Isolation and structure elucidation of a neuropeptide from three species of Namib Desert tenebrionid beetles

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The corpora cardiaca of three Namib Desert beetles, *Onymacris plana, O. rugatipennis* and *Physadesmia globosa* (family Tenebrionidae), contain hyperlipaemic and/or hypertrehalosaemic factors as shown by heterologous bioassays in migratory locusts and American cockroaches. The compounds have been isolated by reversed-phase high performance liquid chromatography from the three species. The retention times of the compounds for all three species were identical. Amino acid composition data, in combination with determination of the primary structure by pulsed-liquid phase sequencing employing Edman chemistry after enzymically deblocking the N-terminal pyroglutamate residue, reveals an identical octapeptide for the three investigated species: pGlu-Leu-Asn-Phe-Ser-Pro-Asn-TrpNH_2. This peptide, code-named Tem-HrTH, is a member of the large adipokinetic hormone/red pigment-concentrating hormone family and was previously found in two other tenebrionid beetles which are only very distantly related to the Namib beetles. Thus, it appears that during evolution of tenebrionid beetles only one AKH/RPCH-family peptide was recruited.

Die corpora cardiaca van die Namibwoestynkewers, *Onymacris plana, O. rugatipennis* en *Physadesmia globosa,* (familie Tenebrionidae), bevat hiperlipemiese en/of hipertrehalosemiese faktore, soos aangetoon deur heteroloë biotoetse in treksprinkane en Amerikaanse kakkerlakke. Hierdie verbindings is geïsoleer uit die drie spesies deur omgekeerde fase hoë doeltreffendheid vloeistofchromatografie. Die retensietye van die verbindings vir al drie spesies was identies. Aminosuursamestellingsdata, tesame met die bepaling van die primêre struktuur deur 'pulsed-liquid phase' volgordebepaling met behulp van die Edmanproses, nadat die N-terminale piroglutamaatresidu ensiematies gedeblokkeer is, lewer 'n identiese oktapeptied op vir die drie spesies wat ondersoek is: pGlu-Leu-Asn-Phe-Ser-Pro-Asn-TrpNH_2. Hierdie peptied, genaamd Tem-HrTH, is 'n lid van die groot adipokinetiese hormoon/rooi pigment konsentrerende hormoon-familie, en is voorheen gevind in twee ander kewers van die Tenebrionidae, wat slegs verlangs verwant is aan die Namibkewers. Dit wil dus voorkom of slegs een peptied van die AKH/RPKH-familie verwerf is tydens die evolusie van die kewers.

It is well known that peptides represent the largest single class of neuroregulatory substances in mammals (Snyder 1980; Iverson 1983). Peptidergic neurosecretory cells synthesize and release specific chemical messengers, the neuropeptides. A famous example of the intimate functional association between nervous and endocrine systems and the spatial separation between the site of production of the neuropeptide and its release, is the mammalian hypothalamo-hypophyseal system. In insects a similar system has been discovered: peptides are produced in the median and/or lateral neurosecretory cells of the brain and, after axonal transport, stored in the neurohaemal organ, called the corpus cardiacum (Scharrer & Scharrer 1944). In addition, the corpora cardiaca contain intrinsic glandular neurosecretory cells. These were identified as the site of synthesis and storage for the locust adipokinetic hormone, a neurohormone stimulating lipid mobilization during flight (Stone, Mordue, Batley & Morris 1976; Gäde 1990a for a review). As we know now it belongs to a large family of structurally related but functionally diverse peptides found mainly in insects, but also in Crustacea. In insects these peptides regulate energy metabolism by affecting mainly the energy balance of the fat-body, whereas in crustaceans they regulate colour change (Wheeler, Gäde & Goldsworthy 1988; Gäde 1990a). Structural data have been obtained from representative species of most of the main orders of insects. They all possess octa- nona- and decapeptides which are characterized by being blocked N-terminally by a pyroglutamate residue and C-terminally by an amide; at position 4 they have an aromatic phenylalanine or tyrosine residue and at position 8 the aromatic amino acid tryptophan (Gäde 1988a, 1990a, 1992). With the exception of peptides found in the dipteran species *Phormia terraenovae* (Gäde, Wilps & Kellner 1990) and *Drosophila melanogaster* (Schaffer, Noyes, Slaughter, Thorne & Gaskell 1990), and in various beetles of the superfamily Scarabaeoidea (Gäde 1991a; Gäde, Lopata, Kellner & Rinehart 1992), all family members lack a residue which is charged under physiological conditions.

Considering the huge number of beetle species, the information on their neuropeptides is still scarce. The Colorado potato beetle, *Leptinotarsa decemlineata* (family Chrysomelidae), contains two octapeptides which are identical to the two hypertrehalosaemic peptides sequenced from the corpora cardiaca of the American cockroach, *Periplaneta americana* (Gäde & Kellner 1989). The cockchafer, *Melolontha melolontha* (family Scarabaeidae, subfamily Melolonthiinae), the fruit beetles, *Pachnoda marginata* and *P. sinuata* (family Scarabaeidae, subfamily Cetoniinae) and the dor-beetle, *Geotrupes stercorosus* (family (Geotrupidae), all have a unique octapeptide with the amino acid residues tyrosine and aspartic acid at positions 4 and 7 respectively (Gäde 1991a; Gäde *et al.* 1992). Corpora cardiaca extracts from two members of the family Tenebrionidae, the mealworm beetles *Tenebrio molitor* and *Zophobas rugipes*, have been shown to elicit increases in haemolymph carbohydrates in cockroaches and haemolymph lipids in locusts (Gäde 1984, 1989). The *T. molitor* extract also activates fat-body