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

G. Gäde
Zoology Department, University of Cape Town, Private Bag, Rondebosch, 7700 Republic of South Africa

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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

glycogen phosphorylase (Gäde 1988b), induces hypertrehalosaemia in intact mealworms and is responsible for carbohydrate release from the fat-body *in vitro* (Rosinski & Gäde 1988). The active principles of both species co-elute during isolation on reversed-phase high performance liquid chromatography and have identical amino acid compositions and primary structures (Gäde 1989; Gäde & Rosinski 1990). An identical octapeptide was found recently in the corpora cardiaca of the primitive cockroach, *Polyphaga aegyptiaca* (Gäde & Kellner 1992).

Members of the tenebrionid family are especially abundant in the Namib Desert. Therefore, it was decided to investigate the species *Onymacris plana* (Peringuey), *O. rugatipennis* (Haag) and *Physadesmia globosa* (Haag), which belong to the tribe Adesmiini and are closely related to one another (Penrith 1986), but are very distant from *T. molitor* and *Z. rugipes* (Watt 1974; Doyen & Tschinkel 1982). The objectives were to elucidate the primary structures of the putative AKH-family members in the Namib Desert beetles and to find out whether a family-specificity existed. In the present study interest was not focused on investigating the physiological function of the peptides.

Materials and Methods

Experimental insects

All specimens used in this study were adults. The three Namib Desert beetle species were collected near the Desert Ecological Research Unit in Gobabeb, Namibia, and were a gift from Dr. M.K. Seely. They were transported by air to Cape Town and kept for a few days at 25°C with a photoperiodic regime of 14 h light — 10 h dark. They were fed fresh lettuce daily.

For bioassays (see below) adult 15–25-day-old male migratory locusts, *Locusta migratoria*, and adult male American cockroaches, *Periplaneta americana*, were used: they were kept and fed as described previously (Gäde 1991b; Gäde *et al.* 1992).

Preparation of corpora cardiaca extracts

The head capsule of the beetles was opened dorsally by a superficial cut. The tiny corpora cardiaca, which are localized just posterior to the brain, were dissected and placed in a 1,5 ml Eppendorf centrifuge tube containing 200 µl of ice-cold distilled water. The glands were disrupted by sonication on ice for two 20-s periods. After centrifugation at 12 000g for 5 min, the supernatant was saved and the pellet extracted by sonication with 200 µl of 80% methanol. After centrifugation (as above) the resulting supernatant was combined with the first supernatant and dried by vacuum centrifugation. The sample was resuspended in 200 µl 80% methanol, centrifuged as above and the supernatant again dried. The resulting material was either taken up in water (for bioassays) or in 20% B (solvent B: 0,1% trifluoroacetic acid in 60% acetonitrile, for liquid chromatography).

Isolation of peptides by liquid chromatography

Reversed-phase high performance liquid chromatography (RP-HPLC) in the gradient mode was used for isolation on a Nucleosil 100 C18 column (i.d. 4 mm, length 250 mm; 5 µm particle size); substances were separated mainly by their hydrophobicity. Thus, more hydrophobic substances were eluted by a higher percentage of the organic modifier acetonitrile. Details of the method and equipment used have been described previously (Gäde 1985, 1989) (see also Figure 1). Peak fractions were collected manually, dried by vacuum centrifugation, resuspended in an appropriate amount of water to give 0,5 gland equivalents and used for bioassays in locusts by injecting a 10-µl dose into five assay insects.

Amino acid composition data, enzymic deblocking and pulsed-liquid phase sequencing

The dried, purified material which had biological activity was used for obtaining structural information. Amino acid composition data were collected only for *O. plana* because there was sufficient material present only for this species. An aliquot was hydrolysed with 5,7 mol dm^{-3} hydrochloric acid. Precolumn derivatization with phenylisothiocyanate (PITC) and detection of the phenylthiocarbamoyl (PTC) amino acids at 254 nm following separation of the derivatives by RP-HPLC (Spherisorb ODSII, 3 µm material, 125 × 4,6 mm column) was essentially according to Heinrikson & Meredith (1984).

