

Effect of two ecdysteroids on the isolated abdomen of the diapausing giant silk worm, Samia cynthia pryeri

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(Received July 20, 1991)

Abstract

Effects of two ecdysteroids, α -ecdysone and 20-hydroxyecdysone (β -ecdysone), on the isolated abdomen from diapausing giant silk worm, *Samia cynthia pryeri* were investigated. α -ecdysone did not induce apolysis of cuticle of the isolated abdomen, while 20-hydroxyecdysone induced apolysis. On the fat body transplanted isolated abdomen, α -ecdysone induced apolysis as well as β -ecdysone. These results indicate that a real active substance of the target organs is 20-hydroxyecdysone in living insects, and α -ecdysone secreted from prothoracic glands changes into 20-hydroxyecdysone in some of the internal organs involved fat body previous to the activation of the target organs.

Introduction

It has been known that the molting, metamorphosis and the activation of the insect are controlled by the activity of the prothoracic glands that synthesize and secrete the prothoracic gland hormone (FUKUDA, S. '44, WILLIAMS, C. M. '46, '47, '52).

Prothoracic gland hormone was isolated from male pupae of the silk-worm, *Bombyx mori*, as a crystal and it was designated ecdysone (BUTNANDT, A. and KARSON, P. '54). Furthermore, its chemical structure became clear by HUEBER, R. and HOPPE, W. ('65). Another activation substance, 20-hydroxyecdysone, for the molting and metamorphosis or activation of diapause of insect was isolated from silk-worm (HOFFMEISTER H. and GRUETZMACHER, H. F. '66).

Although these two ecdysteroids, α -ecdysone and 20-hydroxyecdysone, both revealed strong activity with various bioassay systems, the one that is released from the prothoracic glands of silk-worm is not 20-hydroxyecdysone but α -ecdysone (CHINO, H. et al. '74). Which ecdysteroid is a real active agent for the target organs of insect? There is a number of report on this problem. In many of them, it is considered

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that the ecdysteroid that activates directly the target organs is 20-hydroxyecdysone (GILBERT, L. I. and KING, D. S. '73, GIBERT, L. I. et al. '77, '80a, SRIDHARA, S. et al. '78, KOOLMAN, J. '82, '90, KARLSON, P. '83). α -ecdysone released from prothoracic glands changes to 20-hydroxyecdysone in an adipose tissue and/or Malpighian tubules at first then 20-hydroxyecdysone activates the target organs through the formation of the combination with the acceptor sites of the target organs. This view, however, not always seems to base on the direct evidences. The direct evidence that α -ecdysone does not activate the target organs seems to be lacking.

In this study, the direct effect of α -ecdysone and 20-hydroxyecdysone on the epithelial tissue of the isolated abdomen from which all the internal organs is removed prepared from the diapausing giant silk-worm, *Samia cynthia pryeri* was investigated.

Material and Methods

Those employed for this study were the diapausing pupae of the giant silk-worm, *Samia cynthia pryeri*.

Only normal pupae were used in this study. The pupae were obtained from the garden belong to the laboratory. They were fed with *Ailanthus altissima*. The pupae were stored in an incubator kept 26°C dark condition. In this condition the pupae were kept diapausing.

To prepare the isolated abdomen, following procedure was employed. i.e. a pupa was cut between the second abdominal segment and the third abdominal segment. Then the internal organs, intestine and fat bodies etc., were removed carefully with a pincette, then the isolated abdomen was sealed on a slide glass after washing interior with insect physiological saline. Next, the apex of the tail was cut to make a pore about 2mm diameter. Through this pore the abdomen was filled by various test media or transplanted various tissues. Finally, the preparation was finished with a paraffin sealing of the tail pore.

The results of following experiments were observed.

Isolated abdomens were filled with the α -ecdysone containing medium.

Isolated abdomens were filled with the 20-hydroxyecdysone containing medium.

Fat tissue transplanted abdomens were filled with the α -ecdysone containing medium.

Fat tissue transplanted isolated abdomens were filled with 20-hydroxyecdysone containing medium.

The concentrations of the two ecdysteroids in the medium were 5, 10, and 20 μ g/ml respectively. Used α -ecdysone and 20-hydroxyecdysone were obtained from the Sigma Chemical Company U. S. A.

