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Testosterone, progesterone, and FSH levels in *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) and its host *Galleria mellonella* L. (Lepidoptera: Pyralidae)

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Abstract: Various vertebrate-type steroid and gonadotropin hormones have been identified in invertebrates, including insects; however, studies in this area are not sufficient to presume a generalized role for these hormones. We identified testosterone, progesterone, and follicle-stimulating hormone (FSH) in the whole-body homogenates of *Galleria mellonella* L. (Lepidoptera: Pyralidae) and its parasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae). Testosterone concentration significantly decreased from early instars to last instars and pupal stages of *G. mellonella*; however, no significant difference was observed in progesterone levels at different stages. FSH concentration reached the highest level at the pupal stage of *G. mellonella*. No significant difference was observed in testosterone, progesterone, and FSH concentrations in young and mature adults of the parasitoid *P. turionellae* for both sexes. We also monitored the changes in testosterone, progesterone, and FSH levels over 2, 6, and 24 h in parasitized *G. mellonella* pupae. During the experimental periods no significant difference in hormone levels was found between controls and parasitized *G. mellonella* pupae.

Key words: Testosterone, progesterone, FSH, *Pimpla turionellae*, *Galleria mellonella*, chemiluminescent immunoassay

Introduction

In recent years, increasing attention has been paid to the endocrine systems of insects in order to determine whether they are much more complex than previously supposed (Swevers et al., 1991; Meunpol et al., 2007; De Loof, 2008). In insects, juvenile hormone (JH) and 20-hydroxyecdysone (20-HE) take part in arranging a huge number of conditions, in particular reproduction, metamorphosis, immune defense reactions, and aging (Kramer, 1985; Riddiford, 1993). In addition to the generally known insect hormones, various vertebrate peptide and steroid hormones have been characterized in insects and different invertebrate species (De Clerck et al., 1983, 1984, 1988; De Loof and De Clerck, 1986; Denlinger et al., 1987; Bradbrook et al., 1990; Darvas and Szekacs, 1997; Keshan and Ray, 2000; Meunpol et al., 2007). The most dominant vertebrate-type steroid hormones characterized in different insect tissues are estrone, estradiol, androsterone, testosterone, progesterone, and corticosteroids (De Clerck et al., 1983, 1984, 1988; Lafont, 1991; Das, 1994; Darvas and Szekacs, 1997). Moreover, in the brains of *Periplaneta americana* L. (Blattaria: Blattidae), *Locusta migratoria*

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L. (Orthoptera: Acrididae), and *Sarcophaga bullata* (Diptera: Sarcophagidae), the vertebrate-type gonadotropin hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), were also discovered (Verhaert and De Loof, 1986, 1988; Theunis et al., 1989). All of these studies revealed that the occurrence of vertebrate-type hormones could be demonstrated by different techniques in whole-body extracts, hemolymph, and gonads and that the amounts of identified vertebrate hormone-like substances were high enough to estimate a hormonal and functional role in insects (Takac et al., 1993). Despite a number of studies that report the existence of vertebrate hormones or hormone-like substances in insects, their accurate functions and modes of action are still unclear.

Parasitoids are important biologic control agents that integrate into host physiology by affecting processes such as development, immunity, and metabolism in order to ensure survival of their progeny (Zhu et al., 2009). Developmental effects, including lengthening of larval and pupal molting time or precocious development, are under hormonal control according to the mode of action of common insect hormones (i.e. JH, 20-HE, and prothoracicotropic hormone) (Wani et al., 1997; Khafagi and Hegazi, 2001; Zhu et al., 2009). Such host regulatory effects have been attributed to maternally derived parasitoid-associated agents such as calyx fluid, polydnavirus, venom, virus-like particles, and teratocytes (Beckage and Gelman, 2004). *P. turionellae* is a solitary idiobiont endoparasitoid wasp species that uses prepupae and host pupae from an extremely wide range of lepidopteran species (Kansu and Uğur, 1984) and is devoid of symbiotic viruses. The role of *P. turionellae* venom and/or parasitism in suppressing the immune defenses of its host, *G. mellonella*, has been studied (Ergin et al., 2006; Uçkan et al., 2010; Er et al., 2010, 2011). Additionally, alteration in total protein levels in the larval and pupal stages of *G. mellonella* after parasitism by *P. turionellae* has been previously demonstrated (Sak et al., 2011).