Active material from all three species was enzymically deblocked using L-pyroglutamate aminopeptidase as described previously (Gäde, Hilbich, Beyreuther & Rinehart 1988), with the exception that the enzyme was purchased from Sigma Chemical Co., U.S.A.. The deblocked peptides were separated from the intact molecules by RP-HPLC (for details, see legend to Figure 3), manually collected, dried by vacuum centrifugation, and used for sequencing. Automated Edman degradation was performed with a pulsed-liquid phase sequencer (Model 477A; Applied Biosystems, Foster City, CA, U.S.A.) connected to an on-line phenylthiohydantoin (PTH) amino acid analyser (Model 120A; Applied Biosystems). Sequencing reagents and solvents were from Applied Biosystems. Sequencing and PTH analysis were carried out with standard programmes.

An aliquot of the purified peptide from *O. rugatipennis* was treated with the enzyme carboxypeptidase A to determine whether the C-terminus of the peptide was blocked or not. The method used was essentially the same as described earlier (Siegert & Mordue 1986; Gäde *et al.* 1990). After enzymatic treatment, the material was applied to RP-HPLC (Nucleosil 100 C-18 column, dimensions and solvents as given in legend to Figure 1; but gradient ran from 30 to 56% B in 20 min).

Bioassays

Convenient and widely used heterologous bioassays were used to monitor biological activity of the extracts from corpora cardiaca of the Namib beetles and of fractions during isolation. In locusts the amount of vanillin-positive material in the haemolymph is determined by a simple colorimetric assay as a measure of the amount of lipids mobilized from the fat-body and transported into the haemolymph (Zöllner & Kirsch 1962). In cockroaches, a similar assay is conducted to measure the amount of

anthrone-positive material in the haemolymph, representing released trehalose from the fat-body (Spik & Montreuil 1964).

Synthetic peptides

The two adipokinetic peptides from *Locusta migratoria* (acronyms Lom-AKH-I and Lom-AKH-II; according to Raina & Gäde 1988), the two hypertrehalosaemic peptides from *Periplaneta americana* (Pea-CAH-I and Pea-CAH-II), the red pigment-concentrating hormone of Crustacea (RPCH), and the hypertrehalosaemic peptide II from the stick insect *Carausius morosus* (Cam-HrTH-II), came from Peninsula Laboratories (Belmont, CA, U.S.A.). The hypertrehalosaemic peptide from *Tenebrio molitor* (Tem-HrTH), was a gift from Novabiochem (Läufelfingen, Switzerland). For structures of peptides see Table 1.

Results and Discussion

In order to detect the biologically active fractions later during isolation, crude methanolic extracts of corpora cardiaca of the three beetles under investigation were assayed for their ability to increase lipids in locusts and/or carbohydrates in cockroaches. Owing to the limited amounts of material available from some species both assays were not always conducted. As depicted in Table 2, the extract from *O. plana* and *O. rugatipennis* showed bioactivity in both the heterologous bioassays, and the extract from *P. globosa* had hypertrehalosaemic activity. Thus, these experiments suggested that all three beetle species contain hypertrehalosaemic/adipokinetic material in their corpora cardiaca.

Table 1 Structures of AKH/RPCH-family peptides used in this study

Peptide name	Structure
Lom-AKH-I	pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-ThrNH$_2$
Cam-HrTH-II	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-Gly-ThrNH$_2$
Lom-AKH-II	pGlu-Leu-Asn-Phe-Ser-Ala-Gly-TrpNH$_2$
Scg-AKH-II	pGlu-Leu-Asn-Phe-Ser-Thr-Gly-TrpNH$_2$
Pab-RPCH	pGlu-Leu-Asn-Phe-Ser-Pro-Gly-TrpNH$_2$
Tem-HrTH	**pGlu-Leu-Asn-Phe-Ser-Pro-Asn-TrpNH$_2$**
Pea-CAH-II	pGlu-Leu-Thr-Phe-Thr-Pro-Asn-TrpNH$_2$
Pea-CAH-I	pGlu-Val-Asn-Phe-Ser-Pro-Asn-TrpNH$_2$

The first isolation experiments were carried out in 1989 on material from *O. plana* which was sent to Germany from South Africa by Dr. S. Nicolson.

