On the Release and Action of the Hypertrehalosaemic Hormone from the Cockroach *Nauphoeta cinerea*

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Synthetic Hypertrehalosaemic Hormone, *Nauphoeta cinerea,* Activation of Fat Body Glycogen Phosphorylase, Increase of Blood Carbohydrates, Release of Hypertrehalosaemic Hormone

The corpora cardiaca of the cockroach *Nauphoeta cinerea* contain a hypertrehalosaemic hormone (HTH) which is chemically characterized as a blocked decapeptide. The synthetic HTH shows the same chromatographic behaviour as the material isolated from corpora cardiaca. The synthetic peptide causes hypertrehalosaemia and fat body glycogen phosphorylase-activation in *N. cinerea* as well as in the American cockroach, *Periplaneta americana* in a dose-dependent fashion. It is calculated that one gland from *N. cinerea* stores about 50 pmol of HTH. Roughly 10% of the total available hormone in the gland is released *in vitro* during exposure to an elevated potassium saline which causes depolarization of the neurosecretory cells.

Introduction

In 1961 the presence of a factor in the corpora cardiaca of the American cockroach *Periplaneta americana,* was demonstrated which was capable of elevating haemolymph trehalose levels in that species [1]. Recently, two myoactive peptides (designated M I and M II) were isolated from the corpora cardiaca of *P. americana* [2] and their primary structures elucidated [3]. These peptides proved to be identical to the hypertrehalosaemic factors of the American cockroach isolated by Gäde [4–6] and sequenced by Scarborough *et al.* [7]. Both peptides are octapeptides (M I: pGlu–Val–Asn–Phe–Ser–Pro–Asn–Trp–NH_2; M II: pGlu–Leu–Thr–Phe–Thr–Pro–Asn–Trp–NH_2). They have been shown to increase the concentration of the second messenger cyclic AMP in the fat body marginally when injected *in vivo* [8], but not *in vitro* [9], and to activate fat body glycogen phosphorylase *in vivo* [8] and *in vitro* [9].

Another hypertrehalosaemic peptide (HTF II) was isolated [4, 10] and sequenced [11] from the corpus cardiacum of the Indian stick insect, *Carausius morosus*. It is a decapeptide (HTF II: pGlu–Leu–Thr–Phe–Thr–Pro–Asn–Trp–Gly–Thr–NH_2) with close homology to M I and M II.

Last year, a new peptide with hypertrehalosaemic activity was found in the corpus cardiacum of the cockroach, *Nauphoeta cinerea* [12]. It was isolated and purified by reversed-phase high performance liquid chromatography (RP-HPLC), identified as a decapeptide by its amino acid composition data [13] and, subsequently, assigned its primary structure by fast atom bombardment mass spectrometry (FABMS) (HTH: pGlu–Val–Asn–Phe–Ser–Pro–Gly–Trp–Gly–Thr–NH_2) [14]. The structure is similar to those of other insect hypertrehalosaemic peptides and all these compounds belong to the so-called adipokinetic/red pigment-concentrating hormone family of arthropod peptides (see [15]). An identical HTH has been found in the corpus cardiacum of another cockroach species, *Blaberus discoidalis* [16].

In our previous studies we assayed the increase of haemolymph carbohydrates and the activation of fat body glycogen phosphorylase not in *N. cinerea* itself, but in the American cockroach [12, 13]. Furthermore, we had always used crude extracts of corpora cardiaca or, at best, HPLC-purified fractions. After elucidation of the primary structure it was possible to synthesize the peptide. With the availability of synthetic HTH it was feasible to study the ability of known amounts of this compound to activate glycogen phosphorylase and elicite hypertrehalosaemia in the haemolymph. To get insights into the action of HTH in *N. cinerea* itself, we analyzed these effects in *N. cinerea* as well as in the American cockroach. In addition, to determine whether HTH plays a physiological role in *N. cinerea,* we developed a rapid and simple method to demonstrate release of HTH from the corpus cardiacum.

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Materials and Methods

Insects

Adult cockroaches, *Nauphoeta cinerea,* of both sexes were a gift from Dr. B. Lanzrein (University of Bern, Switzerland). Adult American cockroaches, *Periplaneta americana,* of both sexes were supplied by Professor Dr. K. Hansen (Universität Regensburg, FRG) or from Thompson Company (Düsseldorf, FRG) and male adult migratory locusts, *Locusta migratoria* were purchased from a commercial dealer. All animals were kept in our insectary at about 25 °C with a LD of 14:10 h light cycle and were reared as described previously [12, 13].

