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Regulation of juvenile hormone titer in African locust

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Summary. In the African locust, the titer of C-16 JH in hemolymph (determined by GC-MS), reflects the rate of hormone biosynthesis (determined by RCA) in normal adult females. Severance of the nervi corporis allati-I (NCA-I) results in a low C-16 JH biosynthesis without affecting physiological events dependent on JH. In NCA-I-transectioned animals, JH titer is higher than in control locusts. JH catabolism does not seem to be involved in this high titer of hormone associated with a very low rate of JH production. In sham-operated females, the bulk of injected [^{3}H]C-16 JH quickly disappeared from the hemolymph but JH was retained in the bloodless body. After severance of the NCA-I, the remaining radiolabeled JH in the hemolymph increased. These results suggest the role of tissue JH-binding or of JH excretion in regulating its level in the locust.
Key words. Juvenile hormone; biosynthesis; catabolism; tissue binding; titer; disconnected copora allata, locust.

Many physiological events in insect life are affected by juvenile hormone (JH). Hemolymph JH titers reflect the net difference between JH biosynthesis, JH catabolism JH excretion and JH binding; events whose relative contribution to the regulation of JH titer remain uncertain. A major mechanism for the regulation of JH titers in larval insects is believed to be catabolism in the hemolymph[1,2]. On the other hand, several authors[3,4] have suggested that changes in the rate of biosynthesis are a major contributor to JH titer change. We have now studied both biosynthesis and JH titer in *Locusta migratoria* females, and our data support the second view in normal adult females. Nevertheless, in adult female locusts whose corpora allata (CA) were denervated, we have shown that neither JH biosynthesis nor JH catabolism were major contributors to changes in JH titer. We suggest that binding of JH by tissues and/or JH excretion could be important factors in controlling JH hemolymph titer.

The pattern of biosynthetic activity of the CA of adult female locusts has been studied with a radiochemical assay in vitro[5] and shown to correlate with ovarian events during the first gonadotrophic cycle[6,7]. After transection of nervi corporis allati I (NCA-I) on the first day of adult life, CA showed a very low rate of synthetic activity[7]. This activity was constant within the 15 days of the observation period. The low biosynthetic activity of the CA after NCA-I transection did not affect ovarian development[7], whereas allatectomy of the young females suppressed ovarian development[8]. Severance of the NCA-I at the beginning of the fourth-larval stadium prevented an increase in JH biosynthesis during the whole of the stadium; but this decreased synthesis did not alter the nature of the next molt which was typically larval[7].

To investigate more fully the apparent discord between CA activity and JH-induced physiological changes after NCA-I severance, we investigated other physiological situations which have been shown to be JH dependent. So, we compared male sexual behavior, male accessory gland activity, and the yellowing that accompanies sexual maturation in operated and sham-operated locusts. We observed that after the CA had been denervated on the first day of adult life, JH production was much lower than in sham-operated males (fig. 1), but all the physiological events under the control of JH occurred at the same time in operated and sham-operated males. Secretions of the male accessory gland began on the 4th day. Mating behavior occurred on days 10–12, but spermatophores containing spermatozoa began to be produced only on the 16th day; inseminated females

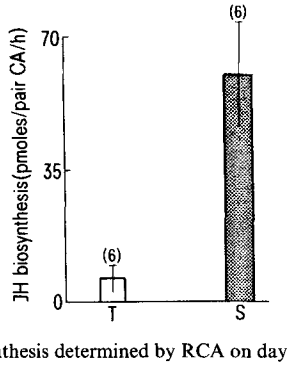

Figure 1. JH biosynthesis determined by RCA on day 21 in NCA-I-transectioned males (T) and sham-operated males (S). The operation was performed on the first day of adult life. Mean ± SEM. Number of animals in parentheses.