A crude methanolic extract of 25 corpora cardiaca was fractionated on C-18-RP-HPLC. As shown in Figure 1A, three distinct UV absorbance peaks (numbered 1 to 3), with retention times of 14,6; 28 and 36,6 min respectively, were clearly visible. Only the material eluting at 36,6 min caused hyperlipaemia in locusts when aliquots representing 0,5 gland equivalents were injected into five locusts each (increase of haemolymph lipids of 7,5 ± 2,2 mg/ml; the other two fractions caused non-significant increases of 2,2 ± 1,3 and 3,2 ± 2,1 mg/ml). Compared to the retention times of known synthetic peptides of the AKH/RPCH-family (Figure 1B), the beetle's active material eluted close to Lom-AKH-I, but the retention time was not identical. Although we were working on the isolation and characterization of the peptides from two other tenebrionid beetles, *Tenebrio molitor* and *Zophobas rugipes*, at this stage we had no structural data yet and, therefore, were not able to compare the retention time of the Namib beetle material with the synthetic peptide from the two other tenebrionids.

In Cape Town in 1990–92 it was possible to isolate corpus cardiacum material from all three Namib beetle species using a simpler RP-HPLC gradient programme and to compare the retention time with the synthetic peptide (acronym Tem-HrTH) isolated previously from *T. molitor* and *Z. rugipes* (Gäde & Rosinski 1990). These data are

Table 2 Adipokinetic and hypertrehalosaemic activity of crude methanolic extracts (0,5 gland equivalents) from corpora cardiaca of three Namib beetles in locusts and cockroaches respectively. Insects were injected as indicated. Total haemolymph lipid concentration in adult male locusts and total haemolymph carbohydrate concentration in adult male cockroaches are expressed as mg/ml of haemolymph, and results are means ± *S.D.* The significance of the difference between values before and after injection is indicated by * $p \leq 0{,}01$ and ** $p \leq 0{,}001$ using paired *t* tests

	Acceptor insect							
	Locusta migratoria Blood lipids (mg/ml)				*Periplaneta americana* Blood carbohydrates (mg/ml)			
Treatment	*n*	0 min	90 min	Difference	*n*	0 min	120 min	Difference
Control: distilled water	8	10,5 ± 1,5	11,8 ± 1,7	1,3 ± 1,1	8	14,1 ± 2,8	16,7 ± 2,5	2,6 ± 1,3
O. plana	6	11,1 ± 1,7	19,3 ± 2,8	8,2 ± 2,4**	6	13,1 ± 1,9	23,2 ± 3,1	10,1 ± 1,8**
O. rugatipennis	5	15,2 ± 8,6	30,2 ± 4,1	15,0 ± 7,2*	5	14,1 ± 1,4	37,4 ± 4,5	23,3 ± 3,7**
P. globosa	5	–	–	–	5	12,9 ± 1,8	25,1 ± 3,6	12,2 ± 3,6**
Synthetic Lom-AKH-I, 10 pmol	8	12,4 ± 2,0	45,6 ± 6,8	33,2 ± 5,4**	–	–	–	–
Synthetic Pea-CAH-I, 10 pmol	–	–	–	–	6	13,7 ± 2,5	36,2 ± 4,1	22,5 ± 5,1**

