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0014-4754/92/050425-06$1.50 + 0.20/0

Adipokinetic hormones: Cell and molecular biology

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Abstract. Adipokinetic hormones AKH I (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2) and AKH II (pGlu-Leu-Asn-Phe-Ser-Trp-Gly-Thr-NH_2) are synthesized by neurosecretory cells (NSC) of the corpora cardiaca (CC) in the locust, *Schistocerca gregaria*. These NSC constitute a homogeneous 'peptide factory' as each cell synthesizes both AKH I and AKH II. This homogeneity makes the CC an excellent system in which to study aspects of neuropeptide biosynthesis. This report summarizes recent findings on AKH inactivation and metabolism, as well as on AKH prohormone processing and biosynthesis.
Key words. Corpus cardiacum; neuropeptide; adipokinetic hormone; prohormone processing; *Schistocerca gregaria*.

Introduction

Adipokinetic hormone (AKH) is the name given to a biological activity detectable in extracts taken from the locust corpora cardiaca (CC), major neuroendocrine structures in insects [1, 18]. In recent years there has been a great deal of progress at the molecular level concerning these and related hormones in other insects and several primary structures are known. The CC of the locust have moreover provided us with a model preparation in which to investigate the molecular biology of neuropeptide biosynthesis.

The adipokinetic activity found in the locust CC is produced by two different but related peptide hormones called AKH I and AKH II. The primary structure of AKH I [40] is: pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2; the sequence of the related AKH II peptide from *Schistocerca* is pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-NH_2 [36]. In recent years structurally related hormones have been discovered in the CC of other insects. A selection of the insect hormones with the AKH signature is given in table 1.

While in the locust the adipokinetic hormones liberate diacylglycerols from the fat body into the circulation and are in general involved in the activation of lipid metabolism [21], this is not the case for the similar peptides of other insect species. For example, in the cockroach *Periplaneta americana*, the AKH-like peptides MI and MII [28, 44] are involved in stimulating carbohydrate metabolism [36]. In many cases two forms of an AKH-related peptide are found in the CC. The biological significance of this is not clear because only one function can be attributed to the two forms. It seems unlikely that the CC would manufacture two different peptides unless they had distinct and exclusive functions. Functional studies, however, have proven more difficult than chemistry and the gap between our knowledge of the biological activities of the AKH peptides on the one hand and their molecular structures on the other hand continues to widen. It has widened further recently because molecular cloning and protein sequencing techniques have been applied to the study of AKH biosynthesis [11, 20, 34]. This has led to the identification of the AKH precursors and novel peptides of as yet unknown function that are co-synthesized, co-stored and co-released with the AKHs. These novel peptides are parts of the AKH precursors and are named AKH Precursor Related Peptides or APRPs [11].

This brief essay will add very little to our understanding of the functional significance of either the adipokinetic hormones or the co-synthesized APRPs. Our main purpose is to illustrate how useful the CC of the locust have been in allowing us to probe the molecular and cellular biology of an insect peptide hormone. We have for example been able to conveniently study AKH biosynthesis and AKH inactivation. Moreover, peptidergic systems may offer new potential targets for insect control [24, 25, 27, 31]. The AKH system will therefore be useful for testing novel compounds which interact with the molecular machinery of peptide biosynthesis, action and inactivation.

AKH: Localization and site of synthesis

The CC are the major organs of the insect neuroendocrine system that store neurohormones and release them into the circulation. As their name implies, they are intimately associated with the heart vessel and are therefore well placed to distribute hormones rapidly throughout the animal. The neurohormones secreted by the CC are synthesized by neurosecretory cells contained both in the brain and within the CC themselves. In the locust the neurosecretory cells intrinsic to the CC are clustered together in the so-called glandular lobes. The neurosecretory cells of the brain send axons to the CC through two pairs of nerves and these axons arborize in separate lobes called the storage lobes.

The adipokinetic hormones are located in and synthesized by the intrinsic neurosecretory cells of the glandular lobes [6, 9]. These are typical neurosecretory cells appearing to be short axonal-neurons with cell bodies approximately 50 μm in diameter. Several thousand of them are packed closely together in the glandular lobes and each contains large numbers of electron dense secretory granules [16, 30] which have been shown by differential centrifugation to contain adipokinetic activity [39]. Several lines of evidence show that individual neurosecretory cells of the CC make both AKH I and AKH II. This can be seen clearly by immunocytochemical labelling of the glandular lobe using antibodies with high specificity for AKH I and AKH II [10]. All cells of the glandular lobe in the adult locust are labelled by AKH I specific antibodies (fig. 1), and all cells are also labelled with AKH II specific antibodies [8]. With respect to AKH I and AKH II biosynthesis therefore, the neurosecretory cells of the CC are a

