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Water scorpions (Heteroptera, Nepidae) and giant water bugs (Heteroptera, Belostomatidae): Sources of new members of the adipokinetic hormone/red pigment-concentrating hormone family

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ABSTRACT

Two novel octapeptide members of the AKH/RPCH family have been identified from the corpora cardiaca (CC) of two species of water bugs. The giant water bug *Lethocerus indicus* (family: Belostomatidae) contains a peptide code-named Letin-AKH with the sequence pGlu-Val-Asn-Phe-Ser-Pro-Tyr-Trp amide, and the water scorpion *Nepa cinerea* (family: Nepidae) has the peptide code-named Nepci-AKH with the sequence pGlu-Leu/Ile-Asn-Phe-Ser-Ser-Gly-Trp amide. The sequences were deduced from the multiple MS^N electrospray mass data from crude CC extracts. Synthetic peptides were made and co-elution on reversed-phase high performance liquid chromatography (RP-HPLC) with the natural peptide from crude gland extract confirmed the accuracy of the deduced sequence for Letin-AKH and demonstrated that Nepci-AKH contains a Leu residue at position 2 and not an Ile residue. A previously characterized member of the AKH/RPCH family was identified in the stick water scorpion *Ranatra linearis* by mass spectrometry: Grybi-AKH (pGlu-Val-Asn-Phe-Ser-Thr-Gly-Trp amide) has the same mass (919 Da) as Nepci-AKH and differs in two positions from Nepci-AKH (residues 2 and 6). The apparent function of the peptides is to achieve lipid mobilization in the species under investigation; indications for this came from conspecific bioassays using the appropriate synthetic peptides for injecting into the insects. This function is very likely linked to dispersal flight metabolism of water bugs. Swimming activity in *N. cinerea* also results in an increase in lipid concentration in the hemolymph.

1. Introduction

In insects, peptides of the adipokinetic hormone (AKH)/red pigment-concentrating hormone (RPCH) family have been detected in every major order [8,9].

Although the peptides have diverse and multiple functions, the major action is to regulate the mobilization of stored energy substrates (triacylglycerols, glycogen and proline) during times of intense metabolic activity such as flight, swimming or running [8,12,17]. Previously, we showed that *Notonecta glauca*, a backswimmer belonging to the heteropteran family Notonectidae, uses an endogenous octapeptide (code-named Anaim-AKH) during bouts of natural swimming and flight [14]. A recent analysis of another heteropteran

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family (Corixidae) showed a novel member of the AKH/RPCH family, code-named Corpu-AKH, in the water boatman, *Corixa punctata*; Corpu-AKH is structurally similar to Anaim-AKH with only a Leu^2/Val^2 exchange [18]. Corixidae are well known for their dispersal flight ability and our functional data indicated that the AKH may be used to control lipid-based flight metabolism and, certainly, swimming activity [18]. There is another large clade of water bugs, the Nepoidea, which consists of the water scorpions, Nepidae, and the giant water bugs, Belostomatidae. The two major subfamilies of the Nepidae are the Ranatrinae and Nepinae with their representatives *Ranatra linearis* and *Nepa cinerea*, respectively. In both species both pairs of wings are well developed and functional, but in general *R. linearis* is better known to undertake flights than *N. cinerea* [20]. However, when studying small lakes and also temporal water bodies in northern Germany, *N. cinerea* can be found almost in each of them, suggesting that dispersal by flight should be quite common [Rolf Niedringhaus, personal communication]. On the other hand, the giant water bugs, which are represented in this study by a member of the subfamily Belostomatinae, are known to be attracted to light traps and may fly several kilometres [2]. They are also a classical study object for the isolation and characterization of all types of proteins in their flight muscles [25]. Although both Nepidae and Belostomatidae have their forelegs modified as raptorial limbs to catch prey, both clades can swim; Belostomatidae swim best, and *R. linearis* better than *N. cinerea* [20].

The current investigation was prompted by this dispersal behavior and our recent success in finding a novel AKH/RPCH member in a water bug [18]. We, therefore, studied primarily the complement of AKH/RPCH family peptides in the corpora cardiaca of water scorpions and giant water bugs and, secondly, performed some biological and functional assays to decide which substrate mobilization may be controlled by these peptides.

