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Head critical period and juvenile hormone titre during the last larval instar of the cockroach, *Periplaneta americana*

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Abstract. From neck ligation experiments with last instar larvae of the cockroach *Periplaneta americana* it was concluded that the head critical period is reached around day 17, which corresponds to 59% of the last larval stage. At the same stage the juvenile hormone III titre in the hemolymph dropped to undectable levels.
Key words. Head critical period; juvenile hormone; moult regulation; *Periplaneta americana*.

Insect moulting is a process which is regulated by different hormones in cooperation with the nervous system. It is generally accepted that a glandotropic neuropeptide (ecdysiotropin, prothoracicotropic hormone, PTTH) originating from the brain stimulates the prothoracic gland to produce and release ecdysone, which is metabolized in the hemolymph to the moulting hormone, 20-hydroxyecdysone. In normal, uninjured animals, after triggering of the ecdysone release in the prothoracic gland by a surge of ecdysiotropin into the hemolymph the gland becomes independent from the brain, and the physiological processes of the next moult are initiated. This period of ecdysone synthesis and secretion becoming independent of the brain is called the 'head critical period'. It results in the commencement of the next moult[1]. Juvenile hormone (JH), in relation to moult in insects, is known to be critical for the regulation of differentiation and metamorphosis. The presence of species-specific titres of JH in the haemolymph at the onset of the ecdysteroid rise that causes each moult determines the character of a moult as larval or pupal. Whereas in holometabolous insects[2] JH is still produced by the corpora allata in the early part of the final instar, the titre of JH differs in hemimetabolous insects in quantity and in the time-course of its production[3]. For example, in the cockroach *Nauphoeta cinerea*[4], the cricket *Teleogryllus commodus*[5], and in *Locusta migratoria*[6] JH levels are low to undetectable early in the final instar. In the cockroach *Diploptera punctata* a brief period of juvenile hormone synthesis occurs at the beginning of the last larval stage[7].

In *Periplaneta americana*, the determination of JH-titres with biological tests only has been published[8]. However, there are no reports of recent determinations of JH-titres in this cockroach with more highly sensitive methods. Data on ecdysiotropic activity in hemimetabolous insects are also quite scarce at present. The head critical period may serve as a reliable indicator for the time of ecdysiotropin release into the hemolymph.

Materials and methods

The method of determining the head critical period is either through decapitation or through neck ligation. We chose the method of neck ligation. Newly moulted larvae of the last larval instar (nymphs) of *Periplaneta americana* were selected, and the neck ligated at different times after the moult. Before ligation, the larvae were starved for two days to empty the gut. After ligation, larvae were kept in Petri dishes on moist filter paper to avoid desiccation. Since ligated larvae are not able to eclose, apolysis was controlled by scraping with forceps on the dorsal middle line of the pronotum and at the abdominal segments. Additionally, larvae in apolysis are characterized by a reduced locomotor activity. Animals which moulted to a larva (and were thus shown to have been penultimate instar larvae) or which had amputations were excluded from the calculations. Untreated control larvae were kept in groups of maximally 20 larvae in containers like the ligated larvae, in a climate chamber (27 °C, 60% humidity, light/dark period 12/12 hours). The length of the larval stage in the control larvae was 28.9 ± 3.4 days ($n = 89$). The survival time of neck ligated larvae which did not show signs of moulting was 9.8 ± 4.4 days ($n = 451$) under the conditions used.

Hemolymph sample preparation and quantification of JH III by combined gas chromatography selected ion monitoring mass spectrometry (GC-MS-MIS) was done by the method of Rembold and Lackner[9]. The ethyl ester of the hormone being assayed was used as an internal standard. Dimethyl-3,3,4,4,5,5,6,6,6-nonafluorohexyl-chlorosilane (DMNFHSCI) was purchased from Fluka (Buchs). The limit for determination of JH III