There is a large body of literature on the effects of parasitism in altering host endocrine titers in many host–parasitoid systems (Beckage, 2008; Zhu et al., 2009). However, virtually nothing is known about the existence of vertebrate-type steroids and gonadotropins in parasitoid *P. turionellae* and its natural host, *G. mellonella*. Therefore, in the current study we aimed to determine stage- and sex-related vertebrate-type hormone profiles of *P. turionellae* reared on *G. mellonella* and the effects of parasitism on host hormone profiles.

Materials and methods

Host and parasitoid insects

A stock laboratory culture of the host insect, *G. mellonella*, was collected from black honeycombs provided by apiarists in the villages of Balıkesir, Turkey. The host colony was reared with honeycombs at 25 ± 2 °C (Uçkan et al., 2004) to sustain their natural medium in bee hives. Individuals of the parasitoid *P. turionellae* were cultivated by parasitizing *G. mellonella* pupae at 25 ± 2 °C, 60 ± 5% relative humidity, and a photoperiod of 12 L : 12 D under laboratory conditions (Uçkan et al., 2004). Adult male and female *P. turionellae* specimens were fed with a 30% (v/v) honey solution and kept in wire cages of 20 × 23 × 21 cm.

Parasitization of *G. mellonella* pupae

Parasitization of *G. mellonella* was carried out on newly molted host pupae by exposing individual pupa (130 ± 10 mg) to a 20-day-old *P. turionellae* female. Parasitized insects were held under the same conditions described above with the control groups until sample preparation for hormone analysis.

Preparation of samples

Analyses were carried out on the following developmental stages: young (<20 days old) and mature (>20 days old) adult females and males of *P. turionellae*, early instars in the feeding phase (8–12 g) and last instars (more than 25 g), and 1- to 2-day-old pupae of *G. mellonella*. Additionally, 1- to 2-day-old parasitized *G. mellonella* pupae at 2, 6, and 24 h after parasitization were used in experiments. The whole insects in the experimental groups were homogenized, diluted with deionized water, and centrifuged at 10,000 rpm for 10 min. The pooled supernatants were used in hormone analyses. For each experiment 5 host pupae, larvae, and *P. turionellae* male and female adults were used, and controls were performed in 3 replicates in a given

period. The hormone level amounts were converted to the value per insect.

Hormone levels

Testosterone, progesterone, and FSH levels were determined using a competitive chemiluminescent immunoassay with the Bayer ADVIA Centaur Immunoassay System, which is a 2-site sandwich immunoassay using 2 monoclonal antibodies. Measurements were performed according to manufacturer instructions.

Statistical analysis

Data were subjected to one-way ANOVA and the differences between means were detected by Tukey's honestly significant difference post hoc tests using SPSS 12.0. Obtained data were described as statistically significant at $P < 0.05$.

Results and discussion

Hormone levels and developmental changes in *P. turionellae* and its host *G. mellonella*

Experiments showed that the whole-body homogenates of *G. mellonella* contained testosterone, progesterone, and FSH (Table 1). The amounts of testosterone in early instars, last instars, and pupae of *G. mellonella* were 164.51, 30.88, and 43.11 ng/dL, respectively (Table 1), and differed significantly among stages ($F = 8.956$, $df = 2, 6$; $P < 0.05$). Testosterone levels decreased significantly as larvae progressed from early instars to last instars and pupal stages (Table 1). However, there was no significant difference in progesterone levels between the larval and pupal stages of *G. mellonella* ($F = 0.497$, $df = 2, 6$; $P > 0.05$). FSH levels were 4.28, 1.60, and 8.33 mIU/mL in early instars, last instars, and pupae of *G. mellonella*, respectively (Table 1). The highest FSH titer occurred in the pupal stage, and statistical analyses revealed significant differences between larval stages and pupae ($F = 13.426$, $df = 2, 6$; $P < 0.05$).

