A unique charged tyrosine-containing member of the adipokinetic hormone/red-pigment-concentrating hormone peptide family isolated and sequenced from two beetle species

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An identical neuropeptide was isolated from the corpora cardiaca of two beetle species, *Melolontha melolontha* and *Geotrupes stercorosus*. Its primary structure was determined by pulsed-liquid-phase sequencing employing Edman chemistry after enzymically deblocking the *N*-terminal pyroglutamate residue. The *C*-terminus was also blocked, as indicated by the lack of digestion when the peptide was incubated with carboxypeptidase A. The sequence of this peptide, which is designated Mem-CC, is pGlu-Leu-Asn-Tyr-Ser-Pro-Asp-Trp-NH₂. It is a new member of the adipokinetic hormone/red-pigment-concentrating hormone (AKH/RPCH) family of peptides with two unusual structural features: it is charged and contains a tyrosine residue at position 4, where all other family members have a phenylalanine residue. Structure–activity studies in the migratory locust (*Locusta migratoria*) and the American cockroach (*Periplaneta americana*) revealed that the peptide was poorly active, owing to its structural uniqueness.

INTRODUCTION

Members of the adipokinetic hormone/red-pigment-concentrating hormone (AKH/RPCH) family comprise structurally related but functionally diverse peptides found in the neurosecretory corpora cardiaca of insects as well as in the neurosecretory X-organ–sinus gland complex of the eyestalk of crustaceans. The functions of these peptides cover a wide range. In crustaceans they are involved in colour change and in insects in lipid and carbohydrate regulation, as well as in the inhibition of protein biosynthesis in the fat-body [1–4]. Certain studies on bioactivities *in vitro*, and immunohistochemical localization of specific neurons and nerve fibres other than the intrinsic neurons of the corpus cardiacum, suggest also a role as neuromodulators or neurotransmitters for these peptides [5–7]. The presence of such peptides has been shown by convenient bioassays in all major orders of the insects [8]. Structural data have been obtained mostly from locusts, grasshoppers, cockroaches, crickets, stick insects and two species of moths [8,9], but recently the primary sequences of three species of flies [10–12] and of the primitive insect orders of dragonflies [13] and praying mantis [14] have been elucidated. All these species contain octa-, nona- and decapeptides that are characterized by being *N*- and *C*-terminally blocked, and possessing a pyroglutamate residue at position 1, a phenylalanine residue at position 4 and a tryptophan residue at position 8. With the exception of the peptide found in the dipteran species *Drosophila melanogaster* [12] and *Phormia terraenovae* [11] all family members are uncharged.

Although almost 50% of all known insect species belong taxonomically to the order Coleoptera (beetles), structural information on AKH/RPCH peptides in this group is still rather limited. Previously, we had shown that two neuropeptides from the corpora cardiaca of the Colorado potato beetle, *Leptinotarsa decemlineata* (Family Chrysomelidae), which are very likely involved in stimulating proline synthesis in the fat-body, are identical in structure with two hypertrehalosaemic neuropeptides

sequenced from the cockroach *Periplaneta americana* [15]. Recently, we reported a novel structure for the neuropeptide isolated from the corpora cardiaca of the tenebrionid beetles *Tenebrio molitor* and *Zophobas rugipes* [16].

Our research has provided some indications that the corpora cardiaca of certain cockchafer and rosechafer species (*Melolontha hippocastani*, *Pachnoda marginata*, *Pachnoda sinuata*; Family Scarabaeidae) may contain a novel member of the AKH/RPCH family of peptides as judged by retention time in reverse-phase h.p.l.c. and amino acid composition data [17]. Thus, when a new mass outbreak of the cockchafer, this time the species *Melolontha melolontha*, occurred, we collected sufficient material to report in the present study the complete structure elucidation of the corpora cardiaca neuropeptide and compare the data with those of the dor beetle, *Geotrupes stercorosus* (Family Geotrupidae).

