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Activity of Juvenile Hormone Acid in Brainless, Allatectomized Diapausing Cecropia Pupae

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Pupal diapause in the cecropia silkmoth is terminated by activation of the prothoracic glands. Implantation of adult male cecropia corpora allata or injection of juvenile hormone-I (JH-I) or juvenile hormone-I acid (JH-I acid) causes initiation of development in debrained, allatectomized diapausing pupae. The subsequent development results in formation of second pupae or pupal–adult intermediates. The dose–response patterns for JH-I acid and JH-I are nearly identical. These data suggest that JH-I acid activates prothoracic glands and in addition acts like a morphogenetic hormone.

Diapause in saturniid pupae results from the programmed inactivation of the brain–prothoracic gland system and is terminated by activation of the prothoracic glands (PG) which leads to the secretion of ecdysone (Williams, 1952). Removal of the brain from such pupae keeps them in a state of permanent diapause (dauerpupae) which can be terminated by implantation of active brains or active corpora allata (Williams, 1952, 1959; Ichikawa and Nishiitsutsuji-Uwo, 1959). Juvenile hormone (JH), farnesol, methyl farnesoate, and various other compounds with JH activity are also effective in terminating pupal diapause (Gilbert and Schneiderman, 1959; Krishnakumaran and Schneiderman, 1965). Besides the presumed activation of the prothoracic glands, JH and JH analogs also prevent normal metamorphosis and hence dauerpupae treated with sufficient JH often develop into second pupae or pupal–adult intermediates.

Recently, it was discovered that corpora allata (CA) of adult male cecropia lack an active JH acid methyltransferase and *in vitro* secrete virtually no JH but instead secrete JH-I and JH-II acid (M. Peter, unpublished observation). Therefore, the earlier observations that adult male CA can activate PG and cause pupal–adult development were puzzling since JH acid was reported to be inactive when bioassayed (Slade and Zibitt, 1972) and has generally been considered to be an inactive precursor or catabolite with no known hormonal activity. To resolve this paradox, we injected various concentrations of JH-I acid into brainless, allatectomized diapausing cecropia pupae. We report here that JH-I acid does indeed terminate diapause as well as induce formation of second pupae or pupal–adult intermediates.

MATERIALS AND METHODS

Diapausing cecropia pupae were purchased from Lepidoptera Company, Chicago, Illinois. Brain and corpora allata–corpora cardiaca (CA–CC) complexes were surgically removed (Williams, 1959) from diapausing pupae, chilled at 4–6° for 2–3 months. After surgery, the pupae were kept in plastic containers in a room maintained at 27° and 70–80% relative humidity with a 16:8 light–dark cycle. None of these animals showed any signs of development at the end of 1 week and were thereafter considered dauerpupae and used for our experiments. The age of the pupae at the time of experimentation varied from 7 to 90 days after brain–CA–CC complex removal. The state of development of the experimental animals was evaluated 35 days after treatment.

Racemic JH-I was obtained from Eco-Control, Cambridge, Massachusetts, and purified by high-resolution liquid chromatography. JH-I acid, prepared by alkaline hydrolysis of JH, was provided by Dr. K.

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Dahm. JH-I was diluted in olive oil and the more polar JH-I acid in 10% ethanolic Ringer.

RESULTS AND DISCUSSION

In view of the interesting sexual dimorphism in CA activity of adult cecropia we tested whether corpora allata from 1- to 3-day-old adult males and females differ in their ability to terminate diapause in brainless pupae. The results shown in Table 1 reveal that male CA are far more active than female CA. All animals which developed after implantation of two pairs of male CA were pupal–adult intermediates and three of the five animals with one pair of implanted male CA metamorphosed into intermediates. In contrast, of those animals which received two pairs of female CA only one initiated development and it metamorphosed into a normal adult. Such differences in prothoracicotropic and morphogenetic activities between male and female CA were not reported earlier either for cecropia (Williams, 1959) or for *Philosamia* (Ichikawa and Nishiitsutsuji-Uwo, 1959).

Next we injected various dosages of JH-I and JH-I acid ranging from 1.0 to 100.0 μg/pupa. The results showed that in general JH-I acid was almost as active as JH-I in terminating diapause and many of the recipients developed into second pupae or pupal–adult intermediates (Table 2). At lower concentrations (1.0–25 μg/pupa) JH-I seemed to have more activity but we are not certain whether this is a genuine difference or simply due to factors associated with the solvent's dispersal in the animal or to the greater metabolic instability of JH-I acid. The response to JH-I acid could not be due to the presence of JH-I as a contaminant, because if such contamination existed it was less than 1% (Dr. K. Dahm, personal communication). Nearly all the animals which received 100.0 μg/pupa of either JH-I or JH-I acid broke diapause and a number of them developed into second pupae. All the control pupae remained in diapause and continued to remain so even after 6 months.

It is unlikely that JH-I acid directly stimulated epidermal cells to develop in these dauerpupae because injection of 100 μg of the acid into isolated abdomens ($N = 5$) produced no response. Isolated abdomens which received adult male CA or JH-I failed to develop, but injection of 10.0 μg of β-ecdysone promoted adult development. These results clearly indicate that JH-I acid (and JH-I) activated the PG in the brainless, allatectomized pupae. However, it should be noted that the doses of JH-I and JH-I acid used in the present study are likely to be above physiological levels and evidence

TABLE 1
ACTIVATION OF DEVELOPMENT IN BRAINLESS, ALLATECTOMIZED[a] DIAPAUSING CECROPIA PUPAE BY IMPLANTATION OF CORPORA ALLATA (CA)

Implant (pair per host)	No. hosts[b]	No. developed	Resulting developmental response			
			No. adults	No. pupal–adult intermediates	No. 2nd pupae	Average score[c]
2 adult male CA	20	10	0	10	0	3.0
1 adult male CA	9	5	2	3	0	2.0
2 adult female CA	6	1	1	0	0	0.0
Sham implantation	47[d]	0	0	0	0	—

[a] Surgical procedures led also to removal of the corpora cardiaca.