Chemicals

Biochemicals for the phosphorylase assay were obtained from Boehringer (Mannheim, FRG), chemicals for the HPLC-solvents and all other chemicals (analytical grade) came from Merck (Darmstadt, FRG). The *N. cinerea* hypertrehalosaemic decapeptide (HTH) was custom-synthesized by Dr. S. Kyin (University of Illinois, Biotechnology Center, Urbana-Champaign, USA). Natural HTH from *N. cinerea* corpora cardiaca was purified by RP-HPLC as outlined elsewhere [13].

Bioassays

Adipokinetic activity was measured by injection of samples (10 µl) into male (14- to 25-day-old) acceptor locusts as described earlier [17]. Hypertrehalosaemic activity was assayed by injection of material (10 µl) either into adult male acceptor American cockroaches [17] or into adult acceptor *N. cinerea.* Haemolymph sampling in the latter species is somewhat difficult since the haemolymph clots rapidly. The samples were collected by bending the cockroaches as nearly in half as possible between the thorax and abdomen. This allows the haemolymph to accumulate in the abdominal sinus (T. K. Hayes, personal communication). An intersegmental membrane of the dorsal abdomen was then pierced and a 1 µl capillary filled with the extruding haemolymph and immediately blown into concentrated sulphuric acid. The second haemolymph sample was taken 2 h post-injection.

Concentrations of total haemolymph lipids (vanillin-positive material) or carbohydrates (anthrone-positive material) were analyzed according to previously published methods [18, 19].

Fat body glycogen phosphorylase of adult *N. cinerea* and of adult female *P. americana* was assayed 15 to 20 min after injection of known amounts of synthetic HTH as outlined in detail elsewhere [8] by determining glycogen breakdown spectrophotometrically [20]. All values for active phosphorylase (in the absence of AMP) are given as the percentage of total phosphorylase activity (in the presence of AMP).

Release experiments

The ability of the corpora cardiaca to release hypertrehalosaemic *(N. cinerea)* and adipokinetic *(L. migratoria)* hormones was investigated by incubating the glands in saline with low and high potassium concentration as depicted in Fig. 1. Whole corpora cardiaca from adult *N. cinerea* and adult *L. migratoria* were dissected with utmost care, rinsed with saline I (see below), 4 glands (experiment 1) or 10 glands (experiment 2) placed into wells of a microtitre plate filled with 200 µl (experiment 1) or 250 µl (experiment 2) of either saline I (140 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM $NaHCO_3$, 5 mM trehalose, 90 mM sucrose and 5 mM Hepes, pH 7.2) [2] or in saline in which the potassium concentration was elevated to 50 mM, replacing an equimolar concentration of sodium (saline II) and incubated in a shaking water bath at 25 °C. After 60 min 80 µl (experiment 1) or 120 µl (experiment 2) of both salines were removed, dried by vacuum centrifugation (Speed-Vac, Savant) and chromatographed (see below). Appropriate fractions were collected manually, dried as above, dissolved in 50 µl (experiment 1) or 80 µl (experiment 2) of distilled water and used for bioassays by injection of a 10 µl dose into 5 or 8 receptor animals. For convenience, the salines from the *N. cinerea* experiment were injected into adult male American cockroaches and the carbohydrate concentration of the haemolymph measured, whereas the salines from the *L. migratoria* experiments were tested for hyperlipaemic effects in locusts.

RP-HPLC

The dried material from the release experiments was dissolved in 25 µl of 25% solvent B (0.1% trifluoroacetic acid in 60% acetonitrile), vigorously mixed and applied to a Nucleosil C-18 column. Details of the equipment used and the conditions employed are described elsewhere [10] and in the legend of Fig. 2.

Results

Comparison of natural and synthetic peptides

As depicted in Fig. 2 (A *versus* B) natural HTH isolated from the corpora cardiaca of *N. cinerea* showed a single absorbance peak at 210 nm with a retention time of 12.0 min and co-migrated with the peak of the synthetic HTH prepared according to the sequence assigned previously by FABMS [14]. Co-injection of the natural and synthetic peptides gave a single larger peak with no hint of resolution (Fig. 1C).

