Hormonal control of reproduction in the female pyralid moth *Plodia interpunctella* (Hübner) (Lepidoptera: Phycitidae)

E. Shaaya*, D. Silhacek†, P. Shirk†, H. Rees§, G. Zimowska† and S. Plotkin*

Abstract

The differentiation and growth of ovaries of pupae of *Plodia interpunctella* were analysed using immunofluorescence microscopy, and correlated with changes in external morphology and ecdysteroid titre. The data indicated that ovarian development progresses in two distinct, ecdysteriod-coordinated steps. The first occurs during the first three days of the pupal period and is initiated by an increasing titre of ecdysteroids (identified as mainly ecdysone) starting eight hours after the moult. Once ovarian development is initiated, specialised cell types, e.g. nurse cells, follicle cells and ocytes, differentiate and develop to a point just before yolk protein synthesis and uptake by the ocyte. The second stage in ovarian development is signalled by the reattainment of a baseline level of ecdysteroids during the final three days of the pupal period. Follicle development occurs during this stage. Subsequent development appears to proceed sequentially without further regulation by ecdysteroids. After eclosion, the ocytes are chorionated and the mature eggs are layed. Mating accelerates the final stages of egg maturation and egg laying, an effect that can be mimicked by juvenile hormone treatments.

Introduction

The hormonal regulation of postembryonic ovarian development is only partially understood in Phycitidae. In these moths, which have short-lived adults, ovaries complete all or most of egg maturation before adult eclosion. Placing adult ovarian development within the parameters of metamorphosis requires that the process progress directly to completion, without arrested follicular stages and that regulation of vitellogenesis be controlled by the hormones regulating metamorphosis.

Egg maturation and vitellogenesis in late pharate adults of the Indianmeal moth were blocked with ecdysteroid levels maintained in late pharate adults by treatment with 20-hydroxy ecdysone (20-HE) (Shirk et al. 1990). This effect of 20-HE was shown to be independent of juvenile hormone activity because treatment with 20-HE and juvenile hormone did not restore vitellogenesis or egg maturation. To more critically assess the role of ecdysteroids in normal females of the Indianmeal moth, we measured changes in the ecdysteroid titre during pharate adult development and correlated the changing ecdysteroid titre with the progress of adult ovarian development and the onset of vitellogenesis. Since there exists in general a negative correlation in adult insects between sexual activity and life span (Partridge 1986; Ayerty 1975; Norris 1933), we also investigated the endocrine basis for this, by investigating the effect of juvenile hormone on life span and egg laying in virgin and mated females of *P. interpunctella*.

* The Volcani Center, ARO, Bet-Dagan 50250, Israel.
† USDA, ARS, Insect Attractants, Behaviour and Basic Biology Laboratory, P.O. Box 14565 Gainesville, FL 32604, USA.
§ Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, England.

Material and Methods

Insect cultures

The *P. interpunctella* colony was reared according to Silhacek and Miller (1972), in a 16 hour light: 8 hour dark photoperiod at 30°C and 70% r.h. For ecdysteroid studies, moulted white pupae (±1.5 h) were collected to obtain synchronised cohorts and then kept in a long-day photoperiod, as described above, until used for experiments. As a source of pupae for the continuous darkness condition, newly moulted white pupae were collected and placed in total darkness until the appropriate age. Insect age is expressed in hours from the time of pupation.

Ecdysteroid extraction and quantification

For each time point one to six pupae were homogenised in 70% methanol at 4°C. The homogenate was centrifuged 10 min at 2600 g and 4°C. The supernatant was transferred to another tube, and the pellet was re-extracted in cold 70% methanol. The supernatants were combined and stored for a minimum of 24 hours at –20°C. Following cold storage, the samples were centrifuged 10 min at 2600 g and 4°C. The supernatants were dried using a stream of nitrogen gas and heating at 30°C. Dried samples were stored at –20°C and were reconstituted with 100% methanol for assaying. Aliquots of each sample were taken for quantitative ecdysteroid analysis by RIA as described by Shaaya et al. (1986). The 20-HE rabbit antiserum, DLII, was a gift of Professor J. Koolman (Marburg/Lahn, Germany), and the rabbit complement serum, HLA-ABC, was purchased from Sigma (St. Louis, Mo.). Radiolabelled [3H]20-HE (89 Ci/mmol) was purchased from NEN (Boston, Mass.), and 20-HE purchased from Sigma (St. Louis, Mo.). Quantification of each sample was based on evaluation of triplicates using different concentrations. A standard curve was based on competitive binding with various concentrations of 20-HE.

