Invited Trends Article

The Revolution in Insect Neuropeptides Illustrated by the Adipokinetic Hormone/Red Pigment-Concentrating Hormone Family of Peptides

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The last decade has seen a surge in the knowledge on primary structures of insect neuropeptides. Particularly successful were isolations and sequence determinations of more than 30 members of the adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family of peptides. This brief overview describes the techniques used to obtain data on purification and structure such as high performance liquid chromatography, Edman sequencing and mass spectrometry. Moreover, a short account on the precursors and on the multiple functions of the peptides of the AKH/RPCH family in various crustacean and insect species is given.

Introduction

Peptidergic neurosecretion plays a major role in cellular communication in almost all animals and many neuropeptides have been structurally elucidated. Therefore, it is not surprising that the expression "peptide revolution" was coined (see Reichlin, 1980). In insects, too, many physiological, developmental and behavioural processes are affected by peptides produced in neurosecretory cells. These neuropeptides can be classified into three major categories (Gäde, 1996):

1. Peptides which modify spontaneous muscle contractions, such as the myokinins, myotropins, tachykinins, sulfakinins, pyrokinins and FMRF amide-related peptides.

2. Peptides which control reproduction, growth and development, such as prothoracicotropic hormones, allatotropins and allatostatins, eclosion hormone, diapause hormone and folliculostatins and gonadotropins.

3. Peptides which regulate physiological homeostasis and metabolism, such as diuretic and antidiuretic peptides and adipokinetic and hypertrehalosaemic hormones.

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Most of these peptides are stored in neurohaemal organs in very small quantities (a few pmoles per insect). Therefore, the primary structure of the first insect peptide, the pentapeptide proctolin, was only published about 10 years after the first isolation studies were undertaken (Starratt and Brown, 1975) after the compound was isolated from 125 kg of whole cockroaches. In 1976, the adipokinetic hormone Lom-AKH-I from locusts was structurally characterised (Stone *et al.*, 1976). The latter authors used as starting material the tissue where the peptide was synthesised and stored, the corpora cardiaca, and thus started the isolation process with a less contaminated source. Furthermore, structural information was achieved by utilising the new emerging techniques for peptide characterisation, in this case mass spectrometry. The last decade in particular has seen a surge in the identification of neuropeptides, mainly due to refinements in physical-chemical techniques. To date, about 180 primary structures of insect neuropeptides are known (Gäde, 1996) but each month new ones are elucidated. This explosion of structural information could only happen after major progress had been made in improving existing methodologies for isolating peptides by liquid chromatography and for sequencing peptides and detecting amino acid derivatives; This short review article outlines briefly the methods for purification and structure determination, using the metabolic

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peptides of the adipokinetic hormone/red pigment-concentrating hormone (AKH/RPCH) family as examples. Thereafter, this family is briefly introduced by discussing some of their functions and modes of action.

Methods

A prerequisite for detecting the peptides during isolation is a reliable and relatively fast bioassay. Here the adipokinetic test is described briefly. When extracts of corpora cardiaca of locusts were injected into a resting locust, an increase in the concentration of lipids (diacylglycerols) in the haemolymph was measured (see Gäde, 1990a). These observations lead to the development of a simple bioassay which in our laboratory is routinely performed as follows: at time zero a 1 µl sample of haemolymph from *Locusta migratoria* is taken with a microcapillary. Subsequently, the locust is injected with 10 µl of the solution to be analysed (a corpus cardiacum extract or a fraction from HPLC separation), and a second 1 µl haemolymph sample is taken 90 min post-injection. Analysis of the lipid content in the haemolymph samples is achieved by the sulphophosphovanillin method. The developed pink colour is easily read in a simple filter photometer at about 450nm, and the amount of lipid quantified by the use of a standard curve. For further reading on this adipokinetic bioassay and on a hypertrehalosaemic one, the reader is referred to Stone and Mordue (1980) and Gäde (1990a).

