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JUVENILE HORMONE REGULATION OF THE LEFT COLLETERIAL GLAND IN INTACT AND OVARIECTOMIZED *BLATTELLA GERMANICA* (L.) (DICTYOPTERA: BLATTELLIDAE)

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Abstract—The left colleterial glands of intact and ovariectomized females were shown to accumulate protein in response to juvenile hormone III. Their distal tubule diameter was highly correlated with their protein content. While intact females excreted the oöthecal proteins to produce an egg case, ovariectomized females retained these proteins without apparent loss from the gland. In overiectomized females the colleterial gland therefore became larger and contained more protein than in intact females. The size and protein content of the left colleterial gland of ovariectomized females were arrested after the insects were allatectomized, and both were induced by injections of synthetic juvenile hormone III. Thus, the left colleterial gland can be used as a cumulative record of circulating juvenile hormone within an individual ovariectomized female, much as the basal oöcytes are used in intact females, and it can be used to distinguish betwen females that had increased juvenile hormone synthesis and those that had not.

Key Word Index: Colleterial gland; juvenile hormone; corpora allata; ovariectomy; cockroach; *Blattella germanica*

INTRODUCTION

The colleterial glands of cockroaches are accessory sex glands that synthesize and secrete the components that form the oötheca. They are paired organs whose left portion secretes all the structural proteins, calcium oxalate, phenolic tanning glucosides and a polyphenol oxidase and the right portion secretes only a β-glucosidase that cleaves the glucoside to form a quinone which results in a hardened oötheca (Brunet and Kent, 1955). The accumulation of proteins in the left colleterial gland has been shown to be related to presence of active corpora allata and/or juvenile hormone in both *Periplaneta americana* (Bodenstein and Sprague, 1959) and *Blattella germanica* (Zalokar, 1968). In ovariectomized *P. americana* females the glucoside accumulates beyond levels needed to produce a normal oötheca, yet without any apparent excretion of either glucoside or proteins (Willis and Brunet, 1966). Iris and Sin (1988) showed that in *P. americana*, ecdysteroids reduced RNA synthesis and induced excretion of proteins from the left colleterial gland. Given these findings, and evidence that in several species the mature ovary contains ecdysteroids (see Hagedorn, 1985; Tobe and Stay, 1985), we hypothesized that proteins would continue to accumulate in the left colleterial gland in the absence of the ovaries. If so, the left colleterial gland could be used as a cumulative record of circulating juvenile hormone as does oöcyte size in intact females, and complement the relatively instantaneous radiochemical assay of juvenile hormone synthesis.

The corpora allata of both intact and ovariectomized *B. germanica* adult females attain similar high rates of juvenile hormone synthesis, but in ovariectomized females the glands exhibit only a single cycle of activity followed by variable but generally high juvenile hormone synthesis; the onset of their activity is also highly variable, with some corpora allata remaining relatively inactive for at least 28 days after the adult moult (Gadot *et al.*, 1991). Therefore, using only the *in vitro* radiochemical assay of juvenile hormone synthesis, it could not be established whether relatively inactive corpora allata from older ovariectomized females had previously undergone a cycle of activity or if they had never experienced high activity. A second indicator of corpus allatum activity in intact *B. germanica* is the gland's volume, but in ovariectomized females it increases gradually and does not reflect juvenile hormone synthesis (Chiang *et al.*, 1991). We sought to establish an independent assay of the cumulative juvenile hormone experienced by ovariectomized

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females that could serve the same role as does oöcyte length in intact females. We hypothesized that the accumulation of proteins in tubules of the left colleterial gland could be used to distinguish between females that had undergone a cycle of high corpora allata activity and those that had not.

MATERIALS AND METHODS

Insects were maintained at 27°C under a 12 h light–12 h dark photoperiodic regime with Purina dog chow and water provided *ad libitum*. Females were ovariectomized early in the last instar. Upon adult emergence the sexes were separated and housed in groups (Gadot *et al.*, 1989a). Females were grouped with males for at least 8 h daily and only females that mated between days 4 and 9 were used. Details of the experimental protocols are described in Gadot *et al.* (1991). Allatectomy was done on ovariectomized newly emerged females except where indicated otherwise.

Colleterial glands were dissected under saline (Kurtti and Brooks, 1976) and frozen at −20°C until assayed. Individual glands were disrupted in 500 μl saline using a Kontes Micro Ultrasonic cell disruptor. Protein was measured using the Bio-Rad protein assay kit with bovine serum immunoglobulin as the protein standard. Absorbance was read at 595 nm.

