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Shrimp endocrinology. A review

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Abstract

Despite the growing importance of shrimp aquaculture, the study of shrimp endocrinology is lagging behind the effort invested in the study of crayfish, crabs, and lobster endocrine glands and their hormones. Fortunately, there is an increasing number of laboratories interested in the specific study of metabolism and endocrinology of cultured species of shrimp. Recent advances in the sequence elucidation of sinus gland (SG) neuropeptides and in their mode of action in the penaeids is encouraging. Small molecules like ecdysteroids and methyl farnesoate have increasingly become the subject of applied research in shrimp aquaculture. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Despite the growing importance of shrimp aquaculture in a worldwide context, the specific study of shrimp endocrinology is lagging behind the effort made to understand the intricacies of crustacean endocrinology, directed mainly at the study of crayfish, crabs, and lobsters. Of course, much of what has been learned about these latter decapods can be extrapolated to the shrimp, but there is a substantial need to deepen our knowledge of shrimp physiology and, especially, endocrinology.

Due to overexploitation of the wild population of marine shrimps with the consequent decrease in productivity, shrimp farming has increased remarkably in the last 20 years, surpassing an annual production of 700,000 MT. The input of research in the fields of physiology, biochemistry, endocrinology, nutrition, pathology, and genetics has resulted in new technological advances that have improved the economic gains of shrimp aquaculture.

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Previous excellent reviews by Quackenbush (1986) on general crustacean endocrinology, by Keller (1992) on crustacean neuropeptides, by Chang (1992) on shrimp endocrinology, and by Fingerman (1995, 1997) on endocrine mechanisms, are recommended. This review will concentrate on developments of the last 10 years, mainly in penaeid shrimps, but other crustaceans will also be considered when necessary for comparison purposes. For the nomenclature of penaeid shrimps, I have adopted the proposed classification of Pérez Farfante and Kensley (1997), but to avoid confusion, the new name (in parentheses) follows the old one.

2. Ecdysteroids

The Y-organs are the source of the molting hormone, secreted as a precursor, ecdysone, to the hemolymph to be converted into the active hormone, 20-OH-ecdysone, by a 20-hydroxylase activity present in the epidermis and other organs and tissues (Fig. 1). Crustacean ecdysteroids are very polar (20-OH-ecdysone has six hydroxyl and one keto groups) and there is no evidence for carrier proteins in the hemolymph. Thus, they circulate freely and can enter cells by simple diffusion, but in addition, as demonstrated by Spindler et al. (1984), there is also an energy-dependent and carrier-mediated process of hormone entry (at least in crayfish and crabs). Receptor binding sites have been found in the DNA of *Metapenaeus ensis* (Chan, 1998), lending further evidence to the hypothesis that steroid hormones mediate their action by differential transcription of specific genes.

The circulating titer of 20-OH-ecdysone varies impressively along the molt cycle. Immediately after ecdysis (Stage A), it is very low and generally remains so during intermolt. A dramatic surge takes place at stage D_1–D_2 followed by a precipitous drop just before the actual molt (Fig. 2).

The Y-organ has been studied mainly in brachyuran and macruran species, but Dall (1965) described the Y-organ in the shrimp, *Metapenaeus* sp., and Bourguet et al. (1977) isolated and described it in the shrimp, *Penaeus (Marsupenaeus) japonicus*. It is located in the anterior branchial chamber in all crustaceans but it can appear as a compact mass in crabs or as a less compact mass in crayfish and lobsters (Lachaise et al., 1993).

Experiments with eyestalk (ES)-ablated animals and with Y-organs incubated in vitro in the presence of sinus gland (SG) extract have led to the conclusion that the SG

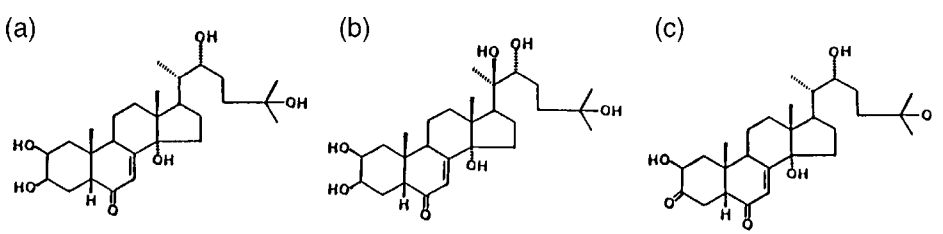

Fig. 1. Structure of crustacean ecdysteroids: (a) ecdysone, (b) 20-OH-ecdysone, (c) 3-dehydroecdysone. (Modified from Chang, 1997).

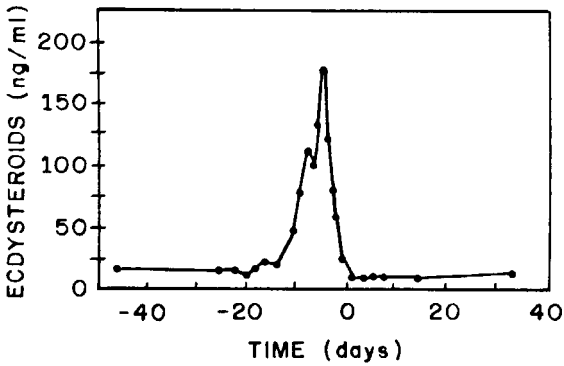

Fig. 2. Ecdysteroid titers of *S. ingentis* hemolymph before and after molt (taking place on day 0). (Modified from Chang, 1992).

contains a peptidic factor that regulates the synthesis of ecdysone in the Y-organ (Chapter II). Synthesis and/or secretion rates of ecdysone by the Y-organ may be the predominant mechanism of controlling hemolymph ecdysteroids (Chang, 1992).

Spindler et al. (1987) observed in the shrimp, *Palaemon serratus*, low levels of ecdysteroid content in eggs at extrusion time, and increasing levels toward hatch. Similarly, in the shrimp, *Sicyonia ingentis*, Chang et al. (1992) observed that the content of ecdysteroids in embryos at spawning is negligible but that the concentration increases significantly as embryonic development proceeds. This is likely due to endogenous synthesis of hormone.

Blais et al. (1994) have shown that the major ecdysteroid produced in vitro by the Y-organ of the penaeid shrimp, *P. (Litopenaeus) vannamei*, is 3-dehydroecdysone, but the major circulating ecdysteroid in premolt animals is 20-OH-ecdysone. Hemolymph ecdysteroid levels increased in parallel with the rise in ecdysteroid production, peaking at the late premolt stage D_1. Ecdysteroids in the incubation media were quantified at different stages of the molt cycle by enzyme immunoassay after HPLC separation. Three major immunoreactive compounds were identified, 20-OH-ecdysone, ecdysone and 3-dehydroecdysone. This last one appeared to be the predominant secretory product, and it was inferred that the other compounds could arise from its metabolism by contaminating epidermis in the preparation.

In the alpheid shrimp, *Alpheus heterochaelis*, exposed to micromolar concentration of 20-OH-ecdysone during 5 days, the winter molt cycle was shortened by 18 days, or 65%. At the same time, claw transformation was accelerated. This latter effect could imply that ecdysteroids have a modulating role in morphogenesis (Mellon and Greer, 1987). Likewise, Knowlton (1994) has proposed the existence of a morphogenetic factor residing in the ES in view of the metamorphic arrest seen in alpheid shrimp larvae when both ES are ablated during Stage II.

Chan (1998) has cloned a cDNA from the shrimp *M. ensis*, encoding a nuclear receptor superfamily, homologous to the insect ecdysone-inducible E75 gene. Its deduced amino acid sequence has all the five domains typical of a nuclear receptor and it is expressed in the epidermis, ES, and nervous tissue of premolt shrimp. As yet, there

is no information on what the ligand of this receptor may be and this author prefers to classify it as an orphan steroid receptor.

3. Molt-inhibiting hormone (MIH)

The surgical extirpation of the ES results in a shortened molt cycle interval, while the implantation of the ES contents restores this interval. A factor has been implied that normally inhibits the molting process and it has been named the MIH. In brachyurans and macrurans, ES ablation also results in a considerable increase in circulating ecdysteroids. The neurosecretory system of the ES consists of a group of peptidergic neurons clustered in the medulla terminalis X-organ (MTXO) and their bulbous axonic terminals that constitute the SG, which is a neurohemal organ that releases into the hemolymph a number of peptide hormones, including the MIH. The reviews by Skinner (1985) and by Chang (1985) are recommended.

