DOES cAMP CONTROL PROTEIN SYNTHESIS?

Ivan HORAK and Klaus KOSCHEL
Institut für Virologie und Immunbiologie der Universität Würzburg, D-8700 Würzburg, FRG

Received 24 August 1977

1. Introduction

It has been suggested that protein phosphorylation might be involved in the regulation of protein synthesis [1–5]. Recently, Datta et al., have proposed that the activity of the cAMP-independent protein kinase, which phosphorylates the initiation factor eIF-2 [4–6], is controlled by a cAMP-dependent protein kinase [7]. These authors [7] postulate a model in which protein synthesis is regulated by cAMP, and suggest that the inhibition of cell growth by cAMP (review [8]) might be a consequence of protein synthesis inhibition.

C6 cells derived from chemically induced rat glioma [9] have the advantage of a physiologically responding in vivo system to catecholamines. Isoproterenol raises the intracellular cAMP level in C6 cells to extremely high values from about 10–20 pmol/mg protein up to 2000–3000 pmol/mg [10,11]. Thus, such a system can be useful in testing the role of the intracellular cAMP level on protein synthesis in vivo.

2. Experimental

C6 cells (ATCC, CCL-No. 107) were grown in medium containing 5% fetal calf serum [9]. Fresh lymphocyte cultures prepared from mouse spleens were cultured according to Mishell and Dutton in medium without serum [14]. The intracellular cAMP concentration was measured with the method of Gilman [13]. For protein synthesis determination, cells were labelled with 0.1 μCi/ml ^{14}C-labelled protein hydrolysate (Amersham) and ^{14}C-labelled amino acid incorporation into trichloroacetic acid insoluble precipitate was measured.

Isoproterenol was purchased from Serva, Heidelberg; Prostaglandin E1 was a generous gift from Dr J. Pike, Upjohn Co.

3. Results and discussion

The experiment shown in fig.1a indicates that the enhancement of the intracellular cAMP level up to 1000-times does not affect the total protein synthesis in C6 cells. Furthermore, no difference has been detected in the phosphorylation pattern of ribosome-associated and ribosomal phosphoproteins between isoproterenol treated and control cells when the cells were labelled with $^{32}P_i$ and analyzed on polyacrylamide gels as described [12] (data not shown).

Identical results have been obtained with mouse lymphocytes (fig.1b) in which the basal cAMP level (15 pmol/mg protein) was elevated 4–5 times 60 min after addition of prostaglandin E1. The possibility that C6 tumor cells represent a special case having different translational controls which do not respond to cAMP seems therefore unlikely.

If C6 cells were treated with both isoproterenol, 10^{-4} M, and 3-isobutyl-1-methylxantine (IBMX), 10^{-3} M [15,16] there was no rapid decrease of the intracellular cAMP after 60 min and the high level of cAMP can be maintained for several hours. We have observed a slight inhibition (10% after 2 h) of protein synthesis in this experiment. However, the same inhibition is seen in controls treated only with IBMX. IBMX treatment alone did not raise the cAMP level in C6 cells. A need for the prolonged enhancement of the intracellular cAMP concentration to affect the protein synthesis is unlikely but cannot be excluded. Precautions must be taken in the evaluation of experi-