Colleterial gland tubule diameter was measured with an ocular micrometer in a dissecting microscope, using the mean of 3–5 measurements of the distal tubules from each gland. Juvenile hormone III (80% pure; Sigma) was injected between the first and second sternites in 1 μl olive oil (0.8 μg juvenile hormone III/μl).

The *in vitro* radiochemical assay for juvenile hormone biosynthesis was adapted from Pratt and Tobe (1974) as described in Gadot *et al.* (1989b) except that corpora allata–cardiaca complexes were dissected from cold-anaesthetized females.

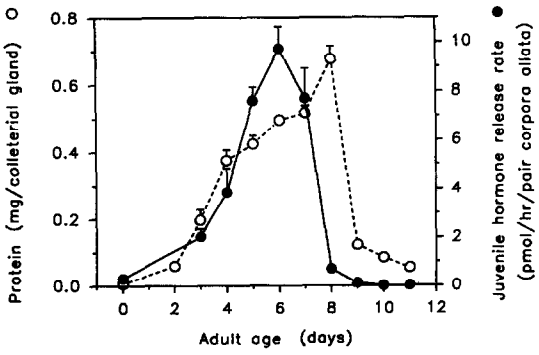

Fig. 1. Mean total protein accumulation in the left colleterial gland and juvenile hormone biosynthetic rates in intact *B. germanica* females through the first oviposition.

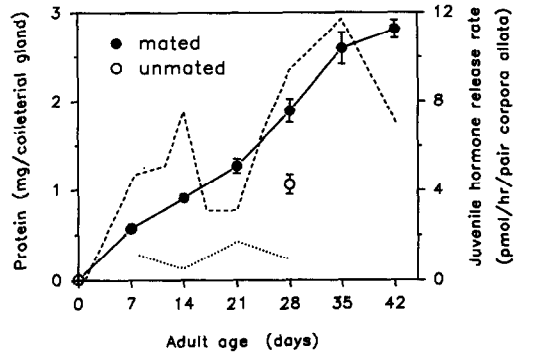

Fig. 2. Mean total protein accumulation in the left colleterial gland in ovariectomized *B. germanica* females. Each point represents at least 10 determinations. Vertical bars show SEM. Dashed and dotted lines show juvenile hormone synthesis in mated and unmated females, respectively (from Gadot *et al.*, 1991).

RESULTS

The left colleterial gland of intact females that had mated on day 6 accumulated approx. 0.7 mg protein before ovulation. The *in vivo* accumulation of proteins reflects the temporal pattern of juvenile hormone synthesis by the corpora allata measured *in vitro* (Fig. 1). The significant loss of proteins on day 9 marks ovulation and formation of the oötheca.

Ovariectomized females that mated by day 9 accumulated large amounts of oöthecal proteins by day 42 and it appeared that, in the absence of ovulation and oviposition, oöthecal proteins were retained in the left colleterial gland (Fig. 2). The relationship to juvenile hormone biosynthesis is less obvious than in intact females because the left colleterial gland is not evacuated and corpus allatum activity in these females does not return to the low levels that are seen in intact females (Figs 1 and 2). Ovariectomized females that did not mate by day 28 in daily exposure to males were assayed on that day; they accumulated significantly lower amounts of colleterial gland proteins than those females that had mated by day 9 (t-test, $P < 0.005$). The corpora allata of ovariectomized females that mated always synthesized higher amounts of juvenile hormone than those of unmated females which consistently exhibited low activity from days 8–28 (Fig. 2).

The diameter of the distal tubules and total protein content of the left colleterial gland were highly correlated ($r^2 = 0.96$) within individual intact and ovariectomized females (Fig. 3). Therefore, in subsequent assays we used distal tubule diameter as a valid measure of left colleterial gland protein content. The diameter of distal tubules in intact females increased in relation to juvenile hormone synthesis by the corpora allata in both the first and second gonotrophic cycles with the colleterial glands remaining small in gravid females (Fig. 4). In contrast, distal tubule diameter in ovariectomized mated females increased gradually as did protein content in their colleterial gland (Figs 2 and 4).

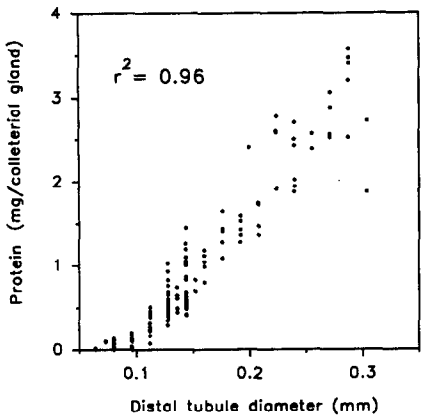

Fig. 3. Correlation between the protein content in the left colleterial gland and the distal tubule diameter within the same intact or ovariectomized females.

