The Relationship Between Adenosine 3': 5'-Cyclic Monophosphate (cAMP) and Interferon Activity in Mouse Cells

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(Accepted 18 January 1980)

SUMMARY

Three mouse cell lines (L, 3T3, and SV 3T3) were studied with respect to the elevation of cellular cAMP levels following interferon treatment, and the effect of stimulators of cAMP levels on the antiviral activity of interferon. Interferon treatment resulted in increased cAMP levels in L and 3T3 cells but not in SV 3T3. The antiviral activity of interferon in cells treated with epinephrine and 1-methyl-3-isobutyl xanthine (stimulators of cAMP levels) was potentiated in L cells, but not in 3T3 cells and was lost in SV 3T3 cells.

INTRODUCTION

Interferon is a glycoprotein that renders cells incapable of supporting the replication of a wide range of animal viruses. Since it has some properties similar to that of glycopeptide hormones (Friedman, 1977) it is possible that, as with these hormones, cAMP is involved in its intracellular action. If interferon operates through such a mechanism, then cAMP alone, or analogues of cAMP or substances that increase the intracellular concentration of cAMP, should be able to mimic the antiviral action of interferon.

Interferon has been found to elevate intracellular cAMP levels. Mécès et al. (1974) reported that interferon treatment of L cells increased the incorporation of 3H-adenine into cAMP. Weber & Stewart (1975), Meldolesi et al. (1977), Stanwick et al. (1978) and Tovey et al. (1979) found that interferon could increase intracellular cAMP levels. Cyclic AMP, however, appears to have an inconsistent effect on virus growth. Friedman & Pastan (1969) reported that cAMP could potentiate but not mimic interferon action in chick embryo fibroblast cells. Allen et al. (1974) found a similar effect in mouse L cells, using cAMP or cAMP analogues. Kost & Hayes (1974) and Weber & Stewart (1975) reported similar results. Stanwick et al. (1978) found that elevated cAMP levels had an additive effect with interferon treatment in inhibiting virus growth.

A variety of effects of cAMP on virus growth have been reported. Biron & Raska (1973) found that dibutyryl cAMP (db cAMP) inhibited adenovirus type 2 in some cells but not in others. Soltysiak & Maassab (1977) on the other hand reported that db cAMP could stimulate influenza growth in primary kidney cells. There were no effects on other viruses in the same cells or on influenza virus in other cell types. Stanwick & Nahmias (1979) reported that db cAMP and theophylline inhibited HSV type 1 but stimulated cytomegalovirus growth in human cells, while Robbins & Rapp (1979) reported that cAMP inhibited the growth of measles virus in culture.

The purpose of this study was to determine, with several mouse cell lines, whether the
effects of cAMP and interferon on the growth of the vesicular stomatitis virus (VSV) were consistent regardless of cell line used, and whether interferon consistently potentiates cAMP.

METHODS

Chemicals. Adenosine 3′: 5′-cyclic monophosphate (cAMP), L-epinephrine and 1-methyl-3-isobutylxanthine (MIX) were supplied by Sigma (St. Louis, Mo., U.S.A.). 3H-cAMP was obtained from New England Nuclear (Lachine, Quebec, Canada). Foetal calf serum (FCS) and tissue culture media were obtained from Flow Laboratories (Rockville, Md., U.S.A.).

Cells. L929 cells (American Type Culture Collection, ATCC) were grown and maintained in Eagle’s minimum essential medium (MEM). 3T3 (ATCC) and SV40-transformed 3T3 cells (SV 3T3), obtained from Flow Laboratories, were grown and maintained in Dulbecco’s modified Eagle’s MEM. Growth media contained 10% FCS, 2 × 10^5 units per litre of penicillin and 0.2 g per litre of streptomycin. Compounds used to treat cells were diluted in serum-free medium.

Viruses. The Indiana strain of vesicular stomatitis virus (VSV) was propagated in chick embryo fibroblast cells. Newcastle disease virus (NDV) obtained from ATCC was grown in the allantois of embryonated hens’ eggs.

Interferon. Interferon was produced by induction of L cells with NDV as previously described (Weber & Stewart, 1973). It was further purified through a Biogel P-150 column. Protein was measured by the method of Lowry et al. (1950). Interferon was titrated in L cells in terms of reduction of VSV plaques (O’Shaughnessy et al. 1972), and in L, 3T3 and SV 3T3 cells in terms of VSV yield reduction (Baron et al. 1968); L cells were used to titrate virus progeny. Two preparations of interferon were used in this study; that just described, with a specific activity of 0.5 × 10^5 reference units (NIH standard) per mg protein, and a preparation kindly supplied by Dr K. Paucker (Medical College of Pennsylvania), with a specific activity of 1 × 10^7 units per mg protein.

Determination of cAMP levels. Confluent cell monolayers grown on plastic Petri dishes (Falcon) were treated with 5% trichloroacetic acid (TCA) after which cells were removed from the surface using a rubber policeman, and homogenized in a volume of 2 ml with 20 strokes in a Ten Broeck tissue grinder. The samples were then acidified with 0.2 ml of 1 N-HCl and extracted five times in 3 vol. of water-saturated ether. The samples were lyophilized and resuspended in 1/10 the original volume in 50 mM-tris-HCl with 8 mM-theophylline and 5 mM-2-mercaptoethanol. The samples were assayed for cAMP activity by the method of Brown et al. (1972).

