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The Effect of The Neurosecretory Material in The Median Neurosecretory Cells on Egg Development of The Endoparasitic *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)

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Abstract

The purpose of this study is to examine the effects of the neurosecretory material (NSM) in median neurosecretory cells (MNC) on reproduction of endoparasitic *Pimpla turionellae* L.. For this purpose, the egg maturation and amount of the NSM in MNC of the insects were examined by the serial cross sections of the brain. Egg maturation was determined by measuring the terminal oocyte length in the ovariole. The sections of insect brains were stained by paraldehyde fuchsin to determine the amount of the NSM in the neurosecretory granules of the MNC. The amount of the NSM in MNC was at a certain level in the first day of egg development. While terminal oocyte was reaching the maximum length, amount of NSM in MNC decreased the minumum level. During the egg laying phase, the amount of the NSM in MNC reached the maximum level. These observations give us the idea that the neurosecretory material in median neurosecretory cell of this insect may be related to the egg development.

INTRODUCTION

The median neurosecretory cells of the brain were shown as the control center of reproduction on *Calliphora erythrocephala* [1], *Aedes aegypti* [2], *Musca domestica* [3], *migratoria* [4] and *Protophormia terraenovae* [5]. These cells in different orders were studied during the reproduction period of female insect [6-9]. Formerly, it was specified that the brain cells which are stained purple by paraldehyde fuchsin techniques include neurosecretory material [10]. There are many studies on these neurosecretory cells with stained purple by paraldehyde fuchsin [4,5,9,11]. These cells were searched concerning egg maturation on *Calliphora erythrocephala* [1], *Lepismodes inquilinus* [12], *Musca domestica* [6], *Labidura riparia* [8] and *Lymantria dispar* [13]. The fact that, the neurosecretory material which is produced in the median neurosecretory cells of the brain reaches to the corpora allata by passing through the corpora cardiaca via the nervi corporis cardiac is well known. In CA of some insects, the NSM which is stainable with paraldehyde fuchsin positively, was presented [14]. In insects, corpora allata are known to be

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involved in metabolic activities [15-21].

As seen above, the relationship between the neuroendocrine system of the insects belonging to different orders and the reproduction activities were studied and various results were obtained. Previously the head endocrine system of *P.turionellae* had been given histologically and morphologically [22], but the physiological relationships between the endocrine system and the reproduction activity had not been mentioned. On the insects merged to Hymenopter order, it is rare to encounter studies that show the correlation between the brain neurosecretory cells and the reproduction. Especially, there is no study about the reproduction period which is related to the correlation between the neurosecretory material in median neurosecretory cells and the egg development of endoparasitic *P. turionellae.*

This study represents that the role of the neurosecretory material of the median neurosecretory cells in the ovarian development of the endoparasitic *P. turionella* was observed. Thus, it was aimed to enlighten the relationship between the neurosecretory material in median neuro-secretory cells and reproduction in *P. turionellae.*

MATERIALS AND METHODS

Experimental Animals:

P. turionellae was reared at the Experimental Animals Laboratuary of Ankara University Biology Department between 2003-2005 years. The female experimental animals were obtained from the stock culture. The continuity of the stock culture was supplied from the greater wax moth, *Galleria mellonellae,* reared in the semi-synthetic diet (Bronskill, 1961). All *P. turionellae* females were kept at temperature of 25 ± 2°C with relative humidity of 75 ± 5 percent in a 12 : 12 (L : D) photoperiod. They were fed with cotton pieces absorbed with 50% honey solution and a pupa of *G. mellonellae* was given to each insect every two days in order to satisfy their host haemolymph needs. All the experimental virgin females were reared without males to eliminate possible effects of mating [23]. They were selected on the 0, 3, 6, 9, 12, 15, 18, 21, 24 and 27th days, after adult emergence. All experimental females were selected in the same body size to eliminate the difference in terminal oocyte length [24].