Dose-response relationships for hypertrehalosaemia and phosphorylase activation in N. cinerea

Synthetic HTH was injected into acceptor *N. cinerea* cockroaches in a variety of doses in order to demonstrate complete dose-response relationships for hypertrehalosaemic and phosphorylase-activating responses. A dose of 5 pmol of HTH is sufficient to elicite a maximal hypertrehalosaemic effect and about 3 pmol are needed for a significant response ($p = 0.01$, Student's t-test, Fig. 3A). The maximal increase in haemolymph carbohydrate levels was only about 7 mg/ml.

A much lower dose of HTH is needed to activate the glycogen phosphorylase of the fat body from *N. cinerea* fully when compared to the effect on blood carbohydrates (Fig. 3B *versus* 3A); 0.5 pmol cause already a maximal activation and 0.2 pmol of HTH are sufficient for a significant response ($p = 0.05$, Student's t-test, Fig. 3B). In non-injected or water-injected insects the phosphorylase is about 30% active whereas about 60% of the enzyme is in the active form when maximal stimulated.

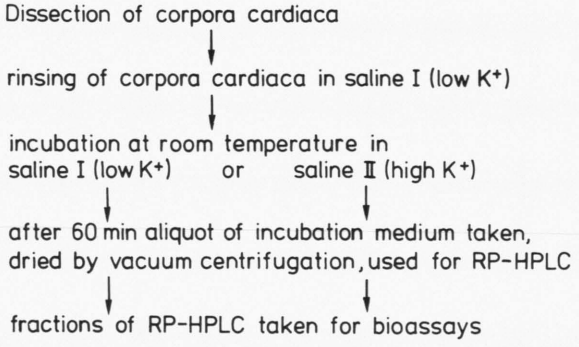


Fig. 1. Diagrammatic scheme of the release experiments. For further details see text.

Dose-response relationships for hypertrehalosaemia and phosphorylase activation in P. americana

For comparison, synthetic HTH was also administered by intrahaemocoelic injection into acceptor *P. americana*, and complete dose-response curves were established for both hypertrehalosaemic and phosphorylase-activating responses. The maximal increase in the levels of haemolymph carbohydrates was much higher in *P. americana* than in *N. cinerea* (26 mg/ml *versus* 7 mg/ml; Fig. 4A *versus* Fig. 3A). This maximal response in *P. americana* is achieved upon injection of 6 to 8 pmol of HTH. A significant

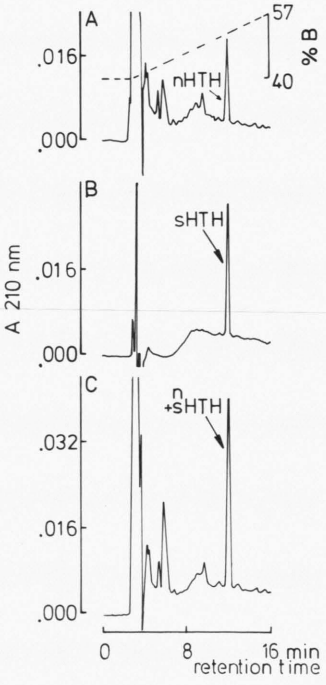


Fig. 2. The separation of natural and synthetic hypertrehalosaemic peptide from *N. cinerea* (HTH) using RP–HPLC. The figure shows chromatograms of
A. natural hypertrehalosaemic material of 8 pairs of *N. cinerea* corpora cardiaca chromatographed previously on HPLC;
B. 400 pmol of synthetic HTH (sHTH);
C. natural hypertrehalosaemic material (equivalent to 8 gland pairs) plus 400 pmol sHTH.
The analyses were performed on a Nucleosil C-18 column (*i.d.* 4.6 mm, length 250 mm) 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 40 to 65% B within 22.5 min at a flow rate of 1 ml/min. The gradient lag time after injection (0 min) was 2.25 min. The elution was monitored at 210 nm.

