Allatotropic activity of the median neurosecretory cells of *Galleria mellonella* (Lepidoptera) larval brain[1]

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Summary. The brain allatotropic hormone (ATTH) is released in *Galleria mellonella* from the Median neurosecretory cells located in the pars intercerebralis. These cells show the ability to elicit supernumerary larval molts upon implantation into sensitive host larvae, and the ability to in vitro stimulate the juvenile hormone synthesis in corpora cardiaca-corpora allata glands of *G. mellonella*.

Key words. Median neurosecretory cells; allatotropic hormone; juvenile hormone; corpora cardiaca-corpora allata glands; *Galleria mellonella*.

Neurohormonal regulation of juvenile hormone (JH) synthesis in corpora allata (CA) has been widely studied[2–5]. It has been shown that stimulation of JH synthesis can be mediated by the allatotropic neurohormone (ATTH) and inhibited by the allatostatic neurohormone; both hormones originate in the brain[6–10]. Brain cells in which they are synthetized have not been yet identified in any insect species.

For two insects species there is indirect evidence that the ATTH-cells are located in the pars intercerebralis; in *G. mellonella* larval brain cautery of MNC resulted in a loss of the brain ATTH activity[2], while in *Locusta migratoria* adult female brain electrostimulation of MNC substantially enhanced JH synthesis in CA[11].

The aim of the present investigation was to provide direct evidence that MNC of *G. mellonella* larval brain are the source of ATTH.

Material and methods. Wax moth, *Galleria mellonella*, was bred as described earlier[3]. The material comprised 3-day-old penultimate instar (VI_3) and 1-day-old last instar (VII_1) larvae either untreated or chilled (in order to stimulate the allatotropic activity of the brain)[12] at 0 °C for 3 h. Immediately after chilling the larvae were transferred back to 30 °C and used for experiments 18–24 h later.

Surgical procedures. Dissection of brain and corpora cardiaca-corpora allata complex (CCCA) as well as implantation of brain was performed as described by Sehnal and Granger[3]. Isolation of MNC from brain was carried out in MEM medium (Gibco, cat. no. 072-1300).

The brain was sectioned at the level of the deutocerebrum, and the neurolamella was separated from the neuropile by gentle pressing of the brain, starting from the top to the midline. Thereafter 8 bluish-opalescent neurosecretory cells, well visible under a stereoscope, were separated from the surrounding tissue, and with the use of a suction micropipette either transferred to an incubation vial or implanted into *G. mellonella* VII_1 larva. In order to obtain a brain deprived of MNC, the neurolamella over the pars intercerebralis was sectioned, and the MNC were removed with a thin needle. In each series of experiments the accuracy of the MNC removal was checked by staining some brains with paraldehyde-fuchsin[13]. Most of the brains examined contained no stainable MNC; in only a few cases 1–2 cells were present.

The in vivo ATTH assay was performed according to Sehnal and Granger[3]. As recipients, water-anesthetized *G. mellonella* VII_1 larvae were used. Implantation was made of: clusters of MNC obtained from 2 or 4 brains of chilled VII_1 larvae, 2 or 4 intact brains of chilled VII_1 larvae and 2 or 4 brains deprived of MNC.

The in vitro ATTH assay was performed as described earlier[14]. The clusters of MNC obtained from 4 or 8 brains of chilled VII_1 larvae, or 4 or 8 intact brains of chilled VII_1 larvae, or else 4 or 8 brains deprived of MNC were incubated in 200 µl of sterile MEM for 1 h. The post-incubation medium was centrifuged and the supernatant was passed through a Sephadex G-15 column; the eluate was collected in a glycolized glass vial and freeze-dried, whereupon its allatotropic activity, i.e. ability for in vitro stimulation of JH synthesis, was tested on 4 pairs of CCCA of VI_3 larvae. Because of their close anatomical relationship, the CC and CA could not be surgically separated, and thus the effect of ATTH on separated CA could not be tested. Immediately after dissection the glands were transferred in 98 µl of the stock medium (99.5 ml Hanks saline and 0.5 ml MEM containing 2 mg sodium acetate and 2 mg sodium propioniate as substrate for JH synthesis, 20 mM Hepes, pH 6.7)[15] to a vial with the tested ATTH-containing material. 1 µl of ethanolic solution of EPPAT (O-ethyl-S-phenyl-phosphoramidothiolate) was added to the vial to inhibit the JH esterase activity[16]; the final concentration of the inhibitor was 1×10^{-4} M. After 15-min preincubation 1 µl (methyl-^{3}H)-methionine, specific radioactivity 59.2 Ci/mmole (Amersham) was added; the final concentration of methionine and final specific radioactivity

