Stimulation of Juvenile Hormone Biosynthesis *in vitro* by Locust Allatotropin

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In *Locusta migratoria* the gonotrophic cycles are regulated by juvenile hormone. The cyclical changes of juvenile hormone synthesis in locust corpora allata seem to be regulated by a neurohormonal factor. Such an allatotropin could be extracted from corpora cardiaca and brains of *Locusta migratoria*. It is a small pronase-sensitive and heat-stable peptide. Extract of one corpus cardiacum stimulates corpus allatum biosynthetic activity *in vitro* 5 to 20-fold.

Introduction

As in most insects in *Locusta migratoria* larval and adult development depends on juvenile hormone (JH). This hormone is synthesized and released into the haemolymph by the corpora allata (CA). The regulation of biologically active JH concentrations can occur at several different levels, *i.e.* synthesis, transport, sequestration, catabolism, and excretion [1–3]. Previous investigations in adult female *Locusta migratoria* indicate synthesis to be a prominent factor in the regulation of the JH titre [4]. In young female locusts the CA exhibit a low synthetic activity. This JH biosynthesis increases through previtellogenesis and reaches a maximum during vitellogenesis. At the end of an oogenic cycle JH synthesis decreases to low previtellogenic levels and rises again with the onset of the next oogenic cycle. An allatotropic factor originating in the brain and released by the corpora cardiaca (CC) is suspected to be responsible for this cyclic regulation of CA activity.

Materials and Methods

Locusts were kept at 30 °C with a constant photoperiod of 14 h and were fed wheat shoots, lettuce and oats. For preparation of extracts 50–100 CC and supraoesophagial ganglia were collected from females containing maturing oocytes, homogenized in 80% methanol and centrifugated at $8000 \times g$ for 5 min. Low molecular weight material was removed by chromatography of the supernatant on Sephadex G-10 in 80% methanol. The front peak was collected and dried with nitrogen. Prior to incubation the extracts were redissolved in incubation medium and insoluble material removed by centrifugation. JH synthesis in isolated CA was measured as described previously [4, 5]. Each pair of CA was placed in 50 µl of locust medium [11] containing [methyl-^{14}C]-methionine (Amersham-Buchler, FRG) with a final specific activity of 40.5 mCi mmol^{-1}. After incubation at 30 °C for 4 h a 25 µl aliquot was directly applied onto a thin layer plate and 10 µg JH III added as a carrier. After chromatography the JH containing spot was scraped off and the radioactivity determined in a liquid scintillation counter.

Digestion of extracts was performed with pronase (Boehringer, Mannheim) and trypsin (Serva, Heidelberg) (final concentration 1 mg/ml) at 37 °C for 2 h. The digestion was stopped by the addition of methanol. Extraction of the remaining allatotropin took place as described before.

Results and Discussion

Extracts from locust CC contain a factor which stimulates CA activity *in vitro*. This stimulation can be demonstrated by determining the biosynthesis of JH *in vitro* by isolated CA as described previously [4, 5]. The extract of 1.0 CC equivalents taken from a maturing female locust causes an increase of JH biosynthesis by a factor of 15 (Fig. 1). Diluted extracts are less potent. However, a significant response is only obtained by the addition of a dose larger than 0.1 CC equivalents. This indicates that the allatotropic factor is present only in small

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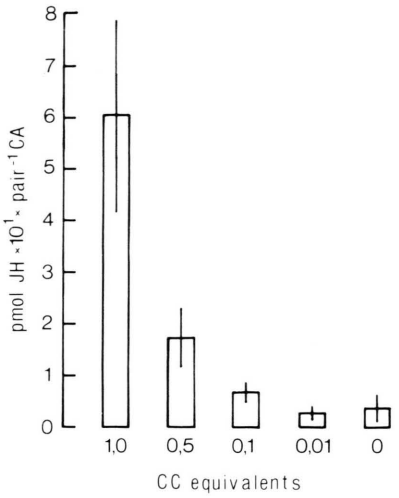

Fig. 1. Dose response of CA by extracts of CC from maturing female locusts (all data S. E. M., N = 5).

Table I. Assays of untreated, heat-treated (100 °C, 5 min) and enzyme digested allatotropin containing extracts.

Treatment	JH synthesis [pmol pair^{-1} CA]
1. Experiment:	
Control with allatotropin	19.5 ± 4.8
Control without allatotropin	2.1 ± 0.7
Allatotropin heated	18.6 ± 7.0
Allatotropin trypsin digested	19.7 ± 8.3
2. Experiment:	
Control with allatotropin	39.7 ± 16.8
Control without allatotropin	8.3 ± 4.1
Allatotropin pronase digested	10.7 ± 5.1

amounts or/and has a short half life. The addition of more than 1.0 CC equivalents does not always result in a further increase of JH synthesis. It cannot be excluded that other factors present in the extracts interfere with the JH biosynthesis. In our test system we used CA pairs taken from adult female locusts within 24 h after emergence. Such CA have very low biosynthetic activity with a rather small individual variation, while in older locusts a higher but considerably scattered activity is found [4]. Furthermore, those previous results indicate that CA biosynthetic activity is naturally turned on within a few days after emergence. The allatotropic factor contained in the tested extracts is obviously a small peptide which is heat-stable and pronase-sensitive, while trypsin has no effect (Table I). We estimate its molecular weight to be around 2000 Dalton. For a more detailed biochemical characterization the allatotropic factor has to be further purified, *e.g.* by HPLC. The crude extracts tested may also contain other active principles such as the adipokinetic hormone and a hyperglycemic factor [6, 7].

The allatotropic factor is also present in locust brains. Brain extracts made from immature and maturing females and assayed in the manner described gave the following results: 0-day females, 8.8 ± 6.1 pmol JH; vitellogenic females, 57.7 ± 11.1 pmol JH; controls, 2.6 ± 0.3 pmol JH (all data are S. E. M. of 4 h incubations, equivalents of 5 brains added to each incubation). These results suggest that the allatotropin is produced by the brain and released by the CC. A large number of histological studies relating paraldehydfuchsin stainable material in neurosecretory cells of the pars intercerebralis and its transport along the nervi corporis cardiaci I to the CC with CA activity support our observation. After injection of antibodies against locust brains and CC a cessation of the JH dependent oogenesis in *Locusta migratoria* was reported [8]. Electrocoagulation of the internal cardica tracts markedly suppressed CA biosynthetic activity in locusts [9]. However, electrostimulation of the cerebral neurosecretory cells enhances JH biosynthesis in the migratory locust [10]. Apparently the allatotropic factor described is released from these cells after electrical stimulation.

How the isolated allatotropin modulates JH biosynthesis remains to be clarified. We expect the allatotropin to be a short term modulator of CA activity, while growth of the CA glands and possible nervous stimuli may cause an overall and more persistent increase in JH biosynthesis. However, further purification and characterization of the locust allatotropin is necessary to answer these questions.

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