The peptidic nature of MIH has been established for many brachyuran and macruran species. Aguilar et al. (1996) isolated the MIH from the SG of the Mexican crayfish, *Procambarus bouvieri*, and compared its sequence with the other four known peptides at the time, from *Homarus americanus*, *Carcinus maenas*, *Callinectes sapidus*, and *P. (Litopenaeus) vannamei*. Their lengths vary between 72 and 78 residues and their molecular masses between 8 and 9 kDa. All have six cysteines that form three disulfide bonds.

Sun (1994) was the first to clone a cDNA encoding a MIH-like neuropeptide from the white shrimp, *P. (Litopenaeus) vannamei*. Total RNA was isolated from the MTXO and used to clone this cDNA by the 3′ and 5′ rapid amplification method (RACE). The deduced polypeptide consists of a 72-residue mature peptide and a 30-residue region of a propeptide (Fig. 3A). This neuropeptide has been termed MIH-like because it has been cloned with the aid of a primer whose encoded sequence is shared by CHH and probably, by VIH of several species, and because it has not been tested in a bioassay. By means of in situ hybridization, she was able to show that the MIH-like mRNA is exclusively localized in the MTXO of the ES, but in the brain, the MIH-like gene transcript was detected in the neurosecretory cells, the giant cells and the lateral cell bodies (Sun, 1995). This could mean that the MIH-like neuropeptide could have specific functions in the nervous system, in addition to its hormonal function.

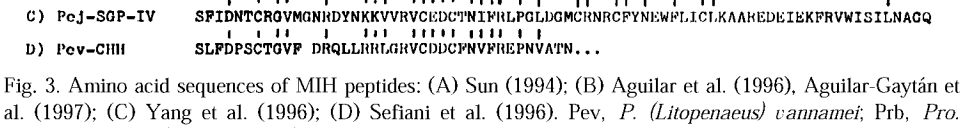

Fig. 3. Amino acid sequences of MIH peptides: (A) Sun (1994); (B) Aguilar et al. (1996), Aguilar-Gaytán et al. (1997); (C) Yang et al. (1996); (D) Sefiani et al. (1996). Pev, *P. (Litopenaeus) vannamei*; Prb, *Pro. bouvieri*; Pej, *P. (Marsupenaeus) japonicus*. Note that Pej-SGP-IV has an extra amino acid (Gly) at position 12 (Type II). Identical or similar (in charge or hydrophobicity) residues have been united by dashes.

A somewhat different situation has been the cloning of a cDNA encoding the MIH from the Mexican crayfish, *Pro. bouvieri*. (Aguilar-Gaytán et al., 1997). In this case, the peptide was isolated, sequenced, and bioassayed (Fig. 3B) (Aguilar et al., 1996). It is a 72-residue peptide with blocked amino- and carboxyl-termini, and six cysteines forming three disulfide bonds.

Yang et al. (1996) isolated and sequenced a peptide with MIH activity (termed Pej-SGP-IV) from the SG of the kuruma prawn, *P. (Marsupenaeus) japonicus*. It inhibited ecdysteroid synthesis in vitro by Y-organs of the crayfish, *Pro. clarkii*. It is a 77-residue peptide with both free amino- and carboxyl-termini (Fig. 3C) and it had very little hyperglycemic activity. Ohira et al. (1997) have succeeded in cloning a cDNA correponding to the *P. (Marsupenaeus) japonicus* MIH and have shown by Northern blot analysis that specific hybridization is present only with RNA extracted from the ES but not from other tissues, but the levels of mRNA did not decrease significantly at the premolt stages, which suggests that the synthesis and secretion of MIH might be regulated post-transcriptionally.

By means of immunohistochemistry, Shih et al. (1998) have localized neurosecretory cells in a cluster of the MTXO of the kuruma prawn, *P. (Marsupenaeus) japonicus*, that react with an antiserum raised against the synthetic C-terminal decapeptide of Pej-MIH (V-W-I-S-I-L-N-A-G-Q-OH), conjugated with bovine serum albumin. Another antiserum was raised against the synthetic C-terminal decapeptide of Pej-CHH (termed Pej-SGP-III): E-E-H-M-A-A-M-Q-T-V-NH_2, conjugated with bovine serum albumin. Three kinds of neurosecretory cells were recognized: those that reacted with only one of the two antisera and very few that reacted with both. The cells that reacted with the CHH-antiserum where more abundant than the ones that reacted with the MIH-antiserum. Whether this means that there is colocalization of the two peptides in one neurosecretory cell or that these antisera recognize other peptides is not known. In other species, colocalization between CHH and GIH (gonad-inhibiting hormone, also known as VIH) was found by De Kleijn et al. (1992) in *H. americanus*, and by Rotllant et al. (1993) in *H. gammarus*, but Klein et al. (1993) could not find colocalization of MIH and CHH in *C. maenas*.

A peptide with both MIH and CHH activity was isolated from the SG of *P. (Litopenaeus) vannamei* by Sefiani et al. (1996). By mass spectrometry, its molecular mass was determined to be 8627 Da, but its sequence was determined to only 38 residues (Fig. 3D). Using an antiserum directed against the lobster CHH_A, the hyperglycemic activity of a SG extract was completely suppressed but the MIH activity was not affected unless the immune complexes were removed by protein A. This could mean that different epitopes of the peptide display different activities and that the site responsible for the MIH activity is not masked by the binding of the antibody, but this hypothesis has to be confirmed experimentally.

There are other instances in which peptides with both MIH and CHH activity have been described. Chang et al. (1990) purified a peptide from the SG of the lobster *H. americanus*, that had both MIH and CHH activities which could not be dissociated by various chromatographic steps.

Recently, Gu and Chan (1998a,b) have cloned a cDNA encoding a putative MIH from the ES of *M. ensis*. The deduced amino acid sequence consists of 77 residues,

preceded by a signal peptide of 28 residues. This sequence is very similar to the sequence of Pej-MIH, with conserved position of the six cysteine residues. It is expressed in the ES and in the brain, in postmolt, intermolt, and premolt stages. This MIH gene consists of at least two introns localized in the nucleotide sequences corresponding to the signal peptide and the mature peptide.

According to the review of De Kleijn and Van Herp (1995), the analysis of the preprohormones of shrimp MIHs shows that they belong to type II precursors in that they do not contain a CPRP (CHH-Precursor Related Peptide), but consist of a signal peptide, and the sequence of the mature peptide, and have a glycine residue at position 12 of the latter.

MIH is able to inhibit ecdysteroid secretion by Y-organs in vitro in a dose dependent manner and this is the standard way to measure its activity (Mattson and Spaziani, 1985). The injection of serotonin (5-OH-tryptamine, 5HT) or stress lower hemolymph ecdysteroid levels and these effects are canceled by ES ablation in the crab *Cancer antennarius*, or by inhibiting the synthesis of 5HT or blocking its receptors. Thus, it is concluded that MIH release is mediated by 5HT (Spaziani et al., 1994). On the other hand, elevated levels of circulating ecdysteroids exert a feedback effect by inhibiting MIH release from the SG, but not its synthesis in the MTXO. On the basis of these data, Spaziani et al. (1994) propose a cycle of MIH regulation: sensory input via 5HT would release MIH that would inhibit ecdysteroid synthesis and release by the Y-organ, and MIH release would be subjected to a negative feedback by elevated ecdysteroid titers.

