Titer of Juvenile Hormone III in *Drosophila hydei* during Metamorphosis Determined by GC-MS-MIS

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The titer of juvenile hormone III (JH-III) has been determined by the use of combined gas chromatography-mass spectroscopy (GC-MS) in whole body extracts of the larvae, prepupae, pupae and adults of *Drosophila hydei*. A characteristic JH-III titer curve was established by use of the hormone derivatives. No other juvenile hormone homologs were detected.

JH-III shows a broad peak maximum of about 30 pmol/g fresh weight during the last larval instar, whereas only traces of the hormone are detectable at pupariation. Prepupae and pupae exhibit about the same JH-III level. In older pupae and in the pharate adults there was no JH-III detectable but it reappeared soon after emergence. Low values of JH-III are found in young male and female flies. The JH contents rise to distinct peaks in older and reproductive adults, both in male and female animals.

Introduction

Insects need juvenile hormone not only for larval growth but also for metamorphosis to the imago [1]. In most hemi- and holometabolous insects it is also necessary for the induction and maintenance of reproductive processes [2]. Main sources of its biosynthesis are the corpora allata.

The structure of JH-I was elucidated by Roeller *et al.* [3] in the lepidopteran *Hyalophora cecropia*, that of JH-II by Meyer *et al.* [4] in the same species. Judy *et al.* [5] discovered and determined JH-III in the moth *Manduca sexta*, Bergot *et al.* [6] identified JH-0 in the eggs of this lepidopteran.

Appearance and titer of JH have been followed in hemi- and holometabolous insects. Sensitive tests for the biological effects of JH are the *Galleria* [7] and the *Tenebrio* [8] assays.

The use of coupled GC-MS analysis for juvenile hormone measurements was first described by Trautmann *et al.* [9] and a similar method by Mauchamp *et al.* [10]. Rembold *et al.* [11] and Bergot *et al.* [12] made use of JH-derivatives. Their detection is highly sensitive, specific and reliable, also at extremely low JH titers, the border of detection being around few nanograms of hormone.

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A radiochemical assay for demonstration of JH synthesis *in vitro* was described by Pratt *et al.* [13]. *In vitro* studies with JH precursors were practized by Feyereisen *et al.* [14]. Baehr *et al.* [15] and Strambi [16], describe radioimmunoassays for the juvenile hormones.

Despite of many trials, JH could not be detected in dipterans for a long time and only for a few species data are available [17–20]. In the fruitfly, JH is indispensable for development and metamorphosis [11] and is later needed for vitellogenesis and yolk formation [22–24]. We now present evidence of JH-III to be the only homolog present in third instar larvae, prepupae, young pupae and adults of *Drosophila hydei* and a titer curve of the hormone.

Materials and Methods

Cultural conditions

Strains of the fruitfly *Drosophila hydei* were reared according to Berendes [34]. Flies were kept in plastic cages (50 × 50 × 50 cm) at 23–25 °C and 40–60% relative humidity under long day conditions (16/8 h).

Oviposition

8–18 day old flies were used for oviposition. The time for egg laying was confined to two hours to make sure that animals used in experiments were at a similar stage of development. The eggs hatched and developed under conditions mentioned above.

Biological samples

Larvae, prepupae and pupae of the desired age were collected, washed, dried on cellulose filters and weighed. The adults were immobilized by cooling them to 4 °C and thus could be easily sampled. They were separated according to their sex under CO_2-anesthesia. Until further use, all samples were kept at −70 °C.

JH measurements

Purification of biological samples and their derivatization to the 10-hydroxy-11-nonafluorohexoxy-(35), or the nonafluorooctoxy-(36) compounds, either as such or as their 10-heptafluorobuturyloxy esters, has been described. Alternatively, 10-nonafluorohexyldimethylsilyl-11-methoxy-JH was prepared. The technique of quantification by use of the corresponding JH-ethyl ester and the details of GC-MS-MIS measurements have also been described [11].

Table I. JH-III titers of different developmental stages of *Drosophila hydei*, in pmol/g fresh weight (FW) and in fmol/animal. "Traces" means limit of detection (0.08 pmol). *Abbreviations:* L = larvae; PP = prepupae; P = pupae; A = adults.

