ADENOSINE 3',5'-MONOPHOSPHATE PHOSPHODIESTERASE ACTIVITY DURING DEVELOPMENT OF THE INSECT *CERATITIS CAPITATA*

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Summary. Cyclic AMP phosphodiesterase activity was determined in homogenates of the insect *Ceratitis capitata* at several stages of development. These phosphodiesterase activities are interpreted on the bases of cyclic AMP concentrations and adenyl cyclase activities previously determined at the same stages of development. Variations of both enzyme activities are discussed in relation to the hormonal regulation of post-embryonic development in insects. The experimental results point to the possible existence of two sets of enzymes exhibiting different affinities for the naturally occurring cyclic nucleotides.

The function of adenosine 3',5'-monophosphate as a hormonal messenger (1) requires the presence of both adenyl cyclase, which synthesizes the compound, and 3',5'-AMP phosphodiesterase, which removes it again. The significant role played by cyclic nucleotides in vertebrates has been studied in great detail; however, its presence and function (2-4) in insects have largely been ignored. Adenyl cyclase and cyclic AMP dependent protein kinase activities in insects have been specially reported in relation to development phenomena (5-9). Sutherland and Rall (10) described the phospho - diesterase that catalyzes the apparently nonreversible hydrolysis of the 3'-bond as the major physiological pathway for the termination of the cellular effects of cyclic nucleotides. Phosphodiesterases occur widely in biological systems and they have been found also in insects (11-13). Thus, cyclic AMP phosphodiesterase shares with the cyclase the function of the hormonal control of the intracellular cyclic AMP levels and the consequent effects.

In this paper the variations of cyclic AMP phosphodiesterase activity during development of the Dipterous *Ceratitis capitata* are reported.

MATERIALS AND METHODS

Ceratitis capitata (Wiedeman) was used during the larval, pharate adult and adult stages of development. Diet, temperature

and humidity conditions of culturing were carefully controlled as reported previously (14).

^{3}H-Cyclic AMP (sp.act.27.5 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, U.K. All reagents were obtained from Sigma Chemical Co., St.Louis, USA.

Insects were directly homogenized with two volumes of cold 40 mM Tris buffer, pH 8, for 5 min in a glass homogeniser fitted with a Teflon pestle. Total homogenates were centrifuged twice at 800 g for 10 min and supernatants were used for measuring phospho diesterase activity.

Phosphodiesterase activity was determined in triplicate by the radioisotope assay described by Rojakovick and March (13). The incubation mixture contained: 25 µl, 1.6×10^{-7}M ^{3}H-cyclic AMP in 40 mM Tris pH 8; 25 µl,20 mM $MgCl_2$ in 40 mM Tris pH 8; 50 µl, supernatant (1.5-2 mg proteins). Assays were carried out in glass tubes. The reaction was initiated by the addition of the substrate and allowed to proceed for 12 min at 35º The reaction was stopped by placing the tubes in boiling water for 3 min. After cooling in ice, the samples were centrifuged at 800 g for 10 min and 25 µl aliquots of supernatants were analyzed by paper chromatography on Whatman 3MM. Chromatograms were developed in a 1M ammonium-acetate -95% ethanol (3:7) solvent system for 16 hr using cyclic AMP and 5'-AMP as reference markers. UV visualized 5'-AMP areas were cut out and placed into counting vials with 20 ml of the scintillation mixture (10.5 g PPO, 0.45 g methyl-POPOP, 150 g naphtalene,dioxane to 1500 ml and water to 1800 ml). Radioactivity was measured in a Nuclear Chicago 6766 liquid scintillation spectrometer.

The validity of the phosphodiesterase assay was confirmed by adding *Crotalus adamanteus* venom as a source of phosphomono - esterase. No difference was observed in the rate of cyclic AMP conversion to adenosine in either the presence or absence of the venom, indicating that the phosphodiesterase action is the rate limiting step (15).

Proteins were determined by the Lowry´s method (16).

Analytical results are given as nmoles 5'-AMP formed per mg of proteins per min. Extreme values of the three analytical values are represented.

Radioimmunoassay method was used for the determination of cyclic AMP as described previously (4).

RESULTS AND DISCUSSION

3',5'-AMP phosphodiesterase activity was determined during larval and pharate adult stages of development of the insect *Ceratitis capitata*. Enzyme activities are given in fig.1 in which a general pattern of the development of the insect is also given.

Phosphodiesterase activity shows a sharp peak at second and third instars of the larval stage whereas the enzyme activity values exhibited by the pharate adult stages of development are very low.

Insect development is controlled by the coordinated activity of the neuroendocrine system. Larval and metamorphic molts depend on the ecdysial gland secretory activity that is under control of the brain prothoracotropic hormone. The character of the molt is determined by the juvenile hormone secreted by the corpora allata; molting in the presence of both ecdysone and juvenile hormone retains immature characteristics, but molting induced by ecdysone alone involves tissue differentiation toward adult characteristics. In this connexion, a close relationship between ecdysone and

