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Hormonal Control of Induction and Termination of Diapause in *Pieris rapae* L.

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Non-diapausing larvae and diapausing pupae of *Pieris rapae* L. were treated with 20-HE and JH, and the concentration of 20-HE and JH in body were also measured to determine mechanisms of diapause induction and termination.

When early last instar larvae of non-diapausing stage were injected with 20-HE or JH-I, diapause was not induced in both cases. 20-HE concentration of pre-diapausing last instar larvae was very low, and 20-HE peak of non-diapausing two-day-old pupae was not present in diapausing pupae. There was no difference in concentration of JH-I between diapausing and non-diapausing stages. When early and late diapausing pupae were injected with 20-HE, JH-I, or JH-III, diapause was terminated by 0.1-0.5 μg of 20-HE, but not by JH-I, or JH-III.

The above results indicate that diapause of *Pieris rapae* was induced by decrease of 20-HE concentration and also terminated by gradual accumulation of 20-HE during diapause process.

KEY WORDS: *Pieris rapae* L., Diapause, 20-Hydroxyecdysone, Juvenile hormone

Insects decide whether they will enter the diapause by recognizing the environmental change at specific stage. The mechanisms of neuroendocrinal control on diapause are diverse with species. The increase of juvenile hormone (JH) at certain stage was reported to be involved in induction and termination of larval diapause in *Diataea grandiosella* (Yin and Chippendale, 1973, 1979; Yage and Fukaya, 1974) and several lepidopteran species (Chippendale, 1977; Takeda, 1978). However, JH concentration of *Ostrinia nubilalis* drastically decreases at the onset of diapause, indicating that JH appears to be involved only in diapause induction (Chippendale and Yin, 1979; Bean and Beck, 1980). Also it was reported in *Pieris brassicae* that when insects were treated with short day condition (9L:15D, 20°C), secretion of prothoracicortropic hormone (PTTH) was inhibited and resulting low cooncentration of 20-hydroxyecdysone(20-HE) in body induces pupal diapause (Seuge and Veith, 1976). However, JH was also reported to be involved in pupal diapause of *Mamestra brassicae* (Yagi, 1975). Thus, the mechanisms of diapause induction and termination were represented to be specific with different species.

The present work is to determine the hormones and its control mechanism involved in pupal diapause of *Pieris rapae* L..

Materials and Methods

Insects

Adult cabbage white butterfly, *Pieris rapae* L., were collected in the vicinity of Seoul and mated in a vinyl house. Hatched larvae were reared on keil at 27±1°C and 70-75% RH under photoperiod of 14L:10D. Sexes were segregated according to Richards and Davis (1977).

Induction of diapause due to photoperiod and humidity

Newly ecdysed 3rd instar larvae were reared at 20°C, 70-75% RH and 10L:14D. Non-diapause pupae were emerged to adult within 6.5 to 7 days after pupation and emergence to adult was determined by the color of wing scale on cuticle of 4-day-old pupae. Pupae were regarded to enter the diapause if white color was not detected on wing scale of cuticle within 14 to 15 days after pupation.

Treatments of 20-HE, JH-I, and JH-III

20-HE(Sigma Chemical Co., 2,3,14,20, 22,25-hexahydroxycholest-7-en-6-one) was dissolved in distilled water to be concentration of 0.1 μg/μl. JH-I(Sigma Chemical Co., Methyl (2E, 6E)-(10R)-10,11,-Epoxy-7,11-Diethyl-3-Methyl-2,6-Dodecadienoate) and JH-III(Sigma Chemical Co., Methyl (2E,6E)-(10R)-10,11--Epoxy-3,7,11-Trimethyl-2,6-Dodecadienoate) were each dissolved in 100% acetone to be concentration of 0.1 μg/μl.

Larve were topically treated with 20-HE, JH-I and JH-III, but pupae were injected with same chemicals between second and third abdominal segments with Hamilton microsyringe and then sealed with liquid paraffin.

Determination of 20-HE and JH levels

20-HE was used as the standard after being dissolved in distilled water. Twenty insects were taken at each stage from 3rd instar larvae and homogenized in motar and centrifuged at 10,000 rpm at 4°C for 15 min. The superatant was filtered through gauze to remove fat according to Lafont *et al.* (1980). Filtered supernatant was partially purified through Waters Sep-Pak C_{18} cartirdge with different percentage of methanol. The supernatant was put in the cartridge and passed through it with different percentage of methanol. 500 μl each was extracted and ecdysteroid content in each fraction was measured using High Performance Liquid Chromatography (HPLC) at the wavelength of 254 nm. The results showed that 60% methanol fraction contains highest concentration of ecdysteroid. Solvent used was acetonitrile:water:acetic acid (20:75:5, v/v/v) and μ Bondapak C_{18} reverse phase column was used.

