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Precocious induction of vitellogenin with JH III in the twospotted stink bug, *Perillus bioculatus* (Heteroptera: Pentatomidae)☆

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Abstract

The effect of juvenile hormone (JH) III on the hemolymph composition of vitellogenin was examined in *Perillus bioculatus*. Adult females were treated topically with JH III, and the premature presence of vitellogenin in the hemolymph was then detected using electrophoresis and Western blot analyses. JH III treatment resulted in a dose-dependent early production of vitellogenin that was detectable 48 h before vitellogenin was present in non-treated insects. Vitellogenin was not observed in the hemolymph of JH III-treated adult males. The techniques reported here may be useful for the detection, isolation and characterization of compounds with JH-like activity in *P. bioculatus* and other species of Heteroptera (which are thought to have JH-like substances other than the JHs with known chemical identity). These same techniques may also provide a method for researchers to investigate the interactions of JH-like compounds and other substances, such as ecdysteroid, in the regulation of vitellogenesis in Heteroptera.
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Keywords: Bioassay; Reproduction; Predator; Induction; Heteroptera; Juvenile hormone; Vitellogenesis; Vg

1. Introduction

In insects, the process of vitellogenesis, biosynthesis, distribution and packaging of the yolk protein precursor known as vitellogenin (Vg), is under hormonal control, and includes ecdysteroids and juvenile hormones (JHs) (Gäde et al., 1997). In Heteroptera, vitellogenesis is primarily regulated by JH, with the role of ecdysone considered to be less significant (Davey, 1997). JHs are a group of acyclic sesquiterpenoids secreted by the corpora allata that regulate many aspects of reproduction, as well as other aspects of insect physiology, including metabolism, development, metamorphosis and diapause (Simonet et al., 2004). JH has been shown to stimulate Vg biosynthesis in female fat body and follicle cells and uptake from the hemolymph by oocytes in the growing follicles (Nation, 2002). While the chemical identity of several JHs has been determined, isolation and characterization of JH in Heteroptera has not been accomplished, although evidence suggests that it is different from the JH of other insect orders (Kotaki, 1996; Davey, 1997).

Recently, vitellogenesis has been developed as a biomarker to monitor effects caused by endocrine-disrupting agents (Hahn et al., 2002). In many insects, there is a direct relationship between the synthesis of Vg and the presence of JH. Therefore, with the discovery of the anti-allatal compounds precocenes, bioassays were developed in which a chemical allatectomy was followed by treatment with compounds that were assessed for their ability to restore JH activity (Venugopal and Kumar, 2000). Unfortunately, one heteropteran species, *Perillus bioculatus* has proven to be detrimentally sensitive to treatment with low levels of precocenes, causing ca. 50% death and neurological abnormalities

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in the surviving adults (unreported data), leading us to search for other means in which to study JH-like activity in this insect.

P. bioculatus is a native North American predator of the Colorado potato beetle and is a proven beneficial predator used in augmentative biological control programs (Hough-Goldstein et al., 1996). The hemolymph of female *P. bioculatus* contains a major vitellogenin with a molecular mass of 528 kDa that consists of 3 apoproteins with masses of 177, 84 and 59 kDa. (Adams et al., 2002). Vg levels in the hemolymph start to increase in females 2.5 days after adult emergence and reach peak levels at 5.5 days after adult emergence (Adams et al., 2002). Treatment of female *P. bioculatus* adults with JH III has been shown to increase the amount of vitellogenic protein detected in the ovaries, and supported the conclusion that the JH III treatment positively affected vitellogenin synthesis (Adams, 1997; Adams et al., 2002). In addition, extended treatment of larval stages of the dipteran, *Chironomus thummi* resulted in a small number of individuals initiating vitellogenesis precociously in the subsequent pupal stage (Laufer et al., 1986). Therefore, it seemed probable that treatment of *P. bioculatus* with JH III might cause a change in the timing as well as the amount of production of vitellogenin. The purpose of this study was to determine if such a change occurred and if that change would be suitable to assist in the investigation of hormonal regulation of vitellogenesis, tissue competency and characterization of JH-like compounds in *P. bioculatus.*