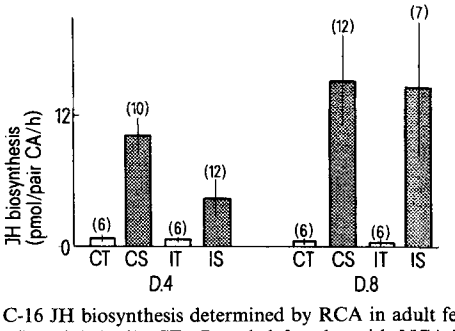

Figure 2. C-16 JH biosynthesis determined by RCA in adult females on days 4 (D.4) and 8 (D.8). CT, Crowded females with NCA-I-transectioned on the first 3 h of the fourth larval stadium. CS, Crowded sham-operated females. IT, Isolated females with NCA-I transectioned in the first 3 h of the fourth larval stadium. IS, Isolated females, sham-operated in the first 3 h of the fourth larval stadium. Means ± SEM. Number of animals in parentheses.

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had viable progeny. On the 16th day, the males became yellow, which is characteristic of sexual maturation. Moreover, JH is also involved in determining locust phase polymorphism[8]. CA activity was much reduced in adults with transectioned NCA-I in both isolated and crowded conditions (fig. 2); sham-operated isolated locusts exhibited nearly as high a biosynthetic activity as corresponding crowded locusts (fig. 2). The low biosynthetic activity of denervated CA from the isolated animals did not affect the tendency of the isolated animals to maintain the solitary form. During the 5th larval stadium and the adult phase, operated, isolated locusts became green (solitary color) and their adult solitary index (F/C) is characteristic of the solitary phase (fig. 3).

Some of us have suggested that CA of control animals synthesize an 'excess' of JH, but that the threshold sensitivity of target tissues may be compatible with the reduced activity of the denervated CA[7]. Using gas chromatography-mass spectrometry (GC-MS) and radiochemical assay (RCA), we have now correlated the activity of CA with the hemolymph JH titer in operated and sham-operated adult female locusts. In sham-operated females, GC-MS determination shows that the JH titer increases during the first gonadotrophic cycle (fig. 4B). The activity of the CA of corresponding ages, as determined by RCA, reflects hemolymph JH titer fluctuations (fig. 4A). These results suggest that in sham-operated female locusts, JH biosynthesis may be an important contributor to changes in JH titer. In females with NCA-I-transection, C-16 JH titer was higher than in controls (fig. 4B). This result is very surprising since the biosynthetic activity of denervated CA was very low during the whole ovarian maturation stage (fig. 4A). Nevertheless, the high titer in operated locusts is in agreement with the observed maintenance of JH-dependent physiological events. In this experimental situation, JH biosynthesis by the CA does not appear to be the main determinant of hemolymph JH titer. The existence of a source of JH biosynthesis other than the CA could explain such a peculiar situation. However, we analyzed the JH titer in the hemolymph of 10-day-old adult females which had been allatectomized on day 1; we could not detect any JH by GC-MS. This result confirms that the CA are the exclusive source of JH in adult *Locusta* females.

After injecting racemic [10-^{3}H]-C-16 JH (NEN) (2 pmoles in 5 µl 50% ethanol solution, 11 Ci/mmole) into the hemocoele, we tried to estimate JH catabolism, excretion, and binding. During

Recovery of [10-^{3}H] C-16 JH (2 pmoles in 5 µl 50% ethanol solution, 11 Ci/mmole) at various times following injection into the hemocoele of 9-day-old females

Incubation (min)	Hexane (dpm)	Methanol (dpm)	Total (dpm)	HS ratio
A) Control females				
1	41841	7777	49618	0.84
5	33248	14112	47360	0.70
10	27073	18856	45926	0.59
20	25888	22116	48004	0.53
40	15087	28494	43581	0.35
60	15964	29791	45755	0.35
B) NCA-I transectioned females				
1	36516	8907	45423	0.80
5	24396	11976	36372	0.67
10	29924	18294	48218	0.62
20	23262	21090	44352	0.52
40	17506	28092	45598	0.38
60	15056	27675	42731	0.35

Each locust was homogenized in 6 ml of methanol/water (70/30); JH was then partitioned with 4 ml of hexane. Radioactivity in methanol and hexane was determined by liquid scintillation counting in 5 ml Ready sol MP (Beckman). The only labeled compound in hexane, detectable by HPLC analysis on an ODS column with methanol/water (80/20), had the same retention time as C-16 JH and the total radioactivity of the hexane phase was recovered in this compound. HS ratio was defined as the proportion of hexane-soluble radioactivity out of the total recovered radioactivity.

