Crustacean hyperglycemic hormone family Neurohormones: A prevailing tool to decipher the physiology of crustaceans

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An advanced understanding of neurohormonal activation and inhibition of numerous functions is vital for two important reasons. First, basic research gradually elucidates the mechanisms by which activation of many neurohormonal systems causes further alteration in various gene expressions. Second, pharmacologic agents can be designed and used to interfere with the target specific components of neurohormonal systems. Future approaches will probably emphasize on specific characteristics of neurohormonal systems, comprising targeting neurohormones and the intracellular second messenger systems. Accordingly, the objective of this review is to define the mechanisms of neurohormonal activation of various functions in crustaceans and to deliver a comprehensive description. This review will also serve as a vital reference for researchers by providing the structural and physiological basis for current and future research in the field of crustacean neurobiology.

[Keywords: Crustacean Hyperglycemic Hormone, Crustaceans, Neurohormones, Crustacean neurobiology]

Pleiotropic Hormone Family and Neuroendocrine Regulation

Immunocytochemical and physiological evidences suggests that the CHH family neuropeptides are synthesized in the X-organ. The neuropeptides are then transported to the sinus gland that extant flanked by the medulla externa and medulla interna in the crustaceans eyestalks1,2. CHH family neuropeptides function as intermediate between neural signaling and hormone signaling responsible for the activation or inhibition of various physiological functions in malacostracan crustaceans3,4. In all cases, these neuropeptides synthesize by neural cells lead distally via axons recuperated in tractus and secrete under the suitable stimuli5,6. Several in-vitro and in-vivo approaches have shown that in addition to eyestalks, the CHH family neuropeptides including CHH (crustacean hyperglycemic hormone), MIH (molt inhibiting hormone), VIH (vitellogenin inhibiting hormone), MOIH (mandibular organ inhibiting hormone) are also synthesized by the pericardial organs, thoracic, abdominal and cerebral ganglia which might be significant in neuroendocrine regulator of different physiological developments7,8. CHH family neuropeptides are pleotropic in nature and plays vital role in various processes including hyperglycemia, molting, regeneration, lipid metabolism, water uptake etc1. These neuropeptides have a another endocrine gland as their target, for example the production of ecdysteroids is repressed by MIH which binds to its cognate receptor on Y-organ, vitellogenesis (process of yolk formation) is explicitly inhibited by VIH while MOIH impedes the secretion from mandibular organ1,9,10,11. CHH family neuropeptides have certain structural resemblances and intricate
physiology due to the multiple processes that may overlap and influence each other. CHH neuropeptide family of neurohormones can be separated into two groups based upon the prehormone sequence available. One group of peptides encompassing CHH often called as CHH-I or type-I which is responsible for the hyperglycemic activity and, the other CHH-II comprising MIH, MOIH and VIH accountable for various other physiological activities. The work published on the endocrine regulation of growth and development in various crustacean species has augmented considerably during the last few years. Possible stimulating and inhibiting effect of these neuropeptides can occur at diverse physiological stages but findings on the precise molecular pathways by which the neuroendocrine system can be controlled are remains unknown rare. Mechanisms that regulate both the synthesis and secretion of these neurohormones in the nervous system are not fully understood. This can occur at different physiological stages like fluctuating the secretion of hormones or meddling with the interactions of hormone with the membrane bound receptors. These regulations are essential to comprehend the regulation of molting, reproduction and various other processes (Figure1). An extensive array of isoforms have been described to be crustacean hyperglycemic hormone family for crustacean species, especially CHH isoforms when present in the crustaceans can be also affect molting and reproduction in addition to its core function as stress hormone. Crustaceans constitute a major part of all the known animal species in nature and are one of the most pervasive clusters of invertebrates, populating all sorts of aquatic habitats. Many crustaceans species have been preferred as experimental species for assessing various effects of these neuropeptides in copious studies; in spite of this, utmost in vitro bioassays used for recognizing neurohormones and their receptors on various endocrine organs have been developed with vertebrate (particularly mammalian) cell lines. Hormonally regulated functions affected by CHH family neuropeptides have been earlier appraised in decapod crustaceans by various groups of scientists. Goal of the present review is to appraise the state of the art, highlighting from a general fact of view the precarious phases and mechanisms of the neuroendocrine regulated processes of crustaceans possibly pretentious by Crustacean hyperglycemic hormone (CHH), Molt inhibiting hormone (MIH), Vitellogenin inhibiting hormone (VIH).

