Pergamon

PII: S0022-1910(97)00102-9

J. Insect Physiol. Vol. 44, No. 1, pp. 31–38, 1998

0022-1910/97 $19.00 + 0.00

Feeding and Activation of Corpora Allata in the Cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae)

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Received 6 April 1997; revised 10 July 1997

Adult females of the cockroach *Blattella germanica* have clearly-defined feeding cycles related to oogenesis. In the first cycle, food ingestion precedes volumetric increase in the corpora allata, which in turn precedes juvenile hormone production, whereas starved females do not develop the corpora allata and produce very low amounts of juvenile hormone. When the second gonadotropic cycle is provoked by removing the ootheca, the first event observed is an increase in food consumption, followed by an increase in corpora allata volume and activity. However, this increase in corpora allata volume (and activity) does not occur if females are starved, thus indicating that the ootheca in the genital chamber inhibits primarily feeding, and indirectly corpora allata development and activity. Corpora allata volume in isolated heads from starved and decapitated females was able to increase to levels similar to fed controls, but this increase was abolished by allatostatin treatment. We suggest that a factor produced in the thoracico–abdominal compartment, which reaches the head mainly through a nervous pathway, is released during starvation and inhibits corpora allata development. This factor may stimulate allatostatin production or release, or may well be allatostatin itself.

Feeding Corpora allata Juvenile hormone *Blattella germanica* Allatostatin

INTRODUCTION

In most insect species availability of food is critical for oogenesis (see Wheeler, 1996 for review). This is especially evident in cockroaches, where pioneering studies had shown that starvation suppresses oocyte growth in *Blattella germanica* and *Leucophaea maderae* (Roth and Stay, 1962; Engelmann and Rau, 1965, respectively). In *B. germanica* further work confirmed the critical importance of food for reproduction (Kunkel, 1966; Cochran, 1983; Durbin and Cochran, 1985; Piulachs, 1988), and the influence of dietary protein contents upon food consumption and oogenesis (Hamilton and Schal, 1988; Cooper and Schal, 1992).

The invention of a radiochemical system to measure rates of juvenile hormone synthesis by corpora allata incubated *in vitro* (Pratt and Tobe, 1974) shed light on the relationships between feeding and oocyte growth. In *Periplaneta americana* (Weaver and Pratt, 1981; Weaver, 1984), *Diploptera punctata* (Woodhead and Stay, 1989), *L. maderae* (Aclé *et al.*, 1990), and *B. germanica* (Schal *et al.*, 1993) the suppression of oogenesis observed in protein-deprived females correlated with reduced rates of juvenile hormone production. The method for measuring juvenile hormone production also revealed that nerve transection increased, to some extent, corpora allata activity in starved specimens of *P. americana* (Weaver, 1984), *D. punctata* (Woodhead and Stay, 1989) and *B. germanica* (Schal *et al.*, 1993). However, juvenile hormone production in females of *B. germanica* with denervated corpora allata that were fed protein-deficient diets was much lower than that observed in females that were fed normal diets (Schal *et al.*, 1993). The whole data suggest that the relationships between nutrition and corpora allata function may be complex and involve nervous as well as humoral factors.

Here we used the species *B. germanica* as the model to study the effects of feeding upon corpora allata development and function. Observations of the dynamics of feeding, corpora allata volume and juvenile hormone synthesis led to ligature and decapitation experiments.

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These, in turn, led us to postulate the involvement of YXFGL-NH_2 allatostatins, neuropeptides well known for their inhibitory properties upon the corpora allata, which have been isolated from various species of insects (Stay *et al.*, 1994), including *B. germanica* (Bellés *et al.*, 1994).

MATERIALS AND METHODS

Insect rearing and study of the gonadotropic cycles

We used virgin or mated females of *B. germanica* obtained from a colony fed on Panlab dog chow and water, and reared in the dark at 30 ± 1°C and 60–70% r.h. Both, mated and virgin females, undergo similar corpora allata activity and feeding cycles (Gadot *et al.*, 1989; Lee and Wu, 1994). For studies carried out within the first gonadotropic cycle, freshly moulted females were isolated and used at appropriate ages, and were assessed wherever possible by measuring the basal oocyte length (Bellés *et al.*, 1987).

The second gonadotropic cycle was experimentally provoked by removing the ootheca from the genital atrium or by severing the ventral nerve cord (see Roth and Stay, 1959) on mated females that had formed the ootheca 24–48 h earlier. Intact females with the ootheca attached and sham-operated females (see below) were used as respective controls. To assess the influence of food consumption, starved females with the ootheca removed or with the nerve cord severed were also studied. Food consumption, corpora allata volume, basal oocyte growth and juvenile hormone biosynthesis were the parameters measured. The second gonadotropic cycle, either spontaneous or provoked, lasts between 10 and 12 days in our rearing conditions. However, since our interest focused on the activation of the corpora allata, which occurs between days 6 and 8 of the second cycle, the experimental specimens were monitored until day 8.

Food consumption, and crop and midgut weight

To study the rhythm of feeding during the first and second gonadotropic cycles, food consumption was determined with the method of Cochran (1983) with minor variations. Food was weighed on a Sartorius 2004 MP balance (0–166 g, d = 0.01 mg). Individual specimens were provided with water *ad libitum* and a weighed food portion (fresh weight of initial food, FW). After 24 h the food remaining was transferred to an oven at 60°C, left there for 24 h and then weighed again (dried weight of final food, DW). In parallel, a similar amount of food was placed in a control box containing only the water vial, and a total of 4–6 of these observations were carried out at every experimental session. The water lost to evaporation (evaporation factor, EF) estimated from these control experiments was used as a correction factor. With these parameters, the dry weight of food consumed (food consumption, FC) was then calculated from the formula: $FC = FW - [DW + (FW \times EF)]$.