2. Materials and methods

2.1. Insects

Adult specimens of both sexes and unspecified age of the water scorpions, *N. cinerea* (subfamily: Nepinae) and *R. linearis* (subfamily: Ranatrinae), were collected from a sluggish flowing stream or stagnant, vegetation-rich ponds in the vicinity of Debrecen (Hungary) in August/September 2004 and 2005; another batch of *N. cinerea* was netted from a small lake close to Lingen (Germany) in September 2006. Adult giant water bugs, *Lethocerus indicus* (subfamily: Lethocerinae), of both sexes and unknown age were a gift of Dr. Kevin Leonard (European Molecular Biology Laboratories, Heidelberg, Germany) and were originally collected in Thailand. For bioassays, adult specimens of both sexes and unknown age of *L. niloticus* from either Guinea-Bissau (commercially supplied by Riverzoo Farm) or from Namibia (collected in a small lake close to Ondangwa during December 2006) were used. For heterologous bioassays, adult 10- to 18-day-old male migratory locusts, *Locusta migratoria*, were used; their rearing is outlined elsewhere [6].

2.2. Tissue preparation and peptide isolation

Corpora cardiaca (CC) were dissected with the help of a stereomicroscope. The glandular material was placed in 80% methanol, and extracts were prepared as outlined previously [16]. Such dried extracts were either sent to České Budějovice (Czech Republic) for mass spectrometric analyses (see below), used for conspecific and heterologous bioassays (see below) or used for purification of the active peptides on reversed-phase high performance liquid chromatography (RP-HPLC) as outlined previously ([4]; see also legend to Fig. 4).

2.3. Bioassays

The heterologous bioassay in locusts was performed as described previously [3]. Conspecific bioassays were performed on *N. cinerea* and *L. niloticus* 48–72 h after capture; the body mass of the water bugs used in the assays was 163.2 ± 38.9 mg (mean ± S.D., $n = 13$) for *N. cinerea* and 6.0 ± 0.3 g (mean ± S.D., $n = 8$) for *L. niloticus*. Bugs were removed from the water and wrapped in wet tissue paper 2 h prior to experimentation at about $22 \pm 1\,^{\circ}C$ to prevent swimming activity and keep the bugs under resting conditions (see [14]). Immediately prior to taking a hemolymph sample, the bugs were dried using tissue paper, and 1 μl hemolymph samples were collected into 200 μl of concentrated sulphuric acid for determination of resting levels of metabolites. Hemolymph was sampled with a glass microcapillary after penetrating the cuticle ventro-laterally at the mid of the abdomen. The substance to be tested (dissolved in 10 or 20 μl of distilled water) or distilled water (as negative control) was then injected into the abdomen, the water bugs rewrapped in wet tissue paper and, 90 min after injection, a second sample of hemolymph was taken. The hemolymph was mixed thoroughly with the sulphuric acid. Half the volume of the mixture (100 μl) was used for measuring the total anthrone-positive material (=carbohydrates) and the remaining mixture served to determine the total vanillin-positive material (=lipids) as described previously [3]. For activity experiments, specimens of *N. cinerea* were kept in a small beaker with natural water which was then placed on a shaker for continuous motion for 1 h (see also [14]).

Statistical analyses of the data were performed as stated in the text and in the footnotes of the tables.

2.4. Liquid chromatography and mass spectrometry

Liquid chromatography (LC)/mass spectrometry (MS) analysis was performed on an LCQ mass spectrometer (Thermo Electron, San Jose, USA) equipped with an electrospray ion source operated at 4.2 kV and capillary temperature at 210 °C. Five microliters of aqueous 0.1% formic acid, containing about 0.8–1.2 gland pair equivalent of each water bug species, was injected into a 150 mm × 1 mm i.d. RP-C18 Luna HPLC column (Phenomenex, Torrance, USA). A gradient elution with solvents A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile, was used from 20 to 55% B within 10 min at a flow rate of 50 $\mu l\ min^{-1}$.

The primary sequence of the peptides was deduced from the electrospray MS^N spectra obtained by the collision-induced