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ALLATOSTATIC AND ALLATOTROPIC FACTORS IN THE BRAIN OF THE DESERT LOCUST, *SCHISTOCERCA GREGARIA*

by

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SUMMARY

A polyclonal antibody against allatostatin-5 (Dip-AST 2) of *Diploptera punctata* was used to demonstrate allatostatin-like material in the brain of *Schistocerca gregaria*. Immunoreactivity was found in cells of the pars lateralis and in axons running to the corpus allatum (CA). A methanolic brain extract of 7000 brains was fractionated by HPLC on a reversed phase C18 column. Using a sensitive juvenile hormone biosynthesis bioassay, we detected several fractions that influence juvenile hormone synthesis by the corpora allata of the cockroach *Diploptera punctata*. Four fractions have strong allatostatic activity and three fractions have allatotropic activity.

Key words : neuropeptide, corpora allata, juvenile hormone.

INTRODUCTION

The corpora allata (CA) of insects are the major sites of production of juvenile hormone (JH) which plays a central role in the control of metamorphosis and reproduction in most insects (TOBE and STAY, 1985). These small glands are connected to the brain by two pairs of nerves : the nervi corporis cardiaci I (NCA I), which originate in neurosecretory cells of the pars lateralis and the NCA II, which originate in cells of the suboesophageal ganglion (RADEMAKERS, 1977 ; KONINGS *et al.*, 1989) (Fig. 1). JH biosynthesis and release has been the subject of extensive research and two groups of signalling substances have been reported : the allatotropins (WILLIAMS,1961 ; GIRARDIE, 1967), responsible for stimulation of JH biosynthesis and the allatostatins (TOBE, 1980) responsible for inhibition. Recently, seven allatostatins were isolated form the cockroach, *Diploptera punctata* (WOODHEAD *et al.*, 1989, 1994 ; PRATT *et al.*, 1991), two from the cockroach *Periplaneta americana* (WEAVER *et al.*, 1994), five from the fly *Calliphora vomitoria*

