J. FAC. SCI., SHINSHU UNIVERSITY, Vol. 22, No. 2, 1987

Effect of injection of ecdysone on development of diapausing pupae with 20°C-incubation in Samia cynthia pryeri

By AKIRA KOENUMA

Department of Biology, Faculty of Science, Shinshu University, Matsumoto, Japan

(Received Nov. 3, 1987)

Abstract

Termination of pupal diapause occurs with activation of brain-prothoracic gland system in *Samia cynthia pryeri*. It does not occur with 20°C-incubation but occurs with 26°C-incubation. To investigate the cause of failure of development in these pupae with 20°C-incubation, a comparison of the histological change of the lateral B-type neurosecretory cells in pars intercerebralis during 20°C-incubation with that during 26°C-incubation and effect of injection of ecdysone on development of the pupae with 20°C-incubation were observed. Histologically, phloxinophil granules of the lateral B-type neurosecretory cells began to decrease at second day of 20°C-incubation as well as those with 26°C-incubation. From this result, it seems to be suggested that activation of release of prothoracicotropic hormone of brain with 20°C-incubation occurs as well as that with 26°C-incubation. Diapausing pupae developed to imagines with injection of both α-ecdysone and β-ecdysone. From present study, it is probable that failure of development of the pupae to imagines with 20°C-incubation is not resulted from failure of activation of the pupal brain with 20°C-incubation but it is resulted from inoccurrence of secretion of prothoracic glands with 20°C-incubation.

Introduction

Endocrinological mechanism which controlled pupal diapause of cecropia silkworm, *Hyalophora cecropia* has been studied by WILLIAMS, C.M. ('46, '47, '52). He presented the system that pupal diapause of cecropia silkworm was induced by decrease of hormonal activities of "brain-prothoracic gland system" and it was terminated by increase of the hormonal activities of the system.

In another wild silkworm, *Samia cynthia pryeri*, its pupal diapause was controlled by a similar "brain-prothoracic gland system" (KOENUMA, A. '85).

In both cases, development of pupae to imagines was induced directly by ecdysone which were secreted from prothoracic glands, and the prothoracic gland of diapausing pupa itself was stimulated by prothoracicotropic hormone, PTTH, secreted from brain. In regard to the source of PTTH, ISHIZAKI, H. presented four paired of mid-dorsal neurosecretory cells in *Bombyx* brain ('86). This result resembles with AGUI's result in tobacco hornworm, *Manduca sexta*. But in the tobacco hornworm the source of the PTTH was lateral neurosecretory cells (AGUI, N. *et al.* '79). In the pupa of *Samia cynthia pryeri*, the lateral B-type neurosecretory cells in pars intercerebralis were guessed as the source of PTTH because they changed the feature of their cellular components with the change of the ability to terminate diapause of the recipient pupae by transplantation (KOENUMA, A. '85).

When the diapausing pupae of *Samia cynthia pryeri* have been exposed to a low temperature, below 5°C, for a long period, over about 90 days, then they are transferred to an incubator keeping to above 26°C, they can develop to imagines in an laboratory. But they can not develop to imagines in an incubator keeping to 20°C. However, when the pupae being incubated with 20°C for 15 days are transferred to an incubator keeping to 26°C, all of them develop to imagines (KOENUMA, A. '85). In regard to the cause of the difference of development between 26°C-incubation and 20°C-incubation, two alternative working hypotheses have been proposed. They are assumed as no activation of brain occurs with 20°C-incubation on the one hand, and as no activation of prothoracic gland occurs but the activation of brain occurs with 20°C-incubation on the other hand (KOENUMA, A. '85).

In this study, a comparison of the results of histological observations of lateral B-type neurosecretory cells between 20°C-incubation and 26°C-incubation, and the injection experiments of ecdysone during 20°C-incubation were carried out to verify the above hypotheses.

Material and Methods

Pupae used in this study were those of *Samia cynthia pryeri*. They were kept in a refrigerator with 5°C for 90 days and over before beginning of incubation.

Following experiments were carried out.

(1) *Comparison of histological change of lateral B-type neurosecretory cells in pars intercerebralis of pupal brain between* 20°C-*incubation and* 26°C-*incubation*

Histological change of lateral B-type neurosecretory cells in pars intercerebralis of pupal brain in 20°C-incubation was compared with that of the pupa in 26°C-incubation. The cells were observed with GOMORI's chrome-haemato-

xilin-phloxin staining preparations of $10^{\mu m}$ thick paraffin sectioning samples.