The body homogenates of the parasitoid *P. turionellae* displayed various concentrations of testosterone, progesterone, and FSH in all developmental stages and sexes tested (Table 2). Testosterone levels were 68.57 and 27.61 ng/dL in young and mature adult females and 93.85 and 51.82 ng/dL in young and mature adult males, respectively (Table 2). Testosterone levels were consistently higher in males than females. Moreover, young adults of both sexes of *P. turionellae* had higher levels of testosterone than mature adults. However, no significant differences were observed among the treatments regarding testosterone levels ($F = 3.301$, $df = 3, 8$; $P > 0.05$) (Table 2). Progesterone was also present in considerable amounts in *P. turionellae* males and females, and the titers of progesterone in the investigated stages and sexes of *P. turionellae* were similar ($F = 2.439$, $df = 3, 8$; $P > 0.05$). A significant level of FSH was measured by immunoassay in the investigated stages and sexes of *P. turionellae*. FSH levels were 9.88 and 9.58 mIU/mL in young and mature adult females and 13.87 and 8.81 mIU/mL in young and mature adult males, respectively (Table 2). However, no significant difference was observed in FSH titers ($F = 0.780$, $df = 3, 8$; $P > 0.05$).

Table 1. Immunoassay results of *G. mellonella*: amount of steroids (testosterone and progesterone) and gonadotropin (FSH) measured per insect.

Stage	Testosterone (ng/dL) (Mean ± SE)[a]	Progesterone (ng/dL) (Mean ± SE)[a]	FSH (mIU/mL) (Mean ± SE)[a]
Early instars (feeding phase)	164.51 ± 34.53 **a**	1 ± 0.11 **a**	4.28 ± 0.96 **a**
Last instars	30.88 ± 1.08 **b**	0.38 ± 0.11 **a**	1.60 ± 0.66 **a**
Pupae	43.11 ± 25.19 **b**	0.71 ± 0.67 **a**	8.33 ± 1.10 **b**

[a]Numbers in columns followed by the same letter are not significantly different ($P > 0.05$).

Table 2. Immunoassay results of *P. turionellae*: amount of steroids (testosterone and progesterone) and gonadotropin (FSH) measured per insect.

Stage	Sex	Testosterone (ng/dL) (Mean ± SE)[a]	Progesterone (ng/dL) (Mean ± SE)[a]	FSH (mIU/mL) (Mean ± SE)[a]
Young adult	Female	68.57 ± 12.2 **a**	1.94 ± 0.23 **a**	9.88 ± 2.67 **a**
Mature adult	Female	27.61 ± 9.94 **a**	0.55 ± 0.11 **a**	9.58 ± 4.29 **a**
Young adult	Male	93.85 ± 21.23 **a**	1.75 ± 0.6 **a**	13.87 ± 0.5 **a**
Mature adult	Male	51.82 ± 15.63 **a**	1.51 ± 0.45 **a**	8.81 ± 0.78 **a**

[a]Numbers in columns followed by the same letter are not significantly different ($P > 0.05$).

Recent reports have dealt with the occurrence of vertebrate-type steroids in a broad range of different insect orders (De Clerck et al., 1983, 1984, 1988; De Loof and De Clerck, 1986; Denlinger et al., 1987; Bradbrook et al., 1990; Darvas and Szekacs, 1997; Keshan and Ray, 2000). De Clerk et al. (1983) showed the presence of both testosterone and progesterone in hemolymph from *S. bullata* larvae. Previous studies showed that progesterone concentration in both females and males of *Nauphoeta cinerea* Oliver (Blattoidea: Epilamprinae) and *L. migratoria* varied during adult life, and progesterone concentration in hemolymph was higher in males than in females (Novak et al., 1987; Takac et al., 1993). However, our study revealed no sex-specific differences in testosterone and progesterone levels. According to earlier studies, vertebrate-type steroid concentrations have been shown to differ with respect to the developmental stage of the insect in whole-body extracts; however, the correlation between hormone titer and physiological and developmental states could not be clarified (Bradbrook, 1990). Further research should be done to establish whether testosterone or progesterone function as sex hormones in insects as they do in vertebrates (De Clerck et al., 1983).

