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Changes in binding of JH-III in hemolymph of adult female *Locusta migratoria*

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Summary. Hemolymph from adult female *Locusta migratoria migratorioides* was analyzed for binding of juvenile hormone III (JH-III) after allatectomy and transection of the *nervus corporis allati* 1 (NCA-I). These operations did not affect the apparent dissociation constant of the binding (Kd = 3.3 10^{-8} M). The concentration of binding sites exhibited fluctuations in relation to age and type of operation: an increased concentration of binding sites in females with disconnected corpora allata and a decreased concentration in allatectomized females. The changes in concentration of binding sites was not due to differences in water content or hemolymph volume in operated animals. The hemolymph protein concentration was reduced after NCA-I transection and even more after allatectomy. However, variations in protein concentration did not correlate with changes in concentration of JH-III binding sites. The changes in binding site concentration were related to changes in JH-titer.
Key words. locust; corpora allata; JHBP; hemolymph protein content; hemolymph volume.

In insect hemolymph, juvenile hormone (JH) has been shown to associate specifically with binding proteins (JHBP)[1,2]. The JHBP facilitate JH transport from the corpora allata to the target tissues and prevent degradation by carboxyesterases or unspecific binding to the body wall and tissue membranes. Furthermore, it has been suggested that JHBP may be involved in recognition of the target cell[3,4].

On the basis of affinity, specificity and binding capacity, 2 classes of JHBP have been identified in Lepidoptera: low affinity high molecular weight lipoprotein and high affinity low molecular weight protein[1]. Moreover, in insects like *Locusta migratoria* with only JH-III (C-16) as natural hormone[5], hemolymph contains a third class of JHBP with high affinity and high molecular weight[6].

By protecting JH against carboxysterases, the concentration of JHBP may have a significant influence on the JH titer. In *Manduca sexta* larvae, direct correlation between relative hemolymph JH titer and the level of JHPB was noted only at the beginning of the fourth and the fifth instars. During the rest of the fourth instar JH and JHBP concentrations appeared to be inversely related[2]. In the present study, fluctuations in JHBP concentrations were investigated in batches of female *Locusta migratoria* known to contain different JH titers: 1) adult females with increasing JH titers during oocyte maturation[7], 2) allatectomized females without JH after removal of the JH source[7] and 3) females with corpora allata disconnected from the *nervus corpori allati* I (NCA-I). NCA-I transection results in higher JH titers than in controls, despite low rates of JH biosynthesis by these corpora allata[7].

Allatectomy and NCA I transection were performed on day 1 adult females as previously described[8]. Hemolymph samples were collected into capillary pipettes after cutting the neck membrane. Hemocytes were removed by centrifugation (10,000 g, 5 min, 4 °C) and hemolymph from 5 animals was pooled.

Native gradient PAGE (slab gels 4–20%, c = 2.7%, 2 mm thick, used horizontally with 0.11 glycine titrated with Tris pH 8.9, 24 h with constant 200 V at 10 °C) of hemolymph from controls, allatectomized and disconnected corpora allata females, revealed qualitatively similar patterns (results not shown). According to de Bruyn et al.[9], a band with molecular weight of 575,000 Dalton can be identified as JHBP. Lower staining intensity was observed for the JHBP band in hemolymph from allatectomized females, but the total protein content was also lower[10].

To investigate changes in JHBP content of different hemolymph samples, we used Scatchard plot analysis. Increasing amounts of JH-III (Calbiochem) were incubated with a constant amount of hemolymph and the hormone-protein complex precipitated with polyethylene glycol (PEG)[11]. The concentration of JH-III in ethanolic solution was checked spectroscopically at 220 nm (E = 13,800)[12]. Carboxyesterase and protease activities were inhibited by Para-oxon (10^{-4} M) and PMSF (Phenyl methyl sulfonyl fluoride 10^{-4} M) respectively. JHBP of *Locusta* bind efficiently and specifically to 10R JH-III[6,13]. A very small amount of hemolymph (1 µl in 800 µl TMK buffer) was used to bind about 50% of racemic [10 ^{3}H] JH-III (NEN Corporation) at the concentration of 10^{-9} M.

