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Ecdysteroid Titer during Metamorphosis and the Effect of Ecdysteroid on Oocyte Development in *Phormia regina*

검정금파리의 변태기에 따른 엑디스테로이드와 난세포성숙에 미치는 엑디스테로이드의 효과

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ABSTRACT The ecdysteroid titers of representive developmental stages of the blackblow fly, *Phormia regina*, were determined by radioimmunoassay and the effect of ecdysteroid on the oocyte maturation was investigated. Prior to every molts ecdysteroid levels began to increase sharply, suggesting ecdysteroid was the major component for egg-larval, larval-larval, and larval-pupal transformation. A difference in the levels of ecdysteroid between male and female was observed during adult life span. Following the protein meal, ecdysteroid in the females increased rapidly to a maximum at 96 hr of age when terminal oocyte fully matured. Effect of ecdysteroid on oocyte development was determined for control and ecdysone-treated female flies after the liver-feeding. The growth of oocyte in the flies treated by 1 μg of ecdysone, along with the control flies, was not facilitated. When the flies treated by 5 μg of ecdysone, however, duration of oocyte maturation was shorter than those of other two groups. This can be suggested that oocyte development in *P. regina* is due to the critical level of ecdysone.

KEY WORDS Ecdysteroid, *Phormia regina*, radioimmunoassay, moult, oocyte development

초 록 검정금파리의 변태에 따른 엑디스테로이드를 Radioimmunoassay법으로 측정하고, 난세포성숙에 미치는 엑디스테로이드의 효과를 조사하여 얻은 결과는 다음과 같다. 산란직후 존재하였던 난내 엑디스테로이드는 발생과정 중 감소하다가 부화 직전 다시 증가하였으며, 유충기와 용기의 성장 변태시 엑디스테로이드함량의 변화를 보면 유충-유충과 유충-용으로의 탈피시에 일시적인 증가현상을 나타냈다. 특히 용화 후 48시간에 높은 엑디스테로이드의 농도를 보였는데 이는 큐티클분비와 경화작용과 밀접한 관계가 있는 것으로 생각된다. 성충기에서는 수컷의 경우 엑디스테로이드가 거의 검출 되지 않은 반면, 암컷에서는 단백질원 섭식 후 96시간에 최고의 함량을 나타내어 난성숙도와 일치하는 변화를 보였다. 엑디스테로이드 처리와 난성숙도와의 관계를 보면, 1μg 처리군은 대조군에서와 같은 성숙도를 나타내 차이를 보이지 않았으나, 5μg처리군에서는 대조군에서 보다 12시간 빠르게 난세포성숙이 완료되어, 엑디스테로이드 처리시 임계농도 이상에서는 난세포조기성숙에 직접적인 영향을 미치는 것으로 나타났다.

검 색 어 검정금파리, 엑디스테로이드, 난세포성숙, 탈피, 방사선면역측정법

Insect growth, development, and metamorphosis are dependent on the endocrine controls of juvenile hormone, ecdysteroid and neurohormones. According to the typical model, which

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includes larval-larval, larval-pupal, and pupal-adult moult, a successful moult is influenced by the presence and absence of the hormone at critical times prior to the moult. This model was primarily based on ligation, transplantation, extirpation, and parabiosis experiments and was later confirmed by determination of the titer of juvenile hormones and ecdysteroids at representive developmental stages in various insect species(Whisenton et al. 1987, Zdarek & Denlinger 1987).

On the other hand, female adults often contain large amounts of ecdysteroids in the ovaries although they are no longer able to moult. After oviposition, ecdysteroids are recovered in the eggs. Ovaries and newly laid eggs of many insects contain high and recovered in the eggs. In some cases, ovarian ecdysteroids are secreted into the maternal haemolymph, where they might play a role in the females(Kaplanis et al. 1973, Mizuno & Ohnishi 1975, Lagueux et al. 1977).