MATERIALS AND METHODS

Insects

Cockchafers (*Melolontha melolontha*) were collected in Horrenberg (near Heidelberg, Germany) and dor beetles (*Geotrupes stercorosus*) were caught near Bonn, Germany. For bioassays, adult (15–30-day-old) male migratory locusts (*Locusta migratoria*) were used. Locusts were reared under crowded conditions at 32 °C with a 14 h-dark/10 h-light cycle and were fed twice daily with fresh grass supplemented with dried oat flakes. Adult male cockroaches (*Periplaneta americana*) were also used for bioassays. Cockroaches were mass-reared at 27 °C and had the same photoperiod as the locusts; they were fed with Pro Nutro and has access to water *ad libitum*.

Isolation of corpora cardiaca peptides

Immediately after transportation of cockchafers and dor beetles to the laboratory corpora cardiaca were carefully dissected under a stereo microscope, collected in distilled water and frozen

Abbreviations used: AKH, adipokinetic hormone; RPCH, red-pigment-concentrating hormone; Pea-CAH-I, *Periplaneta americana* hypertrehalosaemic hormone I; Lom-AKH-I, *Locusta migratoria* adipokinetic hormone I; Mem-CC, *Melolontha melolontha* corpus cardiacum peptide; Ges-CC, *Geotrupes stercorosus* corpus cardiacum peptide; Tem-HrTH, *Tenebrio molitor* hypertrehalosaemic hormone; Pht-HrTH, *Phormia terraenovae* hypertrehalosaemic hormone.

Fig. 1. Isolation of cockchafer (*Melolontha melolontha*) peptide

The chromatogram shows the separation of a methanolic extract from ten corpora cardiaca. The analysis was performed on a Nucleosil C_{18} column, which 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 43 to 53 % solvent B within 20 min at a flow rate of 1 ml/min. The elution was monitored at 214 nm. Peak fractions numbered 1, 2 and 3 were collected, dried by vacuum centrifugation and dissolved in 100 μ l of distilled water, and a 10 μ l sample of each was injected into each of five locusts and cockroaches respectively for bioassays. A slight hypertrehalosaemic activity of fraction 1 is indicated by ●.

at -25°C . Methanolic extracts were prepared as in ref. [18]; they were applied to a Nucleosil 100 C_{18} column for reverse-phase h.p.l.c. in batches of 10–40 glands, using the equipment described previously [14,19]. Peak fractions as indicated in the Figures were collected manually, and the combined purified material from a total of 140 (cockchafer) and 40 (dor beetle) corpora cardiaca respectively was used for the determinations of the amino acid composition and for primary structure elucidation.

Amino acid composition

H.p.l.c.-purified portions (equivalent to 100 corpora cardiaca of the cockchafer and 15 corpora cardiaca of the dor beetle) of the neuropeptide were dried *in vacuo* (Vacuum Concentrator; Bachhofer G.m.b.H., Reutlingen, Germany) and hydrolysed with 5.7 M-HCl for 24 h. Precolumn modification with phenyl isothiocyanate and detection of the phenylthiocarbamoyl-amino acids at 254 nm after separation of the derivatives by reverse-phase h.p.l.c. (Spherisorb ODS II; 3 μ m material; column size 125 mm $\times$ 4.6 mm) were essentially as described in ref. [20].

Enzymic deblocking and pulsed-liquid-phase sequencing

The dried purified peptide material from 40 corpora cardiaca of cockchafers and 15 corpora cardiaca of dor beetles was enzymically deblocked by using L-pyroglutamate aminopeptidase as described previously [21]. The deblocked peptides were separated from the intact molecules by reverse-phase h.p.l.c. (for

conditions see Fig. 3), collected manually, dried by vacuum centrifugation, and used for pulsed-liquid-phase sequencing. Automated Edman degradation was performed with a pulsed-liquid-phase sequencer (model 477A; Applied Biosystems, Foster City, CA, U.S.A.) connected to the on-line amino acid phenylthiohydantoin analyser (model 120A; Applied Biosystems). Sequencing and phenylthiohydantoin analysis were carried out with standard programmes.

C-Terminal analysis

A portion of the h.p.l.c.-purified cockchafer peptide (equivalent to ten corpora cardiaca) was treated with carboxypeptidase A as described in ref. [22]. H.p.l.c. equipment and solvents were the same as in refs. [14] and [19] but the gradient ran from 30 to 56 % solvent B in 20 min.

