# VARIATION OF THE LEVELS OF CYCLIC AMP AND CYCLIC GMP DURING DEVELOPMENT OF THE INSECT *CERATITIS CAPITATA*

R.E.Catalán, M.P.Castillón and A.M.Municio

Department of Biochemistry.Faculty of Sciences
Complutensis University. Madrid. Spain

Received June 29, 1976

Summary. Changes in the levels of adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) during development were studied in the Dipterous *Ceratitis capitata*. The developmental patterns were different to each other. Cyclic AMP showed a sharp maximum in the larval stage to decrease afterwards during adult development. Changes of cyclic GMP exhibited an opposite pattern, although its levels were always higher than those of cyclic AMP.

Cyclic AMP and its regulating enzymes have been recently implicated in a variety of physiological processes in different insects (1-12). Cyclic AMP acts essentially within the cell by the activation of a series of protein phosphokinases which have been described in a great variety of tissues (13-21).

On the other hand, the concentrations of cyclic GMP in most tissues 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 (22). In intact cell systems the two cyclic nucleotides produced similar effects when applied in high concentration under unphysiological conditions in several systems (23). 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 (24) or from heart and skeletal muscle (25). An interesting exception was seen with a preparation of protein kinase from lobster muscle, where cyclic GMP was as effective as cyclic AMP (26).

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 as intracellular mediators of different stimulatory extracellular signals which in varying combinations could produce somewhat different qualitative or quantitative responses (27,28). Thus,

the opposing influences of cyclic GMP and cyclic AMP upon lymphocyte proliferation may be mediated through the effects of these agents on phosphorylation of specific nonhistone chromatin proteins (29).

This paper is concerned with a study on the levels of both cyclic AMP and cyclic GMP 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 other conditions of culture were carefully controlled as previously cited (30). Frozen insects were homogenized at 0°C in 6% trichloroacetic acid and the precipitated protein was removed by centrifugation. Supernatants were extracted eight times with 10 volumes of water-saturated ether that was afterwards removed by blowing air through the solution at 60-70°C. The final pH was 7.5 (31).

Cyclic GMP was measured by means of a radioimmunological method (32,33). The standard curve was obtained by incubating various con - centrations of unlabelled cyclic GMP and 8-($^{3}$H)cyclic GMP with the specific antiserum (34). Simultaneously, insect preparations were incubated with labelled cyclic GMP and the antiserum. Separation of the protein-bound cyclic GMP from the unbound nucleotide was achieved by ammonium sulphate precipitation and centrifugation. The precipitate containing the antibody bound-complex was dissolved in water and the activity determined by liquid scintillation using Instagel. The dose-response curve was linearized and the final results calculated through a computer program using a Hewlett-Packard computer 2116B according to the logit-log method of Rodbard *et al.* (35). The validity of the assay procedure was confirmed by the linearity of sample dilution and use of internal standards. Recovering assays averaged 80%. The intraassay coefficient of variation was 5-9% and the interassay coefficient of variation was 6-13%.

Cyclic AMP was measured by a competitive binding protein assay previously described (36).

Nucleotide determinations through the development of the insect were carried out on triplicate samples of 100-individual homogenates. Extreme values of the three analytical values are represented in the Figure 1.

Proteins were determined by the Lowry's method (37).

RESULTS AND DISCUSSION

Cyclic AMP has been suggested to be implicated in the action of insect hormones (2, 38-41). However, there is not many information available in insects on the function of cyclic GMP (28,42,43)

On the other hand, a different metabolic behaviour has been reported in the various stages of development of the insect *Ceratitis capitata* . Prompted by these results and those described on the cyclic AMP-dependent protein kinase and protein binding activities during the larval and pharate adult stages of this insect (21), a simultaneous analysis of the levels of cyclic AMP and cyclic GMP in a series of stages of development of the insect was carried out. Results given in figure 1 are expressed as pmol of cyclic nucleotide per mg of protein.

