CYCLIC NUCLEOTIDE CYCLASE VARIATION DURING DEVELOPMENT OF THE INSECT *CERATITIS CAPITATA*

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Summary. Guanylate and adenylate cyclase activities were estimated in homogenates of the insect *Ceratitis capitata* at various stages of development. Guanylate cyclase activity was notably higher than adenylate cyclase activity in agreement with both cyclic nucleotide ratio and cyclic nucleotide-dependent protein kinase ratio reported in arthropod tissues. Variations in both enzyme activities during development were coincident in the adult development, while in other biological stages, as the larval development and puparium formation, the most significant changes affected to the activity of guanylate cyclase.

The existence of cyclic AMP, adenylate cyclase, cyclic AMP phosphodiesterase and cyclic AMP-dependent protein kinase has been demonstrated in some insects (1-12). In a previous study on the cyclic AMP-dependent protein kinase and protein binding activities during development of the Dipterous *Ceratitis capitata*, the presence of several protein kinases with different cyclic nucleotide dependency was suggested (8).

On the other hand, guanylate cyclase that catalyzes the formation of guanosine-3',5'-monophosphate (cyclic GMP) from GTP has been found in several tissues throughout the animal kingdom (13-18). Soluble guanylate cyclase activity from various mammalian tissues (13-16, 19-21) as well as particulate guanylate cyclase activity from rat small intestine (18) and lung (21) and sea urchin sperm (15,22) have been studied. In contrast to adenylate cyclase, guanylate cyclase is not activated by fluoride in broken cell preparations and the activity in cell free systems from liver and heart is unaffected by glucagon, insulin, epinephrine, ACTH, and other hormones (23,24). In most tissues the concentrations of cyclic GMP are generally at least tenfold lower than those of cyclic AMP and the excretions of cyclic AMP and GMP are generally controlled independently by hormonal effects (25).

Cyclic AMP and cyclic GMP are probably involved in a number of biological systems in promoting different cellular events that

in most instances appear to be strikingly contrasting. The two cyclic nucleotides could act cooperatively in a monodirectional system, either as positive effectors of different sequential steps or as intracellular mediators of different stimulatory extracellular signals which in varying combinations could produce somewhat different qualitative or quantitative responses (26).

Concerning the activation of kinases, cyclic GMP in high amounts produces in some cases the same maximum stimulation as cyclic AMP. Cyclic GMP has been found virtually without effect on a cyclic AMP-dependent protein kinase from adipose tissue (27) or from heart and skeletal muscle (28). An interesting exception was seen with a preparation of protein kinase from lobster muscle, where cyclic GMP was as effective as cyclic AMP (29); the two fractions of protein kinase activity could be separated from lobster muscle, which has quite different affinities for cyclic GMP and cyclic AMP. In intact cell systems the two cyclic nucleotides produced similar effects when applied in high concentration under unphysiological conditions in several systems (30).

This paper is concerned with a study on the relative adenylate and guanylate cyclase activities during the development of the insect *Ceratitis capitata*.

MATERIALS AND METHODS

Ceratitis capitata (Wiedemann) was used at the larval, pharate adult and adult stages of development and diet, temperature and humidity conditions were carefully controlled as reported (31).

Insects were directly homogenized in a Potter-Elvehjem Teflon-glass homogenizer with 8 volumes of 0.25 M sucrose containing 0.02 M Tris-HCl buffer (pH 7.4), 1mM EDTA and 10 mM 2-mercaptoethanol. Total homogenates were centrifuged for 10 min at 2°C at 10,000g. The supernatants thus obtained were employed as enzyme preparations.

Guanylate cyclase activity was determined in triplicate by a procedure slightly modified from that of Nakazawa and Sano (32). The standard reaction mixture contained 60 nmol of (8-^{3}H)GTP (0.02 µCi/nmol), 0.3 µmol of cyclic GMP, 0.9 µmol of $MnCl_2$, 30 µmol of tris-HCl buffer, pH 7.8, and 100 µg of enzyme protein in a total volume of 0.15 ml. The mixture was incubated for 15 minutes at 30°C with constant shaking and the reaction was stopped by heating for 2 min in a boiling bath and chilled.

The whole reaction mixture was applied on a neutral aluminium oxide column (33). The column was washed with 5 ml of 0.05 M tris-HCl buffer, pH 7.4. The washing containing cyclic GMP was directly drained onto a Dowex 1X2 column (34)that was washed afterwards with 10 ml of 0.05 N formic acid. Cyclic GMP was subsequently eluted from the column with 6 ml of 0.2 M ammonium formate in 4.0 N formic acid. By this procedure more than 85% of cyclic GMP was recovered. The radioactivity of samples was determined with Instagel using a Nuclear Chicago liquid-scintillation spectrometer. Analytical results are given as pmol of cyclic GMP formed per mg of protein per minute. Extreme values of the three analytical values are given in figure 1.