Synthetic peptides

The novel cockchafer and dor-beetle peptide was synthesized on a solid-phase peptide synthesizer by Novabiochem G.m.b.H. (Sandhausen, Germany), and is commercially available now. Synthetic Pea-CAH-I (nomenclature according to Raina & Gäde [23]), the hypertrehalosaemic peptide of the American cockroach, and synthetic Lom-AKH-I, the adipokinetic peptide of the migratory locust, came from Peninsula Laboratories (Belmont, CA, U.S.A.). The [Asn⁷] analogue of the cockchafer peptide was custom-synthesized by Professor D. Botes (Department of Biochemistry, University of Cape Town) and the synthetic hypertrehalosaemic hormone from tenebrionid beetles (Tem-HrTH; see ref. [16]) came from Novabiochem.

Bioassays

The bioassays in cockroaches and locusts were performed as described in ref. [24]. Haemolymph sampling, injection of material into adult cockchafers and determination of their haemolymph carbohydrate and lipid concentrations were conducted as in the above species.

Phosphorylase activity in the fat-body of *Melolontha melolontha* was measured as described in ref. [25].

RESULTS

Biological activity

Crude extracts of cockchafer corpora cardiaca were assayed for lipid- and carbohydrate-mobilizing activity in acceptor locusts and cockroaches respectively (Table 1). A small but significant increase in the concentrations of the respective blood metabolites was shown in both assay systems, but quite high dosages of corpora cardiaca extract had to be injected. Furthermore, no dose-dependent response could be elicited. Compared with the maximal possible responses in locusts and cockroaches achieved by injecting the endogenous adipokinetic and hypertrehalosaemic peptides (synthetic Lom-AKH-I and Pea-CAH-I respectively), the increase with cockchafer material amounted only to 20–30 % (Table 1). Conspecific injections of the cockchafer corpora cardiaca extract revealed no increase in blood lipids or in carbohydrates (Table 2). The cockchafer extract was also not able to increase the activation state of the enzyme glycogen phosphorylase in the fat-body: in controls (injection of 10 μ l of doubly distilled water) $61.7 \pm 11.7\%$ ($n = 4$) of the total phosphorylase was in the a-form, and after the injection of 0.2 gland equivalent $58.7 \pm 4.4\%$ was in the a-form. Owing to the small amount of corpora cardiaca material available from the dor beetle, no attempt was made to analyse biological activity of crude extracts from this species.

Table 1. Adipokinetic and hypertrehalosaemic activity of crude methanolic extract of corpora cardiaca from the cockchafer 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 blood, and results are means $\pm$ s.d. The significance of the difference between values before and after injections is indicated by * $P < 0.05$, † $P < 0.01$ and ‡ $P < 0.001$ using paired t tests.

Treatment	Acceptor insect ...		<i>Locusta migratoria</i>			<i>Periplaneta americana</i>		
			Blood lipids (mg/ml)			Blood carbohydrates (mg/ml)		
	<i>n</i>	0 min	90 min	Difference	<i>n</i>	0 min	120 min	Difference
Control (10 μ l of distilled water)	6	7.8 $\pm$ 1.4	8.9 $\pm$ 1.5	1.1 $\pm$ 1.3	6	14.2 $\pm$ 1.8	17.0 $\pm$ 2.1	2.8 $\pm$ 1.6
Cockchafer extract (CC equivalents)								
0.5	6	7.4 $\pm$ 1.0	8.6 $\pm$ 1.4	1.2 $\pm$ 1.7	6	12.6 $\pm$ 1.5	19.8 $\pm$ 4.4	7.2 $\pm$ 3.8†
1.0	4	12.2 $\pm$ 2.8	18.5 $\pm$ 2.8	6.3 $\pm$ 2.5*	7	13.1 $\pm$ 1.9	20.1 $\pm$ 2.9	7.0 $\pm$ 1.7†
2.0	8	8.6 $\pm$ 2.7	13.8 $\pm$ 4.1	5.2 $\pm$ 4.1†	7	14.1 $\pm$ 1.8	21.4 $\pm$ 2.9	7.3 $\pm$ 1.7†
Synthetic Lom-AKH-I (10 pmol)	6	8.0 $\pm$ 0.8	39.9 $\pm$ 9.5	31.9 $\pm$ 9.1†	—	—	—	—
Synthetic Pea-CAH-I (10 pmol)	—	—	—	—	6	15.2 $\pm$ 2.4	37.6 $\pm$ 5.3	22.4 $\pm$ 3.3†

Table 2. Conspecific adipokinetic and hypertrehalosaemic activity of crude methanolic extract of corpora cardiaca from the cockchafer

Insects were injected as indicated. Total haemolymph lipid concentration and total haemolymph carbohydrate concentration in adult cockchafer are expressed as mg/ml of blood, and results are means $\pm$ s.d.

