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Isolation and primary structure of two peptides with cardioacceleratory and hyperglycemic activity from the corpora cardiaca of *Periplaneta americana*

(invertebrate neuropeptide/peptide family/metabolic effects/amino acid sequence)

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ABSTRACT **Two cardioacceleratory peptides from the corpora cardiaca of *Periplaneta americana* have been purified by gel filtration and reversed-phase liquid chromatography. Based on analysis of the intact factors and their chymotryptic fragments, we have assigned the primary structure of these octapeptides as pGlu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2, designated periplanetin CC-1, and pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2, designated periplanetin CC-2. They represent new members of a family of invertebrate peptides that includes locust adipokinetic hormone and crustacean red-pigment concentrating hormone. Both peptides show adipokinetic activity in grasshoppers and hyperglycemic activity in cockroaches. One of these peptides (CC-2) has provocative sequence homology with the NH_2-terminal portion of glucagon.**

The insect corpora cardiaca (CC) are major neurohemal organs that are analogous to the vertebrate hypothalamo-hypophyseal system. Not only do the CC store and release products synthesized in the brain, but also they contain intrinsic glandular cells producing a variety of bioactive factors affecting developmental, metabolic, and myotropic processes (1). Many of these factors appear to be peptides; only one, adipokinetic hormone (AKH) from *Locusta migratoria*, has been identified (2).

Corpora cardiaca of the cockroach *Periplaneta americana* have proven a rich and accessible source of bioactive factors. Previous studies have demonstrated that CC homogenates and partially purified fractions affect the cockroach heartbeat (3–6) and elevate the concentration of hemolymph trehalose (the main sugar in blood of most insects) (7–9). These factors appear to be peptides (3, 7), but it is difficult to assess how many are actually distinct substances or whether some of them have multiple activities (8, 10–12).

Amino acid compositions for three cardioacceleratory peptides from cockroach CC, but no sequence information, have been reported to date. These include neurohormone D, a peptide of $M_r \approx 1000$ (6), and factors "2a and 2b," small peptides of $M_r \approx 1000$–2000 (11). Factor 2b was reported to have hyperglycemic activity that diminished upon purification, while a factor similar to 2b also has been reported to have hyperglycemic activity (ref. 13 as cited in ref. 9).

In this paper we report the isolation and sequence determination of two structurally related octapeptides from the CC of *P. americana* that have cardioacceleratory and hyperglycemic activity in the host insect.

MATERIALS AND METHODS

Insects. Cockroaches (*P. americana*) were raised at 28°C and 50% relative humidity under a 16-hr light/8-hr dark photo regime and were fed on dry dog food. Corpora cardiaca with corpora allata attached were dissected from 0- to 6-wk-old cockroaches and collected in saline (5 mM $CaCl_2$/1 mM $MgCl_2$/5 mM KCl/140 mM NaCl/4 mM $NaHCO_3$/5 mM trehalose/20 mM Hepes, pH 7.0) at 0°C prior to freezing (−20°C). A total of ≈4000 cockroach CC were used.

Heart Bioassay. Aliquots of all fractions from purifications were assayed for bioactivity by using a semiisolated heart preparation (6). Heart rate was monitored with an impedance converter (UFI model 2991) connected to a frequency integrator and recorder. A heart was selected on the basis of frequency (≤60 beats per min) and regularity and then was bathed in saline to stabilize (30 min). Test samples to be assayed were applied to the heart in a volume of 50 μl.

Carbohydrate and Lipid Bioassays. Hemolymph carbohydrate levels in *P. americana* and hemolymph lipid levels in the grasshopper *Schistocerca nitens* were determined as reported (14). In both assays values were calculated as percentage increase over saline-injected controls.

Extraction and Preliminary Purification. The CC were homogenized in 5 M acetic acid (1.0 ml per 1000 CC) with a 0.5-ml glass homogenizer. The homogenate was centrifuged (10 min at 10,000 × *g*), and the pellet was reextracted. The combined supernatants were applied to a Sephadex G-25 column (1.3 × 100 cm) and eluted with 5 M acetic acid at 6.5 ml/hr at 21°C. To each fraction was added 100 μg of bovine serum albumin prior to drying in a Speed Vac concentrator (Savant). Alternatively, supernatants of CC extracts were applied to a Sep-Pak C_{18} cartridge (Waters), which was washed and then eluted with 30% 1-propanol/H_2O into tubes, each containing 100 μg of bovine serum albumin.

