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[COMMUNICATION]

Comparative Activity of Juvenile Hormones I, II, and III in Promoting Egg Maturation in the Moth, *Heliothis virescens* (Noctuidae)

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ABSTRACT—Adult females of the moth, *Heliothis virescens* (Noctuidae), were decapitated at embryogence and injected with various doses of juvenile hormone (JH) I, II, or III in 5 μl corn oil. All three hormones promoted dose-dependent egg maturation while corn oil only did not. JH I and II appear to be more active than JH III. Egg maturation was inhibited by treatment of females with 50 μg precocene II. This inhibitory activity could be reversed by JH therapy. Compactin, an inhibitor of HMG-CoA reductase (an enzyme necessary for the formation of a JH precursor), had no effect on JH-mediated egg maturation in *H. virescens*.

INTRODUCTION

Oogenesis in insects may be divided into distinct phases such as previtellogenic development of oogonia, vitellogenesis, and choriogenesis, during which various biochemical and morphological changes occur in the oocyte. These phases are regulated to varying degrees by hormones such as juvenile hormone (JH), 20-hydroxyecdysone (20-OHE), and neurosecretions [1]. During choriogenesis, follicle cells surrounding the developing oocyte synthesize and deposit an eggshell, the chorion, at the end of vitellogenesis [2]. Nijhout and Riddiford [3] documented the necessity of corpora allata (CA) and its secretion, JH, for egg maturation in the moth, *Manduca sexta*. Similarly, egg maturation in decapitated adult *Heliothis virescens* was restored with JH III therapy [4]. Additionally, Ramaswamy *et al.* [4] reported on the dose-dependent inhibitory effect of precocene II on JH-dependent egg maturation in *H. virescens*. In a recent report, *Helicoverpa zea* females were also shown to require JH for egg maturation [5]. It is further reported that CA of *H. zea* females are capable of producing JH I, II, and III *in vitro*, with the latter 2 being quantitatively predominant [5].

The current study was conducted to determine the comparative activity of JH I, II, and III in promoting egg maturation in *H. virescens*. An additional objective of this study was to determine if the inhibitory effect of precocene II could be reversed with JH therapy.

MATERIALS AND METHODS

Heliothis virescens larvae were reared on a wheat germ diet (Bioserv, Frenchtown, New Jersey) in plastic cups. Female pupae were placed under a 14 : 10 L : D reversed photoperiod in cages in an environmental chamber at 25±2°C, 60% RH. Adults emerge only during scotophase and, consequently, pupae were observed every 15 min for adult emergence throughout scotophase beginning one hour before light off [6]. Adults emerging within each 15 min period were grouped and their time of emergence denoted time zero. Moths used within each test were from the same generation; however, the tests were conducted separately over several generations.

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A group of adults within 15 min of emergence was anesthetized with CO_2, and moths were decapitated with iridectomy scissors. Immediately after decapitation, females were injected with various doses of JH I, II, or III (Sigma, St. Louis, MO) dissolved in 5 μl generic corn oil purchased from a local grocery store (Kroger). A group of decapitated females treated with 5 μl corn oil only served as the treated controls. Another group was anesthetized and left intact, and unfed, to serve as the untreated controls. All treatments were performed in a dark room with red Kodak safety lights [4]. Treated moths survived for 5 days under these conditions. Upon recovery from anesthesia, moths were placed in 500 ml opaque plastic cups lined with moist Kimwipes in the environmental chamber noted above and dissected at 27 hr after emergence to count chorionated oocytes. These data were subjected to regression analysis to determine the dose-response activity of the 3 hormones. The data were subjected to the Generalized Linear Models (GLM) procedure [7] to determine if there were any differences in slopes and intercepts of the three hormones, indicative of differential potencies.

Another group of adults at emergence was anesthetized and injected with 50 μg precocene II (Sigma) in 5 μl corn oil. A second group of females was injected with 5 μl corn oil and served as controls. Twenty four hours later, the precocene-treated feamels were divided into 2 groups, one receiving 10 μg JH III in 5 μl corn oil and the other receiving corn oil only. The control group received another dose of 5 μl corn oil. After treatment, females were left unfed in plastic cups with moistened Kimwipes. The number of eggs chorionated by such females 48 hr (24 hr after JH treatment) after emergence was determined. These data were subjected to analysis of variance (ANOVA) and Student-Newman-Keuls' (SNK) multiple range test.