Treatment	Acceptor insect ...	<i>Melolontha melolontha</i>							
		Blood lipids (mg/ml)				Blood carbohydrates (mg/ml)			
		<i>n</i>	0 min	90 min	Difference	<i>n</i>	0 min	120 min	Difference
Control (10 μ l of distilled water)		6	7.8 $\pm$ 1.3	8.0 $\pm$ 1.2	0.2 $\pm$ 0.4	6	12.0 $\pm$ 4.5	11.8 $\pm$ 4.7	-0.2 $\pm$ 1.1
Cockchafer extract (CC equivalents)									
0.5		6	8.1 $\pm$ 1.3	7.9 $\pm$ 1.1	-0.2 $\pm$ 0.4	6	9.0 $\pm$ 3.4	8.4 $\pm$ 3.8	-0.6 $\pm$ 0.6
1.0		6	7.8 $\pm$ 1.3	7.9 $\pm$ 1.4	0.1 $\pm$ 0.3	6	9.9 $\pm$ 1.9	9.8 $\pm$ 2.5	-0.1 $\pm$ 1.1

Fig. 2. Isolation of dor-beetle (*Geotrupes stercorosus*) peptide

The chromatogram shows the separation of a methanolic extract from ten corpora cardiaca. For all other details see the legend to Fig. 1.

Isolation of beetle corpora cardiaca peptides

A methanolic extract of ten corpora cardiaca from the cockchafer was fractionated on an analytical reverse-phase h.p.l.c column in a gradient mode with trifluoroacetic acid as ion-pairing reagent (Fig. 1). Under the conditions employed, there were three major distinct absorbance peaks at 214 nm (numbered 1, 2 and 3 in order of elution). None of these fractions displayed adipokinetic activity in locusts. Hypertrehalosaemic activity, measured in cockroaches, appeared to be associated only with the first absorbance peak, which had a retention time of 8.5 min, but the activity was slight.

When an extract of ten corpora cardiaca from the dor beetle was subjected to h.p.l.c. analysis, the chromatogram showed only one distinct absorbance peak (numbered 1; Fig. 2). This material and material from the regions numbered 2 and 3 were assayed for lipid- and carbohydrate-mobilizing activity in the respective acceptor insects. None of the materials elevated the concentrations of blood lipids in locusts, but the material associated with peak 1 had a slight hypertrehalosaemic effect in cockroaches (carbohydrates before injection 13.6 ± 1.7 mg/ml, 120 min after injection 17.9 ± 2.2 mg/ml, $n = 5$, $P < 0.01$; for comparison, injection of 10 pml of Pea-CAH-I increased the carbohydrates from 13.7 ± 2.5 mg/ml before injection to 36.2 ± 4.1 mg/ml after injection, $n = 5$, $P < 0.001$).

The peaks numbered 1 in both species investigated here had been previously seen at the same retention time when extracts of corpora cardiaca from three other beetle species from the Family Scarabaeidae (*Pachnoda marginata*, *Pachnoda sinuata*, *Melolontha hippocastani*) were separated on h.p.l.c. [17]. The material from *Pachnoda marginata* was identified as a peptide with an unusual amino acid composition compared with other

members of the AKH/RPCH family [17]. Therefore, despite the unsatisfactory results in the bioassays in this present study, I decided to purify sufficient material of peak 1 to obtain amino acid composition and sequence data.

Amino acid composition data

With the above separation method the peak 1 material of 100 corpora cardiaca from the cockchafer and 15 glands from the dor beetle was isolated and subjected to hydrolysis in HCl. As summarized in Table 3, the same seven amino acid residues were present in both peptides. Although the peak 1 material was shown to result in a distinct absorbance peak when monitored with a fluorimeter (set at an excitation wavelength of 276 nm and emission at 350 nm; Shimadzu fluorescence monitor RF 535; results not shown), the presence of tyrosine made it difficult to decide whether an additional tryptophan residue was present in the analyses.