For anautogeneous dipteran insects, the cycle of egg production is tied to the consumption of a blood meal. The regulation of the various physiological components in oocyte maturation and vitellogenesis is dependent on the interactive function of ecdysteroid, juvenile hormone, etc. In all these insects, egg production occurs in the adult stage, and yolk protein synthesis is stimulated mainly by ecdysteroid and juvenile hormone(Adams & Filipi 1988).

In the black blowfly, *Phormia regina* Meigen, earlier works on the oocyte development during the first reproductive cycle were focused on the feeding behavior of adult female and the role of the corpus allatum in relation to juvenile hormone synthesis, vitellogenin synthesis, and vitellogenesis(Mjeni & Morrison 1973, Mazzini et al. 1987, Liu et al. 1988, Zou et al. 1989). Recently, Yin et al.(1990) reported that ecdysteroid regulates the vitellogenin biosynthesis, while juvenile hormone regulates the uptake of vitellogenin by the developing oocytes. In *Musca domestica* (Adams & Filipi 1988), treatment of 20-hydroxyecdysone elevated vitellogenin to control levels if the corpus cardiacum-allatum complex present. Tsuchida et al. (1987) reported that the development of ovary in *B. mori* is induced by ecdystroids, and the ovary seems to undergo progressive development that accompanies vitellogenin accumulation. Nevertherless, little data on activity of ecdysteroid during postembryonic development and direct effect of ecdysteroid treatment on the oocyte maturation in *P. regina* was shown. In this study, the examination was carried out the changes of ecdysteroid titer during egg, larval, pupal, and adult stages of *P. regina* and a direct effect of ecdysteroid on the oocyte maturation during the first reproductive cycle of adult female.

MATERIALS AND METHODS

Insects

Phormis regina colonies were reared with an artificial diet as described by Stoffolano(1974). Hatching of the 1st instar larvae occurred 24hr after oviposition. Newly hatched larvae kept in a growth chamber at 25 ± 2℃, 50% r.h. and 16hr light-18hr dark photoperiod. During development, eggs, larvae, pupae, and adults were collected and used for determining the activity of ecdysteroid. After adult emergence, all files were maintained on a sugar-water only for the first 72hr of their adulthood. At 72hr of age, fresh beef liver was provided for about 1 hr for "liver-fed" flies. After the liver feeding bout, flies were again maintained on sugar and

water.

Radioimmunoassay of Ecdysteroid

The eggs were collected at 8 hr intervals after oviposition. Groups of 1st to 3th instar larval stages were collected by selecting larvae that had hatched or moulted into the 1st, 2nd, and 3rd instar within an interval of 20 hr. Pupae were collected every 16 hr after pupariation from larvae. The radioimmunoassay (RIA) was performed by Borst and O'Connor's method(1974). Egg samples were homogenated in 100 ml of chloroform: methanol(2 : 1). After adding 40 μl of water to the chloroform: methanol homogenate, the egg preparation was centrifuged at 3,000 g for 10 min. Methanol phase was removed, dried, and assayed for ecdysteroid.

Haemolymph of larval, pupal, and adult samples were extracted three times with 80% methanol. Pooled methanolic extracts were dired separately, added 100 μl of tritiated α-ecdysone and 100 μl of anti-serum solution. After 12 hr at 4℃, 200 μl of saturated ammonium sulfate was added and the mixture centrifuged at 3,000 g for 10 min. The precipitate was washed with 400 μl of 50% saturated ammonium sulfate, then dissolved in 25 μl of distilled water and mixed with 500 μl of Aquasol Ⅱ. Radioactivity was determined with a Beckman LS 250 Scintillation counter. The assay was able to detect 20 pg of ecdysone(Sigma Chem. Co.) and remained linear from 20 to 1,000 pg. All data should be read as ecdysone equvalents using by standard curve.