4. Crustacean hyperglycemic hormone (CHH)

The CHHs are the most abundant neuropeptides in the SG. Their central role on the regulation of carbohydrate metabolism has been reviewed by Keller and Sedlmeier (1998). Frequently, they are represented by two or more isomorphs, as shown for *P. (Marsupenaeus) japonicus*, where Yang et al. (1995, 1997) described five 72-residue peptides with a free amino-group and an amidated carboxyl-group, and six cysteines that coincide in position with the other known CHHs from different crustaceans. They termed these peptides Pej-SGP-I, II, III, V, and VI (Fig. 4A). Peptide Pej-SGP-IV

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A) Pej-SGP-I      SLFDPSCTGVFDRQLLRRLGRVCDDCFNVFREPNVATECRSNCYNNPVFRQCMAYVVPAHLHNEHREAVQMV-NH2
   Pej-SGP-II     SLFDPSCTGVFDRQLLRRLGRVCDDCFNVFREPNVAMECRSNCYNNPVFRQCMEYLLPAHLHDEYRLAVQMV-NH2
   Pej-SGP-III    SLFDPACTGIYDRQLLRKLGRLCDDCYNVFREPKVATGCRSNCYHNLIFLDCLEYLIPSHLQEEHMAAMQTV-NH2
   Pej-SGP-V      LVFDPSCAGVYDRVLLGKLNRLCDDCYNVFREPNVATECRSNCFYNLAFVQCLEYLMPPSLHEEYQANVQMV-NH2
   Pej-SGP-VI     LVFDPSCAGVYDRVLLGKLNRLCDDCYNVFREPNVATECRSNCFYNLAFVQCLEYLLPPSLHEEYQANVQMV-NH2
B) Mee-CHH        SLFDPSCSGVFDRELLGRLNRVCDDCYNVFRDPKVAMECKSNCFLNPAFIQCLEYLLPEDLHEEYQSHVQVV(NH2)
C) Pes-CHH        ANFDPSCTGVYDRELLGRLSRLCDDCYNVFREPKVATECRSNCFYNPVFVQCLEYLIPADLHEEYQAHVQTV-NH2
D) Scg-ITP        SFFDIQCKGVYDKSIFARLDRICEDCYNLPREPQLHSLCRSDCFKSPYFKGCLQALLLIDEEEKFNQMVEIL(NH2)
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Fig. 4. Amino acid sequences of CHH peptides: (A) Yang et al. (1997); (B) Gu and Chan (1998b); (C) Huberman et al. (submitted); (D) Meredith et al. (1996). Pej, *P. (Marsupenaeus) japonicus*; Mee, *M. ensis*; Pes, *P. (Litopenaeus) schmitti*; Scg, *Sch. gregaria* Ion Transport Peptide. In B and D, the amidated carboxyl-terminus has been deduced from the cDNA. Identical or similar (in charge or hydrophobicity) residues have been united by dashes.

proved to correspond to a MIH (see above). These peptides, which show slight sequence differences among them, are clearly encoded by different genes and are not the product of alternative splicing of mRNA.

In the shrimp, *M. ensis*, Gu and Chan (1998a,b) have cloned and sequenced several cDNAs encoding the preproCHH-like peptides. These cDNAs consist of a signal peptide, a CHH-precursor-like peptide (CPRP) and the CHH-like peptide. The signal peptide and the CPRP are the shortest among all the CHHs known at present. The peptides are expressed in the ES but not in any other tissue. By screening the genomic DNA library from one shrimp and by genomic Southern blot analysis, they found at least six copies of the CHH-like genes, arranged in a cluster in the chromosome, and with an individual size of 1.5 to 2.1 kb. Each of these genes is formed by three exons and two introns. The first intron separates the signal peptide, and the second intron separates the mature peptide in the coding region. The sequence of the CHH-like peptide is 74 residues long, and its six cysteines coincide with the other known shrimp CHHs. It is probably amidated because its deduced sequence includes the codons for glycine, lysine, and termination. In other words, the mature peptide should end in a valyl-amide (Fig. 4B) and be 72 residues long.

It is interesting to note that De Kleijn et al. (1995) found by Northern blot analysis of preproCHH mRNAs in tissues of the lobster *H. americanus*, that the CHH-encoding mRNAs are also expressed in the ventral nervous system. Whether this extends to shrimps has not been demonstrated.

According to the review of De Kleijn and Van Herp (1995) and Yang et al. (1996), the products of these genes belong to the type I precursors inasmuch as they contain a signal sequence, a CPRP, and they lack a glycine residue at position 12 of the mature hormone.

In the Caribbean shrimp, *P. (Litopenaeus) schmitti*, Huberman et al. (submitted) have isolated, purified, sequenced, and bioassayed a peptide with CHH activity which is 72 residues long, with a free amino end, an amidated carboxyl-terminus and six conserved cysteines (Fig. 4C).

Interestingly, an ion transport peptide (ITP) that stimulates Cl^-, Na^+, K^+, and fluid absorption and inhibits H^+ secretion, has been cloned and characterized from the corpus cardiacum of the locust, *Schistocerca gregaria* (Meredith et al., 1996; Phillips et al., 1998), which has great sequence similarity to the known CHHs from different crustaceans (Fig. 4D), but crustacean MIH and CHH had neither stimulatory nor antagonistic actions on the ITP bioassay. It is possible that the CHH-like peptides are common not only to crustaceans but to arthropods in general.

In the CHHs of the lobster *H. americanus*, of the crayfish *Pro. clarkii*, and of the crayfish *Pro. bouvieri*, two isomorphs of the CHH have been found that are differentiated from each other by the presence of a D-Phenylalanine in the third position in the minor isomorph, while in the major isomorph there is a L-Phenylalanine in this same position (Soyez et al., 1994; Yasuda et al., 1994; Aguilar et al., 1995; Huberman and Aguilar, 1998). Up to now, this situation has not been found in shrimps, despite the search for D-amino acids in these organisms.

Hyperglycemic hormones from the shrimp *P. (Marsupenaeus) japonicus* have been shown to inhibit protein and mRNA synthesis in vitro in ovarian fragments of *P.*

semisulcatus (Khayat et al., 1998). This exemplifies the pleiotropic activities of crustacean hormones, through the fact that CHH-family peptides can influence ovarian physiology in these animals. As Webster (1993) has demonstrated the presence of CHH receptors in various tissues, including oocyte membranes in the crabs *C. maenas* and *Can. pagurus*, this confirms that CHH isomorphs may have specific activities in different tissues.

CHH may participate in lipid metabolism, besides its obvious role in carbohydrate metabolism, as shown by Santos et al. (1997) in the crabs *Chasmagnathus granulata* and *C. maenas*, and in the crayfish *Orconectes limosus*. Eyestalk ablation led to a decrease of total hemolymph lipids in *Cha. granulata* and of free fatty acids in *C. maenas*, while the injection of CHH reversed this effects. CHH increased the release in vitro of free fatty acids and phospholipids from *O. limosus* hepatopancreas.

A more complex role of CHH in metabolic control is evidenced by its significant specific binding to different organs like hepatopancreas, heart, epidermis and Y-organ (Kummer and Keller, 1993; Webster, 1993). It is also possible that different isomorphs of the CHH have different receptors and functions. In lobster muscle, Goy (1990) has shown that CHH produces an elevation of cyclic GMP, by activation of a membrane cyclase and not by inhibition of a phosphodiesterase.

5. Vitellogenesis-inhibiting hormone (VIH)

In crustacean females, the late phase of gonadal maturation to form mature ova is named vitellogenesis (Adiyodi, 1985). This process comprises the synthesis or deposition, or both, of yolk or vitellus. The major component of this nutritive material is the lipoprotein vitellin, derived from a precursor called vitellogenin that can be synthesized in extraovarian tissues or in the ovaries.

Since its description by Panouse (1943, 1944), removal of one ES has been used for years to induce an accelerated ovarian maturation and spawning in different species of shrimps used as broodstock in aquaculture. This effect has been attributed to the presence of a vitellogenesis-inhibiting factor present in the MTXO-SG neurosecretory system, and the search for a VIH has been less successful than the study of MIH and CHH. As it has been shown that the action of this hormone is on both male and female gonads, it is more appropriate to name it GIH.

The shrimp, *S. ingentis,* is very useful for the assay of the GIH because it undergoes several cycles of reproduction without intervening molt cycles during the summer months. Chang et al. (1992) injected females of the shrimp *S. ingentis* following a spawn, with SG extracts obtained from nonreproductive female shrimps and observed a significant inhibition of ovarian development and spawning. They are trying to isolate and purify the responsible peptide by means of HPLC. In the shrimp, *P. canaliculatus*, ES-ablated females spawn more frequently than intact females, but the number of eggs and the hatching success is better in the intact animals (Choy, 1987).

