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Incubation Temperature on the Termination of Diapause in Cynthia Pupa

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Abstract

Termination of diapause of the pupa of *Samia cynthia pryeri* is induced by activation of the brain-prothoracic gland system. When pupae kept to 5°C-storage were incubated with 26°C, they developed to imagines. When the incubation temperature was 20°C, they did not develop. But when the pupae were incubated with 20°C for 15 days then with 26°C, they were able to develop. The failure of development with 20°C-incubation was able to consider as the failure of secretion of ecdysone from the prothoracic glands, because the pupa developed with ecdysone injection. Experiments were carried out to ascertain whether or not the failure of development with the 20°C-incubation ascribed the failure of the activation of prothoracic glands. When brain was removed from the pupa after the 15-day incubation with 20°C then the pupa was incubated with 26°C, no pupa developed. When the pupae with the 20°C-incubation were stimulated by electric pulses (1V/mm, 1helz) for 3days, all survived pupae developed. These results show that the failure of development with 20°C is not ascribe the failure of activation of the prothoracic glands. It seems to ascribe the failure of PTTH secretion from the pupal brain.

Introduction

Near Matsumoto city in Nagano prefecture, pupae of one of the giant silkworm, *Samia cynthia pryeri* winter with a diapausing state. It has been known that development of these diapausing pupae is induced by activation of the brain-prothoracic gland system (WILLIAMS, C. M. '46, '47, '52). When the diapausing pupae stored for long period in a 5°C refrigerator are incubated with 20°C, they cannot develop to imagines whereas they can develop to imagines with the 26°C-incubation. In the case in which the diapausing pupae are incubated with 26°C after 15 days and over of the preceding 20°C-incubation, they develop to imagines very well (KOENUMA, A. '85). The cause of the failure of development of the diapausing pupa with the 20°C-incubation can be

regard as the failure of secretion of ecdysone, because the diapausing pupae injected with α or β ecdysone develop with any incubation temperature (KOENUMA, A. *et al.* '86, KOENUMA, A. '87).

B-type neurosecretory cells (NSCs) of the pars intercerebralis in the supraoesophagial ganglion are filled with chromophilic granules in cytoplasm during long chilling, whereas these cells contain little granule during the storage of the pupa with a warmer temperature (KOENUMA, A. '85, 85). These granules disappear at the third day during incubation (KOENUMA, A. '85, KOENUMA, A. *et al.* '86). These B-type NSCs have been considered as the source cells of PTTH, for they have a relation with the termination of diapause of the pupae that the termination of the diapause does not occur with an empty state while the termination of the diapause occurs with the disappear of the chromophilic granules during incubation (KOENUMA, A. '85). So the behavior of these B-type cells is equal during both the 26°C-incubation and the 20°C-incubation, that it is probable to consider that PTTH is equally released from these cells during both incubations. Nevertheless, the fact that the response of the prothoracic glands for secretion of ecdysone is different with the incubation temperature suggests a possility that the reactivity of the prothoracic glands for PTTH is quite lower during the 20°C-incubation than during the 26°C-incubation. Results of the experiments carried out to verify above possibility will be reported in this paper.

Material and Methods

Pupae used in these experiments were those reared in the campus of Shinshu University, Matsumoto, Nagano. The chilled pupae were those of *Samia cynthia pryeri* stored for 100 days and over in a refrigerator keeping 5°C. The nonchilled pupae were those stored for a long period since their pupation in an incubator keeping 26°C.

In one experiment the chilled pupae were divided to four groups. The pupae in the first group were extipated of their brains at the fifteenth day of the 20°C-incubation and then they were transferred to the 26°C-incubator. The pupae of the second group were also extirpated of their brains at the fifteenth day of the 20°C-incubation but they remained in the 20°C-incubator. The pupae of the third group were intact and incubated with 20°C. The pupae of the last group were also intact and they were incubated with 20°C for fifteen days and then incubated with 26°C.

In the other experiment the chilled pupae were stimulated electric pulses through a pair of Ag-AgCl electrodes inserted the pupae at the fifteenth day of the 20°C-incubation and then they were incubated with 20°C. To investi-

gate whether the prothoracic glands would be stimulated directly or not, the decerebrated pupae were stimulated by the same stimuli. The method for stimulation was as follows: One electrode was inserted at the top of the head of the pupa and the other electrode was inserted at the back of the fifth abdominal segment of the pupa. Electric pulses were supplied from an electronic stimulater made of the Nippon Kohden Co. with an electronic separator made of the Nippon Kohden Co. The stimuli were those electric pulses of which direction was antidromic, and of those strength was 1 volt per 1mm, and their frequency was 1 helz. The duration of each pulse was 1 ms. The duration of the stimulation was three days.

Results

Table 1 shows the results of the experiment in which the chilled pupae were removed their brains at the fifteenth day of the incubation with 20°C., then one group of them continued the incubation with 20°C while the other group was transferred to the 26°C-incubator.

