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Predicted versus expressed adipokinetic hormones, and other small peptides from the corpus cardiacum–corpus allatum: A case study with beetles and moths

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ABSTRACT

This mass spectrometric study confines itself to peptide masses in the range of 500–1500 Da. Adipokinetic hormones (AKHs) that are predicted from the genome of the red flour beetle, *Tribolium castaneum*, and the silk moth, *Bombyx mori*, are shown to exist as expressed peptides in the corpora cardiaca (CC) of the respective species as evidenced by various mass spectrometric methods. Additionally, some related species were included in this study, such as the tenebrionid beetles *Tribolium brevicornis* and *Tenebrio molitor*, as well as the moths *Spodoptera frugiperda*, *Spodoptera littoralis*, *Mamestra brassicae* and *Lacanobia oleracea*, to investigate whether AKH peptides are structurally conserved in the same genus or family. Interestingly, the AKH peptide of *T. brevicornis* is identical to that of *T. molitor* but not to the ones of its close relative *T. castaneum*. Moreover, other peptides in *T. brevicornis*, such as various FXPRL amides (=pyrokinins), also match the complement in *T. molitor* but differ from those in *T. castaneum*. All the CC of beetles lacked the signal for the mass of the peptide corazonin. All moths have the nonapeptide Manse-AKH expressed in their CC. In addition, whereas the silk moth has the decapeptide Bommo-AKH as a second peptide, all other moths (all noctuids) express the decapeptide Helze-HrTH. In *M. brassicae* and *L. oleracea* a novel amidated Gly-extended Manse-AKH is found as a possible third AKH. The noctuid moth species also all express the same FLRF amide-I, corazonin, and a group-specific isoform of a γ-PGN-(=γ-SGNP) peptide. In *L. oleracea*, however, the latter peptide has a novel sequence which is reported for the first time, and the peptide is code-named Lacol-PK.

1. Introduction

Neuropeptides represent the largest single class of regulatory compounds in invertebrates, as well as in vertebrates. In insects there are various neuropeptide families, the members of which mostly occur in multiple forms and display pleiotropic actions [12]. One of the largest such family is the adipokinetic hormone (AKH) peptide family, of which a

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plethora of information has been gathered during the last 30 years [11,14]. These neuropeptides are synthesized in neurosecretory cells of the insect's corpus cardiacum (CC). They are from 8 to 10 amino acids long, and are structurally characterized by a blocked N-terminus (pGlu) and C-terminus (carboxyamide), by the occurrence of aromatic amino acids at positions 4 (Phe or Tyr) and 8 (Trp), and by a Gly residue at position 9 [11,14]. The majority of AKHs are not charged under physiological conditions.

The major function of these AKHs is to mobilize stored products (fat, glycogen or proline) by the activation of phosphorylases or lipases, but many other functions such as myostimulation, inhibition of the synthesis of RNA, fatty acids and protein in the fat body, and stimulation of substrate oxidation in the flight muscles are known to be regulated by AKH. They may also be involved in the insect immune response [14]. Many isoforms of AKHs have been identified and these peptides are present in all major insect orders [19,20].

Recently, the genome of certain insect species has been elucidated and the data are available, e.g. for *Tribolium castaneum* [http://hgsc.bcm.tmc.edu/projects/tribolium/] and for *Bombyx mori* [http://silkworm.genomics.org.cn/index.jsp; http://sgp.dna.affrc.go.jp/; http://www.ncbi.nlm.nih.gov/]. The search for structural homologs of adipokinetic peptides in these databases reveals the presence of such peptides, but this information does not indicate that the peptides are, indeed, expressed in these species. Our knowledge on AKHs in the honey bee *Apis mellifera* may support this idea that genomic presence does not guarantee expression of the encoded protein/peptide. With conventional protein chemical methods it was shown that the Italian race of the honey bee, *A. mellifera ligustica*, contains a peptide with hypertrehalosemic activity and which is structurally identical to the adipokinetic peptide of the tobacco hornworm moth, *Manduca sexta*, i.e. Manse-AKH, a nonapeptide (pELTFTSSWG amide) [40,55]. The genome of the honey bee, which was sequenced from DH4 strain of *A. mellifera*, did not, however, reveal the presence of a precursor for Manse-AKH. In contrast, an octapeptide with the sequence pELNFSTGW amide, code-named Schgr-AKH-II and first sequenced from locusts [18,49], was found to be encoded in the honey bee genome [27]. The DH4 strain of honey bee was principally *A. mellifera ligustica* and a mixed product that included the SMR (suppression of mite resistance) trait (D. Weaver, B. Weaver Apiaries, personal communication).