Scatchard plot analysis of [10 ^{3}H] JH-III binding to hemolymph

Water content (determined by the ratio (fresh weight − dry weight)/fresh weight) and hemolymph volume (determined by labeled inulin dilution) in day-1 allactectomized females, day-1 NCA-1 transected females and control females. Means ± SEM; n, number of individual determinations

Females	Water content (%)	Hemolymph volume (µl)
Control	70.07 ± 0.68 (11)	794.73 ± 72.00 (11)
NCA-transected	71.85 ± 1.07 (12)	915.25 ± 67.64 (12)
Allatectomized	69.93 ± 1.08 (13)	774.58 ± 75.03 (12)

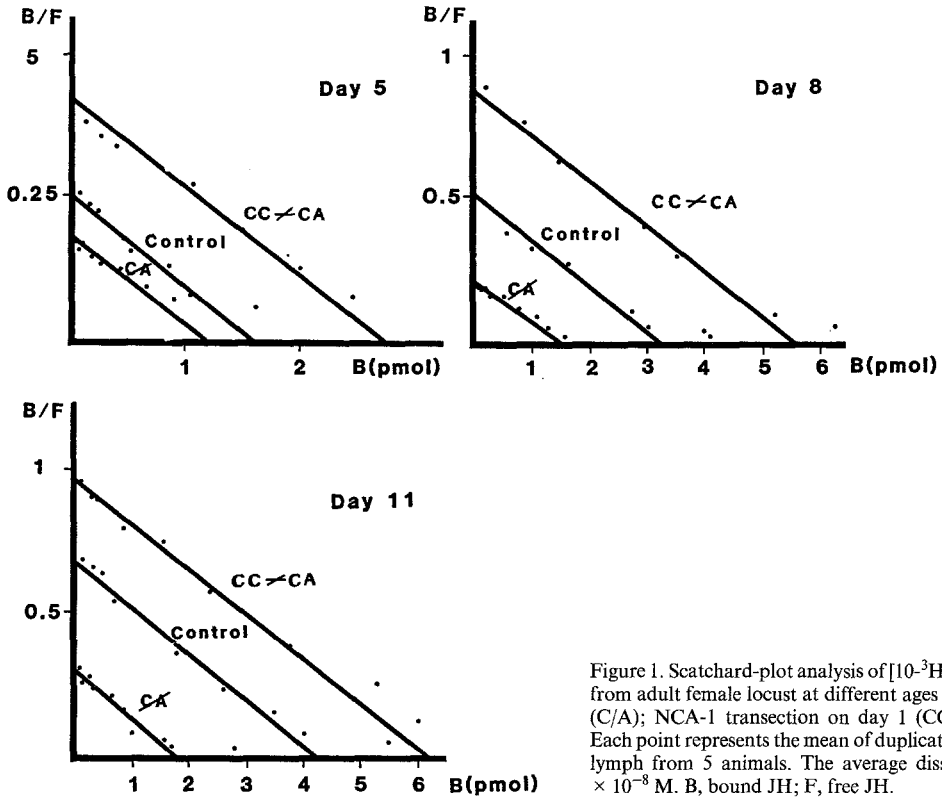

Figure 1. Scatchard-plot analysis of [10-^{3}H] JH-III binding to hemolymph from adult female locust at different ages and after allatectomy on day 1 (C/A); NCA-1 transection on day 1 (CC ≠ CA) or control (Control). Each point represents the mean of duplicate experiments on pooled hemolymph from 5 animals. The average dissociation constant (Kd) is 3.3 × 10^{-8} M. B, bound JH; F, free JH.

showed the same apparent dissociation constants (Kd) values (3.3 10^{-8} M) for hemolymph of control, allatectomized and NCA-I transected females of different ages (fig. 1). This result, together with the observations using native pore PAGE suggested that JH binds to the same JHBP in hemolymph of control and operated females.

From Scatchard plots, the concentration of binding sites can be calculated. Large variations exist at different ages and after different microsurgical operations (fig. 2). In control females, the concentration of binding sites increased between day 5 and day 11. Allatectomized females and females with disconnected corpora allata also exhibited an increasing concentration of binding sites during the first 11 days, but these operations induced important changes in concentration of binding sites. After allatectomy, the JHBP concentration is lower than in controls, but after NCA-I transection, JHBP concentration is higher than in controls.

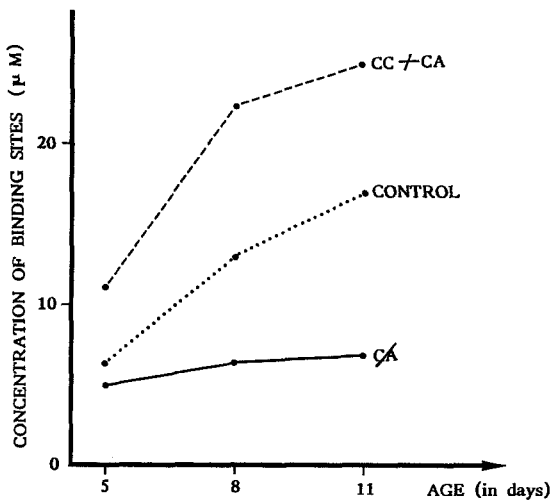

Figure 2. Changes in concentration of binding sites calculated from Scatchard plot analysis (fig. 1) for day-1 allatectomized females (C/A), day-1 NCA-1 transected females (CC ≠ CA) and control females (Control) during the first 11 days of adult life.