For feeding rhythm studies the criterion of the basal oocyte length cannot be used to assess the physiological age, and food consumption during the gonadotropic cycle showed considerable day-to-day variation and asynchrony, mostly due to differences in the onset of feeding, which in turn influenced the length of the cycle. However, in all cases food consumption was low after adult emergence, peaked around the middle of the gonadotropic cycle, and declined thereafter. For data presentation, the date of adult emergence and that of the formation of the first ootheca were used as references, and the day-to-day measurements of food consumption were realigned by using the mean intervals between these two reference points (following Cochran, 1983) and the data were referred to a scale of 8 days, which is the most common duration of the first gonadotropic cycle under our rearing conditions (Bellés *et al.*, 1987).

Corpora allata volume and oocyte length

All micrometrical measurements were carried out with an ocular micrometer adapted to the dissecting stereomicroscope. Corpus allatum volume (V_{CA}) was estimated by the formula $V_{CA} = 4/3\pi x_1 x_2 x_3$, where x_1, x_2 and x_3 are the radii of the three principal axes. For each specimen, both corpora allata were measured and averaged. Basal oocyte length was measured on six ovarioles chosen at random in the ovary pair, and the values were averaged.

Quantification of juvenile hormone synthesis

Individual pairs of corpora allata were incubated in 100 μl of TC 199 medium (Flow, Ayrshire, Scotland, UK), containing L-methionine (0.1 mmol), Hank's salts, Hepes buffer (20 mmol) plus Ficoll (20 mg ml^{-1}), to which L-[^{3}H-methyl] methionine (Amersham, Bucks, UK) had been added to achieve a final specific activity of 7.4 GBq $mmol^{-1}$. Details of the method for juvenile hormone III determination *in vitro* in *B. germanica* are given in Piulachs and Couillaud (1992). Routine quantification of juvenile hormone III produced *in vitro* was carried out in standard 2 h incubation periods. At the end of the incubations, juvenile hormone III was determined in the medium plus homogenized glands.

Head ligature, decapitation and ventral nerve cord severance

All the operations were carried out on specimens anaesthetized with carbon dioxide. Head ligated specimens were prepared by putting a ligature round the neck, which was then gently tightened and knotted, taking care that the neck cuticle was not severed. Decapitation was carried out following the same procedure, but in this case the ligature was firmly tightened and knotted, and the neck was then sectioned posterior to the ligature. In all cases, the corpora allata remain isolated within the head capsule. In head ligated specimens the haemocoelic isolation of the head with respect to the thoracico–abdominal compartment was assessed at the end of the experiment by injecting methylene blue (0.2% in aqueous

solution) in the abdomen and observing the diffusion of the dye 5 min later. Those specimens showing dye within the head were discarded. In the experiments to provoke the second gonadotropic cycle (see above), the nerve cord was severed with fine forceps introduced through a lateral slit between the fifth and the sixth abdominal sternites. Sham-operations were performed according the same procedure, but without severing the nerve cord.

Injection of allatostatin into isolated heads

We used one of the allatostatins of *B. germanica* (BLAST 2: Asp-Arg-Leu-Tyr-Ser-Phe-Gly-Leu-NH_2), which was synthesized and characterized as described in Bellés *et al.* (1994). It was injected with a Hamilton microsyringe at a volume of 0.3 μl in Ringer saline containing 3% acetonitrile. Specimens were anaesthetized with carbon dioxide, and prepared with a lax ligature placed round the neck. The needle of the syringe charged with the allatostatin was introduced to the head through the occipital foramen, and the ligature was tightened around the needle. Then the allatostatin was gently injected, after which the needle was removed and the ligature was immediately knotted. Finally, the neck was sectioned posterior to the ligature.

RESULTS

Rhythm of feeding in the first gonadotropic cycle

Food consumption by virgin females during the first gonadotropic cycle [Fig. 1(A)] was low after imaginal ecdysis (day 0, 0.50 ± 0.29 mg/day, n = 12), increased progressively to a maximum between days 3 and 4 (11.00 ± 1.00 mg/day, n = 9), and then rapidly decreased until the formation of the ootheca (day 7), when the values were similar to those measured in freshly emerged specimens. Food consumption in females transporting the ootheca [day 8: Fig. 1(A), and also 2 to 3 days after the formation of the ootheca, not shown] was uniformly low, between 1 and 2 mg per day.

In addition, crop and midgut were weighed on the same days, in order to obtain a food transit reflection of the feeding rhythm studied above. Results on crop weight [Fig. 1(B)] show increasing values from day 0 (0.72 ± 0.13 mg, n = 10) to day 3 (3.15 ± 0.65 mg, n = 9), and a drop from day 4 onwards, to reach values similar to those observed on day 0. Midgut weight [Fig. 1(C)] also shows a cyclic profile, although in this case the more apparent change was observed between day 0 and 1, then the weight slowly increased until day 4 (1.60 ± 0.09 mg, n = 11), and decreased on days 5, 6 and 7 (0.66 ± 0.07 mg, n = 8).

Feeding, corpora allata activity and oocyte growth

In another set of experiments we studied the effects of feeding upon corpora allata volume, juvenile hormone synthesis and oocyte growth. For this purpose, normally fed females were compared to starved females.