The development of column packing materials of micron size which could sustain high mechanical force without collapsing was a prerequisite for the introduction of high performance (pressure) liquid chromatography (HPLC). Such materials are characterised by their toleration of a high flow rate of the passing liquid at a high pressure. HPLC is mainly used in its reversed-mode (RP-HPLC) for the separation of peptides: it is a form of partition chromatography in which the starting mobile phase is more polar than the stationary phase. The latter is silica whose silanol groups are chemically derivatised with organosilanes such as octadecyl (C-18) for example. In peptide purification such C-18, but also C-8 and C-4, supports are widely used. When the column is developed in a gradient mode, the polar solvent is often water, whereas the hydrophobic solvent is acetonitrile. To increase the resolving power for certain peptides, ion-pairing reagents such as trifluoroacetic acid or heptafluorobutyric acid are added to the solvents.

Since the corpora cardiaca, which are the source of synthesis and storage for the AKH/RPCH peptides in insects, contain these peptides in relatively high quantities (which is in contrast to other insect neuropeptides), these tissues are routinely dissected and subsequently extracted in 80% methanol. The methanolic extracts are subjected to RP-HPLC in a gradient mode and excellent separation of the AKH/RPCH peptides occurs (Gäde *et al.*, 1984). Thus, in a single-step purification scheme the peptides are sufficiently pure for structural work to assign a sequence. If, however, whole heads are used at the start of the purification, much more contamination is introduced and more purification steps are necessary (Hayes *et al.*, 1986; Liebrich *et al.*, 1995). For further reading on liquid chromatography methodology the reader is referred to Esch *et al.* (1983); Schooley *et al.* (1990); Serwe and Meyer (1994).

Sequencing of peptides is achieved by the Edman degradation process, in which the N-terminal amino acid of a peptide is cleaved off the peptide and this residue is subsequently derivatised and identified. For this, automated sequencers have been built whose technology has been constantly improved. The current generation of gas phase or pulsed liquid phase sequencers have built-in facilities for on-line RP-HPLC separation of the derivatised amino acids in the microbore fashion, and are thus able to operate in the range of about 10 pmol (Lottspeich *et al.*, 1994). For Edman degradation a free N-terminal amino acid is needed. The peptides of the AKH/RPCH family, however, are all blocked at the N-terminus by a pyroglutamate residue. This residue has to be cleaved off enzymatically by pyroglutamate aminopeptidase; in this process a new peptide, the des-pyroglutamate fragment, is produced. This has a free N-terminus, and thus can be automatically sequenced.

Besides the blocked N-terminus, members of the AKH/RPCH family of peptides contain a further post-translational modification: they are also blocked at the C-terminus by an amide group. This cannot be detected by Edman degradation and therefore mass spectrometric methods are used to clarify the sequence. Since some amino acids have

the same mass (for example: leucine, isoleucine and hydroxyproline: 113 mass units), a combination of Edman degradation and mass spectrometry has to be employed. Mainly, mass spectrometry is used to measure the mass of a peptide accurately, thereby confirming sequencing results achieved with other methods. However, modern mass spectrometry is also able to assign sequences of smaller peptides. Members of the AKH/RPCH family with a length of maximally 10 amino acids, combined with the features of blocked N- and C-termini, are ideal candidates for mass spectrometric investigation. For example, the peptides from the moth *Manduca sexta* (Ziegler *et al.,* 1985) and from the stick insect *Carausius morosus* (Gäde and Rinehart, 1987a) have been analysed by tandem mass spectrometry in the fast atom bombardment mode (FAB). For this technique, the peptide is taken up in a glycerol matrix which is subsequently bombarded with a beam of argon or xenon atoms resulting in protonated $[M + H]^+$ or deprotonated $[M - H]^+$ ion signals of the peptide, depending whether positive or negative mass spectra were generated. Thus in the first double-focusing spectrophotometer the peptide is ionised and the "parent" ion filtered to reach the second mass spectrometer. In the free-field region of the two instruments the ion is fragmented by collision with the helium or argon atoms, called collision activated dissociation, and the produced "daughter" or product ions are detected and analysed in the second double-focusing mass spectrometer. This methodology also successfully detected another post-translational modification in a stick insect AKH/RPCH family peptide: here, we found an unusual glycosylation site, namely at the tryptophan residue (Gäde *et al.,* 1992a).