(2) *Injection of ecdysone into the pupae during* 20°C-*incubation*

Diapausing pupae kept at 5°C for long period were injected with either α-ecdysone or β-ecdysone. Injection of ecdysone was carried out at fifteenth day of 20°C-incubation. And they were represented as control II.

The α-ecdysone used was made in Sigma Pharmaceutical Co. Ltd. The β-ecdysone used was made in Lohoto Pharmaceutical Co. Ltd. They were prepared for injection medium to dissolve in ethanol then to dilute to contain 10% ethanol with EPHRUSSI-BEADIE's salt solution. Their final concentration of ecdysone were 1 $^{mg}/1\ ^{ml}$ medium. Each pupa was injected with $10^{\mu l}$ of the medium into the dorsal portion of the second or third abdominal segment. In control experiment, $10^{\mu l}$ of the medium without ecdysone was injected into the pupae and then they ware incubated with 20°C. The decerebrated pupae were also injected with ecdysone. The pupae from which brain were once removed then transplanted into themselves were injected with ecdysone, and they were represented as "Sham" operations.

Effect of injection was judged according to occurrence of normal pharate imagines. The case in which the pupa developed into normal pharate imago was represented as +, and the case in which the pupa showed no change was represented as −.

Results

(1) *Comparison of histological change of the lateral B-type neurosecretory cells in pars intercerebralis of the pupal brain between* 26°C-*incubation and* 20°C-*incubation*

Histological change of the lateral B-type cells during incubation are present in figure 1. The change of the cells during 26°C-incubation is present in "A", "C" and "E" on the one hand, and on the other hand that during 20°C-incubation is present in "B", "D" and "F". "A" shows the cells before the beginning of 26°C-incubation, and "B" also shows the cells before the beginning of 20°C-incubation. In these figures, the lateral B-type neurosecretory cells are shown with arrows. It is shown that one of the cells in each figure contains a lot of phloxinophil granule.

The lateral B-type neurosecretory cells at third day of 26°C-incubation are shown with arrows in figure "C". It is shown that the decrease of granules in the cell occurs.

"D" shows the lateral B-type neurosecretory cells at second day of 20°C-incubation. It is also shown that decrease of the granules occurs.

In figure "E" a lateral B-type neurosecretory cell at 6-th day of 26°C-

incubation is shown with an arrow. Little granule is contained.

"F" shows a lateral B-type neurosecretory cell with an arrow. It is shown that little granule is contained.

When a comparison of change of the granule in the lateral B-type cells during 26°C-incubation with that of the cells during 20°C-incubation, the histological change of the granule in the B-type neurosecretory cell of both cases is quite similar.

(2) *Injection of ecdysone into the pupae during* 20°C*-incubation*

Results of injection of α-ecdysone to the pupae of *Samia cynthia pryeri* during 20°C-incubation are present in Table 1. In this table, a case in which the intact pupae were injected with saline solution without any ecdysone is represented as "Control I", and a case in which the intact pupae were injected with α-ecdysone is represented as "Control II". It is shownt hat those pupae injected with α-ecdysone during 20°C-incubation all developed into imagines.

Results of injection of β-ecdysone to the pupae of *Samia cynthia pryeri* during 20°C-incubation are presented in Table 2. A case in which the intact

Table 1 Effect of α-ecdysone injection on development of chilled pupae in 20°C-incubation (*S. cynthia pryeri*)

pupae	duration 20°C-pre-incubation	injection of α-ecdysone	No. of pupae	development +	development −	died
control I	0	−	10	0	10	0
-Br	0	+	10	10	0	0
-Br	15 days	+	10	10	0	0
Sham	0	+	10	10	0	0
Sham	15 days	+	10	9	0	1
Control II	15 days	+	10	10	0	0

Table 2 Effect of β-ecdysone injection on development of chilled pupae in 20°C-incubation (*S. cynthia pryeri*)

pupae	duration 20°C-pre-incubation	injection of β-ecdysone	No. of pupae	development +	development −	ided
control I	0	−	7	0	7	0
−Br	0	+	7	7	0	0
−Br	15 days	+	7	7	0	0
Sham	0	+	7	7	0	0
Sham	15 days	+	7	7	0	0
control II	15 days	+	7	7	0	0

pupae were injected with saline solution without ecdysone is represented as "Control I", and another case in which the intact pupae were injected with β-ecdysone is represented as "Control II" as well as in Table 1. In this table, it is also shown that those pupae injected with β-ecdysone all developed into imagines.