JH-I, II, III standard (Sigma Chemical Co.) was used by being dissolved in 100% acetone, twenty insects were taken at each step from 3rd instar larvae and JH was transformed into diol form. Insects were homogenized in motar with 5 ml of 60% methanol. The supernatant was separated into methanol phase and n-hexane phase by addition of 5 ml n-hexane. Upper phase (n-hexane phase) was taken and concentrated using Labconco freeze dryer and incubated overnight at 40°C in sulfuric-dioxane (900 μl dioxane + 150 μl of 0.12 M H_2SO_4). This solution was neutralized by addition of 1 ml of 0.01 N NaOH and frozen, dried and mixed with 0.5 ml of acetonitrile: water(50:5, v/v) to make H.P.L.C. sample. All accessory systems in Water ALC-100 LC were the same as ecdysteroid measurement and solvent was acetonitrile: water(50:50, v/v).

Results

Induction of diapause by the treatments of 20-HE and JH-I

Larvae reared at 28°C under photoperiod of 14L: 10D were locally treated with 20-HE or JH-I to determine the diapause induction. Newly ecdysed 5th instar larvae were treated with 0.2-1.0 μg of 20-HE. The results showed that all larvae were normally pupated and not diapaused in all concentration tested(Table A). As the larvae were treated with 0.2-0.5 μg of JH-I, a number of pupae ecdysed gradually decreased with increasing concentrations of hormone injected. The larvae which were not pupated showed intermediate form between larvae and pupae. This intermediate form showed larval form in head and abdomen but pupal form in other parts, and all stopped ecdysing and died within 17-30 hrs. Pupal diapause was not induced even in ecdysed pupae (Table 1B).

Measurement and comparison of 20-HE concentration between diapuse and non-diapause stages

Concentration of 20-HE was measured two

Table 1. Effects of 20-hydroxyecysone and juvenile hormone-I on diapause induction in last instar larvae of *Pieris rape* L.*

A. 20-hydroxyecdysone

Dose (μg)	No. applied	No. ecdysed	No. died	No. diapause induced
0**	10	10	0	0
0.2	20	20	0	0
0.3	20	20	0	0
0.4	20	20	0	0
0.5	20	20	0	0
0.6	20	20	4	0
1.0	20	17	16	0

B. juvenile hormone-I

Dose (μg)	No. applied	No. ecdysed	No. intermediate***	No. diapause induced
0****	10	10	0	0
0.2	20	19	0	0
0.3	20	17	3	0
0.4	20	11	9	0
0.5	20	4	16	0

* Larvae were reared under 14L:10D photoperiodic condition during the larval and pupal stages at 28°C. ** Distilled water was used as a control of 20-hydroxyecdysone. *** Larval-pupal intermediate produced during ecdysis did not develop as pupa and died within 30 hours. **** 100% acetone was used as a control of juvenile hormone-I.

times during the period of 3rd instar larva to pupa day 6. In non-diapause stage, 20-HE increased until last larval instar and drastically decreased at early prepupal stage and then reached the peak at middle stage of prepupa. Also, the 20-HE reached the peaks at day 2 and day 5 after pupation. On the while, 20-HE of diapausing insects decreased from 4th larval instar to early prepupae and then reached the peak at middle stage of prepupae, but showed no peaks on day 2 and day 5 after pupation as in non-diapause stage (Fig. 1).

Comparison of JH-I concentration between diapause and non-diapause stages

JH-I concentration in whole body was also measured tow times during the period of 3rd larval instar to pupa day 1 of diapause and non-diapause insects. JH-I of diapause insects showd a little higher value than that of non-diapause insects during the period of larval instar to pupa day 1, but there was no distinct difference between two stages (Fig. 2).

Concentration change of 20-HE during diapausing period

Concentration of 20-HE in body was measured at 20 day intervals during 120 days of early diapause to late diapause. Newly ecdysed pupae were maintained below the temperature of 5°C. The result showed that pupae maintained a relatively cnstant concentration of 30-40 ng/insect until pupa day 70 and then gradually increased to 60 ng/insect at pupa day 120 (Fig. 3).