A group of adults within 15 min of emergence was anesthetized and injected with 50 μg compactin in 5 μl distilled water. A second group was injected with 5 μl distilled water only, while a third group of moths was left untreated. Moths were left unfed in cups in the environmental chamber as described above. The number of eggs matured by the three groups of females was determined 27 hr later. These data were subjected to ANOVA.

RESULTS AND DISCUSSION

Injection of three hormones to decapitated females restored egg maturation in a dose-dependent manner (Fig. 1a). Linear relationships between response and dose for each hormone were assumed (note that the linear relationship for JH II with an r^2 of 0.582 may be questioned), and the data were subjected to the SAS-GLM procedure. A significant difference in the intercept of the 3 regression lines was found (Fig. 1a; $P=0.0064$), suggesting that the 3 hormones exhibited differences in the amount necessary to elicit activation of egg maturation; however, there was no significant difference in the slopes (Fig. 1a; $P=0.2203$). JH I and II were significantly more effective than JH III ($P<0.05$; GLM-Least Significant Differ-

TABLE 1. Egg maturation responses of virgin, decapitated females of *Heliothis virescens* to various doses of synthetic juvenile hormones (Mean ± SE Oocytes/Female) (n=21–38)

TREATMENT	JH I	% MORTALITY	JH II	% MORTALITY	JH III	% MORTALITY
Undecapitated	59.2 ± 6.1	0	59.2 ± 6.1	0	59.2 ± 6.1	0
Oil	1.42 ± 1.40	0	1.42 ± 1.40	0	1.42 ± 1.40	0
0.01 μg	17.5 ± 4.82	0	13.6 ± 2.90	0	1.24 ± 1.00	0
0.05 μg	56.6 ± 17.00	20	44.1 ± 13.04	4.8	19.7 ± 1.56	4.4
0.10 μg	46.1 ± 12.13	5.8	44.8 ± 13.63	14.3	5.8 ± 2.43	0
0.50 μg	65.8 ± 19.40	9.1	85.7 ± 18.01	9.5	19.7 ± 7.89	20.8
1.00 μg	117.45 ± 20.72	0	80.1 ± 14.30	16.7	42.2 ± 8.10	0
5.00 μg	78.7 ± 12.03	4.4	70.8 ± 16.03	13.6	38.8 ± 9.75	16.7
10.00 μg	58.4 ± 16.34	21.7	110.3 ± 13.84	16	100.6 ± 17.10	4.8

