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ABSTRACTS

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New insights into structure and function of peptides of the Adipokinetic Hormone peptide family in insects

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This State-of-the-Art lecture reviews the Adipokinetic hormone/Red pigment-concentrating hormone (AKH/RPCH) family of peptides from the time that the first peptide members were sequenced in decapod crustaceans and insects (1972 and 1976, respectively), to date when approximately 70 different AKH structures from insects have been sequenced or deduced through mass spectrometry or nucleotide information. AKHs are neuropeptide hormones produced in the corpora cardiaca of insects and released into the haemolymph to bind to extracellular domains of specific G-protein coupled receptors (GPCRs). Initially shown to be engaged in energy metabolism in insects, it is now accepted that the AKH peptides are pleiotropic. For example, previous research demonstrated that AKH is myostimulatory and involved in immune responses, as well as activating salivary enzymes and anti-oxidative mechanisms. In addition, we will look specifically at recent data of the effect of AKH on the crop in flies.

Because AKHs are involved in so many key biological activities, this peptide is a probable target for combatting so-called pest insects via a specific biorational approach. It is, therefore, important to investigate the complement of AKH peptides in different insect species to ascertain sequence specificity and to investigate the secondary structures of these octa-, nona- and decapeptides via NMR and modelling. Further, it is necessary to also study the GPCR structures, and interactions with the AKH peptide ligands through receptor-binding assays and computational modelling. Such a data composite would supply information about potential targets for peptide mimetics.

This lecture reviews rare structural modifications to the basic general plan of an AKH and provides the experimental support/evidence for these findings. For example, one of the Indian stick insect's AKH peptide is modified with a C-glycosylation at the C-2 atom of the indol ring of Trp. In three other insect species, the AKH peptide is modified at the sixth amino acid residue: Pro^6 is hydroxylated in the green stinkbug, Thr^6 is phosphorylated in a Protea beetle, and Thr^6 is sulphated in a twig wilter. These are modifications that cannot be deduced from nucleotide data, and these peptides were identified as AKHs from their biological activity in mobilising stored energy in the respective insects.

Many AKH GPCRs have been cloned and characterised following the drive for whole-genome sequencing of organisms. One such AKH receptor was recently characterised from the kissing bug; knock-down of this AKH receptor resulted in a statistically significant change in the expression of certain genes in the fat body that are involved with lipid metabolism.

Future research will intensify ligand/receptor modelling studies including the usage of peptidomimetics in order find greener insecticides to combat pest insects such as locusts, moths and pine weevils, for example.

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Structure-activity relationship of adipokinetic hormones in *Hippotion eson*

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The adipokinetic hormone (AKH) family of peptides is large and well-known for their regulatory role in energy metabolism in insects. Members of this peptide family have characteristic structural features, amongst others, a chain length of 8–10 amino acid residues, Trp^8, and blocked termini: pGlu at the N terminus and an amidated C terminus. In most insects only one G protein-coupled receptor for AKH peptides is identified, and through strategic amino acid substitutions in the ligand, one can glean information on the importance of specific residues for receptor interactions and for eliciting biological activity in a specific insect.

We previously demonstrated a record number of five peptides belonging to the AKH family in the corpora cardiaca of the sphingid moth, *Hippotion eson*: two octa-, two nona- and one decapeptide. Each of these endogenous AKHs, including the decapeptide Manse-AKH-II: pGlu-Leu-Thr-Phe-Ser-Ser-Gly-Trp-Gly-Gln amide, is active in an in vivo lipid-mobilising assay. We further demonstrated that Lacol-AKH (pGlu-Leu-Thr-Phe-Thr-Ser-Ser-Trp-Gly-Gly amide), from a noctuid moth, also elicited a maximal biological response in *H. eson* (even though this peptide has 3 amino acid substitutions compared to Manse-AKH-II). On the other hand, the water bug's Lacsp-AKH (with 4 amino acid substitutions compared with Lacol-AKH: pGlu-Val-Asn-Phe-Ser-Pro-Ser-Trp-Gly-Gly amide), was not active in *H. eson*. This presented us with an opportunity to investigate which of the substitutions could not be tolerated well by the G protein-coupled receptor for AKHs in *H. eson*.

Lacol-AKH was, thus, used as a lead peptide on which a series of AKH analogs were based to represent: (a) single amino acid replacements (according to the substitutions in Lacsp-AKH) and also Ala as replacement residue, (b) shorter chain lengths, (c) modified termini, and (d) a replacement of Trp in position 8. These analogs were tested in in vivo adipokinetic assays to gain insight into the ligand–receptor interaction in *H. eson*. Our results show that the second and third amino acids are important for biological activity in the sphingid moth. Analogs with an [*N*-Ac-Glu^1] or Ala^1 (instead of a $pyroGlu^1$), or a free C terminus, or Ala^8 were not active in the bioassays, while shortened Lacol-AKH analogs showed very reduced activity (below 25 %). Our recent preliminary data set shows that analogs with Glu^1 or Gln^1 have 21 and 54 % activity, respectively, and it is deduced that these residues could be cyclised in vivo to pyroGlu, and hence elicit biological activity. The single residue replacement peptide with Ala^4 significantly affected peptide activity and a response of only 9 % was recorded, whereas the Ala^6 analog mobilised lipids albeit with an activity of 25 %. Ala^5 or Ala^7 analogs were better tolerated by the *H. eson* GPCR (86 and 61 % activity, respectively). This information is important for the consideration of peptide mimetics to combat specific lepidopteran pest insects.

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