Proc. Indian natn. Sci. Acad. **B50** No 3 pp 257-265 (1984)

Stimulatory Effect of Ovariectomy on Food Consumption, Digestion and on the Activity of Median Neurosecretory Cells in the Red Cotton Bug, *Dysdercus cingulatus* Fabr.

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(Received 13 March 1984)

Ovariectomy performed in adult *Dysdercus cingulatus* 24 hr after emergence resulted in hyperactivity of the neurosecretory cells of pars intercerebralis. An increased rate of food consumption in relation to neurosecretory activity was also noticed in castrated insects. Coupled with this, increase in the activity of two midgut enzymes—protease and invertase—was also noticed in the ovariectomised insects. Injection of a methanol-extract of ovaries into ovariectomised insects reduced food consumption as well as midgut enzyme activity to a significant level.

It is suggested that castration results in the removal of some ovarian factor (probably ecdysone) which inhibits the activity of neurosecretory cells of the pars intercerebralis. The hormonal principle from these cells stimulates food consumption and the increased amount of food consumed influences the enzyme activity through a secretogogue mechanism.

Key Words: **Ovariectomy,** *Dysdercus cingulatus,* **Protease, Invertase, Neurosecretory cells**

Introduction

A major portion of the digested food of female insects serves as raw material for vitellogenesis. Engelmann (1970) is of the opinion that in insects endocrine glands can be restrained from their hormone production by a depletion in the food reserves or in the absence of feeding stimuli. In certain insect species, adult ovaries are known to synthesise ecdysone (Hagedorn et al. 1979) under the influence of median neurosecretory cells (Richards 1981). In *Dysdercus cingulatus* hormonal factors from the neurosecretory cells of the pars intercerebralis (PINSC) stimulate food consumption and the proteinaceous food thus ingested stimulates digestive enzyme activity through a secretogogue mechanism (Muraleedharan & Prabhu 1978, 1979a). Absence of the same hormonal factors prevents egg maturation in the same species (Jalaja 1974). In this context, it is of

interest to elucidate whether this humoral influence on vitellogenesis is direct or through its indirect influence on food consumption and subsequent digestion. In the present study an attempt is made to investigate the effect of ovariectomy on food consumption and enzyme activity as well as on secretory activity of PINSC.

Materials and Methods

Dysdercus cingulatus was reared on soaked cotton seeds under a 12:12 light/dark photoperiod, temperature 29±3°C and r.h. 90±3 as described earlier (Muraleedharan & Prabhu 1978).

Within 2 hr of emergence, females were isolated and their ovaries extirpated after a day. Mildly-anaesthetised insects were stuck by the dorsal surface to a wax dish using plasticine ribbons. The abdominal region of the insect was fully bathed in cold sterilised insect Ringer (Ephrussi & Beadle 1936) throughout the operation. All instruments used for surgery were sterilised by boiling and kept immersed in 70% ethyl alcohol prior to the operation. A diagonal slit was made on one side of the 8th abdominal sternal region using a scalpel made from a piece of a sharp razor blade. If the slit is correctly located, part of the ovary comes out. With the aid of a pair of fine curved tweezers (Trident) all the ovarioles on both sides can be pinched off one by one at the pedicel region. A speck of anti-tanning and anti-biotic mixture (phenyl thiourea, streptomycin and penicillin in the ratio 2:1:1) was applied to the wound. The insect was freed from the plasticine cradle and allowed to recover. Controls were sham-operated retaining the ovaries. Fully recovered animals were allowed to feed and mate. Mortality was about 10% and occurred mostly on the day after the operation.

Weight of food ingested by the experimental and control insects were determined at 24 hr intervals as described earlier (Muraleedharan & Prabhu 1978). Determinations were performed on the insects during seven consecutive days after ovariectomy. If there was any mortality on any day, the values for that set were discarded.

The method followed for the preparation of enzyme extract was the same as in earlier studies (Muraleedharan & Prabhu 1978). Midgut protease activity was determined using the original method of Davis and Smith (1955) and invertase activity was estimated employing the method of Noelting and Bernfeld (1948). Both these methods with relevent modifications as reported earlier (Muraleedharan & Prabhu 1979a, b) were followed. Activity of both these enzymes was estimated in the experimental and control insects during seven consecutive days after ovariectomy.