Table 1 Effect of decerebration at the 15th day of the 20°C-pre-incubation of the chilled pupae on development (*S. cynthia pryeri*)

pupae	duration 20°C-pre-incubation	temperatue of incubation	No. of pupae	development		died
				+	−	
control I	15days	20°C	10	0	10	0
-Br	15days	20°C	7	0	7	0
-Br	15days	26°C	7	0	7	0
control II	15days	26°C	10	10	0	0

In this table the control I represents the result of the 20°C-incubation for the intact chilled pupae, and the control II represents the result of the 20°C-incubation for the intact chilled pupae followed by the 26°C-incubation. The chilled pupae removed their brains at the fifteenth day during 20°C-incubation did not develop by any incubation, 20°C or 26°C-incubation.

Table 2 shows the result of the second experiment in which the one group of the chilled pupae was stimulated by electric pulses for three days at the fifteenth day of the 20°C-incubation and it was incubated with 20°C and another group of the chilled pupae was stimulated by electric pulses for three days at the fifteenth day after the removal of their brains. In these experiments, if prothoracicotropic hormone were released during the 20°C-incubation, development of the decerebrated pupae which were carried out brain removal at the fifteenth day of the 20°C-incubation should be resulted from the 26°C-

incubation following to the 20°C-incubation. But the results were different from this expectation. Therefore, this working hypothesis that prothoracicotropic hormone is released from the pupal brain during the 20°C-incubation was not adequate.

Table 2 Effect of electric stimulation on the development to the 20°C-pre-incubated chilled pupae (*S. cynthia pryeri*)

pupae	duration 20°C-pre-incubation	temperature of incubation	No. of pupae	development +	development −	died
control I	15days	20°C	10	0	10	0
El. stim.	15days	20°C	10	6	0	4
-Br+El. st.	15days	20°C	5	0	3	2
control II	15days	26°C	10	10	0	0

In the former group six pupae out of ten developed into imagines and the rest of four pupae were killed. In the latter group, three pupae out of five survived without development and two pupae were killed..

In this table control I represents the result of 20°C-incubation of the intact chilled pupae, and control II represents the results of the 20°C-incubation of the intact chilled pupae followed by the 26°C-incubation.

Discussion

The diapausing pupae of one of the wild silkworm, *Samia cynthia pryeri*, stored with a low temperature are able to develop to imagines by the 26°C-incubation, while they continue diapause with the 20°C-incubation. In the previous papers, it was concluded that the failure of development of the diapausing chilled pupae with 20°C-incubation was not derived from the failure of activation of the PTTH-production cells of the pupal brain but the failure of secretion of ecdysone from prothoracic glands (KOENUMA, A. '86, '87). In these experiments, if prothoracicotropic hormone, PTTH, were released into the haemolymph of the pupa during the 20°C-incubation, the pupae which were incubated with 26°C with the brain removal at the fifteenth day of the 20°C-incubation should develop. But the results were different from this expectation. Therefore, above assumption became invalid.

There are observations that B-type neurosecretory cells, B-NSCs, of pars intercerebralis of this pupa show the same histological change during both the 26°C-incubation and the 20°C-incubation (KOENUMA, A. '86, '87). In the previous papers, this histological change was considered as the evidence of the release of the hormone into the haemolymph (KOENUMA, A. '86, '87).

However, it was shown from the results of these experiments that the release of the hormone into the haemolymph did not occur during the 20°C-incubation. Instead, it was suggested that the hormone still remained within the brain during the 20°C-incubation.

When the pupa was stimulated by electric pulses during the 20°C-incubation, the pupa developed with the 20°C-incubation. This fact means that the hormone, PTTH, was released from the pupal brain into the haemolymph by the electric stimulation during the 20°C-incubation.

The facts that the diapausing chilled pupa did not develop with the 20°C-incubation but it developed with the 26°C-incubation suggest that PTTH was released from the pupal brain by the sitimulation of the 26°C-incubation in the latter case.

Hitherto, on developmental initiation of the diapausing cynthia pupa, the following process is able to assume: the first step; The brain of the diapausing pupa accumulates any nerve secretory substance, n. s. s., in the neurosecretory cells, NSCs, of pars intercerebralis by a prolonged exposure to low temperature:the second step; n. s. s. moves to any releasing site of the brain with the 20°C-incubation: the third step; n. s. s. is released from the releasing site into the haemolymph with the 26°C-incubation: the fourth step; Released n. s. s. changes to PTTH, then it activates prothoracic glands: the fifth step; Ecdysone is secreted from the prothoracic glands then developmental initiation occurs.

It is not possible to know from these experiments where PTTH releasing site is. But there is a possility that corpus allatum is the releasing site. An evidence showing that corpus allatum is the releasing site of PTTH has been reported by AGUI *et al.* ('79). To clarify the PTTH releasing site of the cynthia pupa, some more detailed experiments are required.

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