During the last decade, even more sensitive mass spectrometric techniques have been developed – matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) and electrospray ionisation mass spectrometry (ESI-MS). Both techniques are mainly used for molecular weight measurements. They have an outstanding sensitivity (about 1 pmol) and even large biopolymers up to about 300 kD can be determined when a time of flight mass analyser is used. The MALDI-MS has been used for sequence assignments as well. For detailed accounts of the principles of the spectrometric methods the reader is referred to the following articles: Shimonishi and Takao (1990); Rinehart *et al.* (1990); Weigt *et al.* (1994); Bahr *et al.* (1994); Metzger and Eckerskorn (1994).

The AKH/RPCH Family

In the 1960's peptides were discovered in the American cockroach and in locusts regulating carbohydrate and lipid breakdown, respectively. We now know that these peptides are members of a large family of structurally related peptides which are found in crustaceans and insects. On the basis of the first members of this family to be fully characterised, an adipokinetic hormone from locusts (Stone *et al.,* 1976), now called Lom-AKH-I according to the nomenclature proposed in Raina and Gäde (1988), and a chromatotropic peptide from prawns (Fernlund and Josefsson, 1972), the red pigment-concentrating hormone (codename: Pab-RPCH), such peptides became known under the acronym AKH/RPCH family peptides. The peptides are present in the neurosecretory X-organ/sinus gland complex of the eyestalks of crustaceans and the intrinsic neurosecretory cells of the corpora cardiaca of insects. Both structures are neurohaemal organs, analogous to the vertebrate hypothalamo/hypophyseal system, and it can thus be inferred that the peptides can be released from the neurohaemal organs into the circulation and, thus, act as true hormones. However, release has been demonstrated in only a few cases, for example during flight in locusts, blowflies and the moth *Manduca sexta* (see Gäde, 1992).

With the above described techniques in hand, structural data of peptides have been obtained from representative species of most of the main orders of insects and four species of crustaceans (see Table I for references). Interestingly, although the crustacean species analysed belong to different infraorders and superfamilies, only one form of peptide, Pab-RPCH, is present which does not vary between species. In crustaceans, apparently, the AKH/RPCH family member is conserved, whereas insect species show a high variability in structure of these peptides and quite often contain two (or even three) different AKH/RPCH family members. Common structural features of this family are the following:

1. They consist of 8 to 10 amino acids, thus are short peptides, either octa-, nona- or decapeptides.

Table I. Primary structures of peptides of the AKH/RPCH family.

