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Role of the Brain in Juvenile Hormone Synthesis and Oöcyte Development: Effects of Dietary Protein in the Cockroach *Blattella germanica* (L.)

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We demonstrate links between the protein content of diets, food intake, the resultant body mass, juvenile hormone synthesis by the corpora allata, and oöcyte maturation in *Blattella germanica*. Oöcyte development and oviposition were suppressed in starved females as well as in females fed deficient artificial diets (low- or high-protein contents). On all diets, as well as in starved females, oöcyte growth was significantly potentiated by hydroprene, indicating that the suppressed oöcyte growth on deficient diets was largely due to juvenile hormone deficiency. Also, under all dietary treatments, including in starved females, transection of the nervi corporis cardiaci (NCC)-I and II significantly potentiated juvenile hormone biosynthesis, oöcyte development and oviposition compared with the respective sham-operated females. These results clearly show that intact nerves are a major route of allatostatic signals from the brain which in turn are regulated by food quality. However, a partial suppression of juvenile hormone synthesis and smaller oöcytes in females with denervated corpora allata that were fed protein-deficient diets (starved, 0%, 5%) or high-protein diets (78%) compared with females that were fed normal diets (25%, dog and rat foods) highlights the importance of humoral signals in allata activity. Oöcyte size and juvenile hormone biosynthetic rates were also significantly lower in adult females that were fed a 7.5%-protein diet as nymphs compared with females fed a 25%-protein diet. Denervation of the corpora allata resulted in potentiation of their activity, but to significantly lower rates in females that were raised on the low-protein diet as nymphs, further supporting the importance of the nutritional milieu in corpus allatum activation.

Both food intake and body mass varied directly with the protein content of the diet, confounding the conclusion that corpus allatum activity was affected by signals related to the dietary protein content. To dissociate food consumption and body mass from corpora allata activity and oöcyte growth, 2% trypsin synthesis inhibitor was added to a 7.5%-protein diet. Neither body mass nor total food consumption were changed relative to control females, but oöcytes were significantly small on this diet. Together with data showing that oöcyte maturation can be induced with hydroprene in protein-deprived or even in starved females, these data argue that signals associated with the protein content of the diet partially lift brain inhibition of the corpora allata.

Corpora allata Juvenile hormone Cockroach Protein Nutrition Brain disinhibition *Blattella germanica*

INTRODUCTION

Links between feeding, the resultant body mass and the internal nutrient milieu, and endocrine function including fertility, have been proposed for a wide range of animals, including insects. Thus, starvation (in some cases with concomitant water deprivation) or nutrient deficiency result in significantly lower rates of juvenile hormone biosynthesis in orthopterans (*Schistocerca americana gregaria*, Tobe and Chapman, 1979; *Locusta migratoria*, Couillaud and Girardie, 1985), coleopterans (*Leptinotarsa decemlineata*, Khan *et al.*, 1982), dipterans (*Phormia regina*, Zou *et al.*, 1989) and dictyopterans (*Periplaneta americana*, Weaver and Pratt, 1981; Weaver, 1984; *Leucophaea maderae*, Acle *et al.*, 1990). Among other insects, including cockroaches, measurements of basal oöcytes (Roth and Stay, 1962a) and

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implantations of corpora allata or treatment of starved insects with juvenile hormone (Bell, 1971) provide further evidence that starvation retards or suppresses the activity of the corpora allata.

The signals that mediate the process of corpus allatum activation after feeding are poorly understood. Independently, or together, the quantity of ingested food, a critical body mass, the quality of ingested food, or the resultant internal nutritional milieu may all be important cues in activation of the corpora allata. Ingested food may effect the distention of the alimentary tract, resulting in greater juvenile hormone synthesis either through removal of a central nervous system inhibition of the corpora allata or through direct stimulation of the allata. A critical body mass is an important determinant of the time of moulting in some insects (Nijhout, 1975) and it has been shown to influence normal reproduction in many vertebrates. Both hypotheses (alimentary feedback and critical body mass) would argue that the quantity, rather than the quality, of ingested food is important in corpora allata activation. Because non-nutritive bulk (e.g. cellulose) contributes significantly to the quantity of ingested food (Hamilton and Schal, 1991) and fats contribute significantly to body mass and obesity in various animals, these hypotheses would predict that the corpora allata of females that consumed equally of a high cellulose diet or of a low-protein diet would be activated normally. Recent evidence from *Diploptera punctata* indicates that the corpora allata of protein-deprived adult females produce less hormone than the corpora allata of normal females (Woodhead and Stay, 1989). However, because the resultant body mass of protein-deprived females was also significantly lower, and food intake was not measured, the reduced activity of the corpora allata might also be attributable to smaller body mass or lower food consumption.

Engelmann (1965) proposed that nutritional signals affect the activity of the corpora allata both through intact nerves and directly by the internal nutritional milieu. Signals from the brain to the allata may be both stimulatory and inhibitory and may depend on both the quality and quantity of ingested food. In the cockroaches *P. americana* and *D. punctata*, denervation of the corpora allata in starved or protein-deprived females restores juvenile hormone biosynthetic activity to normal levels, indicating that quantitative and/or qualitative signals may affect hormone synthesis by lifting nervous inhibition of the corpora allata (Weaver, 1984; Woodhead and Stay, 1989). However, in *L. maderae* allatal denervation in starved females, or implantation of active corpora allata, stimulated egg maturation only partially, suggesting to Engelmann (1965) that the corpora allata were directly inhibited by the nutritional haemolymph milieu. Herein we show, by measuring oöcyte maturation and juvenile hormone synthesis, that in *Blattella germanica* the nutritional milieu can affect the corpora allata by a route other than nerves between the brain and the corpora allata. We further demonstrate that the degree of brain inhibition of corpora allata activity is dependent upon the quality of the food.

In oviparous *B. germanica*, as in all cockroaches examined to date, the cyclic maturation of oöcytes parallels and is dependent upon juvenile hormone III synthesis by the corpora allata (Belles *et al.*, 1987; Gadot *et al.*, 1989b). A period of increasing juvenile hormone synthesis and pheromone production (Schal *et al.*, 1991) is followed by mating on days 4–6. Grouped females normally oviposit on day 9 and retain the egg-case externally attached to the genital vestibulum for 3 weeks. The German cockroach is an excellent oviparous model for this work because ovarian maturation is highly sensitive to starvation and protein deprivation (Roth and Stay, 1962b; Kunkel, 1966). In this regard, this cockroach acts as many anautogenous dipterans.

