JOUR. FAC. SCI., SHNSHU UNIV., vol. 8, pp. 81—90, June, 1973

The Effect of the Transplantation of the Postcritical Brain of the Pupa upon the Termination of the Pupal Diapause in Samia cynthia pryeri.

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(received April 28, 1973)

Abstract

The effect on the termination of the diapause of the recipient pupa of the transplantation of the precritical brain for activation of the developing pupa was compared with that of the postcritical brain in a cynthia silkworm, *Samia cynthia pryeri*.

There was no relation between the developmental fate of the non-chilled pupa transplanted the precritical brain and that of the donor pupa, while the termination of the diapause of the non-chilled pupa transplanted the postcritical brain took place only in the cases in which the diapause of the donor pupa was continuing. The inhibition of the termination of the diapause of the recipient chilled pupa occurred in the cases in which the pupa was transplanted the postcritical brain before the critical period for the activation of the own brain, whereas it did not occur in the cases in which the pupa was transplanted after the critical period of the own.

This suggests that the pupal brain of this insect has a something inhibitory factor for the activation of the prothoracic glands after the critical period for the activation in developing pupa.

Introduction

It has been clarified by WILLIAMS,[7~10] and by FUKUDA[1] in two closely related lepidopterous insects, *Hyalophora cecropia* (*Platysamia cecropia*) and *Samia cynthia pryeri* (*Philosamia cynthia cynthia*) respectively, that the onset and the termination of the pupal diapause depend closely on the endocrinological activity of their brain-prothoracic glands system.

There are several reports on the critical period for the activation of the brain of the diapausing pupa to terminate the diapause (WILLIAMS, C. M.,[7~10] VAN DER

KLOOT, W. G.,[6] KOENUMA, A.[3,4]). It has also been reported that no development occurred in the diapausing non-chilled pupa which was transplanted the brain isolated from the incubating chilled pupa after the critical period, whereas the development occurred in the pupa which was transplanted the brain isolated from the incubating chilled pupa before the critical period (KOENUMA, A.[3]).

VAN DER KLOOT[6] supposed of the intercerebral neurosecretory cells as the source of the brain hormone for the activation of the prothoracic glands, but he did not observe directly the change of the neurosecretory cells in correlate with the diapause break. TAKEDA[5] reported that the disappearance of the granules in the "B" cells of the intercerebral neurosecretory cells occurred correspond to the diapause break in the prepupa of a lepidopterous insect, *Monema flavescens*. He, however, did not describe the change with the critical period for the effect of the removal or transplantation of the brain on the diapause break. The attempt to find out the change in the intercerebral neurosecretory cells which would occur in relation with the critical period of the brain for the diapause break in cynthia pupa has not been succeeded in obtainning the satisfactory result at present. Therefore, it can not always to be excluded a possibility that an inhibitory factor will be secreted from the postcritical brain of the pupa. Present experiments are attempted to investigate the inhibitory effect of the postcritical brain upon the termination of the diapause in cynthia pupa.

Material and Methods

The pupae used in this study were those collected near Matsumoto city in the late September 1971, and stored in 26°C without any treatment. The "chilled pupae" were those kept in 5°C for over 8 weeks before the experiment, and the "non-chilled pupae" were those stored in 26°C until the experiment. On the late July of the next year, one out of the 30 pupae which were rest of the pupae used for experiments developed into an imago.

Following experiments and observations were carried out.

(1) Development of the decerebrated chilled pupae were observed, when the removal of the brain was carried out at various periods of incubation of the chilled pupae. In this case the brain-cardiaca-allata complex was removed.

(2) When a brain-cardiaca-allata complex, Br-CC-CA, which was isolated from the chilled pupa at various period of incubation was transplanted in to the dorsal portion of the fourth abdominal segment of the non-chilled pupa, development of the recipient pupa was observed.

(3) When a Br-CC-CA which was isolated from the chilled pupa at 13th day of incubation was transplanted into the dorsal portion of the fourth abdominal segment of the chilled pupa at 5th day of incubation, development of both donor

and recipient was observed.