2. Materials and methods

2.1. Insect rearing

The *P. bioculatus* originated from a colony at the USDA-APHIS PPQ-MPPC (Mission, TX, USA) and were maintained at the Biological Control of Insects Research Laboratory (Columbia, MO, USA) for ca. 30 generations. Experimental rearing conditions were 25±2 °C, 45±10% RH, and a photoperiod of 16:8 (L:D) h, maintained in a walk-in growth chamber. Groups of ca. 400 eggs, defined as a cohort, were obtained over 4 days from approximately 240 females of a colony maintained on 2nd and 3rd instar larvae of *Trichoplusia ni*. First and second instar nymphs were reared in groups of ca. 300 within clear 2.4 l round plastic containers fitted with a filter paper liner, rolled 20 cm × 18 cm vinyl netting, moist dental wicking and covered with cheesecloth. Fresh 2nd and 3rd instar larvae of *T. ni* were added daily. Third through fifth instar nymphs were reared in similar containers, but at a density of 20 per container and fed 3rd instar larvae of *T. ni.* Preliminary data from test insects showed that eggs hatched in 5–6 days, nymphs molted every 2–4 days for second and fifth instar nymphs, respectively. Additionally, the survival to adult was ca. 87%. Late fifth instar nymphs were observed every 2–4 h and adult eclosion (separating from the nymphal exuviae) was recorded. Individuals were sexed at adult emergence, and females were randomly selected from each cohort to be used for control or JH treatments. After treatment, adult females were isolated in half pint paper containers lined with filter paper, containing moist dental wicking, 3rd instar *T. ni*, and covered with a Petri dish lid.

2.2. Bioassay

Late fifth instar and newly emerged female adults that did not have a hardened cuticle (within 4 h of adult eclosion) were used for the treatments. Insects were anesthetized with CO_2 for no longer than 1 min. JH treatments and acetone controls were applied topically on the cuticle surface in various locations. JH III or JH I (Sigma-Aldrich Chemical Co., St. Louis, MO, USA and SciTech, Czech Republic, respectively) was dissolved in acetone at a dosage of 0.5 to 20 μg (1 to 3 μl total volume) per insect. Ten insects were treated with the same dose, and each experiment was repeated at least twice with a separate cohort of insects. Observations of movement, feeding and mortality were made every 4 h following treatment for all individuals in order to assess insect viability. Hemolymph was collected at 12 h intervals ranging from 12 to 48 h after adult emergence.

2.3. Sample collection

The meso- and metathoracic legs were severed at the coxal base of an anesthetized adult, followed by light pressure applied to the abdomen, before hemolymph samples of 1–10 μl were collected in a microcapillary pipette held at the point of amputation. The hemolymph was transferred to chilled microtubes, the volume recorded, fast frozen in liquid nitrogen, and stored at −80 °C. Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C to remove hemocytes and other tissue fragments before analysis.

2.4. Electrophoretic procedures

Individual hemolymph samples were analyzed under denaturing conditions with seven percent acrylamide sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the mini-Protean® 3 electrophoresis system (Bio-Rad, Hercules, CA, USA). One microliter of hemolymph was placed into 9 μl of purified water and mixed with 10 μl of loading buffer (0.042 M Tris pH 6.8, 4.2% glycerol, 4.2% 2-mercaptoethanol, 2% SDS, 0.002% Bromophenol Blue). The mixture was incubated at 100 °C for 10 min before loading onto the gel. Samples were run at 120 V for 1.5 h. For staining of the separated proteins, the gel was incubated for 10 min in staining solution (0.05% Coomassie® Brilliant Blue R250 (Biorad), 50% methanol, 10% acetic acid) followed by overnight incubation in destaining solution (7% methanol, 5% acetic acid).

For Western blot analysis, duplicate gels were run with 0.2 μl of each hemolymph sample, and the proteins were