Verification that the increase in diameter of the distal tubules and protein accumulation in the left colleterial gland were in response to juvenile hormone was done by removing the corpora allata and with juvenile hormone replacement therapy. Allatectomy of newly emerged (day 0) ovariectomized females resulted in no significant increase in tubule diameter by days 21 and 42 (Fig. 4). Allatectomy of ovariectomized females on day 7 resulted in a cessation in protein accumulation within a week of the allatectomy. The importance of the corpora allata in protein synthesis is most clearly seen in the divergence between ovariectomized females that were or were not allatectomized on day 7 (Fig. 4). It is also important to note that the size of the distal tubules in ovariectomized females that were allatectomized on either day 0 or day 7 did not decrease between days 21 and 42 (Fig. 4). This indicated that proteins were retained in the colleterial gland for long periods of time in these ovariectomized females apparently without significant excretion or resorption.

Ovariectomized females that were allatectomized on day 0 and injected with 1 μl olive oil as control on either day 0 or day 7 did not exhibit any significant

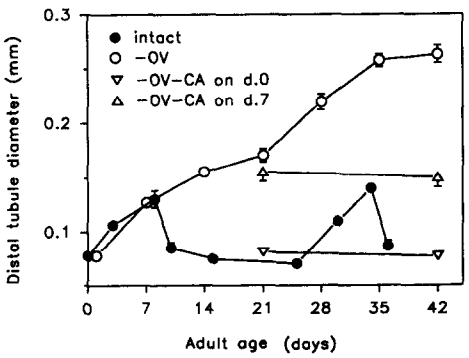

Fig. 4. Mean diameter of the distal tubules of the left colleterial gland, showing the effect of ovariectomy and allatectomy compared to normal intact females through two ovarian cycles. Each point represents at least 10 determinations. Vertical bars show SEM.

Table 1. Protein content in the left colleterial glands of *B. germanica* females that were ovariectomized as last instar nymphs, allatectomized as newly-ecdysed adults and injected with juvenile hormone III or olive oil

Age injected	Age assayed	Injection (μg JH III)	Mean protein ±SEM (mg)	*N*
0	7	Sham	0.015 ± 0.009	9
7	28	Sham	0.028 ± 0.022	10
7	28	0.8	0.481 ± 0.057	9
21	28	0.8	0.484 ± 0.125	10

increases in colleterial protein content by days 7 and 28 respectively (Table 1). In contrast, ovariectomized–allatectomized females that were injected with 0.8 μg juvenile hormone III on either day 7 or 21 produced significantly more proteins in the left colleterial gland (Table 1). The magnitude of the response by the left colleterial gland to this dose of juvenile hormone was the same regardless of the age of the female.

DISCUSSION

The size of the basal oöcytes is used in many insects as a reliable, cumulative measure of circulating juvenile hormone and corpus allatum activity (Tobe and Stay, 1985) and several quantitative gonadotrophic bioassays have been described in *B. germanica* using the growth of basal oöcytes (e.g. Kunkel, 1973). In *B. germanica*, the pattern of oöcyte development has been related precisely to the juvenile hormone biosynthetic activity of the corpora allata *in vitro* (Belles *et al.*, 1987; Gadot *et al.*, 1989a, b). Like oöcyte development, the secretory activity of the left colleterial gland is induced by juvenile hormone and both protein and glucoside content of the gland were suggested as quantitative indirect bioassays for juvenile hormone in both nymphs and adults of *P. americana* (Willis and Brunet, 1966; Shaaya and Bodenstein, 1969). Bodenstein and Sprague (1959) used the appearance of adult *P. americana* colleterial glands transplanted into nymph abdomens as a bioassay of juvenile hormone in the nymph.

Protein content in the left colleterial gland may be used to bioassay cumulative exposure to juvenile hormone in ovariectomized females if it is related to juvenile hormone biosynthesis and only if, in the absence of oviposition, there is negligible loss of proteins from the colleterial gland. The temporal pattern of accumulation of oöthecal proteins in normal mated females was related to the pattern of juvenile hormone synthesis by the corpora allata (Fig. 1). This was expected because the corpora allata and/or juvenile hormone are known to play an essential role in regulating the synthesis of colleterial proteins in several cockroaches [see Iris and Sin (1988) for references]. In *B. germanica* incorporation of [^{14}C]glycine into colleterial proteins *in vitro* declined during oviposition, remained low during pregnancy, increased again shortly before parturition and

attained high levels gain during oöcyte maturation (Zalokar, 1968). Our measurements of distal tubule diameter show an identical pattern (Fig. 4). The pattern, however, requires verification in each species, as highlighted by the recent disagreement regarding the cyclicity of colleterial protein synthesis in *P. americana* (Iris and Sin, 1984, 1988; Weaver and Pau, 1987).