(4) When a Br-CC-CA which was isolated from the chilled pupa at 13th day of incubation was transplanted into the dorsal portion of the fourth abdominal segment of the chilled pupa at 13th day of incubation, development of both donor and recipient was observed.

The temperature of incubation was 26°C in each case.

Results

1. *Development of the chilled pupa which was removed the brain (Br-CC-CA) at a different period of incubation at 26°C.*

Table 1 shows the devlopment of the decerebrated chilled pupae which were kept in 26°C after the removal of the brain at various periods of incubation. In this table and following tables, the degree of the development of the pupa is represented as following symbols; ⧻ as the case in which antenna and thoracic legs and the imaginal character of the newly formed skin were clearly differentiated, ⧺ as the case in which although the imaginal character of the newly formed skin was still obscure antenna and thoracic legs were formed, + as the case in which the newly formed skin was separable from the shell, —as the case in which development of the pupa did not occur.

Table 1 development of the decerebrated pupae (*Samia cynthia pryeri*)

expr.	No. of pupae	chill	period of decerebration	survival	development			
					⧻	⧺	+	—
			day	days				
A	9	+	0	45—126	0	0	0	9
B	10	+	5	28— 65	0	0	0	10
C	18	+	6— 8	30— 90	0	8	1	9
D	14	+	9—12	41—124	0	8	3	3
E	15	+	13—15	27—115	0	4	4	7
F	5	+	....	45—120	4	0	0	1

Pupae of the F group were not decerebrated and they were incubated intactly.

As it was clearly shown in this table, no development occurred in the pupae which were removed the brain within five days from the beginning of the incubation in spite of the prolonged exposure to the low temperatures. In the chilled pupae without the removal of the brain, four pupae out of five developed into imagin during 45 and 58 days of incubation, while one did not develop even after 120 days. In the pupae which were removed the brain between 6 and 15 days after the beginning of the incubation, over the half of them developed in some degree, but in this group no one finished the metamorphosis. In these cases there seemed to be a large individual difference in development. This result seems to suggest that on 5th day of the incubation at 26°C the brain of the chilled pupa still secreted the hormone too little to activate the prothoracic glands. The critical period of the secretion of the brain of the chilled pupa for the sufficient activation of the prothoracic glands seems to be between 6 and 15 days after the beginning of the incubation at 26°C, and there seems to be a large individual difference.

2. *Development of the non-chilled pupa which was transplanted the brain isolated from the chilled pupa at a different period of the incubation in 26°C.*

In table 2, there is a result of the experiment in which non-chilled pupa was transplanted a brain isolated from the chilled pupa at a different period of the incubation at 26°C. In the non-chilled pupa implanted the brain isolated before the incubation, one out of 7 pupae developed into a complet imago, and three pupae developed in some degree. In the case in which the non-chilled pupa was implanted the brain of the chilled pupa isolated at 5th day of the incubation, one pupa out of 18 pupae developed into a complet imago and 14 pupae developed in some degree. When the non-chilled pupa was transplanted the brain of the chilled pupa isolated on 5th to 8th day of incubation, two out of 8 pupae developed into imagin, and four pupae initiated the adult development. When the non-chilled pupa was transplanted the brain isolated from the chilled pupa on 9th to 12th day of the incubation, 8 pupae out of 10 pupae did not develop, although two pupae developed into imagin.

Table 2 the effect of the transplantation of the brain of the chilled pupa on the development of the non-chilled diapausing pupa

expr.	No. of pupae	period of isolation of donor brain	survival	development			
				⧻	++	+	−
		day	days				
G	7	0	35−125	1	1	2	3
H	18	5	23− 78	1	7	7	3
I	8	6− 8	45−128	2	3	1	2
J	10	9−12	54−134	2	0	0	8

Since the critical period for the activation of the prothoracic glands by the secretion of the pupal brain has been largely different with individual, comparison in development was carried out between the donor and the recipient. Table 3 showed the results of such observations. The donor which was removed the brain before 5th day of the incubation always continued the diapause, and in these cases the results are not present in this table. From this table it was evident that