CHH: Key regulator of stress response

The crustacean hyperglycemic hormone (CHH), stimulated by various environmental signals, is involved in variety of key physiological processes further pronounced in many crustaceans species such as lobsters, crayfish and penaeid shrimp. The pleotropic nature of CHH has been demonstrated in many crustacean species ever since the first amino acid sequence was determined from the shore crab, Carcinus maenas by manual microsequencing. The CHH involves in many physiological processes like blood glucose regulation, molting and inhibition of methyl farnasoate synthesis, lipid metabolism regulation, vitellogenesis and ovarian maturation, and water uptake. The CHH is most abundant neuropeptide in XO/SG neurosecretory system in crustaceans and it is also characterized from some other neuroendocrine cells such as pericardial organs, second root of thoracic ganglia and subesophageal ganglia. Primary structure of CHH has been reported in various crustacean species which revealed the presence of various isoforms of CHH in a particular species. CHH-A and CHH-B, the two isoforms of CHH and 12 crustacean precursor-related peptides (CPRP) were detected in the sinus gland extract of American lobster, Homarus americanus. CHH-A was also detected from pericardial organ (PO) and stomatogastric ganglion (STG) while one full length CPRP was also found in pericardial organ. In Carcinus maenas, one CHH was characterized from sinus gland.
extracts consists of 75 amino acid residues (8.5KDa) whereas a CHH with 73 amino acid residues (8.6KDa) and two CPRP were isolated from PO.

*Molt Inhibiting Hormone: Regulation of growth & development*

In crustaceans, various processes comprising molting and reproduction are expedited by well characterized signaling molecules known as ecdysteroids. Packed ecdysteroid into the eggs from maternal source is used during early stage of embryonic growth. Ecdysis is prompted by the augmented basal level of ecdysteroids just after premolt which causes exuviation of the old exoskeleton. This furthermore causes an upsurge uptake of water which consequences the development of a bigger cuticle which is critical for development of the organism. Production of growth hormone (ecdysteroids) is controlled by MIH acting on the non-neural endocrine organ, the Y-organs that are located in the anterior cephalothorax of crustaceans. In the case of crustaceans, dramatically fluctuation of hemolymph ecdysteroid concentration during various molting stages is principally exerted by molt inhibiting hormone. Presence of MIH was also suggested by the fact that eye-stalk ablation leads to prompt rise in ecdysteroid level in the hemolymph and hence, induces advanced shedding of exoskeleton. During intermolt, the ecdysteroid is upheld at low circulating level by MIH and hence, eyestalk ablation results in the shortening of intermolt period and increases the number of molt cycles. MIH wields an inhibitory effect on the Y-organ by binding to its cognate membrane receptor (guanylyl cyclase) and hence, reduces the synthesis and production of ecdysteroids. The facts from several studies suggests that the responsiveness of receptor guanylyl cyclase to MIH is change just prior to premolt which increases the ecdysteroid secretion by the Y-organ and hence, causes an impulsive surge in circulating ecdysteroid levels.

Molt inhibiting hormone blocks the facultative production and secretion of ecdysteroid by initiating an explicit transcription factor that blocks phantom expression in the Y-organ. MIH interacts with guanylyl cyclase on the Y-organ membrane and conquers the ecdysteroid biosynthesis by articulated cAMP-dependent nitric oxide synthase (NOS) and NO-dependent guanylyl cyclase (GC-I). The impact of MIH on Y-organ does not strictly follow the above model and it is evident that the molecules other than Molt inhibiting hormone have straight or circuitously an influence on the regulation of the synthesis of growth hormone.