Age [h]		JH-III [pmol/g FW]	JH-III [fmol/animal]
L	60– 72	0.00	0.00
	72– 84	0.18	0.15
	84– 96	0.14	0.21
	96–108	0.17	0.31
	108–120	0.23	0.61
	120–132	0.29	0.91
	132–144	0.15	0.52
	144–156	0.24	1.25
	156–168	traces (0.08)	traces (0.38)
PP	2	traces (0.08)	traces (0.24)
	4	traces (0.08)	traces (0.25)
	8	traces (0.08)	traces (0.21)
	12	traces (0.08)	traces (0.23)
P	24	traces (0.08)	traces (0.18)
	48	traces (0.08)	traces (0.27)
	72	0.00	0.00
	96	0.00	0.00
A♀	24	0.21	0.71
	120	0.62	2.45
	240	0.65	2.64
A♂	24	0.32	0.77
	120	0.34	0.87
	240	0.74	1.71

Table II. Increase of body weight of *Drosophila hydei* stages. Abbreviations as in Table I.

Age [h]		Weight [mg/animal]
L	68	0.26 ± 0.07
	72	0.42 ± 0.14
	76	0.39 ± 0.17
	80	0.61 ± 0.16
	84	1.01 ± 0.21
	92	0.76 ± 0.09
	100	1.23 ± 0.47
	120	2.63 ± 0.11
	144	4.41 ± 0.09
	162	4.93 ± 0.25
	168	4.86 ± 0.35
	174	4.10 ± 0.39
PP	0–2	4.69 ± 0.69
	4	4.84 ± 0.59
	8	3.98 ± 0.39
	12	4.40 ± 0.70
P	24	3.29 ± 0.71
	48	3.44 ± 0.38
	72	3.64 ± 0.48
	96	3.55 ± 0.48
A♀	1	3.40 ± 0.45
	5	3.91 ± 0.31
	10	4.03 ± 0.39
	15	4.02 ± 0.43
	17	3.54 ± 0.53
	20	3.73 ± 0.36
A♂	1	2.43 ± 0.17
	5	2.41 ± 0.28
	10	2.33 ± 0.39
	15	2.64 ± 0.34
	17	2.26 ± 0.47
	20	2.27 ± 0.52

Results

In whole body extracts of all developmental stages of *Drosophila hydei* only JH-III was detected in quantifiable amounts (Table I). Due to increase of body weight (Fig. 1, Table II) the course of the titer curves is somewhat different dependent on whether the JH values are referred to gram fresh weight, or to sample size (Figs. 2, 3). None of the other JH homologs could be found within the limit of detection of our method. An MIS plot of a probe with 0.51 pmol JH-III/g biological sample is shown in Fig. 4 as an example for the signal/noise ratio.

There are three striking JH peaks in *Drosophila hydei* development. The first in 120–132 h, mid third instar larvae, a second, 12 h later in 144–156 h late third instar larvae and the third and highest peak in the 10 day-old male and female adults.

Second instar larvae of 60–72 h lack demonstrable amounts of JH-III. Relatively stable JH-III values of about 0.18 pmol/g FW are found in the 72

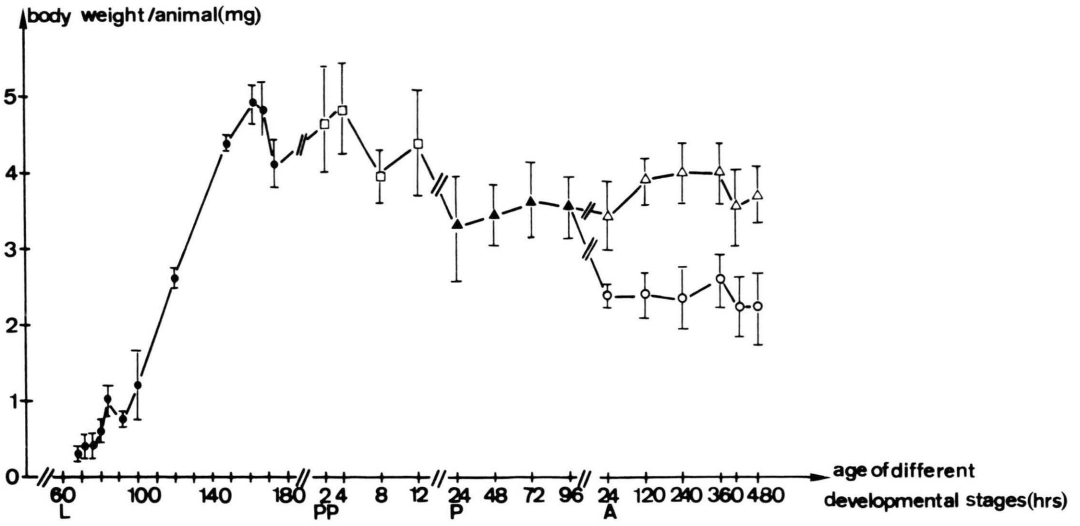

Fig. 1. Increase of body weight of different developmental stages during life cycles of *Drosophila hydei*. The sign – // – on the abscissa indicates different developmental stages: ● larvae; □ prepupae; ▲ pupae; △ female adults; ○ male adults.

