Crustacean neuropeptides: Structures, functions and comparative aspects

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Abstract. In this article, an attempt is made to review the presently known, completely identified crustacean neuropeptides with regard to structure, function and distribution. Probably the most important progress has been made in the elucidation of a novel family of large peptides from the X-organ-sinus gland system which includes crustacean hyperglycemic hormone (CHH), putative molt-inhibiting hormone (MIH) and vitellogenesis (= gonad)-inhibiting hormone (VIH). These peptides have so far only been found in crustaceans. Renewed interest in the neurohemal pericardial organs has led to the identification of a number of cardioactive/myotropic neuropeptides, some of them unique to crustaceans. Important contributions have been made by immunocytochemical mapping of peptidergic neurons in the nervous system, which has provided evidence for a multiple role of several neuropeptides as neurohormones on the one hand and as local transmitters or modulators on the other. This has been corroborated by physiological studies. The long-known chromatophore-regulating hormones, red pigment concentrating hormone (RPCH) and pigment-dispending hormone (PDH), have been placed in a broader perspective by the demonstration of an additional role as local neuromodulators. The scope of crustacean neuropeptide research has thus been broadened considerably during the last years.
Key words. Crustaceans; neuropeptides; hormones; neurosecretion; neuromodulators.

Introduction

Historically, studies on crustaceans have contributed considerably to the development of the concept of neurosecretion and neuroendocrine control. Important early discoveries were those of Koller, Perkins and Brown between 1928 and 1933. They demonstrated that chromatophore control in the shrimp was caused by a substance originating in the nervous system[7,30,48]. In 1972, this substance, red pigment concentrating hormone (RPCH), was isolated and sequenced by Fernlund and Josefsson[17]. This was the first invertebrate neuropeptide whose structure was fully elucidated. Other early landmark discoveries include the neuroendocrine basis of light adaptation of the compound eye[28], blood sugar regulation[1], molt inhibition[8] and heart regulation[2,3]. Subsequently, many other physiological effects of 'factors' from the nervous system became known. Crustaceans also proved to be useful models for morphological studies on peptidergic neurosecretory structures. In this context, the description of the sinus gland in the eyestalk, a classical neurohemal organ, by Hanström in 1937[22] and recognition of the pericardial organs as neurohormal structures by Alexandrowicz in 1953[2] and Alexandrowicz and Carlisle in 1953[3] should be mentioned.

In this article, a review of the current state of knowledge concerning completely identified crustacean neuropeptides will be presented. These peptides account for a number of physiological effects that have been known for a long time. However, there can be little doubt that many more neuropeptides await complete characterization. It can be predicted that crustaceans will continue to yield important contributions to neuropeptide research. To the comparative endocrinologist, they offer some interesting specific problems not encountered in other invertebrates, e.g. chromatophore control, inhibitory control of molting, sexual differentiation and specific mechanisms of hydromineral regulation in relation to their different habitats (sea-/freshwater, terrestrial). Is this situation reflected in the occurrence of crustacean-specific neuropeptides? Certain peptides, e.g. crustacean hyperglycemic hormone (CHH), molt-inhibiting hormone (MIH) and vitellogenesis-inhibiting hormone (VIH), have thus far been found only in crustaceans, but further research is likely to show that they occur in other invertebrate groups as well. Other peptides, e.g. pigment-dispersing hormone (PDH) and crustacean cardioactive peptide (CCAP), were first described in crustaceans but have recently been found also in insects. Still others were originally found in insects, e.g., proctolin, or are members of widely distributed peptide families, e.g. FaRPs, enkephalins. Thus, research on crustaceans does not only yield results to explain physiological regulation in this particular group, but also contributes to our knowledge concerning the distribution of neuropeptides and peptide families among invertebrates in general, and perhaps most important, points to versatility and multiple functions of neuropeptides as hormones, transmitters and modulators.

The CHH/MIH/VIH peptide family

This family includes neuropeptides which are synthesized by large perikarya in the so-called medulla terminalis X-organ (XO) in the eyestalk of decapod crustaceans or in a corresponding position in the protocerebrum of isopods. They are transported via an axonal tract to the principal neurohemal organ, the sinus gland (SG), where they are stored and released. This association with a

typical neurosecretory pathway indicates that they act as blood-borne neurohormones. In contrast to other neuropeptides, they seem to be confined to this eyestalk site and there is little evidence that they have an additional role as neurotransmitters and/or modulators elsewhere in the nervous system. The prototype peptide is the crustacean hyperglycemic hormone (CHH) of the shore crab *Carcinus maenas*, whose structure was the first to be elucidated[26]. Its complete prohormone structure has also been deduced by cDNA cloning[78]. This peptide is, obviously, the 'diabetogenic' factor found in extracts of brachyuran eyestalk 45 years ago[1]. In figure 1, *Carcinus*-CHH is shown together with two more recently elucidated homologous peptides from the crayfish, *Orconectes limosus*[25] and the lobster, *Homarus americanus*[9], the latter having been described as a molt-inhibiting peptide which also possesses hyperglycemic activity. The sequence similarity shows that these hormones constitute an authentic peptide family across species boundaries. Sequence homology is 81% between *Orconectes limosus*- and *Homarus*-MIH/CHH and 61% between *Carcinus*- and *Orconectes*-CHH. The blocked termini (N-terminal pGlu and C-terminal Val-NH_2) are identical, as are chain length and positions of Cys-residues. Assignment of disulfide bonds has been carried out in *Carcinus*-CHH only[26] and it is assumed that the same arrangement applies to the other CHHs. Recently, a CCH of an isopod species, *Armadillidium vulgare*, has been sequenced and found to be very similar to the decapod hormones (G. Martin, personal communication). It is of considerable interest that sequence differences confer striking limitations on interspecific hyperglycemic activity. *Carcinus*-CHH is, e.g., almost ineffective in *Orconectes* while the same dose of its own hormone produces drastic hyperglycemic, and vice versa[27]. The receptor in *Orconectes* does not seem to bind the brachyuran hormone effectively, and vice versa. The *Homarus*-CHH, displaying a higher degree of homology, is quite effective in *Orconectes*.