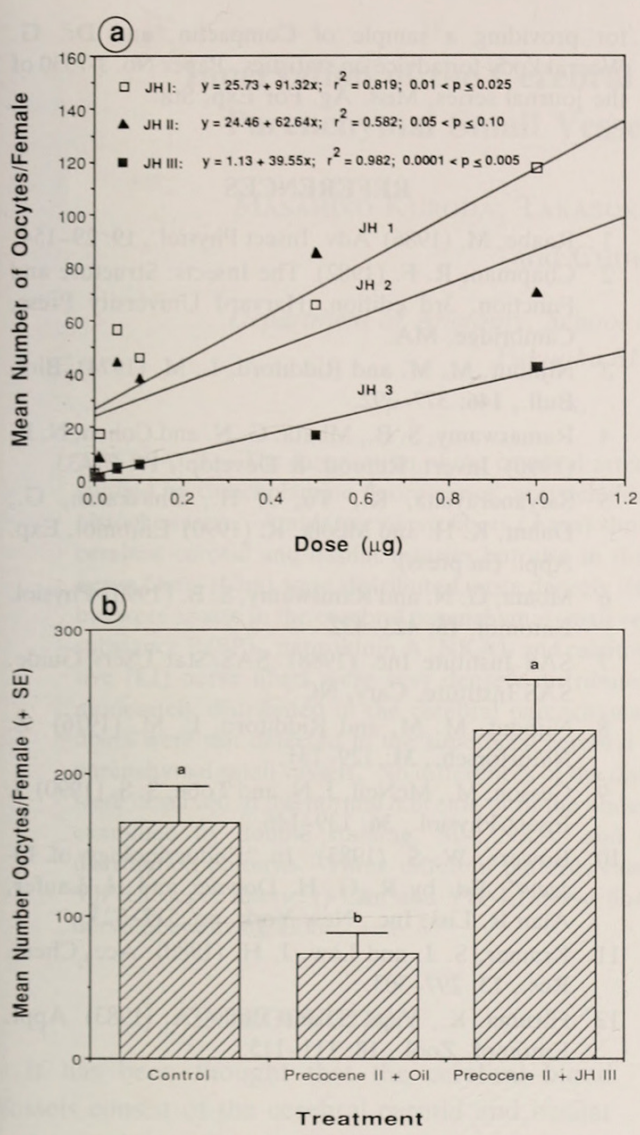

FIG. 1. a: Linear regression lines for JH I, II, and III (n = 39 at each dose); b: Responses of virgin female *Heliothis virescens* to treatment with corn oil (control; n = 34), precocene II + oil (n = 24), or precocene II + juvenile hormone III (n = 43). See text for details on dose and time of treatment.

ence) in promoting egg maturation. However, JH III elicited the most significant correlation of dose-response activity (Fig. 1a). Further increase in dose beyond 1 µg of JH I resulted in suppression of egg maturation (Table 1). However, increasing the dose of JH II or III to 10 µg resulted in a further increase in chorionated eggs. This suppression of egg maturation by JH I could not be accounted for by any increased mortality in treated moths because data presented in Table 1 are only from moths that were alive 24 hr after JH treatment. Note, however, that there were varying levels of mortality in treated versus control moths (Table 1). The reason for the overdose effect of JH I needs to be determined.

The greater sensitivity of *H. virescens* females to JH I and JH II is intriguing in light of the fact that in *Helicoverpa zea*, JH I comprises only about 10% of the three JHs synthesized by CA *in vitro*; JH II and JH III comprise the rest in approximately similar amounts [5]. Similarly, in *M. sexta*, while JH I and JH II are more active than JH III for egg maturation, the CA synthesize approximately equal amounts of JH II and JH III [3, 8]. In another noctuid moth, *Pseudaletia unipuncta*, CA *in vitro* synthesize predominantly JH II followed by JH I and JH III [9]. In light of the greater sentivitity to JH I and JH II in *H. virescens*, CA in this species may be hypothesized to synthesize more JH I and II than JH III. This may be resolved by determining JH biosynthesis *in vitro* by CA from *H. virescens*. Alternatively, the different JHs may have different physiological roles. Additionally, as suggested by Satyanarayana *et al.* [5] and Cusson *et al.* [9], *in vitro* synthesis of JH by excised CA may not reflect CA activity *in vivo*. Currently, studies are being conducted to determine the physiological role of JH in vitellogenesis and choriogenesis in *H. virescens* and will be reported on later.

Injection of 50 µg precocene II into virgin females immediately after emergence significantly depressed egg maturation compared with control individuals (Fig. 1b), corroborating earlier findings in *H. virescens* [4]. Injection of 10 µg JH III 24 hr after precocene treatement resulted in a reversal of the inhibitory effect of precocene II as evidenced by the resurgence in egg maturation 24 hr later in such individuals (Fig. 1b). This further supports the idea that egg maturation in imaginal *H. virescens* is controlled by JH. Such reversal of the inhibitory effect of precocene by JH therapy has been considered to be proof of anti-JH activity of precocenes for the physiological process under study [10].

The fungal metabolite, compactin (ML-236B), is an inhibitor of HMG-CoA reductase, an enzyme necessary for the reduction of 3-hydroxyl-3-methylglutaryl-CoA to mevalonic acid, a precursor of JH in insects [11]. Injection of 50 µg of this

compound in 2 doses has been shown to exhibit anti-JH activity in larvae of *Mamestra brassicae*, the cabbage armyworm [12]. Injection of 50 μg compactin in 5 μl water in a single dose to newly emerged *H. viescens* females resulted in production of 163.7 ($\pm$17.7 SE; n=40) eggs, which is not significantly different from 173.6 ($\pm$24.1 SE; n=26) and 142.9 ($\pm$18.5 SE; n=41) eggs matured by water injected or untreated control females, respectively ($P>0.25$, one way ANOVA). These data suggest that compactin is ineffective in inhibiting the JH-dependent egg maturation process in *H. virescens*. The reasons for this inactivity could be three-fold: 1) mevalonic acid for JH synthesis may be present in the CA in sufficient quantities at adult emergence; and, consequently, inhibition of HMG-CoA reductase after adult emergence has no effect on JH biosynthesis; 2) compactin is ineffective in inhibiting mevalonic acid synthesis in *H. virescens*; or 3) the dose injected was insufficient and did not reach a critical level. These alternative hypotheses remain to be tested.

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