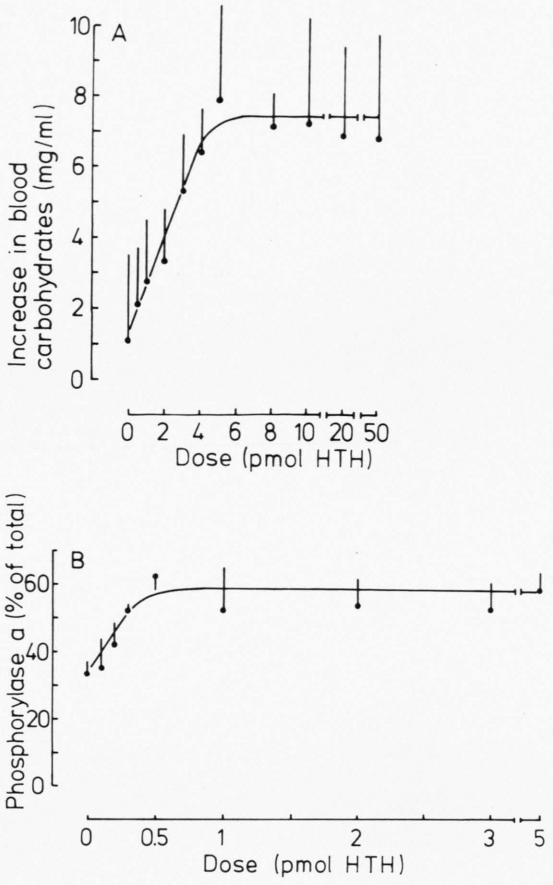


Fig. 3. Hypertrehalosaemic effect (A) and fat body glycogen phosphorylase activation (B) in response to different doses of synthetic HTH. At least 5 individuals of *N. cinerea* were used as acceptor insects. Values are given as means ± S.D.

hypertrehalosaemic effect ($p = 0.001$, Student's t-test) is evident when 0.5 pmol of HTH are injected (Fig. 4A).

The glycogen phosphorylase of the fat body from *P. americana,* which is about 20% activated in control insects, is activated fully (above 60%) by injection of roughly 0.5 pmol of HTH (Fig. 4B). For a significant activation above control values about 0.3 pmol of HTH are needed ($p = 0.001$, Student's t-test).

Release of adipokinetic hormones I and II from the corpora cardiaca of L. migratoria in vitro

Our newly developed simple and rapid method to monitor release of neurohormonal material from corpora cardiaca was first tested on the glands from migratory locusts. Four pairs of corpora cardiaca were incubated in saline solutions with either low (saline I) or high (saline II) potassium concentration. After an incubation time of 60 min an aliquot of both media (here simply called saline I or saline II) was applied onto a HPLC C-18 column. For saline I, one small distinctive absorbance peak at 210 nm was observed which had a retention time of 15.6 min (Fig. 5B). When saline II medium was chromatographed, a much higher absorbance peak with a retention time of 15.7 min was found and, additionally, a second,

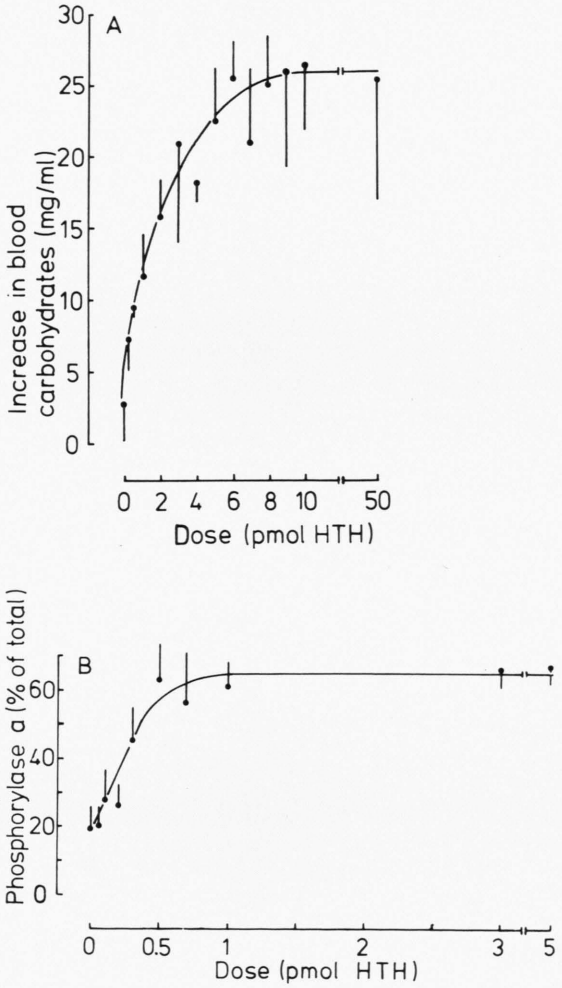


Fig. 4. The effect of increasing doses of synthetic HTH on carbohydrate mobilization (A) and phosphorylase activation (B) in *P. americana.* Values are given as means ± S.D. for at least 6 observations.