These peptides constitute a family not only across species boundaries, but also within single species. It has long been noted that several different peptide forms may be obtained by HPLC-separation of SG-extracts from single species. In *Homarus*, up to four fractions with hyperglycemic activity have been found[63,72]. The structural basis of such 'isoforms' has recently been elucidated for two *Homarus* CHHs, A and B[73]. CHH A is identical to the previously reported MIH/CHH of *Homarus americanus*[9]. CHH B differs in 7 positions (sequence homology 90%). These results have been obtained by isolation of two cDNA clones, using the polymerase chain reaction with degenerated oligonucleotides which were derived from partial amino acid sequences. They suggest the expression of at least two CHH genes in *Homarus americanus*. However, it is felt that allelic polymorphism in the lobster population cannot be ruled out, since RNA pooled from a larger number of individuals has been used for cDNA synthesis in this study[73].

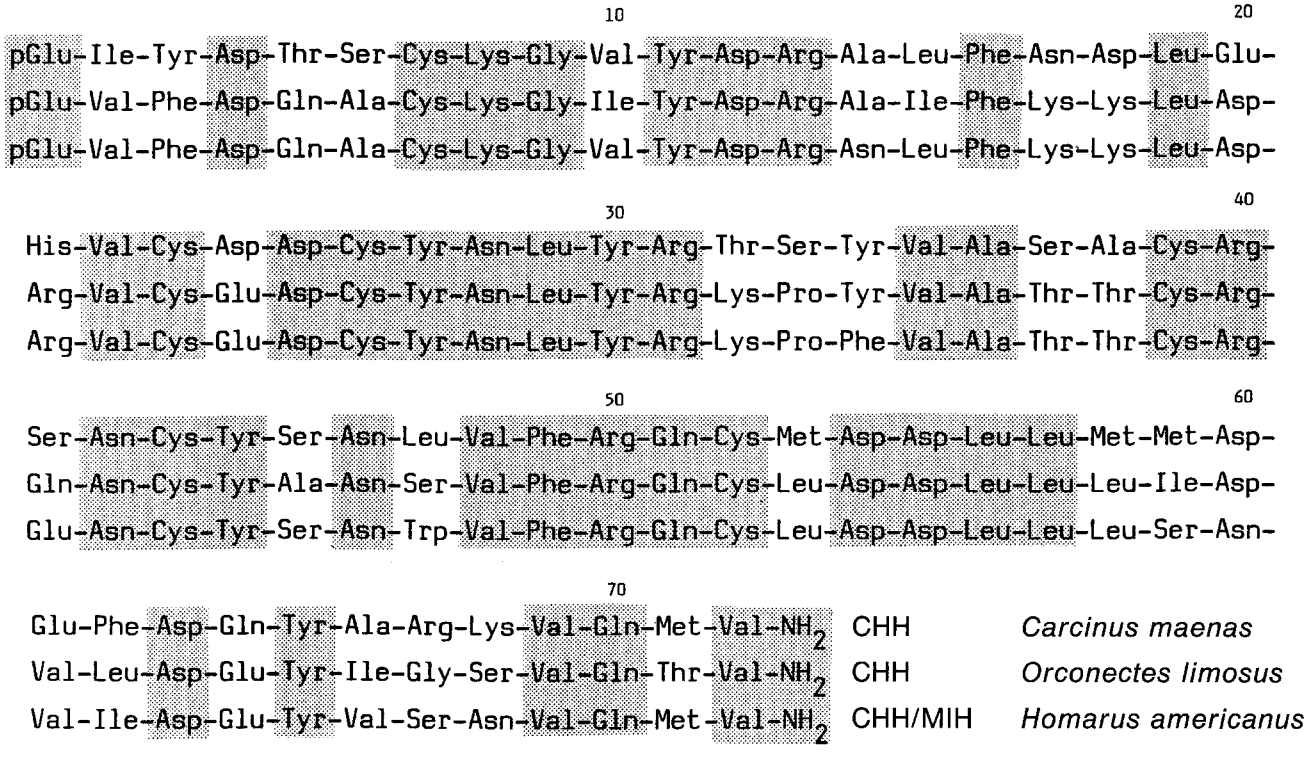

Figure 1. Crustacean hyperglycemic hormones (CHHs) from three decapod species. References: *Carcinus m.*[26]; *Orconectes l.*[25]; *Homarus a.*[9]. The latter was also named MIH due to molt-inhibiting activity. The C-terminal Val-NH_2 in the *Homarus*- CHH/MIH, missing in the original structure[9] was added according to recent data[73]. Conserved sequences are shaded.