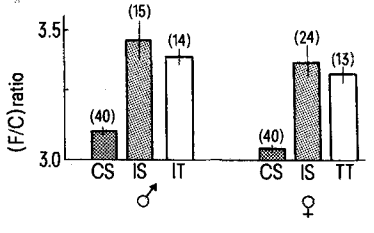

Figure 3. Femur to head capsule (F/C) ratio in groups of locusts (this ratio is an indicator of gregarious vs solitary phases). NCA-I was severed within the first 3 h of the fourth larval stadium. F/C ratio (length of hind femur/maximum width of head) was determined on 4-day-old adults. CS, Crowded sham-operated locusts. IS, Isolated sham-operated locusts. IT, Isolated NCA-I-transectioned locusts. Means ± SEM. Number of animals in parentheses.

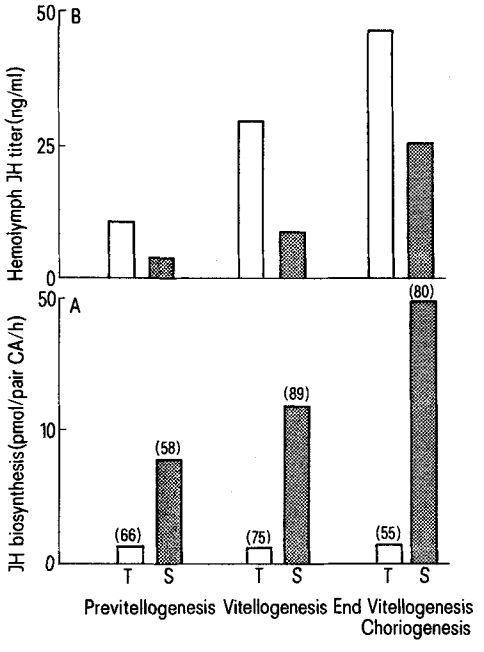

Figure 4. Hemolymph JH titer determined by GC-MS (fig. 4B) and rate of JH biosynthesis determined by RCA (fig. 4A) in adult female locusts during the first gonadotrophic cycle. The NCA-I were transected (T), or a sham operation (S) performed, on the first day of the adult life. Hemolymph from adult females was collected by centrifugation of cold-anesthetized locusts. To get the large amount of hemolymph necessary for GC-MS determination, the oocyte maturation cycle was divided arbitrarily into three periods and blood was collected and pooled. Each pool contains hemolymph from more than 50 females. After protein precipitation with acetonitrile, a hexane extract was successively purified on Sep pak C-18 (Waters Ass.)[12], on an aluminium oxide column, and by HPLC on an ODS column with methanol/water (80/20). Gas chromatographic separation of extracts was performed as previously described[13] on a 25-m CP-Sil 5CB column (Chrompack). The mass spectrometer (Nermag) was used in the positive chemical ionization mode (CI+) with ammonia as reagent gas. Ions with mass 252 (MH^+-CH_3 OH+NH_3) and 235 (MH^+-CH_3OH) were chosen for selected ion monitoring. An internal standard ([10–^{3}H]iso-C-18 JH[12], a gift from Dr D. Schooley, Zoecon) was used to determine extraction efficiency. Calibration curves were obtained with standard C-16 JH (Calbiochem). Only C-16 JH is synthesized by locust CA[13,14] and detected in locust hemolymph[15,16]. Activity of CA was determined using RCA[5]. Pool composition was the same as in JH titer determination.