Isolation by Reversed-Phase Liquid Chromatography (RP-LC). Prepurified extracts of CC were further purified by gradient elution RP-LC (see Fig. 1) with a Spectra-Physics model 8700 pump and model 8300 UV detector (254 nm) in series with a Kratos model 773 UV detector (220 nm); a Hewlett-Packard model 3357 data system provided integration and retention data. Those fractions with cardioacceleratory activity were rechromatographed separately in a 25 × 0.46 cm column of 10-μm C_{18} support (Aquapore RP-300; Brownlee Labs, Santa Clara, CA) with 9% 1-propanol in 0.1% CF_3COOH/H_2O (for CC-1) and 12% 1-propanol (for CC-2) as eluents. However, the factors obtained from purification of an early batch as in Fig. 1 were instead next analyzed by using a slow gradient of CH_3OH in 0.1% CF_3COOH/H_2O as in Fig. 2, and 0.5-min fractions were collected through the major peaks. Fractions from RP-LC

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Abbreviations: AKH, locust adipokinetic hormone; RPCH, crustacean red-pigment-concentrating hormone; CC, corpora cardiaca; RP-LC, reversed-phase liquid chromatography; CC-1, periplanetin CC-1; CC-2, periplanetin CC-2.

were collected in polypropylene tubes; 25 μg of bovine serum albumin was added prior to solvent removal.

Molecular Size Estimation. High-speed gel permeation chromatography was performed with a 30 × 0.75 cm column of Spherogel TSK 2000 SW (Beckman) eluted with 40% CH_3CN/0.1% CF_3COOH in water. The column was calibrated with synthetic peptides having 33, 18, 12, 8, and 5 amino acid residues.

Enzymatic Digestion. Prior to preparative digestion with chymotrypsin (EC 3.4.21.1), samples of peptide were rechromatographed with a 10 × 0.46 cm column (5-μm Vydac 218 TP; gradient from 0% to 30% CH_3CN in 0.1% CF_3COOH/H_2O at 1%/min) to remove bovine serum albumin and to afford the peptide in a small volume. After removing solvent, chymotrypsin (Sigma, 1 μg) was added in 100 μl of 0.2 M NH_4HCO_3. Digests were monitored and worked-up by RP-LC as specified above; completion of digestion of native peptides was estimated by monitoring digests of several synthetic crustacean red-pigment-concentrating hormone (RPCH) analogs. Synthesis of RPCH analogs and their assumed chymotryptic fragments and isomers was achieved by solid-phase techniques (15). Synthetic peptides were purified by preparative RP-LC, and structures were confirmed by amino acid analysis or fast atom-bombardment mass spectrometry (tetrapeptides).

Amino Acid Composition and Sequence Analysis. Aliquots of each peptide or chymotryptic fragment (100–250 pmol) were hydrolyzed (5 μl of 6 M HCl containing 7% distilled thioglycolic acid) and analyzed as described by Böhlen and Schroeder (16). Mass spectra were recorded on a Hewlett–Packard model 5985A GC/MS data system using a direct chemical ionization technique with NH_3 as reagent gas at a source temperature of 300°C. Studies with fast atom-bombardment mass spectrometry used a Phrasor Scientific fast atom gun modified to fit the model 5985A spectrometer. Xenon was used as the bombarding species.

RESULTS

Isolation Procedure. Typically, 1000 pairs of CC were extracted and initially purified by gel filtration with Sephadex. Cardioacceleratory activity was detected in fractions that corresponded to the low molecular weight region (M_r 800–1500) (data not shown). With later batches, preliminary purification was performed by using a Sep-Pak C_{18} cartridge.

The bioactive zone from Sephadex chromatography or Sep-Pak filtration was separated by RP-LC using gradient elution (Fig. 1). Two bioactive zones were observed and appeared to coincide with prominent UV-absorbing peaks. These bioactive zones were rechromatographed with a different system using CH_3OH rather than CH_3CN; bioassay of 0.5-min fractions revealed that biological activity was coincident with UV absorbance of the major component for each factor (Fig. 2). Final purification of later batches was effected by using 1-propanol rather than methanol with the isocratic conditions described in *Materials and Methods*.