MATERIALS AND METHODS

Insects and diets

Stock colonies of the German cockroach were reared at 27°C under a 12 h light–12 h dark photoperiodic regime and provided water and pelleted dog-food (number 1780, Purina) *ad libitum*. In these conditions females have a consistent 9-day preovulatory period; they oviposit eggs that are approximately 2.5 mm in length. Newly emerged (day-0) adult females were starved with access to water for 24 h. Operations were conducted on day-1 and females were immediately placed in groups on experimental diets and water under the same conditions. Males were not provided because their effects on the female's corpus allatum activity and oöcyte growth are minor by day 6 (Gadot *et al.*, 1989a) and females normally do not mate before day 5 under these conditions (Schal, unpublished).

Three different diets were used: (1) dog chow (as above), containing 21% protein; (2) rat chow (number 5012, Purina), containing 22% protein; and (3) a defined artificial diet modified from Hamilton *et al.* (1990) and Cooper and Schal (1992a) containing 25% soybean protein, 46.4% dextrin, 20% α-cellulose, 3% corn oil, 0.6% vitamins, 4% Wesson's salts and 1% cholesterol. All dietary ingredients were obtained from Bio-Serv (Frenchtown, NJ). To obtain any diet ranging from 0%- to 60%-protein, the protein and carbohydrate fractions were interchanged (see Results). To obtain a 78%-protein diet the cellulose content was decreased to 5% in a carbohydrate-free diet. To alter food consumption, the carbohydrate content of a 7.5%-protein diet was reduced to accommodate a cellulose content of 60%. To manipulate the availability of dietary protein without substantial alteration of the diet, 2% trypsin synthesis inhibitor (type I-S from soybean, Sigma) was added to a 7.5%-protein diet containing 20% cellulose.

Hamilton *et al.* (1990) showed that in *Supella longipalpa* nymphal reserves significantly affected adult female reproduction. We found similar preliminary results for *B. germanica* (Cooper and Schal, 1992a, b). "Low-

protein" adult females were obtained either by maintaining newly emerged adults on a 0%-protein diet for 15 days prior to experimentation, or by feeding newly-ecdysed last-instar nymphs on a 7.5%-protein diet. Nymphs and adults were maintained in groups of 10 which were discarded when any sign of cannibalism was evident. The last nymphal stadium of 25- and 7.5%-protein diets was 11.0 ± 0.04 days ($n = 283$) and 12.1 ± 0.11 days ($n = 219$), respectively, with no cannibalism on either diet. On a 5%-protein diet the duration of the last instar was 14.2 ± 0.34 days ($n = 92$), but 32% of the insects were cannibalized.

Juvenile hormone biosynthesis

Juvenile hormone synthesis was measured with the *in vitro* radiochemical assay of Pratt and Tobe (1974) as modified by Feyereisen and Tobe (1981). The corpora allata–corpora cardiaca were dissected out of cold-anaesthetized females and incubated in methionine-free medium 199 with Hanks' salts, L-glutamine and 25 mM HEPES buffered at pH 7.2 (Gibco Laboratories, Grand Island, NY) to which 20 mg/ml Ficoll was added. After all dissections were completed, the glands were transferred to fresh medium containing 75 μM L-[methyl-^{3}H]methionine (NEN, sp. act. 7.4 GBq/mmol) per incubation. The conditions described by Gadot *et al.* (1989b) for *B. germanica* were adopted. Before extraction of the medium according to Feyereisen and Tobe's (1981) partition assay, the glands were removed. Because a simple linear relationship was found between juvenile hormone synthesis and release, indicating that *B. germanica* corpora allata did not accumulate appreciable quantities of juvenile hormone (Gadot *et al.*, 1989b), and juvenile hormone III was identified as the only homologue from *B. germanica* (Camps *et al.*, 1987) we apply the term "juvenile hormone synthesis" to radioactivity extracted from the medium with isooctane (HPLC grade, Fisher Scientific).

Food intake, body mass and oöcyte size

Daily food intake by females was measured gravimetrically as described by Hamilton and Schal (1988). Females were maintained in groups of three in 9 × 2 cm petri dishes in a humidity-controlled incubator (50% RH) under the same temperature and photoperiodic conditions. Fresh body mass of females was measured on day 6.

Basal oöcytes were measured using an ocular micrometer in a dissecting microscope.

Surgical techniques and juvenile hormone analogue treatment

Day-1 females were anaesthetised on ice for 5 min. Because in *B. germanica* the corpora cardiaca and allata are broadly joined by the nervi corporis allati (NCA)-I, transection of these nerves proved difficult. Instead, the nervi corporis cardiaci (NCC)-I and -II were transected. The cervical membrane was incised to expose the retrocerebral complex and the nerves cut with slightly bent, sharpened pins. Antibiotics were not used as more than 95% of the females survived this procedure. Sham-operated females were used as controls.

To uncouple the dependence of both corpus allatum activation and vitellogenesis on dietary proteins, we treated insects with the juvenile hormone analogue *S*-hydroprene. Hydroprene (100 μg, Sandoz Crop Protection Corp.) was topically applied in 1 μl acetone to the mesonotum of day-1 females. The basal oöcyte lengths of treated females were measured on day 6.

RESULTS

Oöcyte maturation on various levels of dietary proteins

Figure 1 shows the relationship between basal oöcyte length on day 6 and the level of dietary proteins, as well as oöcyte lengths on two commercial laboratory diets. Oöcyte maturation on our standard 25%-protein diet was not significantly different from that on either rat-food or dog-food, indicating that this artificial diet is adequate for further endocrinological studies. Mean oöcyte lengths in starved 6-day-old females fed a protein-free diet were not significantly different from those in newly-eclosed females ($P > 0.05$, ANOVA, Games–Howell multiple comparison of means). As the level of protein in the diet increased, so did basal oöcyte length up to 20%-protein. A plateau of maximal oöcyte size was found between 20 and 35% dietary protein. Further increases in protein content, up to 78% of the diet, resulted in smaller basal oöcytes.