Soyez et al. (1987) isolated a 7500 Da peptide from the SG of *H. americanus*, and assayed its GIH activity in vivo in the shrimp *Pal. varians*, by measurement of oocyte diameter. Quackenbush and Keeley (1988) assayed a factor isolated from the ES of the

shrimp *P. (Litopenaeus) setiferus* by studying its effect on the incorporation of radiolabeled leucine into fiddler crab vitellogenin by ovary fragments incubated in vitro, and precipitated with a specific antibody vs. fiddler crab vitellogenin. This factor inhibited the incorporation of radiolabeled leucine into vitellogenin. A specific inhibitory effect of a crude ES extract on vitellin synthesis in the ovary was shown by Quackenbush (1989) in *P. (Litopenaeus) vannamei.*

Two isomorphs of the GIH were isolated and sequenced by Soyez et al. (1991) from the SG of the lobster *H. americanus.* Both consisted of 77 residues and MWs of 9135. The difference between these two peptides has not been established.

A 8388 Da peptide was isolated by Aguilar et al. (1992) from the SG of the crayfish *Pro. bouvieri* which had a depressing activity on the vitellogenin synthesis of cultured *P. (Litopenaeus) vannamei*'s ovaries. Its amino acid composition and partial sequence, clearly indicate that this peptide is a member of the CHH-MIH-GIH family. CHH and MIH from *Pro. bouvieri* did not inhibit vitellogenin synthesis in this same bioassay. This is another indication that the GIH is not species specific.

De Kleijn and Van Herp (1998) suggest that GIH might have a molt-inhibiting function because female lobsters molt only after hatching of their larvae when the CHH and GIH hemolymph levels are low. Thus, high levels of GIH may prevent molting during the immature stages of reproduction, while CHHs may prevent molting during the mature stages of reproduction, tuning between them the synchronization of reproduction and molting during the reproductive cycle.

6. Methyl farnesoate

MF is an unepoxidated sesquiterpene structurally related to the juvenile hormone JH III of insects, synthesized by the crustacean mandibular organ (MO). Le Roux (1968) first described the crustacean MO and Laufer et al. (1987a) identified the secretory product of this gland as methyl farnesoate and found this hormone in the hemolymph of the spider crab, *Libinia emarginata.* In this same crab, they found that the in vitro rate of production of MF by the MO was higher in females undergoing oocyte development and oogenesis. It has been proposed that MF stimulates reproduction in both males and females (Laufer et al., 1987b,c). Liu and Laufer (1996) have isolated and characterized three SG neuropeptides from the spider crab, *L. emarginata*, that inhibit the synthesis of MF in the MO, but at the same time have hyperglycemic activity when injected into destalked fiddler crabs, *Uca pugilator.* Moreover, Wainwright et al. (1996) and Liu et al. (1997a) have characterized MO-IHs from the crabs *Can. pagurus* and *L. emarginata*, respectively. Liu et al. (1997b) have cloned a cDNA corresponding to the preprohormone of MO-IH of *L. emarginata.* The molecular masses of approximately 8400 Da and the similar amino acid compositions of these three neuropeptides are consistent with the hypothesis that the MO-IH are members of the CHH-family of SG neuropeptides.

Chang et al. (1992) used a radiolabeled photoaffinity analog of MF, [^{3}H]-farnesyl diazomethyl ketone ([^{3}H]-FDK) and found specific binding to a 36 kDa protein in the hemolymph of the shrimp, *S. ingentis*, but there was no binding to proteins of other tissues. Unlabeled MF could displace this binding. The same procedure was used by

Prestwich et al. (1990) to characterize an analogous MF binding protein of 42 kDa in the hemolymph of the lobster, *H. americanus*. In the hemolymph of the spider crab, *L. emarginata*, Li and Borst (1991), have also demonstrated the presence of MF binding proteins. In this same crab, Takác et al. (1998), using photoaffinity labeling with [^{3}H]-FDK, have found MF binding proteins in the ovaries, testes, and accessory glands, in addition to the hemolymph. The gonadal tissues from reproductive animals bound twice as much MF as those from non-reproductive animals.

MF may play a role in crustacean reproduction as surveyed by Laufer and Sagi (1991) and Laufer et al. (1993) and act as a gonadotropin and also as a morphogen. Most of their studies have used the spider crab, *L. emarginata*, as test subject. In females, the in vitro secretory rates of MF by MO were closely related to the stage of ovarian growth, being highest in vitellogenic animals (about 3.30 ng/h per gland as compared with 0.5 ng/h per gland in intermolt juvenile females), and implants of MO into juvenile females stimulate ovarian development. There are different male morphotypes: those with most active reproductive behavior, the large abraded individuals, had the highest amount of MF in the hemolymph (67.2 ng/ml as compared to 29.6 ng/ml in unabraded and 10.7 ng/ml in small-clawed males). These large abraded males have also larger reproductive organs, claws, and MOs than the large unabraded and the small males (Homola et al., 1991). Collectively, these data point towards reproductive and morphogenetic functions of MF in crustaceans (Sagi et al., 1993).

The nature of the MO inhibiting factor, tentatively called the MO-inhibiting hormone (MO-IH) has been explored by Laufer et al. (1986), Wainwright et al. (1996), and by Liu et al. (1997a,b). It is water soluble, heat stable and diffusible from the ES in culture. An ES extract will inhibit the synthesis of MF in an in vitro culture of juvenile female *L. emarginata*'s MO by 50–60%. The pigment dispersing hormone (PDH) inhibited MF synthesis by the MO of the crayfish, *Pro. clarkii* (Landau et al., 1989), and cGMP appeared to be a second messenger for the SG factor that produced an inhibition of MF synthesis by the MO of the lobster, *H. americanus* (Tsukimura et al., 1993). On the other hand, the red pigment concentrating hormone (RPCH) stimulates MF synthesis by MO of *Pro. clarkii* (Landau et al., 1989), a situation mimicked by the calcium ionophore A23187, which is interpreted as the effect of RPCH is mediated by the influx of Ca^{2+} into the MO, while in the presence of lanthanum (an ionic calcium channel blocker) in the culture medium, MF synthesis is strongly inhibited (Laufer et al., 1987c).

The culturing in vitro of ovary tissue of the shrimp, *Penaeus (Litopenaeus) vannamei*, in the presence of MF has resulted in a significant increase in the size of the oocytes. This can be interpreted as the involvement of MF in early events related to secondary vitellogenesis. MF has been reported to increase fecundity in cultured shrimp, *P. (Litopenaeus) vannamei* (Laufer, 1992; Laufer et al., 1997). The ovarian enhancement effect of MF in vivo may be due, indirectly, to stimulation of Y-organs to synthesize and secrete ecdysteroids that then accumulate in the ovaries.

7. Chromatophorotropins

Crustaceans have four types of chromatophores: erythrophores, melanophores, xanthophores, and leukophores. These cells are loaded with pigment granules that concen-

trate or disperse as ordered by specific chromatophorotropins. Fernlund and Josefsson (1972) isolated and characterized the first crustacean neuropeptide hormone from the shrimp *Pandalus borealis*. This was the red pigment concentrating hormone (RPCH) with the sequence pELNFSPGW–NH_2. The RPCH of crustaceans proved to be an analog of the adipokinetic hormone (AKH) of insects. While AKH is very variable in insects (Gäde, 1991), RPCH is invariable in all crustaceans investigated to date (Keller, 1992). In the shrimps *Pal. squilla* and *P. (Farfantepenaeus) aztecus*, RPCH showed pigment concentrating activity on leukophores as well as on erythrophores, while in the shrimp *Crangon crangon* it acted on leukophores, erythophores, and melanophores (Rao and Riehm, 1988a,b).

The site of synthesis of RPCH is the neurosecretory cells of the MTXO, but lately, immunoreactive RPCH-like substances have been found in the brain and thoracic ganglia of the crayfish *O. limosus* and the crab *C. maenas* (Mangerich et al., 1986) and in the stomatogastric nervous system of the crab *Can. borealis* (Nusbaum and Marder, 1988). This has prompted the hypothesis that RPCH, in addition to its direct role in promoting the movement of granules in erythrophores, may be acting as a neurotransmitter and neuromodulator in the nervous system of crustaceans.

In the shrimp, *S. ingentis*, Prestwich et al. (1991) demonstrated the presence in neural tissues of two proteins which reacted with photoaffinity analogs of the RPCH. This has been the first report of peptide hormone-binding proteins in an invertebrate and provides further evidence of a role for RPCH as a neurotransmitter in the CNS.

In 1976, Fernlund isolated and sequenced an octadecapeptide from the eyestalks of the shrimp *Pan. borealis* which had distal retinal pigment dispersing activity. It also had pigment dispersing activity in epidermal chromatophores and was named PDH (now α-PDH).