smaller one with a retention time of 17.3 min (Fig. 5C). As shown in Fig. 5D synthetic AKH I and AKH II-L had identical retention times (15.7 and 17.2 min, respectively) to the compounds found in the release experiments. On the other hand, separation of control salines (without incubation of corpora cardiaca) on HPLC revealed no absorbance peaks between 15 and 18 min (Fig. 6B and 6C); the

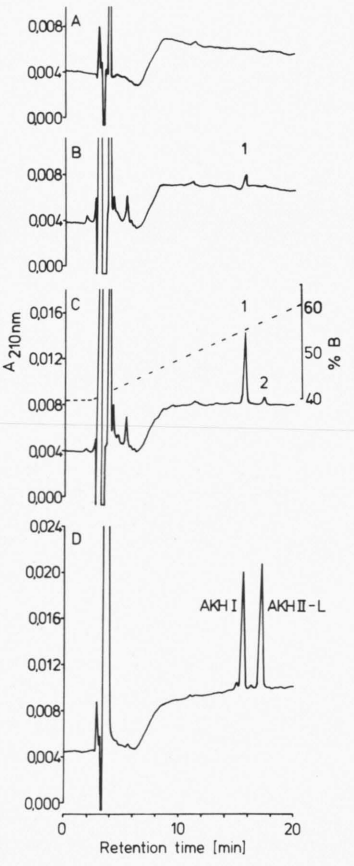


Fig. 5. Chromatographic evidence for release of adipokinetic hormones I and II from corpora cardiaca of *L. migratoria* upon incubation in saline with high potassium concentration. For further explanation see Fig. 1 and text.
A. HPLC control run; injection of 50 µl of solvent B (25%);
B. HPLC run of saline I from the incubation of 4 locust glands;
C. HPLC run of saline II from the incubation of 4 locust glands;
D. HPLC run of 50 pmol of synthetic AKH I and AKH II-L.
The fractions labelled 1, 2, AKH I and AKH II-L were collected and used for the adipokinetic bioassay (see Table I).

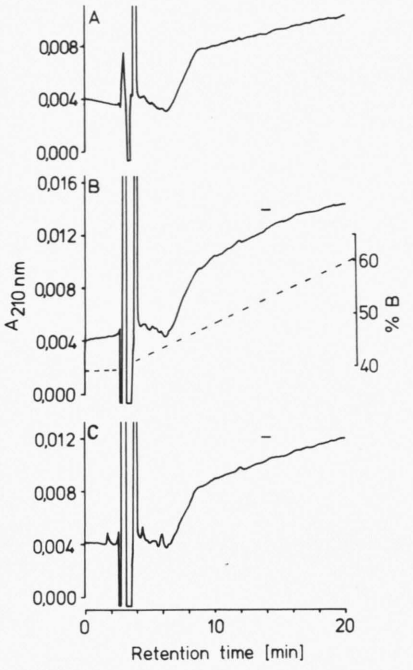


Fig. 6. HPLC runs of different salines
A. control run; injection of 50 µl of solvent B (25%);
B. injection of the equivalent of 100 µl of saline I;
C. injection of the equivalent of 100 µl of saline II.
The marked fractions were collected and used for the adipokinetic (see Table I) and hypertrehalosaemic (see Table II) bioassay.

chromatograms were almost identical to the one where only solvent B was applied to the column as a control (Fig. 6A). Further evidence that the released substances are indeed the adipokinetic hormones came from our bioassays. When the HPLC-fractions were tested for hyperlipaemia in locusts, the material from saline I as well as both fractions from saline II caused an adipokinetic effect (Table I).