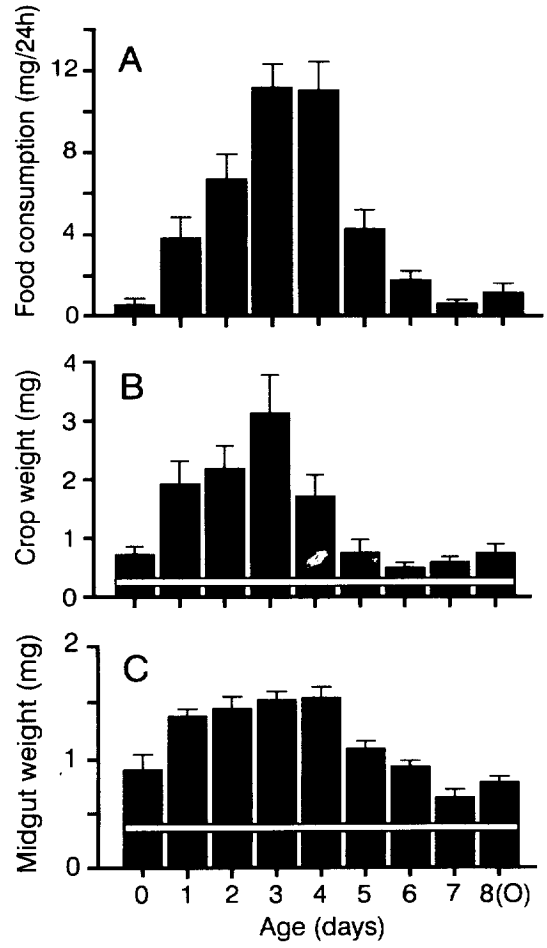

FIGURE 1. Food consumption (A), crop weight (B) and midgut weight (C) during the first gonadotropic cycle of virgin females of *B. germanica*. Results are expressed as $\bar{x}$ ± SEM (n = 9–12). The white space close to the base of (B) and (C) indicates the interval of variation of dry weight of empty crop and midgut, respectively (n = 25 in both cases). 8 (O): 8-day-old females transporting an ootheca.

Normally fed females matured the first batch of basal oocytes and produced the first ootheca around the day 8 of adult life. Conversely, starved females died around day 10 of adult life without showing any ovarian development (Fig. 2, inset).

Concerning corpora allata volume, fed females showed a cycle that can be divided into three stages (Fig. 2): (i) days 0–2, when there were no significant changes, (ii) days 2–6, when a steady increase was observed to a maximum of 1.88 ± 0.05 nl (n = 7) on day 6, and (iii) days 6–8, when a sharp decrease occurred, to values as low as 0.70 ± 0.08 nl (n = 7) on day 8, which are similar to those of freshly ecdysed adults. In contrast, corpora allata of starved females (Fig. 2), decreased slightly (from 0.70 ± 0.08 nl, n = 8, on day 0, to 0.56 ± 0.03 nl, n = 7, on day 8).

Determinations of juvenile hormone synthesis on days 2, 4, 6 and 8 (Fig. 2) in both fed and starved females gave results approximately parallel to those of corpora allata volume. Fed females showed the expected cyclic pattern, whereas starved females gave minimal values,

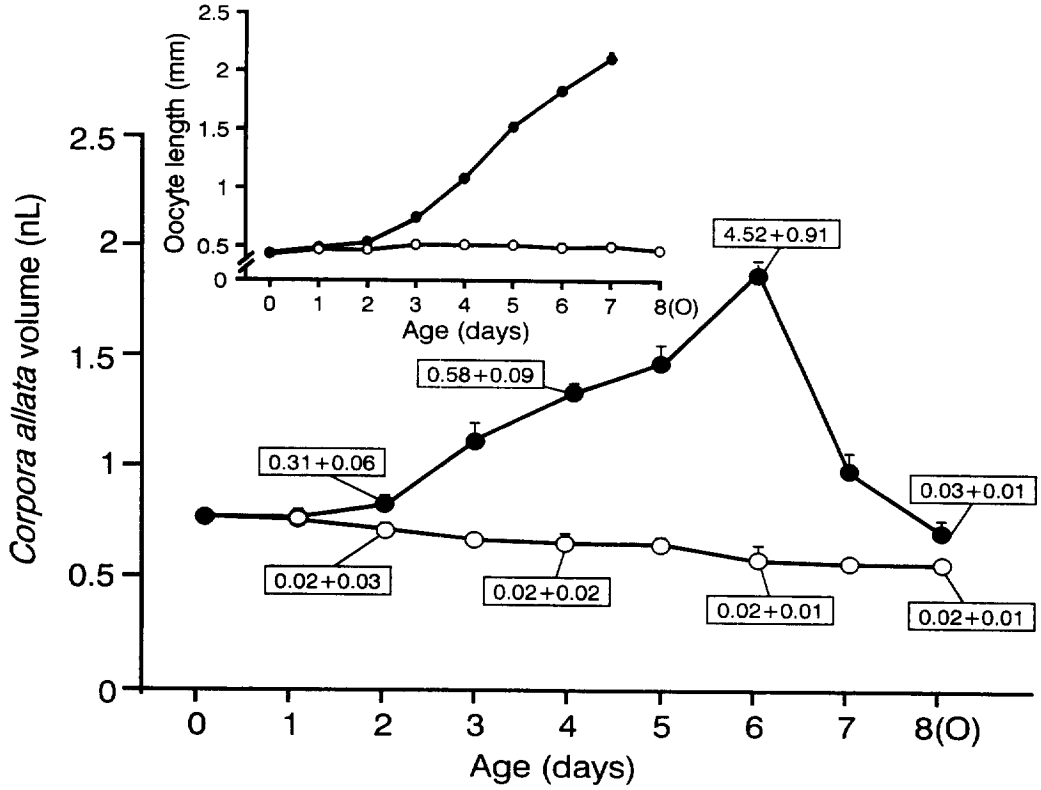

FIGURE 2. Corpora allata volume in fed (●) and starved (○) virgin females of *B. germanica* during the first 8 days of adult life. For each specimen both corpora allata were measured and averaged. The values in the squares besides days 2, 4, 6 and 8 correspond to juvenile hormone biosynthesis (in pmol h^{-1} × pair of corpora allata). The inset shows the basal oocyte length in both groups of experimental females. In all cases results are expressed as $\bar{x} \pm$ SEM (n = 7–11). 8 (O): 8-day-old specimens transporting an ootheca (in the case of fed females).

close to the limit of detection of the method, irrespective of the day of measurement (Fig. 2).