Attempts at the isolation and characterization of a molt-inhibiting hormone (MIH) from the XO-SG-system of *Carcinus maenas*, based on a bioassay in which the inhibition of ecdysteroid synthesis in Y-organs in organ culture was measured, have previously led to the conclusion that this neuropeptide must be related to CHH[77]. This has been confirmed by the recent complete sequencing of MIH[76]. Appropriately, it has been designated "putative" MIH as its function as a physiological molt regulator in vivo has not yet been demonstrated. The alignment with CHH from the same species (fig. 2) clearly shows that it is a member of the CHH-family, although the relationship is not as close as between different CHHs. It differs in chain length (78 vs 72 residues) and both termini are unblocked. Sequence homology is only 28%, and it is noteworthy that identical positions are mostly single and dispersed, not occurring in contiguous, conserved partial sequences as observed between different CHHs (fig. 1). To align the Cys-residues, a gap was left between residues 10 and 11 in the CHH sequence, suggesting a deletion in the CHH gene. I am aware that this is highly questionable, since two N-terminal residues, Asp and Ile, are shifted out of alignment. Elucidation of the real relationship will only be possible by gene or cDNA cloning. A strikingly similar relationship between two XO-SG-peptides is revealed by the recent sequencing of vitellogenesis-inhibiting hormone (VIH) from *Homarus americanus*[62]. Alignment of the sequence with the CHH (CHH A according to Tensen et al.[73]) from *Homarus* resembles the relationship between *Carcinus* CHH and MIH (fig. 2). Similarly, VIH is longer, has unblocked termini, and the sequence homology is only 28%, consisting mostly of single, dispersed identical positions. Here a better case can be made for the insertion of a gap in the CHH sequence to align the Cys-residues, since the residues Gly^9 and Val^{10} in CHH would otherwise not be aligned. In this case, maximal homology is achieved by the gap. This may reinforce the idea that the same situation applies to the *Carcinus* peptides.

Alignment of *Carcinus* MIH and *Homarus* VIH (fig. 2) clearly shows that both peptides are more closely related to each other (48% homology) than either is related to the CHH from the same species. Thus, we are dealing here with a distinct type of peptide, as shown by similarity across species boundaries, that has diverged considerably from its intraspecific sister peptide, the CHH.

A database search (GenBank and NERF) revealed no homology of the sequences of the members of the CHH/MIH/VIH family with that of any other known peptide. Thus far, they seem to be unique to the Crustacea.

Observed physiological effects of peptides of this family include hyperglycemia, inhibition of ecdysteroid synthesis, inhibition of vitellogenin synthesis (= gonad inhibition) and a secretagogue, digestive enzyme releasing action on the hepatopancreas[57]. Not surprisingly, in view of the sequence homologies, overlapping biological effects have been observed. A case in point is the MIH of *Homarus americanus* which also causes hyperglycemia[9].

