The Structurally Similar Neuropeptides Adipokinetic Hormone I and II Are Derived from Similar, Very Small mRNAs*

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The grasshopper neuropeptides adipokinetic hormone (AKH) I and II were among the first of an extensive family of structurally similar arthropod hormones and neuroregulators to be isolated and sequenced. This paper reports the cloning of cDNAs derived from the unusually small mRNAs (550 bases) which code for the precursors of AKH I and II from Schistocerca nitans. Sequence analysis of the cDNAs indicates that AKH I and II are derived from small precursor proteins (63 and 61 amino acids) which are 55% identical in amino acid sequence. Each contains a 22-amino acid hydrophobic leader sequence followed by the AKH I or II sequence and an additional 28-amino acid carboxy-terminal peptide of unknown function. Significant homology at the nucleic acid level (64% identity) is confined to the coding region of the mRNA sequences. Preliminary DNA blot analyses suggest that a single gene codes for each, and that the genes for AKH I and II may be linked. Genomic blots from various tissues fail to suggest that the high level of expression of AKH II in the corpora cardiaca is due to tissue specific gene amplification.

The red pigment concentrating hormone-adipokinetic hormone (AKH) family is a particularly large group of arthropod neuropeptides with similar but distinct structures (Table I). Two members of this family, AKH I and II, are found in grasshoppers. These peptides are localized in a few neurons scattered throughout the central nervous system and occur in very high concentration in the glandular lobes of the corpora cardiaca, which is a small (approximately 1 µl) neurohemal organ located just below the brain (Mayer and Candy, 1969; Schooneveld et al., 1983). The AKH peptides serve a variety of functions. When released from the corpora cardiaca they circulate in the hemolymph and stimulate lipid release from fat cells. Studies of AKH bioactivities and the distribution of AKH-like immunoactivity suggest that AKHs contained in neurons are released to modulate the activity of other neurons, skeletal muscle, gut, and heart (Marder et al., 1987; O'Shea et al., 1987a).

The study of AKH related nucleic acids is of interest for several reasons. First, this family of peptides presents an excellent opportunity to investigate neuropeptide evolution. The number of recognized, structurally distinct red pigment concentrating hormone-AKH family members continues to grow as related peptides are isolated from different insects, and it is possible that the family extends beyond the arthropods (Schooneveld et al., 1987; Sasek et al., 1985). Second, the corpora cardiaca offers unique advantages for studying neuropeptide biosynthesis. It contains fairly high concentrations of the small mRNAs encoding AKH I and II precursors, and protein synthesis continues efficiently in organ culture (Hekimi and O'Shea, 1987; Schaffer and Noyes, 1987b). The high concentration of the peptide precursors along with their distinctive dimeric structures (Hekimi et al., 1989) simplifies the study of their vesicular sorting and processing. Finally, red pigment concentrating hormone-AKH peptides should provide favorable model systems for studying the physiological consequences of regulation of neuropeptide biosynthesis, since they function in relatively simple arthropod neural networks with the potential of identifiable neurons (Schaffer and Noyes, 1987a). To begin exploring these interesting features of AKH I and II biosynthesis and the evolution of their family, we have characterized the AKH mRNAs in Schistocerca nitans, and begun the study of the genes from which they are derived. Schulz-Allen et al. (1989) recently reported a similar AKH I cDNA structure from Schistocerca gregaria.

EXPERIMENTAL PROCEDURES

Tissue Collection and Preparation of Nucleic Acid—All tissue was obtained from an inbred colony of S. nitans grown under crowded conditions. Tissue was dissected under insect saline (140 mM NaCl, 5 mM CaCl₂, 5 mM KCl), immediately frozen in a dry ice/ ethanol bath, and stored at −70 °C until nucleic acids were extracted.

RNA was extracted from 1944 corpora cardiaca (715 mg of tissue) processed in lots of 200 corpora cardiaca/1.5-ml microcentrifuge tube using the modified guanidinium chloride procedure described by Iliara et al. (1986). Total RNA (662 µg) was pooled and poly(A)⁺ RNA was isolated using microcrystalline oligo(dT)-cellulose (New England Biolabs) in a batchwise procedure (Aviv and Leder, 1972). The yield of poly(A)⁺ RNA was 13.7 µg. Brain RNA was prepared using the same procedure on 600 mg of tissue collected from several animals.