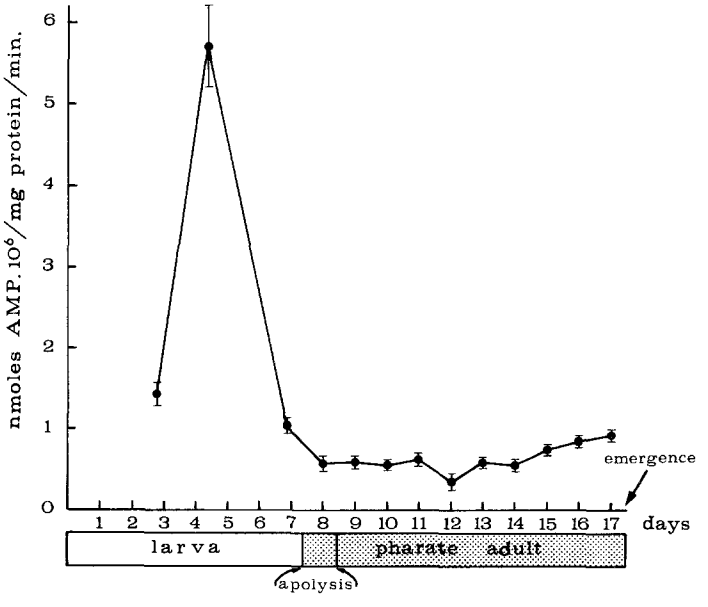

Figure 1. Cyclic AMP phosphodiesterase activity of homogenates of the insect *Ceratitis capitata* at several stages of development.

juvenile hormone action and several enzyme activities has been evidenced (17-22).

In a previous paper (5), the increase of the adenyl cyclase activity in the pharate adult stage of *Ceratitis capitata* was related to the ecdysone action in agreement with the stimulation of the enzyme in the wing epidermis promoted by the injection of ecdysone into chilled *Hyalophora gloveri* (6). Thus, the results given in fig.1 does not show any correlation between this hormone and phosphodiesterase activity; on the other hand, the possibility exists that the maximum in the phosphodiesterase activity exhibited during larval molts be related to the juvenile hormone action.

Figure 2 shows the changes of both cyclic AMP concentration and adenyl cyclase/phosphodiesterase activity ratio during development of the insect. The maximum levels of cyclic AMP coincide with the increase of the enzyme activity ratio during the larval development to decrease sharply at the pharate adult stage. After this coincidence, the increase of adenyl cyclase activity continued (5) whereas the phosphodiesterase activity remained practically unchanged (fig.1), what is reflected in the concomitant increase of

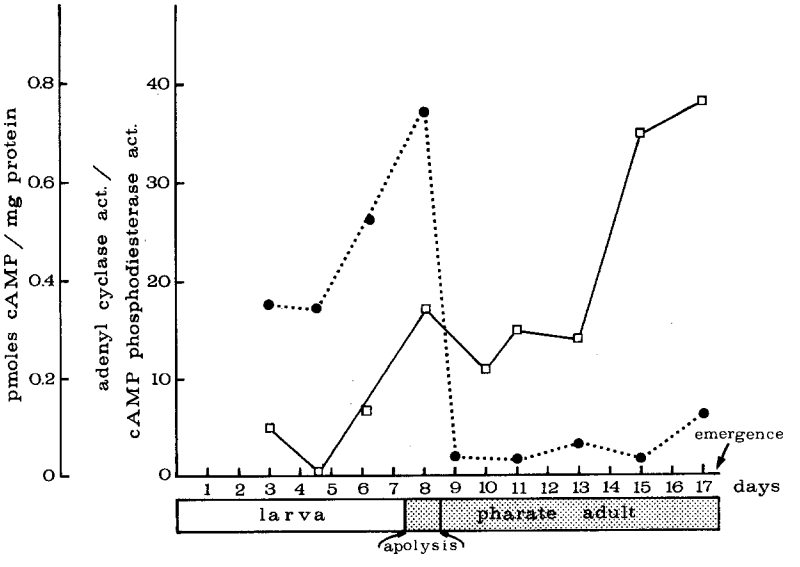

Figure 2. Cyclic AMP levels (---●---) and adenyl cyclase/phospho - diesterase activity ratio in homogenates of the insect (—□—) *Ceratitis capitata* at several stages of development.

the enzyme activity ratio. Only at the highest values of the enzyme activity ratio, in coincidence with the last adult development and adult emergence, a minor rise of cyclic AMP levels was detected (fig.2).

This practical absence of correlation between levels of cyclic AMP and adenyl cyclase/phosphodiesterase activity ratio during the pharate adult stage of development can be interpreted taking into account previous reports on regulation of protein kinase by concentrations of cyclic AMP (9) and specificity of enzyme activities on cyclic nucleotides. Studies on regulation of cyclic AMP-dependent protein kinase activity at various stages of development of *Ceratitis capitata* by varying concentrations of exogenous cyclic AMP showed different patterns according to the development stage; thus, increasing levels of cyclic AMP produced the usual activating effect on the larval protein kinase preparations whereas protein kinase activity was inhibited in preparations of recently emerged adult insect at low concentrations of cyclic AMP (9). It was clear that the development of the insect was accompanied by a progressive disappearance of the regulatory ability of cyclic AMP on the kinase activity albeit an increase of the kinase activity dependent upon the endogenous levels of the cyclic nucleotide was simultaneously shown. This fact was interpreted on the basis of a kinase activity dependence on a cyclic nucleotide different from cyclic AMP.

On the other hand, multiple forms of phosphodiesterase have been shown to exist from a large number of sources and under a wide variety of conditions (23-27); these forms differ mainly in their substrate affinities (28-31) and function (32-33). Present evidence points to the possible existence of two different sets of enzymes for the two naturally occurring cyclic nucleotides (13,34-36) and, in general, there is a greater affinity of phosphodiesterase for cyclic GMP than for cyclic AMP.

The reported significant increase (5) of the adenyl cyclase activity (measured through the ability to synthesize cyclic AMP from exogenous substrate) at the end of the pharate adult stage induces only a low rise of the cyclic AMP level and it is not paralelled by the phosphodiesterase activity. This fact leads also to the hypothesis that cyclic GMP would be more specifically synthesized by the cyclase during the pharate adult stage of development; thus, cyclic GMP would exert the regulatory effect postulated on the protein kinase activity and would affect selectively the phosphodiesterase activity.

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