Code name	Species	Sequence	Reference
Lom-AKH-I	*Locusta migratoria*	pQLNFTPNWGTamide	Stone *et al.* (1976); Siegert *et al.* (1985)
	Schistocerca gregaria		Stone *et al.* (1976)
Phm-AKH	*Phymateus morbillosus*	pQLNFTPNWGSamide	Gäde *et al.* (1996)
Del-CC	*Decapotoma lunata*	pQLNFSPNWGNamide	Gäde (1995b)
Cam-HrTH-I	*Carausius morosus*	pQLTFTPNW°GTamide	Gäde *et al.* (1992a)
Cam-HrTH-II	*Carausius morosus*	pQLTFTPNWGTamide	Gäde and Rinehart (1987a)
	Sipyloidea sipylus		Gäde (1989b)
	Extatosoma tiaratum		Gäde and Rinehart (1990)
Phl-CC	*Phymateus leprosus*	pQLTFTPNWGSamide	Gäde and Kellner (1995)
Taa-HoTH	*Tabanus atratus*	pQLTFTPGWGYamide	Jaffe *et al.* (1989)
Hez-HrTH	*Heliothis zea*	pQLTFSSGWGNamide	Jaffe *et al.* (1988)
Rom-CC	*Romalea microptera*	pQVNFTPNWGTamide	Gäde *et al.* (1988)
Bld-HrTH	*Blaberus discoidalis*	pQVNFSPGWGTamide	Hayes *et al.* (1986)
	Nauphoeta cinerea		Gäde and Rinehart (1986)
	Leucophaea maderae		Gäde and Rinehart (1990)
	Gromphadorhina portentosa		Gäde and Rinehart (1990)
	Blattella germanica		Gäde and Rinehart (1990)
			Veenstra and Camps (1990)
Plc-HrTH-I**,II	*Platypleura capensis*	pQVNFSPSWGNamide	Gäde and Janssens (1994)
	Munza trimeni		Gäde and Janssens (1994)
	Cacama valavata		Veenstra and Hagedorn (1995)
	Diceroprocta semicincta		Veenstra and Hagedorn (1995)
	Magicicada sp.		Raina *et al.* (1995)
Mas-AKH	*Manduca sexta*	pQLTFTSSWGamide	Ziegler *et al.* (1985)
	Heliothis zea		Jaffe *et al.* (1986)
	Bombyx mori		Ishibashi *et al.* (1992)
Psi-AKH	*Pseudagrion inconspicuum*	pQVNFTPGWamide	Janssens *et al.* (1994)
	Ischnura senegalensis		Janssens *et al.* (1994)
Lia-AKH	*Libellula auripennis*	pQVNFTPSWamide	Gäde (1990b)
	Ceratogomphus pictus		Janssens, Kellner and Gäde, unpublished
	Pantala flavescens		Janssens, Kellner and Gäde, unpublished
Emp-AKH	*Empusa pennata*	pQVNFTPNWamide	Gäde (1991a)
	Sphodromantis sp.		Gäde (1991a)
Ani-AKH	*Anax imperator*	pQVNFSPSWamide	Gäde *et al.* (1994b)
	Aeshna subpupillata		Janssens, Kellner and Gäde, unpublished
	Anotogaster sieboldii		Janssens, Kellner and Gäde, unpublished
Pea-CAH-I	*Periplaneta americana*	pQVNFSPNWamide	Witten *et al.* (1984); Baumann and Penzlin (1984); Scarborough *et al.* (1984); Siegert and Mordue (1986)
	Blatta orientalis		Gäde and Rinehart (1990)
	Leptinotarsa decemlineata		Gäde and Kellner (1989)
	Trinervitermes trinervoides		Liebrich *et al.* (1995)
	Mastotermes darwiniensis		Liebrich *et al.* (1995)
Grb-AKH	*Gryllus bimaculatus*	pQVNFSTGWamide	Gäde and Rinehart (1987b)
	Acheta domesticus		Woodring *et al.* (1990); Cusinato *et al.* (1991)
	Gryllodes sigillatus		Gäde (1992b)
	Romalea microptera		Gäde *et al.* (1988)
Tem-HrTH	*Tenebrio molitor*	pQLNFSPNWamide	Gäde and Rosinski (1990)
	Zophobas rugipes		Gäde and Rosinski (1990)
	Onymacris plana		Gäde (1994)
	Onymacris rugatipennis		Gäde (1994)
	Physadesmia globosa		Gäde (1994)
	Polyphaga aegyptiaca		Gäde and Kellner (1992)
	Decapotoma lunata		Gäde (1995b)

Table I. Continued.