To isolate DNA from individual animals, nuclei were prepared from testes as described by Kleist and Smith (1988) and digested with 100 µg/ml protease K overnight at 50 °C. DNA was purified by repeated phenol/chloroform extractions, digested with RNase A and protease K, and recovered by ethanol precipitation (Maniatis et al., 1982). DNA from brain and corpora cardiaca was isolated from the guanidinium chloride supernatants recovered from the RNA isolation procedure above. Following removal of RNA by precipitation with 0.55 volume of ethanol, an additional 0.45 volume of ethanol was added to the guanidinium chloride supernatant and the DNA was allowed to precipitate overnight at −20 °C. The DNA was collected by centrifugation, digested with RNase A and protease K, and extracted with phenol.

cDNA Library Construction and Screening—A cDNA library was constructed by Stratagene (La Jolla, CA) from 5 µg of the corpora cardiaca poly(A)⁺ RNA isolated from S. nitans. cDNA was synthesized as described by Huynh et al. (1985) and packaged in three

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J06170 and J06171.

The abbreviations used are: AKH, adipokinetic hormone; kb, kilobases.

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Structure of AKH I and II cDNAs

Table I

<table>
<thead>
<tr>
<th>Genus</th>
<th>Name</th>
<th>Sequence</th>
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<tr>
<td>Pandalus</td>
<td>RPC</td>
<td>pGLu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH₂</td>
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<tr>
<td>Locusta/Schistocerca</td>
<td>AKH I</td>
<td>pGLu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH₂</td>
</tr>
<tr>
<td>Schistocerca</td>
<td>AKH I</td>
<td>pGLu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH₂</td>
</tr>
<tr>
<td>Locusta</td>
<td>AKH II</td>
<td>pGLu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH₂</td>
</tr>
<tr>
<td>Romalea</td>
<td>Ro I</td>
<td>pGLu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH₂</td>
</tr>
<tr>
<td>Periplaneta</td>
<td>M I (C.CJ)</td>
<td>pGLu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH₂</td>
</tr>
<tr>
<td>Periplaneta</td>
<td>M II (C.CII)</td>
<td>pGLu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH₂</td>
</tr>
<tr>
<td>Blaberus/Nauphoeta</td>
<td>HTH</td>
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<tr>
<td>Curculio/Romalea</td>
<td>HTH-G/Ro II</td>
<td>pGLu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH₂</td>
</tr>
<tr>
<td>Manduca/Heliothis</td>
<td>HTH</td>
<td>pGLu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH₂</td>
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<tr>
<td>Heliothis</td>
<td>HézHrTH</td>
<td>pGLu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH₂</td>
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</tbody>
</table>

* C.C., corpora cardiaca.
1 HTH, hypertrehalosemic hormone.
2 HTF, hypertrehalosemic factor.

RESULTS

Selection of AKH I Recombinants—Initial experiments were done with small amounts of total corpora cardiaca RNA because of limited tissue supplies. Screening a genomic library before a corpora cardiaca cDNA library was desirable because the gene library was constructed more rapidly, but the large Schistocerca genome requires a rather long nearly perfect oligonucleotide probe for a reliable screen. It seemed such a probe might be designed based on RNA priming experiments followed by direct cDNA sequencing (Noyes et al., 1979). A mixture of four 11-mers, chosen to complement the mRNA at the carboxyl terminus of AKH I (see Table II), was used to prime the synthesis of cDNA from 1.6 μg of total corpora cardiaca RNA. Although the AKH I message is fairly abundant in the corpora cardiaca, total RNA gives a complex mixture of products in which AKH I cDNA is a minor product at best. A variant of this procedure which omits dCTP from the reverse transcription reaction gives a much simpler set of products including an AKH-derived 23-nucleotide cDNA (Table II) (Schaffer and Noyes, 1987b). Although the reaction conditions were designed to produce a cDNA which extended up to the first G in the mRNA sequence 5' to the priming site, the product actually isolated was produced by a reverse transcriptase pause, probably induced by secondary structure in the message (see below). Presumably because of this pause, the cDNA contained an A at position 21 rather than a G as the AKH I cDNA sequence predicts. Doing this experiment in the presence and absence of dideoxycytidine 5'-triphosphate appears to be an effective way of distinguishing between enzyme pauses and substrate induced stops. In the case of enzyme pauses the size of the product is not influenced by the presence of the dideoxynucleotide which otherwise is added to the cDNA making it one nucleotide longer. This one base increase in size was seen when the two reactions were carried out with corpora cardiaca poly(A)⁺ RNA.