Staining of the Neurosecretory Cells and Ovaries

All females were decapitated at the same time (14.^{00}-17.^{00}) to eliminate the possible diurnal changes in the neurosecretory system [25,26]. The dissected brain of *P. turionellae* was fixed in aqueous Bouin's solution for 12-18 hours, sectioned serially at 7 mμ thickness after being embedded in paraffin. These sections of the brain were stained with paraldehyde fuchsin after potassium per-manganate oxidation [27]. The rest of the body of the decapitated animals was dropped into isotonic insect saline and was left for no longer than 3 hrs. The ovaries were dissected out, for microscopic calculation, under a binocular microscope and were fixed in Bouin's fixative (4 hrs). Dissected ovaries were sectioned serially at 10 mμ. The sections were stained with Ehrlich's haemotoxylin and eosin.

Measurements:

In order to point out the changes in the intensity of NSM on the experiment days, the paraldehyde fuchsin positive granules in MNC were counted. The NSM in the cell was numbered 0 to 5 increasingly according to its staining intensity [3]. An average value for each day investigated was obtained by dividing the sum of the staining intensity of each cell into the number of cell of each the day. These calculations were done for each insect separately.

The lengths of the terminal oocyte were measured with an ocular micrometer. The developmental stages of the oocyte growth were recorded as the length of the biggest terminal oocyte in ovariole [28]. All measurements were made in the largest area in the cross sections of the terminal oocytes. Only one biggest terminal oocyte length from each of at least 10 animals in each studied day was measured and their averages were recorded. The measurements in the terminal oocytes were done by Fisher's (1948) method of significance control test (P < 0.05) between two means.

RESULTS AND DISCUSSION

Terminal Oocyte Growth:

The larger oocytes in any ovariole represent a longitudinally arranged series in which the terminal oocyte nearest the base of the ovariole is the oldest and largest oocyte representing successively earlier stages in the oocyte maturation [28]. In all *P. turionellae* females, there is no differentiation in the ovariole on the day of emergence (Table 1). The terminal oocytes continue to increase in length between the 3rd day and 15th day. Between the 12th day and 15th day, the length of the terminal oocyte reaches to maximum level which is called egg maturation stage. Between the days 15th and 24th, the length of the terminal oocyte decreases to minimum. These days on which the terminal oocyte length decreased to minimum are called the egg laying stage.

The Neurosecretory Materials in Median Neurosecretory Cells

The NSM intensity in median neurosecretory cells had different levels during the reproductive period of the females of *P. turionellae* (Table 2). The NSM intensity in median neurosecretory cells of newly emerged insects was on a limited scale and the level was low. This intensity level slightly increased on the first days depending on the oocyte maturation that showed fluctuation on the following days. The NSM intensity which decreased to the minimum level before the first egg laying days, reached a highest intensity on the first egg laying days. After the first egg laying days, the NSM intensity

Table. 1.The terminal oocyte lengths of *P. turionellae* on different days of egg maturation.

Age at Dissection (Day)	Insect Number of Terminal Oocyte Length Measured	Terminal Oocyte[1]	
		Numbers	Length(μm) ± SD
0	15	-[2]	-[2]
3	13	13	445.38 ± 17.95[a3]
6	10	10	621.67 ± 28.16[a]
9	14	14	801.03 ± 19.63[bc]
12	14	14	935.58 ± 17.65[b]
15	10	10	1092.63 ± 23.10[b]
18	10	10	777.34 ± 39.46[a]
21	10	10	470.96 ± 40.25[ac]
24	11	11	736.39 ±31.13[a]
27	10	10	837.82 ± 33.14[bc]

[1] Only one terminal oocyte was measured in each female

[2]There is no terminal oocyte in ovariole on the day of emergence

[3]Means compared vertically. Means net followed by the same superscript are significantly different from each other at P < 0.05 by Fisher's T test.

Table 2. The intensity of the neurosecretory material in the median neurosecretory cells of *P. turionellae* on different days of egg maturation.