Desmoucelles-Carette et al. (1996) cloned the cDNAs encoding the precursors of PDH in the shrimp *P. (Litopenaeus) vannamei*, and found that they consist of a 22- or

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Alpha-type
Pab-PDH       NSGMINSILGIPRVMTEA-NH2
Paj-PDH-I     NSGMINSILGIPRVMTEA-NH2
Paj-PDH-II    NSGMINSILGIPKVMADA-NH2

Beta-type

Pej-PDH-I     NSELINSLLGIPKVMTDA-NH2
Pej-PDH-II    NSELINSLLGLPKFMIDA-NH2
Pea-PDH       NSELINSLLGIPKVMNDA-NH2
Paj-PDH       NSELINSLLGLPKVMTDA-NH2
Pev-PDH-I     NSELINSLLGIPKVMNDA-NH2
Pev-PDH-II    NSELINSLLGLPKVMNDA-NH2
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Fig. 5. Amino acid sequences of shrimp PDH peptides: Pab, *Pan. borealis* (Fernlund, 1976); Paj, *Pan. jordani* (Rao and Riehm, 1993); Pej, *P. (Marsupenaeus) japonicus* (Yang et al., 1999); Pea, *P. (Farfantepenaeus) aztecus* (Phillips et al., 1988). Identical or similar (in charge or hydrophobicity) residues have been united by dashes.

23-amino acid signal peptide, a 34-aminoacid PDH-Precursor Related Peptide (PPRP) and the 18-amino acid mature PDH. The deduced mature PDHs sequences were present in two variants differing only in a substitution of a Leu by an Ile in one of them. The signal peptide appears to be highly variable, but the PPRP has low variability among different species, which suggests that it may have an, as yet unknown, physiological function.

Recently, Yang et al. (1999) have characterized two PDHs from the shrimp *P. (Marsupenaeus) japonicus*. Both are octadecapeptides with a free amino-terminus and an amidated carboxyl-terminus. Their respective sequences are: Pej-PDH-I-NSELINSLL-GIPKVMTDA-NH_2 and Pej-PDH-II-NSELINSLLGLPKFMIDA-NH_2. These were designated as β-PDHs because of important differences with *P. borealis*: at position 3, Gly is found in α-PDH and Glu in β-PDHs (Fig. 5).

8. Other factors

Biogenic amines and peptide neuroregulators are known to modulate the release of some neuropeptide hormones from the SG (Lüschen et al., 1993). The hyperglycemic effects of serotonin (5-OH-tryptamine, 5-HT), epinephrine (E) and dopamine (DA), have been shown to be indirect, that is, through release of CHH from the SG, because it is suppressed in ES-ablated animals. In shrimps, DA was shown by Kuo et al. (1995) to produce hyperglycemia in intact *P. monodon* but not in bilaterally ES-ablated animals. DA stimulates the release of CHH mainly through D_1 receptors, because the effect of SKF38393 (a D_1 receptor agonist) was similar to the effect of DA, while SCH23390 (a specific D_1 receptor antagonist) depressed the response to DA. Rothe et al. (1991) have found that crustacean enkephalin inhibits the release of CHH in *C. maenas*.

The kinin peptide family, hitherto confined to insects, has been described lately by Nieto et al. (1998) in a crustacean. They have isolated and purified three myotropic neuropeptides from the CNS of *P. (Litopenaeus) vannamei* that belong to the tachykinin family. One of them, APSGFLGMR-NH_2 (Pev-tachykinin) is present, with some amino acid substitutions, in various vertebrates and invertebrate classes. The other two, ASFSPWG-NH_2 (Pev-kinin 1) and DFSAWA-NH_2 (Pev-kinin 2) are the first crustacean kinins. In insects, kinins are involved in regulation of diuresis in Malpighian tubules. Both Pev-kinins 1 and 2 were active in cricket tubules but whether they are involved in diuresis in crustaceans is not known. Pev-kinin 2 had myotropic activity when tested on the hindgut preparation of *Astacus leptodactylus*, but Pev-kinin 1 and Pev-tachykinin were inactive.

The presence of a pheromone in shrimps has been discussed by Takayanagi et al. (1986a,b) who observed that female shrimp *Paratya compressa* would delay ovarian development unless males were present, but maturation would take place if an extract of the male testis or vas deferens were added to the water.

Injections of progesterone and of 17-α-OH-progesterone are able to induce ovarian maturation in the shrimp *M. ensis* (Yano, 1985) and stimulate vitellogenin secretion in *P. (Marsupenaeus) japonicus* (Yano, 1987). Vertebrate-type steroids have been found by Quinitio et al. (1994) in *P. monodon*, where the levels of estradiol-17β and progesterone

in the hemolymph, ovaries and hepatopancreas were closely related to the stage of ovarian maturity. High levels of estrogen during vitellogenesis may suppress molting in this shrimp, while stimulating vitellogenin production. For an extensive review on vertebrate-type hormones in crustaceans, see Fingerman et al. (1993).

9. Conclusions

The study of shrimp endocrinology has seen an important advance in the characterization of SG neuropeptides. Some of these have been sequenced directly, and some indirectly through the corresponding cDNAs. The CHH–MIH–GIH family of neuropeptides in *P. (Marsupenaeus) japonicus* (Yang et al., 1997), and in *M. ensis* (Gu and Chan, 1998a,b) is composed of at least six members, most of which, surprisingly, have hyperglycemic activity, and at least one in each species has molt-inhibiting activity. Not surprisingly, some of these peptides are pleiotropic, like the hyperglycemic hormone of *P. (Marsupenaeus) japonicus*, that can inhibit protein and mRNA synthesis in ovarian fragments of *P. semisulcatus* (Khayat et al., 1998). Could this peptide be the equivalent of the vitellogenesis inhibiting hormone? The hyperglycemic hormone of the crab *L. emarginata* has MO inhibiting activity in the same species (Liu and Laufer, 1996), but it is not known if this effect is present in shrimps.

The studies of "vertebrate-type" hormones (like opioids, vasopressins, gastrin/cholecystokinin, calcitonin, substance P, eicosanoids and steroids) have been done mainly in lobsters, crabs, and crayfish (Fingerman et al., 1993) and there is need to confirm their presence and functions in shrimps. The interesting finding of circulating gastrin/cholecystokinin in post-prandial *P. (Marsupenaeus) japonicus* and in *P. (Litopenaeus) stylirostris* by Van Wormhoudt et al. (1989) suggests a possible role of these peptides in the regulation of crustacean digestive processes.

There have been intermittent reports of the presence of a vitellogenesis stimulating hormone (VSH) in the SG, in the brain, and in the thoracic ganglion of different crustaceans, but up to now it has not been characterized, and it should be one important aspect of shrimp endocrinology for future research.

In an attempt to isolate ecdysteroid and ecdysone inducible genes in shrimp, Chan (1998) cloned a *M. ensis* cDNA encoding a nuclear receptor superfamily member that is homologous to the insect ecdysone-inducible E75 receptor gene, which suggests that crustaceans and insects may share a similar regulatory mechanism for the control of molting at the molecular level.

The findings that have led to the conclusion that methyl farnesoate is a hormone in crustaceans, like specific binding proteins in hemolymph, inhibiting factors in the SG, highest circulating levels coinciding with maximal vitellogenesis, and specific esterases, have attracted the attention of many researchers that have used it to increase productivity in shrimp aquaculture, but the regulation of shrimp reproduction is multifactorial, based on the interaction of several endocrine organs and their products, and it is doubtful if a single molecular approach will offer an acceptable solution to this goal.

In order to increase the productivity of female shrimp broodstock in aquaculture, it is customary to ablate one ES, with the consequence of a steady decrease in the number of

nauplii per spawn and in their survival to postlarvae. The broodstock has to be substituted every two or three months in order to maintain an acceptable productivity. The ES ablation creates a sudden 50% decrease in the factors that originate in the SG with the corresponding metabolic consequences. It is not known if the remaining SG will hypertrophy to compensate for this loss. One important task for shrimp endocrinologists will be to substitute ES ablation with a more physiological hormonal treatment that will accomplish the same increase in productivity without the corresponding reproductive exhaustion.

The basics of shrimp endocrinology have begun to unravel, but there is need to increase our knowledge of the hormonal regulation of shrimp growth and reproduction at the molecular level. One important step in this direction would be to produce recombinant shrimp neuropeptide hormones that would permit large scale in vivo and in vitro studies of different parameters of metabolism.

