Interferon and Cyclic-3'5'-Adenosine Monophosphate: Potentiation of Antiviral Activity

by

Robert M. Friedman and Ira Pastan
National Cancer Institute
and
National Institute of Arthritis
and Metabolic Diseases
Bethesda, Maryland 20014

Received July 2, 1969

Abstract: The antiviral activity of chick interferon was increased by incubation with 10^{-2}M cyclic-3'5'-adenosine monophosphate. No potentiation of interferon action was noted when incubation was performed with several analogues of cyclic-3'5'-adenosine monophosphate.

The mechanism of action of interferon is of great interest since this substance may be useful in the prevention and treatment of certain viral infections. Interferon, however, is difficult to prepare in amounts necessary for therapy in man and a substance which would increase the efficacy of interferon might be therapeutically useful . Interferon is a polypeptide and resembles polypeptide hormones in some of the characteristics of its action (1). Since cyclic-3'5'-adenosine monophosphate (CAMP) is important in the mechanism of action of various polypeptide hormones (2) we investigated the possible role of CAMP in interferon action. We report that CAMP increases the antiviral activity of interferon although CAMP itself has no antiviral activity.

All experiments and assays were performed employing Semliki Forest virus in primary chick embryo fibroblast monolayers (CEF) prepared as previously described (3). Both the plaque inhibition assay and the virus yield inhibition assay employed to test interferon activity have been previously described in detail (4). All experiments reported were performed by incubating CEF with interferon and CAMP for 1 hr. at 37°. Partially purified chick interferon was

prepared by the method of Fantes (5). The pool used in these studies contained 180 μg of protein/ml and 10,000 units of interferon/ml. A unit of interferon inhibited Semliki Forest virus (SFV) growth by 50%.

In a series of experiments we found that incubation of CEF with interferon and CAMP decreased the yield of virus plaques obtained over a range of interferon dilutions as compared to the number obtained by treatment of CEF with interferon alone. The results shown in Fig. 1 are typical of 6 out of 7 similar experiments. At each dilution tested the cells treated with 10^{-2}M CAMP for 1 hr. together with interferon had fewer plaques than did controls treated with interferon alone. CAMP treatment alone did not inhibit virus plaque yield and was without effect on RNA or protein synthesis of CEF.

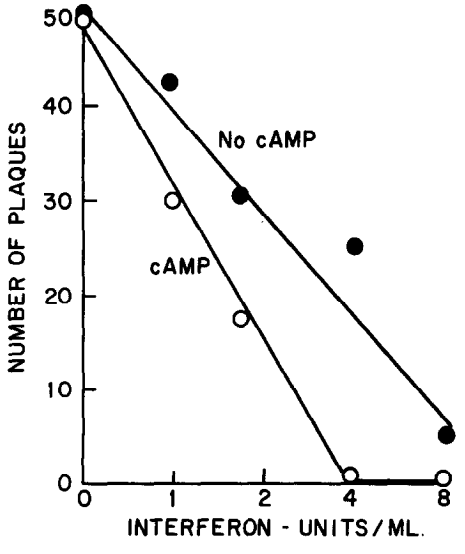

Fig. 1. Potentiation of interferon action by cyclic-3'5'-adenosine monophosphate (CAMP)-virus plaque inhibition assay. Monolayers of chick cells were treated with dilutions of chick interferon with or without 10^{-2}M CAMP. After 1 hr. the cells were washed five times and infected with 30 to 60 plaque forming units of Semliki Forest virus. After virus adsorption the plates were overlayed with agar and nutrient medium. Final plaque readings were made after 72 hrs.

CAMP was also active when tested in a virus growth inhibition assay. Here cells were treated with interferon together with CAMP for 1 hr., washed, and then infected with SFV at a virus:cell multiplicity of 20:1. At the end of the virus growth cycle (8 hrs. after infection) the cultures were frozen and then thawed and assayed for virus growth.

The results of a typical experiment, one of four with similar results, are shown in Fig. 2 where it may be seen that incubation of CAMP with 1, 10, or 100

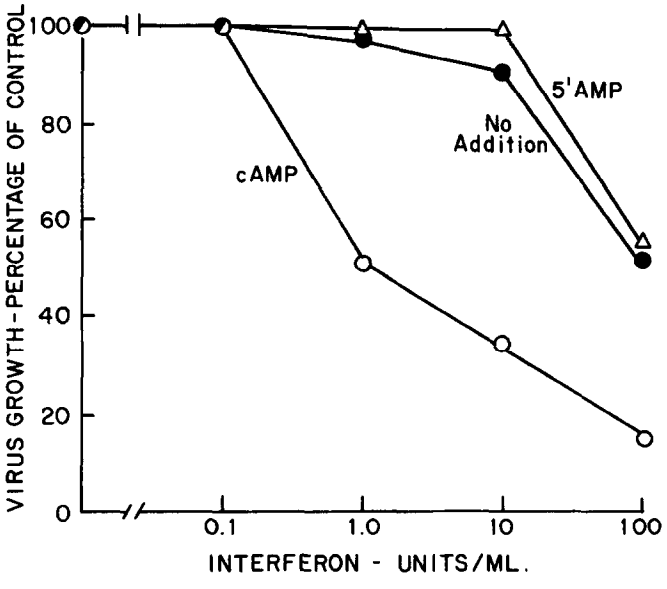

Fig. 2. Potentiation of interferon action by cyclic-3'5'-adenosine monophosphate (CAMP)-virus yield inhibition assay. Monolayers of chick cells were treated with dilutions of chick interferon in the presence or absence of 10^{-2}M CAMP or 5'adenosine monophosphate (5'AMP). After 1 hr. the cells were washed and infected with Semliki Forest virus at a virus to cell multiplicity of 20:1. After 1 hr. the cells were washed and new medium added; 8 hrs. after infection the cells and media were frozen, thawed, and assayed for infectious virus. The virus yields are presented as a percentage of controls treated with medium only, or 10^{-2}M CAMP or 5'AMP. No significant differences were found in the yield of virus obtained from these controls.