This pattern of oöcyte development on various levels of dietary protein was also reflected in the pattern of oviposition (Fig. 2). Females fed a protein-free diet did not oviposit in 60 days of observation. On a 5%-protein diet oviposition was delayed by more than 3 weeks relative to females fed the standard 25%-protein diet. As for oöcyte maturation, a plateau in the mean day of

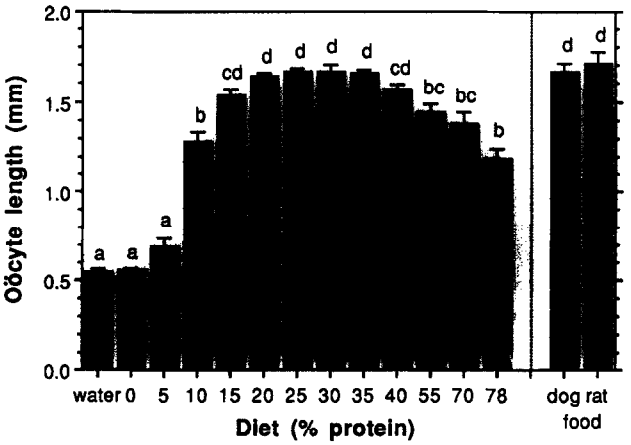

FIGURE 1. Average (±SEM) basal oöcyte length of 6-day-old adult *B. germanica* females either starved or fed various diets. All females were starved on the day of the imaginal moult and then placed in the respective treatment groups on day 1. At least 10 (to 30) females were used per treatment. Treatments with different letters are significantly different from each other ($P < 0.05$, ANOVA, Games–Howell multiple comparison of means).

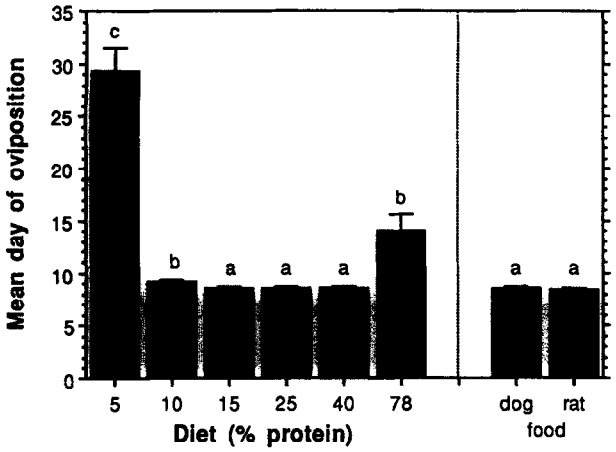

FIGURE 2. Average (±SEM) day of oviposition in *B. germanica* females fed various diets. All females were starved on the day of the imaginal moult and then placed in the respective treatment groups on day 1. At least 30 (to 44) females were used per treatment. Starved and protein-deprived (0%-protein) females did not oviposit in 60 days of observation. Treatments with different letters are significantly different from each other ($P < 0.05$, ANOVA, Games–Howell multiple comparison of means).

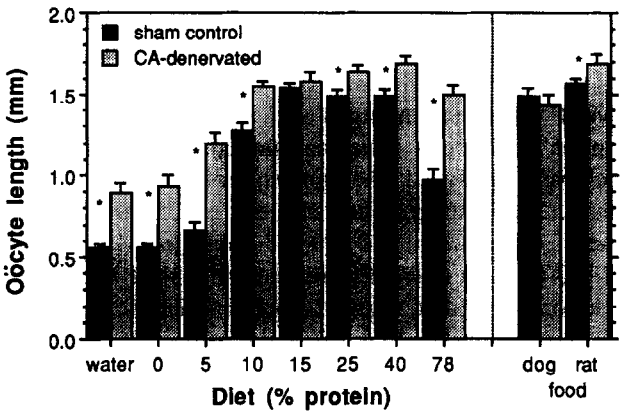

FIGURE 4. Average (±SEM) basal oöcyte length of 6-day-old adult *B. germanica* females after NCC-I and -II transection or sham-denervation. Females were either starved or fed various diets. All females were starved on the day of the imaginal moult and then operated and placed in the respective dietary groups on day 1. At least 13 (to 32) females were used per treatment. Corpus allatum-denervated groups with significantly larger oöcytes than the respective sham-operated controls are denoted by (*) ($P < 0.05$, Mann–Whitney *U*-test).

oviposition occurred on diets containing between 15- and 40%-protein. Females fed a 78%-protein diet exhibited delayed oviposition and 21% died without forming an egg case. Females fed rat food since adult emergence oviposited 8.5 ± 0.08 days after eclosion compared with 8.6 ± 0.10 days in females fed dog food ($P = 0.344$, Mann–Whitney *U*-test).

Stimulation of oöcyte maturation by a juvenile hormone anologue

To determine whether the reduced oöcyte growth and delayed oviposition on low-protein diets were due to suppressed juvenile hormone synthesis and/or a reduced

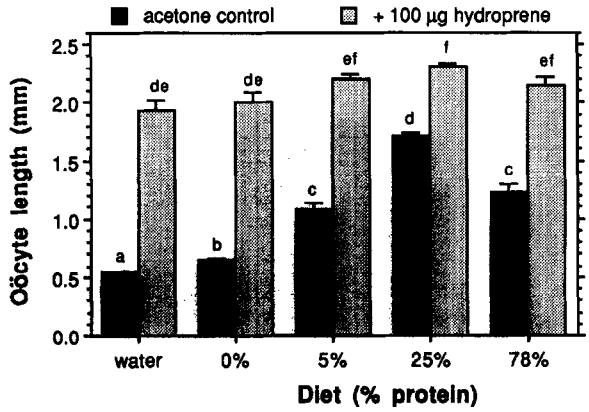

FIGURE 3. Average (±SEM) basal oöcyte length of 6-day-old adult *B. germanica* females after treatment with hydroprene or acetone and either starved or fed various diets. All females were starved on the day of the imaginal moult and then treated and placed in the respective dietary groups on day 1. At least 16 (to 21) females were used per treatment. All hydroprene-treated females had significantly larger oöcytes than the respective acetone-treated controls ($P < 0.05$, Mann–Whitney *U*-test). Treatments with different letters are significantly different from each other ($P < 0.05$, ANOVA, Games–Howell multiple comparison of means).

capacity (nutritional, metabolic) to support oöcyte maturation, we supplied intact virgin females on different diets with an exogenous source of juvenile hormone. Oöcyte growth by day 6 was significantly potentiated by hydroprene in females fed each of the experimental diets as well as in starved females (Fig. 3), indicating that the suppressed oöcyte growth on low-protein diets was largely due to juvenile hormone deficiency. Only starved juvenile hormone analogue-treated females died (21%, 4 of 19), while 10, 29 and 12% of the treated females that were fed 5-, 25- or 78%-protein diets, respectively, oviposited during the 6-day period.