Table 3 development of the donor chilled pupa and of the recipient non-chilled pupa in the transplantation of the brain

donor				recipient		
pupa	period of decerebration	survival	develop	pupa	survival	develop
	day	days			days	
C 1	6	90	−	I 1	84	++
C 2	6	90	−	I 2	56	+++
C 3	6	66	−	I 3	80	++
C 5	8	80	−	I 5	80	+
C 6	8	30	−	I 6	30	++
C 8	8	42	−	I 8	45	+++
D 2	9	70	−	J 2	54	+++
D 9	12	122	−	J 9	60	+++
C 4	8	66	+	I 4	84	−
C 7	8	42	++	I 7	128	−
D 1	9	41	++	J 1	60	−
D 3	9	60	+	J 3	124	−
D 4	10	124	+	J 4	60	−
D 5	11	70	++	J 5	70	−
D 6	11	56	++	J 6	56	−
D 7	11	56	++	J 7	56	−
D 8	12	122	++	J 8	123	−
D10	12	122	+	J 10	134	−

Four of the recipient pupae developed into the complete imagin.

development of the recipient pupa occurred when development of the donor pupa did not, while development of the recipient pupa did not occur when development of the donor occurred. These tendencies suggest that the brain of the chilled pupa before the critical period is competent for the diapause break of the recipient non-chilled pupa, and the brain after the critical period becomes incompetent for the diapause break rapidly.

3. *Develoment of the donor and recipient pupae in the brain transplantation experiments in which the brain of the chilled pupae of 13th day of the incubation was transplanted into the chilled pupa at a different period of the incubation.*

Development of the donor and recipient pupae in the brain transplantation experiments in which the brain of the chilled pupa of 13th day of the incubation was transplanted into the chilled pupa on either 5th or 13th day of the incubation is present in table 4. In the development of the chilled pupae extirpated of their brain on 13th day of the incubation, three out of 13 pupae developed to form the antennae and thoracic legs, four pupae developed slightly, while the rest six pupae did not develop during the incubation for 27 to 115 days. When the chilled pupae were implanted the brain of the chilled pupa of 13th day of the incubation,

Table 4 development of the chilled pupae which were removed or transplanted the pupal brain during incubation

expr.	No. of pupae	chill.	−Br	+Br	surviv.	development			
						+++	++	+	−
			day	day	days				
K	13	+	13	···	27−115	0	3	4	6
L	7	+	···	5	41− 75	0	0	1	6
M	6	+	···	13	41− 87	3	2	1	1

Three pupae of the M group developed into the complete imagin.

Table 5 development of the donor pupae and of the recipient pupae in the brain transplantation experiments in which the brain of the chilled pupa at 13 day of incubation was transplated either to the chilled pupa at 5 day of incubation or to the chilled one at 13 day of incubation

donor				recipient			
pupa	−Br	surviv.	develop	pupa	+Br	surviv.	develop
	day	days			day	days	
K 4	13	87	−	L 4	5	75	+
K 1	13	81	−	L 1	5	67	−
K 2	13	87	++	L 2	5	75	−
K 3	13	87	++	L 3	5	75	−
K 5	13	87	+	L 5	5	75	−
K 6	13	41	−	L 6	5	41	−
K 7	13	27	+	L 7	5	41	−
K 8	13	87	++	M 1	13	49	+++
K 9	13	115	−	M 2	13	51	+++
K10	13	87	−	M 3	13	51	+++
K11	13	96	−	M 4	13	87	++
K12	13	41	+	M 5	13	48	+
K13	13	41	+	M 6	13	41	−

Three of the recipient pupae developed into the complete imagin.

an inhibitory effect against the development of the host seemed to be produced. In the development of the chilled pupa implanted the brain of the chilled pupa of 13th day of the incubation on 13th day of the incubation of the host, a somewhat acceleratory effect for the development seemed to be produced. These results suggest that the postcritical brain of the chilled pupa has a tendency to inhibit the development of the chilled pupa which is earlier than the activation of its own brain, and such inhibitory effect is incompetent to the development of the chilled pupa which is later than the activation of its own brain.

