Juvenile hormones in *Thermobia domestica* females: Identification and quantification during biological cycles and after precocene application

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Summary. JH I and JH III immunoreactive substances were detected in the hemolymph of imaginal females of the primitive insect *Thermobia domestica*. Periodic changes in the levels of these hormones were investigated in correlation with molting and reproductive cycles in inseminated, virgin and precocene-treated females. The presumed influence of JH III on the various phases of vitellogenesis is discussed, also taking into account the periodic changes of the hemolymphatic ecdysteroid levels.

Key words. Firebrat; *Thermobia domestica;* hemolymph; primitive insects; juvenile hormones; precocene application; vitellogenesis; molting cycle; reproductive cycle.

The firebrat *Thermobia domestica* (Insecta, Thysanura) is an apterygote insect which maintains its ability to molt throughout adult life, the reproductive cycles being correlated with molting cycles[1,2]. The juvenilizing activity of the corpora allata (CA) of *T. domestica* was previously demonstrated by CA implantations in *Antheraea polyphemus* pupae[3]; moreover, cauterization of the CA[4] or chemical allatectomy by precocene II in *Thermobia* females[5] has indicated that the juvenile hormone (JH) synthesized by the CA is also involved in vitellogenesis control. Biological tests verified the presence of a JH substance in total extracts of *Thermobia*[6], but physical-chemical techniques (gas-liquid chromatography + mass spectrometry: GLC + MS) gave contradictory data in JH identification: only JH I[7] or only JH III[8].

Using radioimmunoassay (RIA) we tried to identify and to quantify hemolymphatic JH(s) in *Thermobia* during the biological cycles in three different situations: in inseminated, virgin and precocene-treated females.

Material and techniques. Thermobia domestica (Packard) were reared at 37°C and 80% r.h. Adult females, 8–9 months old, present regular cycles which last an average of 11 days. The reproductive cycles start with egg-laying on day 6 and continue until the 6th day of the following cycle. An insemination at the beginning of each molting cycle is required for complete maturation of the terminal oocytes; without insemination all vitellogenic oocytes are resorbed. From each anesthetized female 1–2 µl of hemolymph were collected by a small pin prick at the collum. In order to obtain samples of 160 µl, it was necessary to pool the hemolymph of 100–160 females on the same day of the intermolt. The hemolymph was immediately added to 500 µl of methanol/diethylether 1/1 (v/v) at −35°C until analysis. JHs were quantified by radioimmunoassay (RIA)[9]. Known quantities (3000 dpm) of tritiated JH I, JH II and JH III (NEN 10 Ci/mM) were added per sample prior to extraction. The JHs were extracted with hexane.

Purifications were made successively by silica column chromatography C18 Sep pak® chromatography (Waters S.A.) and reverse phase HPLC, using a 30 cm long, 0.7 mm I.D. µ bonda-pack® column (Waters S.A.). The solvent system, a mixture of methanol/water 4/6 (v/v), was introduced into the HPLC at a flow rate of 1 ml/mn. UV analysis was performed at 218 nm. Fractions of 800 µl were collected after HPLC. The immunoreactivity of each fraction was measured in duplicate with antisera specific for JH I, JH II or JH III: The RIA was performed with iodinated tracers directly in the HPLC solvent. The tritiated JH of each fraction was measured to check for JH elution time and to calculate the recoveries. Because each JH was eluted into two or three fractions, the comparison of tritiated contents and immunoreactivity of each fraction allowed us to validate the RIA by dilution test.

In order to investigate the effects of an anti-allatotropic substance, precocene[5], on the hemolymphatic JH levels, two sets of 160 females were treated with a single application of 10 µg of precocene II (6,7-dimethoxy-2,2-dimethylchromene, Calbiochem) dissolved in 1 µl of pure acetone. Applications were made on days 1 or 3 and RIA analysis performed 36 h later.

Results. The RIA analysis of the hemolymph of *T. domestica* females detected two substances recognized by anti-JH I antisera and by anti-JH III antisera. The analysis was made on fractions 1 to 20 after HPLC separation. Specific anti-JH I antisera reacted uniquely with hemolymphatic material having the same retention time as standard JH I or tritiated JH I. Dilution tests of these fractions were positive, strongly indicating the presence of JH I.

Small amounts of specific anti-JH II antisera bound with a hemolymphatic material whose HPLC elution corresponded to JH I and only weakly with material whose HPLC elution corresponded to JH III. Dilution tests of these fractions were negative. Because of the cross reactivity of the JH II antisera with JH I (43% for the JH I concentrations measured with anti-JH I) this binding is thought to be caused by a substance different from the JH I recognized by anti-JH II antisera, but not by anti-JH I antisera.

Dilution tests proved it was not JH II. This substance, closely resembling JH, remains unidentified.

Specific anti-JH III antisera bound with a hemolymphatic material whose HPLC elution was the same as standard JH III or tritiated JH III. Dilution tests were positive, indicating that this substance is almost certainly JH III.

