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Stage dependent influences of polydnaviruses and the parasitoid larva on host ecdysteroids

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ABSTRACT

In the solitary egg–larval parasitoid *Chelonus inanitus* (Braconidae) both polydnavirus and the parasitoid larva manipulate host development. Parasitization leads to a premature drop in juvenile hormone titre and a precocious onset of metamorphosis in the 5th larval instar. The *C. inanitus* bracovirus (CiBV) alone causes a reduction in host ecdysteroid titres at the pupal cell formation stage and prevents pupation. Here we report three new findings. (1) We show that parasitization causes a reduction in haemolymph ecdysteroid titre immediately after the moult to the 5th instar; similarly low values were seen in nonparasitized larvae after the moult to the 6th instar. These data along with parasitoid removal experiments indicate that the low ecdysteroid titre after the moult is a very early sign of the upcoming metamorphosis. (2) In vitro experiments with prothoracic glands and brain extracts showed that CiBV affects both prothoracic glands and prothoracicotropic hormone after the stage of pupal cell formation. (3) In the haemolymph of parasitized larvae the ecdysteroid titre increased in the late cell formation stage, i.e. immediately before egression of the parasitoid. In vitro experiments showed that late 2nd instar parasitoids release ecdysteroids and are thus very likely responsible for the rise in host ecdysteroids.

1. Introduction

Ecto- and endoparasitic wasps in most cases influence growth and development of their hosts, and in various systems manipulations of host hormones have been documented (Lawrence and Lanzrein, 1993; Beckage, 2002). Prothoracicotropic hormone (PTTH), ecdysteroids and juvenile hormones are the major hormones regulating insect development and metamorphosis. Briefly, PTTH, produced in the brain, stimulates the synthesis and release of ecdysone and/or 3-dehydroecdysone by the prothoracic gland; ecdysone is then converted to 20-hydroxyecdysone (20E), the most widespread moulting hormone. Details of the control and biochemical nature of the ecdysteroidogenic pathway are reviewed in Gilbert et al. (2002). Juvenile hormones produced in the corpora allata dictate the character of the moult. According to current models for lepidopteran metamorphosis larvae must attain a critical size to allow juvenile hormones to decline and PTTH to be released. A slight increase in ecdysone and/or 20E in the absence of juvenile hormones then induces pupal commitment which is associated with wandering or burrowing/digging. Thereafter, a second release of PTTH induces a much higher increase in 20E leading to pupation. An overview of endocrine changes in lepidopteran larvae is given in Edwards et al. (2001). In *Bombyx mori*, an additional element related to the onset of metamorphosis has been identified: it was found that a very low titre of ecdysteroids shortly after the moult to the last instar is a prerequisite for the corpora allata to become inactive (Gu and Chow, 1996). This low ecdysteroid titre would then represent the earliest known endocrine signal preceding larval–pupal metamorphosis. It was proposed that changes in the juvenile hormone levels in the penultimate instar larvae modify the prothoracic glands in a way that they are inactive shortly after the moult to the last instar (Gu and Chow, 1997). Additional data obtained with wing imaginal dics of *B. mori* suggest that pupal commitment proceeds through two stages, from a reversible state that begins around the head capsule slippage stage of the penultimate instar and is associated with a low ecdysteroid titre after the moult to the irreversible state in the last instar at wandering (Koyama et al., 2004).

Koinobiont endoparasitoids which develop inside a developing host are exposed to host's hormones and often manipulate host hormones. In many cases it is not clear which factors interfere with host hormones as polydnaviruses, venom, teratocytes and the parasitoid larva are all possible players. Polydnaviruses occur in several subfamilies of Braconidae (bracoviruses) and Ichneumonidae (ichnoviruses); they replicate in the calyx cells of the ovaries of the female wasps and are injected along with the parasitoid egg

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into the host (Webb et al., 2000; Wyler and Lanzrein, 2003). In several systems polydnaviruses alone or together with venom have been shown to play a role in reducing host ecdysteroids and in inducing a developmental arrest before pupation (Dover et al., 1987, 1988; Tanaka and Vinson, 1991; Soller and Lanzrein, 1996; Grossniklaus-Bürgin et al., 1998; Pennacchio et al., 1998, 2001; Cusson et al., 2001). Whether this is due to effects on the activity of the prothoracic gland and/or PTTH production is unclear in most cases. Emergence of the parasitoid larvae from the host is often associated with an increase in host ecdysteroids and in some cases the parasitoid larvae appear to cause this increase (Brown et al., 1993; Gelman et al., 1998, 1999); but it is unknown how this is accomplished.

Chelonus inanitus successfully parasitizes all stages of eggs (Kaeslin et al., 2005a). Up to the 3rd instar of the host it acts as a conformer and affects host growth only very slightly. However, in the course of the 4th instar some effects become manifest: parasitized larvae weigh less than nonparasitized larvae and the head capsule is smaller (Grossniklaus-Bürgin et al., 1994; Lanzrein et al., 2001). Massive effects are seen in the 5th instar of the host when parasitized larvae enter metamorphosis precociously as compared with nonparasitized larvae which enter metamorphosis in the 6th instar; the parasitoid larvae then emerge from developmentally arrested precocious prepupae (Grossniklaus-Bürgin et al., 1994). Endocrine analyses along with parasitoid removal and implantation experiments and calyx fluid/venom injections led to the following conclusions. The parasitoid larva, in the presence of *C. inanitus* bracovirus (CiBV), causes the precocious onset of metamorphosis and influences host juvenile hormones and juvenile hormone esterases (Pfister-Wilhelm and Lanzrein, 1996; Steiner et al., 1999). CiBV on the other hand is responsible for the developmental arrest in the prepupal stage (Soller and Lanzrein, 1996) and acts on host prothoracic glands and haemolymph ecdysteroids at the stage of pupal cell formation (Grossniklaus-Bürgin et al., 1998). Venom alone in physiological doses has no effect on *Spodoptera littoralis* development but strongly synergizes the effect of the CiBV (Soller and Lanzrein, 1996).