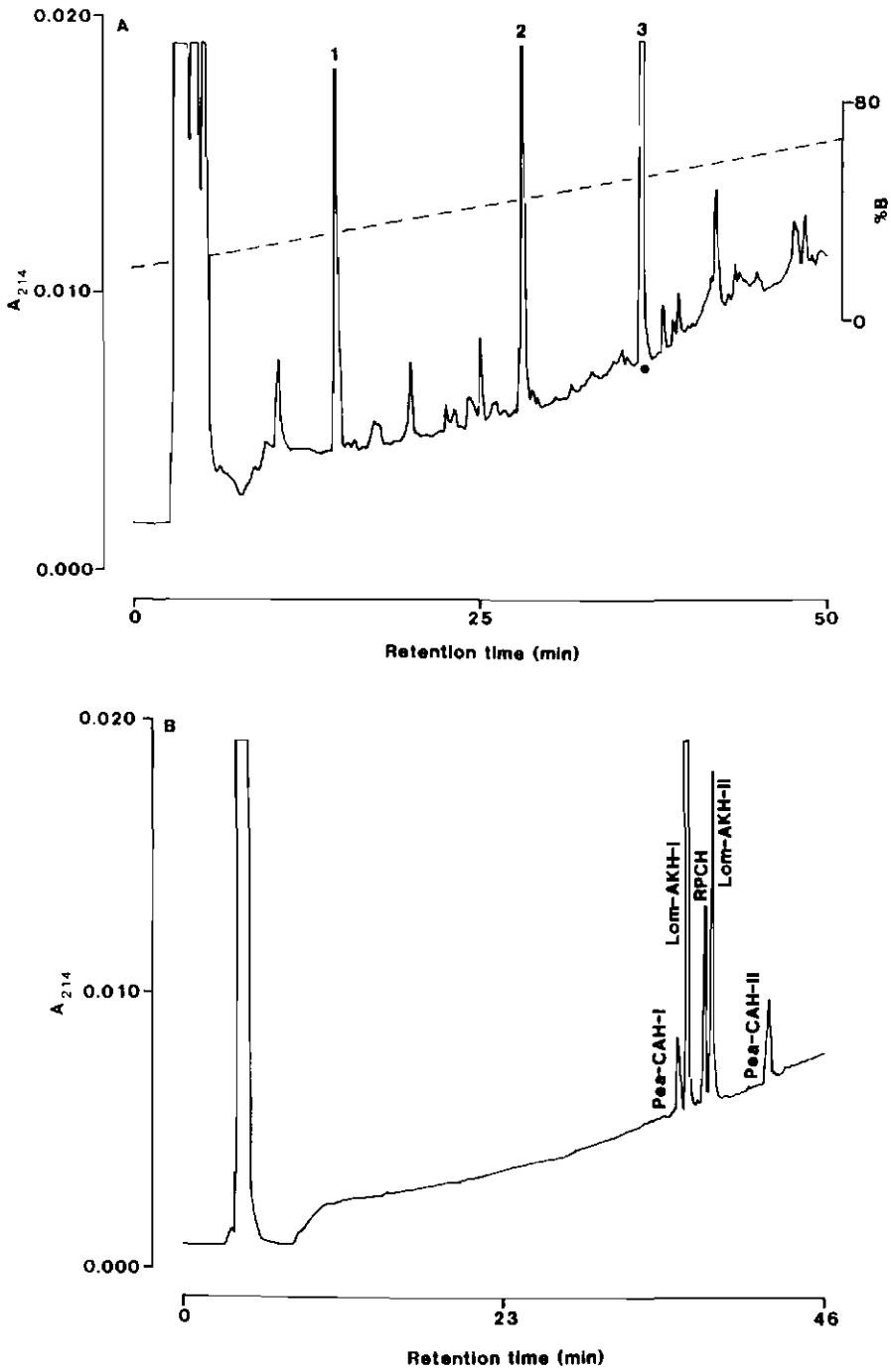

Figure 1A Separation of a methanolic corpus cardiacum extract (25 gland equivalents) from *O. plana* on reversed phase HPLC. Vacuum-dried peak fractions (numbered 1, 2, 3) were taken up in 500 µl distilled water and 10 µl were injected in 5 locusts each to assay total blood lipid concentration. The biologically active fraction is marked by a black dot. **Figure 1B** Separation of five synthetic peptides of the AKH/RPCH-family. Chromatographic conditions: A Nucleosil C-18 column (i.d. 4,6 mm × 250 mm length) plus 20 mm guard column of the same material was eluted with a linear gradient of 0,11% trifluoroacetic acid (solvent A) and 0,1% trifluoroacetic acid in 60% acetonitrile (solvent B). The gradient ran from 20% to 80% B within 60 min at a flow rate of 1ml/min. The elution was monitored with a UV detector at 214 nm and fractions, if applicable, were manually collected.