In table 5, there is a relation of the development between the donor and the recipient in each transplantation experiment. No development occurred in most recipient pupae except one when each of them was transplanted the brain of the donor which was incubated for 13 days on the 13th day of the incubation of the recipient. In this exceptional case in which the pupa developed slightly, the transplanted brain will be still inactive even at 13th day of the incubation, since the development of the donor pupa did not occur. When the chilled pupa which was incubated for 13 days was transplanted the brain of the chilled pupa which was incubated for 13 days, development of the recipient occurred regardless with the development of the donor. In this case, no tendency of the inhibitory effect against the development of the recipient was observed.

Discussion

WILLIAMS[7~10] discovered that the onset and termination of the pupal diapause in the cecropia silkworm, *Hyalophora cecropia*, were under control of a series of the endocrinological events of the brain and the prothoracic glands of the pupa. In these endocrinological events, the onset of diapause occurred in correlate with the decrease of the secretory activity of the prothoracic glands, which was resulted from the failure of the brain in secreting a hormone, and the termination of diapause occurred in correlate with the increase of the secretory activity of the prothoracic glands, which was resulted from the release of the brain hormone.

On the onset and termination of the pupal diapause in another lepidopterous insect, cynthia silkworm, *Samia cynthia pryeri*, which related closely to cecropia silkworm, a quite similar mechanism was evidently proved by FUKUDA.[1]

The prolonged exposure of the diapausing pupa to the low temperatures before the incubation was commonly required for the termination of the diapause in both species. When the chilled pupa was incubated, there has been the critical period in which the brain was indispensable to the diapause break. (WILLIAMS[7~10], VAN DER KLOOT[6], KOENUMA[3,4]) The results of these experiments in table 1 seem to show that the critical period of the brain for the diapause break in cynthia pupa is essentially consistent with those reported previously.

According to WILLIAMS[7~10], the diapausing non-chilled pupa which was implanted the brain obtained from the developing chilled pupa earlier than the critical period initiated the adult development, while the one implanted the brain obtained the developing pupa later than the critical period did not initiate the adult development. Similar phenomena were obtained from cynthia pupa. (KOENUMA[3]) As it is evident in the tables 2 and 3, results of these experiments is consistent fundamentally with those obtained previously.

WILLIAMS[9] regarded the difference of the effect of the pupal brain on the diapause break between the precritical period and the postcritical period as the difference of the endocrinological nature of the brain, for the brain initiated the secretion of the brain hormone shortly before the critical period and then it failed rapidly the secretory activity after the critical period. According to VAN DER KLOOT,[6] the endocrinological activity of the brain of cecropia silkworm derives from the activity of the 26 neuro secretory cells in the pars intercerebralis. He discovered that the activation in the spontaneous electrical activity of the pupal brain occurred shortly before the critical period. Since he has not describe the evidence that the decrease of the electrical activity of the brain occurred after the critical period, the activation in the spontaneous electrical activity of the brain does not always seem to represent the activation in secreting activity of the brain. In cynthia pupa, the spontaneous electrical activity of the brain increased remarkablly prior to the initiation of the adult development. In addition to this, the diapause break took place in the nonchilled pupa implanted the brain obtained from the developing pupa before the spontaneous electrical activity of the brain increased, while the diapause break did not always take place in the pupa implanted the brain obtained from the pupa after the spontaneous electrical activity increased. On the latter case, when the transplanted brain was not still endocrinologically inactive, the brain was competent to initiate the adult development of the host. (KOENUMA[3,4]) In these cases, they could not discriminate from the difference in the spontaneous electrical activity. If the change of the spontaneous electrical activity of the pupal brain is correlated with the change of the endocrinological activity of the brain, it will not always to be attributable only to the failure of secreting activity in the pupal brain that the adult development of the implanted pupa does not occur.