Initial Characterization. Our estimation of M_r 800–1500 for CC-1 and CC-2 is in agreement with that found for CC factors in previous studies (6, 11). We assumed at this point that one of our factors was identical to neurohormone D, whose amino acid composition and physicochemical properties are remarkably similar to two other invertebrate peptides, AKH and RPCH (2, 17) (Fig. 3). Moreover, synthetic AKH has been reported to have cardioacceleratory activity (18). The amino acid composition of neurohormone D differs from that of RPCH (Fig. 3) by only two residues (one extra Asx, one Val, and no Gly or Leu), assuming the Trp was lost under the hydrolysis conditions used (6). The electrophoretic immobility of neurohormone D was suggestive of both amidated COOH terminus and Asx residues as well as a blocked NH_2 terminus, so we speculated that the sequence of neurohormone D (and most likely one of our factors) might be pGlu-Val-Asn-Phe-Ser-Pro-Asn-Trp-NH_2, ([Val^2,Asn^7]RPCH). This seemed reasonable because AKH has Asn residues at positions 3 and 7; replacement of Leu with Val is a conservative change. While this investigation was in progress, a similar analysis led to the same speculation (19).

Our structural elucidation procedures were greatly aided when synthetic [Val^2,Asn^7]RPCH was shown to have chromatographic properties identical with CC-1 as well as potent bioactivity (Table 1). We further investigated the apparent molecular weight of our factors as compared to certain synthetic peptides. AKH, RPCH, and [Val^2,Asn^7]RPCH ran slightly faster than would be expected on Sephadex G-25 but were highly retained on TSK 2000 SW size-exclusion chromatography. On the later column, AKH, RPCH, CC-1, and CC-2 were eluted with the inclusion volume (marked with tryptophan). This anomalous behavior may be attributed to their uncharged, hydrophobic structures.

Amino acid analysis of CC-1 (Table 2) gave values identical to the composition expected for our proposed sequence, [Val^2,Asn^7]RPCH. The amino acid composition of CC-2 was more reminiscent of the decapeptide AKH than RPCH but

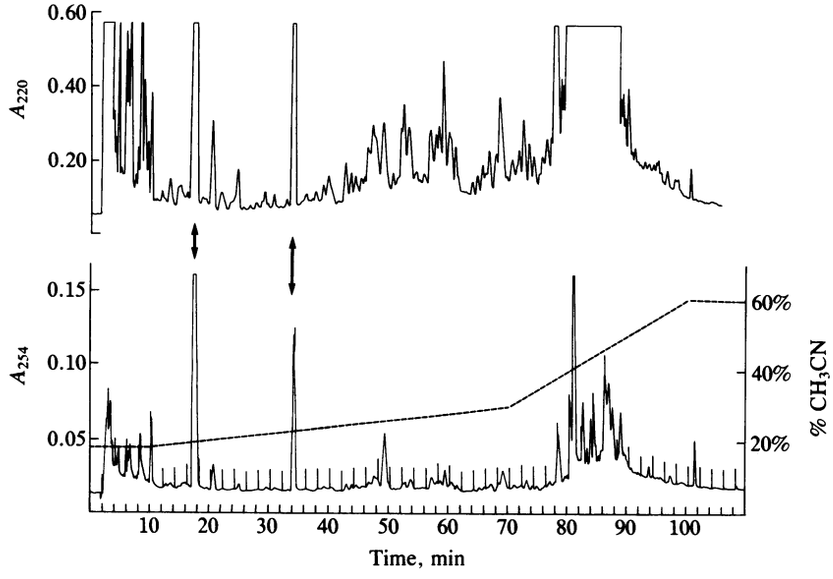

FIG. 1. Fractionation of the factors from a typical batch of 1000 CCs using 5-μm Vydac 218 TP packing (25 × 0.46 cm) eluted initially with 18% CH_3CN in H_2O containing 0.1% CF_3COOH and then with a gradient from 10 to 70 min going to 30% CH_3CN (0.2%/min increase) to elute the factors, followed with a faster gradient (1%/min increase) to 60% CH_3CN to purge the column. The eluent was monitored at 220 (*Upper*) and 254 (*Lower*) nm; strong cardioacceleratory activity was associated with major peaks eluted at 17 min (CC-1) and 34 min (CC-2).

