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Carbohydrate and lipid metabolism in cockroach (*Periplaneta americana*) fat body are both activated by low and similar concentrations of *Peram*-AKH II

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ABSTRACT

Injection of 0.1 pmol of the octapeptide *Peram*-AKH II (pGlu-Leu-Thr-Phe-Thr-Pro-Asn-TrpNH$_2$) elicits a significant hypertrehalosemic response in the American cockroach, *Periplaneta americana*; a maximal effect is obtained with 1 pmol. The latter amount also lowers the level of neutral lipid (NL) and phospholipid (PL) in the hemolymph. The evidence supports the idea that *Peram*-AKH II promotes the liberation of fatty acids from hemolymph phospholipid, and indirectly diacylglycerol in the same compartment. The fatty acids are then transported into the fat body where they are converted into triacylglycerol for storge. Because lipolysis and trehalose synthesis are initiated by a common concentration of *Peram*-AKH II it is reasonable to suggest that the physiological function of *Peram*-AKH II involves the participation of both metabolic pathways.

1. Introduction

The mechanism by which the cockroach hypertrehalosemic hormones (HTH), *Peram*-AKH I and II, increase hemolymph trehalose levels in *Periplaneta americana* is well known. However, unlike the locust adipokinetic hormones (*Locmi*-AKH I and II) which facilitate diacylglycerol formation as a source of energy for flight muscle at the onset of flight [3], the physiological role of *Peram*-AKH I and II in the cockroach remains speculative. In addition to the hypertrehalosemic effect these hormones also induce a marked hypolipemia accompanied by lipogenesis in the fat body [23]. The physiological significance of this change in the distribution of lipid is not understood. It is of interest therefore to understand the nature of any special relationship between carbohydrate and lipid metabolism particularly that which might shed light on the physiological function supported by the shift in lipid metabolism.

Early work on the hypertrehalosemic response employed crude extracts of the corpus cardiacum but because those extracts contained several active factors the action of any given factor was unclear. More recent studies have employed synthetic hormones with the result that specific effects of individual neuropeptides can be identified with certainty. In

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our previous studies [23], doses of 100 pmol of *Peram*-AKH were employed; this amount exceeds the estimated amount of either of the AKHs present in the corpus cardiacum under *in vivo* conditions [30]. Because a potential causal relationship between carbohydrate activation and lipid metabolism is of interest it is essential to establish whether activation of both pathways is elicited by similar doses of hypertrehalosemic peptide within a range considered to be physiological, i.e. a fraction of the neuropeptide present in the corpus cardiacum which lies within a range that produces a concentration-dependent response. An extensive study of the dose-response relationships of hypertrehalosemic hormones belonging to the AKH/RPCH neuropeptide group, including detailed information on those native to *P. americana*, has been carried out by Gäde [10,11]. Hayes and Keeley [15] provide similar information for the hypertrehalosemic hormones of the related species, *Blaberus discoidalis*. Because it is important to understand the dose-response relationship that links *Peram*-AKH II to trehalose synthesis *and* its effects on hemolymph and fat body lipid we have determined the approximate amount of hormone required to generate a maximal response by both pathways under the particular conditions used in these experiments. Finally, we consider whether the increase in fat body triacylglycerol is, in part at least, a consequence of the removal of lipid from the hemolymph.

We have chosen to follow a different convention in naming the hormones used in this study. The hypertrehalosemic hormones belong to the AKH/RPCH family of hormones and for this reason will be referred to, generically speaking, as adipokinetic hormones (AKH) rather than hypertrehalosemic hormones. The structural identity, as before, is indicated by the prefix identifying the species from which the peptide has been derived.

