposition. In these two crustaceans, ecdysteroids and vertebrate steroids occur all through the molting and ovarian cycles. But the level of ecdysteroids peak during premolt stage, whereas, the vertebrate steroids show high concentration in the hemolymph during the intermolt stage, coinciding with active vitellogenesis. Molecular studies by the characterization of their nuclear receptors could only adduce direct evidence in this regard. In conclusion, it may be said that there is a multiplicity of hormonal action in the control of female reproduction in crustaceans, and that the eyestalk neuropeptides could coordinate the actions of the gonadotrophic hormones by their inhibitory/ restraining activity. Further study is important to delineate the step-wise activity of these hormones either synergistically or independently on the gonadal system.

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