illustrated in Figures 2A–E. It became clear that all Namib beetle species showed only one distinct absorbance peak (at 15,2 min) which had biological activity when tested in *P. americana* (Figures 2A–C). These peaks had the same retention time as the synthetic Tem-HrTH (Figure 2E). However, identical retention time on RP-HPLC does not necessarily mean that the chemical structures are identical, as was shown previously for RPCH and the second adipokinetic peptide from *Schistocerca* species (Scg-AKH-II), which co-elute on RP-HPLC (Gäde 1990b). The compounds are both octapeptides with the same primary structure except for Pro^6 in RPCH instead of Thr^6 in Scg-AKH-II (see Table 1).

Structural information had to be obtained for the unequivocal assignment of a structure for the Namib beetle compound. As a first step the elution from RP-HPLC of one of the extracts (from *P. globosa*) was monitored with a

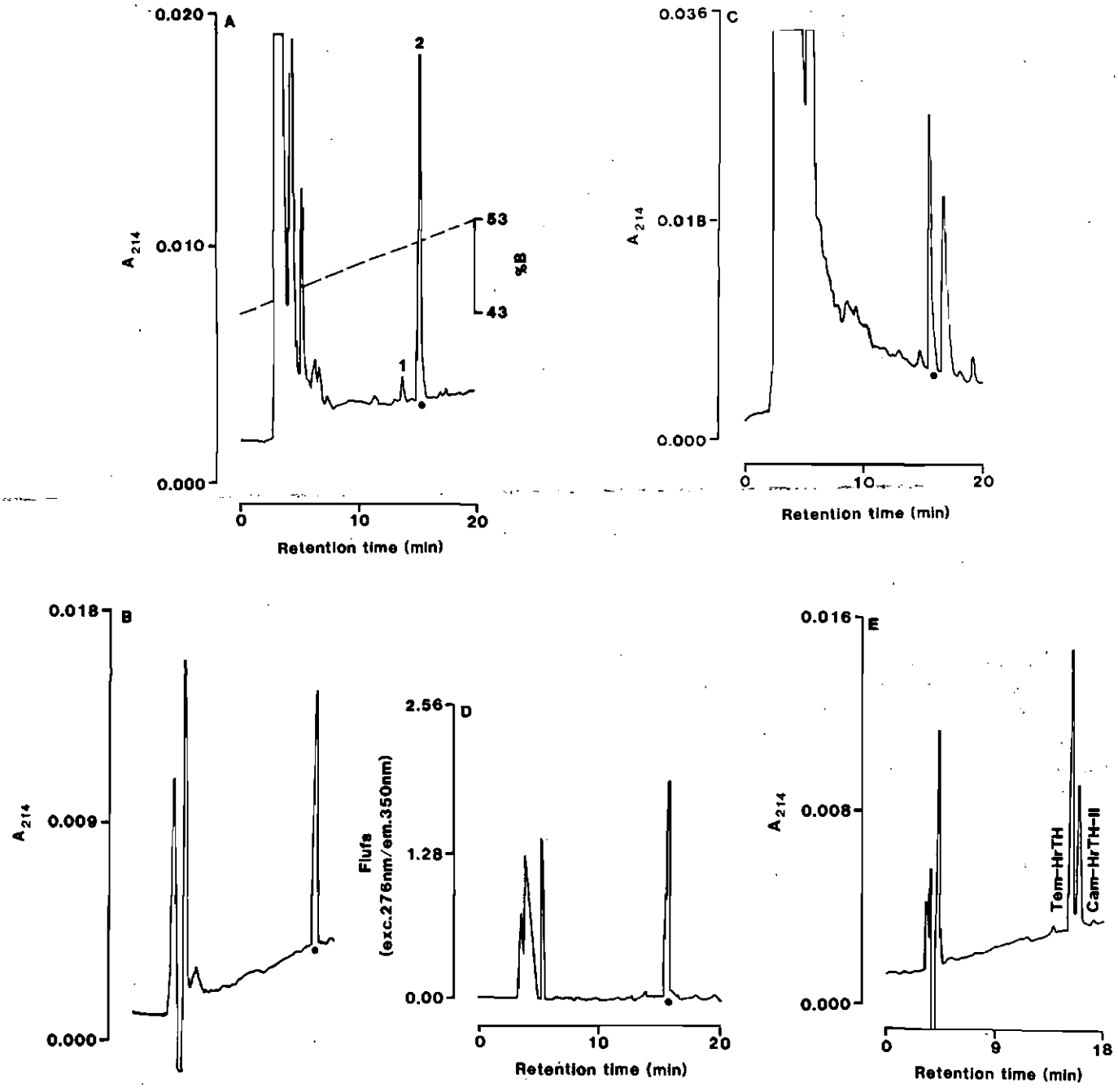

Figure 2 Separation of methanolic corpora cardiaca extracts from various Namib beetle species (A–D)) or synthetic AKH/RPCH-family peptides (E) on reversed-phase HPLC. **A.** *O. plana;* 11 gland equivalents, **B.** *O. rugatipennis;* 12 gland equivalents, **C.** *P. globosa;* 34 gland equivalents, **D.** as C, but fluorescence monitored, **E.** synthetic *Tenebrio* and *Carausius* peptide. Chromatographic conditions: see legend to Figure 1. The gradient ran from 43% to 53% B in 20 min. In D the eluant was monitored with a fluorimeter at 276 nm (excitation)/350 nm (emission).

fluorimeter at an excitation wavelength of 276 nm and an emission wavelength of 350 nm. Under these conditions the presence of the aromatic amino acids Trp or Tyr is indicated in a molecule. As shown in Figure 2D the UV-absorbing peak at 15,2 min was also present when fluorescence was monitored. Thus, the compound had either a Trp or Tyr residue in its structure.