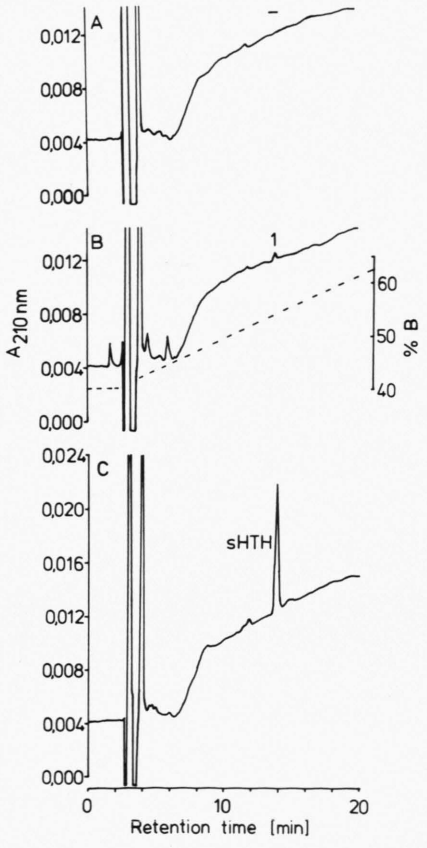
Release of hypertrehalosaemic peptide HTH from the corpora cardiaca of N. cinerea in vitro

Release experiments similar to locust corpora cardiaca were performed using corpora cardiaca from *N. cinerea*. In the first experiment 4 glands were placed in salines with either low (saline I) or high (saline II) potassium levels. Only the HPLC-chromatogram of saline II showed a very small peak (Fig. 7B), whereas saline I produced no absorbance peak in this region (Fig. 7A). The retention time of the absorbance peak, numbered 1 (Fig. 7B), was identi-

Table I. Total haemolymph lipid concentration in adult male migratory locusts before and 90 min after injection of material from different fractions after HPLC (see Fig. 5 and 6). The values shown are the means ± S.D. The significance (p) was evaluated by Student's t-test.

Injection (10 µl dose)	Blood lipids [mg/ml] n	0 min	90 min	Difference	p	Remarks
Controls:						
saline I	5	17.8±3.5	21.9± 2.8	4.1± 2.2	n.s.*	see Fig. 6B
saline II	5	19.1±2.8	24.1± 4.1	5.0± 3.5	n.s.	see Fig. 6C
Release experiment:						
saline I, peak 1	5	20.5±8.4	46.0±15.6	25.5±10.4	≦ 0.05	see Fig. 5B
saline II, peak 1	5	21.2±9.0	49.1±12.9	27.9± 7.9	≦ 0.01	see Fig. 5C
peak 2	5	14.5±1.6	30.4±10.8	15.9±10.2	≦ 0.05	see Fig. 5C
AKH I, 50 pmol	5	14.1±2.4	42.3± 8.4	28.2± 8.6	≦ 0.001	see Fig. 5D
AKH II, 50 pmol	5	15.8±3.3	38.8± 4.3	23.0± 1.4	≦ 0.001	see Fig. 5D

* n.s. = not significant.



cal to that of synthetic HTH (Fig. 7C). In a second release experiment the number of corpora cardiaca incubated with different salines was increased to 10. In this case, the absorbance peak with a retention time of 13.4 min (peak 1) from saline II was much higher than in the previous experiment (Fig. 8B *versus* 7B) co-migrating again with synthetic HTH (Fig. 8C). The height and the shape of the peaks were almost identical; thus, roughly 50 pmol of HTH were detected in the release experiment. No release of HTH material was found when the corpora cardiaca were incubated with saline I (Fig. 7A). To further corroborate the identity of the released compound, the material was used for eliciting a hypertrehalosaemic response in *P. americana*. In both experiments the appropriate fractions from the HPLC runs

Fig. 7. Chromatographic evidence for release of the hypertrehalosaemic peptide (HTH) from corpora cardiaca of *N. cinerea* upon incubation in saline with high potassium concentration.
A. HPLC run of saline I from the incubation of 4 cockroach glands;
B. HPLC run of saline II from the incubation of 4 cockroach glands;
C. HPLC run of 50 pmol of synthetic HTH.
The fractions labelled with a stripe, 1, and HTH were collected and used for the hypertrehalosaemic bioassay (see Table II).