Ecdysone Treatment

β-ecdysterone(β-ecdysone; 2β, 3β, 14α, 20β, 22, 25-hexahydroxy-7-cholesten-6-one, Sigma Chem. Co., E2003) was dissolved in 10% ethanol of reagent grade to the concentration of 1 μg/5 μl and 5 μg/5 μl(Fraenkel & Hollowell 1979). Each fly fed on liver at 72 hr of age was topically treated with 5 μl of ecdysone solution on the side of the thorax using a microapplicator. Each control fly recieved a single dose of 5 μl of 10% ethanol. Carbon dioxide and chilling were used to immobilize the flies and facilitate handling. Effect of ecdysone on oocyte development was determined by measuring the terminal oocyte length which reflects the state of oocyte development.

RESULTS AND DISCUSSION

Titer of ecdysteroid during development

The moulting hormone, ecdysteroid, is one of the most important regulators in insect development. Circulating, increased levels of ecdysteroids are known to regulate preecdysal events and subsequent moulting.

In this study, changes in the titer of ecdysteroids during egg, larval and pupal development of *P. regina* are shown in Fig. 1. During the egg stage ecdysteroid titer at the 0 hr just after oviposition was 0.5 pg/μg, decreased at 8 and 16 hr and then increased again at the time of the hatching. These findings agreed with those of Hsiao and Hsiao(1979) on *Galleria mellonella*, by Kaplanis et al. (1976) on *M. sexta*, and by Wentworth and Roberts(1984) on *S. bullata*. These ecdysteroids are thought to originate from the parent's ovaries which may produce ecdysteroids. The peak of ecdysteroids just before hatching could be explained that the eggs synthesize ecdysteroids from functional embryonic prothoracic glands to stimulate new larval cuticle formation.

The most dramatic events in insect development are the larval-larval and larval-pupal

moult. The ecdysteroid titer during the fourth and fifth larval instars of *M. sexta* was characterized by large increases lasting approximately 24 and 60 hrs, respectively, and these peaks occurred just before each ecdysis. After pupation, the ecdysteroid titer increases, reaching a maximum between days 7 and 9, and then it declined dramatically until day 14(Bollenbacher et al. 1981). In *S. bullata*, the ecdysteroid titer, which was high at the time of pupariation, dropped and then rose several days later at the onset of adult development(Zdarek & Denlinger 1987). Dean et al. (1980) reported the haemolymph ecdysteroid titer of the last larval and pupal stadia of *Calpodes ethlius*. During the last larval stadium, four significant ecdysteroid peaks were present. The first peak occurred 12 hr after ecdysis and correlated temporally with nucleolar activity, RNA synthesis and organelle formation in the fat body and epidermis. Another peak, at 78 hr, started to increase when the prothoracic glands no longer required the influence of the brain to produce ecdysone for pupation, and marked the first critical period. Moray and Tarnoy (1978) also suggested that in the last instar of *G. mellonella* two peaks of ecdysteroid titers were observed, one when larvae started to spin and another just before the pupation. In *P. regina* ecdysteroid levels began to increase sharply prior to every larval moulting and pupariation(Fig. 1). There was a clear correlation between the type of moult and the ecdysteroid content in the apolysed and freshly ecdysed insects. In a variety of insects including *G. mellonella*(Bollenbacher et al. 1978), *C. ethlius*(Dean et al. 1980), *S. bullata*(Wentworth et al. 1981), and *D. melanogaster*(Bainbridge & Bownes 1988), high levels of ecdysteroids just before moult were found. The highering of ecdysteroid levels in *P. regina* may be due to synthetic activity of hormone and release it into haemolymph to induce larval-larval moult.