Here the following open questions were addressed. (1) We investigated whether CiBV affects both PTTH and the prothoracic glands to prevent pupation. For this purpose we analyzed the effect of brain extracts from nonparasitized and X-ray parasitized larvae on prothoracic glands in vitro. (2) We addressed the question whether *S. littoralis* passes through two stages of pupal commitment and whether parasitization interferes with this process. For this purpose we measured haemolymph ecdysteroid titres in penultimate and last instar nonparasitized and parasitized larvae and analyzed how parasitoid removal affects host ecdysteroid titres and metamorphosis. (3) We investigated whether parasitoid emergence is associated with an increase in host ecdysteroids and whether *C. inanitus* larvae contain and release ecdysteroids.

2. Materials and methods

2.1. Insects and X-ray irradiation of wasps

S. littoralis (Noctuidae) was reared at 27 ± 1 °C at a photoperiod of 14 h and fed an artificial diet. Adult *S. littoralis* larvae and diet were kindly provided by Syngenta AG, Stein, Switzerland. *S. littoralis* is a natural host of *C. inanitus* (Braconidae), which is a solitary egg–larval parasitoid. For parasitization, 27–32 h old eggs (kept at 20 °C until parasitization) were used. Parasitization was always verified by dissection of a few host eggs, and an egg clutch was only used when 1–3 *C. inanitus* eggs were found per dissected egg. The parasitoid larva hatches approx. 16 h after parasitization (Kaeslin et al., 2005a) and remains in its first instar while the host develops from the first to the early 5th instar. During its long lasting 1st instar the parasitoid larva undergoes extensive morphological changes (Grossniklaus-Bürgin et al., 1994; Kaeslin et al., 2006). In the feeding phase of the host's 5th instar, the parasitoid is in its 2nd instar and feeds on host haemolymph. The host then precociously enters metamorphosis, digs into the soil, constructs a pupal cell and becomes a prepupa; the parasitoid emerges as a freshly moulted 3rd instar larva from the precocious prepupal host. Nonparasitized larvae, on the other hand, pass through six larval instars. For details about parasitoid and host rearing and development see Grossniklaus-Bürgin et al. (1994). To study the role of CiBV in the absence of a parasitoid larva, female wasps were irradiated with X-ray (146 Gy ± 10%) as described by Soller and Lanzrein (1996). These wasps parasitize normally and inject CiBV and venom but the parasitoid is nonviable. Larvae developing from eggs parasitized with X-ray irradiated wasps are designated as X-ray parasitized. X-ray parasitized larvae, similarly to larvae developing from eggs injected with calyx fluid and venom, pass through six larval instars, dig into the soil, construct a pupal cell and then become developmentally arrested in the prepupal stage (Soller and Lanzrein, 1996).

2.2. Preparation of brain extracts and in vitro incubation of prothoracic glands

Larvae were anaesthetized on ice and brains of 10–24 larvae per assay were extirpated in medium 199 HBS (Bioconcept, Switzerland) and homogenized in an Eppendorf tube with a pestle in the same medium at a concentration of 0.1 brain/μl. Immediately after homogenization, brain extacts were heat-treated at 100 °C for 2 min to minimize proteolysis of PTTH (Bollenbacher et al., 1979) and then cooled in ice water and centrifuged at 15 000 × *g* for 5 min. The resulting supernatant was used for the in vitro assays with prothoracic glands without further purification. If not used immediately, extracts were stored at −80 °C. The reproducibility of this extraction protocol and the stability of the PTTH activity in the extract have been documented by Bollenbacher et al. (1979). Crude brain extracts and recombinant PTTH have been shown to have identical effects in *Manduca sexta* (Gilbert et al., 2000) while in *B. mori* brain extracts were slightly more active (Dedos et al., 1999). Prothoracic glands were prepared as described (Grossniklaus-Bürgin et al., 1998) and then rinsed for 30 min in medium 199. Individual gland pairs were incubated in 30 μl of medium 199 in microculture dishes during 3 h in the dark at 27 °C. For the activation studies with brain extracts, one prothoracic gland of a larva served as the control and the contralateral gland as the test gland as described by Bollenbacher et al. (1979). The control gland was incubated in 30 μl medium 199 and the test gland in 30 μl medium 199 containing brain extract at a concentration of three brain equivalents in 30 μl. At the end of the incubation, the gland was removed and 60 μl methanol was added to the medium. Ecdysone quantities were then measured by radioimmunoassay (see below). To evaluate activation of prothoracic glands by brain extracts, the ecdysone release by the control gland was defined as 100%. Kruskal–Wallis tests were performed with the difference in ecdysone production between induced and uninduced glands (controls). For ANOVA, the difference in ecdysone production (induced gland–uninduced gland) was log transformed to fit a normal distribution. Data were checked for normality and heterogeneity of variances. Statistical analyses were performed using the JMP IN 3.2.1. statistics package (Sall and Lehmann, 1996).

2.3. Measurement of ecdysteroids in prothoracic gland culture medium, haemolymph, whole body homogenates of parasitoids and parasitoid medium

Ecdysteroids were quantified by radioimmunoassay according to the method of Borst and O'Connor (1974). The antibody H 22, a