By protein chemical methods, Schgr-AKH-II had been found in the CC of a number of other hymenopteran species [16,41]. Surprisingly, when honey bee tissues (including CC) from adult workers of the dark European race, *A. mellifera carnica* [4] or whole heads from a mixture of the races *A. mellifera ligustica* and *A. mellifera carnica* [27] were screened by mass spectrometric techniques, neither Schgr-AKH-II nor Manse-AKH were detected. Thus, it appears that the peptide Schgr-AKH-II encoded in the honey bee genome may not be expressed (at least in the tissues investigated), or is expressed in amounts too small to be detected by mass spectrometry, and it is unclear whether Manse-AKH is indeed synthesized in the CC of honey bees.

In the present study, we sought to determine whether the AKHs that are predicted from the genome of *T. castaneum* and *B. mori* can be identified by mass spectrometric analysis (i.e. by mass data and sequencing information) in extracts prepared from the CC of these insects. Furthermore, we have expanded the group of insects under investigation to include some related species to investigate whether AKHs are structurally conserved in the same genus or insect family. For these purposes, the beetle *Tribolium brevicornis*, thought to be closely related to *T. castaneum* [43], and also a perhaps more distantly related tenebrionid, *Tenebrio molitor*, were included in the study. Many neuropeptides from the CC of *T. molitor* are known [21,53] and serve as confirmation of the methods employed. Similarly, we analyze not only the AKH and some other peptides of the CC–CA of *B. mori*, but also from additional lepidopteran species, viz. *Spodoptera frugiperda*, *Spodoptera littoralis*, *Mamestra brassicae* and *Lacanobia oleracea*, with the aims of: (i) looking for three different classical AKHs in *S. frugiperda* CCs which may be expressed, based on a cDNA study [1]; (ii) to check the hypothesis that closely related species contain the same (or similar) AKH peptides, hence the inclusion of *S. littoralis*; and (iii) to study the neuropeptide complement from a comparative point of view in species where some information on AKHs already exists (*M. brassicae*; [7]) or is known from larvae but not adults (*L. oleracea*; [2]).

To achieve these aims, CCs are first screened by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry to detect the presence of AKHs. Sequence information is gathered from electrospray—or MALDI-TOF MS/MS data. We also report on masses of other prominent ions and use that information together with the data on AKHs to speculate on the relationship of certain species.

2. Materials and methods

2.1. Insects

CCs were dissected from adult specimens of indeterminate age. All species under investigation, except *B. mori* and *S. frugiperda*, were reared in the insectaries of the Animal Services and Invertebrate Supply Team, Central Science

Abbreviations: AKH, adipokinetic hormone; Anoga, *Anopheles gambiae*; Bommo, *Bombyx mori*; CA, corpora allata; CAP_{2b}, cadioactive peptide 2b; CC, corpora cardiaca; CID, collision-induced dissociation; DH, diapause hormone; ESI, electrospray ionization; FXPRL amides, pyrokinins; γ-PGN, γ-PBAN-encoding gene neuropeptide; γ-SGNP, γ-suboesophageal ganglion neuropeptide; Helze, *Heliothis zea*; HrTH, hypertrehalosemic hormone; Lacol, *Lacanobia oleracea*; LC, liquid chromatography; Leuma, *Leucophaea maderae*; Locmi, *Locusta migratoria*; MALDI, matrix-assisted laser desorption/ionization; Mambr, *Mamestra brassicae*; Manse, *Manduca sexta*; Melcin, *Melittea cinxia*; Melme, *Melolontha melolontha*; MRCH, melanization and reddish-coloration hormone; Ms, myosuppressin; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MT, myotropin; NVP, NVP-motif containing peptide; PBAN, pheromone-biosynthesis-activating neuropeptide; Peram, *Periplaneta americana*; Phote, *Phormia terraenovae*; PK, pyrokinin; PSD, post-source decay; Pyrap, *Pyrrhocoris apterus*; RT, retention time; Scade, *Scarabaeus deludens*; Schgr, *Schistocerca gregaria*; Spofr, *Spodoptera frugiperda*; Spoli, *Spodoptera littoralis*; tBME, *t*-butylmethyl ether; Tenmo, *Tenebrio molitor*; TFA, trifluoroacetic acid; TOF, time-of-flight; Trica, *Tribolium castaneum*.