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References

Adiyodi, R.G., 1985. Reproduction and its control. In: Bliss, D.E., Mantel, L.H. (Eds.), The Biology of Crustacea vol. 9 Academic Press, New York, pp. 147–215.

Aguilar, M.B., Quackenbush, L.S., Hunt, D.T., Shabanowitz, J., Huberman, A., 1992. Identification, purification and initial characterization of the vitellogenesis-inhibiting hormone from the Mexican crayfish *Procambarus bouvieri* (Ortmann). Comp. Biochem. Physiol. 102B, 491–498.

Aguilar, M.B., Soyez, D., Falchetto, R., Arnott, D., Shabanowitz, J., Hunt, D.F., Huberman, A., 1995. Amino acid sequence of the minor isomorph of the crustacean hyperglycemic hormone (CHH-II) of the Mexican crayfish *Procambarus bouvieri* (Ortmann): presence of a D-amino acid. Peptides 16, 1375–1383.

Aguilar, M.B., Falchetto, R., Shabanowitz, J., Hunt, D.F., Huberman, A., 1996. Complete primary structure of the molt-inhibiting hormone (MIH) of the Mexican crayfish *Procambarus bouvieri* (Ortmann). Peptides 17, 367–374.

Aguilar-Gaytán, R., Cerbón, M.A., Cevallos, M.A., Lizano, M., Huberman, A., 1997. Sequence of a cDNA encoding the molt-inhibiting hormone from the Mexican crayfish *Procambarus bouvieri* (Crustacea, Decapoda). Asia Pacific J. Mol. Biol. Biotechnol. 5, 51–55.

Blais, C., Sefiani, M., Toullec, J.-Y., Soyez, D., 1994. In vitro production of ecdysteroids by Y-organs of *Penaeus vannamei* (Crustacea, Decapoda): correlation with hemolymph titers. Invert. Reprod. Dev. 26, 3–11.

Bourguet, J.P., Exbrayat, J.M., Trilles, J.P., Vernet, G., 1977. Mise en evidence et description de l'organe Y chez *Penaeus japonicus* (Bate, 1881)(Crustacea Decapoda, Natantia). C.R. Acad. Sci. Paris 285, 977–980.

Chan, S.-M., 1998. Cloning of a shrimp *(Metapenaeus ensis)* cDNA encoding a nuclear receptor superfamily member: an insect homologue of E75 gene. FEBS Lett. 436, 395–400.

Chang, E.S., 1985. Hormonal control of molting in decapod crustacea. Am. Zool. 25, 179–185.

Chang, E.S., Prestwich, G.D., Bruce, M.J., 1990. Amino acid sequence of a peptide with both molt-inhibiting and hyperglycemic activities in the lobster, *Homarus americanus*. Biochem. Biophys. Res. Commun. 171, 818–826.

Chang, E.S., 1992. Endocrinology. In: Fast, A.W., Lester, L.J. (Eds.), Marine Shrimp Culture: Principles and Practices. Elsevier, Amsterdam, pp. 53–91, Chapter 4.

Chang, E.S., Hertz, W.A., Prestwich, G.D., 1992. Reproductive endocrinology of the shrimp *Sicyonia ingentis*: steroid, peptide, and terpenoid hormones. NOAA Tech. Rep. NMFS 106, 1–6.

Chang, E.S., 1997. Chemistry of crustacean hormones that regulate growth and reproduction. In: Fingerman, M., Nagabhushanam, R., Thompson, M.-F. (Eds.), Recent Advances in Marine Biotechnology. Endocrinology and Reproduction vol. 1 Oxford and IBH, New Delhi, pp. 163–178.

Choy, S.C., 1987. Growth and reproduction of eyestalk ablated *Penaeus canaliculatus* (Olivier, 1811)(Crustacea: Penaeidae). J. Exp. Mar. Biol. Ecol. 112, 93–107.

Dall, W., 1965. Studies on the physiology of a shrimp, *Metapenaeus* sp. (Crustacea: Decapoda: Penaeidae). II. Endocrines and control of molting. Aust. J. Mar. Freshwater Res. 16, 1–12.

De Kleijn, D.P.V., Coenen, T., Laverdure, A.M., Tensen, C.P., Van Herp, F., 1992. Localization of messenger RNAs encoding crustacean hyperglycemic hormone and gonad inhibiting hormone in the X-organ sinus gland complex of the lobster *Homarus americanus*. Neuroscience 51, 121–128.

De Kleijn, D.P.V., De Leuw, E.P.H., Van den Berg, M.G., Martens, G.J.M., Van Herp, F., 1995. Cloning and expression of two mRNAs encoding structurally different crustacean hyperglycemic hormone precursors in the lobster *Homarus americanus*. Biochim. Biophys. Acta 1260, 62–66.

De Kleijn, D.P.V., Van Herp, F., 1995. Molecular biology of neurohormone precursors in the eyestalk of crustacea. Review. Comp. Biochem. Physiol. B 116, 573–579.

De Kleijn, D.P.V., Van Herp, F., 1998. Involvement of the hyperglycemic neurohormone family in the control of reproduction in decapod crustaceans. Invert. Reprod. Dev. 33, 263–272.

Desmoucelles-Carette, C., Sellos, D., Van Wormhoudt, A., 1996. Molecular cloning of the precursors of pigment dispersing hormone in crustaceans. Biochem. Biophys. Res. Commun. 221, 739–743.

Fernlund, P., Josefsson, L., 1972. Crustacean color change hormone: amino acid sequence and chemical synthesis. Science 177, 173–175.

Fernlund, P., 1976. Structure of a light-adapting hormone from the shrimp *Pandalus borealis*. Biochim. Biophys. Acta 439, 17–25.

Fingerman, M., Nagabhushanam, R., Sarojini, R., 1993. Vertebrate-type hormones in crustaceans: localization, identification and functional significance. Zool. Sci. 10, 13–29.

Fingerman, M., 1995. Endocrine mechanisms in crayfish with emphasis on reproduction and neurotransmitter regulation of hormone release. Am. Zool. 35, 68–78.

Fingerman, M., 1997. Crustacean endocrinology: a retrospective, prospective and introspective analysis. Physiol. Zool. 70, 257–269.

Gäde, G., 1991. The adipokinetic neuropeptide of Mantodea: sequence elucidation and evolutionary relationships. Biol. Chem. Hoppe-Seyler 372, 193–201.

Goy, M.F., 1990. Activation of membrane guanylate cyclase by an invertebrate peptide hormone. J. Biol. Chem. 265, 20220–20227.

Gu, P.-L., Chan, S.-M., 1998a. Cloning of a cDNA encoding a putative molt-inhibiting hormone from the eyestalk of the sand shrimp, *Metapenaeus ensis*. Mol. Mar. Biol. Biotech. 7, 214–220.

Gu, P.-L., Chan, S.-M., 1998b. The shrimp hyperglycemic hormone-like neuropeptide is encoded by multiple copies of genes arranged in a cluster. FEBS Lett. 441, 397–403.

Homola, E., Sagi, A., Laufer, H., 1991. Relationship of claw form and exoskeleton condition to reproductive system size and methyl farnesoate in the male spider crab, *Libinia emarginata*. Invert. Reprod. Dev. 20, 219–225.

Huberman, A., Aguilar, M.B., 1998. D-amino acids in crustacean hyperglycemic neurohormones. In: Jollès, P. (Ed.), D-amino acids in sequences of secreted peptides of multicellular organisms. Birkhäuser Verlag, Basel, pp. 73–83.

Keller, R., Sedlmeier, D., 1998. A metabolic hormone in crustaceans: the hyperglycemic neuropeptide. In: Laufer, H., Downer, R.G.H. (Eds.), Endocrinology of selected invertebrate types vol. II A.R. Liss, New York, pp. 315–326.

Keller, R., 1992. Crustacean neuropeptides: structure, functions and comparative aspects. Experientia 48, 439–448.

Khayat, M., Yang, W.-J., Aida, K., Nagasawa, H., Tietz, A., Funkenstein, B., Lubzens, E., 1998. Hyperglycemic hormones inhibit protein and mRNA synthesis in in vitro-incubated ovarian fragments of the marine shrimp *Penaeus semisulcatus*. Gen. Comp. Endocrinol. 110, 307–318.