Termination of diapause due to injection of 20-HE, JH-I or JH-III

Early diapaused (pupa day 17-20) and late diapaused (pupa day 105-110) insects were injected with 20-HE, JH-I or JH-III to determine the effect of hormones on diapause termination. Termination of diapause did not occur in early diapause phase due to JH-I and JH-III but occurred in 0.1-0.5 μg of 20-HE and reached the peak at 0.3-04 μg concentration (Table 2A). Ratio of diapause termination could not be measured because all pupae died at concentrations above 0.5 μg. Diapause was not broken down in late diapause phase due to JH-I and JH-III. However, diapause was broken down by the injection of 0.1-0.5 μg 20-HE and shows 100% diapause termination at concentration of 0.2 μg lower than that in early diapause phase (Table 2B).

Discussion

Diapause of *Pieris rapae* was induced by photoperiod and temperature. Complete diapause induction was accomplished under the 8-10 photophase/day at 20°C and also all insects were diapaused below 20°C at photoperiod of 14D:10L. These results indicate that diapause is induced by simultaneously suitable condition of photoperiod and temperature which are in accord with natural environmental diapause condition. Above two conditions should be maintained at least for over

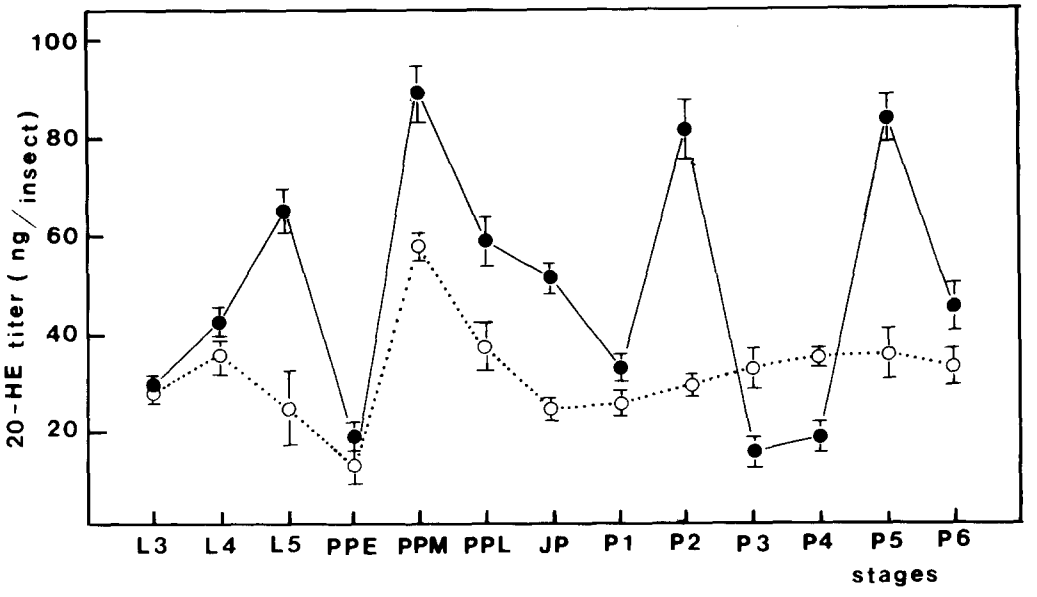

Fig. 1. 20-hydroxyecdysone titer in the whole body of diapause (○) and non-diapause (●). (L, larval instar; PP, pharate pupae; JP, just pupae; P, pupal stages)