Comparison of the concentration of binding sites with the JH titer[7] suggests a correlation between these parameters. Increasing concentration of binding sites during the first 11 days seems to be related to increased JH titers. Furthermore, allatectomized females which contained no JH have rather low concentrations of binding sites. On the other hand, females with corpora allata disconnected from the brain have higher concentrations of binding sites, which is correlated with higher JH titers in these animals[7].

Changes in concentration of JH-III binding sites may result from changes of JHBP content or changes in hemolymph volume or both. To elucidate this question, we estimated water content and hemolymph volume on day 8 in controls and operated animals. Water content was investigated by calculating the ratio (fresh weight – dry weight)/(fresh weight) after drying animals at 80 °C for 12 h. No differences were found in water content (table). Hemolymph volume was determined with [^{3}H] inulin (NEN) by calculating inulin dilution 30 min after injection. Hemolymph volume did not differ significantly between experimental and control animals (Student's t-test; table). Thus, changes in concentration of JH-III binding sites are the result of changes in JHBP concentration.

Synthesis of JHBP is believed to occur in the fat body[14,15]. In locusts, fat body differentiation and protein synthesis are controlled by JH[16]. After allatectomy of the adult on day 1, the fat body failed to differentiate and the hemolymph protein concentration was dramatically reduced[10,16]. To investigate whether the concentration of JH binding sites is correlated with variation in total protein concentration, we measured the hemolymph protein concentration on day 8 in experimental and control females by the method of Bradford[17]. Our results (fig. 3) confirm a reduced protein concentration in allatectomized females. Females

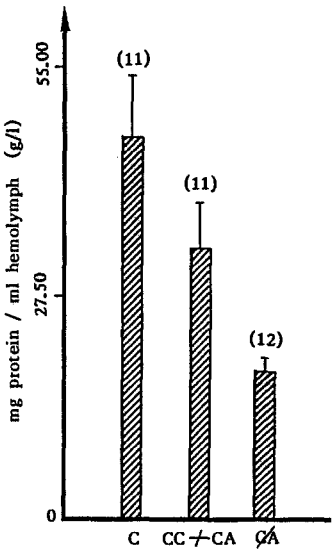

Figure 3. Hemolymph protein concentration on day-8, in day-1 allatectomized females (C/A), day-1 NCA-1 transected females (CC + CA) and control females (Control). Mean ± SEM; n, number of individual measurements.

with disconnected corpora allata show lower protein concentrations than controls but higher than allatectomized females. These results indicate that changes in concentration of JH binding sites do not reflect total protein concentration, suggesting that there is a specific control of JHBP concentration.

It is not yet clear why differences in concentration of JH-III binding sites exist, since the JHBP concentration is far in excess of the physiological JH titers[6,7]. It would also be interesting to know whether JHBP concentration is induced by changes in JH titer. Indeed, further investigations are necessary to establish the significance of JHBP fluctuation and to understand the role of JHBP in maintaining the JH titer in hemolymph.

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Calcitonin gene related peptide stimulates adenylate cyclase activity in rat striated muscle

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Summary. Rat calcitonin gene related peptide (CGRP) and salmon calcitonin (CT) stimulated adenylate cyclase activity in a dose-dependent manner in the rat diaphragm and in the kidney. The ED_{50} value of rat CGRP was lower and that of salmon CT was higher in the diaphragm than in the kidney. These results suggest that CGRP stimulates adenylate cyclase activity in the striated muscle by reacting with sites distinct from the site in the kidney.

Key words. CGRP; calcitonin; adenylate cyclase; neuromuscular junction; neuropeptide.

Amara et al.[1] showed that alternative processing of RNA transcripts from the calcitonin gene resulted in the production of distinct mRNAs encoding the hormone, calcitonin (CT), or a predicted product referred to as calcitonin gene related peptide (CGRP). Its characteristic distribution in the nervous system suggested that CGRP may be a neurotransmitter or a neuromodulator[2,3]. Recently, Takami et al.[4] have found that CGRP coexists with acetylcholine in motor neurons and nerve terminals in neuromuscular junctions of striated muscle. Furthermore, CGRP enhances muscle contraction with a concomitant increase in cyclic AMP in the tissue[5,6]. To investigate further the action of CGRP on the cyclic AMP system, we studied the effect of CGRP on adenylate cyclase activity of the striated muscle, in comparison with the effect of CT, because it has been suggested that CGRP and CT may each cross-react with the specific receptor of the other in other tissues[7].

Male Sprague-Dawley rats (Charles River Japan) weighing about 200 g were decapitated and the diaphragm and kidneys were removed. Each tissue was homogenized in 100 vols of 10 mM Tris-maleate buffer (pH 7.4) by polytron for 20 s. The homogenates were centrifuged twice at 15,000 × g for 10 min with rehomogenization of the pellet in fresh buffer. The final pellets were suspended in 99 vols of the same buffer.

Adenylate cyclase activity was measured in an incubation mix-