Feeding in the second gonadotropic cycle

The second gonadotropic cycle was provoked by removing the ootheca from the genital atrium or by severing the nerve cord. These operations were carried out on mated females 24–48 h after the formation of the first ootheca, and food consumption was measured during the next 8 days. Intact females carrying the first ootheca normally, and ootheca-carrying sham-operated females were used as respective controls.

Results (Fig. 3) show that females either with the ootheca removed or with the nerve cord severed began to feed on the day of the operation, and steadily increased food consumption during the following 5–6 days. Conversely, both control groups showed constantly low feeding rates throughout the period studied.

Corpora allata activity and oocyte growth in the second gonadotropic cycle

Concerning the volume of the corpora allata [Fig. 4(A)], the females with the ootheca removed showed parallel behaviour to those with the nerve cord severed. In both cases, the volume of the corpora allata significantly increased with respect to controls and starved specimens. The differences between the females with the ootheca removed or with the nerve cord severed with respect to the other experimental groups began to be clearly significant 4 days after the beginning of the experiment.

Conversely, juvenile hormone biosynthetic rates [Fig. 4(B)] remained low in all groups until day 8 after the beginning of the experiment, when the rates measured on females with the ootheca removed or with the nerve cord severed were clearly higher than in the other experimental groups (controls and starved specimens).

Data on basal oocyte length [Fig. 4(C)] are consistent with those of the two former parameters. On day 6 after the beginning of the experiment the values measured on females with the ootheca removed or with the nerve cord severed began to be significantly higher with respect to controls and starved specimens, and on day 8 the differences were more apparent.

Time-course of corpora allata development after feeding

The previous results led us to study the links between food consumption and corpora allata development and activity, and we were first interested in minimizing the time involved in the experiments. Therefore, specimens that had been starved during the first three days of adult life were allowed to feed for 1 h. Then, groups of them were dissected at 1 h intervals and crop and midgut weight and corpora allata volume were measured. Results (Fig. 5) indicate that crop weight increases abruptly just

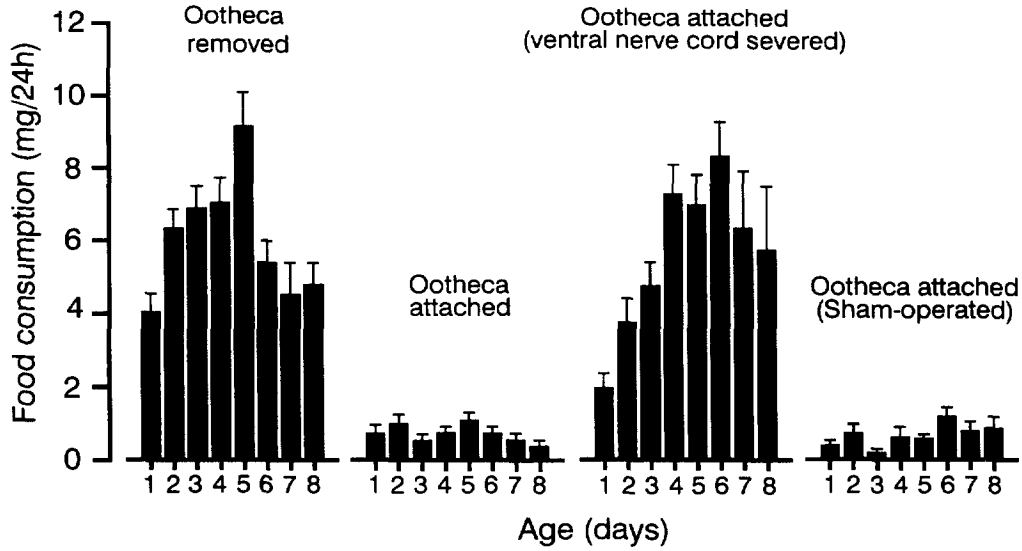

FIGURE 3. Food consumption in mated females of *B. germanica* during the first 8 days of the second gonadotropic cycle. The initiation of the cycle was provoked by removing the first ootheca or by severing the nerve cord. Intact females with the ootheca attached, and ootheca attached sham-operated females were used as controls. Results are expressed as $\bar{x} \pm$ SEM (n = 15–20).

after feeding and decreases thereafter, in parallel with the progressive increase in weight of the midgut. The fluctuations of corpora allata volume were remarkably parallel to those of midgut weight. Therefore, corpora allata volume significantly increased during the first 4 h after feeding (when they reached some 0.75 nl, Fig. 5), and then stabilized. Similar experiments allowing a 15-min pulse feeding showed that corpora allata volume after 4 h was also around 0.75 nl (see next heading and Fig. 6).