Selected AKH family peptides

Genus	Name	Sequence
Schistocerca	AKH I	pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2
Schistocerca	AKH II-S	pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-NH_2
Locusta	AKH II-L	pGlu-Leu-Asn-Phe-Ser-Ala-Gly-Trp-NH_2
Periplaneta	M I	pGlu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2
Periplaneta	M II	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2
Manduca/Heliothis	M-AKH/H-AKH	pGlu-Leu-Thr-Ser-Ser-Trp-Gly-NH_2

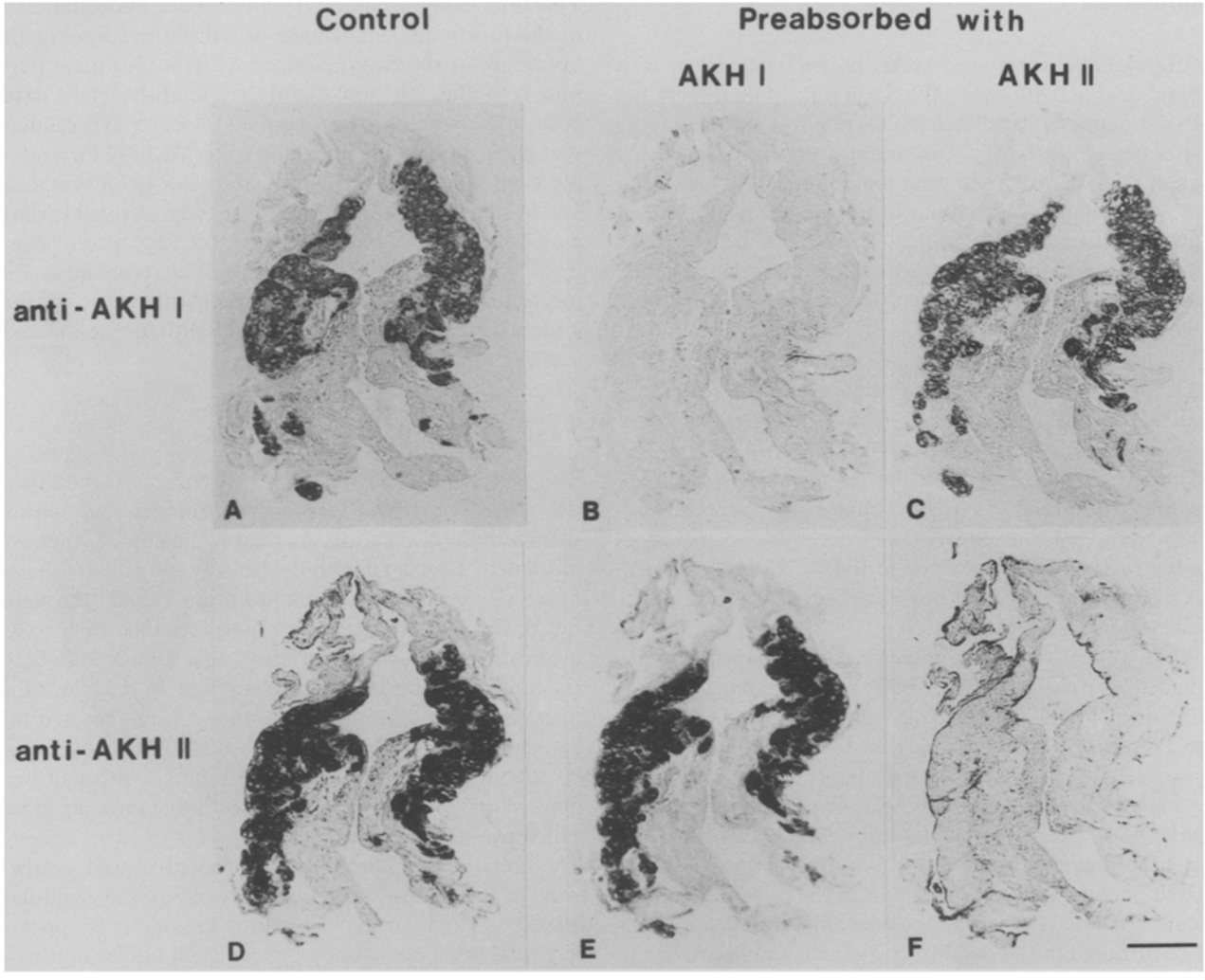

Figure 1. Immunocytochemical evidence for the co-localization of two prohormones (pro-AKH I, or the A-chain, and pro-AKH II, or the B-chain) in the glandular neurosecretory cells of the CC. In *A*, staining with an anti-AKH I-specific antibody reveals the neurosecretory cells of the CC. Specificity is indicated in *B* and *C* by the demonstration of a blockade of staining when the antibody is preincubated with AKH I, but not with AKH II. In the lower panels, a similar series of sections demonstrates the presence of AKH II in the neurosecretory cells of the CC. In these panels, staining blockade occurs when the antibody is preabsorbed with AKH II (*F*), but not with AKH I (*E*). Scale bar, 100 µm.