Code name	Species	Sequence	Reference
Pab-RPCH	*Pandalus borealis*	pQLNFSPGWamide	Fernlund and Josefsson (1972)
	Cancer magister		Gaus *et al.* (1990)
	Carcinus maenas		Gaus *et al.* (1990)
	Orconectes limosus		Gaus *et al.* (1990)
Lom-AKH-II	*Locusta migratoria*	pQLNFSAGWamide	Siegert *et al.* (1985); Gäde *et al.* (1986)
Scg-AKH-II	*Schistocerca gregaria*	pQLNFSTGWamide	Siegert *et al.* (1985); Gäde *et al.* (1986)
	Schistocerca nitans		Gäde *et al* (1986)
	Phymateus leprosus		Gäde and Kellner (1995)
	Phymateus morbillosus		Gäde *et al.* (1996)
	Heterodes namaqua		Gäde (1992b)
	Acanthoproctus cervinus		Gäde (1992b)
	Libanasidus vittatus		Gäde (1992b)
	Anabrus simplex		S.E. Reynolds and D.A. Schooley, unpublished
Mem-CC	*Melolontha melolontha*	pQLNYSPDWamide	Gäde (1991b)
	Geotrupes stercorosus		Gäde (1991b)
	Pachnoda marginata		Gäde *et al.* (1992b)
	Pachnoda sinuata		Gäde *et al.* (1992b)
Ona-CC-I	*Onitis aygulus*	pQYNFSTGWamide	Gäde, unpublished
	Onitis pecuarius		Gäde, unpublished
Ona-CC-II	*Onitis aygulus*	pQFNYSPDWamide	Gäde, unpublished
	Onitis pecuarius		Gäde, unpublished
Lom-AKH-III	*Locusta migratoria*	pQLNFTPWWamide	Oudejans *et al.* (1991)
Miv-CC	*Microhodotermes viator*	pQINFTPNWamide	Liebrich *et al.* (1995)
Poa-HrTH	*Polyphaga aegyptiaca*	pQITFTPNWamide	Gäde and Kellner (1992)
Pea-CAH-II	*Periplaneta americana*	pQLTFTPNWamide	Witten *et al.* (1984); Scarborough *et al.* (1984); Siegert and Mordue (1986)
	Blatta orientalis		Gäde and Rinehart (1990)
	Leptinotarsa decemlineata		Gäde and Kellner (1989)
Taa-AKH	*Tabanus atratus*	pQLTFTPGWamide	Jaffe *et al.* (1989)
Pht-HrTH	*Phormia terraenovae*	pQLTFSPDWamide	Gäde *et al.* (1990)
	Drosophila melanogaster		Schaffer *et al.* (1990)

* There is a hexose substituted at the Trp.
** in all species two peptides are isolated by HPLC; Edman degradation sequencing yielded the same sequence; at the moment the modification on peptide I is not known.

2. They are N-terminally blocked by a pyroglutamate residue, and the C-terminus is blocked by an amide.

3. They contain aromatic amino acids at least at two positions: at position 4 it is mostly phenylalanine but in two cases (Mem-CC and Ona-CC-II) it is tyrosine, whereas at position 8 it is always tryptophan. The two peptides from the dung beetle *Onitis aygulus*, Ona-CC-I and II, however, have an extra aromatic amino acid (tyrosine or phenylalanine) at position 2 (Gäde, unpublished).

4. The amino acid at position 9 is glycine. This is true even for the octapeptides as the amidation of tryptophan at position 8 is contributed by glycine during biosynthesis, as deduced from the genetic code (see below).

5. Most of the peptides are not charged under physiological conditions. Only the peptide Pht-HrTH from certain dipteran species and two members sequenced from scarabaeoid beetles (Mem-CC, Ona-CC-II) have the negatively charged residue aspartic acid at position 7.

The AKH/RPCH family also seems to be a good example for the study of post-translational modifications. Besides the blocked termini, where pyroglutamate is derived from glutamate and the amidation by glycine, one member at least is glycosylated (Cam-HrTH-I) and another, yet un-

known, modification type is present in cicadas (Plc-HrTH-I).

Attempts have been made to use the sequences to speculate about evolutionary relationships. In this context the codon usage was not considered, because only a few AKH/RPCH genes are known (see below), but it was tested whether amino acid substitutions by single base changes are theoretically possible or not (Gäde, 1989a; Gäde *et al.*, 1994a; Gäde, 1995a). The outcome of these analyses is promising and mainly supports current phylogenies in the Odonata and Dictyoptera.

Of course a better parameter for such speculations is the comparison of the preprohormones. To date sequences are known for the precursors of the two adipokinetic hormones of *Schistocerca gregaria* and *S.nitans*, the three adipokinetic hormones of *Locusta migratoria*, of the single AKHs known in *Manduca sexta* and *Drosophila melanogaster* and of the RPCHs of *Carcinus maenas* and *Callinectes sapidus* (see Table II for references).

The organisation of the precursors is fairly similar in all cases: the sequence for a signal peptide is followed by the sequence for the respective AKH/RPCH peptide, a glycine residue for amidation, a processing site and, at the C-terminus, a sequence for another peptide, the so-called precursor-related peptide. The latter sequences encode for the 28-mers in the locust precursors with the exception of the one for Lom-AKH-III. There the precursor-related peptide is 44 amino acids long and falls in the same length category as the ones from *M.sexta* (34-mer) and *D.melanogaster* (46-mer). In the RPCH precursors those peptides are even longer and contain 74 amino acids (Table II).