Isolation of deblocked peptides and primary-structure data

Another aliquot (equivalent to 40 corpora cardiaca of the cockchafer and 15 glands of the dor beetle) of the h.p.l.c.-purified peak 1 material was incubated with pyroglutamate aminopeptidase and subjected to reverse-phase h.p.l.c. (Fig. 3). Complete digestion was reached after 60 min, and a new absorbance peak [labelled Mem-CC(-pGlu)] with a retention time of 12.2 min was observed, 4 min less than the peak of the undigested peptide (retention time 16.2 min). The material of the dor beetle (labelled Ges-CC) was subjected to 60 min digestion by the enzyme. The retention time of the new absorbance peak, Ges-CC(-pGlu), was exactly the same as for the cockchafer peptide (results not shown).

The deblocked peptides of both species were used for sequence analyses by the Edman degradation technique and yielded identical sequences (Table 4). The last amino acid in each analysis was detected in cycle 7 (tryptophan), and the next two cycles gave no degradation product. The sequence is in agreement with the amino acid composition data, but an additional tryptophan residue was found.

Incubation of a sample of the purified cockchafer peptide with carboxypeptidase A in the presence of [Leu]enkephalin resulted in no change in retention time for the beetle peptide when rechromatographed, whereas [Leu]enkephalin was cleaved and a new absorbance peak was observed, indicating that the C-terminus of the beetle peptide was blocked (results not shown). At this stage it was decided to synthesize the new peptide and characterize the synthetic compound in comparison with the natural one.

Comparison of natural and synthetic peptide

The synthetic N- and C-terminally blocked octapeptide, now given the acronym Mem-CC peptide, had the same chromatographic behaviour under different h.p.l.c. conditions [steep or shallow gradient elution as described in legends to Fig. 1 and 3; isocratic elution in 10 mM-sodium phosphate buffer, pH 6.9, containing 30% (v/v) acetonitrile] as the natural peptide from beetle corpora cardiaca and was always co-eluted as a single absorbance peak when co-injected (results not shown).

Bioactivity

Since the new peptide has two structural features that are not common in peptides of the AKH/RPCH family, namely the charged aspartic acid residue at position 7 and the tyrosine residue at position 4, I wanted to know whether these structural

Fig. 3. Enzymic deblocking of the cockchafer peptide

The peak 1 material (designated Mem-CC) from 40 corpora cardiaca previously purified by reverse-phase h.p.l.c. was incubated with pyroglutamate aminopeptidase. The chromatograms show the separation of a sample after 1 min (20 μ l; a) and 60 min (180 μ l; b) of enzymic digestion fractionated on a Nucleosil C₁₈ column with the solvents described in the legend to Fig. 1. The linear gradient ran from 33 to 58 % solvent B within 25 min at a flow rate of 1 ml/min.

Table 3. Amino acid composition of cockchafer and dor-beetle neuropeptide after total hydrolysis

H.p.l.c.-purified material from 100 cockchafer and 15 dor-beetle corpora cardiaca was hydrolysed with 5.7 M-HCl for 24 h and, after modification with phenyl isothiocyanate, the phenylthiocarbamoyl amino acids were quantified by h.p.l.c. Values are given as molar ratios with respect to leucine (= 1.00).

Amino acid residue	Composition (molar ratio)	
	Cockchafer	Dor beetle
Asx	1.91	1.92
Glx	0.97	0.91
Ser	0.83	0.80
Pro	1.12	1.02
Tyr	0.81	0.78
Leu	1.00	1.00

Table 4. Amino acid sequence of cockchafer and dor-beetle neuropeptide by automated Edman degradation

The *N*-terminal pyroglutamate residue of the h.p.l.c.-purified peptide from 40 cockchafer and 15 dor-beetle corpora cardiaca was cleaved off by pyroglutamate aminopeptidase, and the deblocked peptides were analysed.