Daily variations of JH I immunoreactive material (JH I-IM) and JH III-IM during a standard molting cycle are presented in figures 1 and 2. In females reared with males (60 to 80% of these females were inseminated and fecund) JH I-IM was present throughout the cycle with a maximum on day 5 (11.0 ng/ml = 37.4 nM) and a minimum on day 11 (5.8 ng/ml = 19.7 nM). The levels on days 4, 5, 7 and 9 were not significantly different. The JH III-IM levels were always lower than JH I-IM levels, fluctuating cyclically with a maximum on day 3 (5.9 ng/ml = 22.1 nM) and decreasing values on days 7, 9 and 11 (0.9 ng/ml = 3.4 nM on day 9).

In females without males, the JH levels were investigated only during the first 7 days of the molting cycle, because the synchrony of the insects was not satisfactory beyond this period. In these females, variations of the JH I-IM levels were similar to those of inseminated females, but the JH III-IM levels were

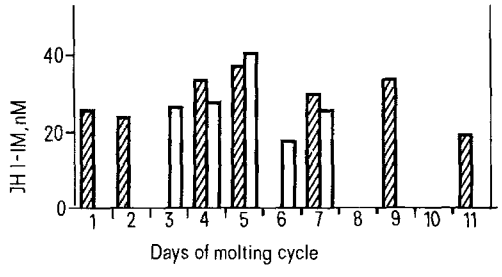

Figure 1. Changes of JH I-IM levels during a standard molting cycle in *Thermobia domestica* females. Open columns: females without males; hatched columns: females kept with males.

lower than in inseminated females (< 0.7 nM on day 2; 3 nM on day 3). In females treated with 10 µg of precocene II at day 1 or 3, JH III-IM was non-detectable 36 h after application; JH I-IM was only present as traces after application at day 1 and its level decreased to only 3 ng/ml = 10.2 nM after application at day 3.

Discussion. Although RIA is not an identification technique, the two substances, JH I-IM and JH III-IM, detected in the hemolymph of adult females of *T. domestica* should be JH I and JH III. Indeed in three different chromatographic systems, both reacting substances had the same behavior as standard JH I and JH III. The dilution tests of the HPLC fractions eluted as JH I or JH III were positive. Chemical allatectomy by precocene II reduced or abolished the JH level in the hemolymph. Recently GLC + MS techniques were employed by some authors to identify JHs in *T. domestica*. In a first investigation using more than 1 kg of insects (representing about 50,000 non-synchronized adults), only JH I was identified, but the presence of low quantities of JH II and JH III could not be rejected[7]. A re-evaluation of this report concluded that only JH III occurs in the hemolymph of *Thermobia* as in most pterygote insects[8]. So the identity of the JH I-IM remains questionable. We do not know anything about the JH II-IM, except that it is distinct from other known JHs; it cannot be excluded as juvenoid, because it is close to JH.

The present investigation on JH and a previous paper on ecdysteroids[10] provide detailed information on the two major morphogenetic hormones in *T. domestica*. Circulating ecdysteroid levels are very low during the first half of the intermolt, they increase slightly on days 7 and 8, then present a maximum on day 9, a few days before ecdysis (fig. 2). As a comparison, JH I-IM is present throughout the cycle and especially on day 9 during the ecdysteroid peak.

Moreover, changes in hormone levels can be considered in correlation with the reproductive cycle. In standard animals, follicle maturation during each ovarian cycle can be divided into three major phases: previtellogenesis (days 6 to 10 of the intermolt), yolk deposition (last day of molting cycle and days 1 to 5 of the next intermolt) and egg-envelope formation (days 5 and 6). Vitellogenesis begins just after the ecdysteroid peak, when the JH III-IM titre is low. This suggests that ecdysteroid might induce the onset of vitellogenesis in *T. domestica*, or make the oocytes competent for yolk sequestration. It should be pointed out that ovary development in these species is exactly synchronous in all females observed at the time of exuviation. Low CA activity has already been observed at the beginning of vitellogenesis in cockroaches and Orthoptera[11,12]; nevertheless very low JH level can induce yolk deposition[13].

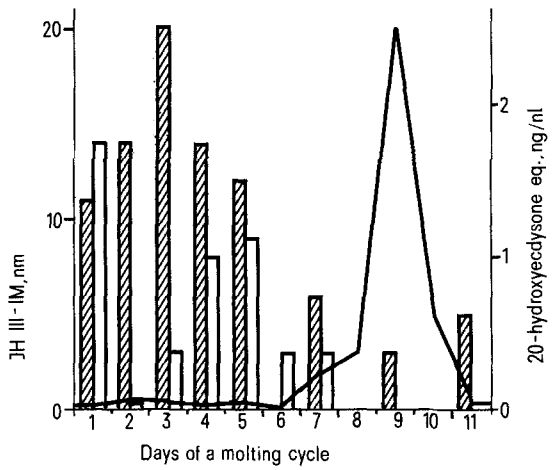

Figure 2. Changes of JH III-IM levels during a standard molting cycle in *Thermobia domestica* females. Open columns: females without males; hatched columns: females kept with males. For comparison the solid line indicates the changes of ecdysteroid levels in the hemolymph, obtained by a previous RIA analysis[10].