The operated abdomens were incubated with 26°C in dark condition for 15 days, then they were fixed with Boin's solution for the histological examinations. The

histological examinations were carried out with the light microscopic observations of the 10 μ m thick paraffin sections stained with haematoxyline-eosine. The effect of two ecdysteroids were determined from the occurrence of apolysis in the abdominal cuticle.

Results

Histology of the isolated abdomens were present in figure 1. From these histological observations it became clear that apolysis of cuticle of the isolated abdomens was induced with both 10 μ g/ml and 20 μ g/ml 20-hydroxyecdysone containing media, while apolysis of cuticle of the isolated abdomens was not induced by α -ecdysone containing media. In the cases that the isolated abdomens were administered ecdysteroids with the fat body transplantation, apolysis was induced by the α -ecdysone containing medium as well as the 20-hydroxyecdysone containing medium.

The effects of the two ecdysteroids on the isolated abdomens are present in table 1. It is shown in this table that α -ecdysone did not induce apolysis of the cuticle in any given dose, but 20-hydroxyecdysone induced the apolysis of cuticle in some isolated abdomens in the doses over the 10 μ g/ml. In the control experiments, no apolysis

Table 1. Effect of the two ecdysteroids on the isolated abdomen of diapausing pupae (*Samia cynthia pryeri*)

substances	dose μ g/ml	Nm of pupae	apolysis		dead
			+	-	
α -ecdysone	5	10	0	10	0
	10	10	0	10	0
	20	10	0	10	0
20-hydroxyecdysone	5	10	0	10	0
	10	10	5	4	1
	20	10	6	2	2
control	0	10	0	10	0

Table 2. Effect of the ecdysteroids on the fat body transplanted isolated abdomen

substances	does μ g/ml	Nm of pupae	apolysis		dead
			+	-	
α -ecdysone	10	5	3	2	0
+ fat body	20	5	3	2	0
20-hydroxyecdysone	10	5	2	2	1
+ fat body					
control	0	5	0	3	2

occurred.

The effects of the ecdysteroids on the isolated abdomens with fat body transplantation are present in table 2. In this table, the remarkable difference from the results of table 1 is that the 10 and 20 μ g/ml doses of α -ecdysone induced apolysis of cuticle in some of the isolated abdomens as well as the 10 μ g/ml of 20-hydroxyecdysone. In the control experiments, no apolysis occurred.

Discussion

Ecdysone (α -ecdysone) and 20-hydroxyecdysone (β -ecdysone) are isolated from silk-worm, *Bombyx mori*, as substances of the prothoracic glands hormone that induce the molting, metamorphosis and activation of diapause (BUTENANDT, A. & KARLSON, P. '54 for α -ecdysone, HOFFMEISTER, H. & GRUETZMACHER, H. F. '66 for β -ecdysone). It is well known fact that these two ecdysteroids have similar physiological activity for insect.

The ecdysteroid that is synthesized by the prothoracic glands and secreted from them is, however, not the 20-hydroxyecdysone but the α -ecdysone (CHINO, H. et al. '74). In fact, since α -ecdysone itself is able to be metabolized to 20-hydroxyecdysone within the insect, it is considered that α -ecdysone secreted from the prothoracic glands changed to 20-hydroxyecdysone within the fat body etc. then this 20-hydroxyecdysone combines with its target organs and activates the target organs (GILBERT, L. I. & KING, D. S. '73, GILBERT, L. I. et al '77, '80a, SRIDHARA, S. et al. '78, KOOLMAN, J. '82, '90 KOOLMAN, Koolman J. and KARLSON, P. '83). But it seems to be found no direct evidence that α -ecdysone itself can not activates the target organs.

In this study, the results show that α -ecdysone did not induce the apolysis of the cuticle of the isolated abdomens, while 20-hydroxyecdysone induced the apolysis of the cuticle of the isolated abdomens. On the other hand, α -ecdysone induced the apolysis in the fat body transplanted abdomens as well as 20-hydroxyecdysone. These facts are well explainable as α -ecdysone which was incapable to combine to the target organs changed to 20-hydroxyecdysone within the transplanted fat body, then this 20-hydroxyecdysone in turn combined with the target organs, and finally induced the apolysis. These results seem to confirm the hypothesis that α -ecdysone secreted from the prothoracic glands is metabolized to 20-hydroxyecdysone within fat body etc., then the 20-hydroxyecdysone really activates the target organs to lead the molting or metamorphosis of the insect.

