Inhibition of Allatotropic Activity and Ovary Development in *Locusta migratoria* by Anti-Brain-Antibodies

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Z. Naturforsch. **35 c,** 1117–1119 (1980);
received July 7/July 30, 1980

Locusta migratoria, Neurosecretion, Allatotropic Factor, Anti-Brain-Antibodies, Ovary Development

Evidence for an immunogenic allatotropic factor in the brain of *Locusta migratoria* is presented. Antibodies to brain material from mature females were raised in rabbits. *In vivo* injection of these antibodies into young locusts inhibited ovary development and resulted in hypertrophic as well as necrotic corpora allata. Antibodies to brains without neurosecretory material were not able to block allatotropic or gonadotropic activity.

In insects, the neurosecretory cells (NSC) of the brain produce hormones which regulate the activity of the corpora cardiaca (CC), corpora allata (CA) and prothoracic glands. One of these factors from the pars intercerebralis (Pi) controls the CA, which produce juvenile hormone (JH) [1, 2]. In *Locusta migratoria* as in many other insects, JH functions as a gonadotropin [2–4]. In absence of neurosecretory material (NSM) from the NSC no ovary development takes place, as shown by surgical methods [5–7]. The chemical structure of the allatotropic factor is unknown; it is assumed to be a peptide or a protein.

In mammalian systems, removal of hormone activity by passive transfer of anti-hormone-antibodies is a useful method of endocrinological research [8–10]. In the present study, this approach is applied to locusts to investigate the endocrine sequence NSC – CA – ovary. Antisera to brain material were produced. The effects of their *in vivo* application to female locusts are described.

Materials and Methods

Animals

Locusta migratoria migratorioides (R & F) were reared at 35 °C, 40% relative humidity, and a 10:14 (L:D) photoperiod. The locusts were kept under crowded conditions.

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0341-0382/80/1100-1117 $ 01.00/0

Antibodies

Antisera to brain material from mature females were produced in rabbits; control sera were raised by using brains from one-day old females without stainable neurosecretory material. The medial part of the brains was dissected without any part of the retrocerebral complex. Per rabbit and injection, 5 brains were homogenized at 4 °C in 100 µl phosphate-buffered saline (PBS). The homogenate was made up to 1 ml with PBS and emulsified with 1.5 ml complete Freund's adjuvant (Difco). Intradermal injections were administered at multiple sites; a booster injection was given after an interval of 10 days with the same antigen preparation. An antiserum was taken from a bleeding 10 weeks after the primary injection. In order to increase antibody concentration, immunoglobulin G (IgG) was prepared from a bleeding taken 16 days after an additional booster injection, which was given 3 months after the primary immunization. The IgG-fraction was obtained from the respective antiserum by ammonium sulfate precipitation at 40% saturation, followed by two consecutive precipitations at 33% saturation. After the last precipitation, the dissolved material was exhaustively dialyzed against PBS.

In vivo injection of locusts

Ninety-five newly emerged females were marked individually. Fourty animals were treated with antiserum, 35 with IgG, and 20 with IgG from control serum. Fourty µl of the corresponding solution was injected into the abdomen by means of a microapplicator. Injections were given daily, starting at day 3 after adult emergence until day 23. To study ovary development, the animals were weighed every day as oviposition is indicated by a marked decrease of weight. At the end of the study the terminal oocytes were measured (normal terminal oocyte length 1–6.5 mm, depending on state of ovary development [11]).

Paraffin sections of Bouin- or Carnoy-fixed brains and retrocerebral complexes were stained with paraldehyde fuchsin (PAF) for light microscopy. The volume of the CA was calculated from complete sections of this gland.

Results and Discussion

After injection of anti-brain-antiserum, ovary development of more than 60% of the animals was