Extirpation of the corpora allata further verified the essential role of juvenile hormone in stimulating the secretory activity of the left colleterial gland. Although both the corpora allata and the colleterial gland are activated in ovariectomized *B. germanica*, there was no significant change in the size of colleterial glands of ovariectomized female that were allatectomized on day 0 (Fig. 4). In females allatectomized on day 7, the diameter of distal tubules of the colleterial gland continued to increase but reached a plateau within a week. Immediate cessation of colleterial protein production was not expected since Pau *et al.* (1986) found that it took 4 days for the concentration of oöthecin-C mRNA, coding for a major oöthecal structural protein in *P. americana*, to drop to consistently low levels after females with mature oöcytes were allatectomized. It subsequently took 6 days for protein synthesis to decrease (see also Bodenstein and Shaaya, 1968). A similar decrease in colleterial glucoside was obtained in decapitated *P. americana* (Willis and Brunet, 1966).

The presence of proteins in the left colleterial gland following administration of juvenile hormone III to ovariectomized females that were allatectomized on day 0, indicated that juvenile hormone induced protein synthesis in the colleterial gland (Table 1). These data also showed that inactive left colleterial glands responded similarly to juvenile hormone on both days 7 and 21. This continued responsiveness (competence) of the left colleterial gland in ovariectomized females is important if they are to be used as a bioassay of juvenile hormone, since activation of the corpora allata is highly variable among ovariectomized females (Gadot *et al.*, 1991). It is important to note however, that we did not examine the first few days of the adult stage, when competence of the colleterial gland may be lower (see Bodenstein and Shaaya, 1968).

There was no visual evidence of colleterial proteins being released to the genital vestibulum in ovariectomized females. The accumulation of protein to more than four times the normal level suggests that little, if any, proteins are secreted out of the gland. This was also indicated by the plateau in colleterial gland size (and protein content) when ovariectomized females were allatectomized on day 7 (Fig. 4). These data, taken together with the highly significant correlation between protein content and the size of the distal tubules of the left colleterial gland, support the validity and effectiveness of using either parameter as an indirect measure of cumulative juvenile hormone titre in the haemolymph and corpus allatum activity.

The steady accumulation of oöthecal proteins in ovariectomized *B. germanica* reflects the profile of juvenile hormone synthesis in these females (Fig. 2). A decline in juvenile hormone synthesis on days 17 and 21 is reflected in a slower increase in the diameter of the distal tubules of the left colleterial gland (Fig. 4). Accumulation of colleterial proteins accelerates again after day 21, reflecting the sharp increase in the rate of juvenile hormone synthesis in ovariectomized females. Iris and Sin (1988) showed in *P. americana* that ecdysteroids reduced RNA synthesis in the left colleterial gland. If the ovary of *B. germanica* is a major source of ecdysteroids, as it is in many insects (see Hagedorn, 1985), and the ovary contributes to haemolymph ecdysteroids levels, than in ovariectomized *B. germanica* ecdysteroids would not be inhibiting colleterial gland RNA synthesis.

Relatively high mean rates and a clear cycle of juvenile hormone synthesis were obtained in ovariectomized *B. germanica* only after females were experimentally synchronized by using the onset of sexual receptivity to select for females with moderately active corpora allata (Gadot *et al.*, 1991). However, in the absence of the ovaries or other indirect measures of cumulative corpora allata activity, it was possible that the moderate decline on days 17 and 21 represented, at least in part, slowly activating corpora allata which had not yet reached their peak juvenile hormone synthesis, rather than a decline in highly active corpora allata. Our present use of the colleterial gland clearly corroborates earlier findings that a true cycle of juvenile hormone synthesis occurred in ovariectomized females. The left colleterial gland contains more proteins on day 21 than on day 14, contrary to the lower amounts predicted if day 21 represented slowly activating corpora allata. This is also illustrated by females that did not mate by day 28; they had significantly less colleterial proteins than 28-day-old females that mated (Fig. 2), reflecting the consistently low rate of juvenile hormone synthesis by their corpora allata (Gadot *et al.*, 1991).

Stay *et al.* (1983) discussed the dangers of using indirect measures of corpora allata activity in ovariectomized females. They showed that haemolymph vitellogenin titres reached high levels in ovariectomized *Diploptera punctata* even though the activity of the corpora allata remained low. Our results with ovariectomized *B. germanica* illustrate that, coupled with the *in vitro* radiochemical assay of corpora allata activity, the colleterial gland can serve as a relative measure of the cumulative juvenile hormone experienced by females, as does the ovary in intact females.

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