A mixed sequence 25-mer based on these RNA priming experiments (Table II) was not adequate for gene library screening but was used to screen each of three cDNA library fractions. After a final wash at 53 °C in 6 × SSC for 3 min, autoradiography overnight showed numerous positive plaques in each fraction. Half of the positives analyzed were readily identifiable as AKH I recombinants. To isolate a near full-length copy of the AKH I mRNA, a radioactively labeled 130-nucleotide probe from the 5'-end of one of these recombinants (nucleotides 121-250, see Fig. 1) was used to screen fraction I of the library. About 1% of the plaques appeared positive for this probe.
The sequences of the synthetic oligonucleotides used in initial priming experiments and screening the cDNA library for AKH I and II are shown along with the known peptide sequences, the possible mRNA sequences, and the actual sequences of the corresponding cDNAs.

### AKH I

<table>
<thead>
<tr>
<th>Codons</th>
<th>Oligonucleotide probes</th>
</tr>
</thead>
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<tr>
<td>5'</td>
<td>pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH₂</td>
</tr>
<tr>
<td>3'</td>
<td>CA₆U₆G₆-C₆U₆C₆-ACX₆G₆-CCX₆-AA₆G₆-UGG-GGX-ACX₆GGX</td>
</tr>
<tr>
<td>11-mer mix</td>
<td>TY₆T₆T₆ACC-CC₆T₆T₆G</td>
</tr>
<tr>
<td>3'</td>
<td>TTT₆A₆G₆-GGG₆TG₆T₆-ACC-CC₆T₆T₆G</td>
</tr>
<tr>
<td>25-mer</td>
<td>AC₆-TTA₆A₆G₆-GGG₆T₆T₆-ACC-CC₆T₆T₆G</td>
</tr>
<tr>
<td>cDNA clone</td>
<td>CAG-CTC-AAC-CCC-ACC-ACC-TGG-GGC-ACC-GCC</td>
</tr>
</tbody>
</table>

### AKH II

<table>
<thead>
<tr>
<th>Codons</th>
<th>Oligonucleotide probes</th>
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<tbody>
<tr>
<td>5'</td>
<td>pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-NH₂</td>
</tr>
<tr>
<td>3'</td>
<td>A₆CX₆U₆U₆U₆C₆-ACX₆G₆-GGG₆GGX</td>
</tr>
<tr>
<td>Probe I</td>
<td>TTT₆A₆G₆-TGG₆CG₆-ACC-CCG</td>
</tr>
<tr>
<td>Probe II</td>
<td>TTT₆A₆G₆-TGG₆CG₆-ACC-CCG</td>
</tr>
<tr>
<td>Probe III</td>
<td>TTT₆A₆G₆-TGG₆CG₆-ACC-CCG</td>
</tr>
<tr>
<td>cDNA clone</td>
<td>CAG-CTC-AAC-CCC-ACC-ACC-GGG-TGG-GGG</td>
</tr>
</tbody>
</table>

**Fig. 1.** Nucleotide and deduced amino acid sequence of AKH I precursor. The numbers refer to the nucleotide sequence. Translation is assumed to initiate at the first Met (position 49) and continue to the first stop codon at position 238 to give a precursor peptide of 63 amino acids. The mature AKH I peptide (115-144) is in **bold** letters. Sequences of consecutive basic amino acids are underlined. The first forms the carboxyl-terminal processing site of AKH I. The significance of the second is unknown. A canonical polyadenylation signal beginning at nucleotide 350 is underlined.

after two 1-h washes at 68 °C in 0.5 x SSC. Two of the recombinants that were selected and sequenced contained the longest, and exactly the same length of, non-poly(A) sequence which is shown in Fig. 1. Another seven AKH I recombinants were sequenced. All contain a poly(A) tail and extend variable lengths toward the 5'-end of the message. All sequences are identical except for minor allelic variations in the 3'-untranslated region. Three of nine clones have an A at position 251 instead of G, and five of nine have a G at position 267. Some recombinants have the additional sequence ATT or ATTAG just before the poly(A) tail. Even in the presence of one of these longer sequences, a canonical polyadenylation signal, AAATAAA, occurs within 21 nucleotides of the poly(A) tail (Proudfoot and Brownlee, 1976).

**AKH I mRNA—RNA blots of corpora cardiaca poly(A)⁺ RNA probed with a single strand DNA synthesized from an AKH I M₁₃ recombinant reveal a single size class of AKH I message centered at 550 nucleotides (Fig. 2). Washing the filters at different stringencies from 2 x SSC at room temperature to 0.1 x SSC at 60 °C did not change the pattern, and never revealed a signal in brain RNA despite the use of 30-fold more poly(A)⁺ RNA.