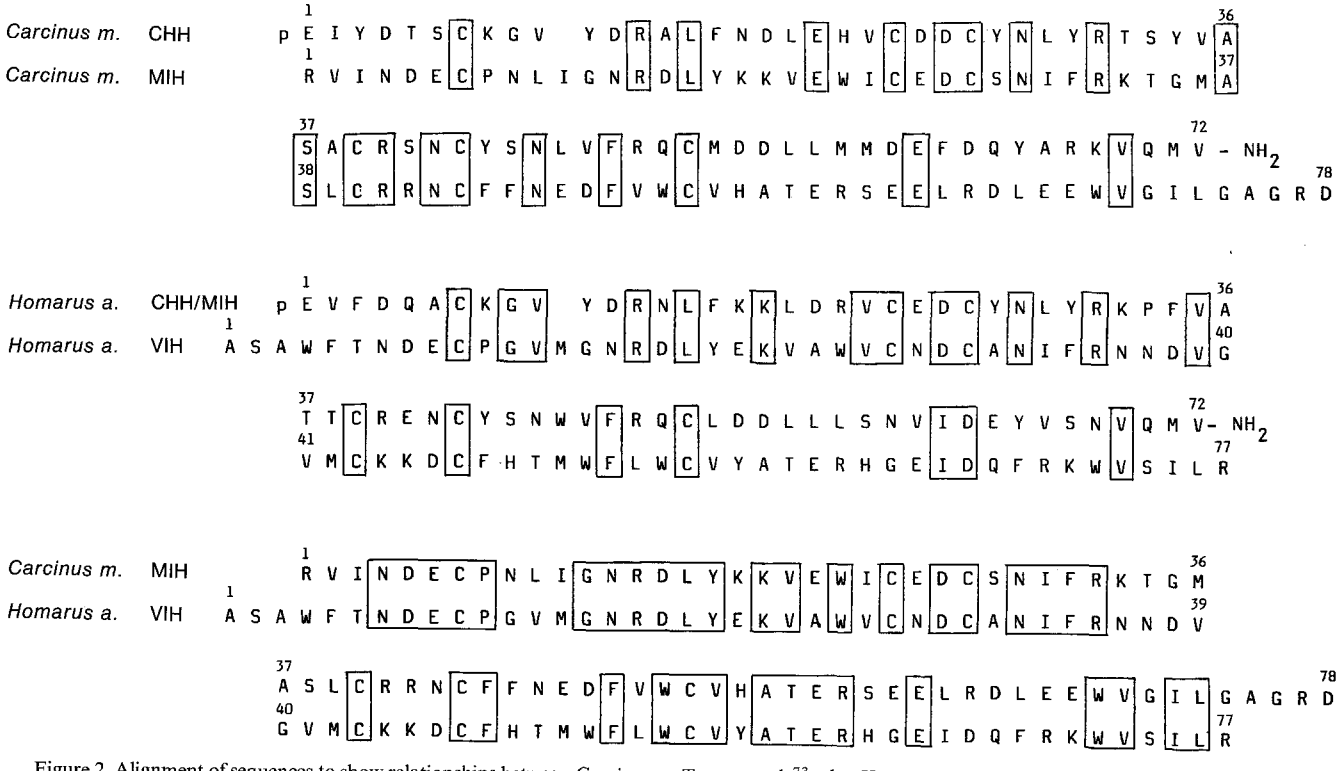

Figure 2. Alignment of sequences to show relationships between *Carcinus* CHH[26] and MIH[76], *Homarus* CHH/MIH[9] and vitellogenesis-inhibiting hormone (VIH)[62] and *Carcinus* MIH and *Homarus* VIH. According to Tensen et al.[73], the *Homarus* CHH shown here is the isoform A. The isoform B differs in seven residues[73]. Identical positions are boxed.

However, the MIH of *Carcinus* is not hyperglycemic in brachyurans[77], but the CHH of this species does inhibit ecdysteroid synthesis in isolated Y-organs, albeit with much lower potency than MIH. Among the peptides from the SG of *Homarus* some display overlapping hyperglycemic and vitellogenesis (= gonad)-inhibiting activity[72], but the typical VIH (fig. 2) appears to be devoid of CHH activity[61].

In conclusion, there is still considerable uncertainty concerning the primary physiological function of the different peptides. To clarify the situation, we need more systematic, comparative studies on the relative potencies of fully identified peptides in a range of standardized bioassays.

Cardioactive and myotropic peptides

The search for cardioactive/myotropic neuropeptides in invertebrates has resulted in a quite dramatic upsurge of reports of novel structures during the last few years. In *Locusta* alone, more than 30 peptides have been identified[23] and the nervous system of molluscs, in particular the bivalve *Mytilus*, has also yielded a large number[44]. The family of known *FMRF*amide-related peptides (FaRPs) has been greatly enlarged by many new members from different sources[50]. What is the crustacean contribution to this burgeoning field? Although the results in crustaceans are not dramatic with regard to the number of identified peptides, the last years have seen considerable progress. This is largely due to renewed interest in the pericardial organs (PO), which are large neurohemal organs in the pericardial cavity that were recognized long ago as release sites of cardioactive peptide, as well as the biogenic amines serotonin, octopamin and dopamine (see Cooke and Sullivan[10] for a review of older work). Improved methodology made the identification of several peptides possible, The currently known crustacean peptides are listed in figure 3. With the exception of orcokinin and the enkephalins, they have been isolated from the POs, which proved to be a particularly rich source, but almost all of them have also been identified by immunocytochemistry in other parts of the nervous system. Generally speaking, their occurrence in the POs, which are highly specialized for neurohemal release, argues for a role of these peptides as circulating neurohormones, but their distribution elsewhere in the nervous system suggests that they have, in addition, a role as locally acting transmitters or neuromodulators (see Keller[27] for review).

Proctolin

This pentapeptide was first isolated and sequenced from the cockroach on the basis of its hindgut-contracting activity[70]. Subsequent studies with synthetic proctolin revealed a rather broad range of stimulatory activities on different types of muscles and neurons, not only in insects, but also in crustaceans and other arthropods. A recent review with emphasis on insects is available[46]. The existence of authentic proctolin in crustaceans was demonstrated by isolation and structural elucidation from the PO of *Homarus*[56] and *Carcinus*[66]. Recently, it has also been identified in the central nervous system of *Limulus*[21]. From an evolutionary point of view, it is interesting that proctolin appears to be unique to arthropods. Convincing evidence of its occurrence as well as physiological actions in other invertebrates (e.g. molluscs) and vertebrates has as yet not been provided[46]. Its presence in the PO clearly suggests a neurohormonal role of proctolin in crustaceans, although attempts to detect it in the hemolymph have, in contrast to results for CCAP and the FaRPs, not been very successful. The perikarya that give rise to the secretory axons and end-