During pupal stage the ecdysteroid titer continued to increase and peaked(5.3 pg/μl, Haemolymph) at 48 hr following the pupariation. The peak in ecdysteroid concentration at 48hr may suggest a closely timed hormonal release which results in cuticle secretion and tanning. A similar peak of ecdysteroid activity has been reported by Shaaya and Karlson (1965) in *Calliphora erythrocephala*, by Hodgetts et al.(1977) in *D. melanogaster*, and by Wentworth et al.(1981) in *S. bullata*. During this time the process of phenolytic tanning is undergoing. This process is initiated by 20-hydroxyecdysone which apparently activates the gene for dopadecarboxylase(Karlson & Sekeris 1966). However, it has been suggested by Seligman et al. (1977), that the hormone exerts its effects in conjunction with an anterior retraction factor and puparium tanning factor; This report has shown that five distinct peaks of ecdysteroid activities occurred during the egg, larval, and pupal stages of development in *P. regina*. The first three presumably initiated egg-larval and larval-larval moult while the fourth was coincident with larval-pupal apolysis and ecdysis. A maximum peak of the fifth was correlated with the synthetic activity for cuticle formation.

Haemolymph ecdysteroid content of adult male and female flies declined to non-detectable level until liver feeding at 72 hr after emergence(Fig. 2). Following the protein meal, the level of ecdysteroid in the female increased rapidly to a maximum at 96 hr of age and then continuously decreased, while male haemolymph ecdysteroid profile maintained trace amount after even liver-feeding. The changes in ecdysteroid content of the female are closely re-

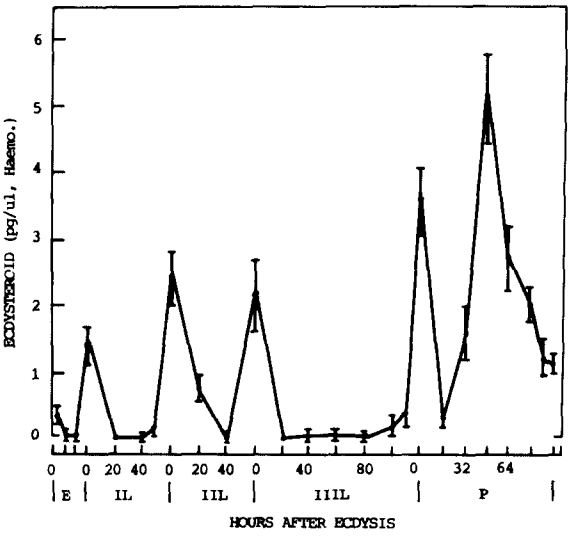

Fig. 1. Ecdysteroid titer during egg-larval-pupal development of *Phormia regina*.
Stages represented are the egg(E), first instar larva (IL), second instar larva(IIL), third instar larva (IIIL), and pupa(P). Each datum point is the mean ± SEM of four to six separate determinations.

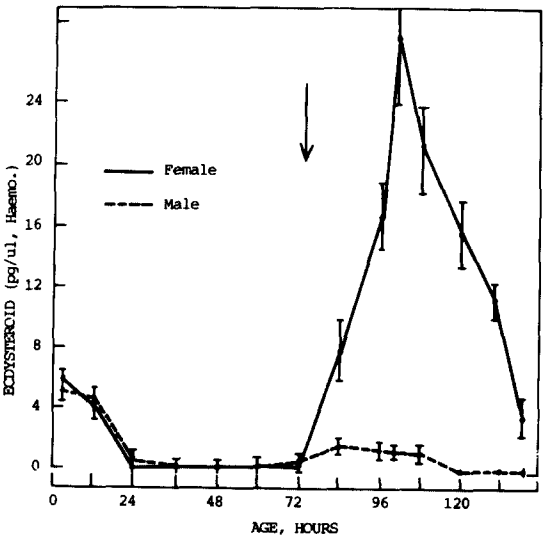

Fig. 2. Ecdysteroid titer of *Phormia regina* adult. Flies were provided with sugar and water only 1st 72hr of adulthood and, at 72hr of age, liver(arrow) was made available in addition to sugar and water. Each datum point is the mean ± SEM of four to eight separate determinations.