The size of the message suggests that the longest clones (371 nucleotides beyond the poly(A)) are essentially full length. This was confirmed by analyzing cDNAs made with reverse transcriptase and corpora cardiaca poly(A)⁺ RNA primed with either the 11-mer mix described above or with a 17-nucleotide primer designed to hybridize to position 47-63 in the known sequence. The poly(A)⁺ RNA, unlike total RNA, gives a very simple set of products with one major species arising from each primer (Fig. 3). The size of each product was determined by comparison to a dideoxy-nucleotide sequencing ladder constructed from an AKH I M₁₃ recombinant. The AKH I message, then, begins with a 62-nucleotide 5'-untranslated region with is open in all reading frames at least for the 48 nucleotides of known sequence. The first Met codon occurs in the frame which codes for AKH I, and this ATG is preceded by an A in the −3 position as expected (Cavener, 1987). Codon usage follows the pattern seen in highly expressed Drosophila messages (Sharp et al., 1988).
Structure of AKH I and II cDNAs

**FIG. 2.** Blot analysis of *S. nitans* RNA. Glyoxylated RNAs from corpora cardiaca (CC) and brain were electrophoresed on 1.4% agarose, transferred to nitrocellulose, and hybridized with a $^{32}P$-labeled (1.4 $\times$ 10^6 dpm/µg, 6.4 $\times$ 10^5 dpm/ml) single strand DNA probe specific for the AKH I mRNA (nucleotides 1-356, Fig. 1). 1st through 4th lanes show results obtained with 1 µg of total RNA, no RNA, 1.1 µg of poly(A)+ RNA from corpora cardiaca, 5th through 8th lanes show results for 1.5 µg of poly(A)+ RNA, 2.9 µg of poly(A)+ RNA, no RNA, and 8.3 µg of total RNA from brain. The sizes, in number of bases, and distances migrated for HaeIII restriction fragments of φX174 DNA are indicated on the left of the figure. Autoradiography was at -70 °C for 18 h.

produced no positives plaques. It seemed unwise to reduce the stringency of the screen since the nucleic acid sequences of AKH I and II were potentially very similar, and if mRNA levels paralleled the levels of mature peptide observed by high performance liquid chromatography of extracts (Schaffer and Noyes, 1987b; Fig. 1) the AKH I clones should be about 5.4 times more numerous than those derived from AKH II. Therefore two mixed sequence oligonucleotides (Table II, probes I and II) were tested for their ability to prime cDNA synthesis from corpora cardiaca poly(A)+ RNA. As with the AKH I 11-mer mix (Fig. 3), none of the oligonucleotides produced significant products from corpora cardiaca poly(A)+ RNA or brain poly(A)+ RNA. Probe II failed to prime corpora cardiaca poly(A)+ RNA, while probe I gave two minor products, one at the position expected if priming was from AKH I mRNA, and one 10 nucleotides larger. It seemed likely that the second product was AKH II-derived, and that modification in the sequences of this mixture might produce a suitable probe. Therefore a unique sequence 20-mer was synthesized incorporating a change in the Gly codon (Table II, probe III).

**FIG. 3.** Determination of the length of the AKH I mRNA. AKH I-specific primers (1 pmol, 7-9 $\times$ 10^6 dpm/pmol) were annealed to 25 ng of RNA and extended with avian myeloblastosis virus reverse transcriptase. Products were electrophoresed on an 8% polyacrylamide, 8 M urea gel and visualized after autoradiography at -70 °C with intensifying screens for 2 h. 1st through 4th lanes show products obtained with no RNA, brain poly(A)+ RNA, corpora cardiaca poly(A)+ RNA, and corpora cardiaca poly(A)+ RNA primed with the AKH I-specific amino-terminal 17-mer, 5'-d(GCACCGCTGCACC-ATCC). cDNA products from the same series of RNAs primed with the AKH II 11-mer mix (Table II) are shown in the 9th through 12th lanes (AKH OLIGO). Dideoxynucleotide sequence analysis of an AKH I-M13 recombinant (nucleotides 1-356; Fig. 1) primed with the $^{32}P$-labeled amino-terminal 17-mer is shown in the 5th through 8th lanes. The numbers at the left mark sizes in number of nucleotides as determined from this dideoxynucleotide sequence ladder. The position of the EcoRI linker between the AKH I cDNA sequence and the M13 vector sequence is also indicated.