units of interferon for 1 hr. significantly increased the antiviral activity of the interferon dilution tested. Again CAMP alone had no effect on virus growth. A product of CAMP breakdown, 5'-adenosine monophosphate (10^{-2}M), had no effect on interferon action (Fig. 2). The following compounds tested at a 10^{-2} and in some cases also at 10^{-3}M were not effective in potentiating interferon action; adenine, adenosine, 3'-adenosine monophosphate, cyclic-3'5'-guanosine monophosphate, N^6-2'-dibutyryl adenosine-3'5'-cyclic phosphate (DBC), adenosine diphosphate, adenosine triphosphate, N^6-monobutyryl adenosine-3'5'-cyclic phosphate (6), and cyclic-3'5'-deoxyadenosine monophosphate (7). The failure of DBC to act was surprising since this compound has been found to be more active than CAMP in other animal tissues (2); however, DBC is not active in stimulating β-galactosidase synthesis in E-coli, whereas CAMP is active (H.Varmis, R. Perlman, and I. Pastan, unpublished result).

Figure 3 illustrates the effect of varying the concentration of CAMP in a virus yield experiment. The concentrations of CAMP employed did not affect the virus yield in the absence of interferon. Incubation of a range of concentrations of CAMP (10^{-4} to 10^{-2}M) with 50 units/ml of interferon for 1 hr. increased the antiviral activity of the interferon as compared to the activity of 50 units/ml of interferon alone. Similar results have been obtained with concentrations of 25 or 10 units/ml of interferon.

The mechanism by which CAMP increases interferon's antiviral activity is not yet clear. In E. coli CAMP increases the rate of synthesis of some inducible enzymes (8). Since experiments with metabolic inhibitors have suggested that interferon requires cell RNA and protein synthesis to establish its antiviral activity (9), it is also possible that CAMP may potentiate the production of an antiviral cell protein induced by interferon treatment.

The potentiation of interferon's action by CAMP may be of practical as well as of theoretical interest, since interferon may be useful in the treatment of viral infections in man. Whether CAMP treatment may potentiate the in vivo antiviral activity of exogenous or endogenous interferon is currently under investigation.

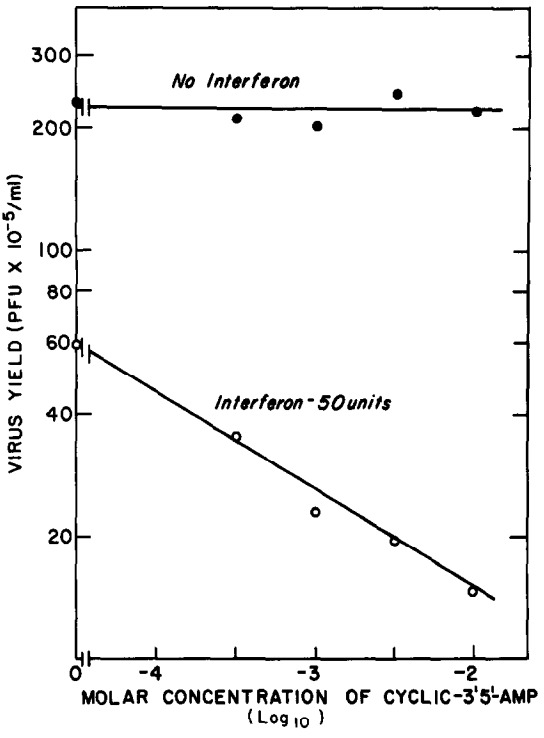

Fig. 3. Effect of concentration of cyclic-3'5'-adenosine monophosphate (CAMP) on interferon action. Chick cell monolayers were treated for 1 hr. with no interferon or 50 units of interferon together with various dilutions of CAMP (0 to 10^{-2}M). The cells were then washed, infected and the virus yields assayed as described in the legend to Fig. 2.

REFERENCES

1. Friedman, R.M., Science, 156, 1760 (1967).

2. Robison, G.A., Butcher, R.W., and Sutherland, E.W., Ann. Rev. Biochem., 37, 149 (1968).

3. Taylor, J., Virology, 25, 340 (1965).

4. Friedman, R.M. and Cooper, H.L., Proc. Soc. Exptl. Biol. Med., 125, 901 (1967). Friedman, R.M., Nature, 201, 848 (1964).

5. Fantes, K.H., Nature, 207, 1298 (1965).

6. Falbriard, J.G., Posternak, Th., and Sutherland, E.W., Biochem. Biophys. Acta. 148, 99 (1967).

7. Drummond, G.I., Gilgan, M.W., Reiner, E.J., and Smith, M., J. Am. Chem. Soc., 86, 1626 (1964).

8. Perlman, R. and Pastan, I., Biochem. Biophys. Res. Comm., 30, 656 (1968); Perlman, R. and Pastan, I., J. Biol. Chem., 243, 5420 (1968).

9. Sonnabend, J.A. and Friedman, R.M. in Interferons (edit. by Finter, N.B.) (North-Holland, Amsterdam, 1966).