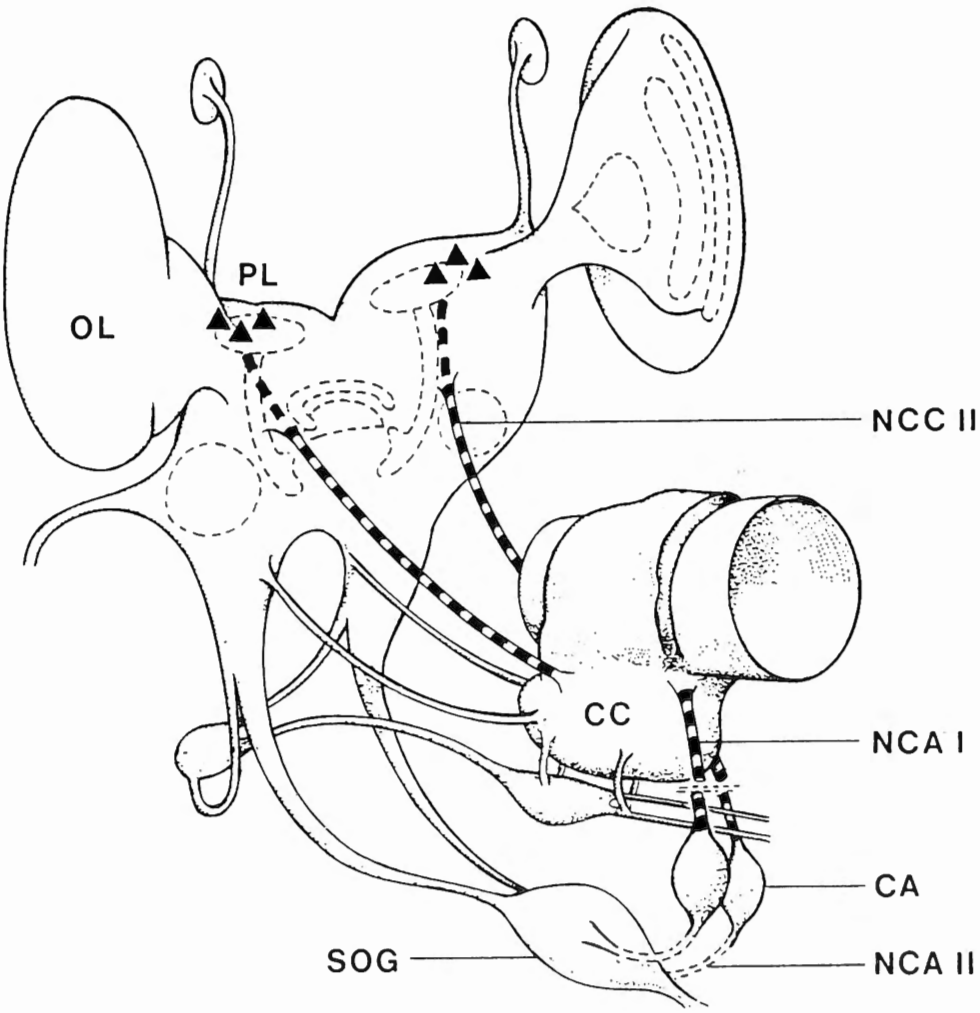

Fig. 1. — Diagram of corpora allata (CA) (KONINGS *et al.*, 1989) of *Schistocerca gregaria* with their connections to the brain via the nervi corporis cardiaci II (NCC II) and the nervi corporis allati I (NCA I) and to the suboesophageal ganglion (SOG) via the nervi corporis allati II (NCA II). Allatostatin immunoreactive cell bodies projecting to CA : closed triangles. PL, pars lateralis ; CC, corpus cardiacum ; OL, optic lobe.

(DUVE *et al.*, 1993) and one from the tobacco hornworm *Manduca sexta* (KRAMER *et al.*, 1991).

In locusts the presence of allatotropic factors (FERENZ and DIEHL, 1983 ; FERENZ 1984 ; GADOT and APPLEBAUM,1985 ; REMBOLD *et al.*, 1986 ; COUILLAUD and GIRARDIE 1990 ; LEHMBERG *et al.*, 1992) and allatostatic factors (VEELAERT *et al.*, 1995) has been reported but none have yet been purified. In this paper we

describe the presence of allatostatic and allatotropic factors in an extract of 7000 brains of *Schistocerca gregaria* and their partial purification by HPLC on a reversed phase C18 column.

MATERIAL AND METHODS

Animals and immunocytochemistry

Schistocerca gregaria (Forskal) was raised according to ASHBY (1972). Brains from adults were dissected in locust saline (NaCl 168 mM, KCl 6.4 mM, $NaHPO_4$ 1.2 mM, $NaHCO_3$, 3.0 mM, $MgCl_2$ 3.6 mM, $CaCl_2$ 2.1 mM). Immunohistochemical staining was accomplished by means of the peroxidase-anti-peroxidase method as described by VANDESANDE and DIERICKX (1976). Tissue preparation and specificity was controlled as described by VEELAERT *et al.* (1995).

Tissue extraction and HPLC purification

7000 brains of *Schistocerca gregaria* were extracted in methanol/water/acetic acid (90:9:1) and concentrated on C18 reversed phase Sep-pak cartridges (Waters Associates, Milford, MA). The Sep-pak cartridges were subsequently eluted with 50 % acetonitrile containing 0.1 % TFA and with 80 % acetonitrile containing 0.1 % TFA. The 50 % eluate was chromatographed on a Delta-Pak C18 column (250 × 10 mm) (Waters Associates, Milford, MA) on a Gilson HPLC system. HPLC conditions were as follows : solvent A, 0.1 % TFA in water ; Solvent B, 50 % acetonitrile in 0.1 % aqueous TFA. Initial conditions : 100 % A, then linear gradient to 100 % B over 180 min ; flow rate 6 ml/min ; detector : 5 absorption units full scale (AUFS) set at 214 nm. Peaks were collected manually every 4 minutes.