Amino acid	Recovery (pmol)	
	Cockchafer	Dor beetle
Leu ¹	649	53
Asn ²	412	36
Tyr ³	514	24
Ser ⁴	155	14
Pro ⁵	386	36
Asp ⁶	171	14
Trp ⁷	118	4

changes have an influence on the bioactivity in the locust (lipid-mobilizing) and cockroach (carbohydrate-mobilizing) test systems. Therefore, we synthesized the [Asn⁷] analogue of Mem-CC; the [Phe⁴,Asn⁷] analogue was already available as a synthetic compound, since this molecule occurs as a natural bioanalogue in the corpora cardiaca of beetles from the Family Tenebrionidae [16]. It is obvious from Table 5 that the [Phe⁴,Asn⁷] analogue (Tem-HrTH) is quite potent in the locust bioassay: a response of almost 50 % (compared with the native locust hormone Lom-AKH-I) is reached with 15 pmol. The [Asn⁷] analogue shows

much less potency: even with 600 pmol injected the full response is not achieved, and 200 pmol is needed for a 50 % response. The new peptide, Mem-CC, is very inactive and shows only about 40 % activity even when 400 pmol is injected.

Similar results were obtained when these peptides were injected into cockroaches (Table 5). Whereas the [Phe⁴,Asn⁷] analogue is very potent and achieves a nearly 100 % response with about 15 pmol, 200 pmol of the [Asn⁷] analogue is needed for a 60 % response, and the Mem-CC peptide gives less than 50 % response when 400 pmol is injected.

Table 5. Adipokinetic and hypertrehalosaemic activity of Mem-CC and analogues

Locusts and cockroaches were injected as indicated. Total haemolymph lipid concentrations were measured in adult male locusts and total haemolymph carbohydrate concentrations were measured in adult male cockroaches before and 1.5 h (locusts) or 2 h (cockroaches) after injections. The Table shows the increase (means $\pm$ s.d.) in blood metabolites in this time expressed as mg/ml of blood. All increases were significant as indicated by * $P < 0.01$ and † $P < 0.001$ using paired t tests. Furthermore, the different responses are expressed as percentages of the response (= 100%) to either endogenous locust (Lom-AKH-I) or cockroach (Pea-CAH-I) hormone.

Treatment	Amount (pmol)	Locusta migratoria		Periplaneta americana	
		Accepter insect ...	Increase in blood lipids (mg/ml)	n	Increase in blood carbohydrates (mg/ml)
Lom-AKH-I	10	6	37.3 $\pm$ 4.8† (= 100 %)	—	—
Pea-CAH-I	10	—	—	7	26.8 $\pm$ 9.0† (= 100 %)
Mem-CC	100	8	4.9 $\pm$ 1.9† (= 13 %)	6	6.5 $\pm$ 3.9* (= 24 %)
	200	7	9.8 $\pm$ 3.7† (= 26 %)	7	9.4 $\pm$ 3.9* (= 35 %)
	400	7	13.7 $\pm$ 8.9* (= 37 %)	8	11.5 $\pm$ 4.7† (= 43 %)
[Asn ⁷] analogue	200	5	19.1 $\pm$ 2.3† (= 51 %)	7	16.8 $\pm$ 6.6† (= 63 %)
	600	7	31.3 $\pm$ 6.0† (= 84 %)	—	—
[Phe ⁴ ,Asn ⁷] analogue (= Tem-HrTH)	15	4	15.7 $\pm$ 2.6* (= 42 %)	8	24.9 $\pm$ 11.3† (= 93 %)
	80	6	27.6 $\pm$ 6.2† (= 74 %)	—	—

DISCUSSION

Peptide structure

The peptide isolated and sequenced from the corpora cardiaca of the two beetles *Melolontha melolontha* and *Geotrupes stercorosus* has the structure pGlu-Leu-Asn-Tyr-Ser-Pro-Asp-Trp-NH₂ and appears to be a new member of the large AKH/RPCH family of peptides, as judged from its blocked N- and C- termini, and the pyroglutamic acid and tryptophan residues at position 1 and 8 respectively. It shows two unusual features: (1) an aspartic acid residue at position 7 and, thus, a charge; another charged peptide with an aspartic acid residue at position 7 has recently been found in *Drosophila melanogaster* [12] and *Phormia terraenovae* [11]; (2) a tyrosine residue at position 4, which is a unique feature not previously found in any other member of the AKH/RPCH family. The change from phenylalanine to tyrosine requires only a single base change and can be explained by point mutation.