Proctolin	Arg-Tyr-Leu-Pro-Thr	(70)
FLI3 (*Homarus*)	Ser-Asp-Arg-Asn-Phe-Leu-Arg-Phe-NH_2	(74)
FLI4 (*Homarus*)	Thr-Asn-Arg-Asn-Phe-Leu-Arg-Phe-NH_2	(74)
DF_2 (*Procambarus*)	Asp-Arg-Asn-Phe-Leu-Arg-Phe-NH_2	(42)
NF_1 (*Procambarus*)	Asn-Arg-Asn-Phe-Leu-Arg-Phe-NH_2	(42)
FaRP *Callinectes s.*	Gly-Tyr-Asn-Arg-Ser-Phe-Leu-Arg-Phe-NH_2	(31)
CCAP	Pro-Phe-Cys-Asn-Ala-Phe-Thr-Gly-Cys-NH_2	(69)
Orcokinin	Asn-Phe-Asp-Glu-Ile-Asp-Arg-Ser-Gly-Phe-Gly-Phe-Asn	
Met-enkephalin (*Carcinus*)	Tyr-Gly-Gly-Phe-Met	(35)
Leu-enkephalin (*Carcinus*)	Tyr-Gly-Gly-Phe-Leu	(35)

Figure 3. A current list of completely identified crustacean cardioactive/myotropic peptides. Orcokinin: unpublished results from the laboratory of the author.

ings in the PO have been mapped by immunocytochemistry in the thorax ganglion of *Carcinus*[15]. In addition, a large number of other proctolin-immunoreactive neurons have been found throughout the central nervous system of *Homarus* and *Procambarus*[59], including neurons that innervate skeletal muscles directly[60]. They are more numerous than any other identified peptidergic cell type. These morphological findings showing proctolin to be associated with all major types of neurons, including neurosecretory ones[59], are already sufficient evidence of an important role of proctolin in crustaceans.

As far as proctolin physiology and pharmacology are concerned, crustaceans are the best-studied group next to insects. Of the many observed effects, only a representative selection can be considered here. The excitatory action on rate and force of heart contraction is well documented. Stimulation of cardiac ganglion motor neurons is at least partially responsible for this[18,71]. In addition, a rather complex and subtle regulation of cardiac outflow is suggested by the finding that proctolin has a selective contracting effect on heart values[32]. Also well established is a positive modulating effect on neurons of the stomatogastric ganglion of *Cancer borealis* and *Panulirus interruptus*, in keeping with the input of fibers with proctolin-immunoreactivity into the STG[24,39]. Proctolin effects on skeletal muscles include long-lasting contracture of the dactyl-opener of *Homarus*, considered to be directly myotropic rather than presynaptic[55], modulation of a crab ventilatory muscle[40] and enhancement of abdominal tonic flexor muscle contractions in the crayfish[6]. The neuropeptide activates calcium channels in the membrane of the tonic flexor muscle[5]. The effect on visceral muscles is exemplified by the potent contracting activity on the crayfish hindgut (own unpublished observations). A modulatory effect of proctolin on afferent responses of a mechanoreceptor has also been demonstrated[47].

Concerning the mode of action of proctolin, the term neuromodulator or co-transmitter appears to be appropriate. This would apply regardless of whether it is locally released or, e.g. from the PO, into the circulating hemolymph to reach the targets. A classical transmitter role has not been found. This aspect is discussed in detail in the review by Orchard et al.[46].

FMRFamide related peptides (FaRPs)

It is now clearly established that members of this peptide family are ubiquitous among metazoa. Subsequent to the initial isolation of FMRFamide from a clam[49], studies on molluscs have yielded a particularly large number of FaRPs, but many have also been found in other animals and the family is likely to grow fast in the near future (for review see Price and Greenberg[50]).

In crustaceans, five distinct FaRPs have been identified (fig. 3), all of them unique. While the fact that they belong to the FLRFamide rather than the FMRFamide subset may indicate a specific crustacean feature, final conclusions will have to await the structural elucidation of other immunoreactive FaRPs that have, for example, been demonstrated in *Homarus americanus* in addition to the fully identified FLI 3 and 4[74]. However, it has been established that little or no authentic FMRFamide is present in the PO of this species[74]. In this context, it is interesting that synthetic FLI 4 is at least 10^3-times more potent on the lobster heart and the dactyl opener muscle than FMRFamide[74].

All five known FaRPs have been isolated from the PO of the respective species. It is an intriguing result that FLI 3 and 4 from the lobster and NF_1 and DF_2 from the related reptantian *Procambarus clarkii*[42] are identical except for an additional N-terminal residue in both FLI 3 and 4. The peptide from the brachyuran *Callinectes sapidus* is quite different.

The PO contain, as has been shown by RIA in *Homarus americanus*, particularly high amounts of FaRPs[29]. This clearly suggests a neurohormonal role, a conclusion which is supported in this case by the demonstration of significant peptide levels of 10^{-11}–10^{-10} M in the hemolymph. Moreover, FaRP-immunoreactive neurons (e.g. motorneurons) occur throughout the entire nervous system, which provides evidence for an additional role as locally acting transmitters or cotransmitters/modulators[29].

Several physiological effects of crustacean FaRPs have been documented. FLI 4 increases force and rate of contraction of the heart and causes contraction and enhances *EPSPs* in the dactyl opener muscle preparation of *Homarus*[74]. The same cardioexcitatory actions were observed in *Carcinus maenas* and *Orconectes limosus*, and in the latter species hindgut-contracting activity was also demonstrated (own unpublished observations). In *Procambarus clarkii*, FLI 3 and 4 proved equally potent in enhancing tension and synaptic transmissions in the deep abdominal extensor muscles[41]. A modulating effect of FLI 3 on neurons of the stomatogastric ganglion of *Cancer borealis* has been reported[79]. The input of FaRP immunoreactive fibers into the stomatogastric ganglion (STG) of the crab[79] suggests that this is a physiological mechanism. The data, although limited, permit the prediction that FaRPs are likely to have a considerable spectrum of activities in crustaceans.

