COMMENTARY

In search of a gonadotropic hormone in Crustacea

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Crustacean reproductive biology is at the crossroads of its possible contribution to the development of aquaculture, which in India is hampered by the lack of appropriate technology in the production and supply of seeds. Aquaculture production of these arthropods is one of the potential commercial ventures with shrimp, lobster and crab yielding high economic benefits. With the advent of aquaculture of edible finfish species, many edible species of crustaceans belonging to the order Decapoda attracted attention from invertebrate endocrinologists, primarily to find means to control reproduction under controlled conditions of hatchery operations. The prominent species for the aquaculture endeavours included shrimp and freshwater prawns, lobsters and crayfishes, and marine and estuarine crabs. Among these decapods, shrimp culture has emerged as a significant worldwide industry. Since crustaceans deposit a high quantity of complex yolk inside the egg, vitellogenesis is the central event of egg development; hence in the hatchery production of commercially significant shrimp seeds, control of vitellogenesis is the primary concern.

In practice, hatchery production of shrimp seeds depends on the procurement of natural brooders from the oceanic waters, and making them spawn under controlled conditions. Such a situation resulted in the over fishing of the brooders, in addition to hiking their price. This problem could be resolved only by induced rematuration of brooders by way of hormonal intervention and manipulation. There has never been a time more appropriate than now to appreciate the recent advancements made on the endocrine regulation of growth and reproduction of the commercially important crustaceans. However, scientists as well as aquaculture practitioners in India and other South East Asian countries in particular, are content to use only the traditional methods such as the eyestalk ablation blind-foldedly to induce egg production and faster body growth. Sustained use of this eyestalk-ablation technique has however underlying relevance to its quick results on ovarian maturation and spawning under controlled conditions, albeit shortcomings and drawbacks on the quality of the seeds produced.

The search for a hormone to control reproduction in crustaceans commenced with the discovery that eyestalk ablation accelerated ovarian maturation in a grass shrimp *Palaemon serratus* by Panouse in 1943 (ref. 1). This eyestalk inhibitory factor was later found to be acting in opposition to a stimulatory neurohormone secreted from the brain. These two hormonal factors were subsequently identified to be the gonad-inhibitory as well as gonad-stimulatory hormones (GIH or VIH, for vitellogenesis inhibiting hormone), secreted respectively, from the X-organ/sinus gland complex of the eyestalk ganglia and the neurosecretory cells of the brain and thoracic ganglia in different crustacean species[2].

In recent years, continued attention has been paid to the understanding on the regulatory role of GIH, inasmuch as the eyestalk ablation of several crustacean species resulted in the initiation/acceleration of ovarian maturation. No wonder, eyestalk ablation is a regular world-over practice to induce maturation and spawning in the shrimp hatcheries. By far, the eyestalk neuropeptides are the best-studied hormones with respect to their structural elucidation and physiological functions. In addition to GIH, the X-organ/sinus gland complex is also the seat of other inhibitory neuropeptides to control synthetic activities of the Y-organ and mandibular organ, in addition to a group of diabetogenic hormones. Amino acid sequence studies have uncovered the high degree of homology existing among these neuropeptides, to include them in a single family of neuropeptides, crustacean hyperglycemic hormones (CHH). The structural similarity as well as the physiological overlapping among these super family peptides may suggest the ancestral nature of the CHH neuropeptides, giving origin to the inhibitory peptides such as VIH, moult inhibiting hormone (MIH) and mandibular organ inhibiting hormone (MOIH) by way of gene duplication[3].

The actual mechanism of VIH action has been elusive for a long time since its discovery, but its isolation and *in vitro* testing of its effect on the target organs have unravelled the unique inhibitory pathway to control vitellogenin synthesis. As with other peptide hormones, VIH acts through the second messenger systems involving cyclic nucleotides, Ca^{2+}, and protein kinase C in the signalling pathways that regulate vitellogenin mRNA synthesis in the ovary and hepatopancreas[4]. There is some evidence that VIH could also act on the vitellogenin uptake into the oocytes, by inhibiting the endocytotic process, but more evidence is available to show that VIH primarily inhibits Vg synthesis[5]. On the contrary, both MIH and MOIH are known to act on their respective endocrine glands, such as Y-organ and mandibular organ, prohibiting synthesis of the moulting hormone, ecdysone, and the crustacean juvenoid, methyl farnesoate, the latter possessing diversified functional roles in the control of reproduction and moulting. It could still be possible that VIH acts alternatively through its respective stimulatory hormonal centre. But evidence is wanting.

In addition, the group of CHH-active peptides exhibits a wide range of overlapping functions on the reproductive and moulting physiology in crustaceans. For example, as many as six HPLC-purified CHH peptides of the kuruma prawn, *Marsupenaeus japonicus* are shown to have additional VIH activity on ovarian tissues. CHH from lobsters and shrimp also exhibit inhibitory activity on Y-organ synthesis of ecdysteroids. Similarly, the MOIH of the spider crab, *Libinia emarginata* retains CHH activity, while exerting inhibitory action on methyl farnesoate synthesis by mandibular organ. Although all the CHH family peptides possess CHH activity to some extent, none of the other negative regulatory peptides (MIH, VIH and MOIH) exhibited any inter-functional role between them. This fact strongly supports the contention that CHH is the ancestral peptide molecule to give rise to other inhibitory peptides in the family and not vice versa (see ref. 6).