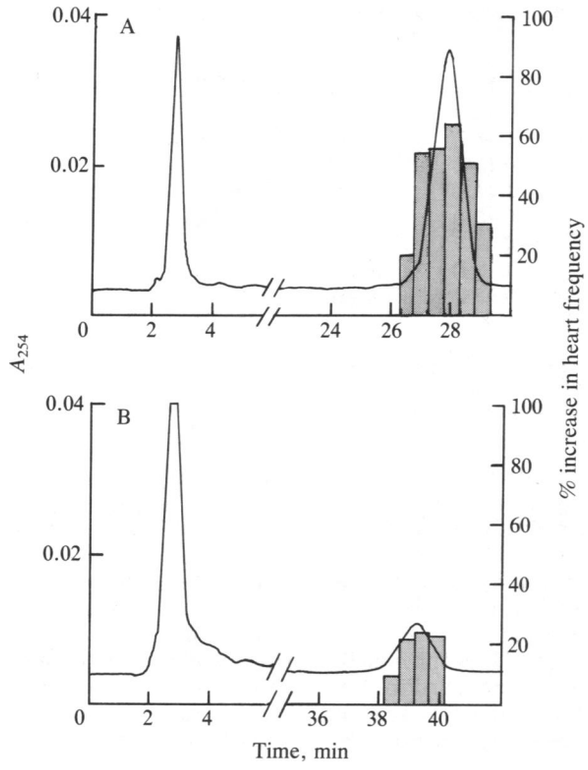

FIG. 2. The two major zones with cardioacceleratory activity from Fig. 1 were rechromatographed to determine whether the biological activity was precisely coincident with UV absorbance (mass). Both factors were analyzed on a 25 × 0.46 cm column of 10-μm Aquapore RP-300 with slow gradients of CH_3OH in 0.1% CF_3COOH/H_2O starting at 32% CH_3OH for CC-1 (*A*) or 35% CH_3OH for CC-2 (*B*) increasing at 0.1%/min. Narrow fractions (0.5 min) were cut in the region of the major UV active peak for the heart bioassay.

lacked one Asx and the Gly of AKH. Using the sequences of AKH and RPCH, we speculated that the structure for CC-2 was pGlu-Leu-Asn-Phe-Thr-Pro-Thr-Trp-NH_2 (i.e., [$Thr^{5,7}$]-RPCH), with the conservation of residues 1–6 of AKH and replacement of Asn^7 with Thr at a position that can accommodate replacement, as seen in comparing AKH and RPCH.

Synthetic [$Thr^{5,7}$]RPCH was shown to have retention behavior identical to CC-2 on C_{18} columns as well as potent bioactivity (Table 1). Although this evidence was quite suggestive that [Val^2,Asn^7]RPCH and [$Thr^{5,7}$]RPCH were in fact CC-1 and CC-2, respectively, unequivocal proof of structure would lie in sequence analysis of the native materials.

Sequence Analysis. Preparative chymotryptic digests were performed on native CC-1 and CC-2 in parallel with digests of synthetic samples of [Val^2,Asn^7]RPCH and [$Thr^{5,7}$]RPCH. The digests were worked up by gradient elution RP-LC, and

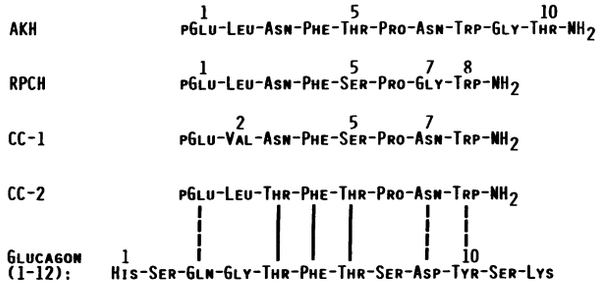

FIG. 3. Primary sequences of several invertebrate peptides and glucagon.

Table 1. Effects of several peptides on the *P. americana* semiisolated heart bioassay

Peptide	Concentration, nM		
	10	100	1000
AKH	+	++	++
RPCH	+	++	++
[Val^2,Asn^7]RPCH	+	++	++
[$Thr^{5,7}$]RPCH	+	+	++
[$Thr^{3,5}$,Asn^7]RPCH	++	++	++
CC-1 (native)	+	++	ND
CC-2 (native)	+	++	ND

The heartbeat frequency was evaluated by distinguishing two ranges: ≤30% (+) and >30% increase (++). ND, not determined.