Crustacean cardioactive peptide (CCAP)

This neuropeptide was first isolated and sequenced from POs of *Carcinus maenas*[68]. Its structure is not related to that of any known peptide. Its occurrence in *Orconectes limosus*[65] and *Homarus americanus* (unpublished studies from our laboratory) has also been established. RIA determinations have shown that CCAP occurs throughout the nervous system, although it is particularly abundant in the PO[69]. Among the ganglia of the ventral nerve cord, the 6th abdominal ganglion displays a particularly high content of CCAP in *Orconectes* and *Homarus* (Stangier et al.[69]; own unpublished observations). Lev-

els may be higher than those of the FaRPs and proctolin. In the lobster, amounts in the ventral nerve cord (thorax-ganglion 4 to abdominal ganglion 5) are approximately 4–5 times higher than those of the FaRPs and proctolin (comparison of own unpublished data with published data on FaRPs[29] and proctolin[56]), while in the 6th abdominal ganglion, the CCAP content is almost 100 times higher. In the PO of *Carcinus*, the CCAP content is approximately 8 times higher than that of proctolin[69].

A hormonal role of CCAP, already suggested by its presence in the PO, is further supported by its release under high (K^+) and by measurable peptide levels in the hemolymph[69]. Both in resting *Carcinus* and *Orconectes*, they amount to approximately 5×10^{-10} M.

Immunocytochemical investigations have revealed CCAP-immunoreactive neurons throughout the nervous system of *Carcinus* and *Orconectes*[13,75]. In agreement with the results of FaRPs[29] and proctolin[59], additional neurohemal release sites in the neural sheath of the ventral nerve cord have been observed[75]. However, the morphology of other CCAP-immunoreactive structures clearly suggests that the peptide functions as a locally acting signal substance in addition to its neurohormonal role.

Studies on the physiological effects of CCAP have been limited thus far. They include an excitatory action on the crustacean heart and on the crayfish hindgut[65]. In the latter, spontaneous contractions are enhanced in force and rate and contractions are induced in quiescent preparations. In intact *Cancer borealis* and *Homarus gammarus*, injection of CCAP showed only limited effects on the heart, whereas the respiratory rhythm displayed sustained enhancement[64].

According to recent studies, CCAP is not unique to crustaceans. It has been identified from the nervous system of *Locusta* by sequencing[67] and immunocytochemical studies have demonstrated a complex system of CCAP neurons throughout the nervous system of the locust, including conspicuous, apparently neurosecretory projections to the heart[14]. A potent hindgut and oviduct contracting activity has also been observed in the insect[67].

Orcokinin

This neuropeptide was recently identified in our laboratory, during the investigation of a hindgut contracting substance, which was separated by HPLC of extracts from the abdominal nerve cord of *Orconectes limosus*, at a retention time not indicative of any of the known crustacean myotropic peptides. The sequence is unlike that of any other known neuropeptide or hormone. It was named orcokinin in reference to both its origin and its biological activity. This peptide resembles proctolin and the enkephalins in being neither N-terminally nor C-terminally blocked. If one considers the known invertebrate neuropeptides, this is rather untypical. At this very early stage of investigation, little can be said with regard to the occurrence of this novel neuropeptide in other crustaceans or other invertebrate groups, or its neuronal association and function. The only clear biological effect known at present is a potent contracting activity on the isolated hindgut of the crayfish. In this respect, its potency is higher than that of FLI 3, CCAP or proctolin. At a concentration of 10^{-9} M, it increases the force of contraction by 300–350%, while the other three peptides cause increases of 80–100% under identical conditions. Its effect on the crayfish heart, however, seems to be less than that of the three above-mentioned peptides (preliminary unpublished data from our laboratory).

Enkephalins

Leu- and Met-enkephalins have not been isolated and sequenced from the PO, but from the thoracic ganglion (ThG) *of Carcinus maenas*[35]. However, there is good evidence that they are produced by ThG-perikarya that project into the PO[15]. In the latter, fibers and neurosecretory terminals with Leu-enkephalin-like immunoreactivity have been demonstrated. This suggests that enkephalin may be released into the circulation[12]. These elements are clearly distinct from CCAP-, proctolin- and FaRP-immunoreactive fibers[12].

The enkephalins have been included here as newly identified crustacean peptides, although, to my knowledge, clear effects on the usual neuromuscular preparations, on the gut and the heart have as yet not been observed. If applied alone, enkephalins did not have any overt effect on the *Carcinus* or *Orconectes* heart nor on the spontaneously contracting crayfish hindgut (own unpublished observations). Whether enkephalins modulate the action of other substances in these preparations remains to be established.

The chromatophorotropins

These are peptides classically known as pigmentary effector hormones in crustaceans. Work relating to this role has been reviewed in detail by Rao[51] and will therefore not be considered here. It is felt, however, that these peptides merit discussion in view of recent results on other functions and on distribution. These studies have placed the chromatophorotropins in a broader perspective, both in functional and evolutionary terms.