Further structural information came from amino acid analysis. Only active material from *O. rugatipennis* was analysed. After hydrolysis in hydrochloric acid the presence of seven amino acid residues was shown (Table 3). As no Tyr residue was detected, although Tyr, contrary to Trp, is not destroyed during acid hydrolysis, the presence of a Trp residue can be inferred by elimination from the spectrometric data above.

The amino acid composition data indicated a Glx residue (glutamic acid or glutamine) which could have been derived from the hydrolysis of pyroglutamic acid pGlu (a cyclicysed glutamic acid). This amino acid had previously been found at the N-terminus of all other AKH/RPCH-family members (see Gäde 1992). A peptide blocked by pGlu cannot be degraded by the Edman degradation owing to the absence of a free N-terminal amino group. Therefore, this residue has to be removed. This can be done with a specific enzyme,

Table 3 Amino acid composition of the corpus cardiacum neuropeptide from *O. rugatipennis* after acid hydrolysis. RP-HPLC-purified material was hydrolysed in hydrochloric acid for 24 h and, after modification with phenylisothiocyanate, the phenylthiocarbamoyl amino acids were quantified by RP-HPLC. Values are given as molar ratios with respect to Leucin (= 1,0)

Amino acid residue	Composition (molar ratio)
Asx (Asn or Asp)	1,92
Glx (Gln or Glu)	0,96
Ser	0,80
Pro	1,09
Leu	1,00
Phe	0,97

pyroglutamate aminopeptidase, which selectively cleaves the pGlu residue. After separation of the deblocked from the native peptide by RP-HPLC, the now open N-terminus can be subjected to automated analysis. This was done for all three Namib beetle peptides. As an example, for the material of *P. globosa*, the deblocked peptide eluted at 26 min (about 5 min earlier than the intact molecule; Figure 3) and is therefore less hydrophobic. It is also clear that the enzyme had cleaved the peptide almost quantitatively, since only a very small absorbance peak of the undigested peptide can be seen at 30,8 min. Sequence analysis of the deblocked