Brains of experimental and control insects were fixed either in Bouin's fluid or in formal saline. These were either stained whole using paradehyde fuchsin (Cameron & Steelee 1959), resorcin fuchsin (Ittycheriah & Marks 1971), performic acid-victoria blue (Dogra & Tandon 1964) or paraffin sections (6μ) were stained with Gomori's Chrome alum-haematoxylin-phloxine (Gomori 1941). Calculation of the index of the neurosecretory material in PINSC were performed subjectively on a 1–3 scale as done by Jalaja and Prabhu (1977). The number of neurosecretory cells were counted and the secretory content on the subjective scale was multiplied by the number of cells. Added value of all the three products gave the index of neurosecretory material in each animal. Highnam (1962) and Engelmann (1970) are of the opinion that the secretory content of neurosecretory cells can not be taken alone as a criterion for their hyperactivity. They recommend that account should be taken of the size of the secretory cells and the nuclei in addition to the secretory content. So in the present studies activity of PINSC were judged in the

Table 1 *Effect of injection of ovarian extract on food consumption and digestive enzyme activity in the castrated females of* D. cingulatus

Time after first injection	Amount of food consumed (in mg)	Protease activity (μg tyrosine liberated/ midgut/hr)	Invertase activity (μg glucose liberated/midgut/hr)
24 hr–EXP	12.00±0.3	178±9	1080±60
24 hr–CON	15.00±0.5	200±7	1350±43
48 hr–EXP	10.50±0.8	170±10	915±50
48 hr–CON	18.50±0.6	220±8	1480±40

Each value represents mean of 8 separate determinations
Mean values of adjacent pairs of experimental and control of each column are significantly different ($P<0.01$)

light of all the three parameters. Measurements of PINSC and their nuclei were done using a calibrated ocular micrometer.

Method followed for the preparation of ovarian extract was the same as that of Imboden and Lanzrein (1982) with slight modifications. Twenty pairs of ovaries dissected out from healthy adults (4 days after adult emergence) were homogenised under cold conditions in 2 ml of methanol-distilled water mixture (3:2). The filtrate was evaporated to dryness in an oven at 45°C. The residue was dissolved in 0.2 ml of the methanol-water mixture (3:2). After mild anaesthesia, each ovariectomised insect (48 hr after operation) was injected with 3 μl of extract through the prothoracic sternal region with the aid of a calibrated and pointed microcapillary with a small rubber cannula attached. After 24 hr, similar injections of a repeated dose were given to the experimental group of operated insects in order to compensate for any metabolic degradation of the active principle in the extract.

Each value is the mean of 8 separate determinations in different experiments. Statistical significance of the data was assessed using the student's '*t*' test.

Results

A significant increase in the amount of food ingested by the ovariectomised insects of different age-groups (1–7 days after castration) compared to that in the respective control groups was noticed (figure 1). Food consumption reaches a peak in both operated and control insects 6 days after castration, though the level was much lower in the controls. A steady increase in food consumption was observed in the operated insects throughout the seven days after castration which is in agreement with the results in controls except for the decrease on the 4th day which could be the effect of egg-laying by controls. Injection of methanol-extract of adult ovaries

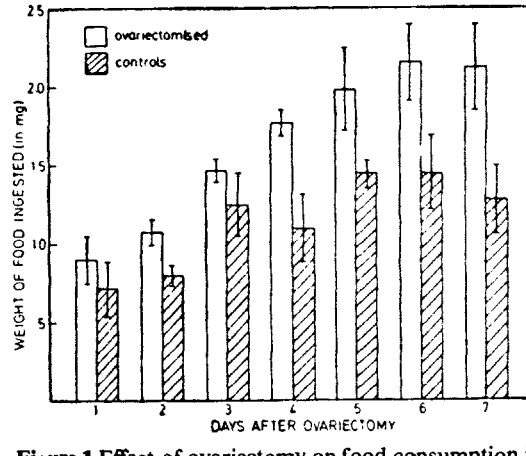

Figure 1 Effect of ovariectomy on food consumption in *D. cingulatus*. Each column denotes mean value from 8 separate individuals and the bars represent±SEM

into the ovariectomised insects brings down food consumption (table 1) and the differences between the values recorded for the experimentals and controls are statistically significant ($P \leqslant 0.01$).