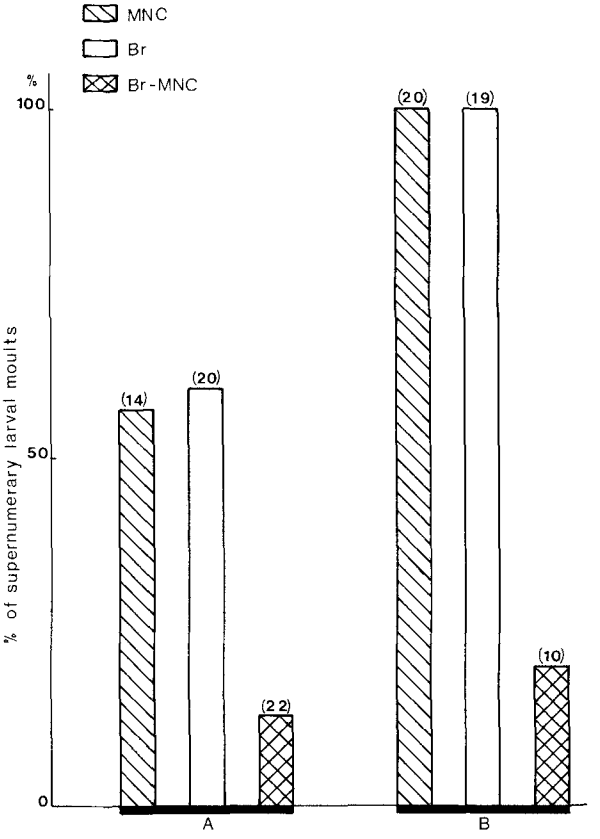

Figure 1. The allatotropic activity of the median neurosecretory cells (MNC) of the chilled *G. mellonella* VII_1 larval brain, tested by in vivo assay. The activity is expressed by the percentage of supernumerary larval molts of recipients upon implantation of: *A* 2 clusters of MNC (from 2 brains) or 2 intact brains (Br) or else 2 MNC-deprived brains (Br-MNC); *B* 4 clusters of MNC (from 4 brains) or 4 intact Br or else 4 Br-MNC. In parentheses, no. of animals tested.