In starved females, as well as in females fed either a low-protein diet or a 78%-protein diet, hydroprene-stimulated oöcyte maturation was only slightly lower than in females fed the 25%-protein diet (Fig. 3). These results suggest that in females fed low- or high-protein diets, oöcyte development is inhibited mainly by juvenile hormone deficiency and only to a minor degree by deficiency in nutrients.

Effects of diets and denervation of the corpora allata on oöcyte maturation and juvenile hormone biosynthesis

Oöcyte maturation and juvenile hormone biosynthesis were compared on selected diets in females with denervated corpora allata and in sham-operated control females. With the exception of the 15%-protein diet and dog food, transection of the NCC-I and II resulted in significantly larger basal oöcytes on day 6 than in the respective sham-operated females under all other dietary treatments, including in starved females (Fig. 4). As with hydroprene stimulation, denervation of the corpora allata potentiated both parameters significantly more in starved females, in protein-deficient females (0%, 5%), or in females on high (78%) protein diets than on intermediate (15%, 25%) protein levels. However, the

basal oöcytes of nerve-transected females were significantly smaller in females fed low-protein diets than in either sham-operated or nerve-transected females fed the standard 25%-protein diet, dog food or rat food (Fig. 4). Together, these data suggest that neurally transmitted signals comprise only one of several mechanisms for regulation of the corpora allata.

Lack of potentiation of oöcyte growth in dog food-fed females with denervated corpora allata appears to be an anomaly. Recently, we have shown that German cockroaches develop more slowly on steam-extruded commercial dog food (Purina number 1780) than on either unextruded or extruded and ground dog food; rat food is normally not subjected to steam extrusion (Cooper and Schal, 1992b). Feeding thus appears to be impeded on whole pellets of dog food. We therefore hypothesized that the slower oöcyte maturation in corpora allata-denervated females was due to reduced consumption on whole pellets of dog food. Indeed, a comparison on day 5 of denervated and sham-operated females that were fed ground dog food for 4 days confirmed that oöcyte maturation was potentiated by NCC-transection (1.52 ± 0.04, $N = 39$ and 1.36 ± 0.03 mm, $N = 43$, respectively, $P < 0.01$, Mann–Whitney U-test.

The pattern of juvenile hormone synthesis by the corpora allata on day 6 was similar to the pattern of oöcyte maturation, with significant potentiation of corpora allata activity in allata-denervated females (Fig. 5). A clear suppression of juvenile hormone synthesis in corpora allata-denervated females that were fed protein-deficient diets (starved, 0%, 5%) or high-protein diets (78%) compared with females that were fed normal diets (25%, dog and rat foods) again highlights the importance of non-nervous humoral signals in endocrine activity.

The corpora allata of females fed the standard 25%-protein diet exhibited higher rates of juvenile hormone

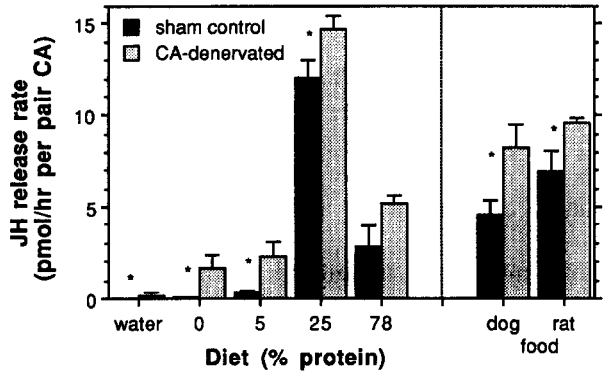

FIGURE 5. Corpus allatum activity of 6-day-old adult *B. germanica* females after NCC-I and -II transection or sham-denervation. Females were either starved or fed various diets. All females were starved on the day of the imaginal moult and then operated and placed in the respective dietary groups on day 1. At least 5 (to 8) females were used per treatment. Corpora allata-denervated groups with significantly greater juvenile hormone biosynthetic rates than the respective sham-operated controls are denoted by (*) ($P < 0.05$, Mann–Whitney U-test). Bars represent SEM.

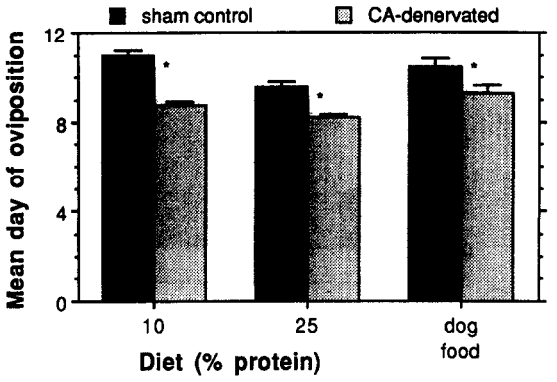

FIGURE 6. Average (±SEM) day of oviposition in *B. germanica* females after NCC-I and -II transection or sham-denervation on various diets. All females were starved on the day of the imaginal moult and then operated and placed in the respective dietary groups on day 1. At least 7 (to 18) females were used per treatment. In all treatments, corpus allatum-denervated groups exhibited significantly faster oviposition than the respective sham-operated controls (denoted by *) ($P < 0.05$, Mann–Whitney U-test).

synthesis than those of females maintained on either dog- or rat-food (Fig. 5), suggesting that our formulated diet was superior to the commercial diets. However, we did not detect any significant differences in the basal oöcyte lengths of either intact females (Fig. 1), or corpora allata-denervated females (Fig. 4) fed these three diets for 5 days. This suggested that there was a gradual increase in the differences among the diets and that these differences might be detected after day 6.