More interestingly, an isoform of the CHH (CHH-B) of the American lobster, *Homarus americanus* has a stimulatory role on vitellogenesis. In this lobster, the

CHH are not only produced in the optic ganglia, but also in other parts of the central nervous system (CNS) such as the brain and thoracic ganglia. Yet again, in the penaeid shrimp *Metapenaeus ensis*, MIH-B, an isoform of MIH, produced in the X-organ/sinus gland as well as the brain, thoracic ganglia and ventral nerve cord increased the expression levels of Vg mRNA in both the ovary and hepatopancreas, in addition to delaying the moult cycle. Similar results have been reported on the blue crab, *Callinectus sapidus.* In addition, a vertebrate-type decapeptide, gonadotropin-releasing hormone (GnRH), has also been discovered in the CNS of the giant freshwater prawn, *Macrobrachium rosenbergii.* Arguably, the stimulatory neuropeptides of crustacean CNS described above could constitute the classical gonad stimulatory hormone of the brain/thoracic ganglia, proposed first by Otsu in 1960 and other workers[6].

Evidently, the neurosecretory cells of CNS, including the eyestalk, in crustaceans produce a wide variety of peptides with different controlling effects on vital physiological processes concerned with growth and reproduction. A hormonal interplay between them is expected to create neuronal networking to delicately interlink various reproductive and moulting activities for apportioning the nutritive storage materials to meet the metabolic demands of these energy-demanding physiological pathways. As the components involved in the hormonal networking pathways are complex and interrelated, delineation of individual pathways to control different activities is difficult to discern in isolation.

Furthermore, other neurotransmitter-like molecules called biogenic amines, synthesized by the CNS cells, seemingly control the above-discussed hormonal interactions between these neuropeptides. Particularly, 5-hydroxytryptamine (5-HT) and dopamine control the release of the neuropeptides into the haemolymph and thereby control each other's activity. The output of these combinatorial interactions finally controls the proximate endocrine glands to produce the appropriate hormone to control oocyte maturation and probably spawning, as in shrimp. Gonadal maturation and spawning in some penaeid shrimp has indeed been achieved by injecting exogenous amines such as 5-HT into the non-reproductive females[7]. Eventually, the injection of biogenic amines could be the first level of ovarian stimulation in the culture of commercially important crustaceans.

While there is much consensus on the functionality of the eyestalk and brain hormones described above, discordant opinions are expressed on the nature of other proximate gonad-stimulatory hormones in crustaceans. Methyle farnesoate, a sesquiterpenoid secreted by the mandibular organ, the ecdysteroids as well as the vertebrate-type sex steroids, such as 17-β estradiol and progesterone have been proposed as the main candidate hormones for gonad stimulatory activity. Recent molecular studies involving Vg gene expression have provided authentic evidence to show that these hormonal factors have stimulatory action on vitellogenin synthesis. Nevertheless, both ecdysteroids and methyl farnesoate have their primary role in moult induction, with the former activating and accomplishing the moulting process, and the latter, inducing the Y-organ to secrete the moulting hormone. In species that brood their eggs in the pleopods, ecdysone controls the embryonic larval moulting and hatching, thus facilitating sequential adult moulting with the female reproductive cycle[8]. In the event of the dual role that these two hormones play on moulting and reproduction, crustaceans employ them in the hormonal coordination of these two vital physiological activities. Truly, many decapods synchronize moulting and female reproduction in a way that egg production could be successfully accomplished without affecting somatic growth.

It then remains to be deduced that vertebrate sex steroids are the only hormones having exclusive controlling effect on ovarian development. This is in conformity with the vertebrate system, much the same way that estrogen and progesterone activate vitellogenin synthesis and meiotic maturation respectively. Nevertheless, information on various hormone receptors, including their expression patterns during various stages of reproductive and moulting activities is important to unravel the mechanisms and control of hormone action on various target organs concerned with egg maturation and moulting.

Taken together, a hierarchical manner of hormone action involving chemically diverse molecules is emerging in crustaceans. The employment of many hormonal factors by crustaceans, unlike other arthropods and vertebrates, is only to operate many signalling pathways in the control and coordination of various cyclic activities pertaining to reproduction and moulting.

With the advent of intensive aquaculture in many South East Asian countries, understanding the controlling mechanisms of reproductive processes becomes imperative. Nevertheless, there is no better time than now to utilize the information available to us for formulating strategies to augment brood stock production of commercially important crustacean species such as shrimp, lobsters and crabs. Looking into the future, it is only futile to continue the reductionist approach of characterizing the action molecules of reproductive control in solving the problem of induced maturation for commercially important crustaceans. The endocrine perspective emerging from this note is to identify the hormonal factors appropriate to the species concerned and use them in the control of their reproduction and moulting.

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