Effect of head ligature and decapitation on corpora allata development

In order to discriminate between nervous and humoral messages, experiments of head ligature (which would impair the traffic of haemolymph messages) and decapitation (which would impair haemolymph as well as nervous messages) were carried out. Therefore, 3-day-old starved females were allowed to feed for 15 min, and then ligated or decapitated, and corpora allata volume was measured 4 h after this operation. Appropriate controls (starved and intact specimens) were also studied. Results (Fig. 6) show that corpora allata volume in starved and ligated specimens was not significantly different (although somewhat greater on average) from that measured in starved and intact, but significantly lower than those from fed, either ligated or intact. In contrast, corpora allata volume in isolated heads from starved and decapitated females was significantly greater than that measured in other starved groups (ligated and intact), and similar to that measured in fed specimens, either decapitated, ligated or intact (Fig. 6). We then carried out selective nerve section of the nerve cord at level of the neck following the same experimental strategy, but no differences in corpora allata volume were observed between operated and controls (results not shown).

Effect of allatostatin on corpora allata development

Results described in the previous section suggest the occurrence of a factor inhibiting corpora allata development and travelling from the thoracico–abdominal compartment to the head, mainly through a peripheral nervous pathway, while the insect is starving. Reasonable candidates for such a role would be allatostatins, given their inhibitory properties upon the corpora allata and their dual occurrence in gut and central nervous system tissues. Therefore, we studied whether exogenous application of one of the allatostatins of *B. germanica* (BLAST 2: Asp-Arg-Leu-Tyr-Ser-Phe-Gly-Leu-NH_2) would counteract the stimulatory effect of decapitation upon corpora allata volume. The peptide was injected into the isolated head of 3-day-old starved and decapitated specimens, and corpora allata volume was measured 4 h later. Results (Fig. 7) show that corpora allata from specimens treated with 1 μg of BLAST 2 did not increase in volume, as controls did. In contrast, the dose of 0.1 μg was ineffective.

DISCUSSION

Monitoring of food consumption has shown that adult females of *B. germanica* have clearly-defined feeding cycles related to reproduction. In general, the feeding profiles reported herein are in agreement with those described by Kunkel (1966), Cochran (1983) or Lee and Wu (1994). Similar bell-shaped profiles have been observed in larvae, with food consumption low at the beginning of each instar, peaking near the middle, and declining afterwards to reach minimal values before the moult (Valles *et al.*, 1996). Our results also show that profiles of crop and midgut weight reflect food consumption, and that feeding cycles match almost exactly those

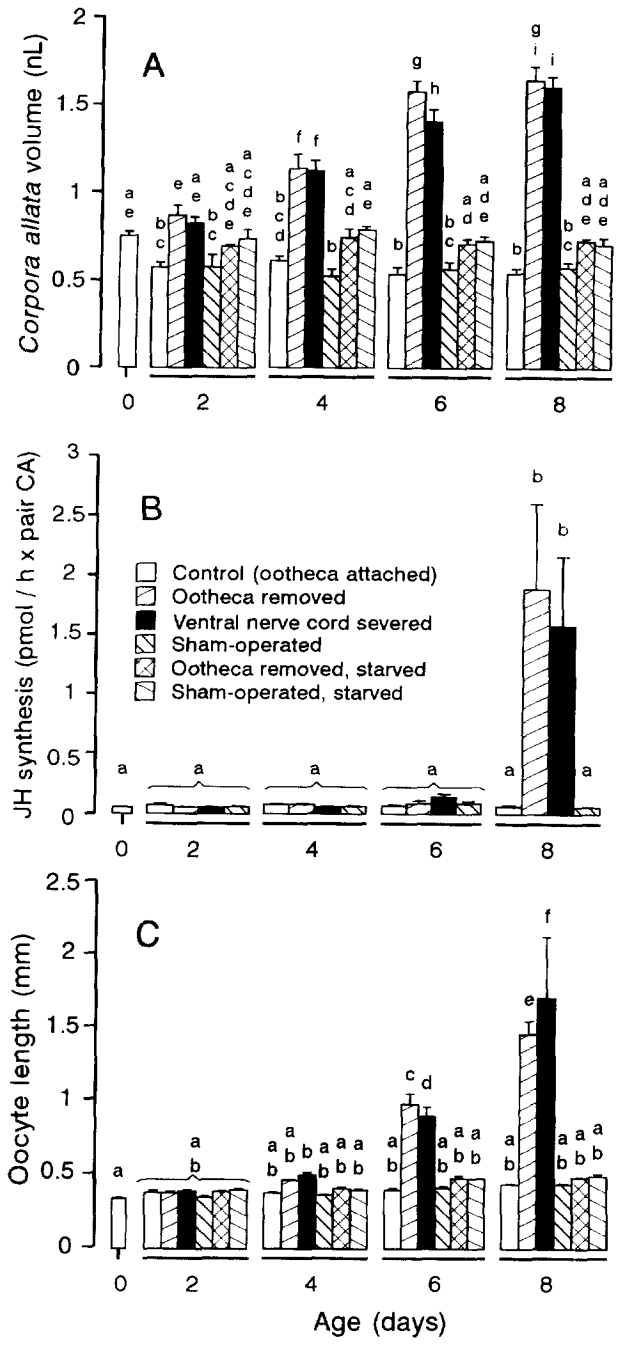

FIGURE 4. Corpora allata volume (A), juvenile hormone (JH) synthesis (B) and basal oocyte length (C) in mated females of *B. germanica* after the formation of the first ooheca and subjected to the experiments indicated in panel (B). For each specimen both corpora allata were measured and averaged. The three parameters investigated were measured every 2 days for 8 days after the treatment (indicated as day 0). In all cases results are expressed as $\bar{x} \pm$ SEM (n = 6–15). Different letters above the columns in each panel indicate significant differences (P = 0.05, ANOVA, one-way, least significance difference).

of vitellogenin production, as reported by Martín *et al.* (1995).