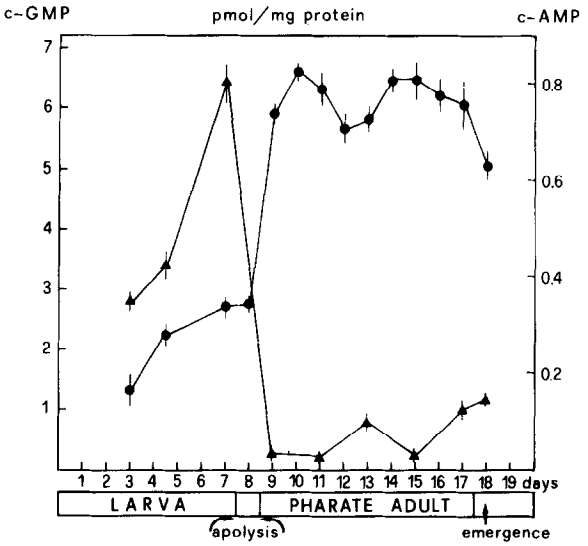


Figure 1. Levels of cyclic AMP (—▲—) and cyclic GMP (—●—) in homogenates of the insect *Ceratitis capitata* at different stages of the development.

Although the ratio of the levels of cyclic GMP and cyclic AMP varies according to the stage of development of the insect, the levels of cyclic GMP were always notably higher than those of cyclic AMP, in agreement with the constant highest activities of guanylate cyclase showed during development of *Ceratitis capitata* (44). These results agree with the fact that guanylate cyclase activity in sperm from

several invertebrates is several hundred-fold greater than in the most active mammalian tissues (45).

Figure 1 shows that the levels of both cyclic nucleotides changed differently during larval development and larval apolysis of the insect. Cyclic AMP levels showed a sharp maximum in coincidence with the third larval instar to decrease notably afterwards in coincidence with the formation of puparium. On the contrary, the levels of cyclic GMP rised gradually during larval development to exhibit the highest values in the pharate adult stage of development. These values of both cyclic nucleotides does not parallel the changes observed in the adenylate and guanylate cyclase activities (44) in the same stages of development of the insect. This fact agrees with the absence of correlation between the levels of cyclic AMP and adenylate cyclase/phosphodiesterase activity ratio during the pharate adult stage (10). It can be interpreted taking into account previous reports on regulation of protein kinase by concentrations of cyclic AMP (21). 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 (21). These facts were interpreted on the basis of a kinase activity dependence on a cyclic nucleotide different from cyclic AMP (21) and are consistent with the view that metabolism of the two cyclic nucleotides is independently controlled in the insect.

All these considerations allow to think over the possibility that certain cellular functions such as larval development and puparium formation may be controlled by the regulatory influences of cyclic AMP while other such as adult development are mediated mainly by cyclic GMP.

These observations are also consistent with the changes in the metabolic behaviour of the Dipterous *Ceratitis capitata* at different development stages previously recorded (46,47).

REFERENCES

1. Sutherland, E. W., Rall, T. W., and Menon, T. (1962) J. Biol. Chem., 237, 1220-1227.
2. Applebaum, S. W., and Gilbert, L. I. (1972) Dev. Biol., 27, 165-175.
3. Rojakovick, A. S., and March, R. B. (1972) Comp. Biochem. Physiol., 43B, 209-215.