homogeneous population of peptide-producing cells – an extremely useful and unusual feature as most neural tissues are typically highly heterogeneous at the cellular level. The locust CC can therefore be considered to be a dedicated peptide factory, much like the ELH-producing bag cells of *Aplysia*[32, 33] or the insulin-producing β-cells. It is as if the glandular lobes contained a clonal cell line in which the hormone-producing genes were specifically and significantly up-regulated. Unlike an artificially constructed cell line, however, the glandular lobes are a real tissue in which there is appropriate gene expression and regulation. We have exploited this system to understand the molecular and cell biology of AKH biosynthesis and its regulation.

AKH: Release and functions

The actions of peptides which share the AKH structural signature are diverse but appear in general to be concerned with increased metabolic activity. For example, some members of the AKH family are involved in stimulating carbohydrate metabolism, others act directly on muscle to favour lipid oxidation and some are cardioacceleratory. In the locust, AKH activity has primarily been studied in the adult in which it regulates metabolism during long-distance migratory flight. Flight appears to be an important stimulus for the release of adipokinetic hormones in adult locusts and the releasing factor may be octopamine, an amine produced in the brain by some of the neurosecretory cells which project to the storage lobe of the CC[22, 23]. This picture of AKH function in the adult unfortunately does not explain the need for two forms of AKH, nor does it help us understand why the AKH peptides are present in all wingless larval stages. Concerning the physiological function of the peptides co-synthesized with the AKHs (the APRPs), we as yet have no direct evidence that they are hormones. The

APRPs, however, are co-released with the AKHs[11] so it seems likely that they are hormones, perhaps with an activity related to flight behaviour in the adult. An as yet untested hypothesis is that the APRPs are diuretic hormones – an idea suggested by the observation that during flight, when the locust is metabolizing lipid, metabolic water is produced. Locusts, like other insects, have impermeable cuticles so this additional water load must be removed by secretion. Indeed, it has been observed that during long-term flight locusts do secrete water, a physiological activity presumably provoked by a diuretic hormone. These arguments suggested to us that the APRPs are involved with water balance in some way. This hypothesis has yet to be tested.

AKH: Inactivation and metabolism

Timely and effective inactivation of neurohormones is necessary to ensure the appropriate temporal organization of the biological function. For neuropeptide-mediated functions, enzymatic degradation is an important means by which such signals are terminated. For neuropeptides in the CNS inactivation is accomplished by degradative cell surface enzymes, and examples of this have been demonstrated for the inactivation of mammalian neuropeptides such as the enkephalin[35], substance P[17] and neurotensin[3], as well as for insect CNS neuropeptides[12, 13]. The fate of neuropeptides such as AKH which act outside the nervous system, however, is less clear. In some cases peptide hormones are internalized by targets or other tissues where they may be degraded intracellularly (e.g. insulin[41]). In other cases, cell surface associated peptidases have been implicated in peptide hormone degradation[42].

We have studied directly the metabolic fate of the two locust AKH peptides after they are released into the circulatory system. Since the AKHs possess chemically blocked N- and C-termini, enzymatic inactivation of these peptides would be expected to be initiated by an endopeptidase. In the CNS of the locust AKH I is apparently inactivated by a membrane bound peptidase derived from nervous tissue[13]. In the circulation it has been suggested that AKH is degraded by intracellular enzymes following internalization by non-neural tissues[19]. Our studies, however, suggest that circulating AKH is inactivated not intracellularly but by a cell surface membrane associated endopeptidase similar to the one detected in the CNS by Isaac[13].

By following the fate of radioactively labelled AKH I and II we have shown that inactivation in the circulation is achieved by an endopeptidase present on the surfaces of the fat body, Malphigian tubules and skeletal muscles. The enzyme cleaves the Asn to Phe bond present in both AKHs, producing peptide fragments incapable of effecting the biological actions of the AKHs. The inactive fragments of AKH I and II C-terminal to the cleavage are then rapidly degraded in vivo from their free amino termini by aminopeptidases, whereas the N-terminal fragment from both hormones is relatively more stable. We were unable to detect endopeptidase activity in the haemolymph, but the haemolymph does contain aminopeptidase activity which is capable of degrading