lated with oocyte development. In 1975 Hagedorn et al. reported that in the mosquito, *Aedes aegypti*, ecdysone is secreted by the ovary into the haemolymph in response to an egg development neurosecretory hormone, the release of which is triggered by ingestion of a blood or protein meal. They considered that the ovarian ecdysone stimulates the production of vitellogenin by the fat body. Legay et al.(1976) proposed that ecdysteroids during oocyte development play a dual role ; one of stimulating oocyte growth and the other of influencing morphogensis of the oocyte. A similar system of the correlation between ecdysteroid and oocyte development has been reported in *B. mori*(Ono et al. 1975), *D. melanogaster*(Postlethwaite & Handler 1979), *S. bullata*(Wentworth & Roberts 1984), *Pieris brassicae*(Beydon et al. 1989), and *P. terraenovae*(Wilps & Zoller 1989).

Effect of ecdysteroid on oocyte development

The effects of ecdysteroid treatments on insects have been forcused on a dual purpose ; one of inhibiting growth and ecdysis in larvae in order to get the basal data for insect pest control(Kubo et al. 1983, 1987) and the other of influencing vitellogenesis and development in adult females(Bownes 1982, Huybrechts & DeLoof 1977). This study was here led to reveal the effect of the ecdysteroid treatment on the oocyte development in *P. regina* adult female. As shown in Table 1, the lengths of terminal oocytes in both control and 1 μg of ecdysone treatment groups were increased gradually until 12 hr after liver-feeding. The more rapid rises occurred during next 24 hr and very rapid rises were observed at 60 hr old to reach a maximum(1,121 ± 61.7 μm). There was no significant differences between two

Table 1. Effect of β-ecdysone on oocyte development in adult female *Phormia regina*

Hours After treatment	Control	Ecdysteroid Treatment	
		1 μg	5 μg
0	117.3 ± 11.4	117.6 ± 10.3	118.4 ± 12.4
4	118.0 ± 19.1	119.4 ± 12.2	256.2 ± 20.2
12	170.2 ± 19.9	196.5 ± 21.4	363.5 ± 29.8
24	387.0 ± 82.6	394.8 ± 15.6	496.1 ± 50.2
36	421.6 ± 24.2	445.6 ± 38.7	894.7 ± 60.1
48	977.4 ± 121.8	1015.8 ± 72.5	1146.0 ± 89.7
60	1121.5 ± 61.7	1124.2 ± 89.2	764.3 ± 246.3
72	230.0 ± 57.7	234.3 ± 68.3	349.8 ± 59.6

Female flies were provided with sugar and water only for the first 72 hr of adulthood and then liver was made available for 1 hr in addition to sugar and water. Ecdysone or ethanol alone was topically applied to female flies after liver-feeding. Oocyte length was measured after liver fed and expressed in μm.

gropus. When flies treated by 5 μg of ecdysone, duration of oocyte maturation was shorter than those of the control and 1 μg ecdysone treated females. This may mean that the critical level of ecdysteroid acts directly on the precoceuos egg maturation. In *D. melanogaster*(Bownes 1982) and *S. bullata*(Huybrechts & DeLoof 1977) it is known that ecdysteroids stimulate the vitellogenin synthesis. Izumi and Tomino(1983) reported that the biosynthesis of vitellogenin in *B. mori* starts immediately after pupation, and Ohno et al. (1975) showed that ecdysteroid could stimulate the biosynthesis of haemolymph proteins including female specific protein. These supported indirectly the present results that the development of ovary in *P. regina* was induced by ecdysone. The present conclusion on the role of ecdysone was in consisent with the observation on the effect of ecdysteroid treatment on the growth of ovary in *B. mori*(Tsuchida et al. 1987). Although these correlations between the dosage of ecdysteroid treatment and the growth of oocyte in size exist, more experiments are necessary to investigate the function of this hormone in the processes of biochemical and genetic levels.

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