Klein, J.M., De Kleijn, D.P.V., Huenemeyer, G., Keller, R., Weidemann, W., 1993. Demonstration of the

cellular expression of genes encoding molt-inhibiting hormone and crustacean hyperglycemic hormone in the eyestalk of the shore crab *Carcinus maenas*. Cell Tissue Res. 274, 515–519.

Knowlton, R.E., 1994. Effects of larval eyestalk extirpation on morphogenesis and molting in the snapping shrimp *Alpheus heterochaelis*. J. Exp. Zool. 270, 162–174.

Kummer, G., Keller, R., 1993. High affinity binding of crustacean hyperglycemic hormone (CHH) to hepatopancreatic plasma membranes of the crab *Carcinus maenas* and the crayfish *Orconectes limosus*. Peptides 14, 103–108.

Kuo, C.M., Hsu, C.R., Lin, C.Y., 1995. Hyperglycaemic effects of dopamine in tiger shrimp, *Penaeus monodon*. Aquaculture 135, 161–172.

Lachaise, F., Le Roux, A., Hubert, M., Lafont, R., 1993. The molting gland of crustaceans: localization, activity and endocrine control (a review). J. Crust. Biol. 13, 198–234.

Landau, M., Laufer, H., Homola, E., 1989. Control of methyl farnesoate synthesis in the mandibular organ of the crayfish *Procambarus clarkii*: evidence for neurohormone with dual functions. Invert. Reprod. Dev. 16, 165–168.

Laufer, H., Landau, M., Borst, D., Homola, E., 1986. The synthesis and regulation of methyl farnesoate, a new juvenile hormone for crustacean reproduction. In: Porchet, M., Andries, J.C., Dhianaut, A. (Eds.), Advances in Invertebrate Reproduction 4. Elsevier Science, Amsterdam, pp. 135–143.

Laufer, H., Borst, D., Baker, F.C., Carrasco, C., Sinkus, M., Reuter, C.C., Tsai, L.W., Schooley, D.A., 1987a. Identification of a juvenile hormone-like compound in a crustacean. Science 235, 202–205.

Laufer, H., Landau, M., Borst, D.W., Homola, E., 1987b. Methyl farnesoate: its site of synthesis and regulation of secretion in a juvenile crustacean. Insect Biochem. 17, 1123–1127.

Laufer, H., Homola, E., Landau, M., 1987c. Control of methyl farnesoate synthesis in crustacean mandibular organs. Am. Zool. 27, 69A.

Laufer, H., Sagi, A., 1991. Juvenile hormone-like compounds and reproduction in male and female crustaceans: with implications for aquaculture. Bull. Inst. Zool. Acad. Sinica 16, 541–551.

Laufer, H. 1992. Method for increasing crustacean larval production. United States Patent 5, 161, 481.

Laufer, H., Ahl, J.S.B., Sagi, A., 1993. The role of juvenile hormones in crustacean reproduction. Am. Zool. 33, 365–374.

Laufer, H., Paddon, J., Paddon, M., 1997. A hormone enhancing larval production in the Pacific white shrimp *Penaeus vannamei*. In: Alston, D.E., Green, B.W., Clifford, H.C. (Eds.), IV Symposium on Aquaculture in Central America: Focusing on shrimp and tilapia. The Latin American Chapter of the World Aquaculture Society, Tegucigalpa, Honduras, pp. 161–162.

Le Roux, A., 1968. Description d'organes mandibulaires nouveaux chez les crustacés decapodes. C.R. Hebd. Acad. Sci. Ser. D, Sci. Nat. 266, 1414–1417.

Li, H., Borst, D.W., 1991. Characterization of methyl farnesoate binding protein in hemolymph of *Libinia emarginata*. Gen. Comp. Endocrinol. 81, 335–342.

Liu, L., Laufer, H., 1996. Isolation and characterization of sinus gland neuropeptides with both mandibular organ inhibiting and hyperglycemic effects from the spider crab *Libinia emarginata*. Arch. Insect Biochem. Physiol. 32, 375–385.

Liu, L., Laufer, H., Wang, Y., Hayes, T., 1997a. A neurohormone regulating both methyl farnesoate synthesis and glucose metabolism in a crustacean. Biochem. Biophys. Res. Commun. 237, 694–701.

Liu, L., Laufer, H., Gogarte, P.J., Wang, M.H., 1997b. cDNA cloning of a mandibular organ inhibiting hormone from the spider crab *Libinia emarginata*. Invert. Neurosci. 3, 199–204.

Lüschen, W.S., Willig, A., Jaros, P.P., 1993. The role of biogenic amines in the control of blood glucose level in the decapod crustacean, *Carcinus maenas* L. Comp. Biochem. Physiol. C 105, 291–296.

Mattson, M.P., Spaziani, E., 1985. Characterization of molt-inhibiting hormone (MIH) action on crustacean Y-organ segments and dispersed cells in culture and a bioassay for MIH activity. J. Exp. Zool. 236, 93–101.

Mellon, DeF. Jr., Greer, E., 1987. Induction of precocius molting and claw transformation in alpheid shrimps by exogenous 20-hydroxyecdysone. Biol. Bull. 172, 350–356.

Mangerich, S., Keller, R., Dircksen, H., 1986. Immunocytochemical identification of structures containing putative red pigment concentrating hormone in two species of decapod crustaceans. Cell Tissue Res. 245, 377–386.

Meredith, J., Ring, M., Macins, A., Marshall, J., Cheng, N.N., Theilmann, D., DiBrock, W.H., Phillips, J.E.,

1996. Locust ion transport peptide (ITP): primary structure, cDNA and expression in a baculovirus system. J. Exp. Zool. 199, 1053–1061.

Nieto, J., Veelaert, D., Derua, R., Waelkens, E., Cerstiaens, A., Coast, G., Devreese, B., Van Beeumen, J., Calderón, J., De Loof, A., Schoofs, L., 1998. Identification of one tachykinin- and two kinin-related peptides in the brain of the white shrimp, *Penaeus vannamei*. Biochem. Biophys. Res. Commun. 248, 406–411.

Nusbaum, M.P., Marder, E., 1988. A neuronal role for a crustacean red pigment concentrating hormone-like peptide: neuromodulation of the pyloric rhythm in the crab, *Cancer borealis*. J. Exp. Biol. 135, 163–181.

Ohira, T., Watanabe, T., Nagasawa, H., Aida, K., 1997. Molecular cloning of a molt-inhibiting hormone cDNA from the kuruma prawn *Penaeus japonicus*. Zool. Sci. 14, 785–789.

Panouse, J.B., 1943. Influence de l'ablation de pedoncle oculaire sur la croissance de l'ovaire chez la crevette *Leander serratus*. C.R. Acad. Sci. Paris 217, 535–555.

Panouse, J.B., 1944. L'action de la glande du sinus sur l'ovaire chez la crevette *Leander*. C.R. Acad. Sci. Paris 218, 293–294.

Pérez Farfante, I., Kensley, B., 1997. Penaeoid and sergestoid shrimps and prawns of the world, Keys and diagnoses for the families and genera. Universal Book Services, Leiden, The Netherlands.

Phillips, J.E., Meredith, J., Audsley, N., Richardson, N., Macins, A., Ring, M., 1998. Locust ion transport peptide (ITP): a putative hormone controlling water and ionic balance in terrestrial insects. Am. Zool. 38, 461–470.

Phillips, J.M., Rao, R.K., Riehm, J.P., Morgan, W.T., 1988. Isolation and characterization of a pigment-dispersing hormone from the shrimp *Penaeus aztecus*. Soc. Neurosci. 14, 534, Abstr.

Prestwich, G.D., Bruce, M.J., Ujváry, I., Chang, E.S., 1990. Binding proteins for methyl farnesoate in lobster tissues: detection by photoaffinity labeling. Gen. Comp. Endocrinol. 80, 232–237.

Prestwich, G.D., Bruce, M.J., Chang, E.S., 1991. Binding proteins for a peptide hormone in the shrimp, *Sicyonia ingentis*: evidence from photoaffinity labeling with red pigment concentrating hormone analogs. Gen. Comp. Endocrinol. 83, 473–480.

Quackenbush, L.S., 1986. Crustacean endocrinology, a review. Can. J. Fish. Aquat. Sci. 43, 2271–2282.