Discussion

When diapausing pupae of *Samia cynthia pryeri* in a low temperature, below 5°C, for long period are incubated at 26°C in a laboratory, they can develop to imagines. On the other hand, when the pupae are incubated at 20°C, they remain in diapause. In regard to the difference in fate of the pupae between 26°C-incubation and 20°C-incubation, Two alternative possibilities have been proposed: on the one hand, there is no activation of brain to secrete prothoracicotropic hormone in 20°C-incubation, and on the other hand, there is no activation of prothoracic glands to secrete ecdysone despite of the activation of brain to secrete of prothoracicotropic hormone (KOENUMA, A. '85).

When histological change of the lateral B-type neurosecretory cells in pars intercerebralis with 20°C-incubation was compared with that of the cells with 26°C incubation, there were quite similar change in course of time between both cases. It was considered that decrease of phloxinophil granules of lateral B-type neurosecretory cells of pars intercerebralis with 26°C-incubation represented secretion of "prothoracicotropic hormone" from those cells, for the decrease of those granules corresponded to the decrease of ability of the transplanted pupal brain to activate the diapausing recipient pupa in course of time (KOENUMA, A. '85). Therefore, the similar histological change in lateral B-type neurosecretory cells in 20°C-incubation suggests that secretion of "prothoracicotropic hormone" seems to occur in this condition of incubation.

When injection of α-ecdysone or β-ecdysone into the diapausing chilled pupae was carried out during 20°C-incubation, the injected pupae developed into imagines in both cases in which the pupae were injected α-ecdysone on the one case and β-ecdysone on the other case. From these results, it is probable that inoccurrence of secretion of ecdysone from prothoracic gland is responsible for failure of development of the pupae in 20°C-incubation. It is obvious that prothoracic gland of the pupae activates in 26°C-incubation because the pupae initiate development without injection of ecdysone. Therefore, it suggests that prothoracic gland does not activate at 20°C while it activates at 26°C.

It has been demonstrated *in vitro* that prothoracic gland secretes not β-ecdysone but α-ecdysone (CHINO, H. *et al.* '74). In present experiments, that

prothoracic gland remained inactive during 20°C-incubation can be suggested from the results that the pupae injected with α-ecdysone initiates adult development.

MEOLA, R. W. and ADKISSON, P. L. ('77) reported that termination of diapause of pupae of *Heliothis zea* did not occur at 21°C but occurred either at 27°C or with injection of α-ecdysone at 21°C. In this case, it means that prothoracic glands of *Heliothis* remain inactive at 21°C. In this regard, present results seem to be resemble to the results in *Heliothis*.

Author express cordial thanks to Mr. KOGURE, HIROMICHI, and Mr. SHINOHARA, YORIFUMI for their helpful assistance during this study.

References

AGUI, N., GRANGER, N. A., GILBERT, L. I. and BOLLENBACHER, W. E. (1979) Cellular location of the insect prothoracicotropic hormone : *in vitro* assay of a single neurosecretory cell. *Proc. Natl. Acad. Sci. USA.* **76**, 5694–5698

CHINO, H., SAKURAI, S., OHTAKI, T., IKEYAMA, N., MIYAZAKI, H., ISHIBASHI, M. and ABUKI, H. (1974) Biosynthesis of ecdysone by prothoracic glands *in vitro*. *Science*, Washington, 183, 529–530

ISHIZAKI, H. (1986) A PTTH pilgrimage from *Leuhdorfia* to *Bombyx*. *Zool. Sci.* **3**, 921–929

KOENUMA, A. (1985) The role of the brain upon the termination of the pupal diapause in a giant silkworm, *Samia cynthia pryeri* Butler. *J. Fac. Sci. Shinshu Univ.* **20**, 21–87

MEOLA, R. W. and ADKISSON, P. L. (1977) Release of prothoracicotropic hormone of developmental ability during diapause in bollworm *Heliothis zea*. *J. Insect Physiol.* **23**, 683–688

WILLIAMS, C. M. (1946) Physiology of insect diapause : The role of the brain in the production and termination of pupal dormancy in the giant silkworm, *Platysamia cecropia*. *Biol. Bull.* **90**, 234–243

WILLIAMS, C. M. (1947) Physiology of insect diapause II. Interaction between the pupal brain and prothoracic gland in metamorphosis of giane silkworm, *Platysamia cecropia*. *Biol. Bull.* **93**, 89–98