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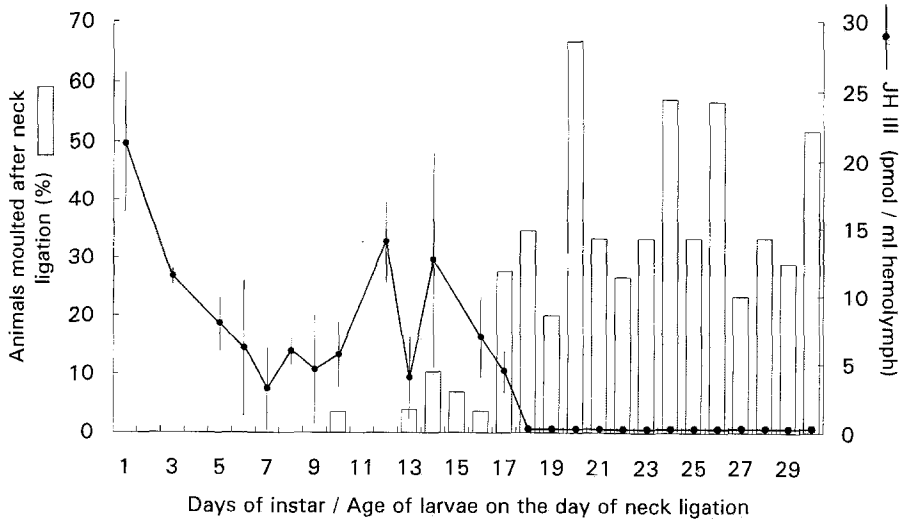

Figure. Moults of last instar larvae of *Periplaneta americana* after neck ligation, expressed as percentage of ligated animals, and hemolymph juvenile hormone titer of nonligated animals in pmol/ml (bars indicate SEM, p = 1%, n = 5–7).

according to this method was 0.05 pmol per injected sample.

Results

The time after which the brain plays no further role in the activity of the prothoracic gland (head critical period) was determined by inspecting the ligated larvae for moulting signs (apolysis) every day. The results are shown in the figure and reveal that animals ligated before day 9 never moulted, while of the animals ligated between the 10th and 16th days of their instar a mean of 4% (n = 203) showed moulting signs. On the 17th day the moulting rate of ligated larvae increased significantly and varied between 30 and 70% until the end of the instar (fig.). In those animals which showed moulting signs like abolition of the old cuticle or even only slits in the dorsal ecdysial line of the pronotum, these signs appeared in the group up to the 16th day at a mean of 6.4 ± 2.4 days after neck ligation (n = 8), and at 4.7 ± 2.8 days (n = 154) after neck ligation in the group older than 16 days. Thereafter they died. This means that the head critical period of the last larval instar is about the 17th day, i.e. a few days after the middle (at a period corresponding to 59%) of the last larval stage.

The titre of juvenile hormone III in the hemolymph declined during the first third of the larval stage (fig.). In the middle of the larval stage it slightly increased again to a transient peak. Between the 17th and 18th days it finally dropped to undetectable levels.

Discussion

The timing of the head critical period in *Periplaneta americana* is in coincidence with that of other cockroach species. In *Diploptera punctata*[10], the head critical period was determined by neck ligation to be on the 12th day, i.e. after 55% of the larval stage. In *Blattella germanica*[11], the head critical period as well as the regeneration critical period were found after artificial synchronisation of the larvae, by feeding after a period of food deprival, to occur on the 3rd day, i.e. at 58% of a 5.4-day stage (4th instar). In younger *Periplaneta americana* larvae the regeneration critical period was found in leg amputation experiments to be at 65% of the larval stage[12].

In *Periplaneta americana*, ecdysteroid synthesis and secretion in the prothoracic gland increases slowly after the 18th day to a peak on day 22 of about 10 ng secreted ecdysteroids during 6 hours of incubation[13–15]. In *Diploptera punctata*, after the middle of the 22-day larval stage, an increase of ecdysone hemolymph titre appears between days 13 and 20, i.e. also immediately after the critical period[10].

Ecdysiotropic activity in the brain of *Periplaneta americana* is present from the 16th day, as shown in experiments in which prothoracic glands were incubated with crude extracts of brains under in vitro conditions[13].

The head critical period of *Periplaneta americana* in the last larval stage is characterized, moreover, by a decrease of the juvenile hormone III titre in the hemolymph below an undetectable level. Also in *Diploptera punctata* in the early days of the final stage the corpora allata produce up to 5 pmol/h juvenile hormone[7]. After the 10th day the corpora allata are inactive in the final stage[16].

In contrast to the available data on *Periplaneta americana* and the cockroaches listed above, in some other insect species the head critical period appears markedly earlier or later in the instar. For example, in the lepidopterous insect *Manduca sexta* the head critical period

was determined on the 4th day, i.e. at a time corresponding to 40% of the 10-day last instar[1]. In the wax moth *Galleria mellonella*[17], (Böhm in preparation) the head critical period was found to be at a period corresponding to about 75% of the last larval stage. In *Rhodnius prolixus*, a hemimetabolous insect like *Periplaneta*, the head critical period was determined to be on the 5th–6th days, i.e. at 26% of the duration of a 21-day stage[18].

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