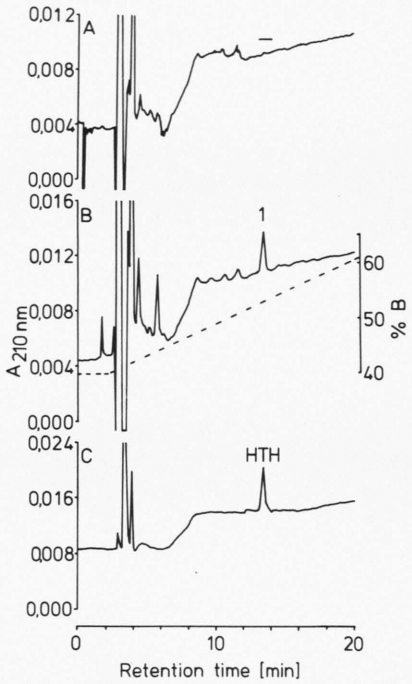


Fig. 8. Quantitative chromatographic evidence for the release of HTH from corpora cardiaca of *N. cinerea*.
A. HPLC run of saline I from the incubation of 10 cockroach glands;
B. HPLC run of saline II from the incubation of 10 cockroach glands;
C. HPLC run of 50 pmol of synthetic HTH.
The fractions labelled with a stripe and 1 were collected and used for the hypertrehalosaemic bioassay (see Table II).

of saline I caused a small hypertrehalosaemic effect; however, levels of blood carbohydrates were almost maximally elevated upon injection of peak 1 material from saline II (Table II).

Discussion

The hypertrehalosaemic neuropeptide HTH from the corpora cardiaca of *N. cinerea* is a blocked decapeptide whose primary structure had been elucidated by FABMS [14]. As a prerequisite for the present study on the quantitative effect of the synthetic peptide in *N. cinerea* we had first to establish that the natural and synthetic compounds were identical. This was achieved by RP-HPLC: in our chromatographic systems natural and synthetic HTH have identical retention times. The results on the mode of action of synthetic HTH show clearly that it acts *via* activation of the glycogen phosphorylase in the fat body. The activation by synthetic HTH is dose-dependent and low doses are already effective. Thus, 0.2 pmol of synthetic HTH are sufficient for a significant activation. The activation is entirely due to the conversion of the inactive form of phosphorylase (phosphorylase b) to the active form (phosporylase a), because the specific activity of the total enzyme is unaffected (unpublished observations). The product of phosphorylase action is glucose-1-phosphate which is converted *via* several steps to trehalose, the

Table II. Total haemolymph carbohydrate concentration in adult male American cockroaches before and 120 min after injection of material from different fractions after HPLC (see Fig. 6, 7 and 8). The values shown are the mean ± S.D. The significance (p) was evaluated by Student's t-test.

Injection (10 µl dose)	n	Blood carbohydrates [mg/ml] 0 min	120 min	Difference	p	Remarks
Controls:						
saline I	5	17.9±2.3	22.8±4.7	4.7±3.2	n.s.*	see Fig. 6B
saline II	5	17.2±2.7	21.1±2.9	3.9±1.8	n.s.	see Fig. 6C
Release experiment 1:						
saline I, fraction 14/15	5	16.2±1.5	22.5±3.8	6.3±3.1	≦ 0.01	see Fig. 7A
saline II, peak 1	5	15.8±1.2	31.9±2.3	16.1±1.8	≦ 0.001	see Fig. 7B
Release experiment 2:						
saline I, fraction 13/14	8	22.5±4.6	30.8±8.3	8.3±5.0	≦ 0.05	see Fig. 8A
saline II, peak 1	8	19.1±2.6	42.7±7.1	23.6±6.1	≦ 0.001	see Fig. 8B
HTH, 50 pmol	5	17.3±1.9	42.0±2.1	24.7±2.5	≦ 0.001	see Fig. 7C

* n.s. = not significant.

main haemolymph sugar in insects. An increase of trehalose in the haemolymph upon injection of low doses of synthetic HTH is shown. However, the doses needed are about 10-fold higher as were necessary to activate glycogen phosphorylase. Furthermore, the maximal change in the blood sugar concentration after injection of synthetic HTH is only about 7 mg/ml; thus, very small when compared to the changes in blood sugar concentration occurring in the American cockroach (see below). This result may be related to the low concentration of glycogen stored in the fat body of *N. cinerea,* but no data are available yet. It is interesting to note in this context that the haemolymph carbohydrate concentration of another cockroach, *Blaberus discoidalis,* is also only increased by about 5 mg/ml when injected with 10 pmol of synthetic HTH, which is the endogenous hormone for that species, too [16]. As both cockroach species, *N. cinerea* and *B. discoidalis,* are far slower when running than *P. americana,* it is possible that there is a correlation between the degree of activity and the maximal release of carbohydrates, thus action of HTH. In other words, insects that use carbohydrates as energy source for "fight and flight" reactions do need quantitatively more readily oxidizable carbohydrates in their haemolymph and this is achieved by HTH.