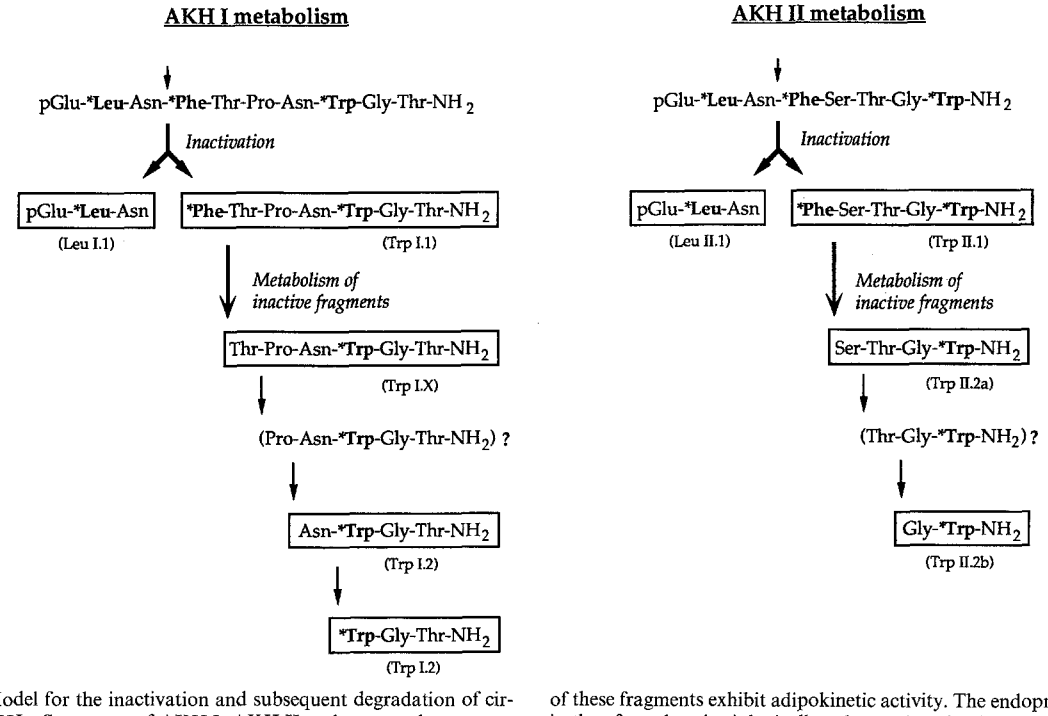

Figure 2. Model for the inactivation and subsequent degradation of circulating AKHs. Sequences of AKH I, AKH II and proposed structures of their respective metabolites are depicted. Note that the initial endoproteolysis produces 2 fragments of each of the respective hormones; none of these fragments exhibit adipokinetic activity. The endoproteolytic step is therefore the physiologically relevant inactivating step. For further details, see Rayne and O'Shea[31].

the primary C-terminal fragment following the action of the inactivating membrane-associated endopeptidase. The molecular processes involved in the inactivation of AKH I and II in the haemolymph are summarized in figure 2.

By comparing the endopeptidase activity found peripherally with the neurally derived activity described by Isaac, we consider that a very similar, perhaps identical, enzyme is involved in terminating neuropeptide action both in the CNS and in the circulation. Moreover, the endopeptidase activity we identify[31] resembles mammalian endopeptidase 24.11 and may be effective against a variety of Phe-containing peptides which are N- and C-terminally protected. Our results support the view that many invertebrate and vertebrate neural hormones are inactivated by a single class of cell surface endopeptidase. It is reasonable to assume therefore that if a specific enzyme inhibitor were designed, a number of biological responses mediated by neuropeptides would be disrupted. Such enzyme inhibitors may therefore be insecticidal.

AKH: Prohormone and precursor biosynthesis

We now have a fairly complete picture of how the locust adipokinetic hormones are made (fig. 3). The model is complicated somewhat by the fact that the immediate precursors of AKH I and II are not linear prohormones (pro-AKH I and pro-AKH II) but dimeric constructs (P1, P2 and P3). This surprising (to us) and unprecedented feature of peptide hormone biosynthesis was discovered by characterizing the hormone precursors by direct protein chemistry as well as molecular cloning.

The unexpected discovery of precursors formed from two prohormone subunits led us to question the precise mode of biosynthesis of the precursor dimers. There are two ways to synthesize a dimer protein like the Ps: 1) from proteins containing more than one copy of the monomer sequence, or 2) from the oxidation of independently translated monomer prohormones. We could not distinguish between these alternatives by performing pulse-chase experiments because we could not detect potential precursor proteins large enough to contain more than one monomer chain and we have not convincingly demonstrated the existence of independent monomer chains prior to dimer formation. Thus on the basis of our cell biological and biochemical experiments, we could not decide between the two formal possibilities for the synthesis of the dimer precursors. This question was addressed using cell-free in vitro translation of CC mRNA and DNA sequencing of positive cDNA clones[34].