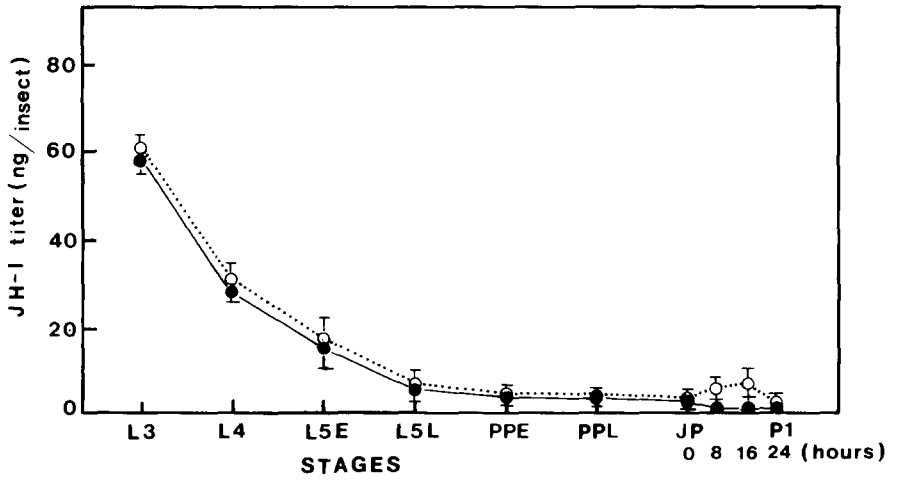

Fig. 2. Juvenile hormone-I concentration at different stages in the whole body of diapause (○) and non-diapause (●). (L, larval instar; L5E, 5th larval instar early; L5L, 5th larval instar late; PP, pharate pupae; JP, just pupae P1, 1 day old pupae.)

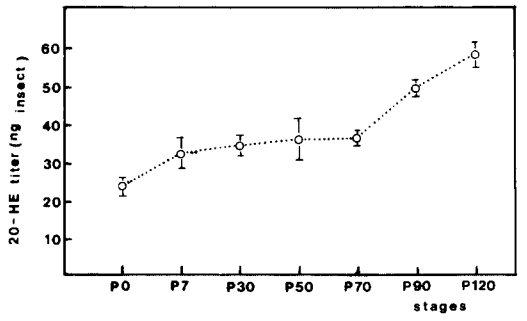

Fig. 3. 20-hydroxyecdysone titer in the whole body during diapause. (P0, P7, P30, P50, P70, P90, P120 represents just pupae, 7, 30, 50, 70, 90, 120 day old pupae respectively.)

8 days from early 3rd instar to induce diapause. Also, when early 5th instar larvae were again treated with normal condition, the ratio of diapause induction drastically decreases, suggesting that 3rd instar larvae recognize the change of environmental condition while 5th instar larvae decide whether they will enter the diapause.

When *Ostrinia nubilalis* was injected with JH analog (ZR-515), diapause was not induced but rather delayed (Yagi and Akaike, 1976; Chipendale and Yin, 1979; Bean and Beck, 1980). However, when *Diatraea grandiosella* was injected with JH analog (ZR-1662), diapause-like-state was induced and also JH is involved in maintenance and termination of diapause (Yin and Chippendale, 1973, 1974, 1979). In the present work *Pieris rapae* was not diapaused by the injection of 20-HE or JH-I, indicating that increasing concentrations of these hormones do not play any role in diapause induction. The formation of larval-pupal intermediate due to the injection of JH was reported in other species (Beck and Shane, 1969; Yagi and Akaike, 1976; Bean and Beck, 1980). Yagi and Akaike (1976) suggested that this intermediate form is a kind of diapause. In *Pieris rapae*, however, all these intermediate forms died within 30 hrs, indicating that injection of JH interferes with normal pupation process.

20-HE in body of diapausing stage is present in similar concentration to that of non-diapausing stage until diapause deciding time, but maintains a low concentration during diapause stage in *mamestra cnfigurata* (Bodnaryk, 1977, 1985), *Heliothis viresceus* (Loeb, 1982), and *Sarcophaga crassipalpis* (Walker and Denlinger, 1980). Also, Ohtaki and Takahashi (1972) reported that 20-HE peak involved in adult development is absent in diapausing pupae. Kono (1973) reported the PTTH of nondiapausing *Pieris brassicae* begins to be secreted from brain just after pupation and reaches the concentration enough to stimulate prothoracic gland at 36 hrs. In *Pieris rapae*, peak of 20-HE occurred at 2-day-old pupa in non-diapausing insect, but not in diapausing insects. These results indicate that 20-HE peak is involved in adult development and therefore diapausing pupae have no such a peak (Fig. 1). Also, there was not much difference in concentration of JH-I, but distinct difference in concentration of 20-HE between diapausing and nondiapsusing insects, indicating that diapause was not induced by JH, but by a rather decrease of 20-HE concentration. JH-II and -III were present in small amounts during larval stage. The concentration of hormones during life cycle might be rhythmically changed even if its width will be small. Unfortunately such data were not reported in any insects. However, based on the physiological change due to the concentration change of hormone, it must deserve consideration.