As expected, starved females do not increase in corpora allata volume, and produce juvenile hormone only at very low rates. Previous reports by Roth and Stay (1962), and Piulachs (1988) had described that starved *B. germanica* cannot develop the ovaries, whereas Schal *et al.* (1993) reported that protein contents in the diet is a critical factor for juvenile hormone production. In other cockroaches, such as *P. americana* (Weaver and Pratt, 1981; Weaver, 1984) *D. punctata* (Woodhead and Stay, 1989) and *L. maderae* (Aclé *et al.*, 1990), starved females also produce low amounts of juvenile hormone.

In normally fed females, corpora allata volume progressively increases from day 2 after adult emergence, whereas rates of juvenile hormone synthesis begin to increase significantly from day 4 (see also Bellés *et al.*, 1987; Gadot *et al.*, 1989; Maestro *et al.*, 1994). The results indicate that food ingestion precedes the increase in volume of the corpora allata, and that the latter precedes juvenile hormone production.

When the second gonadotropic cycle is provoked, either by removing the ootheca or by severing the nerve cord, the first effect observed is an increase in food consumption, followed by an increase in corpora allata volume, and then in juvenile hormone production, again in this order. Our results also show that increase in corpora allata volume does not occur in starved specimens, although the ootheca was removed or the nerve cord severed. Pioneer studies by Roth and Stay (1959, 1962), based on oocyte length measurements, had suggested that the presence of an ootheca in the genital chamber would directly inhibit corpora allata function. However, our data suggest rather that the ootheca in the genital chamber primarily inhibits feeding, and indirectly corpora allata development and activity. A similar sequential mechanism had been proposed for *L. maderae* by Engelmann and Rau (1965), on the basis of observations on food consumption.

At this point, we wondered about the links between nutrient consumption and corpora allata development. To facilitate the experimental approach, we first investigated the time-course of corpora allata development after feeding, and the results showed that 4 h are enough to observe a significant increase in corpora allata volume. Thus, 4 h was taken as the standard experimental period for further studies. Corpora allata volume in isolated heads from starved and decapitated females increased to levels similar to fed controls, which suggested that under starving conditions a factor directly or indirectly inhibiting corpora allata development reached the head from the thoracico–abdominal compartment mainly through a nervous pathway. In addition, the experiments of nerve cord severance at level of the neck suggested that this pathway could be a peripheral nervous network, rather than the nerve cord. On the other hand, it is possible that the increase in corpora allata volume following decapitation were just a reaction to stress, as reported in *P. americana* by Weaver and Pratt (1981) (see also Weaver, 1984). However, the fact that corpora allata volume did not increase in head ligated specimens seems to rule out the traumatic hypertrophy hypothesis.

Finally, we postulated that the inhibitory factor could be related to a YXFGL-NH_2 allatostatin. This hypothesis

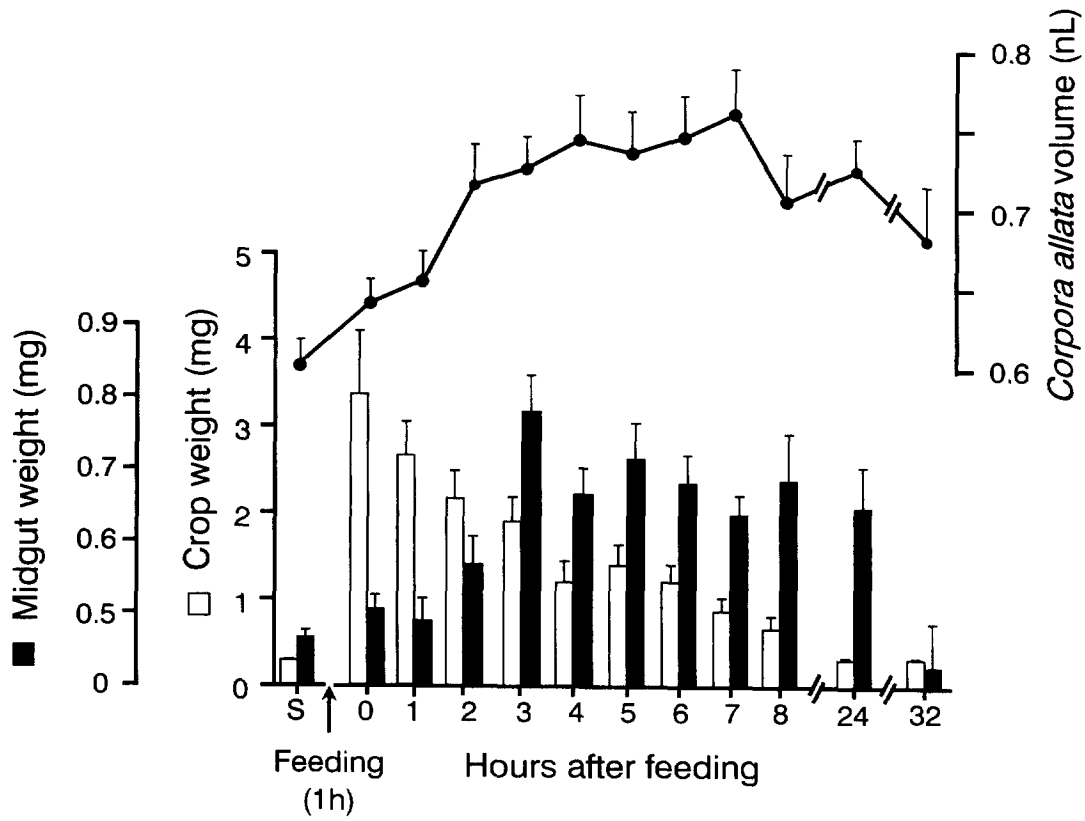

FIGURE 5. Volumetric changes in the corpora allata (•), and weight changes in the midgut (black bars) and in the crop (white bars) in 3-day-old starved females of *B. germanica* after 1 h of feeding. After this feeding pulse, specimens were dissected at 1 h intervals during 8 h, and at 24 and 32 h. For each specimen both corpora allata were measured and averaged. Values from 3-day-old starved specimens (S) are also showed. Results are expressed as $\bar{x} \pm$ SEM (n = 10–15).