The age of oviposition was monitored in groups of corpora allata-denervated and sham-operated virgin females fed 10- or 25%-protein diets, or ground dog food (Fig. 6). In all cases, allata-denervated females oviposited significantly before the respective sham controls, as expected from the data on oöcyte maturation and juvenile hormone biosynthesis rates. Also, as expected from these data, corpora allata-denervated females fed dog food oviposited significantly later than females fed the 25%-protein diet ($P < 0.01$, Mann–Whitney U-test).

Effects of nymphal protein-deprivation on adult corpora allata activity, oöcyte maturation and oviposition

It was possible that the higher rates of juvenile hormone synthesis and oöcyte growth in both corpora allata-denervated and hydroprene-stimulated females fed low-protein diets could be due to metabolic reserves acquired during the previous nymphal stages. Females were fed either 7.5- or 25%-protein diets as last larval instars, and switched to a 5%-protein diet as adults. Oöcyte size and juvenile hormone biosynthesis rates were significantly lower in 6-day-old adult females that were fed the low-protein diet as nymphs (Fig. 7). In both sets of females, denervation of the corpora allata resulted in potentiation of their activity. However, the rates of juvenile hormone synthesis were significantly lower in corpora allata-denervated females that were raised on the low-protein diet as nymphs. It is significant

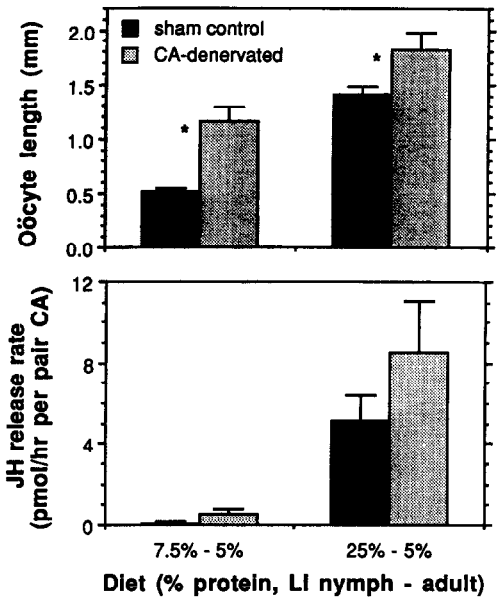

FIGURE 7. Average (±SEM) basal oöcyte length and corpus allatum activity of 6-day-old adult *B. germanica* females after NCC-I and -II transection or sham-denervation. Females were fed either a 7.5%-protein diet or a 25%-protein diet as nymphs, they were starved on the day of the imaginal moult, operated and provided a 5%-protein diet on day 1. At least 4 (to 17) females were used per treatment. Corpus allatum-denervated groups with significantly greater oöcytes or juvenile hormone biosynthetic rates than the respective sham-operated controls are denoted by (*) ($P < 0.05$, Mann–Whitney U-test).

that both parameters (mean oöcyte sizes and juvenile hormone synthesis rates) were similar, respectively, in adult females that were fed either a protein-deficient diet or dog food during the last stadium. However, both parameters were significantly greater in 6-day-old adult females fed the 25%-protein diet as nymphs (Fig. 7) than in females raised on dog-food as nymphs (Fig. 4); hormone synthesis was more than 3-fold higher in allata-denervated adult females fed the 25%-protein diet as nymphs than in females fed dog food as nymphs,

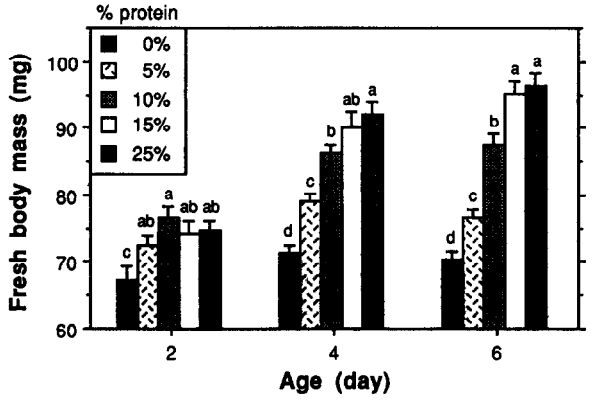

FIGURE 8. Fresh body mass of adult *B. germanica* females fed various diets. All females were placed on the respective diets on the day of the imaginal moult (day 0). Twenty females were used per treatment. The same females were weighed on days 2, 4 and 6. For each day, treatments with different letters are significantly different from each other ($P < 0.05$, ANOVA, Games–Howell multiple comparison of means). Bars represent SEM.

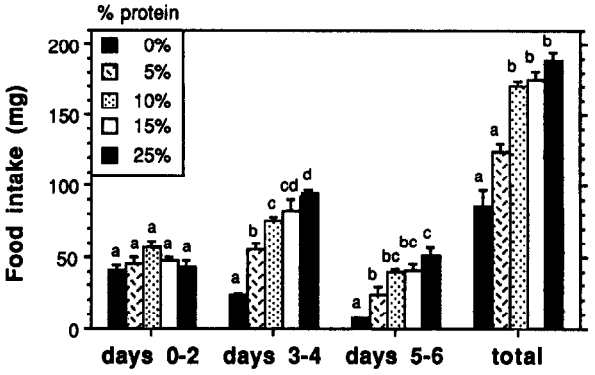

FIGURE 9. Average (±SEM) food intake in adult *B. germanica* females fed various diets. All females were placed on the respective diets on the day of the imaginal moult (day 0). Ten to 15 groups each consisting of 3 females were used per treatment. Net food intake was measured gravimetrically every 2 days. For each period, treatments with different letters are significantly different from each other ($P < 0.05$, ANOVA, Games–Howell multiple comparison of means).

again suggesting that the 25%-protein diet is superior to some commercial diets.