Red pigment concentrating hormone (RPCH)

This octapeptide was first isolated and sequenced from eyestalks of the prawn, *Pandalus borealis*[17]. In the meantime, it has been fully identified from three other decapod species[20]. RPCH is a member of the AKH/RPCH peptide family which consists mostly of adipokinetic hormone(AKH)-related insect peptides. This family has become quite large during the last few years, owing to the continuing discovery of new insect peptides. In figure 4, RPCH and the mollusc peptide APGWamide are shown together with a selection of insect peptides. These were chosen to demonstrate the molecular variation in the insect branch. The list is not complete (almost 20 pep-

RPCH (*Pandalus* and other crust.)	pGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2
APGWamide (*Fusinus/Achatina*)	Ala-Pro-Gly-Trp-NH_2
Tem-HrTH	pGlu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH_2
Lom-AKH I	pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2
Lom-AKH II	pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp-NH_2
Mas-AKH	pGlu-Leu-Thr-Phe-Thr-Ser-Ser-Trp-Gly-NH_2
M I = neurohormone D	pGlu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2
M II	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2
Pht–HrTH	pGlu-Leu-Thr-Phe-Ser-Pro-Asp-Trp-NH_2

Figure 4. Red pigment concentrating hormone (RPCH) shown together with the molluscan APGWamide and a selection of insect peptides of the AKH-family. References: RPCH [17, 20]; APGWamide [33, 34]. The structures of insect peptides and their names are from a paper by Gäde, 1991 [19], with origins as follows: TemHrTH, *Tenebrio molitor*; LomAKH I/KK, *Locusta migratoria*; MasAKH, *Manduca sexta*; MI/II, *Periplaneta americana*; PhtHrTH, *Phormia terraenovae*, *Drosophila*. For details see text.

tides are known at present) and the reader is referred to a recent publication by Gäde [19] for a complete list.

The insect peptides display mostly adipokinetic or hyperglycemic activity (or both), but some of them have been shown to be myotropic and cardioexcitatory. From an evolutionary point of view, the AKH/RPCH-peptides exhibit a number of interesting features. First, the structure of these C-amidated peptides is relatively conserved with regard to chain length. Thus far, only octa-, nona- and decapeptides are known (disregarding APGWamide). Second, there are the invariant residues $pGlu^1$, Phe^4 and Trp^8, and Pro is often, but not always, found in position 6. Concerning the remaining residues, there is, however, a surprisingly high degree of variation among insect AKHs/HGHs.

This poses the obvious question whether there is comparable molecular variation among RPCHs of different crustaceans. Thus far, RPCH has been sequenced from *Pandalus borealis*, *Cancer magister*, *Carcinus maenas* and *Orconectes limosus*, representing three infraorders of decapods [20]. All structures were found to be identical. Less stringent, but nevertheless convincing structural evidence exists for seven other species, suggesting that their RPCHs also have the same primary structure [20]. Another interesting point is that, while several insects possess two AKHs (three in *Locusta*), there seems to be only one RPCH in crustaceans, and it is also worth mentioning that authentic RPCH has thus far not been found in this group, although several very similar octapeptides occur in insects (some differing by only one residue, e.g. in *Tenebrio molitor*, fig. 4). Hypothetically, the conservation of the RPCH structure among crustaceans may be due to a stringent ligand-receptor relationship that has not tolerated the evolution of variants, whereas in insects molecular variation may even have been exploited to create modified functions.

That RPCH may have functions other than its well established hormonal action on chromatophores was first suggested by immunocytochemical evidence. In a study on *Carcinus maenas* and *Orconectes limosus*, RPCH immunoreactivity was demonstrated in neurosecretory eyestalk neurons projecting to the neurohemal sinus gland, but also in neurons in other parts of the nervous system that did not seem to project to a neurohemal area, leading to the conclusion that RPCH has an additional role as a local transmitter or neuromodulator [37]. This was fully confirmed by combined immunocytochemical and neurophysiological studies which demonstrated a dense input of RPCH-immunoreactive fibers into the neuropile of the stomatogastric ganglion of *Cancer borealis* and marked excitatory activity of RPCH [45]. This excitatory activity was further analyzed in detail in the STG of *Panulirus interruptus* [11]. In another study, RPCH was shown to act as an excitatory modulator of swimmeret activity rhythms in the crayfish when perfused into the ganglia of the isolated nerve cord. This was corroborated by demonstration of RPCH-positive perikarya and fibers in the abdominal ganglia [58].

Considering these results, it is interesting to recall earlier results on myotropic and cardioexcitatory activity of some of the members of the AKH-family in insects, e.g. MI and MII [4, 54, 80]. It appears that the potential for myotropic actions is a 'built-in' feature of these peptides. In this context, it is of interest that the small peptide APGWamide, isolated from the snails *Fusinus ferrugineus* and *Achatina fulica*, has myotropic (radula retractor twitch potentiating) activity in *Fusinus* and inhibitory neurotropic effects in *Achatina* [33, 34]. It possesses important structural features of the AKH/RPCH peptides, although it is not clear, owing to its small size, whether it is a real congeneric family member.