2. Materials and methods

2.1. Insects

The cockroaches, *P. americana*, used in this study were raised at 28 ± 2 °C, and a relative humidity of $60 \pm 5\%$, with a photoperiod cycle of 12 h light, 12 h dark. Water and food were provided *ad libitum*. The food provided was a mixture of Purina Cat Chow, sucrose and yeast. In late afternoon on the day prior to use adult male cockroaches, 4–6 weeks after the final moult, were removed from the stock cages, placed in individual petri dishes, and stored overnight in an incubator maintained at 28 °C and a relative humidity of 55%. A ligature was applied to the neck membrane of these cockroaches to clear hormone from the hemolymph and to avoid stress-induced release of hormone due to handling.

2.2. Chemicals

All inorganic and organic chemicals, including *s*-diphenylcarbazide and *s*-diphenylcarbazone, used in this study were of reagent grade and were obtained from a variety of sources. Solvents, including acetyl acetone (2,4-pentanedione) chloroform, ethyl acetate, heptane, isooctane (2,2,4-trimethylpentane), isopropanol and methanol, were obtained from VWR, Toronto. Synthetic hypertrehalosemic hormone, *Peram*-AKH II, was obtained from Pennisula Laboratories, Belmont, CA. A stock solution was prepared in 50% acetic acid based on the analysis provided by the supplier. [9,10-^{3}H(N)]-Oleic acid, 2–10 Ci/mmol was obtained from NEN Life Sciences (PerkinElmer, Markham, ON, Canada) and EM Science silica gel (particle size 0.04–0.063 mm) from VWR, Toronto, ON.

2.3. Injection procedures

For each experiment, a stock solution of hormone (1 nmol 002 μL) was diluted in physiological saline (154 mM NaCl; 8 mM KCl; 2 mM $CaCl_2$; 3 mM $MgCl_2$ and 40 mM HEPES (4-(2hydroxyethyl)-1-piperazineethanesulfonic acid), pH 7.4) to yield the required final concentration in 10 μL. Injection (10 μL), between the sixth and seventh abdominal segments, was performed using a micrometer device fitted with a 1 mL Hamilton syringe and a 30 gauge needle. The controls received 10 L of saline. In experiments with radioactive lipids or fatty acids a 10 μL aliquot of the required ^{3}H-labeled lipid was injected into the cockroach immediately prior to injection of saline or hormone.

2.4. Collection of tissue

Hemolymph and fat body were obtained as previously described [23].

2.5. Determination of hemolymph trehalose

Trehalose in the hemolymph was determined with anthrone reagent [4]. Hemolymph was drawn from the hemocoel by puncturing the propterothoracic membrane at the base of the coxa of the metathoracic leg and a 5 μL sample collected in a glass capillary pre-cut to contain 5 μL. The sample was placed in a polyethylene centrifuge tube containing 1 mL of 5% trichloroacetic acid and the capillary crushed with a glass pestle. Following centrifugation at $1360 \times g$ for 5 min in a clinical centrifuge 0.5 mL of the sample was transferred to a test tube and the tube chilled in a bath of crushed ice. Cold anthrone reagent (2.5 mL) was added and the sample vigorously mixed in the ice bath. The test tubes were placed in boiling water for 15 min, cooled, and the absorbance read at 620 nm with appropriate blanks and a standard prepared from 30 mM trehalose.

2.6. Labelling of lipids in hemolymph

The free fatty acid fraction in hemolymph was labeled with ^{3}H-oleic acid as follows. A sample of oleic acid containing 25 Ci of ^{3}H was dried under nitrogen in a conical centrifuge tube and redissolved in 1 mL of freshly collected hemolymph. Neutral lipid (NL) and phospholipid (PL) for inclusion in hemolymph were labeled with ^{3}H-oleic acid as follows. Three cockroaches were each injected with 50 μL of hemolymph containing 2.5 Ci of ^{3}H-oleic acid and held at 28 °C for 4 h. The cockroaches were homogenized in a Waring blender with 50 mL chloroform:-methanol (2:1, v/v), the mixture centrifuged, and the supernatant collected. The pellet was re-extracted twice in the same