Midgut protease activity was significantly higher in the ovariectomised insects than in the respective controls on all days except 1 day after the operation (figure 2) and higher difference was noticed especially 4–7 days after castration. A steady increase in enzyme activity was recorded in the operated insects throughout the 7 days after ovariectomy. Significant increase in midgut invertase activity was also observed in the ovariectomised insects over that in the controls in all age-groups except 2 days after ovariectomy. Changes in the pattern of invertase activity in the castrated insects of different age-groups was almost similar to that of protease activity (figure 2). In the castrated insects an increase in the enzyme activity (protease and invertase) was noticed which parallel the increasing rate of food consumption (figures 1 & 2). When the ovariectomised insects were injected with methanol-extract of adult ovaries, activities of both the enzymes came down significantly (table 1).

The level of neurosecretory activity in PINSC of the castrated insects is remarkably increased 1–7 days after the operation. Secretory indices in the ovariectomised insects are significantly higher in the different experimental groups ($P \leqslant 0.01$) than those in the corresponding controls (figure 3). A correlated increase in the diameter of both PINSC and also their nuclei were also apparent in the experimental insects. The diameters of the secretory cells and the nuclei in experimental animals varied from 20 to 26 μ and

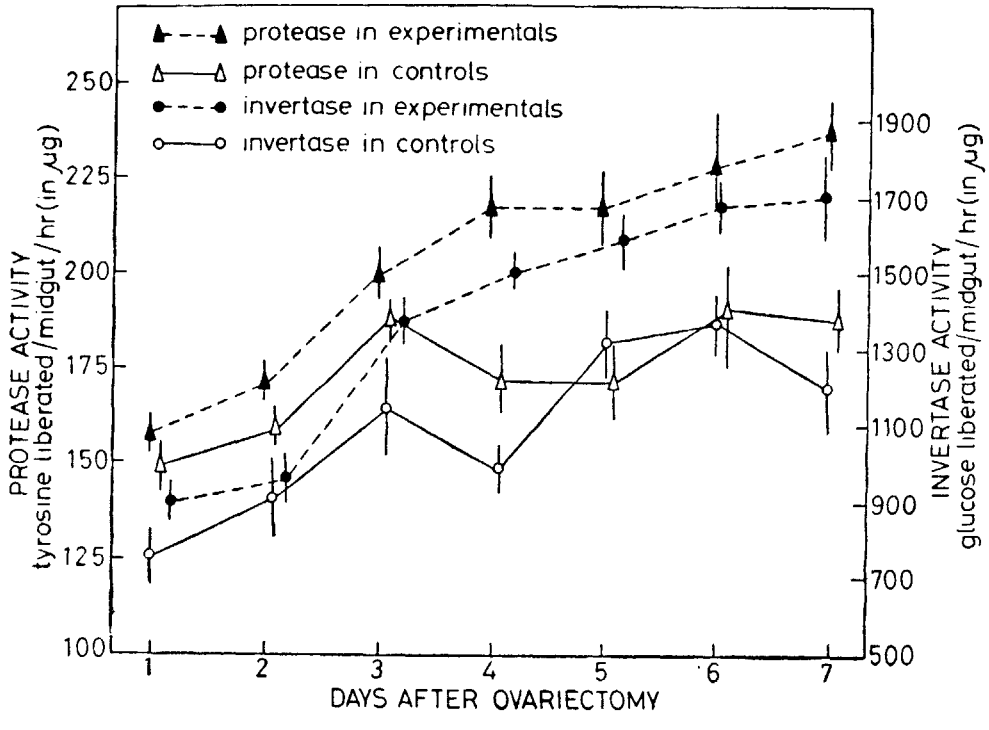

Figure 2 Effect of ovariectomy on midgut protease and invertase activity in *D. cingulatus*. Each point represents the mean value from 8 separate determinations and the vertical lines are ±SEM