Adenylate cyclase activity in the insect homogenates was also determined in triplicate according to the assay mixture: 100 µl of insect homogenate, 20 µmol of tris-HCl, pH 8.0, 1 µmol of $MgCl_2$, 2 µmol of NaF, 2.5 µmol of caffeine and 8.10^{-3} µmol of ATP containing 0.1 µCi of (2-^{3}H)ATP (18 Ci/mmol) in a total volume of 0.2 ml. Measurement of labelled cyclic AMP was carried out after separating by paper chromatography according to Ho *et al.*(35) 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 (36). Analytical results on adenylate cyclase activities were also given in figure 1 as pmol of cyclic AMP formed per mg of protein per minute; extreme values of the three analytical determinations are represented.

RESULTS AND DISCUSSION

A different metabolic behaviour has been reported in the various stages of development of the insect *Ceratitis capitata* (37,38).

Prompted by the results described in a previous study on the cyclic AMP-dependent protein kinase and protein binding activities during the larval and pharate adult stages of this Dipterous (8), a simultaneous analysis of the adenylate cyclase and guanylate cyclase activities in a series of stages of the insect was carried out.

Adenylate cyclase activity showed a small but clear maximum in coincidence with the formation of puparium to rise sharply afterwards during the last days of pharate adult development (39). The enzyme activity measured at the adult emergence was about fivefold higher than that during larval development and larval apolysis of the insect. Guanylate cyclase activity exhibited the highest levels during the second larval instar to decrease notably afterwards in coincidence with the formation of puparium; the enzyme activity

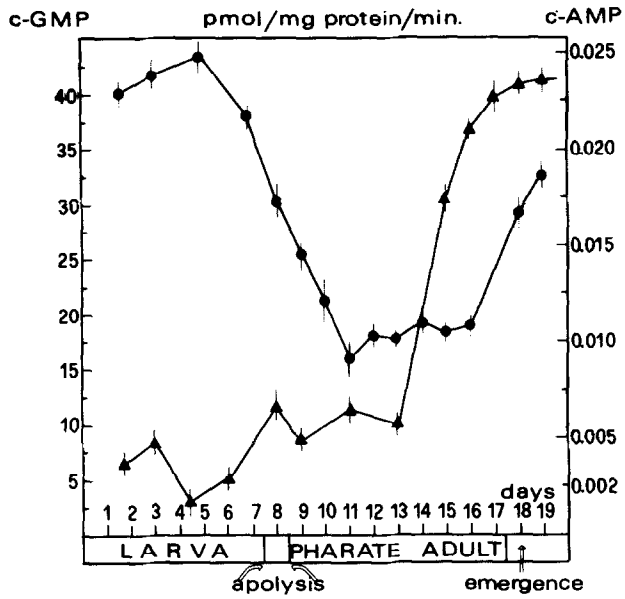

Figure 1. Guanylate cyclase activity (—●—) and adenylate cyclase activity (—▲—) of homogenates of the insect *Ceratitis capitata* at different stages of development.

remained practically unchanged during the whole pharate adult stage and raised sharply at the emergence of the adult from the puparium. Therefore, some remarkable differences between both cyclic nucleotide cyclase activities can be pointed out. Thus, at the larval-pupal apolysis the levels of adenylate and guanylate cyclase activities changed in an opposite way whereas both activities increased almost concomitantly at the emergence of the adult.

Although the changes observed in the enzyme activities does not necessarily imply the parallel variation of the corresponding cyclic nucleotides, it adds new data on the cyclic nucleotide-hormone relationships and their regulatory influences. The concept that cyclic GMP and cyclic AMP provide at least different if not opposing regulatory influences in certain insect systems (40) is in agreement with these findings. The pattern of adenylate cyclase activity during development agrees with the changes in protein kinase activity and cyclic AMP binding activity at the same stages of the insect (8). Nevertheless, the regulation of protein kinase activity by increasing concentrations of exogenous cyclic AMP exhibited very different patterns according to the stage of development of the insect (8) and only larval preparations were able to be

normally stimulated by the increasing levels of cyclic AMP. Also, the gradual disappearance of the regulatory ability of cyclic AMP on the kinase activity after metamorphosis may be related to the progressive loss of synchronism in the development and the conse - quent high increase in the variability of cyclic AMP values observed by de Reggi and Cailla (41).

All these observations allow to take into consideration the possibility that certain cellular functions such as larval development and puparium formation may be controlled by one type of regulatory influences while other such as adult development are mediated by several signals.

Further studies on the cyclic GMP-dependent protein kinase and the protein binding activities during development of *Ceratitis capitata* are in progress.

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