the first h following injection of [^{3}H]-C-16 JH, the total radioactivity recovered from the insect was similar to the amount injected in transectioned as in control females (table). JH catabolism, as determined by the ratio hexane-soluble JH remaining to the total recovered radioactivity, was similar in experimental and control females. Radiochromatograms of homogenized locusts or hemolymph extracts indicated that the only labeled compound in the hexane phase (see legend to the table) had the same retention time as C-16 JH. The half-life of [^{3}H]-C-16 JH was 23 min in operated and sham-operated locusts, in agreement with a previous determination[9]. The half-life of the natural enantiomer of C-16 JH is probably longer than the half-life of the [^{3}H]-C-16 JH[10]. The discrepancy between JH biosynthesis and JH titer in NCA-I-transectioned females therefore does not seem to result from a reduced catabolism.

Only 1 min after injection of [^{3}H]-C-16 JH into the hemocoele, the percentage of labeled hormone recovered in the hemolymph of control animals was low (< 10%). However, the labeled JH was not catabolized because at this time point the recovered radioactivity in the total animal was about 100%, and 80% of this radioactivity was [^{3}H]-C-16 JH (table). This result suggested the retention of a large amount of JH by the tissues and/or the uptake of the JH by the gut. After severance of NCA-I, the percentage of labeled hormone recovered in the hemolymph was 20%, which suggested a reduction of the JH tissue binding and/or the gut uptake. Those factors might be determining agents in the regulation of hemolymph JH titer and could contribute to maintain a high hemolymph JH titer in NCA-I-transectioned females even though JH biosynthesis by the CA was very low.

In conclusion, in *Locusta,* as in *Leptinotarsa*[11] and *Diploptera*[3], JH biosynthesis reflects JH titer in the hemolymph in normal females. Our experiments with allatectomy confirm that in *Locusta* females, the CA seem to be the exclusive source of JH. Moreover, in the locust the retention of JH by the tissues and/or the uptake by the gut were significant and could also be involved in the regulation of the JH titer. Evidence for these processes was found after nervous disconnection of the CA, which reduced JH biosynthesis and uptake of the JH from the hemolymph. The reduced uptake by the tissues and/or the gut promotes JH conservation in the hemolymph and could partially counterbalance the deficiency in JH biosynthesis, and explain the high JH titer and the normal accomplishment of all JH-mediated physiological events.

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Molecular cloning of the fibroin light chain complementary DNA and its use in the study of the expression of the light chain gene in the posterior silk gland of *Bombyx mori*[1]

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Summary. Fibroin light chain (L-chain) mRNA (mol. wt 4.0×10^5 daltons) was purified from the posterior silk gland of the silkworm, *Bombyx mori* (J-131 strain). Double-stranded complementary DNA was synthesized and inserted into the PstI site of pBR322 employing the oligo(dC)-oligo(dG) tailing method. Several recombinant plasmids containing the inserts of about 800 base pairs were isolated. Hybridization-translation assay demonstrated that these clones hybridized specifically with the fibroin L-chain mRNA. One of these clones (pLA23) was used as a probe to investigate relative concentrations of the fibroin L-chain gene and mRNA in the posterior silk glands at different stages of late larval development.
Key words. Silkworm; *Bombyx mori;* silk gland; mRNA; complementary DNA; fibroin light chain; molecular cloning.

A large amount of fibroin is synthesized and secreted in the posterior silk gland of the silkworm at the 5th instar and this system has been considered to be a suitable model for investigating control mechanisms underlying the expression of a specific gene in a specific eukaryotic tissue. The fibroin molecule consists of two polypeptides of different mol. wts linked by disulfide bonds[4-6]. The large polypeptide, termed a heavy chain (H-chain), has a mol. wt of approximately 350,000 and an amino acid composition of the so-called 'fibroin-type', that is, particularly rich in glycine, alanine and serine. On the other hand the