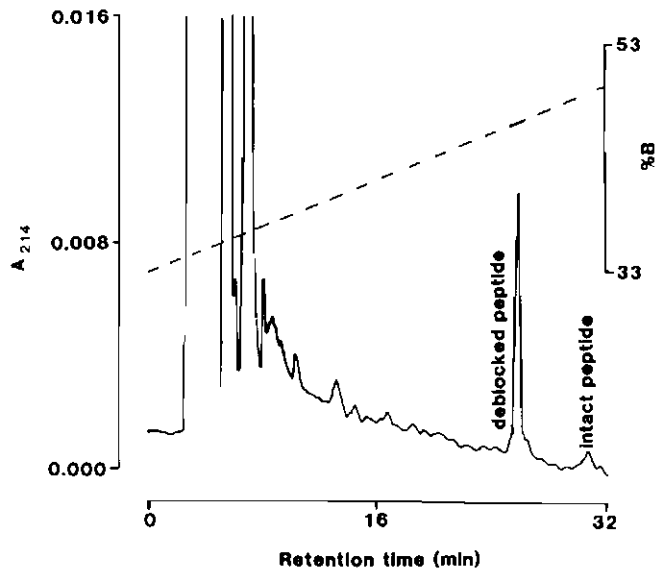

Figure 3 Enzymic deblocking of the amino terminal. The bioactive material from *P. globosa* previously purified by reversed-phase HPLC was incubated with pyroglutamate aminopeptidase. After digestion, the material was fractionated on a column as outlined in legend to Figure 1 with the exception that the gradient ran from 33% to 53% B within 40 min. Note that most of the peptide is digested.

Table 4 Amino acid sequences of Namib Desert beetle corpus cardiacum peptide by automated Edman degradation. The N-terminal pyroglutamate residue of RP-HPLC-purified peptides from the three beetle species' corpora cardiaca was cleaved off by pyroglutamate aminopeptidase, and the purified deblocked peptides were analysed

Amino acid residue	Recovery (pmol)		
	O. plana	*O. rugatipennis*	*P. globosa*
Leu^1	25	45	11
Asn^2	19	29	12
Phe^3	21	35	12
Ser^4	10	12	6
Pro^5	15	21	7
Asn^6	14	23	5
Trp^7	4	5	2

peptides yielded the same result in all cases (Table 4): the first step (cycle) showed the presence of Leu. Thus, Leu is present at position two of the intact peptides when taking into account that the first amino acid, pyroglutamate, had been split off by enzymatic deblocking. The last amino acid detected in the three analyses was always tryptophan in cycle 7; no amino acids were present in the next two cycles. Thus, in accordance with the amino acid analysis and the fluorimetric data all three peptides were octapeptides. However, what was not yet known was whether the C-terminus was free or blocked. The latter is the case with all other AKH/RPCH-family members (see Gäde 1992). Therefore, an aliquot of the active purified material from *O. rugatipennis* was incubated with the enzyme carboxypeptidase A, in the presence of Leu-enkephalin which served as a model substance. Leu-enkephalin is not blocked at the C-terminus and was therefore cleaved by the enzyme and showed a different retention time before (10,5 min) and after (8,3 min) treatment with the enzyme (Figure 4A versus B). The absorbance peak of the beetle peptide, however, did not shift, indicating that the C-terminus was blocked. Although this method does not permit the analysis of the nature of the blockage, it can safely be assumed to be the amide group for the following reasons:

1. It is by far the most common modification at the C-terminus of proteins and peptides (see Schulz & Schirmer 1979),
2. it is universal in AKH/RPCH-family peptides (Gäde 1990a),
3. the synthetic peptide Tem-HrTH, which does contain all the structural elements determined here for the Namib beetle peptides, including the amide at the C-terminus, co-elutes in different RP-HPLC gradients when co-injected with a Namib beetle active fraction (results not shown; see also Figure 2A–C versus Figure 2E).