According to HERMAN and GILBERT,[2] there are two types of neurosecretory cells in the pars intercerebralis of the brain of the cecropia silkworm. In this regard it is not always exclude the possibility that they will bear the different endocrinological function respectively. On the morphological change of the intercerebral neurosecretory cells correlated with the diapause break, there is a TAKEDA's work[5] in a slug moth, *Monema flavescens*. In this work, however, there is no description of the correlation in the change of the neurosecretory cells with the critical period for the diapause break. Therefore, on the relation of the endocrinological activity of the brain with the activation of the prothoracic glands in the mechanism of the diapause break in both cecropia and cynthia pupa, two possible schemata will be illustrated from the various evidences.

Schema A in figure 1 is a WILLIAMS' case in which the brain will secrete the prothoracic glands stimulation factor, PSF, at a definite period of the adult development, and soon after this secretion it will cease the secretion of PSF, while the prothoracic glands will be activated by this PSF. In this case, the postcritical brain will never inhibit the development of the host when it will be transplanted into the developing chilled pupa even at any age of the developing pupa.

Schema B in figure 1 is another case in which the brain will secrete the PSF continuously after a definite period of the adult development, but it will initiate to secrete the other factor, prothoracic inhibitory factor, PIF, with somewhat delay from the initiation of the secretion of PSF. In this schema, it will be supposed that only the inactive prothoracic glands will be activated by PSF. In this case, the result of the transplantation of the postcritical brain to the developing chilled pupa will quite differ with the age of the recipient pupa. When the transplantation will be carried out before the critical period of the recipient pupa, the prothoracic glands of the recipient pupa will be affected by PIF of the transplanted brain. Therefore, the recipient will not develop. When the transplantation will be carried out after the critical period of the recipient pupa, the recipient will develop, since the prothoracic glands of the recipient will not be affected by PIF of the transplanted brain, for the prothoracic glands will be activated by own PSF previously. The results in the tables 4 and 5 showed that the donor brain which was isolated after the critical period inhibited the development of the recipient pupa when the transplantation was carried out before the critical period of the recipient, while the donor brain did not inhibit the development of the recipient when the transplantation was carried out after the critcal period of the recipient. In the latter case, the transplanted brian rather slightly accelerated the development of the recipient. Although the source of the PIF is quite unknown at present, this seems to suggest that the secretion of PSF of the brain does not cease rapidly, but the secretion of PIF of the brain initiate after the critical period. This seems also to agree with the supposition that the prothoracic glands which will be activated by PSF previously will not be affected by PIF.

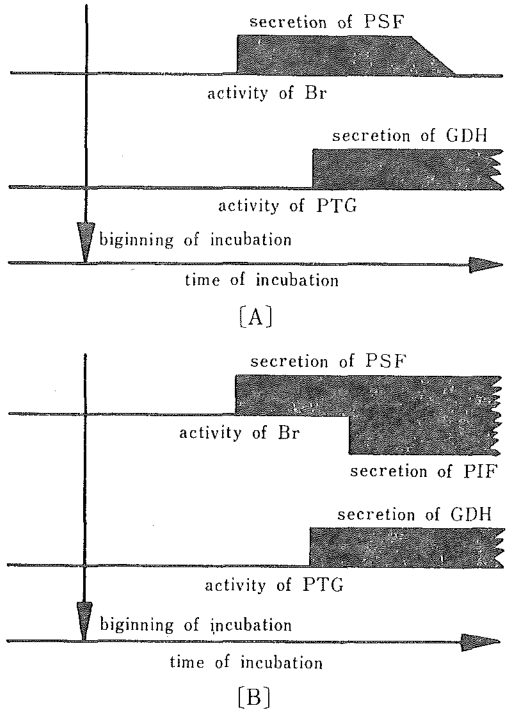

Fig. 1. two possible schemata on the relation between the endocrinological activity of brain and the activation of prothoracic glands in the mechanism of diapause break

In natural, the development will not be inhibited by own PIF, since the prothoracic glands will always be activated by own PSF secreting prior to the secretion of PIF.

The author wishes to express his thanks to Dr. S. FUKUDA for his direction and encouragement in this study. He also wishes to express his gratitude to Professor H. KURASAWA of Shinshu University for his criticism and invaluable help in preparing the manuscript.

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