Some females were fed dog food until the imaginal moult and then switched to a protein-free diet to deplete their nymphal reserves. On day 15 their corpora allata were denervated or sham-denervated and the females were provided with a 5%-protein diet. Females with denervated glands oviposited 21.1 ± 9.6 days later ($N = 9$, one female did not oviposit in 50 days) while none of 8 sham-denervated females oviposited in 50 days. The ability of females raised on dog food as nymphs and a 5%-protein diet after the imaginal moult to oviposit on day 29 (Fig. 2) is therefore most likely due to nymphal nutritional reserves. These results show that the potentiating effects of corpora allata denervation are most pronounced in nutrient-deficient females, in which central nervous system inhibition of the corpora allata is presumably greatest.

Body mass and food intake on various levels of dietary proteins

Fresh body mass varied directly in relation to changes in dietary protein content (Fig. 8). These data suggest that, in addition to the quality of the diet, either body mass or the quantity of diet consumed might also influence corpora allata activity and oöcyte development.

Food intake was monitored gravimetrically between days 2 and 6. Consumption of diets varied directly with the protein content of the diet (Fig. 9). These results clearly show that suppression of corpora allata activity and oöcyte maturation in protein-deficient adults are correlated with, and probably influenced by, the quantity of food consumed, by the resultant body mass, as well as the quality of the adult diet and its effects on the internal nutrient milieu. Thus, before suppressed corpora allata activity is interpreted as a response to a specific nutritional deficiency, it is essential to show that neither food intake nor body mass are reduced in females

TABLE 1. Effects of dietary manipulations on food consumption, body mass and oöcyte development in females

Diet†	*N*	Oöcyte length (mm)	Body mass (mg)	Total food consumed (mg)
Control				
20%-cellulose diet	42	1.20 ± 0.04	98.2 ± 1.43	147 ± 4.63
Experimental				
60%-cellulose diet	22	1.51 ± 0.06*	95.9 ± 2.16	172 ± 6.31*
20%-cellulose/2%-TSI diet‡	42	1.08 ± 0.03*	95.7 ± 2.05	154 ± 3.97

†Newly emerged females were provided with water only for 24 h and then one of the 3 preweighed diets, each containing 7.5% protein, for 5 days. On day 6, fresh body mass, total food consumption, and oöcyte lengths were determined. Values are the mean ± SE.

*Indicate significantly different from the control diet ($P < 0.05$, Student's t-test).

‡TSI = trypsin synthesis inhibitor.

that are fed the deficient diet. This was not possible with diets in which only the protein to carbohydrate ratio changed (Figs 8 and 9).

We employed two approaches to dissociate food consumption and body mass from corpora allata activity and oöcyte growth. First, we increased the cellulose content of a 7.5%-protein diet from 20 to 60%. Body mass was unaffected (Table 1). However, oöcyte size was significantly greater in females fed the higher cellulose diet, as females feeding on cellulose-diluted diets consumed more diet (i.e. more protein).

To control for the elevated food consumption, 2% trypsin synthesis inhibitor from soybean was added to a 7.5%-protein diet containing 20%-cellulose. Neither body mass nor total food consumption were changed relative to control females (Table 1). However, oöcytes were significantly smaller on this diet. Together with data from Fig. 3, showing that oöcyte maturation can be induced with hydroprene in females fed a protein-free diet or even in starved females, these data demonstrate that signals associated with the protein content of the diet partially lift the brain inhibition of the corpora allata.

DISCUSSION

The juvenile hormone biosynthetic activity of the corpora allata in adult female cockroaches is dependent upon and modulated by intrinsic signals originating from the brain, ovary, mating and nutrients, and by extrinsic signals including temperature, pheromones, tactile cues and social conditions such as isolation and crowding (Engelmann, 1970). The degree of dependence of allatal activity on any one of these factors varies widely and appears to be species-specific even in closely related cockroach species. In *B. germanica*, activity of the corpora allata is potentiated more by signals from grouping than from mating (Gadot *et al.*, 1989a). Conversely, stimuli from mating stimulate juvenile hormone synthesis by the corpora allata in *S. longipalpa*, in which isolated and grouped adult females exhibit similar patterns of oöcyte development (Chon *et al.*, 1990). Signals associated with feeding influence oöcyte maturation (Kunkel, 1966; Roth and Stay, 1962a, b) and by inference, corpus allatum activity. In the present study, we documented that in starved adult females with access to water, juvenile hormone biosynthesis and oöcyte maturation are suppressed. Similarly, artificial diets containing either low or high levels of protein inhibit both reproductive parameters. Intermediate levels of dietary proteins, including 25%-protein which approximates the level in most commercial diets, result in juvenile hormone synthesis and oöcyte growth that are at least as high as on control commercial diets. Treatment with the juvenile hormone anlogue hydroprene stimulates oöcyte maturation in females fed all diets, suggesting that in *B. germanica* oöcyte maturation is suppressed on deficient diets mainly due to juvenile hormone deficiency. The effects of denervation of the corpora allata are more pronounced on low- and on high-protein diets than on intermediate levels of dietary protein. However, in all treatments, corpora allata-denervation increases the respective spontaneous rates of juvenile hormone biosynthesis.

Proteins as specific signals modulating corpora allata activity

The quantity and the quality of ingested food and the resultant internal nutritional milieu can affect juvenile hormone synthesis either directly or indirectly by influencing body mass. Protein deficiency in the last larval instar reduced corpus allatum activity in adult female *D. punctata* but these adults also attained significantly lower body mass than control females; food intake was not monitored (Woodhead and Stay, 1989). Thus, in *D. punctata* it is currently unknown to what extent specific signals from protein deficiency restrain the corpora allata independently of feedback directives from body mass and from the quantity of ingested food. Similarly, in *L. maderae*, although the oöcytes of starved virgin females did not mature faster in response to either mechanical distention of the gut (by ligation of the hind-gut or by sealing the anus) or distention of the abdomen (by implantation of paraffin pellets), conclusions are confounded by an observed reduced food intake (Engelmann, 1960).