4. Whitmore, D., Applebaum, S. W., and Gilbert, L. I. (1973) J. Insect Physiol., 19, 349-354.

5. Morishima, I. (1973) J. Insect Physiol., 19, 2261-2265.

6. Castillón, M. P., Catalán, R. E., and Municio, A. M. (1973) FEBS Lett., 32, 113-115.

7. Morishima, I. (1974) Biochim. Biophys. Acta, 370, 227-241.

8. Rojakovick, A. S., and March, R. B. (1974) Comp. Biochem. Physiol., 47B, 189-199.

9. De Reggi, M. L., and Cailla, H. L. (1974) FEBS Lett., 46, 293-295.

10. Catalán, R. E., Castillón, M. P., and Municio, A. M. (1975) Biochem. Biophys. Res. Commun., 65, 385-391.

11. Morishima, I. (1975) Biochim. Biophys. Acta, 391, 75-83.

12. De Reggi, M. L., and Cailla, H. L. (1975) J. Insect Physiol., 21, 1671-1674.

13. Walsh, D. A., Perkins, J. P., and Krebs, E. G. (1968) J. Biol. Chem., 243, 3763-3765.

14. Langan, T. A. (1968) Science, 162, 579-581.

15. Miyamoto, E., Kuo, J. F., and Greengard, P. (1969) J. Biol. Chem., 244, 6395-6402.

16. Kuo, J. F., and Greengard, P. (1969) J. Biol. Chem., 244, 3417-3419.

17. Corbin, J. D., and Krebs, E. G. (1969) Biochem. Biophys. Res. Commun., 36, 328-336.

18. Albin, E. E., and Newburgh, R. W. (1975) Biochim. Biophys. Acta, 377, 389-401.

19. Kuo, J. F., Krueger, B., Sanes, J. R., and Greengard, P. (1970) Biochim. Biophys. Acta, 212, 79-91.

20. Miyamoto, E., Kuo, J. F., and Greengard, P. (1969) Science, 165, 63-65.

21. Catalán, R. E., and Municio, A. M. (1974) Biochem. Biophys. Res. Commun., 61, 1394-1399.

22. Posternak, T. (1974) Annu. Rev. Pharmacol., 14, 23-33.

23. Murad, F., Manganiello, V., and Vaughan, M. (1970) J. Biol. Chem., 245, 3352-3360.

24. Corbin, J. D., and Krebs, E. G. (1969) Biochem. Biophys. Res. Commun., 36, 328-336.

25. Krebs, E. G., Observations published in Ann. Rev. Pharmacol., 14, 25 (1974) by T. Posternak.

26. Kuo, J. F., and Greengard, P. (1969) Proc. Nat. Acad. Sci. U. S. A., 64, 1349-1355.

27. Goldberg, N. D., Haddox, M. K., Nicol, S. E., Glass, D. B., Sanford, C. H., Kuehl, F. A., and Estensen, R., In Advances in Nucleotide Research, Vol. 5, pp. 307-330 (1974). eds. Drummond, G. I., Greengard, P., and Robison, G. A. (Raven Press, New York).

28. Bodnaryk, R. P. (1975) Life Sci., 16, 1411-1416.

29. Johnson, E. M., and Hadden, J. W. (1975) Science, 187, 1198-1200.

30. Castillón, M. P., Catalán, R. E., Jimenez, C., Madariaga, M. A., Municio, A. M., and Suarez, A. (1974) J. Insect Physiol., 20, 507-512.

31. Cooper, R. H., McPherson, M., and Schofield, J. G. (1972) Biochem. J., 127, 143-154.

32. Steiner, A. L., Parker, C. W., and Kipnis, D. M., In Role of cyclic AMP in cell function. Vol. 3, pp. 89-111 (1970), eds. Greengard, P., and Costa, E. (Raven Press, New York).

33. Steiner, A. L., Wehmann, R. E., Parker, C. W., and Kipnis, D. M., In Advances in cyclic nucleotide research. Vol. 2, pp. 51-61 (1972), eds. Greengard, P., and Robison, G. A. (Raven Press, New York).

34. Catalán, R. E., Castillón, M. P., Avila, C., and Vila, T., submitted for publication.

35. Rodbard, D., Bridson, W., and Rayford, P. L. (1969) J. Lab. Clin. Med., 74, 770-781.

36. Catalán, R. E., Vila, T., and Castillón, M. P. (1976) Experientia, in press.

37. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem., 193, 265-275.

38. Berridge, M. J. (1970) J. Exp. Biol., 53, 171-186.

39. Leenders, H. J., Wullems, G. J, and Berendes, H. D. (1970) Exp. Cell Res., 63, 159-164.

40. Fuchs, M. S., and Schaeger, D. A. (1973) Biochem. Biophys. Res. Commun., 54, 784-789.

41. Vandenberg, R. D., and Mills, R. R. (1974) J. Insect Physiol., 20, 623-627.

42. Ishikawa, E., Ishikawa, S., Davis, J. W., and Sutherland, E. W. (1969) J. Biol. Chem., 244, 6371-6376.

43. Kuo, J. F., Lee, T. P., Reyes, P. L., Walton, K. G., Donnelly, T. E., and Greengard, P. (1972) J. Biol. Chem., 247, 16-22.

44. Catalán, R. E., Castillón, M. P., and Municio, A. M. (1976) Biochem. Biophys. Res. Commun., 69, 914-919.

45. Gray, J. P., Drummond, G. I., Luk, D. W. T., Hardman, J. G., and Sutherland, E. W. (1976) Arch. Biochem. Biophys., 172, 20-30.

46. Municio, A. M., García, R., and Pérez-Albarsanz, M. A. (1975) Eur. J. Biochem., 60, 117-121.

47. Municio, A. M., Odriozola, J. M., and Pérez-Albarsanz, M. A. (1975) Eur. J. Biochem., 60, 123-128.