When cDNA was synthesized using this oligonucleotide and corpora cardiaca poly(A)+ RNA, a single, major product of 165 nucleotides was obtained.

Fraction I of the cDNA library was screened with this 20-nucleotide probe. After two final 10 min washes at 50 °C and autoradiography overnight at -70 °C, 0.2% of the plaques were positive. Six of these were sequenced, and all were AKH II-derived. This result suggests that the AKH II mRNA, like the peptide, is about 5-fold less abundant than AKH I mRNA.

The longest of the AKH II cDNA inserts was excised by EcoRI digestion and subcloned into M13. Its sequence is presented in Fig. 4. Four other recombinants that have been sequenced possess different size poly(A) tails and the same
sequence extending variable lengths toward the 5'-end of the message. One of these has a single base change (T to C) at position 186 in the protein coding region, but this does not alter the predicted amino acid sequence of the peptide.

**AKH II mRNA**—The filter shown in Fig. 2 was stripped and hybridized with a single strand probe made from an AKH II M13 recombinant. Hybridization and washing were done exactly as for the AKH I probe. The result was indistinguishable from that shown in Fig. 2 except that, as expected, when the specific activity of the probes and the exposure times were equal, a much weaker signal was obtained from the AKH II message than from AKH I mRNA. Cross-hybridization between AKH I and AKH II recombinants is not detected with these probes when blots are washed at 60 °C in 0.1 M SSC.

The fact that probe III primes the synthesis of a 165-nucleotide cDNA from corpora cardiaca poly(A)+ RNA indicates that the AKH II mRNA extends 40 nucleotides beyond the longest recombinant characterized and has a 5'-untranslated region of about 73 nucleotides. Thus AKH I and II mRNAs are about the same size, although AKH II has a slightly longer 5'-untranslated region than AKH I. Also, AKH I has a longer 3'-untranslated region. Comparison of the two sequences using the computer program LFASTA (Pearson and Lipman, 1988) shows 64% identity with three gaps over a 218-nucleotide region of about 73 nucleotides. Thus AKH I and II coding regions were prepared from M13 recombinants to confirm the identity of the two genes on alkaline DNA blots. Hybridization analyses of testes DNA digested with EcoRI, BamHI, HincII, or HindIII produce similar patterns of positive fragments with either AKH I- or II-specific probes suggesting a single gene for each. The cDNA sequence predicts that the AKH I gene should produce two positive fragments after digestion with HincII and that is the case. When DNA from pooled tissue of many animals is analyzed following EcoRI digestion, three fragments hybridize with the AKH I probe (Fig. 6, AKH I, lanes 5–7). This is the result of restriction fragment length polymorphisms linked to the gene, since the same analysis performed on DNA from single individuals produces one or two positive fragments (Fig. 6, AKH I, lanes 1–4).

Hybridization with a probe specific for AKH II reveals two positive fragments in each enzyme digest. The results for EcoRI are shown in Fig. 6, AKH II. Here, as with other restriction fragments larger than 4 kb, one of the two positive fragments comigrates with an AKH I-positive fragment in the same digest, suggesting that the two genes may be linked. The pattern seen with individual 3 (Fig. 6, lane 3) is consistent with this possibility to the extent that this animal was heterozygous in the size of both AKH I and II EcoRI fragments. The second AKH II fragment runs very close to the 13.5-kb fragment present in all individuals, but its presence was confirmed by examining the autoradiograph. If the two genes are linked, the fact that the second AKH I fragment is a different size than the second AKH II fragment (7 and 14 kb, respectively) requires that in the second allele several EcoRI sites must be different or there must have been a significant
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AKH Biosynthesis—The frequency of cDNA clones in our library, the amount of product obtained from reverse transcription, and the intensity of signals on RNA blots all suggest that, in corpora cardiaca, AKH I mRNA is about five times more abundant than AKH II mRNA. This parallels the levels of the mature peptides. Hekimi and O’Shea (1988) have found that the mature AKH peptides in the corpora cardiaca of the closely related gregaria species are derived from three 8-kDa precursors. These precursors are the three possible dimers of the pro-AKH peptides formed through the single cysteine residue found in each (pro-AKH I dimer, the mixed dimer, and pro-AKH II dimer). Clearly the same structures occur in the corpora cardiaca of Locusta migratoria (Heitter et al., 1989). Data on the relative abundance of the dimeric precursors (Hekimi and O’Shea, 1989; see Fig. 2) suggest a binomial distribution among the possible structures assuming a five to one ratio of initial transcripts. Thus it seems the relative amounts of corpora cardiaca mRNA dictate the relative amounts of mature peptides, and the dimeric intermediates form at random among the possibilities. A simple mechanism compatible with these results is that the pro-AKHS are sorted into vesicles at high concentrations and dimer formation proceeds spontaneously. It remains to be demonstrated, however, exactly when, where, and how the disulfide bond is formed. Certainly the insect corpora cardiaca provides a very convenient experimental system for the study of synthesis and processing (O’Shea et al., 1984; Schaffer and Noyes, 1987a; Hekimi and O’Shea, 1989) that can be exploited to delineate the metabolism of these precursors, and the physiological significance of their dimeric structures.