fractions were collected for identification of fragments by amino acid analysis and mass spectrometry. The peptide maps from digestion of CC-1 and [Val^2,Asn^7]RPCH were identical, whereas the profiles from [$Thr^{5,7}$]RPCH vs. CC-2 were somewhat different (data not shown). The two major chymotryptic fragments from CC-1 were presumed to be pGlu-Val-Asn-Phe and Ser-Pro-Asn-Trp-NH_2. Amino acid analysis of aliquots of the two main fragments of CC-1 supported this assumption, whereas the amino-acid analysis of aliquots of the two major fragments from CC-2 showed compositions of (*i*) Glx (1), Leu (1),Thr (1), and Phe (1) and (*ii*) Thr (1), Pro (1), Asx (1), and Trp (1) (Table 2). These compositions allowed formulation of an alternative hypothesis for the sequence of CC-2—namely, pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2, ([$Thr^{3,5}$,Asn^7]RPCH). A synthetic sample of this analog was found to be inseparable from CC-2 and [$Thr^{5,7}$]RPCH on C_{18} columns. Samples of [$Thr^{3,5}$, Asn^7]RPCH and CC-2 were digested with chymotrypsin; the peptide maps from RP-LC were now identical with respect even to minor fragments. Additionally, we developed RP-LC conditions that gave a partial separation (α = 1.058) of these two synthetic isomers by using a 15 × 0.46 cm phenyl column (5-μm Vydac, eluted with 10% 1-propanol/0.1% CF_3COOH/H_2O). Under these conditions, CC-2 and [$Thr^{3,5}$, Ans^7]RPCH were coeluted at 88.7 min, while [$Thr^{5,7}$]RPCH was eluted at 93.7 min.

We synthesized a number of tetrapeptides for comparison to the chymotryptic fragments and developed isocratic RP-LC conditions allowing their resolution (Fig. 4). The two major fragments from CC-1 had retention behavior on RP-LC identical with synthetic pGlu-Val-Asn-Phe and Ser-Pro-Asn-Trp-NH_2, respectively, whereas the fragments from CC-2 were coeluted with pGlu-Leu-Thr-Phe and Thr-Pro-Asn-Trp-NH_2 rather than with the fragments that would have been expected from our initial hypothesis.

Final proof of sequence of the chymotryptic fragments from CC-1 and CC-2 was obtained by using direct chemical ionization mass spectrometry. Methanol solutions of peptides were evaporated on a polyimide-coated fused-silica capillary, which then was inserted directly in the electron beam. When direct chemical ionization mass spectrometry with ammonia as reagent is used, small peptides generally show MH^+ and $MH^+ + NH_3$ peaks and abundant fragment ions of two major classes: NH_2-terminal amide ions and COOH-terminal ammonium ions (20). In the case of CC-1, the NH_2-terminal fragment (ZVNF, Table 2) afforded a mass spectrum (not shown) identical with that of synthetic pGlu-Val-Asn-Phe; spectra contained fragment ions at m/z 129, 228, and 342 of the NH_2-terminal amide-type and at m/z 379, 280, and 166 of the COOH-terminal ammonium-type (20). Thus, we observed two fragment ions for each amide bond providing complete sequence information. The COOH-terminal fragment of CC-1 (SPNW-NH_2, Table 2) exhibited a mass spectrum containing strong ions (m/z 130, 155, and 172)

Table 2. Amino acid compositions of factors and their chymotryptic fragments

Amino acid*	CC-1	ZVNF	SPNW-NH_2	CC-2	ZLTF	TPNW-NH_2
Asx (B)	1.76(1)	0.98(1)	0.66(1)	0.96(1)	—	0.59(1)
Thr (T)	0	—	—	1.96(2)	1.16(1)	1.37(1)
Ser (S)	1.07(1)	—	1.12(1)	0.35(0)	—	—
Glx (Z)	1.03(1)	0.97(1)	—	1.29(1)	0.87(1)	—
Gly (G)	—	—	—	0.41(0)	—	0.15(0)
Val (V)	1.23(1)	0.85(1)	—	0	—	—
Leu (L)	0	—	—	1.18(1)	0.94(1)	—
Phe (F)	1.06(1)	1.20(1)	—	0.95(1)	1.02(1)	—
Trp (W)	0.70(1)	—	1.02(1)	0.90(1)	—	0.84(1)
Pro (P)	1.12(1)	—	1.11(1)	0.60(1)	—	1.05(1)

All amino acids other than those shown were detected at ≤10 mol% each.
*The single-letter amino acid code, used in identifying the fragments of CC-1 and CC-2, is shown in parentheses. In addition, Asn = N and pGlu = Z.

derived from the indole side chain of Trp, but also displayed MH^+ and prominent ions from cleavage of each of the three amide bonds, proving identity of sequence of this fragment with synthetic Ser-Pro-Asn-Trp-NH_2. Based on sequence of both chymotryptic peptides and the amino acid composition, the sequence of CC-1 can only be as shown in Fig. 3.