The functions of the AKH/RPCH family peptides are quite diverse. In addition to its "classical" effects of triggering pigment concentration in chromatophores ("colour change") and migration of eye pigments, RPCH acts as neuromodulator and/or neurotransmitter in certain crustaceans, as evidenced by immunocytochemical and electrophysiological studies (see Rao and Riehm, 1993).

The "classical" function of AKHs in locusts is to make lipids available for long-distance flights. The fat body stores triacylglycerols. These are broken down to monoacylglycerols, which are subsequently re-acylated to diacylglycerols, which are released from the fat body into the haemolymph. Classical second messenger systems, like cAMP and Ca^{2+}, and protein kinases and a triacylglycerol lipase are apparently involved in this process. In the haemolymph AKHs are responsible for a rearrangement of existing lipoproteins. In resting locusts the predominant species of lipoprotein is the high-density lipophorin. This is loaded with the lipids released by AKH action from the fat body. At the same time the high-density lipophorin associates with an apoprotein, apolipophorin III. The overall result is the provision of a larger but less dense low-density lipophorin. Thus, the lipid-carrying capacity of the haemolymph is increased (see, Goldsworthy and Mordue, 1989; Kanost *et al.*, 1990). In the adult tobacco hornworm moth, *Manduca sexta*, lipids are also used as fuel for flight and the endogenous peptide, Mas-AKH, is released during flight to control this process. Recently, a tritium-labelled, biologically active analogue was synthesised and shown to bind specifically to a membrane fraction prepared from the moth's fat body (Ziegler *et al.*, 1995). This is the first serious attempt to start investigating and characterising the AKH receptor. Hopefully this line of research will in future elucidate the receptor protein.

Other actions of AKH in locusts have been reported. For example, the synthesis of proteins is inhibited (Carlisle and Loughton, 1986). Furthermore, fatty acid synthesis is inhibited (Gokuldas *et al.*, 1988). Since a time-consuming method was used for that bioassay, recent studies used *in vitro* assays, either small fat body pieces (Lee and Goldsworthy, 1995a) or dispersed fat body cells (Lee and Goldsworthy, 1995b), to measure the uptake of radiolabelled acetate into lipids. In such tests, Lom-AKH-III was a very potent inhibitor of acetate uptake; Lom-AKH-I and II were of similar potency to each other, but about 4–7 times less active than Lom-AKH-III. The latter compound also was the most potent of the three endogenous AKH peptides in inhibiting total RNA synthesis *in vitro* in the fat body of *L.migratoria*, which is another task of AKHs (Kodrik and Goldsworthy, 1995).

It is obvious, then, that AKHs have pleiotropic actions in locusts. The same is true in cockroaches. Classically, AKH-family peptides are involved in the regulation of carbohydrate mobilisation (hypertrehalosaemic effect, see Gäde, 1992a). However, more recent work on *Blaberus discoidalis*

Table II. Amino acid sequences of AKH/RPCH peptide precursors as deduced by cDNA cloning*.