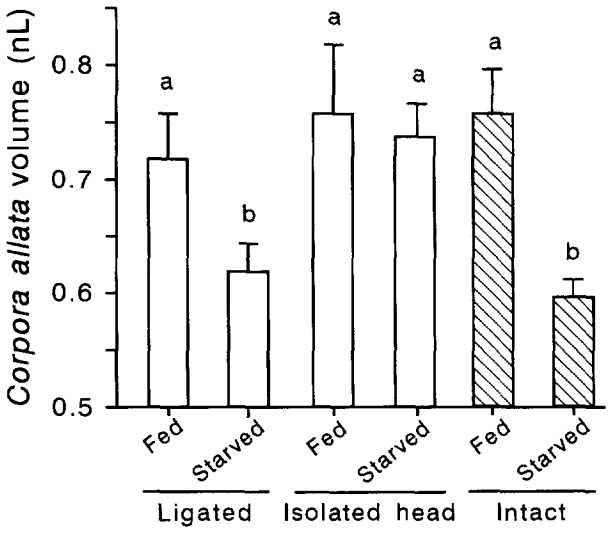

FIGURE 6. Effect of head-ligature or decapitation on corpora allata volume in 3-day-old starved females of *B. germanica* after 15 min of feeding. Specimens were ligated or decapitated just after this feeding pulse, and both corpora allata were measured and averaged 4 h later. Values from both starved and fed intact specimens are also showed. Results are expressed as $\bar{x} \pm$ SEM (n = 10–15). Columns with different letters indicate significant differences (P = 0.05, ANOVA, one-way, least significance difference).

was founded on the fact that these allatostatins inhibit corpora allata function in cockroaches (Stay *et al.*, 1994), including *B. germanica* (Bellés *et al.*, 1994), and that they occur in the central nervous system as well as in gut tissues (*D. punctata*: Reichwald *et al.*, 1994; Yu *et al.*, 1995; *L. maderae*: Duve *et al.*, 1995; *B. germanica*: Maestro *et al.*, unpublished). A dose of 1 μg of BLAST 2, one of the allatostatins described in *B. germanica* (Bellés *et al.*, 1994), injected into the isolated head of starved females, inhibited the expected volumetric increase of corpora allata.

Taken together the data indicate that a factor produced in the thoracico–abdominal compartment, which reaches the head mainly through a nervous pathway, is released during starvation and directly or indirectly inhibits corpora allata development. The experiments of allatostatin treatment, although preliminary, would suggest that this factor may stimulate allatostatin production or release, or be allatostatin itself. The design of a method to measure allatostatin contents in different anatomical compartments (gut, brain, ventral nerve ganglia, etc.) in *B. germanica*, is now in progress in our laboratory. This may allow comparisons of starved and fed females and shed new light on this issue.

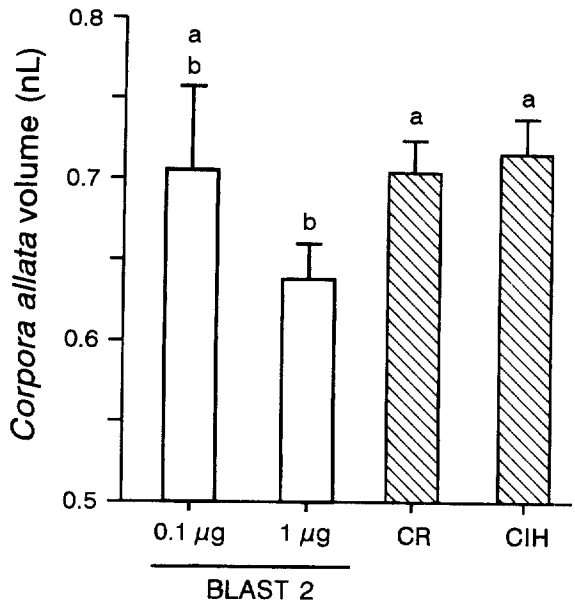

FIGURE 7. Corpora allata volume in isolated heads from 3-day-old starved females of *B. germanica* injected with the allatostatin BLAST 2. For each specimen both corpora allata were measured and averaged. Time elapsed between injection and corpora allata measurement was 4 h. Controls were: corpora allata from isolated heads injected with Ringer saline containing 3% acetonitrile (control Ringer: CR), and corpora allata from isolated heads (control isolated heads: CIH). Results are expressed as $\bar{x} \pm$ SEM (n = 15–20). Columns with different letters indicate significant differences (P = 0.05, ANOVA, one-way, least significance difference).

REFERENCES

Aclé D., Brookes V. J., Pratt G. E. and Feyereisen R. (1990) Activity of the corpora allata of adult female *Leucophaea maderae*: effects of mating and feeding. *Archives of Insect Biochemistry and Physiology* **14,** 121–129.

Bellés X., Casas J., Messeguer A. and Piulachs M. D. (1987) *In vitro* biosynthesis of JH III by the corpora allata of adult females of *Blattella germanica* (L.). *Insect Biochemistry* **17,** 1007–1010.