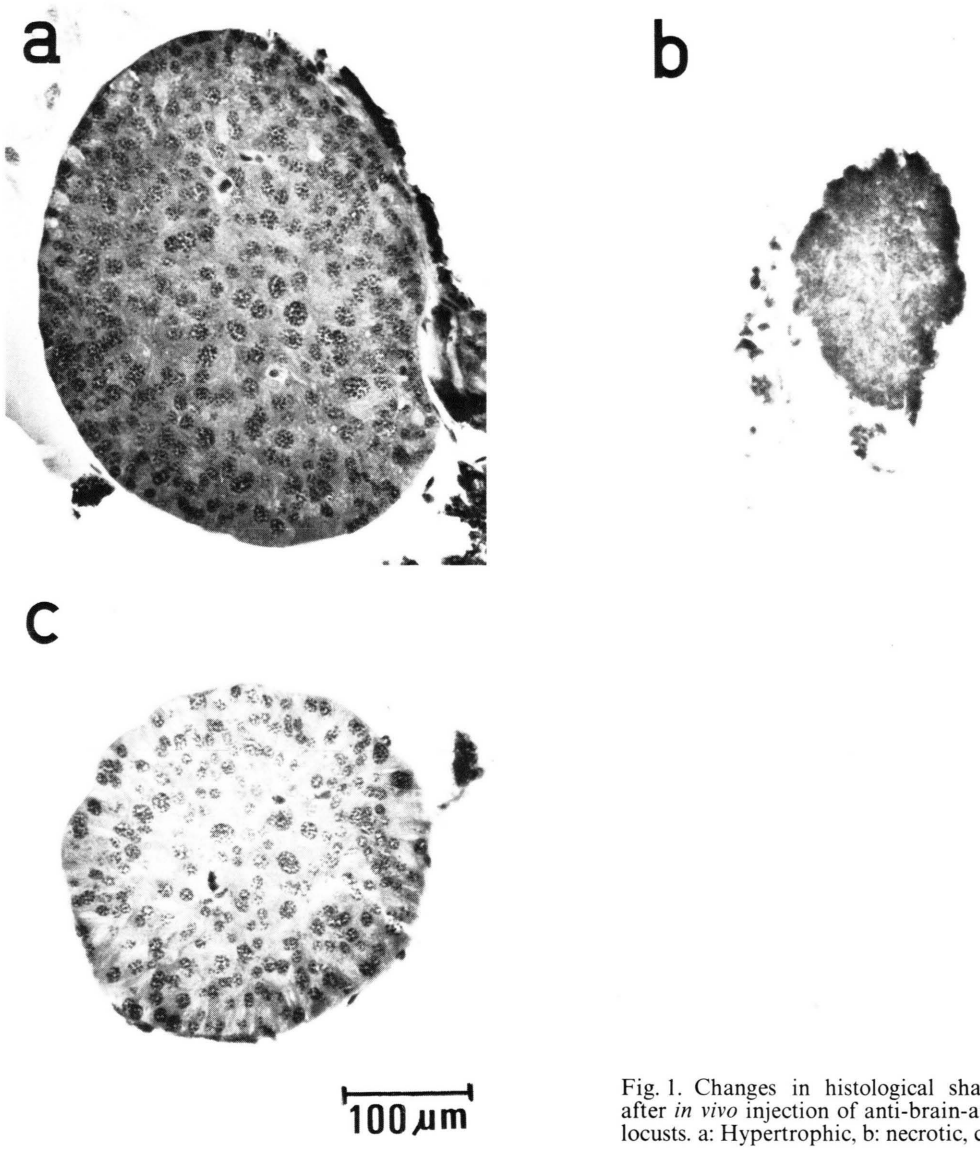

Fig. 1. Changes in histological shape of corpora allata after *in vivo* injection of anti-brain-anti-bodies into female locusts. a: Hypertrophic, b: necrotic, c: control CA.

disturbed or inhibited (Table I). In 25 out of 40 locusts ovary development was delayed for more than a week and most of these females layed only 2–20 eggs. Five out of 40 animals had no ovary development (length of terminal oocytes was less than 1 mm). Measurement of CA-volume showed that nearly half of these glands were affected by the antiserum injection. Eighteen out of 40 CA were 1.5 to 3 times larger than control-CA of animals with the same length of terminal oocytes (Fig. 1 a).

The CA of one animal was necrotic; the volume of the gland was drastically reduced to 1/6 of its original size, and the cell structure was completely disorganized (Fig. 1 b). In order to increase the concentration of specific antibodies, IgG was prepared after an additional booster injection. A fourfold concentration resulted from the conditions of the ammonium sulfate precipitation. Together with the effect of the booster injection, the total increase in concentration of specific antibodies was estimated to be not less than tenfold as compared to the above-mentioned antiserum. Injection of this IgG-fraction clearly enhanced the effect on the ovaries and the CA. In 32 out of 35 animals ovary de-

Table I. Effects of *in vivo* injection of anti-brain-antibodies into female locusts.

	Number of animals	Ovary development			Corpora allata		
		normal	disturbed	inhibited	normal	hypertrophic	necrotic
antiserum [a]	40	15	20	5	21	18	1
IgG-fraction [b]	35	0	3	32	7	16	12
control [c]	20	19	1	0	20	0	0

[a] Antiserum against brains with stainable neurosecretory material;
[b] IgG-fraction of antiserum against brains with stainable neurosecretory material;
[c] IgG-fraction of antiserum against brains without stainable neurosecretory material.

velopment was inhibited (length of terminal oocytes was less than 1–3.0 mm). Most of them had no vitellogenin incorporated and only a few of them displayed a light yellow colour. Three out of 35 females layed a few eggs (2–30) with a delay of more than one week. In 30 out of 35 animals the CA were affected: 18 turned out to be hypertrophic and 12 were necrotic (Table I, Fig. 1 a, b). The NSC and their axons as well as the CC showed no visible change in the amount of stainable neurosecretory material after both the injections.

In the control experiment no effect on the CA and the ovary was observed (Table I and Fig. 1 c), although the same immunization schedule and IgG-purification as with the mature brains had been used. The effects obtained with antibodies to mature brains are therefore due to an immunospecific blocking of active material which is not present in juvenile brains and is most likely neurosecretory material. The presence of an immunogenic allatotropic factor can be concluded. Blockade of the gonadotropic action most likely occurs by blocking of the allatotropic activity. In analogy to the mammalian systems mentioned, the results show that *in vivo* ablation of hormonal activity by passive injection of antibody can be used in an insect as well, thus providing an efficient tool for the study of the postulated endocrine sequence NSC – CA – ovary. The results described suggest a peptide or protein structure for at least one factor involved in allatotropic control. Whether a peptide hormone, a precursor, or a carrier molecule is inactivated by the antibody is unknown thus far. Nevertheless, until contrary evidence is available, the designation "allatotropic factor" seems justified. It should be mentioned that in mammalian systems antibodies to neurohormones have been successfully used for their preparative purification [11–14]. The immunogenicity of the allatotropic factor reported may be useful for a similar approach.

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