North-Holland Publishing Company – Amsterdam

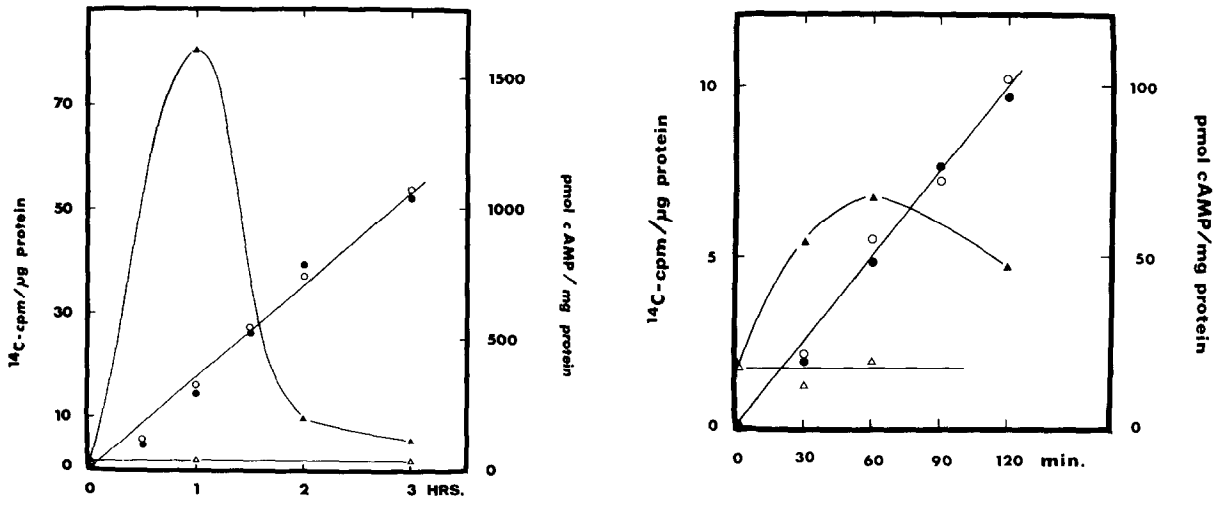

Fig.1. The effect of changes of the intracellular cAMP concentration on the protein synthesis: (a) in C6 cells, (b) in mouse lymphocytes. (A) Logarithmically-growing C6 cells in 60 mm Petri dishes were washed twice to remove serum proteins and incubated in 2 ml growth medium containing ^{14}C-labelled amino acids and 10^{-4} M isoproterenol. At intervals, ^{14}C incorporation into trichloroacetic acid-insoluble precipitate was measured. The intracellular cAMP concentration was monitored in parallel cultures. (B) Fresh lymphocyte cultures were labelled with ^{14}C-labelled amino acids and one-half of the cultures was treated with 10 µg/ml prostaglandin E1. Symbols: Triangles, cAMP concentration; circles, ^{14}C-labelled amino acid incorporation; filled symbols, cells treated with isoproterenol or prostaglandin E1; open symbols, control cells.

ments with IBMX and other phosphodiesterase inhibitors because of their possible secondary effects.

If cAMP plays a direct role in the translation control of protein biosynthesis, as has been proposed [7], one may expect that the oscillations of the intracellular cAMP concentrations should be followed by rapid changes in the rate of protein synthesis. Such oscillations of the cAMP level can be introduced by physiological means in the cultured cells. However, we have failed to detect any changes of protein synthesis in response to the changes of the intracellular cAMP concentrations during the time period studied.

Acknowledgements

We thank Drs Eberhard Wecker and William Hall for critical reading of the manuscript, and Mr Th. Hünig and R. Dörries for help with the lymphocyte cultures. This work was supported by the Deutsche Forschungsgemeinschaft SFB 105.

References

[1] Kabat, D. (1970) Biochemistry 9, 4160–4175.
[2] Eil, C. and Wool, I. G. (1971) Biochem. Biophys. Res. Commun. 43, 1001–1009.
[3] Kaerlein, M. and Horak, I. (1976) Nature 259, 150–151
[4] Balkow, K., Hunt, T. and Jackson, R. J. (1975) Biochem. Biophys. Res. Commun. 67, 366–375.
[5] Levin, D. H., Ranu, R. S., Ernst, V. and London, I. M. (1976) Proc. Natl. Acad. Sci. USA 73, 3112–3116.
[6] Farrell, P. J., Balkow, K., Hunt, T., Jackson, R. J. and Trachsel, H. (1977) Cell 11, 187–200.
[7] Datta, A., Haro, C., Sierra, J. M. and Ochoa, S. (1977) Proc. Natl. Acad. Sci. USA 74, 1463–1467.
[8] Pastan, I. H., Johnson, G. S. and Anderson, W. B. (1975) Annu. Rev. Biochem. 44, 491–522.
[9] Benda, P., Lightbody, J., Sato, G., Levine, L. and Sweet, W. (1968) Science 161, 370–371.
[10] Gilman, A. G. and Nirenberg, M. (1971) Proc. Natl. Acad. Sci. USA 68, 2165–2168.
[11] Schultz, J. P., Hamprecht, B. and Daly, J. W. (1972) Proc. Natl. Acad. Sci. USA 69, 1266–1270.
[12] Horak, I. and Schiffmann, D. (1977) Eur. J. Biochem. in press.

[13] Gilman, A. G. (1970) Proc. Natl. Acad. Sci. USA 67, 305–312.
[14] Mishell, R. J. and Dutton, R. W. (1967) J. Exp. Med. 126, 423–442.
[15] Peytremann, A., Nicholson, W. E., Liddle, G. S., Hardman, J. G. and Sutherland, E. W. (1973) Endocrinology 92, 525–534.
[16] Aschroft, S. J. H., Kandle, P. J. and Täljedal, I. B. (1973) FEBS Lett. 20, 263–266.