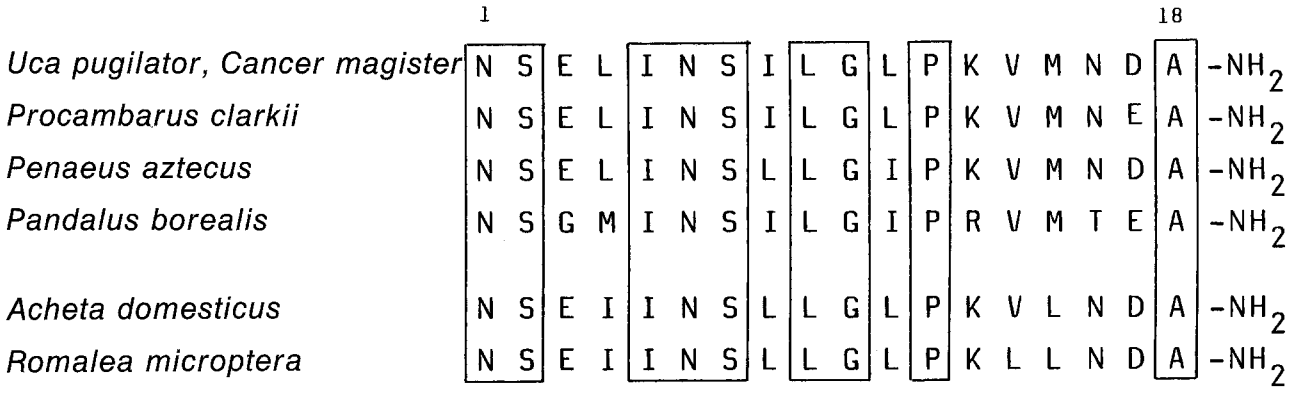

Figure 5. Peptides of the pigment-dispersing hormone (PDH) family from 5 crustaceans and 2 insects. Data taken from Rao and Riehm 1989[52]. Invariant positions are boxed.

Pigment-dispersing hormones (PDHs)

In 1976, Fernlund[16] isolated and sequenced distal retinal pigment hormone (DRPH), an octadecapeptide from eyestalks of *Pandalus borealis*. It was then known as a hormone inducing light-adapting movements of pigment in the crustacean compound eye[28]. Subsequently, it was established that it also causes pigment dispersion in epidermal chromatophores, and the acronym PDH has been adapted for DRPH and homologous peptides (for review see Rao[51]). Structural elucidation of PDH from several crustaceans and two insect species has revealed the existence of a true peptide family. The structures listed in figure 5 were taken from a paper by Rao and Riehm[52]. There is additional evidence that one species may contain more than one PDH[53]. The amino acid exchanges seen in these peptides have a marked influence on interspecific activity, e.g. PDHs of *Procambarus clarkii* and, in particular, *Pandalus borealis*, are much less active on the melanophores of *Uca pugilator* than its own hormone[52]. As in the case of RPCH, immunocytochemical mapping studies first suggested a non-hormonal role for PDH. The association of PDH-immunoreactivity not only with secretory pathways but also with apparently non-secretory neurons (e.g. interneurons) throughout the nervous system of *Orconectes limosus* and *Carcinus maenas* suggested a local transmitter or neuromodulator function[36,38]. In *Panulirus interruptus*, for example, an extensive input of PDH-fibers into the neuropile of the stomatogastric ganglion was demonstrated, which resembled the pattern of proctolin-, FaRP- and RPCH-fibers[43]. In view of the established physiological effects of these latter peptides, this result is particularly suggestive of a neuromodulator role of PDH too. Finally, the occurrence of PDH in insects is interesting in this respect since, as chromatophores resembling those of crustaceans are lacking, the classical hormonal role can be ruled out. At present, however, no physiological effect of PDHs other than pigment movement in chromatophores or eyes has been described.

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Comparative aspects of structure and action of molluscan neuropeptides

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Abstract. A number of neuropeptides were isolated from the ganglia and muscles of molluscs, and their actions were examined. Diverse neuropeptides, in addition to several classical neurotransmitters, were suggested to be involved in the regulation of the anterior byssus retractor muscle of *Mytilus*. A wide structural variety of members of the *Mytilus* inhibitory peptide family was observed in each of the genera *Mytilus, Achatina* and *Helix*. Gly-Trp-NH_2, the C-terminal dipeptide fragment of the neuropeptide AGPWamide, showed a more potent action than the parent peptide in all of the muscles examined. Peptides related to some molluscan neuropeptides were found to be distributed interphyletically. Some neuropeptides containing a D-amino acid residue were found in *Achatina* and *Mytilus*. These aspects of molluscan neuropeptides are thought not to be exceptional.
Key words. Neuropeptide; Mollusca; ABRM; *Mytilus; Achatina; Helix*; D-amino acid residue.

Introduction

Over the last 15 years, a large number of neuropeptides have been isolated from molluscs. According to the structure and the action of the peptides, they have been classified into family groups, such as the FMRFamide family, the SCP family and the myomodulin-CARP family [25]. In collaboration with other laboratories, we have also isolated a number of neuropeptides mostly from muscles and ganglia of molluscs, and have examined their actions on muscles and neurons. Most of the isolated peptides were found to be members of families described previously. However, some comparative aspects of the structure and the action of the peptides were found to be unique. Here, we review those unique aspects.