Quackenbush, L.S., 1989. Vitellogenesis in the shrimp *Penaeus vannamei*: in vitro studies of the isolated hepatopancreas and ovary. Comp. Biochem. Physiol. 94B, 253–261.

Quackenbush, L.S., Keeley, L.L., 1988. Regulation of vitellogenesis in the fiddler crab, *Uca pugilator*. Biol. Bull. (Woods Hole) 175, 321–331.

Quinitio, E.T., Hara, A., Yamauchi, K., Nakao, S., 1994. Changes in the steroid hormone and vitellogenin levels during the gametogenic cycle of the giant tiger shrimp, *Penaeus monodon*. Comp. Biochem. Physiol. 109C, 21–26.

Rao, K.R., Riehm, J.P., 1988a. Chemistry of crustacean chromatophorotropins. In: Bagnara, J.T. (Ed.), Advances in Pigment Cell Research. A.R. Liss, New York, pp. 407–422.

Rao, K.R., Riehm, J.P., 1988b. Pigment-dispersing hormones: a novel family of neuropeptides from arthropods. Peptides 9 (Suppl. 1), 153–159.

Rao, K.R., Riehm, J.P., 1993. The melanotropic peptides. Ann. N.Y. Acad. Sci. 680, 78–88.

Rothe, H., Lüschen, W., Asken, A., Willig, A., Jaros, P.P., 1991. Purified crustacean enkephalin inhibits release of hyperglycemic hormone in the crab *Carcinus maenas* L. Comp. Biochem. Physiol. 99C, 57–62.

Rotllant, G., De Kleijn, D.P.V., Charmantier-Daures, M., Charmantier, G., Van Herp, F., 1993. Localization of crustacean hyperglycemic hormone (CHH) and gonad-inhibiting hormone (GIH) in the eyestalk of *Homarus gammarus* larvae by immunocytochemistry and in situ hybridization. Cell Tissue Res. 271, 507–512.

Sagi, A., Homola, E., Laufer, H., 1993. Distinct reproductive types of male spider crabs *Libinia emarginata* differ in circulating and synthesizing methyl farnesoate. Biol. Bull. 185, 168–173.

Santos, E.A., Nery, L.E.M., Keller, R., Gonçalves, A.A., 1997. Evidence for the involvement of the crustacean hyperglycemic hormone in the regulation of lipid metabolism. Physiol. Zool. 70, 415–420.

Sefiani, M., Le Caer, J.-P., Soyez, D., 1996. Characterization of hyperglycemic and molt-inhibiting activity from sinus glands of the penaeid shrimp *Penaeus vannamei*. Gen. Comp. Endocrinol. 103, 41–53.

Shih, T.-W., Suzuki, Y., Nagasawa, H., Aida, K., 1998. Immunohistochemical identification of hyperglycemic hormone- and molt-inhibiting hormone-producing cells in the eyestalk of the kuruma prawn, *Penaeus japonicus*. Zool. Sci. 15, 389–397.

Skinner, D.M., 1985. Molting and regeneration. In: Bliss, D.E., Mantel, L.H. (Eds.), The Biology of Crustacea vol. 9 Academic Press, New York, pp. 43–146.

Soyez, D., Van Deijnen, J.E., Martin, M., 1987. Isolation and characterization of a vitellogenesis-inhibiting factor from sinus gland of the lobster, *Homarus americanus*. J. Exp. Zool. 244, 479–484.

Soyez, D., Le Caer, J.P., Noel, P.Y., Rossier, J., 1991. Primary structure of two isoforms of the vitellogenesis inhibiting hormone from the lobster *Homarus americanus*. Neuropeptides 20, 25–32.

Soyez, D., Van Herp, F., Rossier, J., Le Caer, J.P., Tensen, C.P., Lafont, R., 1994. Evidence for a conformational polymorphism of invertebrate neurohormones. D-amino acid residue in crustacean hyperglycemic peptides. J. Biol. Chem. 269, 18295–18298.

Spaziani, E., Mattson, M.P., Rudolph, P.H., 1994. Regulation of crustacean molt-inhibiting hormone. Perspect. Comp. Endocrinol., 243–250.

Spindler, K.-D., Dinan, L., Londershausen, M., 1984. On the mode of action of ecdysteroids in crustaceans. In: Hoffmann, J., Porchet, M. (Eds.), Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones. Springer Verlag, Berlin, pp. 255–264.

Spindler, K.-D., Van Wormhoudt, A., Sellos, D., Spindler-Barth, M., 1987. Ecdysteroid levels during embryogenesis in the shrimp, *Palaemon serratus* (Crustacea, Decapoda): Quantitative and qualitative changes. Gen. Comp. Endocrinol. 66, 116–122.

Sun, P.S., 1994. Molecular cloning and sequence analysis of a cDNA encoding a molt-inhibiting hormone-like neuropeptide from the white shrimp *Penaeus vannamei*. Mol. Mar. Biol. Biotechnol. 3, 1–6.

Sun, P.S., 1995. Expresion of the molt-inhibiting hormone-like gene in the eyestalk and brain of the white shrimp *Penaeus vannamei*. Mol. Mar. Biol. Biotechnol. 4, 262–268.

Takác, P., Ahl, J.S.B., Laufer, H., 1998. Methyl farnesoate binding proteins in tissues of the spider crab, *Libinia emarginata*. Comp. Biochem. Physiol. 120B, 769–775.

Takayanagi, H., Yamamoto, Y., Takeda, N., 1986a. An ovary stimulating factor in the shrimp, *Paratya compressa*. J. Exp. Zool. 240, 203–209.

Takayanagi, H., Yamamoto, Y., Takeda, N., 1986b. Ovary-stimulating pheromone in the freshwater shrimp, *Paratya compressa*. J. Exp. Zool. 240, 397–400.

Tsukimura, B., Kamemoto, F.I., Borst, D.W., 1993. Cyclic nucleotide regulation of methyl farnesoate synthesis by the mandibular organ of the lobster, *Homarus americanus*. J. Exp. Zool. 265, 427–431.

Van Wormhoudt, A., Favrel, P., Guillaume, J., 1989. Gastrin/cholecystokinin-like post-prandial variations: quantitative and qualitative changes in the hemolymph of penaeids (Crustacea Decapoda). J. Comp. Physiol. B 159, 269–273.

Wainwright, G., Webster, S.G., Wilkinson, M.C., Chung, J.S., 1996. Structure and significance of mandibular organ-inhibiting hormone in the crab, *Cancer pagurus*. Involvement in multihormonal regulation of growth and reproduction. J. Biol. Chem. 271, 12749–12754.

Webster, S.G., 1993. High-affinity binding of putative moult-inhibiting hormone (MIH) and crustacean hyperglycaemic hormone (CHH) to membrane bound receptors on the Y-organ of the shore crab *Carcinus maenas*. Proc. R. Soc. London B 251, 53–59.

Yang, W.-J., Aida, K., Nagasawa, H., 1995. Amino acid sequences of a hyperglycemic hormone and its related peptides from the kuruma prawn, *Penaeus japonicus*. Aquaculture 135, 205–212.

Yang, W.-J., Aida, K., Terauchi, A., Sonobe, H., Nagasawa, H., 1996. Amino acid sequence of a peptide with molt-inhibiting activity from the kuruma prawn *Penaeus japonicus*. Peptides 17, 197–202.

Yang, W.-J., Aida, K., Nagasawa, H., 1997. Amino acid sequences and activities of multiple hyperglycemic hormones from the kuruma prawn, *Penaeus japonicus*. Peptides 18, 479–485.

Yang, W.-J., Aida, K., Nagasawa, H., 1999. Characterization of chromatophorotropic neuropeptides from the kuruma prawn *Penaeus japonicus*. Gen. Comp. Endocrinol., (in press).

Yano, I., 1985. Induced ovarian maturation and spawning in greasyback shrimp *Metapenaeus ensis*, by progesterone. Aquaculture 47, 223–229.

Yano, I., 1987. Effect of 17-α-OH-progesterone on vitellogenin secretion in kuruma prawn, *Penaeus japonicus*. Aquaculture 61, 46–57.

Yasuda, A., Yasuda, Y., Fujita, T., Naya, Y., 1994. Characterization of crustacean hyperglycemic hormone from the crayfish (*Procambarus clarkii*): multiplicity of molecular forms by stereoinversion and diverse functions. Gen. Comp. Endocrinol. 95, 387–398.