ADENYL CYCLASE VARIATION DURING DEVELOPMENT OF THE INSECT *CERATITIS CAPITATA*

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1. Introduction

Cyclic AMP has been suggested to be the receptor site for neurotransmitters as well as a second messenger which mediates the action of a variety of hormones and neurohormones in different animal tissues. The level of cyclic AMP is balanced by its synthesis from ATP through the activity of adenylcyclase and its degradation by a phosphodiesterase. Thus, the change in the level of the cyclic nucleotide leads to different effects depending upon the type of cell in which the change occurs.

Because the adenyl cyclase enzyme is located at the cell membrane, it is a good candidate for the role of helping the cell to respond to changes in the outside environment. The most striking feature of adenyl cyclase of cells of multicellular organisms is its sensitivity to stimulation by hormones.

Since the initial demonstration of Sutherland [1] that epinephrine stimulates the adenyl cyclase system of liver cells, numerous hormonal substances have been shown to act through altering the activity of cell membrane-bound adenyl cyclase in their respective target tissues. Steroid hormones can directly influence also adenyl cyclase activity [2–4].

Cyclic AMP content of nervous tissue can change in response to synaptic activity; the inhibitory synaptic action of norepinephrine in the rat cerebellum is mediated by cyclic AMP. Activation of adenyl cyclase activity by norepinephrine, 5-hydroxy-tryptamine, acetylcholine and histamine has been achieved in brain homogenates and slices [5]; direct administration of monoamines into the central nervous system causes a prolonged activation of adenyl cyclase exhibiting a dose–activity relationship [6].

In this communication, direct evidence is presented demonstrating a change in adenyl cyclase activity during adult development of the dipterous *Ceratitis capitata*.

2. Materials and methods

C. capitata (Wiedemann) was used during the larval and pharate stages of development and the diet, temperature and humidity conditions of culturing were carefully controlled, as reported previously [7].

Insects were directly homogenized with 1.5 vol of cold 0.25 M sucrose for 5 min in a glass homogenizer fitted with a Teflon pestle. Total homogenates were centrifuged at 900 *g* for 10 min and supernatants were taken off with a plastic syringe. Supernatants were used for measuring the adenyl cyclase activity before one hour. All these operations were carried out at 0–4°.

Adenyl cyclase activity was determined in triplicate according to the assay mixture: 100 μl of insect homogenate, 100 μl buffer (200 mM Tris, 10 mM $MgCl_2$, 20 mM NaF, 25 mM caffeine, pH 8), 10 μl ATP (0.0 mM ATP containing 0.1 μCi of adenosine-[2-^{3}H]5′-triphosphate, specific activity 18 Ci/mmole).

Measurement of [^{3}H]cyclic AMP was carried out by paper chromatography using as development solvent isopropanol–NH_4OH (25%)–water (7:2:1, v/v/v) according to Ho et al. [8]. UV-detected areas on the paper were placed into counting vials with 15 ml of the scintillation mixture (PPO 4 g, POPOP 40 mg and toluene to 1 ℓ).

Proteins were determined by the Lowry's method [9].

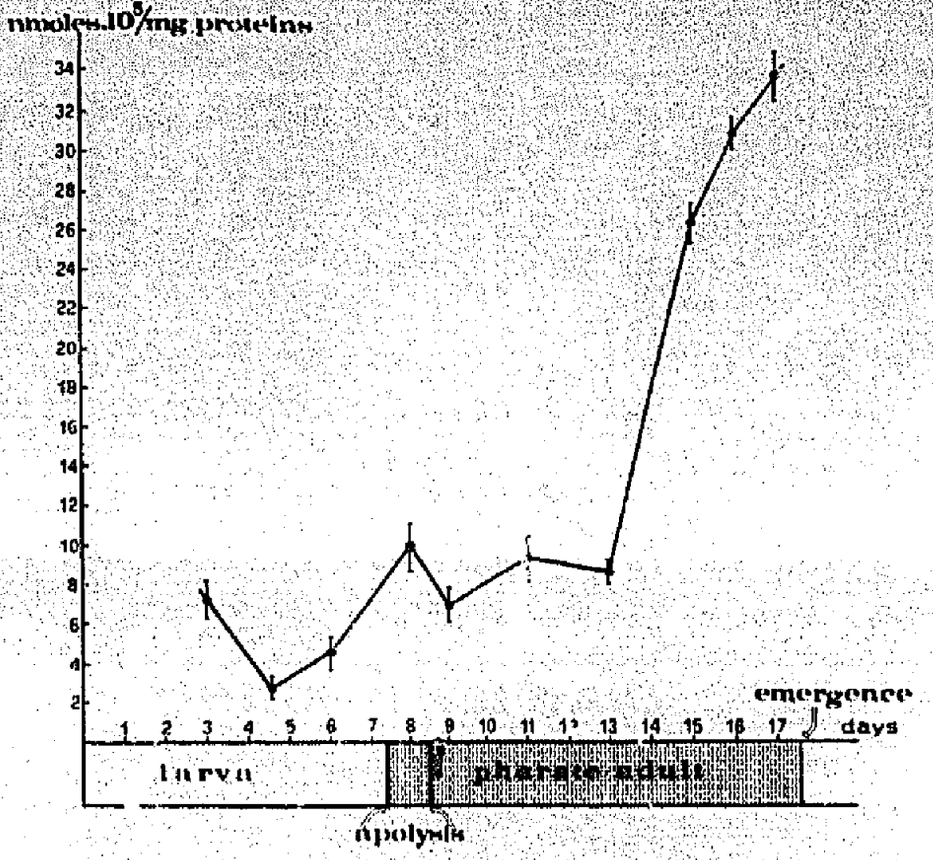

Fig. 1.

Analytical results are given as nmoles of cyclic AMP synthesized per mg of protein; extreme values of the three analytical values are represented in fig. 1.

3. Results and discussion

The assay for adenyl cyclase activity was conducted during larval and adult development [7] through the capacity of the insect homogenates to form [^{3}H]cyclic AMP from [^{3}H]ATP. Results are given in fig. 1 in coincidence with a general view of the ontogeny of the insect. Adenyl cyclase activity shows a slight diminution during larval development and a very sharp rise in the pharate adult stage. This elevation takes place, therefore, in the developing adult stage and it has to be interpreted in relation with the established hormonal control of insect development. Three metamorphosis hormones play vital roles in the regulation of post-embryonic development: brain hormone (BH), juvenile hormone (JH) and moulting hormone (MH). During larval development JH and MH are both secreted thus initiating larval apolysis; transformation to the pupa occurs when JH levels are low enough and MH is secreted alone. The most dramatic biochemical function of MH is to promote protein synthesis.

Therefore, the increase of the activity of adenyl cyclase in the pharate adult stage is one of the metabolic events as a result of the MH action. The direct effect of the moulting hormone is a clear demonstration of adenyl cyclase stimulation by a steroid hormone and it is in agreement with the stimulation of the enzyme in the wing epidermis promoted by the injection of β-ecdysone into chilled *Hyalophora gloveri* [10].

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