By translating mRNA derived from the CC in vitro we were able to determine the size of the complete translational protein from AKH I encoding mRNA. The size of this protein (6.8 kDa) indicated that it could not accommodate more than one copy of the subunit of P1. The identity of the in vitro translated protein was established by showing that its translation is prevented by an anti-

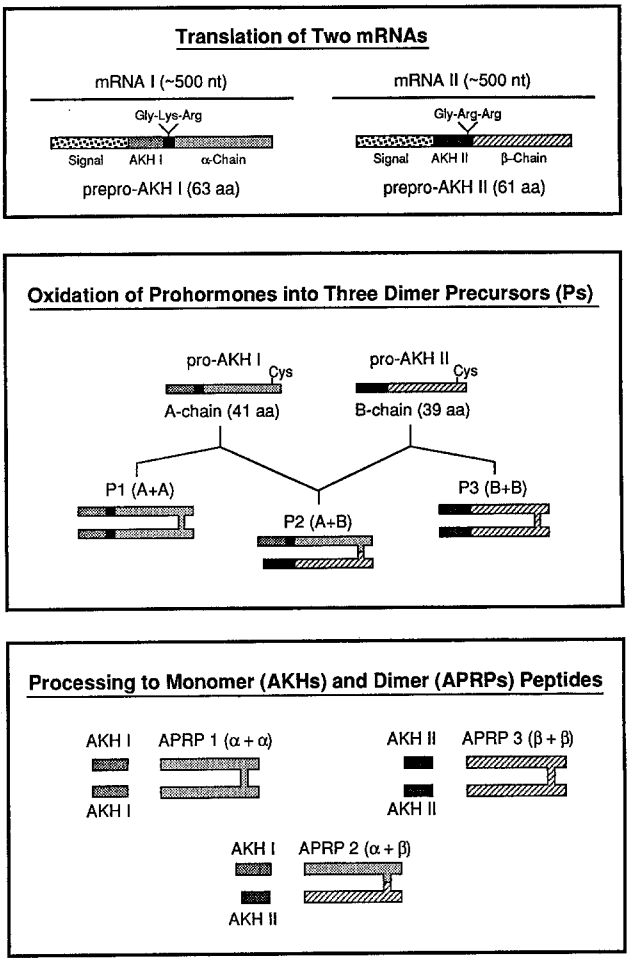

Figure 3. A model of AKH I, AKH II and APRP biosynthesis from three dimer precursors P1, P2 and P3. (nt = nucleotides; aa = amino acids). For further details, see text and O'Shea et al.[26].

sense oligonucleotide which would specifically complement an mRNA encoding the subunit of P1. This experiment (see fig. 3 in Schulz-Aellen et al.[34]) indicated strongly that P1 is therefore produced by the oxidation of independent copies of the P1 subunit rather than by post-translational modification of a larger protein containing more than one subunit copy. Similar experiments have indicated that the heterodimer P2 is also produced by the oxidation of independently translated proteins (Schulz-Aellen et al., in preparation).

Confirmation that the dimeric precursors are produced from independently translated monomers is provided by sequencing of cDNA clones derived from a CC cDNA library. The sequence of the cDNA clone which translates exactly into the subunit of P1 is given in figure 4. The inferred protein has a molecular weight exactly corresponding to the molecular weight of the protein produced during in vitro translation.

During cell-free in vitro translation, normal post-translational or co-translational modifications do not occur. Cell-free translation therefore gives the product of genetic information alone; all other subsequent biosynthetic

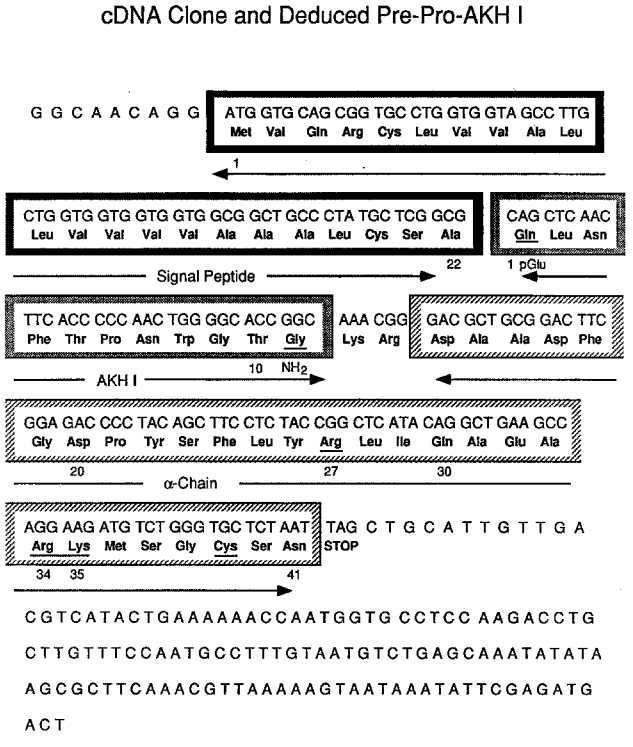

Figure 4. Nucleotide sequence of mRNA-I encoding signal peptide AKH I, a processing site and the α-chain of APRP 1 and APRP 2. This inferred protein sequence derived from a cloned cDNA gives the structure of prepro AKH I. The inferred 41 amino acid sequence starting from Gln is exactly that expected from protein sequencing of the P1 precursor of AKH I. For further explanation, see Schulz-Aellen et al.[34].