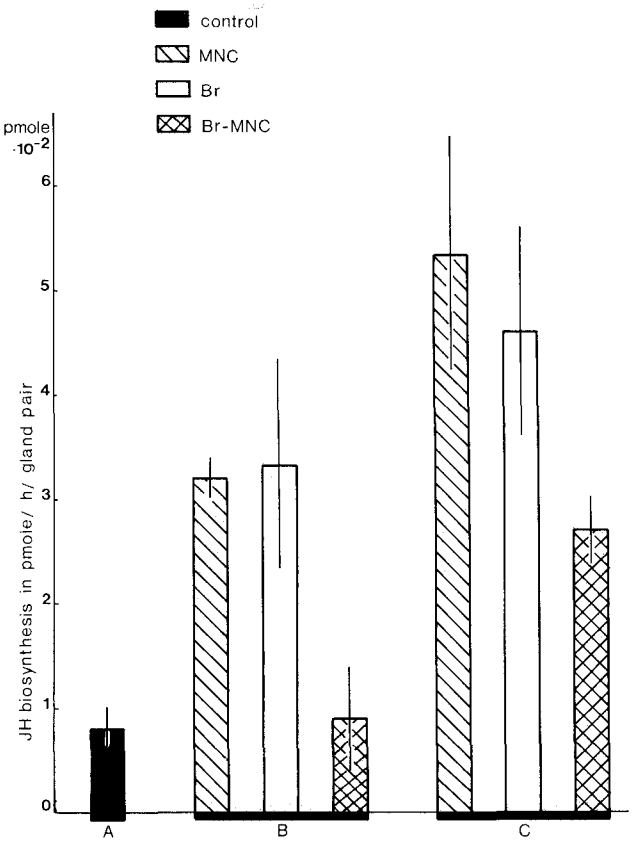

Figure 2. The allatotropic activity of the median neurosecretory cells (MNC) of the chilled *G. mellonella* VII_1 larval brain, tested by the in vitro assay. The activity is expressed by the rate of juvenilae hormone (JH) synthesis by corpora cardiaca-corpora allata (CCCA) of VI_3 larvae in vitro stimulated by allatotropic hormone (ATTH) released from MNC or intact brain (Br) or else from MNC-deprived brain (Br-MNC). *A* control CCCA incubated without ATTH; *B* CCCA incubated with ATTH equivalent to: 1 cluster of MNC for 1 Br or else 1 Br-MNC per gland pair; *C* CCCA incubated with ATTH equivalent to 2 clusters of MNC or 2 Br or else 2 Br-MNC per gland pair. Each point represents the mean of 3–6 replicates ± SD.

were 0.64 μM and 9.86 Ci/mmole, respectively. The incubation was carried out for 3 h at 30 °C with gentle shaking. The radioactivity of released JH was routinely quantified by the partition assay of Feyereisen and Tobe[17].

Results and discussion. In the present studies brains of chilled VII_1 larvae were chosen for identification of the ATTH-cells because it has been demonstrated that the brain exhibits a very high allatotropic activity. Earlier, it was shown that MNC are the putative source of ATTH in *G. mellonella* VII_1 brain; this conclusion was drawn from the observation that brains with a cauterized pars intercerebralis, in contrast to intact brains, did not exhibit ATTH activity, as measured by their ability to elicit supernumerary larval molts (SLM) upon implantation into sensitive last instar larval host[2].

It was found that MNC isolated from the brains of chilled VII_1 larvae exhibited in vivo ATTH activity similar to that of intact brains (fig. 1). Implantation of clusters of MNC obtained from chilled VII_1 larvae or implantation of intact brains of chilled larvae led to about 60% of SLM. When 4 clusters or 4 intact brains were used, 100% of SLM was obtained. Host which had received 2 or 4 brains deprived of MNC molted in a considerable lower percentage, 13% and 20% of SLM, respectively.

The high ATTH activity of MNC from *G. mellonella* chilled VII_1 brain was confirmed by the in vitro assay. JH synthesis in CCCA of VI_3 larvae was markedly activated by ATTH released from isolated MNC incubated in vitro, as shown by figure 2. As compared with control CCCA incubated without ATTH, the rate of JH synthesis in CCCA incubated with ATTH equivalent to 1 and 2 clusters of MNC per gland pair was increased nearly 4 and 7 times, respectively; likewise, this rate was similarly increased on CCCA incubated with ATTH from 1 or 2 intact brains per gland pair. In the case of CCCA incubated with ATTH from 1 or 2 brains deprived of MNC per gland pair, the rate of JH synthesis was lower than in the above-mentioned experimental variants; at the same time, for 2 brains deprived of MNC it exceeded by about 3.4 times the JH synthesis rate in control CCCA incubated without ATTH. The latter fact may be due to the release of ATTH from cell axons and/or to the possibility that some MNC may have been left in the operated brains.

Summing up, direct evidence was adduced for the presence of the ATTH activity in MNC from *G. mellonella* larval brain.

1 Acknowledgments. I am greatly indebted to Wesleyan University (Connecticut, USA) for supporting this study.

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0014-4754/87/080908-02$1.50 + 0.20/0
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