Juvenile hormone biosynthesis assay

Samples of 11 brain equivalents were dried in a Speedvac concentrator and assayed on the CA of *Diploptera punctata* (Eschscholtz) according to TOBE and CLARKE (1985). They were dissolved in methionine-free medium 199 containing Ficoll (20 mg/ml) and L-[methyl]-^{14}C-methionine (2.1GBq/mmol ; Amersham). Following 3-h incubations, samples were extracted and quantified as described by TOBE and CLARKE (1985). Eight glands were tested per group. Inhibition was determined by comparison with control glands, incubated at the same time (parallel incubations), to which no extract had been added. This established the control rates of JH release, and rates of release by treated glands were then expressed as a percentage of the control values.

RESULTS AND DISCUSSION

Allatostatin immunoreactivity is found in three cells of the pars lateralis (Fig. 2) with axons projecting to the CA. These cells belong to a group of cells at the rostro-ventral part of the pars lateralis whose axons contribute to the NCC II and the NCA I (KONINGS *et al.*, 1989).

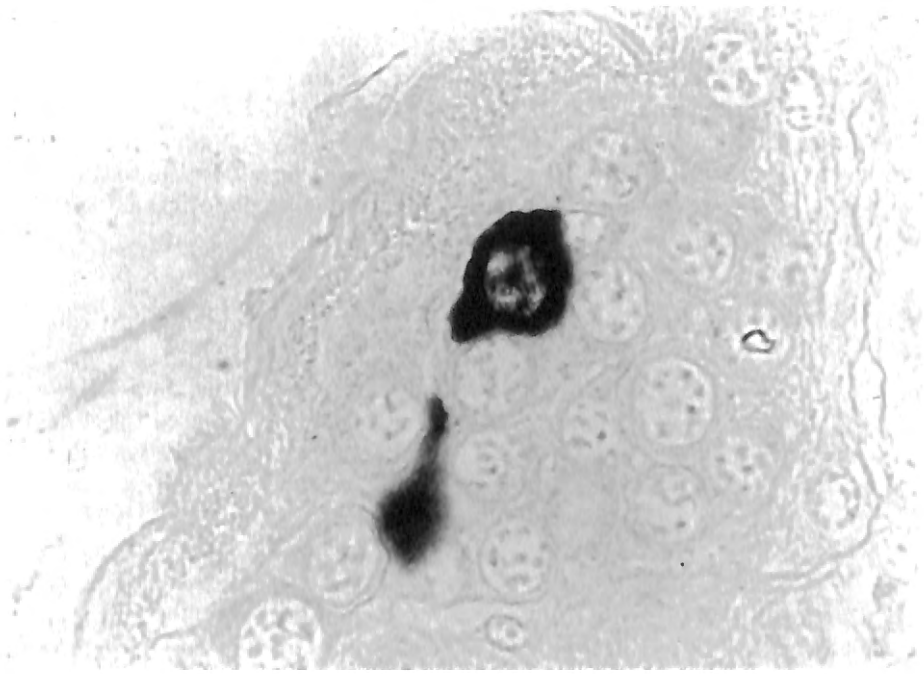

Fig. 2. — Allatostatin immunoreactivity in two cells in the pars lateralis of the brain of *Schistocerca gregaria*, 800 ×.

The inhibition of juvenile hormone biosynthesis by the fractions separated by HPLC is shown in Fig. 3. Fractions 12 till 23 show inhibition. Strong inhibition was found in fractions 15, 17, 18, 20 (more than 70 %). We found stimulation of juvenile hormone biosynthesis in fraction 7, 8 and 37 (more than 30 %).