Authors express hearty appreciation to Professor Dr. Uwa and the every member of the laboratory of Developmental biology for their encouragements and helpful discussions in this study.

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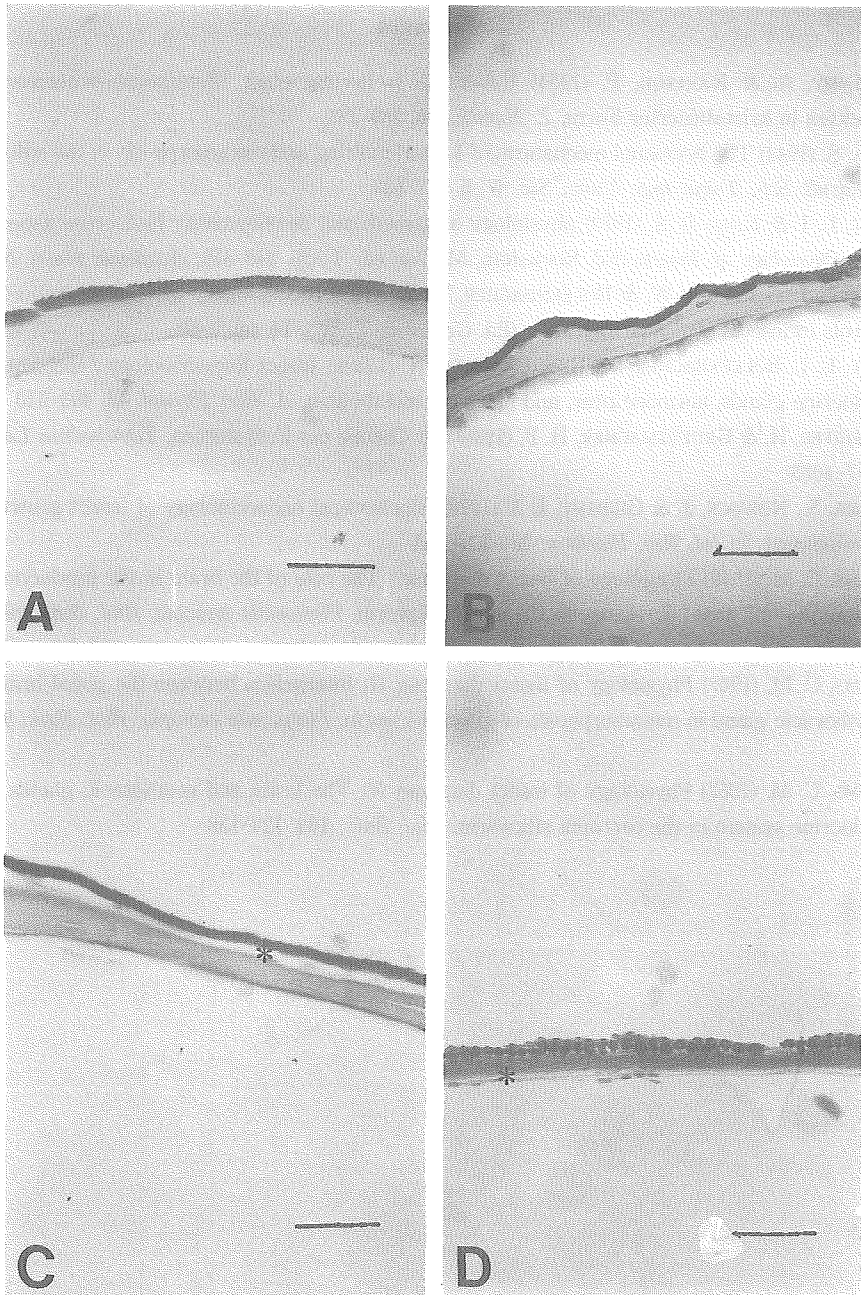


Fig. 1 Histology of the cuticle of the isolated abdomens
A : control, B : $10\mu\text{g/ml}$ α -ecdysone filled abdomen
C : $10\mu\text{g/ml}$ β -ecdysone filled abdomen, D : $10\mu\text{g/ml}$ α -ecdysone filled fat-body transplanted abdomen
* : apolysis of cuticle. A bar in the figure represents $100\mu\text{m}$.