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Rates of juvenile hormone synthesis control caste differentiation in the stingless bee *Scaptotrigona postica depilis*

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Summary. In social insects the expression of caste-specific characters is controlled by juvenile hormone (JH) during definite sensitive periods in preimaginal development. For a number of stingless bee species the existence of such a JH-sensitive period has already been demonstrated. Queen development can be induced by topical JH applications during the cocoon spinning phase of the last larval instar. Neither JH titers nor rates of JH synthesis were known so far for this subfamily of eusocial bees distinguished by a pronounced caste dimorphism. As the pantropically distributed stingless bees with approximately 400 recent species are the largest group of social bees, JH synthesis was studied in one of the species that can be kept under laboratory conditions. An in vitro radiochemical assay was used to measure stage- and caste-specific activities of the corpora allata (CA). For the first time in a eusocial hymenopteran species it was demonstrated how the endocrine system is reacting to trophogenic stimuli capable to induce caste differentiation during larval development. Generally JH synthesis in queen CA was found to be 30–80% higher than in workers during the penultimate and last larval instar, but a strong and distinct caste-specific modulation of JH synthesis was only observed right before the onset of a JH-sensitive period in the cocoon spinning phase of the fifth instar.

Key words: Meliponinae – caste development – corpora allata activity – in vitro juvenile hormone synthesis

Introduction

One of the most impressive examples of insect polymorphism is the evolution of caste syndromes in social insects. Together with polymorphism in aphids, locust phase polymorphism, and seasonal variation of coloration patterns in some butterfly species, the queen/worker castes in bees have been considered as ecomorphs because of the external programming of developmental pathways by factors like photoperiod, temperature and larval nutrition (de Wilde and Beetsma 1982). Primary trigger in the caste development of bees is a trophogenic stimulus based on differential feeding of queen and worker larvae by nurse bees. The transformation of such socio-environmental cues into an endogenous reaction affecting morphogenesis is yet poorly understood. The same is true for the acting morphogen(s) and regulatory functions controlling polymorphic differentiation on the cellular level. The role of juvenile hormone (JH) in this context has become a central issue of research on insect polymorphism during the last 10 years.

In honey bees JH was found to induce queen morphogenesis when applied to worker larvae (Wirtz 1973; Rembold et al. 1974; Copijn et al. 1979; Dietz et al. 1979). A similar situation exists in bumble bees (Röseler 1976) and stingless bees (Velthuis and Velthuis-Kluppell 1975). These three groups of eusocial bees, however, differ from one another with respect to the timing of developmental periods during which exogenous JH most effectively induces queen characters without interfering with concomitant metamorphic processes. In *Apis mellifera* such a caste-relevant JH-sensitive period was found to extend from the fourth into the early fifth instar (Wirtz 1973; Dietz et al. 1979), while in the bumble bee *Bombus hypnorum* it is restricted to the prepupal phase (Röseler 1976). In all stingless bee species studied so far the cocoon spinning phase in the fifth larval instar was recorded to react most sensitively to exogenous JH (Campos 1978, 1979; Campos et al. 1975, 1983).

A general model on the physiological role of JH in insect caste and phase polymorphism was recently proposed by Nijhout and Wheeler (1982). According to this model the presence of JH during JH-sensitive periods programs the multipotent genotype for a specific morphogenetic pathway. The effective JH haemolymph titer depends on the rates of JH synthesis and JH inactivation by specific or unspecific esterases, and on the amount of hormone bound in target tissues. For the queen honey bee Rembold (1987) describes a strongly elevated JH-III content coinciding with the JH-sensitive period. A similar timing of JH titers and sensitive period was found in the bumble bee *Bombus hypnorum* (Strambi et al. 1984). As no data on JH synthesis or titers in the largest group of social bees, the pantropically distributed stingless bees (Meliponinae), did yet exist, corpora allata activity was studied in a trigonine species, *Scaptotrigona postica depilis*, using a radiochemical assay in order to look for caste-specific modulations of JH synthesis. This radiochemical assay (Tobe and Pratt 1974; Pratt and Tobe 1974) allows to measure in vitro rates of JH synthesis based on the incorporation of a radiolabelled methionine methyl group into methyl farnesoate, one of the last steps in JH biosynthesis.