Fast oocyte growth in *T. domestica* coincides with increasing JH III-IM titres. During this period (days 1 to 5) the role of the CA has been experimentally proved: bilateral allatectomy inhibites oocyte growth[4,14]; after precocene II application, vitellogenesis is interrupted, then oocyte resorption occurs[5], while JH III-IM level decreases to zero. So it appears that in Thysanura, as in most insects, vitellogenesis is controlled by CA activity and more precisely by JH III.

The end of vitellogenesis and egg-envelope deposition take place when the JH III-IM titre decreases; consequently these phases are probably not controlled by JH. In several pterygote insects, a decrease in CA activity has also been observed at the end of a gonotrophic cycle[15,16] and the JH titre was weak or null[13]. In *Locusta migratoria* for example, the CA are not necessary for egg-envelope formation[17,18]. The hypothesis of CA inhibition by an ovarian substance (ecdysteroid?) has been put forward[16,19], but in *T. domestica* this hypothesis cannot be supported because ovarian ecdysteroids do not appear to be released into the hemolymph[10].

In *T. domestica*, insemination at the beginning of each intermolt is required for complete vitellogenesis and for egg-envelope formation. In virgin females oocytes degenerate around day 4 and JH III-IM levels are low at the beginning of the next cycle. Such stimulation of the CA by insemination has been described, for example, in cockroaches[16,20,21] and in *Teleogryllus commodus*[22]. In *T. domestica* it has been indicated that the stimulatory factor triggered by insemination is a neurohormone[4].

1 Watson, J.A.L., J. Insect Physiol. *10* (1964) 399.
2 Rohdendorf, E.B., Acta ent. bohemosl. *65* (1968) 341.
3 Watson, J.A.L., Biol. Bull. *132* (1967) 277.
4 Rohdendorf, E.B., and Watson, J.A.L., J. Insect Physiol. *15* (1969) 2085.
5 Bitsch, C., and Bitsch, J., J. Insect Physiol. *30* (1984) 463.
6 De Loof, A., Madhavan, K., Girard, J.E., Schneiderman, H.A., and Meinwald, J., J. Insect Physiol. *22* (1976) 829.
7 Madhavan, K., Ohta, T., Bowers, W.S., and Schneiderman, H.A., Am. Zool. *21* (1981) 733.
8 Schooley, D.A., and Baker, F.C., Intern. CNRS Symposium Strasbourg (1983) 78.
9 Baehr, J.C., Caruelle, J.P., Porcheron, P., and Cassier, P., in: Juvenile Hormone Biochemistry, p. 47. Eds G.E. Pratt and G.T. Brooks. Elsevier, North Holland 1981.
10 Rojo de la Paz, A., Delbecque, J.P., Bitsch, J., and Delachambre, J., J. Insect Physiol. *29* (1983) 323.
11 Tobe, S.S., and Pratt, G.E., J. exp. Biol. *62* (1975) 611.
12 McCaffery, A.R., and McCaffery, V.A., J. Insect Physiol. *29* (1983) 259.
13 Koeppe, J.K., in: Regulation of Insect Development and Behaviour, part II, p. 505. Wroclaw Technical University Press, Wroclaw (1981).
14 Rohdendorf, E., Zool. Jb. Physiol. *71* (1966) 685.
15 Girardie, J., Tobe, S.S., and Girardie, A., C.r. Acad. Sci. Paris *293* III (1981) 443.
16 Lanzrein, B., Wilhelm, R., and Gentinetta, V., in: Regulation of Insect Development and Behaviour, part II, p. 523. Wroclaw Technical University Press, Wroclaw (1981).
17 Joly, L., Goltzene, F., and Porte, A., J. Insect Physiol. *24* (1978) 187.
18 Rembold, H., in: Juvenile Hormone Biochemistry, p. 11. Eds G.E. Pratt and G.T. Brooks. Elsevier, North Holland 1981.
19 Friedel, T., Feyereisen, R., Mundall, E.D., and Tobe, S.S., J. Insect Physiol. *26* (1980) 665.
20 Weaver, R.J., and Pratt, G.E., Physiol. Ent. *2* (1977) 59.
21 Koeppe, J.K., Brantley, S.G., and Nijhout, M.M., J. Insect Physiol. *26* (1980) 749.
22 Loher, W., Ruzo, L., Baker, F.C., Miller, C.A., and Schooley, D.A., J. Insect Physiol. *29* (1983) 585.

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