Age at Dissection (Day)	Insect Number	Total Number of Median Neurosecretory Cells	Intensity of Neurosecretory Material	
			Total	Average[1]
0	22	81	130	1.6
3	22	77	154	**2.0**
6	8	70	126	1.8
9	40	152	213	1.4
12	24	66	396	0.6
15	18	77	108	1.4
18	20	95	475	5.0
21	12	84	118	1.4
24	28	160	144	0.9
27	22	97	223	2.3

[1]The average intensity of the neurosecretory material was found by dividing the total intensity of neurosecretory material to the total median neurosecretory cell number.

decreased considerably. This level of the NSM intensity in median neurosecretory cells during egg maturation can be mentioned as there is a correlation between the median neurosecretory cells and egg maturation on the reproduction period in this insect.

A brain cell has been considered as neurosecretory if stained purple with paraldehyde fuchsin, and only the purple condition has been considered indicative of the presence of NSM and has been called paraldehyde fuchsin positive [10]. The fact that the median neurosecretory cells of insects include NSM was shown by the studies done by means of light and electron microscope [15,21,29,30]. In the mean time the NSM exists also in the median neurosecretory cells of female of *P. turionellae* was marked in the serial sections which were obtained from the head of the insect [22]. In many hitherto studies on insects, the neurosecretory cells have appeared to regulate oocyte growth [31,32] by activating the corpora allata [33,14,4] to produce a hormone essential for protein transfer from haemolymph into the developing oocytes for yolk deposition [34] and perhaps by controlling protein synthesis and metabolism themselves [16,35,36]. In many studies, the brain hormones have already been chosen to show their cyclic activity [13] correlated with the oocyte maturation [1,2,5,37]. In *P. turionellae* females, the day of emergence was characterized by the lack of differentiation in the ovariole. On this day, the amount of the NSM in MNC was at a certain level. During the first days of the terminal oocyte growth, a permanent increase or decrease was not observed at the amount of the NSM in MNC. After the first days of egg maturation, this level showed fluctuation. Indeed, the level which has the minimum value just before the first egg laying days (on 12th day), increased considerably on the first egg laying days (between 18th day and 21st day). This reduction which occurred just before terminal oocytes reached to the maximum length was assessed as there has been no more need to hormones since the egg maturation was completed. It has been known that the NSM in brain passes to cacorpora allata throughout corpora cardiaca. Thus, the changes in the intensity of NSM in cacorpora allata can be explained with the changes in the intensity of NSM in brain. It was specified that the NSM which is secreted from median neurosecretory cells [38], provides oocyte maturing. Adams specified with another researcher group in 1975 that there is a feedback mechanism between the brain cells of *M. domestica* and ovariums. It was also specified that the oocytes which complete the maturation [6], produce a hormone which blocks the secretion activity of brain that is called oostatic hormone. This specification of Adams can explained by the reduction of NSM in MNC of *P. turionellae* on the 12th day. The striking increase in NSM of *P. turionellae* on the first egg laying days also can be explained with the suggestion of Siew (1965) which shows that egg laying activity requires high level of hormone. When injected the brain extract which is obtained from the egg laying females of *Gryllus bimaculatus* to young mature females, it was observed that they also show egg laying activity and abdominal contractions starts [39]. Thus, it was recommended that more neurohormones are required for egg laying and this neurohormone causes abdominal contractions and egg laying movements. After the egg laying phase, the NSM in MNC of *P. turionellae* decreases seriously. The NSM intensity of *P. turionellae* was decreased after the egg laying phase. As a matter of fact the NSM intensity which has a considerably high level during the egg laying phase, decreased after the egg laying activity completed in *L.riparia* [17], in 9-24 hours after the egg laying activity in *Galeruca tanaceti* [36], and in 12-36 hours in *M. domestica* [3]. This decrease might have occurred for the same reasons as the first decrease.

The results obtained from present study shows that the NSM amount in median neurosecretory cells of *P. turionellae* changes during the reproductive activity. It is also estimated that the egg laying and the egg maturation phases of *P. turionellae* are controlled by median neurosecretory cells hormone (or hormones) could be denoted from these results.

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