**VIH: Regulation of reproduction in Crustaceans**

In crustaceans, reproduction is the prominent cellular activity that occurs during the late phase of gonadal maturation is known as vitellogenesis. This process is characterized by the synthesis and accumulation of a precursor of yolk protein, vitellogenin, in the extraovarian tissues or in the developing ovaries. Vitellogenesis is a vital phase in ovarian development which is characterized by the synthesis and deposition of vitellus, a yolk protein largely composed of a lipoprotein vitellin. It was observed that the confiscation of one eyestalk persuades an augmented ovarian maturation and spawning in various crustaceans species. The synthesis of vitellogenin and ovarian maturation in ovary and hepatopancreas is modulated by a neurohormone synthesized and secreted by the XO-SG neurosecretory system located in the eyestalk of crustaceans referred as vitellogenin inhibiting hormone (VIH) or gonad inhibiting hormone (GIH). Gonad-inhibiting hormone is a member of the CHH family neuropeptide but the mechanism of action of these neuropeptides is not well established since only a limited number of neuropeptide have been characterized to date. A significant inhibiting of ovarian development and spawning was observed when female shrimps of *S. ingentis*, following a spawn, was injected with sinus gland extracts obtained from non-reproductive female shrimps. The
eyestalk ablation increases the frequency of brood in *P. canaliculatus* but it was also found that the number of eggs and hatching success is less in ablated animals\(^40\). A first GIH, a 7.5KDa peptide was isolated and purified using two step reverse phase HPLC from the sinus gland extracts of American lobster, *Homarus americanus*, which was tested for its vitellogenin inhibitory activity *in vivo* in the shrimp, *Palaemonetes varians*. The sinus gland peptide-III of kuruma prawn, *Marsupenaeus japonicus* significantly represses the vitellogenin mRNA levels when incubated with the ovarian fragments and the level rationally high in eyestalk ablated animals\(^41\). The cDNA encoding the Pem-GIH was isolated from the eyestalk of *Penaeus monodon* which encodes 79 amino acids mature peptide having features similar to CHH family neuropeptides which decreases the vitellogenin level in the ovary.\(^42\)

The 8.3KDa peptide was isolated and characterize from the sinus gland extract from crayfish, *Procambarus bouvieri* which inhibits the synthesis of vitellogenin. When Pro-GIH was incubated with the cultures ovaries *P. (Litopenaeus) Íannamei*’s, it represses the production of vitellogenin which indicates that the GIH is not species specific. The same bioassay was performed with CHH and MIH from *Pro. bouvieri*, it did not inhibit vitellogenin synthesis in *P. (Litopenaeus) vannamei*’s.

**CHH Family: Advancement in structural biology**

The solution structure of MIH determined from the *Marsupenaeus japonicus* revealed the presence of five \(\alpha\)-helices and no \(\beta\)-strands. Asparagine at 13\(^{th}\) position in the N-terminal alpha-helix and, serine and isoleucine at C-terminal tail are critical to binding with receptor guanylyl cyclase (Figure2). Sequence analysis of putative molt inhibiting hormone gene from *Metapenaeus ensis* revealed the presence of a 315bp coding region which encodes a mature peptide consisting of 77 amino acid residues\(^43,44\). Several MIH sequences have been isolated and characterized from various taxa of crustaceans, with primary sequence ranging from 72 to 80 amino acid residues. These neuropeptides can be broadly divided into two groups (CHH-A and CHH-B) and each group also consists of many isoforms as they shared over 80-95% amino acid sequence identity, however, the total number of isoforms in each group remains unclear. CHH family neuropeptides having distinctive significant sequence characteristics include the presence of 6 cysteine residues which forms three disulfide links at stringently conserved positions accountable for the stabilization of tertiary structure of the molecules\(^43,45\) (Figure2). C-terminus of CHH family neuropeptides is either free or amidated and the information available from its primary structure associated with their physiological significance suggests that it is primarily responsible for their distinct activity. In most of the cases, the primary structure of CHH family peptides were deduced from cloned cDNA sequences and probable terminal alterations are therefore unidentified. Amidation of C-terminus is responsible in conferring hyperglycemic activity by CHH while there are some exceptions where MIH have an amidated C-terminus but the functional significance is still unknown.

Analysis of sinus gland extract from American lobster, *Homarus americanus* revealed the presence of one GIH and two isoforms of CHH (CHH-A and CHH-B) due to the existence of a D-phenylalanine residue in one of the isoforms\(^46\). Although there is scarcity of data from these crustaceans, it is enticing to speculate that two major forms of MIH-like and two major forms of CHH-like exist in decapods.