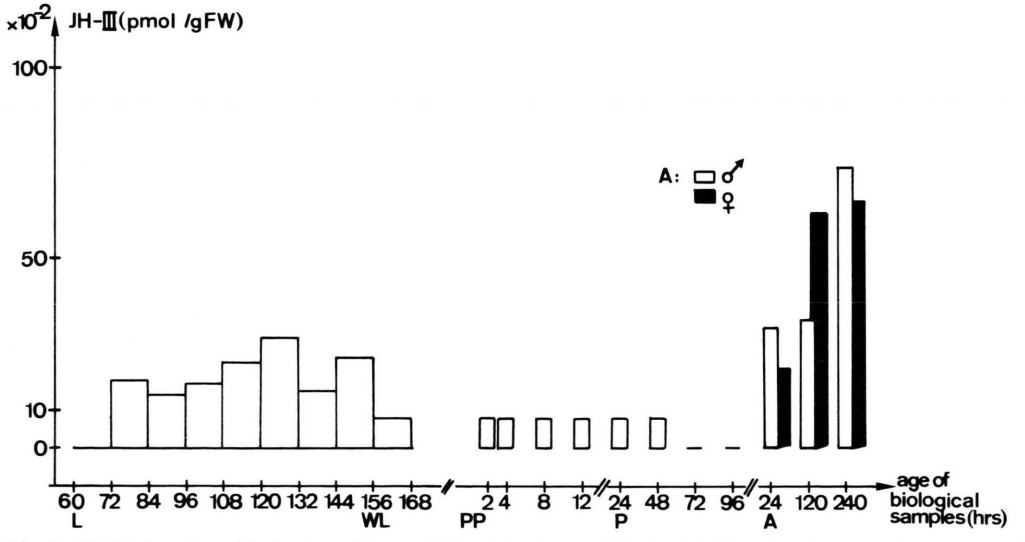

Fig. 2. JH-III titer (pmol/g fresh weight – FW –) in *Drosophila hydei*. The developmental stages are indicated below the bars. The sign – // – indicates different developmental stages. *Abbreviations:* L = larvae; WL = wandering larvae; PP = prepupae; P = pupae; A = adults.

to 120 h old late second and early third instar larvae. The first peak in third instar larvae (120–132 h) has a value of 0.24 pmol/g FW, the second in late third instar larvae (144–156 h) reaches 0.24 pmol/g FW. The oldest larvae (156–168 h), the prepupae and young pupae (24 and 48 h) have traces of JH-III near to the limit of detection (0.08 pmol/g FW), whereas in old pupae and pharate adults no JH-III can be detected.

The JH-III titer rises a third time, constantly from day 1 to day 10 in adult male and female flies. Female *Drosophila* animals have a JH-III maximum of 0.65 pmol/g FW, males reach 0.74 pmol/g FW on day 10.