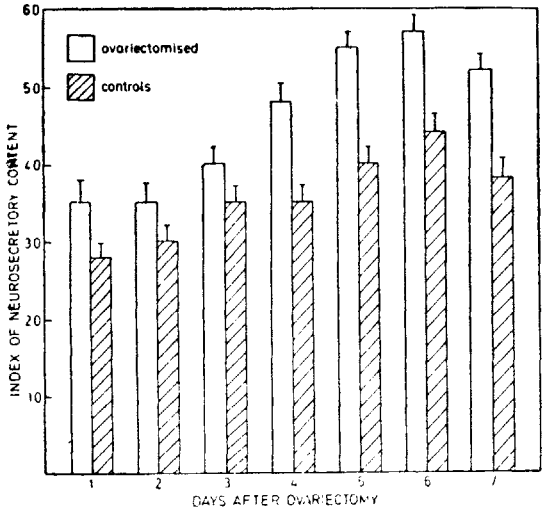

Figure 3 Effect of ovariectomy on neurosecretory index in *D. cingulatus*. Each column represents mean value from 8 separate individuals and the bars ±SEM

7 to 11 μ respectively. On the contrary in the controls, variation in the diameters of PINSC and their nuclei were 19 to 23 μ and 7 to 9 μ respectively. Significant differences in the secretory content of PINSC in the experimentals compared to that in the controls were evident from the photomicrographs as well (figure 4).

Discussion

As Calvez (1980) points out, development of most animals is directly or indirectly controlled by humoral mechanisms which can be partly regulated by exogenous factors like food intake. Results of the present studies demonstrate that in adult *D. cingulatus* whose ovaries have been extirpated, food consumption is much higher than in the sham-operated controls whose ovaries are intact. However, castration does not completely upset the general feeding pattern as noticed in the control insects. This means that ovariectomy has got a stimulatory effect on food consumption in this insect. This is contrary to the observations in *D. koenigii* (Srivastava & Tiwari 1978) in which castration resulted in a drop in the gut contents and hypertrophy of the fat body. Hypertrophy of the fat body is observed in the ovariectomised *D. cingulatus* also (unpublished observation); but a significant increase in food consumption as well. It is also evident from the present experiments that PINSC in the castrated females are hyperactive with rapid secretion and release of the material as evidenced by the increase in the secretory content as well as a simultaneous increase in the diameters of both the cells an nuclei. Median neurosecretory cells were shown to be necessary for adequate ingestion and utilization of food in *Pyrrhocoris apterus* (Slama 1964). Similarly, MNSC ablation in *Sarcophaga bullata* reduce feeding by 22% and causes a corresponding reduction in midgut protease as well (Wilkens 1969). Earlier studies in our own laboratory have shown that in *D. cingulatus* hormonal factors from median neurosecretory cells stimulated food consumption either directly or indirectly (Muraleedharan & Prabhu 1979a). So hyperactivity of PINSC of the castrated *D. cingulatus* and the increased rate of food consumption are fully in agreement with our earlier findings.

A secretogogue regulation of midgut enzyme activity has been reported in *Leucophaea maderae* (Engelmann 1969), *Blatta orientalis* (Gordon 1968), *Glossina morsitans* (Gooding 1974) and in *Rhodnius prolixus* (Garcia & Garcia 1977). In *D. cingulatus* hormonal factors from PINSC stimulate food consumption and the proteinaceous food thus ingested in turn stimulate midgut protease and invertase activity through a secretogogue mechanism (Muraleedharan & Prabhu 1979a). A direct relationship between the activity of brain neurosecretory cells and midgut protease production is also reported in a tick, *Ornithodoros tholozani* (Gabbay & Warburg 1976). Present experiments reveal hyperactivity of PINSC as well as increased

rate of food consumption in the ovariectomised *D. cingulatus*. So there is good evidence that the increased rate of midgut protease and invertase activity observed in ovariectomised *D. cingulatus* is a result of the increased rate of food consumption.