Spectra of fragments of CC-2 were less clear-cut because of the very small samples available: less native CC-2 was isolated than CC-1, adsorptive losses in processing were greater, and yields were lower in digests. The mass spectrum of the NH_2-terminal fragment, although not a precise match with that of synthetic pGlu-Leu-Thr-Phe because of the absence of MH^+ and a few other high-mass ions, did show ions at m/z 129 and 146 characteristic of NH_2-terminal pGlu-, a strong ion at m/z 242 from pGlu-Leu-, and a strong ion at m/z 343 consistent with pGlu-Leu-Thr-. Taken together with the amino acid composition data and the coelution of native and synthetic fragments on RP-LC (Fig. 4), these data are consistent only with the sequence pGlu-Leu-Thr-Phe.

Mass spectral analysis of the COOH-terminal fragment TPNW-NH_2 was the most difficult. We observed an ion at

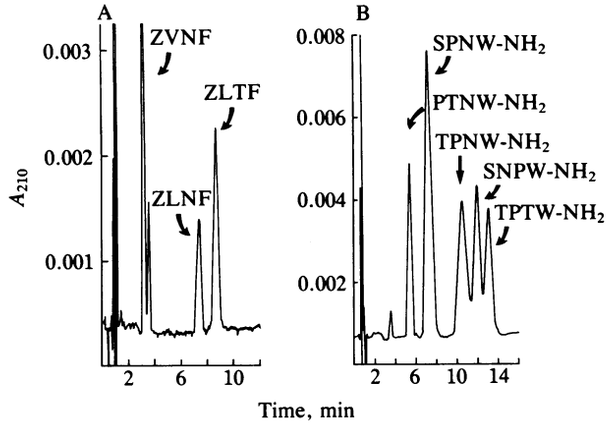

FIG. 4. Separation of a number of synthetic samples of possible chymotryptic digestion fragments (and/or their isomers) using isocratic elution on a 10 × 0.46 cm column of 5-μm Vydac 218 TP. Tetrapeptides are designated by conventional one-letter amino acid abbreviations. (*A*) Possible NH_2-terminal fragments pGlu-Val-Asn-Phe (ZVNF), pGlu-Leu-Asn-Phe (ZLNF), and pGlu-Leu-Thr-Phe (ZLTF) were separated by using 13% CH_3CN in 0.1% CF_3COOH/H_2O. (*B*) Possible COOH-terminal fragments Pro-Thr-Asn-Trp-NH_2 (PTNW-NH_2), Ser-Pro-Asn-Trp-NH_2 (SPNW-NH_2), Thr-Pro-Asn-Trp-NH_2 (TPNW-NH_2), Ser-Asn-Pro-Trp-NH_2 (SNPW-NH_2), and Thr-Pro-Thr-Trp-NH_2 (TPTW-NH_2) were separated by using 5% CH_3CN in 0.1% CF_3COOH/H_2O. Thr-Asn-Pro-Trp-NH_2 (TNPW-NH_2, not shown, an isomer of TPNW-NH_2) does not separate from TPTW-NH_2.

m/z 204 from COOH-terminal Trp-NH_2 and normal Trp fragments at m/z 130, 155, and 172. The partial sequence Asn-Trp-NH_2 was supported by m/z 199 (M^+ − Asn-Trp-NH_2) and by an ion at m/z 216 from Thr-Pro or Pro-Thr. An ion at m/z 330 was consistent with the partial sequence(s) Thr-Pro-Asn or Pro-Thr-Asn. Based on this mass spectrum, we could not have distinguished between Thr-Pro-Asn-Trp-NH_2 and Pro-Thr-Asn-Trp-NH_2. (Even in the mass spectrum of synthetic Thr-Pro-Asn-Trp-NH_2, we could barely observe MH^+ and only a weak ion at m/z 415 diagnostic of Pro-Asn-Trp-NH_2.) However, we could coelute the native fragment on RP-LC with synthetic Thr-Pro-Asn-Trp-NH_2. The synthetic fragment separated easily from Pro-Thr-Asn-Trp-NH_2 on RP-LC (Fig. 4). We conclude that the sequence of CC-2 must be pGlu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2 (Fig. 3), based on sequence analysis of the chymotryptic fragments.

Synthetic periplanetins CC-1, CC-2, analogs, and native CC-1 and CC-2 were compared for their abilities to accelerate the semi-isolated cockroach heartbeat (Table 1). All of the compounds tested were active at 10 nM (~0.5 pmol/assay), but the intrinsic variation in the heart bioassay made quantitative distinction between peptides unreliable.

AKH and RPCH have similar biological activity in organisms of different orders (21–23). We suspected that the periplanetins would have adipokinetic activity because of sequence similarities and because cockroach CC contain adipokinetic activity (12, 24) when assayed in *L. migratoria*. The hyperglycemic activity of AKH and RPCH (23, 25) also suggested a similar role for the periplanetins.