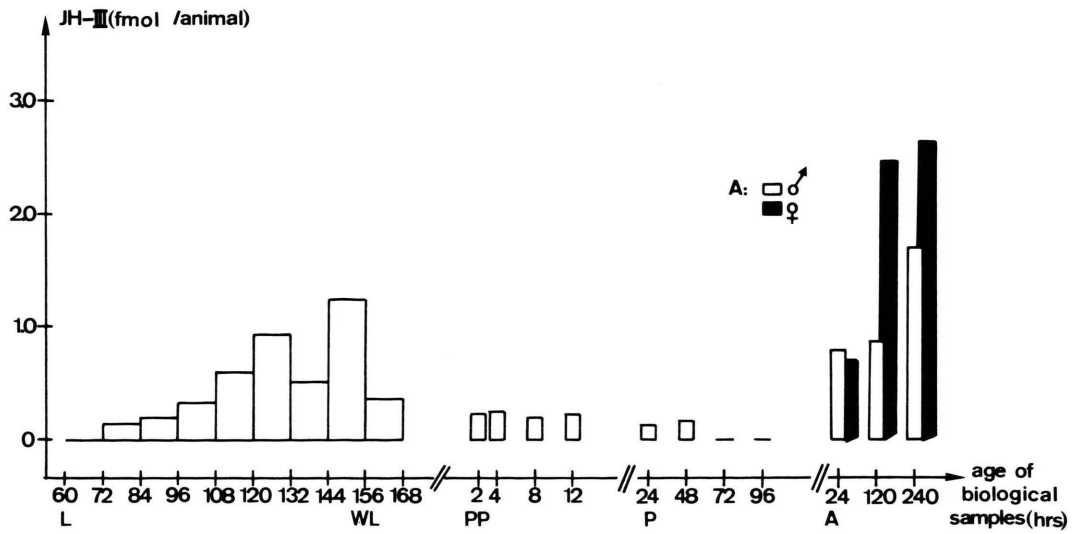

Fig. 3. JH-III titer calculated for the individual animal (fmol/animal) of *Drosophila hydei.* Legend as in Fig. 3.

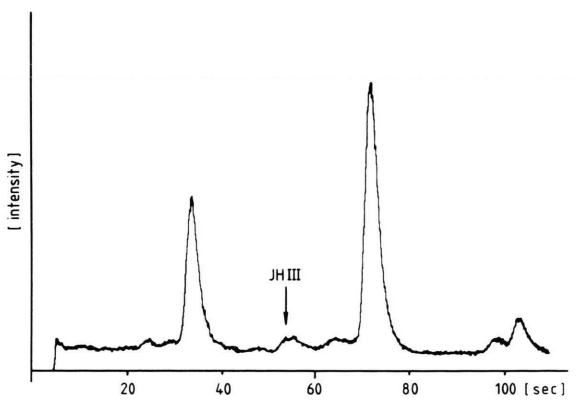

Fig. 4. MIS plot for the fragment *m/e* 376 formed from the 10-nonafluorohexyldimethylsilyl-11-methoxy-JH-III derivative after separation by GC (245° const., capillary column Durabond-1, 0.25 mm *i.d.*, 30 m) – MS in EI mode (0.05 pmol JH-III). For more technical details comp. (12). Retention time of the resp. JH-III ethyl ester (1.2 pmol) is at 73 s.

Discussion

Our present data about the occurrence of JH-III in *Drosophila hydei* confirm our former still preliminary results [37] and allow to draw a titer curve for JH from late second instar larvae up to the adults. The titer is generally lower than in our earlier measurements, however. We can now definitely exclude the existence of other JH homologs.

JH-III therefore is the only naturally occurring JH homolog in *Drosophila hydei* with biological functions as stated for other dipterans as *Musca domestica* [17] and *Sarcophaga bullata* [17] and *Aedes aegypti* [20]. For species of other insect orders JH-III turned out to be the only JH of biological importance, as in *Apis mellifera* (Hymenoptera) [25], *Locusta migratoria* (Orthoptera) [26, 27], *Tenebrio molitor* [9, 28] and *Leptinotarsa decemlineata* (Coleoptera) [4].

The predominance of JH-III in larvae as well as in adults of *Drosophila hydei* indicates that it is indispensable for the hormonal regulation of larval metamorphosis, for vitellogenesis and oogenesis in female flies [22–25, 32]. We explain JH-III levels during third larval instar and in adults as sufficient signals for switching developmental programs.

Only trace amounts of JH were found in prepupae and early pupal stages and no JH at all in older pupae (72 h) and in 96 h pharate adults. Either the turnover of JH is too high and no JH detectable with our technique or it is not even produced.

Extremely low JH concentrations do not seem to be of functional importance for growth, metamorphosis or reproduction in the animal. JH esterase activity in *Drosophila* haemolymph is very high at the beginning of the prepupal stage and then slowly diminishes in pupae, also that of the hypodermis decreases rapidly during pupal life [29].

The JH titer in the animal will therefore certainly drop to zero even if there is still a very low JH biosynthesis in old larvae, prepupae and pupae.

In comparison with the JH-III concentrations in larvae the titer is considerably higher in adults. After 24 h the flies have already exceeded the peak maximum of the larvae. On day 5 when the females already lay eggs whereas the males are still sterile, the former exceed the latter in JH contents. Day 10, however, brings a change in relations. Male flies which now have become mature exceed the females by about 13%. Few acts only are known about the possible role of JH in male insects [30–33].

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