Peptide Processing—Processing of the mature peptides includes the conventional steps of conversion of glutamine to pyroglutamic acid (Fischer and Spiess, 1987), cleavage at double basic amino acid sequences (Lynch and Snyder, 1986), and amide formation from a glycine in the precursor (Bradbury et al., 1982). Cleavage of the signal sequences likely exposes the amino termini of the AKHs. This appears also to be true for SCPB (Mahon et al., 1985), vasopressin (Land et al., 1982), and oxytocin (Land et al., 1983), and a number of larger neuropeptides (Sossin et al., 1989). The COOH-terminal peptides each contain an Arg-Lys sequence which apparently is not a cleavage site in the corpora cardiaca of Schistocerca or Locusta since the intact dimers accumulate in and are released from the corpora cardiaca (Hekimi and O’Shea, 1989; Heitter et al., 1989). This may be a consequence of steric hindrance around the disulfide bond or sequence specificity of the endopeptidase in this tissue. There are certainly many examples of tissue specific processing of neuropeptides (Lynch and Snyder, 1986), so it is possible that this sequence serves as a proteolytic site in other tissues such as neurons.

Evolution of the AKH Family—The AKH RNAs are very small and show clear homology to each other although this is confined to the protein coding region. On the other hand a partial AKH I cDNA sequence from S. gregaria (Schulz-Allen et al., 1989) shows 95.2% homology to the nitsans sequence over the 330 nucleotides of available sequence. This striking homology extending through the 3' untranslated region supports the close relationship between the two species. The structure of the Locusta precursors has not yet been determined but the COOH-terminal peptides show very similar sequences. For example, the AKH II associated COOH-terminal peptide inferred from the cDNA sequence of nitsans...
differs from that of Locusta by only two commonly seen changes (Arg to Lys and Asn to Asp). The COOH-terminal peptides of nitans AKH I and II precursors show significantly more difference suggesting that the two peptides diverged before the separation of these two grasshopper genera. A recently isolated genomic clone predicts the same general precursor structure for Manduca AKH (Bradfield and Keeley, 1989). Features of the precursor include a similar pattern of processing sites and a homologous cysteine in the COOH-terminal peptide, raising the possibility of dimer formation. The Arg-Lys sequence is not present in the moth COOH-terminal peptide and the remaining single Arg does not fit the suggested recognition sequences for cleavage at single Arg residues (Benoit et al., 1987). The predicted peptide, without signal sequence, shows 34% amino acid identity (with one gap) when aligned with the AKH I precursor sequence by LFASTA. Homology at the nucleotide level is restricted to 30 nucleotides encoding the AKH peptides. Searches of the EMBL and GenBank nucleic acid sequence and the NBRF protein database with the related programs FASTA and TFASTA failed to identify any additional related structures. 

AKH Gene Structure—There is an interesting difference between the AKH I and II genes. When the AKH I sequence is used to probe DNA blots, the observed hybridization pattern is easily explained by the presence of a single gene linked to various restriction fragment length polymorphisms in the population. While the AKH I probe detects a single fragment in several different enzyme digests, the AKH II probe invariably shows at least two bands even though the enzymes used do not cleave within the AKH II sequence. Although one of the AKH II-positive fragments co-migrates with an AKH I-positive fragment when the latter exceeds 4 kb, this is not a case of cross-hybridization as demonstrated by recombinant controls run in parallel and by the detection of different size AKH I- and II-positive fragments 2.8 kb and smaller. The data raise interesting possibilities, such as a second neuron-specific AKH II gene, but a simple alternative explanation is that there is a single AKH II gene which contains a large intron causing the mRNA sequence to always be split between two restriction fragments. This issue will be clarified as AKH I and II genomic recombinants are isolated and characterized. 

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