WILLIAMS, C. M. (1952) Physiology of insect diapause IV. The brain and prothoracic glands as an endocrine system in the cecropia silkworm. *Biol. Bull.* **103**, 120–138

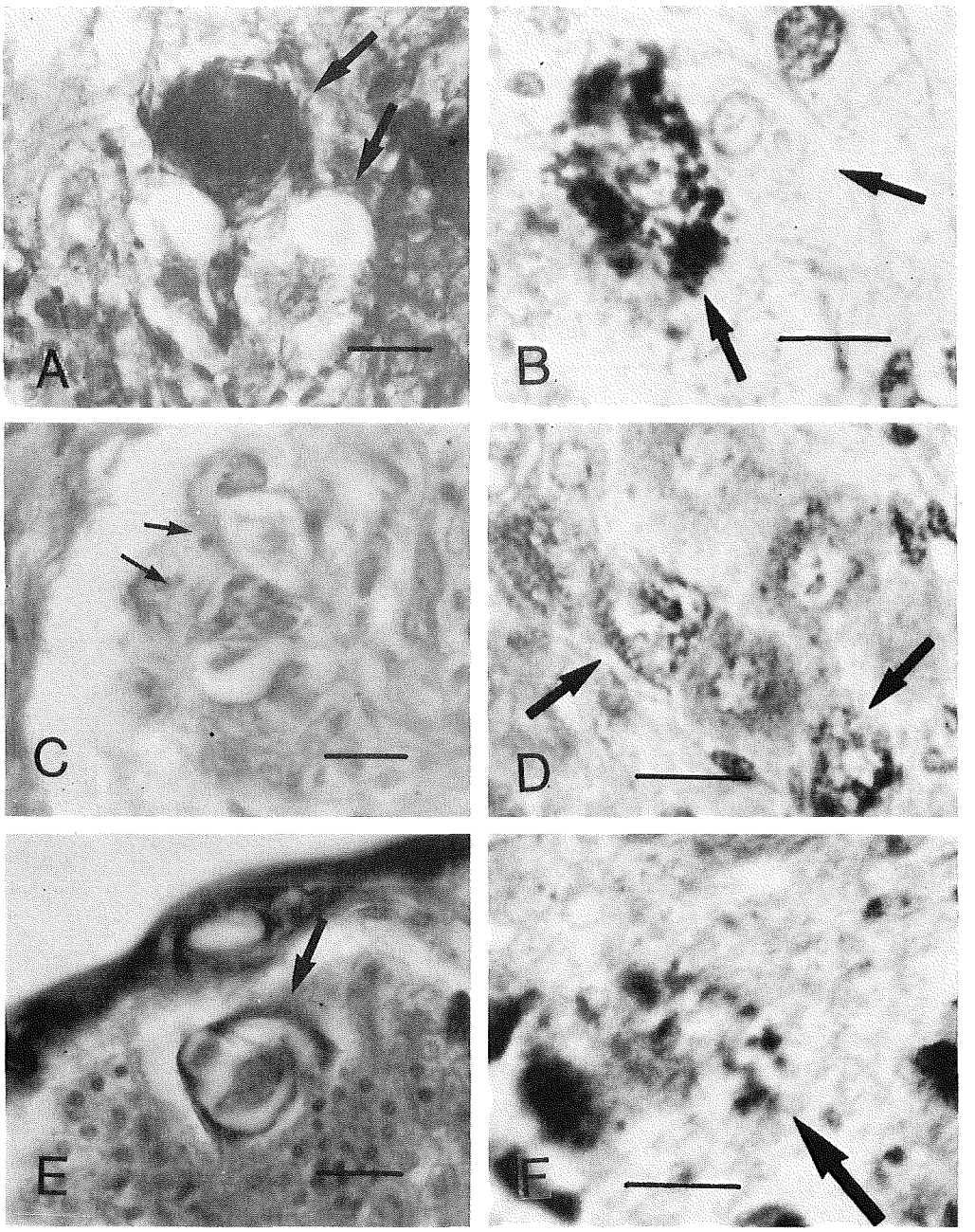

Fig. 1 lateral B-type neurosecretory cells in pars intercerebralis of pupal brain of *Samia cynthia pryeri* during incubation: bar represents 20$^{\mu m}$

A : before beginning of 26°C-incubation
B : before beginning of 20°C-incubation
C : third day of 26°C-incubation
D : second day of 20°C-incubation
E : 6-th day of 26°C-incubation
F : 5-th day of 20°C-incubation