**Chemical interference: A novel tool to boost the growth?**
The studies on the various physiological roles of crustacean CHH family hormones have made pronounced development in contemporary years. Especially, the structure and functional analysis has proved that MIH plays a vital role in several metabolic functions, including growth and reproduction. The growth rate of crustacean can be accelerated by developing competitive inhibitors which can also be used as a powerful research tools. The development of these inhibitors would also be helpful to understand the relational network between CHH family neuropeptide and to assess the potential regulatory roles of MIH in various physiological courses. Furthermore, it has been observed that most of the CHH family peptides display higher specificity towards various biological functions and this factor was also evident from the homology model of CHH where the N-terminal α-helix and C-terminal tail regions are absent which is critical for MIH function. These novel inhibitors for MIH receptor would be specific to competitively inhibit the binding and probably have no measurable affinity towards other CHH family neurohormones. The absence of 3D structure of receptor guanylyl cyclase has made it tough to design the competitive inhibitors and various in-silico tools to build homology models unsuccessful due to very little sequence and structural resemblances. Whether competitive inhibitors will demonstrate as a valuable tool to understand the functions of various neuropeptides and will it be possible to enhance the growth of crustaceans remains to be seen. The structural and functional properties such as structure of GC, CHH and VIH, and their binding sites will be very helpful for the optimization of inhibitors. These informations will certainly help to increase occurrence of molting to attain the improved growth of crustaceans.

**Future perspectives**

In crustaceans, the CHH family neuropeptides have a key part in regulating several activities that impose or favor synchronized responses of quite a few autonomic systems by assimilating external and internal stimuli. As discussed above, the outcomes from several studies evidently point to a crucial role of CHH family neuropeptides on growth and reproduction, and related signaling paths in a physiological pertinent response to diverse circumstances. CHH family could be involved in the regulation of stress, osmoregulation, molting and reproduction. CHH family neurohormones, the most versatile animal hormones are regulatory signaling molecules and probably best example to understand the pleotropic nature where one neuropeptide synchronize many developmental and physiological processes. The multifarious effects of various CHH like neurohormones give an emphasis to characterize the target sites. CHH family of neurohormones affects a remarkable number of processes using guanylyl cyclase receptors on various tissues in crustaceans especially on development and life history, including growth, behavior, reproduction and stress resistance.

While the structure and molecular properties of target sites is unavailable and the underlying these hormones, signaling remains unidentified, the studies on specific inhibitors of the receptor-signaling pathway can offer more perceptions into the various physiological functions and maturation of female reproductive organs. In conclusion, the investigations on GC signaling which is critical, almost all physiological processes including normal growth are need of the hour.

**Acknowledgement**

Authors are grateful to Department of Science and Technology, Govt. of India for providing fellowship to Mr. Sajal Shrivastava under INSPIRE fellowship program and to the management of SASTRA University for providing facilities for this study.
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**Legends/Caption**

**Figure 1.** The various functions of crustacean CHH family neuropeptides. Various environmental factors stimulate the synthesis and secretion of CHH/MIH/MIIH and VIH. MIH inhibits the ecdysteroidogenesis in the Y-organ while stimulates vitellogenesis in the hepatopancreas. VIH inhibits the synthesis of vitellogenin and GSF (gonad stimulating factor) plays a crucial role in ovary maturation. CHH is a pleotropic hormone either stimulates or inhibits various physiological functions including hyperglycemia. Methyl Farnasoate (MF) also known as juvenile hormone and ecdysteroids stimulates the ovary and testis maturation in female and male crustaceans respectively.

**Figure 2.** Multiple sequence alignment of some well characterized molt inhibiting hormone peptides (MIH). The crystal structure of *Marsupenaeus japonicus* MIH was used to derive the structural properties and subsequently the sequences for four α-helices are represented. Six cysteine residues and the three respective disulfide bridges are indicated by numbers. Three residues (Asn13, Ser71 and Ile72) critical for MIH activity is shown below the sequences as asterisk (*). The following crustacean MIH are analyzed: MJ- *Marsupenaeus japonicus*, CM- *Carcinus maenas*; CS- *Callinectes sapidus*; CP- *Cancer pagurus*; CF- *Charybdis feriatus*; OL- *Orconectes limosus*; PC- *Procambarus clarkii*; PJ- *Penaeus japonicus*; ME- *Metapenaeus ensis*; LV- *Litopenaeus vannamei*; PM- *Penaeus monodon*. 