The new beetle peptide shows the most striking resemblance (75 % of its primary structure) to the octapeptides sequenced from prawns (RPCH: pGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH₂) [26], from tenebrionid beetles (Tem-HrTH: pGlu-Leu-Asn-Phe-Ser-Pro-Asn-Trp-NH₂) [16] and from the above-mentioned flies (Pht-HrTH: pGlu-Leu-Thr-Phe-Ser-Pro-Asp-Trp-NH₂) [11,12].

Receptor considerations

The time-course relationships for Mem-CC, the [Asn⁷] analogue and the [Phe⁴,Asn⁷] analogue (= Tem-HrTH) were similar to those observed for the native peptides in the lipid and carbohydrate bioassay in locusts and cockroaches respectively (G. Gäde, unpublished results). Therefore it can safely be inferred that the different relative activities shown in Table 5 very probably reflect structural requirements of the receptor(s) and not simply differences in the transport rates of the various peptides to the fat-body and/or different rates of degradation in the haemolymph. Whereas the naturally occurring analogue, Tem-HrTH, was only 2–10 times less active in the bioassays, the extra phenolic hydroxy group at position 4 changing phenylalanine to tyrosine ([Asn⁷] analogue) resulted in a 20- to more than 60-fold decrease in potency, and this was even potentiated when the

charged Mem-CC peptide was analysed. Thus evolutionary pressure has operated against [Tyr⁴] and/or [Asp⁷] analogues as potent hypertrehalosaemic or adipokinetic peptides. As shown before, the locust receptor(s) seem to be more specific than the cockroach receptor(s) and do not tolerate even conservative exchanges in the sequence of Lom-AKH-I [11,27,28].

Functional considerations

Without knowing the actual losses during purification, a rough calculation shows that the corpora cardiaca of *Melolontha melolontha* and *Geotrupes stercorosus* contain about 30 and 6 pmol of the peptide Mem-CC respectively.

It remains to be established what function(s) Mem-CC serves in the beetles investigated. Both species are powerful flyers, and it is inferred from previous studies on similar peptides in locusts and dragonflies (for example refs. [1 and 13]) that Mem-CC is involved in regulation of metabolic homeostasis. It is thought that beetles of the scarabaeid family use proline as fuel for flight [29–31], as was established previously for the Colorado potato beetle, *Leptinotarsa decemlineata* [32,33]. Thus one possible physiological role of the peptide may be to make available acetyl-CoA, from triacylglycerols, for synthesis of proline in the fat-body. Such action was proposed for corpora cardiaca factors in *Leptinotarsa decemlineata* [33]. These factors appear to be the two peptides of the AKH/RPCH family sequenced from the corpora cardiaca of the Colorado potato beetle [15].

On the other hand, Lom-AKH-I is known for its action on inhibiting protein synthesis in the fat-body [34] and its myotropic effect on the extensor tibialis muscle in locusts [12]. Both effects have still to be measured with Mem-CC.

Evolutionary considerations

Mem-CC is now the fourth AKH/RPCH peptide sequenced from a beetle species. Previously it had been shown that a member of the beetle Family Chrysomelidae, the Colorado potato beetle, contained the same two octapeptides in its corpora cardiaca as the American cockroach, *Periplaneta americana* [15]. A different octapeptide was found in two members of the Family Tenebrionidae, the meal-worm beetle *Tenebrio molitor* and its relative *Zophobas rugipes* [16].

Now I have characterized fully another octapeptide in members of the Families Scarabaeidae and Geotrupidae. Other scarabaeid beetles (*Melolontha hippocastani*, *Pachnoda marginata*, *Pachnoda sinuata*) appear to have the same peptide, as judged from the retention time in reverse-phase h.p.l.c. and amino acid composition data [17]. All these data then point to a Family-specificity of these peptides in Coleoptera. In this context it should be mentioned that Geotrupidae (dor beetles) and Scarabaeidae belong to the same Superfamily (Scarabaeoidea), thus having a common evolutionary history [35].

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