Material and methods

From twelve colonies of *Scaptotrigona postica depilis* kept in the meliponary of the Dept. of Genetics, Ribeirão Preto, Brazil, worker larvae were sampled from the brood combs

and their developmental stage was assessed. The last larval instar was divided into 7 substages. During the first two (L5H and L5F) the larvae are still feeding. The following three (L5S1, L5S2, L5S3) are cocoon spinning stages which were characterized by the progressing voidance of the larval gut. In L5S3 apolysis of the larval cuticle commences, leading over to the prepupal stages (PP1 and PP2). Queen larvae were reared in vitro by transfering first instar worker larvae into queen-sized artificial brood cells made from honey bee wax (Camargo 1972). Each artificial queen cell was supplied with 140 mg of fresh worker larval food. This amount is found in regular queen cells and corresponds to the quadruple supply of a worker cell.

For the study of rates of JH synthesis in *S. postica depilis* larvae, brains with the tightly attached retrocerebral complexes were dissected in a PIPES-buffered balanced salt solution based on honey bee medium (Kaatz et al. 1985). Optic lobes and suboesophagal ganglia were removed. The dissected complexes consisting of the median parts of the brain, corpora cardiaca (CC) and corpora allata (CA) with a piece of supporting oesophagus were rinsed in modified honey bee medium. Brain-CC-CA complexes instead of isolated CA were used since we were mainly interested to follow up the CNS-transduced reaction to the trophogenic stimuli by the retrocerebral endocrine system as a whole, and since the regulatory centers located in the pars intercerebralis are known to modulate CA activity via neural and humoral pathways (Tobe and Stay 1985).

The brain-CC-CA complexes were incubated in vitro in 0.05 ml of honey bee medium (Kaatz et al. 1985) at 30° C for 4 h. The medium was modified for this purpose by subtracting cold methionine from the formulation and substituting BSA by Ficoll (20 mg/ml). Anorganic cationic and anionic conditions, pH and osmolarity of the medium were adjusted to the conditions described for larval honey bee haemolymph (Florkin and Jeuniaux 1973). Media were sterile filtered in a Sterivex-GV unit (Millipore; pore size 0.22 µm) and stored at 4° C. Immediately before use L-(^{14}C-methyl) methionine (NEN, 51.4 mCi/mM) was added to the medium at final concentrations between 0.29 and 0.46 mM (specific activities 17.4–27.7 mCi/mM). The flat-bottomed glass incubation vials had been pretreated with polyethylene glycol (Giese et al. 1977) in order to prevent adsorption of juvenile hormone. At the end of the incubation period the brain-CC-CA complexes were separated from the medium. JH released into the medium during the incubation period was extracted with methanol-chloroform and chromatographed on plastic-backed silica gel plates (Merck 60 F_{254}) with xylene-ethylacetate (4:1) as solvent (Weaver et al. 1980). JH bands were identified by TLC co-separation of cold JH-III (Calbiochem) as an internal standard. JH-III is the only juvenile hormone homologue detected in *Scaptotrigona postica depilis* (Rembold, personal communication). The corresponding bands were cut out of the TLC plates, and the radioactivity was measured by liquid scintillation counting. 6–8 brain-CC-CA complexes had to be coincubated in order to obtain clearly identifiable amounts of radiolabelled JH. For all developmental stages studied in each of the two castes 3–5 replicates were run.