To confirm that pupal diapause of *Pieris rapae* is terminated by increase of 20-HE concentration, pupae of early diapausing and late diapausing stages were injected with different cncentration of 20-HE. The result showed that 0.1 up to 0.5 μg of 20-HE could break up the diapause. However, diapause of late diapausing insects could be efficiently terminated by 0.2 μg smaller than 0.4 μg of early diapausing insects, suggesting that late diapausing insects accumulate already enough 20-HE in body. This result was similar to those of *Sarcophaga argyrostoms* (Gibbs, 1976; Fraenkel and Hsiao, 1968) and *Heliothis punctiger* (Browning, 1981). Hiruma (1979) reported that when diapausing *Mamestra brassicae* were treated with JH analog, pupal diapause was inhibited. However, diapause of *Pieris rapae* was not broken by JH-I or -III. Bradfield and Denlinger (1980) also reported that diapause of *Manduca sexta* could be

Table 2. Number of 17-20(A) and 105-110(B) day-old diapause pupae at 26°C following injection with various amounts of external 20-hydroxyecdysone and juvenile hormone-I, III*

Hormone treated	Dose (μg)	No. injected	No. developed	No. died	Total % of diapuase termination
(A) 20-HE**	0	10	0	0	0
	0.1	10	1	0	10
	0.2	10	3	0	30
	0.3	10	9	0	90
	0.4	10	10	0	100
	0.5	10	6	4	60
	1.0	10	0	10	0
JH-I***	0	10	0	0	0
	0.2	10	0	0	0
	0.4	10	0	1	0
JH-III	0	10	0	0	0
	0.2	10	0	0	0
	0.4	10	0	0	0
(B) 20-HE**	0	10	0	0	0
	0.1	10	1	0	10
	0.2	10	10	0	100
	0.3	10	9	0	90
	0.4	10	5	3	50
	0.5	10	2	8	20
	1.0	5	0	5	0
JH-I***	0	5	0	0	0
	0.3	5	0	0	0
JH-III	0	5	0	0	0
	0.3	5	0	0	0

* All the pupae were reared at 14L:10D photoperiodic condition during the treatments. ** Distilled water was used as a control of 20-hydroxyecdysone *** 100% acetone was used as a control of JH-I, III.

terminated by a large amount of JH analog. However, when *Pieris rapae* were treated with dose above 0.5 μg of JH-I or -III, diapause termination could not be confirmed because most pupae died.

Based on the above results, the brain of *Pieris rapae* was inactivated by the change of external factors such as photperiod and temperature, and so PTTH was not secreted enough to stimulate prothoracic gland. Therefore, drastic decrease of 20-HE in body resulted in diapause induction which has no adult development. Also, when prothoracic gland and brain normally function by external factors, 20-HE concentration increases and diapause is terminated. JH is considered not to involve in diapause of *Pieris rapae*. When last instar larvae and diapause pupae were injected with JH-I, intermediate types were formed but diapause pupae did not show any change, demonstrating that pupation requires absence of JH within the body. Also, since 20-HE seemed to be main hromone concerned with induction and termination of diapause in *pieris rapae*, more works for this hormone were conducted. However, the endogeneous level of JH-I and JH-III during diapause stage might also be needed to understand whole mechanism for diapause. More works on diapause will be carried out.

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배추흰나비(*Pieris rapae* L.)의 휴면 유도 및 타파에 대한 호르몬의 조절

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*Pieris rapae*의 휴면 유도 및 타파기작을 알아보기 위하여 비휴면기 유충과 휴면중인 번데기에 20-HE과 JH을 처리하여 변화를 관찰하는 한편, 체내의 각 호르몬의 농도도 측정하여 보았다.

비휴면기의 종령유충초기에 20-HE과 JH-I을 주입한 결과 어느 경우에도 휴면이 유도되지 않았다. Pre-diapausing 종령 유충에서는 20-HE 농도가 매우 낮았으며, 비휴면기용 2일의 20-HE peak가 휴면기에서는 나타나지 않았다. JH-I의 농도는 두 시기에 있어 뚜렷한 차이가 없었다. 휴면중인 번데기의 초기와 말기에 20-HE, JH-I, JH-III을 주입한 결과 0.1-0.5 μg의 20-HE에 의해 휴면이 타파되었으나, JH-I과 JH-III에 의한 휴면 타파는 일어나지 않았다.

위의 결과로 배추흰나비의 휴면은 20-HE 농도가 저하됨으로 인해 유도되며, 휴면이 진행되는 동안 20-HE 농도가 점진적으로 증가하여 휴면이 타파되는 것으로 생각된다.