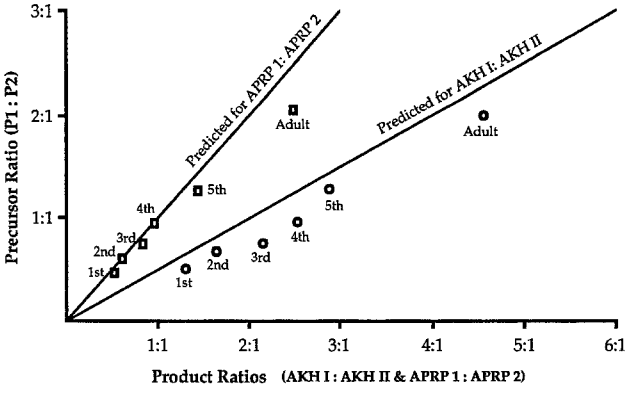

Figure 5. Relationship between relative rates of precursor synthesis (P1 to P2) and the relative amounts of products (AKH I to AKH II and APRP 1 to APRP 2) throughout post-embryonic development. The slopes of these relationships, as predicted from our molecular model and our assumptions about it, are shown as solid lines. The experimentally derived data points are shown as squares (for the APRP ratios) and circles (for the AKH ratios). There is good agreement between the predicted and observed data. Explanation for this is provided in Hekimi et al.[8].

events must therefore depend upon the integrity of the cells and must therefore be studied within the CC. The first event which we can infer directly from a cellular biochemical experiment is that the association between the two precursor subunits (pro-AKH I and pro-AKH II) is an entirely random process in the endoplasmic reticulum. Random coupling of subunits would produce predictable ratios of P1, P2 and P3 dependent only on the relative amounts of the two prohormones. Actual amounts and rates of synthesis of the dimer precursors can be measured directly in pulse-labelling experiments and so the random-coupling hypothesis can be tested. Theoretical calculations and experimental measurements indicate that the predicted amounts of P1, P2 and P3 are very close to the observed amounts and the precursor ratios accurately predict product ratios (fig. 5). Different ratios of the AKHs and APRPs can therefore be precisely regulated in the CC by differential regulation of translation of pro-AKH I and pro-AKH II[8].

To summarize, the dimer precursors of AKH I and II are formed from two prohormones called pro-AKH I (or A-chain) and pro-AKH II (or B-chain) which are encoded by two messenger RNAs called respectively mRNA-I and mRNA-II[7, 26, 34]. The sequence of mRNA-I translates into a prepro-AKH I inferred protein consisting of a 22 amino acid signal peptide, followed by the 41 residue A-chain. The pro-AKH I is AKH I, a Gly-Lys-Arg processing site and a 28 residue peptide called the α-chain. This was followed by a stop codon in the cDNA. The protein inferred from the mRNA-II cDNA clone indicated that a prepro-AKH II protein also consists of a 22 residue signal peptide which is followed by pro-AKH II (Schulz-Aellen et al., in preparation). Pro-AKH II consists of one copy of AKH II, a processing site and a 28 residue peptide called the β-chain. The 28 residue β-peptide shares about 70% homology with the α-chain of pro-AKH I. Amino acid sequences of purified pro-AKH I and pro-AKH II were determined by protein sequencing after pulse-chase experiments identified the precursors of the AKHs. Inferred (from cloned cDNA) and determined (from purified precursors) sequences of the prohormones correspond exactly.

AKH: Processing in vivo and in vitro

The presence of signal or leader peptide sequences upstream from the peptide hormone sequences is common in secreted proteins[38]. It guides the translated protein into the lumen of the endoplasmic reticulum where post-translational processing occurs[43]. An early post-translational processing event is thought to be the removal of the signal peptide, probably co-translationally, transforming the preprohormone into the prohormone. Cyclization of the translated N-terminal glutamine into pyroglutamate appears to be a very early step in the AKH biosynthetic pathway and may also be co-translational. The formation of the dimeric precursors (the Ps) is by the oxidation of the single cysteine residue in the prohormones and also appears to be an early and rapid event because vanishingly little of the monomer prohormones can be detected in the CC. Complete processing of newly formed Ps takes about 7 h. Recent experiments have shown that conversion of Ps to AKHs and APRPs can be

blocked using the ionophore monensin (Rayne and O'Shea, unpublished). Based on the known mode of monensin action, this result indicates that proteolytic processing of Ps takes place in a post-*trans*-Golgi compartment, presumably the secretory granules. Thus the difference in the kinetics of the two stages of AKH biosynthesis (synthesis of precursor dimers and their subsequent processing) and results of the experiments with monensin suggest that the early post-translational events occur in the endoplasmic reticulum, and secondary processing events occur in secretory granules.