In adult *Iphita limbata*, gravid ovaries elaborate a humoral principle which stimulates discharge of neurosecretion from the brain (Nayar 1957). Many workers have reported ecdysone secretion by the ovaries in different species of insects such as *Bombyx mori* (Karlson & Stamm 1956), *Aedes aegypti* (Schlaeger et al. 1974), *Leucophaea maderae* (King & Marks 1974), *Locusta migratoria* (Hoffmann et al. 1975) and *Macrofermes bellicosus* (Bordereau et al. 1976). Humoral head factor stimulates the ovary to release ecdysone in *Aedes aegypti* (Hagedorn et al. 1979). According to Hoffmann et al. (1981) neurosecretion from the brain controls ecdysone synthesis in adult females of *Gryllus bimaculatus*. On the contrary, in *Aedes atropalpus* the brain stimulates juvenile hormone secretion by the corpus allatum, but does not stimulate ecdysone secretion by the ovaries (Shapiro & Hagedorn 1982). In adult *Locusta migratoria*, PINSC may control the synthesis of acdysone by the follicle cell epithelium of the ovaries. The ecdysiotropic neurohormone of adult females appears to be the functional analogue of the prothoracicotropic hormone (PTTH) of larvae and pupae and it is referred to as follicle cell tropic hormone (Charlet et al. 1979). Very low levels of ecdysone were found in ovariectomised *Locusta migratoria* and in the young ovaries of the normal insects (Laguex et al. 1977). However, a substantial increase in ecdysone level can be seen as egg maturation progresses and at the time of egg laying, a marked drop is again apparent. Mwangi and Goldsworthy (1980) are of the opinion that ovaries in *Locusta* exert a humoral effect on protein metabolism. In *Anopheles freeborni* blood meals of sufficient volume influence hormone production by the oocytes, one function being to retain the meal in the gut enabling increased absorption of nutrients (Rosenberg 1980). Egg development neurosecretory hormone (EDNH) or vitellogenin stimulating hormone (VSH) regulates digestion of blood in the midgut of *Aedes aegypti* (Downe 1975). In *Culex pipiens* EDNH regulates the release of VSH (Cole & Gillett 1978, 1979). All these results imply that the neurosecretory hormone has a stimulatory effect on ovarian hormone activity. Results of the present experiments point to the fact that in *D. cingulatus* adults, extirpation of ovaries has a stimulatory effect on food consumption though with less influence on the normal feeding pattern. Injection of methanol-extract of ovaries into the ovariectomised adults reduces food consumption significantly. So it seems that in *D. cingulatus* adults, some methanol soluble factor (probably ecdysone) has an inhibitory effect on the amount of food consumed. The stimulatory effect of PINSC-hormone on food consumption in the same insect has already been established (Muraleedharan & Prabhu 1979a). Since ovariectomy results in hyperactivity of PINSC, it is reasonable to infer that the hormonal effect exerted by ovaries on food consumption is *via* PINSC. In conclusion, it may be suggested that in adult female *D. cingulatus*, some ovarian humoral factor (probably ecdysone) inhibits the activity of PINSC and their reduced rate of activity results in a reduction on food consumption. Such a drop in food consumption reduces the activity of midgut protease and invertase through a secretogogue mechanism.

Acknowledgements

I am thankful to Professor V K K Prabhu of the Department for critically going througn the manuscript and making valuable