Results

Rates of JH synthesis by the CA of *Scaptotrigona postica depilis* larvae fluctuate at rather low activity levels throughout the fourth and fifth instar (Fig. 1a), and in worker larvae they even lie close to the detection limit calculated for this radiochemical assay (Tobe and Stay 1985). Nevertheless, the relatively small standard errors indicate a high degree of reproducibility of the in vitro experiments which, therefore, in the differing release rates of labelled JH reflect the in vivo programming of a stage and caste specific CA activity. In the modified honey bee medium incorporation kinetics for actively JH synthesizing CA of L5S3 worker larvae were linear for incubation periods between 2–6 hours (Fig. 1b). For this reason incubation periods of 4 h were chosen for the analyses of JH synthesis in the different developmental stages. The initial lag phase in the incorporation kinetics probably results from the equilibration of intracellular methionine pools.

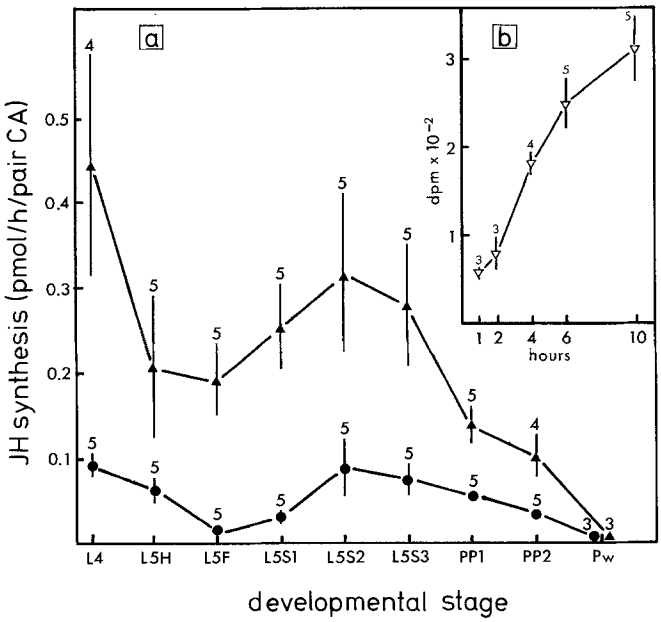

Fig. 1a, b. Rates of in vitro juvenile hormone synthesis by brain-CC-CA complexes of the stingless bee *Scaptotrigona postica depilis*. **a** CA activity in late larval and early pupal stages of queens (▲) and workers (●). *L4*, fourth larval instar; *L5H* and *L5F*, feeding stages in the fifth larval instar; *L5S1–L5S3*, cocoon spinning stages; *PP1* and *PP2*, prepupal stages; *Pw*, white-eyed pupa. **b** Incorporation kinetics of ^{14}C-methyl methionine into JH by corpora allata dissected from L5S3 worker larvae. Mean values, bars = standard error of means, numbers = replicates for each developmental stage

With the transition from the fourth to the fifth larval instar, JH release from both queen and worker CA drops drastically (Fig. 1a). In worker larvae this decrease is much more pronounced and continues until the end of the larval feeding phase, while in L5F queen larvae CA activity remains on the same level as in L5H. By the middle of the spinning phase JH synthesis reaches a maximum in individuals of both castes. The timing of CA activity resulting in this peak of JH synthesis, however, is remarkably different in the two castes. In L5S1 worker larvae the rate of JH release remains on a level only slightly above the minimal release rate in L5F, while in L5S1 queen larvae a steep increase in JH synthesis was recorded.

The fact that rates of JH synthesis are distinctly higher in queen larvae does not necessarily imply a caste-specific pattern of corpora allata activity. This could simply be an epiphenomenon conditioned by the existent differences in