Nevertheless, it is shown for the first time in this study that the endogenous hormone of *N. cinerea,* HTH, activates glycogen phosphorylase and stimulates the release of carbohydrates into the haemolymph at low doses.

When synthetic HTH is injected into the American cockroach, *P. americana,* the effects are very similar to *N. cinerea:* about the same doses are needed for a maximal activation of fat body glycogen phosphorylase and for the increase of blood carbohydrates. However, the quantity of carbohydrates released into the haemolymph is much higher (see Discussion above). These experiments are also interesting from another point of view. In a previous study [13] we had injected different quantities of corpora cardiaca equivalents from *N. cinerea* into *P. americana* and had constructed complete dose-response curves. From these experiments and the ones of the present study, where we injected known amounts of synthetic HTH, we are able to calculate the amount of HTH present per corpus cardiacum of *N. cinerea.* In our former study 0.1 and 0.01 corpus cardiacum equivalents, respectively, were needed for a maximal increase of blood carbohydrates and activation of phosphorylase, respectively [13], whereas 5 pmol and 0.5 pmol, respectively, of synthetic HTH were needed (this study). From these data it can be calculated that one corpus cardiacum of *N. cinerea* contains about 50 pmol of HTH. According to O'Shea *et al.* [2] *P. americana* stores about 46 pmol and 15 pmol, respectively, of the hypertrehalosaemic hormones I and II (M I and M II) per gland.

The study so far has shown that HTH is stored in the corpora cardiaca of *N. cinerea;* it acts *via* activation of glycogen phosphorylase and causes hypertrehalosaemia. For this task the peptide must be released from the corpus cardiacum into the haemolymph and be transported to the fat body. Therefore, we designed experiments to study the release of HTH. We chose an *in vitro* system that has been previously used for detecting release of M I and M II from the corpora cardiaca of *P. americana* [2]. However, the method was rather complicated and time-consuming; therefore, we modified the set-up and present here a very simple but effective method. We first tried this methodology with corpora cardiaca from *L. migratoria,* because it is well-known that the two adipokinetic hormones (AKH I and AKH II-L) are released from the intrinsic neurosecretory cells of the glandular lobe with octopamine serving as a neurotransmitter [21]. We simply caused a massive depolarization in the neurosecretory cells by incubating corpora cardiaca in a saline containing a high potassium concentration besides other ions and sugars. Possibly, the depolarization resulted also in the release of neurotransmitters and, in turn, of the neuropeptides into the surrounding saline. The latter was directly used for separation of HPLC without removing salts and sugars (as was done in [2]) and the absorbance at 210 nm was recorded. It is obviously (Fig. 5C) that two compounds that absorb at 210 nm are released. The evidence that the released materials are AKH I and AKH II-L is as follows. First, the absorbance peaks co-elute exactly with standards of AKH I and AKH II-L (Fig. 5D). Second, the isolated compounds have profound adipokinetic activity when used in the locust bioassay. Finally, the compounds are released in levels apparently proportional to their abundance in the corpus cardiacum; the ratio of compound 1 to compound 2 was about 6–8: 1, and that is also true for AKH I: AKH II-L in a corpus cardiacum extract [4]. When similar experiments were conducted with corpora cardiaca from *N. cine-*

rea, one compound that had an identical retention time as HTH and caused hypertrehalosaemia was detected upon depolarization with an elevated potassium saline. The release of HTH is dependent on the number of corpora cardiaca used for the experiment suggesting that one corpus cardiacum always releases the same amount upon stimulation. From our data we can calculate that roughly 50 pmol of HTH are released by 10 glands. As one gland stores about 50 pmol of HTH (see above), we estimate a release of about 10% of the total available hormone in the gland over a 60 min exposure to the elevated potassium saline. This number is in good agreement to the 5% released of the hypertrehalosaemic hormones from the American cockroach upon 10 min of depolarization [2].

In summary, it was shown that low doses of synthetic HTH causes activation of glycogen phosphorylase in the fat body of *N. cinerea* and result in hypertrehalosaemia. The biological significance of HTH to the cockroach is further corroborated by the fact that it is releasable *in vitro* from the corpora cardiaca upon depolarization with an elevated potassium saline. *In vivo* this would produce a release of HTH directly from the corpora cardiaca into the haemolymph.

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