To determine the molecular composition of the ecdysteroids, methanolic extracts were prepared from females as described above. The ecdysteroids were analysed by gas chromotograph—mass spectrometry with selected ion monitoring [GC-MS(SIM)] as described by Evershed et al. (1987).

Studies with the juvenile hormone analogue, methoprene

Developmentally synchronous pupae 3 days prior to adult eclosion were used to permit enough time for the absorption, distribution in the body, and the action of the juvenile hormone analogue (JHA). The appropriate amount of the JHA was applied topically on the dorsal side of the pupa using a microsyringe. To obtain virgin adults, female pupae were

selected and kept separately. Male adults were kept together during the experiment. Adults age 0–4 hours were used for the experiments. The number of eggs of the test insects was counted daily. Methoprene was a gift of the Zoecon Corporation (Palo Alto, CA).

Results

Ecdysteroid studies

For the studies of the ecdysteroid titres during the pupal and pharate adult stages, females were collected as white pupae, kept in a long-day photoperiod, and collected every 4 hours until adult eclosion (136 hours after pupation). Ecdysteroid titres were determined for each time point (Fig. 1). For the first 8 hours after pupation the titres were < 300 pg/mg wet wt and then increased first to a plateau of 1600–1700 pg/mg wet wt between 16–24 hours and then attained a maximum of 2200–2500 pg/mg wet wt between 28–36 hours after pupation. The titre then began to decline with plateaus at 40–48 hours (1700–1870 pg/mg wet wt) and at 52–60 hours (935–1200 pg/mg wet wt). The ecdysteroid titre fell below 500 pg/mg wet wt by 68 hours and remained below this level for the remainder of pharate adult development. For pupae maintained in continuous darkness instead of in a long-day photoperiod, the profile was essentially the same except that the major ecdysteroid peak between 12 and 68 hours reached a maximum earlier at 24 hours (3130 pg/mg) and was broader than the peak in females kept in photoperiodic conditions.

To determine which ecdysteroids contribute qualitatively to the total titres prior to the onset of vitellogenesis, methanol extracts were made from photo-synchronised female pupae at 2, 28, 52, 76 and 100 hours after pupation. The free ecdysteroid fractions were analysed by GC-MS (SIM). In 2-hour-old pupae, 20-HE comprised 81% of the ecdysteroids measured (Table 1). At 28 hours after pupation, the maximum of the pharate adult peak, the distribution had switched and ecdysone comprised the greater proportion of the ecdysteroids (93%). As pharate adult development progressed, the proportion of ecdysone decreased as the proportion of 20-HE and 20,26-HE increased. By 100 hours after pupation, at the time of initiation of vitellogenesis (Zimowska et al. 1991), no ecdysone was detected; 20-HE and 20, 26-HE comprised all of the ecdysteroids measured. No detectable amounts of 26-HE were found in any of the samples.

JHA studies

When virgin *P. interpunctella* were treated with methoprene as pupae, roughly 3 days prior to adult hatching, the following effects were observed (Table 2). In females receiving 10 and 100 µg methoprene, the mean number of eggs per female increased as compared to control (virgin) females, although it was lower in control (mated) females. The virgin females treated with methoprene started laying eggs earlier, and 100% of the eggs were laid earlier; 3.9 days in the treated females, 3.2 days in control (mated) and 5.2 days in control (virgin) females. This reduction of time necessary for egg laying corresponds to a much shorter life span of 5.5–6 days as compared with 8.8 days in nontreated virgin females and 5.5 days in control (mated).

Discussion

By measuring the ecdysteroids at short time intervals, every 4 hours, for the photo-synchronised Indianmeal moth females, the ecdysteroid profile shows changes in a stepwise fashion with the titre stabilising over several 8-hour plateau periods. In the first 60 hours after pupation, 8-hour plateaus were observed between 0–8, 16–24, 28–36, 40–48 and 52–60 hours. Plateaus in ecdysteroid titres were observed also in the profiles for pupae cultured in constant darkness. Similar stepwise or plateau changes in the ecdysteroid titre have been observed in *Drosophila melanogaster* when short intervals were used between sample collection (Handler 1982). The significance of these 8-hour plateaus was not determined.

Ecdysone was the major component of the early pharate adult ecdysteroid peak. Our data show (Table 1) that high levels of ecdysone, with only minor amounts of other metabolites, are correlated with the initiation of adult ovarian development. As development progressed, the amount of ecdysone progressively decreased as well as the proportion of ecdysone to 20-HE. By 100 hours after pupation, when ecdysteroid titres had declined to <500 pg/mg wet wt, no ecdysone was detected in the samples and 20-HE was the primary ecdysteroid present in the pharate adult females. This switch

Table 1. Quantitative analysis of ecdysteroids from female *Plodia interpunctella* during metamorphosis.