It is difficult to dissociate these parameters (consumption, body mass, corpora allatum activity) in relation to

changes in dietary proteins while maintaining iso-caloric diets and equal aged females (see Figs 8 and 9). However, by diluting the diet with cellulose we were able to increase food consumption and oöcyte maturation without affecting body mass (Table 1). This suggests that body mass, although correlated with corpora allata activity, does not play a major role in regulating juvenile hormone biosynthesis in the adult female. Also, by inhibiting digestive proteases with dietary trypsin synthesis inhibitors we were able to reduce oöcyte growth without affecting either body mass or diet consumption. Together with other data (see Fig. 3), this suggests that neither body mass nor food volume or mass have significant effects on corpora allata activity. Rather, the quality of the diet, specifically its protein content, affects juvenile hormone biosynthesis, as suggested by Engelmann (1965) and Woodhead and Stay (1989).

Our work suggests that nutrient-dependent humoral signals also affect the corpora allata. This idea was first advanced by Engelmann (1965) based on the observation that denervation of the corpora allata in starved *L. maderae* did not restore oöcyte maturation to the level exhibited by fed control females. He further postulated that in females with denervated corpora allata, cyclic changes in blood protein levels might modulate volumetric changes in the corpora allata through humoral signals, and that a richer nutrient milieu in ovariectomized females might support the hypertrophy of the corpora allata in some insects. Pipa (1982) similarly showed that although neural signals were important, transection of both NCA-I in starved *P. americana* females did not stimulate oöcyte growth to the same level as in fed unoperated females, also suggesting that humoral factors might be involved. However, because juvenile hormone synthesis was not determined in either species, it was not known whether these effects were on hormone synthesis or downstream on vitellogenesis.

Diets and the roles of corpus allatum nerves

Our defined artificial diet appears to be superior to commercial dog food and rat food, at least in the short-term studies reported herein. It also appears that dog food is significantly inferior to rat food in supporting both nymphal development and adult reproduction in *B. germanica* (Cooper and Schal, 1992b). These findings have significant implications to previous conclusions from endocrinological studies. In all cockroaches studied to date, the corpora allata have been shown to be restrained to varying degrees by neural signals from the brain (see Feyereisen, 1985; Tobe and Stay, 1985; Khan, 1988). Specific cues such as those from mating (*D. punctata*, Stay and Tobe, 1977; *N. cinerea*, Lanzrein *et al.*, 1981; *S. longipalpa*, Smith *et al.*, 1989; *P. americana*, Weaver and Pratt, 1977; *B. germanica*, Gadot *et al.*, 1989a; *L. maderae*, Acle *et al.*, 1990), feeding (*P. americana*, Weaver and Pratt, 1981; Weaver, 1984; *D. punctata*, Woodhead and Stay, 1989; *L. maderae*, Acle *et al.*, 1990; *B. germanica*, present study) and social stimuli (*B. germanica*, Gadot *et al.*, 1989a) can, together or independently, lift the brain inhibition on the corpora allata. Therefore, transection of the nervous connections between the brain and the corpora allata, which removes this brain inhibition, has generally been considered to "mimic" these species-specific stimuli. Yet in all cockroach species, denervation of the corpora allata potentiates juvenile hormone synthesis and oöcyte maturation beyond the normal levels exhibited by females that experience all the respective disinhibiting cues. This observation appears to be in conflict with the tacit assumption that the experimental conditions are "optimal" and indeed, it suggests that in the presence of as yet unidentified species-specific conditions (internal and/or external signals) the corpora allata should operate at the capacity expressed upon denervation. Our data suggest that certain commercial diets may be deficient for some cockroach species, resulting in varying degrees of corpora allata inhibition in normally grown females.

This hypothesis pertains to a current dispute in the literature. Weaver (1984) showed that transection of the NCA-I in mated *P. americana* reduced both juvenile hormone synthesis and oöcyte growth. In virgin females, both operated and sham-operated females exhibited similar patterns, while in starved virgins corpus allatum denervation enhanced both parameters only "slightly." These results contradicted Pipa's (1982) conclusions that corpora allata-denervation potentiated oöcyte maturation in starved virgin females. Although Pipa (1986) convincingly confirmed and extended his earlier results that the suppression of oöcyte growth in starved females is relieved by NCA-I transection or by insemination, and that critical periods and ages are important for the expression of these differences, Weaver and Edwards (1990) ignored this work and recently concluded that "direct neural modulation of juvenile hormone biosynthesis does not feature highly in the regulation of ovarian maturation in [*P. americana*]." This was based largely on work with fed mated females that exhibited similar mean ovarian cycle lengths whether the corpora allata were denervated or only sham-operated.

In addition to the differences in experimental conditions between the two research laboratories (outlined in Pipa, 1986), our data with *B. germanica* suggest that previously unrecognized differences in diets might have contributed significantly to this discrepancy. In spite of the fact that Pipa's insects were reared at a slightly higher temperature (27–29°C) than Weaver's (27°C), females oviposited appreciably later in the former laboratory [day 12 in Pipa (1985) vs days 8–9 in Weaver and Pratt (1977)]. In Pipa's laboratory cockroaches were fed dog chow, whereas Weaver provided them with a ground mixture of oatmeal, dog chow, peanuts and yeast powder (17:10:4:1, respectively). If the latter dietary mixture is more adequate for *P. americana*, and dog chow is inferior, as in *B. germanica*, the corpora allata of fed mated females in Weaver's experiments would be more disinhibited than those in Pipa's studies.

Furthermore, our data with the German cockroach suggest that nutrient reserves for reproduction may be accumulated during the nymphal instars and buffer short-term deficiencies in the adult diet (Cooper and Schal, 1992a, b). For starvation experiments, Weaver generally collected gravid insects from well-fed stock colonies; these insects would have had ample opportunity to acquire appreciable nutritional reserves. Pipa, on the other hand, selected newly emerged insects, previously fed dog food, for such experiments. These were likely less buffered by nutritional reserves (see Kunkel, 1966; Pipa, 1985). Indeed, Weaver and Pratt (1981) showed that starved females experienced two juvenile hormone peaks and two oviposition cycles before juvenile hormone synthesis was suppressed, and it followed that significant disinhibition would not be evident in well fed females that were starved for only 7–11 days (see Weaver, 1984). Weaver and Pratt (1977) also attributed differences in oöcyte maturation in different laboratories to dietary regimes, but failed to account for such differences in their interpretation of later experimental results.

Our results with *Blattella*, together with inferences from *Periplaneta*, suggest that the quality of laboratory diets may directly or indirectly profoundly affect the interpretation of endocrinological studies. They also strongly suggest that discrepancies in life history parameters for the same species (see Roth, 1981) may be at least in part related to the effects of food quality. Our results highlight the need for detailed descriptions of dietary regimes in such reports.