FSH in vertebrates is a glycoprotein that stimulates gonadal maturing and the synthesis of progesterone or other sex hormones (De Loof et al., 2001). The gonadal maturing in insects is under the control of JH, gonadotropin, and ecdysteroids (De Loof et al., 2001). However, there is no evidence that FSH can act on gonadal maturation in insects (Theunis et al., 1989; De Loof et al., 2001).

Effects of parasitization by *P. turionellae* on hormone levels of *G. mellonella* pupae

The second aim of this study was to investigate the effect of parasitism by *P. turionellae* on the vertebrate-hormone levels of its host, *G. mellonella*. Changes in testosterone, progesterone, and FSH levels in the whole-body homogenates of *G. mellonella* pupae after parasitism by *P. turionellae* are presented in Table 3. In the parasitized host pupae, testosterone titers were higher than those of control at 2, 6, and 24 h after parasitization (Table 3). However, the increase in testosterone levels was not significantly different ($F = 2.781$, $df = 3, 8$; $P > 0.05$). The progesterone level of control pupae was 0.71 ng/dL in nonparasitized control hosts, whereas progesterone-like material could not be detected after 2, 6, and 24 h in parasitized *G. mellonella* pupae (Table 3). FSH levels were 9.87, 15.55, and 13.06 mIU/mL at 2, 6, and 24 h after parasitization by *P. turionellae* (Table 3). During the experimental periods no significant difference in FSH levels was found between control and parasitized *G. mellonella* pupae ($F = 3.66$, $df = 3, 8$; $P > 0.05$).

The experimental results demonstrated that in the *P. turionellae*–*G. mellonella* system no changes were observed in testosterone, progesterone, and FSH levels at 2, 6, and 24 h after parasitization. Earlier studies showed that after parasitism, juvenile hormone and ecdysteroid alterations occurred in several host–parasitoid systems (Gelman et al., 1998; Li et al., 2003;

Table 3. Immunoassay results of *G. mellonella* pupae parasitized by *P. turionellae*: amount of steroids (testosterone and progesterone) and gonadotropin (FSH) measured per insect.

Stage	Testosterone (ng/dL) (Mean ± SE)[a]	Progesterone (ng/dL) (Mean ± SE)[a]	FSH (mIU/mL) (Mean ± SE)[a]
Control	43.11 ± 25.19 **a**	0.71 ± 0.67**a**	8.33 ± 1.1 **a**
2 h after parasitization	103.04 ± 23.46 **a**	0 ± 0 **a**	9.87 ± 2.31 **a**
6 h after parasitization	116.11 ± 17.75 **a**	0 ± 0 **a**	15.55 ± 1.64 **a**
24 h after parasitization	65.91 ± 10.92 **a**	0 ± 0 **a**	13.06 ± 1.49 **a**

[a]Numbers in columns followed by the same letter are not significantly different (P > 0.05).

Beckage, 2008; Zhu et al., 2009). However, this is the first report that represents the effects of parasitism on host testosterone, progesterone, and FSH levels.

The importance of identifying vertebrate hormones in several insect species is controversial, and the discussions focus on the origin of these materials (Takac et al., 1993). Several proposals regarding the possible roles of vertebrate hormones have been suggested. However, the occurrence of vertebrate hormones in different insect tissue extracts does not necessarily describe their physiological roles, and this needs to be proven. Moreover, studies on parasitoid regulation of host endocrinology, especially the less frequently studied vertebrate-type hormones, will facilitate the development of new biological control strategies. In addition, more work needs to be done in other host–parasitoid systems.

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