Name of precursor	Signal peptide	AKH/RPCH peptide	Processing site	Precursor-related peptide	Reference
Scg-AKH-I	MVQRCLVVALLVVVVAAALCSA	QLNFTPNWGT	GKR	DAADFGDPYSFLYRLIQAEARKMSGCSN	Schulz-Aellen *et al.* (1989)
Scn-AKH-I	MVQRCLVVALLVVVVAAALCSA	QLNFTPNWGT	GKR	DAGDYGDPYSFLYRLIQAEARKMSGCSN	Noyes and Schaffer (1990)
Lom-AKH-I	MVQRCALVVLLVVAVAAALCSA	QLNFTPNWGT	GKR	DAADFADPYSFLYRLIQAEARKMSGCSN	Bogerd *et al.* (1995)
Scg-AKH-II	MRQSCALTLMLVVAVCAALSAA	QLNFSTGW	GRR	YADPNADPMAFLYKLIQIEARKLSGCSN	Fischer-Lougheed *et al.* (1993)
Scn-AKH-II	MRQGCALTLMLLVVVCAALSAA	QLNFSTGW	GRR	YADPNADPMAFLYKLIQIEARKLAGCSN	Noyes and Schaffer (1990)
Lom-AKH-II	MTQSCTLTLVLVVAVLAALATA	QLNFSAGW	GRR	YADPNADPMAFLYRLIQIEARKLAGCSD	Bogerd *et al.* (1995)
Mas-AKH	MYKLTFLMFIAFVIIAEA	QLTFTSSWG	GKR	AMTNSISCRNDEAIAAIYKAIQAEAERFIMCQKN	Bradfield and Keeley (1989)
Drm-AKH	MNPKSEVLIAAVLFMLLACVQC	QLTFSPDW	GKR	SVGGAGPGTFFETQQGNCKTSNEMLLEIFRFVQS QAQLFLDCKHRE	Noyes *et al.* (1995)
Lom-AKH-III	MQVRAVLVLAVVALVAVATSRA	QLNFTPWW	GKR	ALGAPAAGDCVSASPQALLSILNAAQAEVQKLI DCSRFTSEANS	Bogerd *et al.* (1995)
Cama-RPCH**	MVRRTGVTLLVVALVVVALVSSVSA	QLNFSPGW	GKR	AAAGSGSSGGVGEAVSALHHSVGGAPGGVVPPG SSSSSGDSCGPIPVSAVMHIYRLIRNEAVRLVQCQD EEYLG	Linck *et al.* (1993)
Casa-RPCH	MVRRSGVTLLVVALLVVTLMSSVSA	QLNFSPGW	GKR	AAGASGSNGGVGEAVSGLHPSVGGAPGGVVPPG SSSPGDSCGPIPVSAVMHIYRLIRSEAVRLVQCQDE EYLG	Klein *et al.* (1995)

* Sequences are not aligned or gaps introduced to achieve highest similarity. Such an attempt can be found in Bogerd *et al.* (1995) for most of the sequences.
** A four letter code is used for the abbreviation of the crustacean species. Otherwise misunderstanding could occur; for example Cam for *Carcinus maenas* cannot be distinguished from Cam for *Carausius morosus*.

showed that the endogenous peptide Bld-HrTH increases mitochondrial haeme synthesis, induces specific expression of the gene for cytochrome P450 and increases protein biosynthesis in the fat body (see Keeley *et al.*, 1994).

Interestingly, mobilisation of the third important fuel for flight in insects besides lipids and carbohydrates, the amino acid proline, is very likely regulated by AKH-family peptides as well. It was shown recently that not only tsetse flies and Colorado potato beetles oxidise proline during flight (see Bursell, 1981), but also certain scarabaeid and meloid beetles (Zebe and Gäde, 1993; Auerswald and Gäde, 1995). Injection of the endogenous AKH-family peptide Mem-CC into the cetoniid beetle, *Pachnoda sinuata*, caused an increase in the haemolymph proline concentration (Auerswald and Gäde, unpublished). Similarly, the novel members of the AKH-family found in the onitid dung beetle, *Onitis aygulus*, Ona-CC-I and II, resulted in hyperprolinaemia when injected into this species (Gäde, unpublished). Interestingly, the latter three peptides are structurally quite similar to each other, containing a Tyr residue either at position 4 (Mem-CC; Ona-CC-II) or 2 (Ona-CC-I); the *Onitis* peptides are unique members of the family containing three aromatic residues (see Table I).

Conclusions

The improvement of certain methods and of instrumentation have facilitated purification of and structural information on insect neuropeptides, especially those of the AKH/RPCH family which contains to date 32 members. A second driving force, however, is a more applied aspect of this type of research. Knowing the primary structure, biosynthesis, processing, release, receptor binding, mode of action and degradation of various insect neuropeptides, to name but a few processes, is a pre-requisite for conducting research into the application of neuropeptides in insect pest management. Some of the current ideas for such achievements are expressed in recent publications (Keeley and Hayes, 1987; Hedin *et al.*, 1994). Clearly knowledge on the precise biological functions of a number of neuropeptides is currently lacking, whereas structural information is available. Thus, after a decade of very successful research into the structures of insect neuropeptides, the coming next years give insect physiologists a great opportunity to further biological knowledge on the identified compounds.

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