[b] Included males and females.

[c] Morphogenetic effects scored according to Williams (1961) on a scale of 0–5, with 0 being a normal adult and 5 a second pupa.

[d] No sign of development was observed even after 6 months.

TABLE 2

EFFECTS OF JUVENILE HORMONE-I AND JUVENILE HORMONE-I ACID ON TERMINATION OF DIAPAUSE AND ON ADULT DEVELOPMENT IN BRAINLESS, ALLATECTOMIZED[a] DIAPAUSING CECROPIA PUPAE

Treatment (μg/pupa)	No. hosts[b]	No. developed	Resulting developmental response			
			No. adults	No. pupal–adult intermediates	No. 2nd pupae	Average score[c]
JH-I 1.0	10	2	2	0	0	0.0
JH-I 10.0	12	3	0	3	0	3.0
JH-I 25.0	20	8	1	7	0	3.1
JH-I 50.0	48	24	0	19	5	4.3
JH-I 100.0	9	8	0	6	2	4.1
JH-I acid 1.0	13	2	1	1	0	1.5
JH-I acid 10.0	16	3	1	2	0	1.7
JH-I acid 25.0	20	3	0	3	0	3.0
JH-I acid 50.0	39	22	3	16	3	3.4
JH-I acid 100.0	9	7	0	3	4	4.6
Olive oil 100 μl	15[d]	0	—	—	—	—
10% ethanolic Ringer 100 μl	15[d]	0	—	—	—	—
No injection	61[d]	0	—	—	—	—

[a] Surgical procedures led also to removal of the corpora cardiaca.

[b] Included males and females.

[c] Morphogenetic effects scored according to Williams (1961) on a scale of 0–5, with 0 being a normal adult and 5 a second pupa.

[d] No sign of development was observed even after 6 months.

is lacking that the CA play any role in activation of the PG during the early period of adult development.

Does JH-I acid directly activate PG or is it first converted to JH-I? Since the CA were also removed from the pupae, the conversion would have had to occur either in the PG itself or in some other tissue and then be transported to the PG. At present we have no evidence to indicate whether such a conversion to JH-I is necessary. The most efficient conversion in whole animals observed so far has been in adult male moths which methylate approximately 6% of the injected JH-I acid to JH-I (Metzler *et al.*, 1972). Adult male accessory sex glands (ASG) convert less than 6% of JH-I acid to JH-I *in vivo* as well as *in vitro* (Shirk, 1978). Therefore, the possibility that some tissues in the pupa first methylate the JH-I acid and release JH-I into the hemolymph to activate the PG seems unlikely due to the almost identical character of the dose–response patterns for JH-I and JH-I acid. In addition, both the lack of sex specificity in the response to JH-I acid and that the male ASG are incapable of methylating JH acids until much later in development (Weirich and Culver, 1979) rule out the ASG as a source of JH. Pupal epidermal cells are, however, capable of methylating JH-I acid in small quantities (<0.01% of that in the medium *in vitro;* Shirk and Bhaskaran, unpublished observation). This ability may be shared by other tissues *in vivo,* including the PG. Hence, we cannot, at present, disregard the alternative that conversion of the acid to the hormone in the PG itself is the primary event in activation of ecdysone secretion by the PG.

The possibility that JH-I is first converted to JH-I acid and the latter then activates PG also merits consideration. Injection of JH into diapausing pupae of *Hyalophora gloveri* induces rapid synthesis and release of esterases, capable of hydrolyzing JH by the fat body (Whitmore *et al.*, 1972). Other tissues such as epidermis

have also been shown to have similar esterase activity (Slade and Zibitt, 1972) and the PG itself may be capable of converting JH-I to JH-I acid. The injected JH-I would thus provide a steady source of JH-I acid.

The conspicuous juvenilizing effect of JH-I acid is also worth noting. At the higher dosages a number of animals formed almost perfect second pupae. Once again, we are not certain whether JH-I acid itself causes juvenilization or is converted to JH-I by peripheral tissues such as the epidermis. In both the *Galleria* wax test (de Wilde *et al.*, 1968) and the *Manduca* black mutant larval assay (Fain and Riddiford, 1975) we found that JH-I acid is at least 2 orders of magnitude lower in activity than JH-I but 2–15 times more active than JH-III (unpublished observations).

Until now, JH acid has been considered an inactive metabolite; however, our findings suggest that it has both prothoracicotropic and morphogenetic properties in brainless, allatectomized cecropia pupae, contrary to its assumed hormonal inactivity. Therefore, we question whether JH acid may play a role in activation of the PG, particularly in the cyclic-AMP-independent activation of PG (Vedeckis *et al.*, 1976) during late larval life of Lepidoptera. The peak of JH esterase activity in larval hemolymph at the end of the feeding period (Vince and Gilbert, 1977) could cause an increase in hemolymph JH acid at this time. The presence of JH acid may also ensure that imaginal tissues will not skip the pupal phase and differentiate prematurely into adult structures (Kiguchi and Riddiford, 1978). We are presently investigating the physiological significance of our findings by studying the events triggered by JH-I acid which lead to activation of the PG and to morphogenetic effects.

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