Bellés X., Maestro J. L., Johnsen A. H., Duve H. and Thorpe A. (1994) Allatostatic neuropeptides from the cockroach *Blattella germanica* (L.) (Dictyoptera Blattellidae) identification, immunolocalization and activity. *Regulatory Peptides* **53,** 237–247.

Cochran D. G. (1983) Food and water consumption during the reproductive cycle of female German cockroaches. *Entomologia Experimentalis et Applicata* **34,** 51–57.

Cooper R. A. and Schal C. (1992) Effects of protein type and concentration on development and reproduction of the German cockroach *Blattella germanica*. *Entomologia Experimentalis et Applicata* **63,** 123–134.

Durbin E. J. and Cochran D. G. (1985) Food and water deprivation effects on reproduction in female *Blattella germanica*. *Entomologia Experimentalis et Applicata* **37,** 77–82.

Duve H., Wren P. and Thorpe A. (1995) Innervation of the foregut of the cockroach *Leucophaea maderae* and inhibition of spontaneous contractile activity by callatostatin neuropeptides. *Physiological Entomology* **20,** 33–44.

Engelmann F. and Rau I. (1965) A correlation between feeding and the sexual cycle in *Leucophaea maderae* (Blattaria). *Journal of Insect Physiology* **11,** 53–64.

Gadot M., Burns E. and Schal C. (1989) Juvenile hormone biosynthesis and oocyte development in adult female *Blattella germanica*: effects of grouping and mating. *Archives of Insect Biochemistry and Physiology* **11,** 189–200.

Hamilton R. and Schal C. (1988) Effects of dietary protein levels on reproduction and food consumption in the German cockroach (Dictyoptera, Blattellidae). *Annals of the Entomological Society of America* **81,** 969–976.

Kunkel J. G. (1966) Development and the availability of food in the German cockroach *Blattella germanica* (L). *Journal of Insect Physiology* **12,** 227–235.

Lee H. -J. and Wu Y. -L. (1994) Mating effects on the feeding and locomotion of the German cockroach *Blattella germanica*. *Physiological Entomology* **19,** 39–45.

Maestro J. L., Danés M. D., Piulachs M. D., Cassier P. and Bellés X. (1994) Juvenile hormone inhibition in corpora allata from ovariectomized *Blattella germanica*. *Physiological Entomology* **19,** 342–348.

Martín D., Piulachs M. D. and Bellés X. (1995) Patterns of haemolymph vitellogenin and ovarian vitellin in the German cockroach and the role of juvenile hormone. *Physiological Entomology* **20,** 59–65.

Piulachs M. D. (1988) Acción gonadotrófica de la hormona juvenil en hembras de *Blattella germanica* alactectomizadas o sometidas a ayuno prolongado. *Miscel.lània. Zoològica (Barcelona)* **12,** 121–124.

Piulachs M. D. and Couillaud F. (1992) Differential stimulation of juvenile hormone III biosynthesis induced by mevalonate and mevalonolactone in *Blattella germanica* (L.). *Journal of Insect Physiology* **38,** 555–560.

Pratt G. E. and Tobe S. S. (1974) Juvenile hormones radiosynthesised by corpora allata of adult female locusts *in vitro*. *Life Sciences* **14,** 575–586.

Reichwald K., Unnithan G. C., Davis N. T., Agricola H. and Feyereisen R. (1994) Expression of the allatostatin gene in endocrine cells of the cockroach midgut. *Proceedings of the National Academy of Sciences U.S.A.* **91,** 11894–11898.

Roth L. M. and Stay B. (1959) Control of oocyte development in cockroaches. *Science* **130,** 271–272.

Roth L. M. and Stay B. (1962) Oocyte development in *Blattella germanica* and *Blattella vaga* (Blattaria). *Annals of the Entomological Society of America* **55,** 633–642.

Schal C., Chiang A. S., Burns E. L., Gadot M. and Cooper M. (1993) Role of the brain in juvenile hormone synthesis and oöcyte development: effects of dietary protein in the cockroach *Blattella germanica* (L.). *Journal of Insect Physiology* **39,** 303–313.

Stay B., Tobe S. S. and Bendena W. G. (1994) Allatostatins: identification, primary structures, functions and distribution. *Advances of Insect Physiology* **25,** 267–337.

Valles S. M., Strong C. A. and Koehler P. G. (1996) Inter- and intra-instar food consumption in the German cockroach *Blattella germanica*. *Entomologia Experimentalis et Applicata.* **79,** 171–178.

Weaver R. J. (1984) Effects of food and water availability, and of NCA-1 section, upon juvenile hormone biosynthesis and oöcyte development in adult female *Periplaneta americana*. *Journal of Insect Physiology* **30,** 813–838.

Weaver R. J. and Pratt G. E. (1981) Effects or starvation and feeding upon corpus allatum and oöcyte growth in adult female *Periplaneta americana*. *Journal of Insect Physiology* **27,** 75–83.

Wheeler D. (1996) The role of nourishment in oogenesis. *Annual Review of Entomology* **41,** 407–431.

Woodhead A. P. and Stay B. (1989) Neural inhibition of corpora allata in protein-deprived *Diploptera punctata*. *Journal of Insect Physiology* **35,** 415–421.

Yu C. G., Stay B., Ding Q., Bendena W. G. and Tobe S. S. (1995) Immunochemical identification and expression of allatostatins in the gut of *Diploptera punctata*. *Journal of Insect Physiology* **41,** 1035–1043.

Acknowledgements—Financial support from DGICYT, Spain (project No PB95-0062, 'Olfaction, feeding and reproduction in the cockroach *Blattella germanica*') and CIRIT, Catalonia (1995 SGR 00059) is gratefully acknowledged.