Our results show that brain extracts of *Schistocerca gregaria* contain allatotropic and allatostatic factors and that it is possible to separate them by HPLC. Nothing is known about the nature of the allatotropic factors, but according to their retention times they must be very hydrophilic. Recently, VEELAERT *et al.* (1995) demonstrated that *Schistocerca gregaria* contains allatostatin (Dip-AST-2 ; DONLY *et al.*, 1993) -like material and that brain extracts of *Schistocerca gregaria* also inhibit juvenile hormone biosynthesis by the CA of *Locusta migratoria* and *Diploptera punctata*.

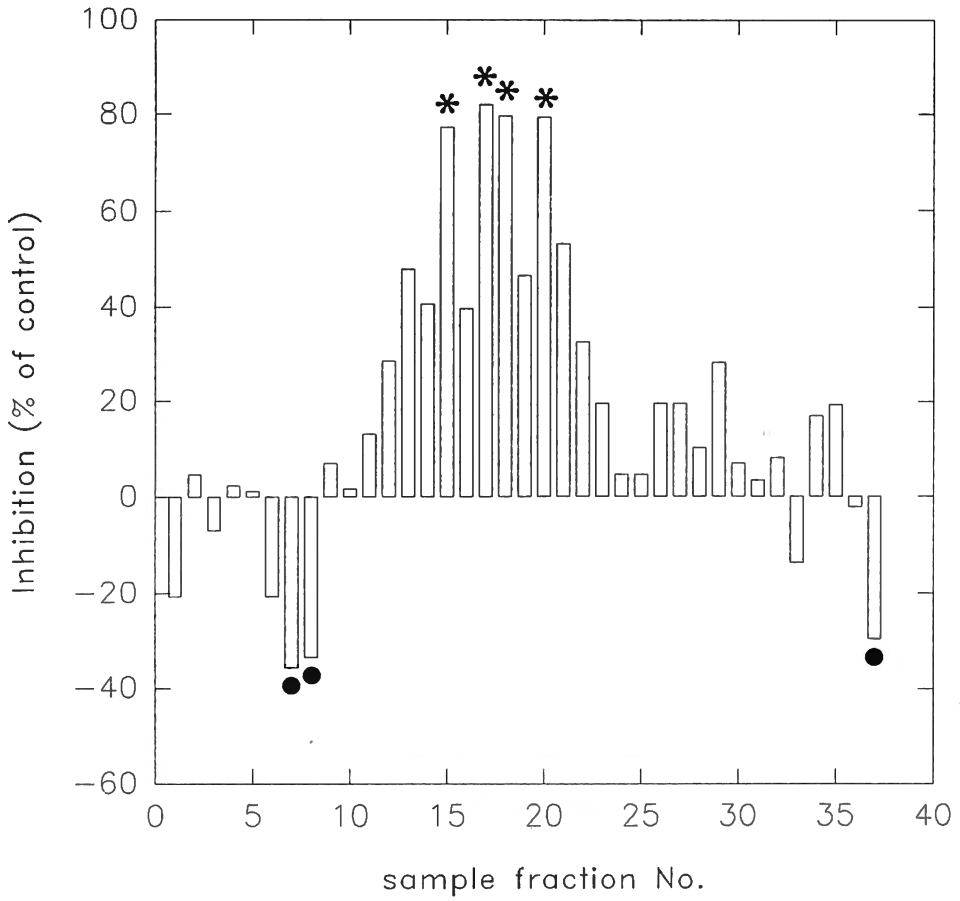

Fig. 3. — Inhibitory effect of 11 brain equivalents of *Schistocerca gregaria* on JH biosynthesis by 2 CA of virgin *Diploptera punctata* cockroaches (n = 8). *, allatostatic ; ●, allatotropic.

Hence, *Schistocerca gregaria* contains an allatostatin-like peptide that might be involved in regulating the production of JH by the CA. Screening the HPLC fractions with the juvenile hormone biosynthesis assay in combination with an ELISA or RIA for allatostatin-like material is probably the best way to purify peptides affecting the CA. Progress is being made in the final purification of the biologically active peptides.

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