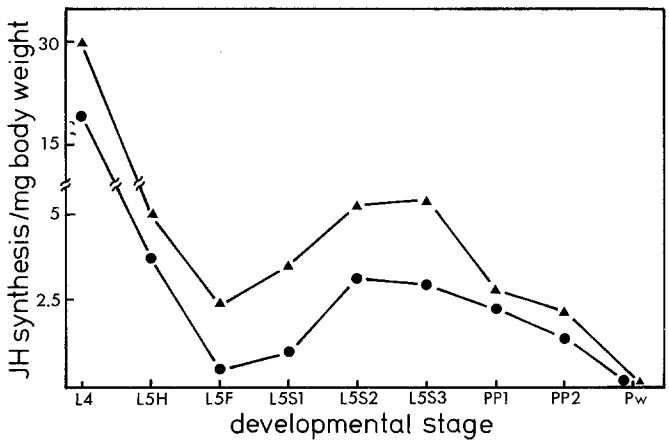

Fig. 2. Rates of JH synthesis corrected in relation to body weight during late larval and early pupal development of *S. postica depilis* queens (▲) and workers (●). For explanations c. Fig. 1

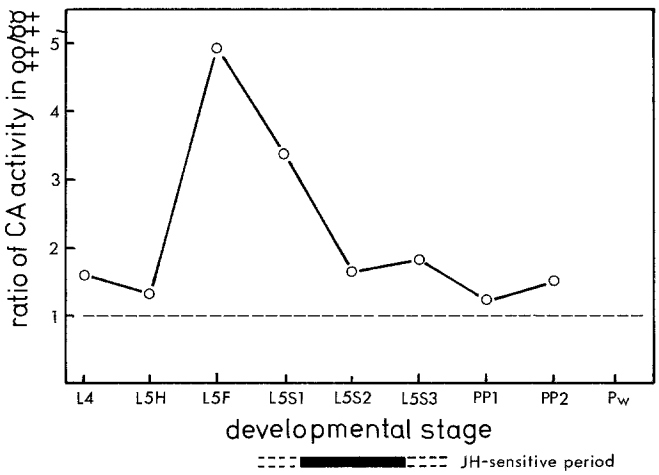

Fig. 3. Queen/worker ratios of JH synthesis of *S. postica depilis*. For explanations c. Fig. 1. The JH-sensitive period for the induction of queen characters in stingless bees is positioned according to the data of Campos (1978, 1979); Campos et al. (1975, 1983) and Velthuis and Velthuis-Kluppell (1975)

body weight and corresponding haemolymph volume between the two castes. But after calculating rates of JH synthesis on a mg body weight basis, relative rates of JH synthesis still remained higher in queens, and the nearly parallel fluctuations in relative CA activity became even more evident (Fig. 2). Obviously, prospective queen larvae synthesize 30–80% more JH per unit body weight than worker larvae during the fourth and in most substages of the fifth larval instar.

Discussion

Problems arising close to the sensitivity limits of the radiochemical JH assay can be circumvented in two ways. The substitution of (methyl-^{14}C)methionine by a tracer of higher specific activity like (methyl-^{3}H)methionine would the method of choice in such a case (Greenberg and Tobe 1985). However, the high rates of radio degradation of (methyl-^{3}H)methionine were prohibitive here since this study had to be carried out in Brazil due to the availability of bee material. Instead, the incubation conditions were carefully optimized by adjusting the composition of the medium as close as possible to haemolymph conditions in bee larvae. This seems to be a crucial point, especially for corpora allata with generally low synthetic activity, and corroborates the findings of Weaver et al. (1980). In a study on rates of JH synthesis in adult honey bees, Bühler et al. (1983) could show as well that medium composition is of pivotal importance since CA activities reflected the course of JH content in whole body extracts much better in a medium approximating haemolymph conditions than in TC-199 with Hank's salts. This vertebrate medium with a comparatively high sodium/potassium ratio is commonly used in CA assays on hemimetabolous insect species, which also have high sodium/potassium ratios in their haemolymph (Florkin and Jeuniaux 1973), but apparently causes problems in studies on hymenopteran species characterized by generally low sodium/potassium ratios, especially in the case of bee larvae.

The low levels of JH synthesis in last instar larvae obtained in this study cannot be attributed to inadequate in vitro conditions for two more reasons. Firstly, the CA activity curves for the two castes of *S. postica depilis* closely correspond to JH-III contents measured in this species (Rembold, personal communication). Secondly, in a number of insect species similarly low rates of JH synthesis have been observed during late larval development (Granger et al. 1982; Couillard et al. 1984).