In a previous study we were unable to document potentiation by day 6 of oöcyte growth in dog food-fed grouped virgin *B. germanica* with denervated corpora allata (Gadot *et al.*, 1989a), but oviposition was significantly accelerated in both virgin and mated operated females relative to sham-operated controls (unpublished). The present study clearly shows that in all dietary treatments corpora allata denervation accelerates both oöcyte growth and juvenile hormone synthesis. Moreover, the divergence of nerve-transected females from sham-operated females increases and is most clearly exhibited at oviposition and on minimally adequate diets. These data with *B. germanica* lend support to Pipa's (1986) conclusions from *P. americana* that a critical period is required to detect the effects of corpora allata denervation, and that this period occurs around the time when neural disinhibition can occur normally in response to species-specific stimuli (insemination and feeding in *P. americana*; insemination, feeding and grouping in *B. germanica*).

Corpora allata nerves and cycles of juvenile hormone biosynthesis

Our results with *B. germanica* bring to the fore a recent proposal on cyclic juvenile hormone synthesis by the corpora allata. In their critique of the roles of corpora allata nerves in *P. americana*, Weaver and Edwards (1990) proposed not only that intact allatal nerves are unnecessary, but also that the presence of oscillating titres of juvenile hormone are unnecessary for the periodic production of oöthecae. This they reason largely on the basis of cyclic production of oöthecae in juvenile hormone analogue-treated allatectomized females. In support of this idea they cite their previous difficulties in correlating juvenile hormone activity with specific reproductive events. Several lines of evidence, some from our current work, challenge this hypothesis.

Clearly, the inability to detect specific reproductive events is due mainly to imprecise staging of insects, as evidenced by cycles of protein synthesis in the left colleterial gland in relation to oöcyte maturation and juvenile hormone synthesis in *P. americana* (Iris and Sin, 1988). Moreover, in ovoviviparous and viviparous females, as well as in oviparous females with non-overlapping growth of basal and penultimate oöcytes, low juvenile hormone titres are necessary to sustain pregnancies and to avoid premature expulsion of oöthecae. This was clearly recognized by Englemann (1960) who concluded that in *L. maderae* with isolated corpora allata "the embryos are always extruded from the brood sac prematurely" and "in the intact animal, therefore, the innervation of the corpora allata assures an activity of the corpora allata at the 'right time'." In many oviparous insects, the process of oviposition is complex, requiring specific substrates and environmental conditions. The corpora allata must be able to respond to such adverse conditions.

Arguments based on phylogenetic considerations that the more primitive oviparous blattids (e.g. *P. americana*) possess an inherently different scheme of corpus allatum regulation from the advanced blaberids (e.g. *D. punctata*, *N. cinerea*, *L. maderae*) (Weaver and Edwards, 1990) are confounded by the fact that the more primitive Cryptocercidae exhibit periods of suppressed ovarian development in relation to brood care (Nalepa, 1988) (and presumably low corpora allata activity) and other oviparous blattellid species [e.g. *B. germanica*, *S. longipalpa* (see Smith and Schal, 1990)] exhibit appreciable neural regulation of the corpora allata as do the blaberids.

Lastly, the arguments we presented above stress the importance of neural and humoral modulation of allata activity in response to environmental and internal signals. This process involves ongoing adjustments in the rate of juvenile hormone synthesis by the corpora allata and necessarily results in cycles of activity. Weaver's (1984) data clearly show "a graded series of sexually suppressed females ... produced by withholding one or more ... stimuli" (feeding, drinking, mating and crowding), indicating that under normal variable conditions some suppression of corpora allata activity would occur, resulting in a juvenile hormone cycle. Our data clearly show this in virgin, isolated, protein-deficient and ovariectomized *B. germanica* females. In formulating their hypothesis, Weaver and Edwards (1990) examined only well-fed mated females, which clearly synthesize hormone and oviposit at nearly maximal capacity. Indeed, under optimal environmental and internal conditions, if

mate finding (pheromone production and mating) is unnecessary, if pregnancy does not occur, and if several oöcytes within each ovariole are vitellogenic concurrently, it is expected that constant high levels of juvenile hormone will maximize oviposition cycles.

Our recent results have shown that in both viviparous and oviparous cockroach species activation of the corpora allata in the adult female occurs primarily through large increases in the size of allatal cells with only minor inconsequential changes in total cell number (Chiang *et al.*, 1989, 1991b). Experimental manipulation, such as ovariectomy, significantly affects the temporal changes in juvenile hormone synthesis (Gadot *et al.*, 1991) and in cell sizes, but not in cell number (Chiang *et al.*, 1991a). We now hypothesize that the insect copes with constant variation in resource quality and availability and responds to its internal state via a process of developmental plasticity of the corpora allata. In starved or protein-limited *B. germanica* (unpublished) and in starved *L. maderae* (Acle *et al.*, 1990), the rates of juvenile hormone biosynthesis *in vitro* in the presence of farnesoic acid or farnesol, respectively, decline as do spontaneous rates, suggesting a general reduction in the levels of all enzymes including the later *O*-methyl transferase and/or 10,11-epoxidase. Although Weaver and Pratt (1981) emphasize a starvation-induced modulation of control points prior to the last two steps in the juvenile hormone biosynthetic pathway in *P. americana*, their data clearly indicate that the corpora allata of starved females exhibit a similar decline in farnesoic acid-stimulated activity as in the other starved cockroach species, but it takes a longer starvation period before the decline in obvious.

Thus, in cockroaches, species-specific signals as well as humoral signals from maturing oöcytes and the nutritional milieu result in a graded and gradual lifting of neural inhibition of the corpora allata, resulting in growth of allatal cells and synthesis of juvenile hormone. Specific neural and humoral signals (e.g. the oöthecae in gravid females, ecdysteroids, starvation, nutrient limitation) suppress corpora allata activity through inhibition of cellular growth. In addition to trophic and inhibitory signals, Chiang *et al.* (1991a) also hypothesized that some cues from the ovaries and egg case act to synchronize the volumetric changes in corpora allata cells.

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