The effects of the periplanetins on hemolymph lipids in grasshoppers and carbohydrate mobilization in cockroaches were examined (Table 3). The well-known variability in both assays (14) does not permit quantitative differentiation amongst peptides showing activity.

Table 3. Carbohydrate-mobilizing effects in *P. americana* and lipid-mobilizing effects in *Schistocerca nitens* after injection of several peptides

Peptide	% increase in hemolymph ± SD	
	Carbohydrates	Lipids (n = 8)
AKH	64 ± 20 (n = 11)	190 ± 56
RPCH	96 ± 35 (n = 11)	77 ± 56
CC-1 (synthetic)	76 ± 29 (n = 12)	162 ± 118
CC-2 (synthetic)	100 ± 33 (n = 12)	147 ± 70
CC-1 (native)	80 ± 37 (n = 12)	91 ± 42
CC-2 (native)	81 ± 28 (n = 12)	83 ± 68
CC-1 acid (synthetic)	34 ± 19 (n = 12)	9 ± 15
CC-2 acid (synthetic)	67 ± 32 (n = 12)	11 ± 12

Each peptide (10 pmol) was injected into *P. americana* while 20 pmol was injected into *S. nitens*; n, number of replications.

DISCUSSION

The cockroach CC contains ≈100 pmol of CC-1 and ≈40 pmol of CC-2. This estimate may be low because of difficulties in handling these hydrophobic peptides. Adsorption to containers resulted in the loss of purified factors, especially CC-2. Although polypropylene tubes mitigated losses as compared with glass, we routinely added bovine serum albumin (when possible) to fractions from purifications. Purified yields of both factors from several batches of 500 or 1000 CCs were reproducibly increased by ≈3-fold when a reversed-phase cartridge filtration was substituted for Sephadex gel filtration.

The sequence analysis of the purified factors was not approached in a classical manner once we realized that both factors were very likely new members of an existing invertebrate peptide family (i.e., AKH and RPCH). We did not attempt Edman degradations on the native factors because sufficient evidence suggested the NH_2 terminus was blocked. The evidence included: (*i*) the probable identity of CC-1 and neurohormone D and the failure of neurohormone D to migrate on electrophoresis or to react with dansyl chloride (6) and (*ii*) comigration of synthetic [Val^2,Asn^7]RPCH with CC-1 under several RP-LC conditions. The proposed structure for CC-1 enabled us to explore alternative methodologies for sequence determination that use synthetic peptide, eliminating the sacrifice of native factors.

Our sequence strategy was straightforward for CC-1 and confirmed our proposed structure unambiguously. Our first working hypothesis for CC-2 was shown to be incorrect, but a second hypothesis was made and confirmed by analysis of the tetrapeptides from chymotryptic digestion. Although proposing a sequence based on amino acid composition and likely homology to a known peptide was successful for CC-1, it is clear that such hypotheses can lead to incorrect assignments if not followed up with rigorous sequence determination. Our initial RP-LC retention behavior and biological comparisons of CC-2 and two isomeric synthetic peptides were not sufficient to prove identity. Caution should be taken in the structural assignments of possible new members of this peptide family, such as AKH-II in *Locusta* (26, 27) and a hyperglycemic peptide from *Carausius* (9), based solely on sequence homology.

The periplanetins, the newest members of the AKH–RPCH family, possess biological activities that are in agreement with previous studies suggesting multiple activities for CC factors (8, 10–12). Separation of hyperglycemic from adipokinetic activity from cockroach CC has been reported (12), but our data suggest this to be incorrect. CC-1 is almost certainly the factor originally given the name neurohormone D (6), and CC-1 and CC-2 are also excellent candidates for the factors affecting carbohydrate metabolism given the names "trehalagon" (28, 29) or "hypertrehalosemic"/"hyperglycemic" factors (9).

At first glance this family of invertebrate peptides does not appear to resemble any known vertebrate peptide classes. Not until after observing elevation of hemolymph carbohydrate levels by CC-1 and CC-2 did we notice sequence homology between CC-2 and the NH_2 terminus of glucagon (Fig. 3). In the light of this homology and reports that cockroach CC contain glucagon-like immunoreactive components (30), it should be interesting to examine the ability of an NH_2-terminal directed glucagon antibody to bind CC-2 or CC-1.

In the last 20 years, several groups have reported that the hyperglycemic action of the CC factor(s) on insect fat body is similar to the action of glucagon on the mammalian liver (28, 29, 31, 32). A "phosphorylase cascade" leading to the breakdown of fat body glycogen and elevation of hemolymph carbohydrate has been implicated in these studies, all of which used crude or semipurified material from the CC. With synthetic samples of the periplanetins, we can now examine the mechanisms involved in hyperglycemic action.