The formation of dimer precursors and the subsequent processing events have been studied directly using in vitro culture methods and the pulse-chase experimental paradigms. Moreover the structural chemistry, including protein sequencing and sizing, were performed on precursor and products (APRPs) isolated and purified from the CC following their identification by pulse-chase experiments. The molecular features of AKH prohormone and dimer precursor biosynthesis which we discovered could not have been inferred from the sequence of cDNA or genomic clones alone. Nucleotide sequences give *deduced proteins* and not the structures of the precursors explicitly. Special features of the CC allowed us to *determine* experimentally rather than deduce the precursor structures. Evidence for their dimeric forms was obtained by performing size exclusion chromatography and protein sequencing on purified precursor in reduced and native form. The dimeric structures could not have been inferred from the sequence of the cDNA alone, neither could the subsequent processing events (see below).

Pro-AKH I (A-chain) and pro AKH II (B-chain) contain the AKH sequences separated by classical processing sites from the 28 residue α and β peptides. The use of this site in processing could perhaps have been correctly inferred from the nucleotide sequences of cDNA clones alone. However, the α and β peptide sequences contain additional potential processing sites (one dibasic and a single arginine, see figs 4 and 6), and their presence might have suggested additional peptides are produced by enzymatic processing within the α and β sequences. We know from protein sequencing, however, that these 28 residues sequences are not processed but remain intact and are present as the three dimeric constructs of APRP 1, 2 & 3.

The conversion of the P1, P2 and P3 dimers into the AKHs and APRPs requires the action of precursor convertases. Many questions remain concerning the number and types of enzymes involved in converting precursors or prohormones into biologically active peptides. For example, how many enzymes are required? How similar are the enzymes in different organisms? How is the synthesis of the convertases regulated? Do the processing enzymes involved in neuronal peptide biosynthesis resemble those which have been isolated from yeast [5] and which appear to have substrate specificities like those which must exist in neurons?

As a first step towards isolating precursor convertases in the CC we are utilizing an in vitro system to reconstitute precursor processing of the AKH I precursor, P1 (fig. 6). In order to perform these experiments we have synthesized the complete 41 amino acid AKH I prohormone. This small protein contains one copy of AKH I, a Gly-Lys-Arg processing site and the 28 residue C-terminal peptide, the α-chain. This synthetic prohormone can be easily oxidized to produce the homodimer P1, the direct precursor of AKH I and APRP 1. When incubated with homogenates of the CC which contain the precursor convertases, synthetic P1 is processed and yields fully processed APRP 1 and intermediates of AKH I processing. The first AKH I processing intermediate produced in this in vitro processing experiment is the C-terminally extended peptide AKH I-Gly-Lys-Arg. This indicates that the first enzymatic action is an endoproteolytic cleavage of P1 between Arg and the N-terminal residue of APRP 1, Asp. The C-terminally extended AKH I is then truncated by two stepwise carboxy-peptidase activities, leading to the production of AKH-Gly-Lys and AKH-Gly. The in vitro conditions under which these events occur do not produce the fully processed amidated AKH I. Experi-

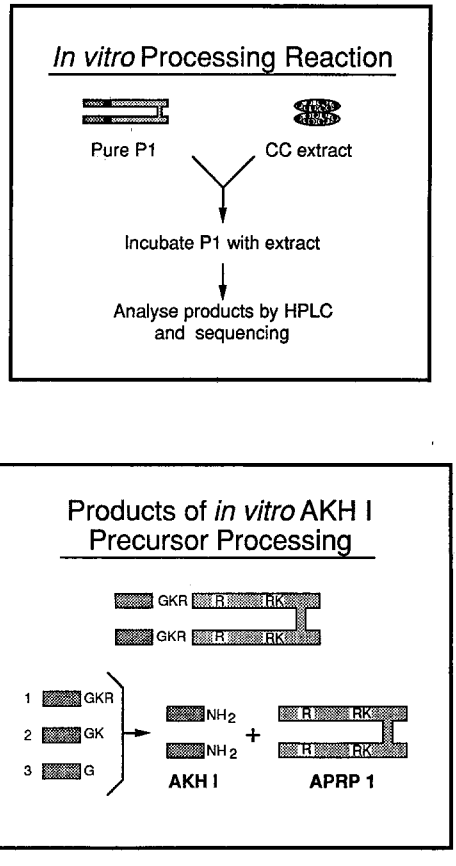

Figure 6. Reconstitution of AKH precursor processing in vitro and analysis of AKH intermediates. In vitro processing reactions are performed as described in the upper panel. The lower panel schematically shows the peptides produced by sequential actions of prohormone convertases present in the CC extracts. Note that potential processing sites (R, RK) are not used. G = glycine; R = arginine; K = lysine.

ments are currently underway to identify those in vitro conditions under which the amidation of AKH-Gly will occur. In other systems[2,15,29] it is thought that two enzymatic steps are involved in converting the C-terminal Gly into the C-terminal amide of the peptide and that amidation requires molecular oxygen, copper and ascorbic acid[4].