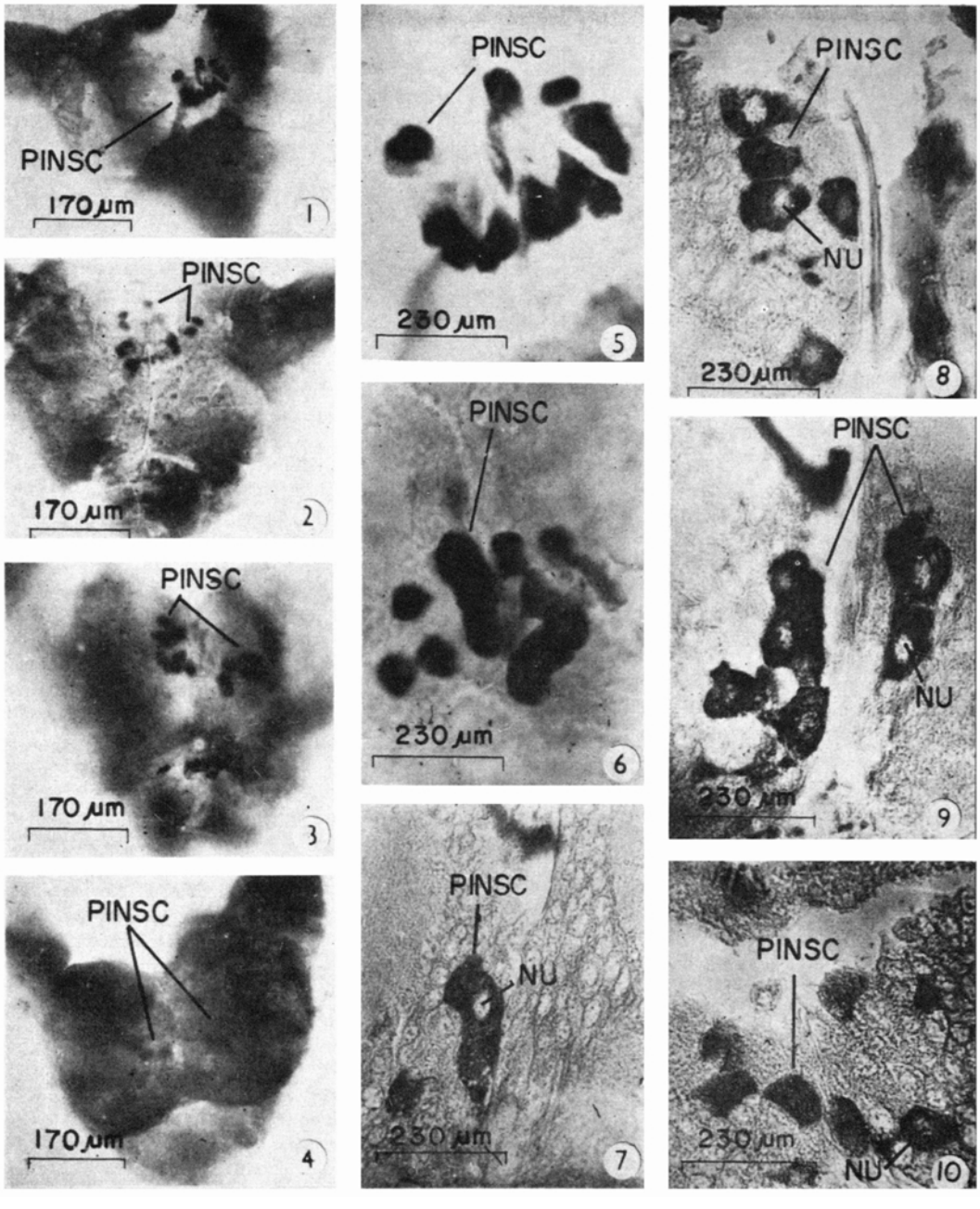

Figure 4 Photomicrographs showing the effect of ovariectomy on the secretory content of the pars intercerebralis neurosecretory cells in *D. cingulatus*. (1) & (2) —one day after ovariectomy and the corresponding sham-operated control respectively (whole mount, Bouin's fluid, paraldehyde fuchsin). (3) & (4) —three days after ovariectomy and the respective control (whole mount, formal-saline, performic acid-resorcin fuchsin). (5) & (6) —one day after ovariectomy and the control respectively (whole mount, performic acid-resorcin fuchsin). (7) & (8) —five days after ovariectomy and the respective control (paraffin section, Bouin's fluid, chrome-alum-haematoxylin-phloxine). (9) & (10) —seven days after ovariectomy and the respective control (paraffin section, Bouin's fluid, chrome-alum-haematoxylin-phloxine).
PINSC = Pars intercerebralis neurosecretory cells; *Nu* = Nucleus

comments. Facilities afforded in the Department by Professor K M Alexander, Head of the Department of Zoology, University of Kerala and a research grant provided by the University Grants Commission are also gratefully acknowledged.

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