This study then reveals that the three Namib beetles, which are closely related (Penrith 1986), synthesize the same AKH/RPCH-family peptide in their corpora cardiaca.

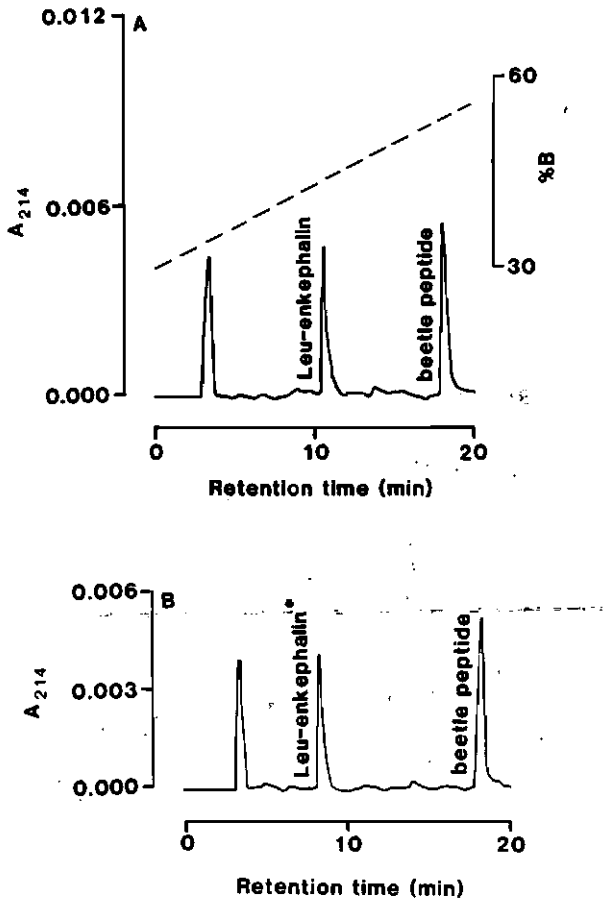

Figure 4 Enzymic deblocking of the carboxy terminal. The bioactive material from *O. rugatipennis* previously purified by reversed-phase HPLC, was incubated in the presence of Leu-enkephalin with carboxypeptidase Before (A) and after (B) digestion, the material was fractionated on a column as outlined in legend to Figure 1 with the exception that the gradient ran from 30% to 56% B within 20 min. Note that after digestion the retention time of the beetle peptide does not shift (no digestion), whereas the retention time of Leu-enkephalin does (B).

Furthermore, an identical peptide is found in two other tenebrionid beetles, namely *T. molitor* and *Z. rugipes* (Gäde & Rosinski 1990). This is surprising because the last mentioned two beetles belong to the subfamily Tenebrioninae, tribe Tenebrionini, whereas the Namib genera belong to the subfamily Tentyriinae, tribe Adesmiini (Doyen & Tschinkel 1982). Although the family Tenebrionidae is one of the largest families in the animal kingdom (comprising about 15 000 described species), and higher classification is still under dispute, it is generally accepted that Tenebrionini and Tentyriini are phylogenetically almost as far apart as possible in this family. Finding an identical peptide, then, in such distantly related members, suggests that very likely all tenebrionids contain this peptide. This may be important to know once techniques have been established to use these small neuropeptides for pest management as proposed recently (Keeley & Hayes 1987; Menn & Borkovec 1989) because some tenebrionid beetles (*T. molitor, Tribolium castaneum*) cause tremendous damage to stored grain products. It is interesting to note that the same peptide, Tem-HrTH, is also found in the corpora cardiaca of the primitive cockroach, *Polyphaga aegyptiaca*. Furthermore, cockroaches of the family Blattidae (Gäde & Rinehart 1990) and the Colorado potato beetle share structurally identical peptides, Pea-CAH-I and II (Gäde & Kellner 1989). At the moment we do not have a plausible explanation as to why highly evolved beetles possess the same peptides as relatively primitive insects such as cockroaches, whereas other highly evolved orders (Diptera, Lepidoptera) do possess specific AKH/RPCH-family members which are not found in other orders.

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