By reconstituting in vitro the conditions for defining the properties of the prohormone or precursor convertases we hope to be able to develop assays which will allow us to purify the enzymatic activities to homogeneity. This we see as the first step in an analysis of the structural and functional characteristics of the enzymes involved in the biosynthesis of the AKH peptides. Again, the special features of the CC, its cellular homogeneity in particular, make us confident that the enzymatic activities which we extract from this tissue are the in vivo enzymatic activities. Uncertainty on this point is a serious shortcoming of approaches to the purification of convertases in which the enzymatic activities are extracted from heterogeneous nervous tissue. Our belief that the enzymatic activities which we have identified in the CC extracts are specific and likely to be the same as those used in vivo is strengthened by the fact that we do not see inappropriate processing in vitro. For example, the potential but unused processing sites in the α-chain of P1, the single Arg and the dibasic Arg-Lys, which are not digested in vivo, are not utilized in our reconstituted in vitro processing experiments (figs 3 and 6).

Conclusions and future directions

The adipokinetic hormones and the tissue from which they are derived have provided an almost ideal model for analysing the cellular and molecular mechanisms of peptidergic systems. Our efforts are now focused on the regulation of AKH biosynthesis and a number of questions remain to be answered by future experiments. For example, while we know that strong differential control over AKH I and AKH II biosynthesis is exerted during development, we do not yet know the molecular mechanism for this regulation. At early stages of postembryonic development pro-AKH I and pro-AKH II are synthesized in approximately equal amounts, but in the adult the ratio between pro-AKH I and pro-AKH II reaches approximately 5: 1. This differential regulation of prohormone biosynthesis may be at the level of transcription, translation or through subsequent post-translational events. A complete answer to questions related to differential regulation will await the analysis of the structures of the AKH I and II genes. Preliminary experiments, however, suggest strongly that some of the regulatory mechanisms are translational, suggesting that the differential expression of RNA binding proteins may explain some of the developmental shifts in prohormone ratios. Analysis of possible transcriptional control awaits the completion of genomic cloning.

The CC, primarily because of its homogeneous cellular composition, has allowed us to perform direct experiments on the mechanisms of prohormone and precursor processing. We hope that the in vitro system we have developed in which most of the post-translational events can be reconstituted will eventually allow us to purify the enzymes involved in producing the AKHs and the APRPs. While this work is of fundamental importance in completing our understanding of peptide biosynthesis, it may also have some practical application. For example, it may be possible to develop specific inhibitors of these important biosynthetic enzymes and such inhibitors may be insecticidal. The development of such inhibitors will require the establishment of specific in vitro assays for the enzymes involved and work towards this goal is currently underway.

Much work remains to be done on the biological actions of the AKHs and the newly discovered APRPs. Although both AKH I and AKH II are known to be involved in lipid metabolism, it is very likely that other functions have yet to be discovered. Moreover, there are likely to be distinct functions for these two related but different hormones. No function has as yet been attributed to the APRPs. It will also be important in the future to uncover the functional significance of the dramatic shifts in peptide ratios which occur throughout postembryonic development. These shifts suggest that there is an enhanced role for AKH II early in development but the functions of AKH II in the pre-adult larval stages have not yet been investigated thoroughly.

The need for further experimental work on the function and pharmacology of the AKH peptides will inevitably lead to investigations of the AKH receptor. The fat body tissue in the locust is likely to be an enriched source of AKH receptor and a variety of experimental strategies could be developed towards the ultimate structural characterization of this important receptor. Such work may also have practical application since peptide mimetics, agonists and antagonists of the receptor may be potential leads towards new types of insecticides which interact with peptidergic systems.

Finally, many of the problems associated with analysing neuropeptide biosynthesis in the CC could be alleviated by the development of an immortal cell line from the neuroendocrine cells of the CC. Work is currently underway towards this goal and we expect that, if it is successful, the problems associated with analysing rare proteins of the CC which may be involved in the regulation of biosynthesis, would be greatly simplified.

In conclusion, we feel that the CC has provided a very fertile ground for the study of a model insect peptidergic system. Work on this system has made important contributions to our basic knowledge of peptidergic systems and has provided fundamentally new insights into how neuropeptides are synthesized. We also look forward to exploring the possibilities that neuropeptidergic systems offer a fertile area of development of novel insecticidal

compounds. Such compounds may, for example, interfere with neuropeptide biosynthesis, its regulation, neuropeptide action or neuropeptide inactivation.

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