Pupal age (hours)	pg/mg wet wt (% of total ecdysteroids)		
	Ecdysone	20-HE	20, 26-HE
2	18(19)	76(81)	ND
28	1640(93)	115(7)	ND
52	16(19)	68(81)	ND
76	4(3)	62(51)	55(46)
100	0(0)	19(68)	9(32)

ND = not detectable.

Table 2. The effect of methoprene on life span and egg production of virgin females of *Plodia interpunctella*.

Dose (ng/pupa)	Total number of eggs	Time (days) needed for laying		Adult lifespan (days)	% of females not laying eggs
		25% of eggs	100% of eggs		
10	57±8	2.4±0.2	3.9±0.5	6.1±0.4	9
100	76±13	2.0±0.2	3.9±0.3	5.5±0.3	5
Control (virgin)	23±8	3.1±0.4	5.2 ±0.5	8.8±0.6	23
Control (mated)	176±20	1.3±0.2	3.2±0.3	5.6±0.5	0

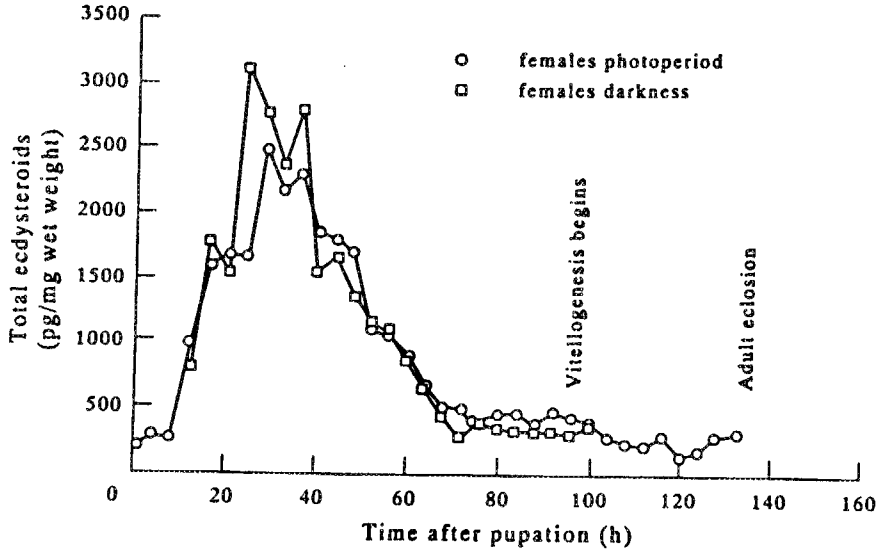

Fig. 1. Changes in ecdysteroid titres (as 20-HE equivalents) during pupal adult development. The pupa were kept either in a long-day photoperiod or in constant darkness.

from ecdysone to 20-HE as the primary ecdysteroid as pharate adult development progresses has also been reported for *H. zea* (Holman and Meola 1978), and *M. sexta* (Warren and Gilber 1986).

The data suggest that the ecdysteroid decline is significant to the initiation of vitellogenesis. Vitellogenesis in *P. interpunctella* begins between 96 and 100 hours after pupation (Zimowska et al. 1991). This is also consistant with the observation that increasing the ecdysteroid titres by treatment with exogenous 20-HE during pharate adult development blocked yolk protein synthesis and egg maturation (Shirk et al. 1990).

Taken together, the data suggest that ecdysteroids play a central role in the regulation of vitellogenesis during pharate adult development. However, the mechanism that leads directly to the initiation of vitellogenesis appears to involve additional regulators because the initiation of vitellogenesis does not occur until 24 hours after the ecdysteroids have completed the decline.

In the present study we were able to demonstrate that methoprene exerts an influence on adult development. The interesting feature is that the JHA reduced the life span of virgins to the control value. Mated females laid many more eggs than virgin females, but virgin females laid a considerable number of eggs when treated with increasing concentrations of methoprene. A similar situation was also recorded for *E. cautella* (Shaaya et al. 1991). Our data suggest that lifespan and egg laying are negatively correlated with each other. Treatment with a JHA acts either via manipulation of the ecdysteroid titre or indirectly as a result of JHA effects on the stage of development of the ovary. The exact mechanism of this regulation is still to be elucidated.

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