It is important to stress that measuring CA activity directly in a radiochemical JH assay results in data much more reliable than indirect parameters like CA volume and numbers or ultrastructural changes in CA cells which are not always correlated with actual rates of JH synthesis (Tobe and Stay 1985). Bueno and Beig (1980) had measured CA volume changes in preimaginal development of *Scaptotrigona postica*. They found significant size differences between queen and worker CA in the larval spinning stage and in early pupal development. Our measurements of CA activity, however, only confirmed the situation in the spinning stage, while in white-eyed pupae of both castes rates of JH synthesis by the CA had dropped to zero, as would be expected from general considerations on the regulation of metamorphosis (Riddiford 1980).

The fact that principally JH synthesis in queen and worker CA runs in a parallel fashion can be understood considering that regulation of metamorphosis is the main functional role of JH in late preimaginal development of holometabolous insects. Other functional aspects will only become apparent once this major "background function" has been filtererd out. Indeed, a pronounced caste-specific modulation in JH synthesis became visible after calculating CA activity ratios of queens versus workers based on the body-weight-corrected data (Fig. 3). This modulation in hormone production activity precisely precedes the JH-sensitive period relevant for queen development in all stingless bee species studied so far, including the very closely related *Scaptotrigona xanthotricha* (Campos et al. 1983).

The basic pattern of CA activity in stingless bee larvae corresponds to that described for *Manduca sexta* (Granger et al. 1982), a lepidopteran species in which the endocrinological events controlling metamorphosis have been studied in great detail. In *Manduca,* rates of JH synthesis decline towards a minimum at the end of the larval feeding phase. With the commencement of gut purge it rises again to reach a peak level in prepupal development. These fluctuations

in JH synthesis and titers (Fain and Riddiford 1975) have been shown to coincide with two JH-sensitive periods of the epidermis, and thus to induce important consequences in the programming of epidermal cells to synthesize a pupal cuticle (Riddiford 1980).

The caste-specific course of late larval JH synthesis, evaluated for the first time in a bee species, therefore has to be interpreted as being composed of a general CA activity pattern responsible for the regulation of metamorphosis, and a superimposed queen-specific peak of JH synthesis. At the end of the feeding period queen larvae apparently do not diminish the level of CA activity as rigidly as worker larvae. In the early spinning phase they then resume JH synthesis faster and at a higher rate than worker larvae. The resulting pronounced short-term modulation of JH synthesis right before a temporal window of JH susceptibility prototypically shows how a hormonal signal should be constructed, whenever an unambiguous decision for a polymorphic developmental pathway is requested. In the case of highly eusocial insects such a strong hormonal signal apparently translates the nutritional switch signal, and thus prevents the occurrence of individuals with intercaste characters (Wittmann and Engels 1987).

The demonstration of such a caste-specific modulation of JH synthesis in eusocial bees gives rise to two questions. Firstly, it remains to be shown whether juvenile hormone itself is the morphogen regulating the expression of polymorphic characters in those target tissues capable of caste-specific differentiation. This question cannot be answered by the classical way of topical JH application but requires the development of an adequate in vitro system for testing the reaction to hormonal stimuli in isolated tissues. Secondly, the possibility to measure CA activity of bee larvae in a radiochemical assay can be used as an interesting tool to gain insight into the steps by means of which the CNS transforms the trophic stimuli into a caste-specific pattern of JH synthesis. Comparisons between rates of JH synthesis in brain-CC-CA complexes and isolated CA should reveal whether worker CA are inhibited or queen CA are stimulated by neural or neurohormonal pathways. In such experiments the addition of farnesoic acid to the incubation medium (Tobe and Pratt 1974, Weaver et al. 1980) will be important in order to determine precursor-independent maximal rates of JH synthesis during critical stages in caste development. Experiments of this type are currently being carried out in our laboratory and hopefully will contribute to better understand the developmental regulation of insect polymorphism including caste syndromes.

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