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1. Raabe, M. (1982) *Insect Neurohormones* (Plenum, New York).
2. Stone, J. V., Mordue, W., Batley, K. E. & Morris, H. R. (1976) *Nature (London)* **263,** 207–211.
3. Unger, H. (1957) *Biol. Zentralbl.* **76,** 204–225.
4. Ralph, C. L. (1962) *J. Insect Physiol.* **8,** 431–439.
5. Davey, K. G. (1964) *Adv. Insect Physiol.* **2,** 219–245.
6. Baumann, E. & Gersch, M. (1982) *Insect Biochem.* **12,** 7–14.
7. Steele, J. E. (1961) *Nature (London)* **192,** 680–681.
8. Brown, B. E. (1965) *Gen. Comp. Endocrinol.* **5,** 387–401.
9. Goldsworthy, G. J. & Gäde, G. (1983) in *Invertebrate Endocrinology: Endocrinology of Insects*, eds. Laufer, H. & Downer, R. G. H. (Liss, New York), Vol. 1, pp. 109–119.
10. Natalizi, G. M. & Frontali, N. (1966) *J. Insect Physiol.* **12,** 1279–1287.
11. Traina, M. E., Bellino, M., Serpietri, L., Massa, A. & Frontali, N. (1976) *J. Insect Physiol.* **22,** 323–329.
12. Holwerda, D. A., Weeda, E. & van Doorn, J. M. (1977) *Insect Biochem.* **7,** 477–481.
13. Jones, J. (1978) Dissertation (Univ. of London, London).
14. Holwerda, D. A., van Doorn, J. & Beenakkers, A. M. T. (1977) *Insect Biochem.* **7,** 151–157.
15. Ling, N., Esch, F., Davis, D., Mercado, M., Regno, M., Böhlen, P., Brazeau, P. & Guillemin, R. (1980) *Biochem. Biophys. Res. Commun.* **95,** 945–951.
16. Böhlen, P. & Schroeder, R. (1982) *Anal. Biochem.* **126,** 144–152.
17. Fernlund, P. & Josefsson, L. (1972) *Science* **177,** 173–175.
18. Stone, J. V. & Mordue, W. (1980) in *Neurohormonal Techniques in Insects*, ed. Miller, T. A. (Springer, New York), pp. 31–80.
19. Greenberg, M. J. & Price, D. A. (1983) *Annu. Rev. Physiol.* **45,** 271–288.
20. Carr, S. A. & Reinhold, V. W. (1982) in *Methods in Protein Sequence Analysis*, ed. Elzinga, M. (Humana, Clifton, NJ), pp. 263–270.
21. Mordue, W. & Stone, J. V. (1976) *Nature (London)* **264,** 287–289.
22. Mordue, W. & Stone, J. V. (1977) *Gen. Comp. Endocrinol.* **33,** 103–108.
23. Van Norstrand, M. D., Carlsen, J. B., Josefsson, L. & Herman, W. S. (1980) *Gen. Comp. Endocrinol.* **42,** 526–533.
24. Goldsworthy, G. J., Mordue, W. & Guthkelch, J. (1972) *Gen. Comp. Endocrinol.* **18,** 545–551.
25. Jones, J., Stone, J. V. & Mordue, W. (1977) *Physiol. Entomol.* **2,** 185–187.
26. Carlsen, J., Herman, W. S., Christensen, M. & Josefsson, L. (1979) *Insect Biochem.* **9,** 497–501.
27. Yamashiro, D., Applebaum, S. W. & Li, C. H. (1984) *Int. J. Pept. Protein Res.* **23,** 39–41.
28. Steele, J. E. (1980) in *Insect Biology in the Future "VBW"*, eds. Locke, M. & Smith, D. S. (Academic, New York), pp. 253–271.
29. Steele, J. E. (1983) in *Invertebrate Endocrinology: Endocrinology of Insects*, eds. Laufer, H. & Downer, R. G. H. (Liss, New York), Vol. 1, pp. 427–439.
30. Tager, H. S., Markese, J., Kramer, K. J., Speirs, R. D. & Childs, C. N. (1976) *Biochem. J.* **156,** 515–520.
31. Steele, J. E. (1963) *Gen. Comp. Endocrinol.* **3,** 46–52.
32. Hanaoka, K. & Takahashi, S. Y. (1977) *Insect Biochem.* **7,** 95–99.