RESULTS

Since plaque assays often gave variable results with 3T3 cells, virus yield reduction assays were used to compare the interferon sensitivity of the three cell lines. The cells were infected with an m.o.i. of 2 to 5 infectious units per cell and the fluids collected after 16 h incubation at 37 °C were titrated by plaque assay in L cells. By this method, interferon activity in 3T3 was found to be 25% of that found in L cells, and in SV 3T3 was 75% of that in L cells.

Intracellular cAMP levels

Monolayers of the three cell lines were grown to confluency in 100 mm Petri dishes and treated with medium containing 10 or 100 units per ml of interferon. At the end of 18 h incubation at 37 °C, the cell monolayers were harvested. Duplicate samples were assayed for cAMP activity and the results shown in Table 1 are typical of at least three separate experiments. One hundred units of interferon consistently potentiated cAMP levels in L
Table 1. Effect of interferon on cAMP levels in L, 3T3 and SV40 3T3 cells

<table>
<thead>
<tr>
<th>Interferon concentration (units/ml)</th>
<th>L cells</th>
<th>3T3 cells</th>
<th>SV40 3T3 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.5 ± 0.5*</td>
<td>4.0 ± 0.7</td>
<td>9.3 ± 2.2</td>
</tr>
<tr>
<td>10</td>
<td>2.2 ± 0.6</td>
<td>3.9 ± 0.8</td>
<td>6.7 ± 1.3</td>
</tr>
<tr>
<td>100</td>
<td>4.2 ± 0.6</td>
<td>5.9 ± 0.8</td>
<td>8.2 ± 0.9</td>
</tr>
</tbody>
</table>

*Confidence interval 95%.

Table 2. Effect of epinephrine and MIX on cAMP levels in various cell lines

<table>
<thead>
<tr>
<th>Concentration of drug</th>
<th>L cells (pmol/10⁶ cells)</th>
<th>3T3 cells (pmol/10⁶ cells)</th>
<th>SV40 3T3 cells (pmol/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine (m)</td>
<td>3.4 ± 0.1†</td>
<td>6.8 ± 0.7</td>
<td>9.5 ± 0.7</td>
</tr>
<tr>
<td>MIX (m)</td>
<td>4.6 ± 0.7</td>
<td>6.8 ± 0.7</td>
<td>9.5 ± 0.7</td>
</tr>
</tbody>
</table>

* Epinephrine and 1-methyl-3-isobutyl xanthine at concentrations shown. † 95% confidence interval.

Effect of epinephrine and 1-methyl 3-isobutyl xanthine (MIX) on intracellular cAMP levels

Since interferon could increase cAMP levels in two cell lines but not in a third, the question was asked whether the SV 3T3 cells were susceptible to potentiation of cAMP levels by treatment with epinephrine and MIX.

Both epinephrine and MIX are cytotoxic, and the extent of this was assessed by chequerboard titrations, with visible c.p.e. by light microscopy taken as an end point. The highest non-cytotoxic dose for each cell line was applied to monolayers for 4 h at 37°C. The results of duplicate samples in two separate experiments (Table 2) show that cAMP levels were increased in L cells (P = 0.01) and SV 3T3 (P = 0.01), but not in 3T3 cells. The cAMP levels in control preparations of L and SV 3T3 cells were fairly consistent between experiments carried out over a period of time. The 3T3 cells, which were maintained in a highly contact inhibited state, were more variable.

Effect of increased cAMP levels on interferon action

Since cAMP has been shown to potentiate interferon action (Allan et al. 1974; Weber & Stewart, 1975), the effect of epinephrine and MIX on the antiviral activity of interferon was studied to determine whether there was a relationship between altered cAMP levels and interferon activity in the three cell lines. The concentrations of MIX and epinephrine used were the same as shown in Table 2 and treatment was for 4 h. Interferon was then applied for 16 h, after which the cultures were infected. Results are shown in Table 3, based on data from two separate experiments, each of which was done in duplicate.
Table 3. Effect of epinephrine and MIX on the antiviral activity of interferon

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Amount of interferon (unit)</th>
<th>% reduction in virus yield on treatment with</th>
<th>Interferon</th>
<th>Interferon, MIX and epinephrine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3T3</td>
<td>2</td>
<td>Interferon</td>
<td>46</td>
<td>55</td>
</tr>
<tr>
<td>SV 3T3</td>
<td>2</td>
<td>Interferon</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>L</td>
<td>2</td>
<td>Interferon</td>
<td>84</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Interferon</td>
<td>47</td>
<td>62†</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>Interferon</td>
<td>5</td>
<td>30†</td>
</tr>
</tbody>
</table>

* Used at concentrations shown in Table 2.
† P = 0.01.

Treatment of the 3T3 cells with epinephrine and MIX significantly increased the virus yield (approx. 50-fold). Interferon was not potentiated in this system since interferon had approximately the same antiviral activity in cells treated with or without MIX and epinephrine.

In the SV 3T3 system, MIX and epinephrine had no appreciable effect on virus yield; however, interferon demonstrated antiviral activity in the control preparations, but the antiviral activity of interferon was eliminated with epinephrine and MIX treatment of the cells. Epinephrine and MIX treatment potentiated the antiviral activity of interferon in L cells.

DISCUSSION

In the work presented here, interferon increased the levels of cAMP in L cells, as found by others (Mécs et al. 1974; Weber & Stewart, 1975; Meldolesi et al. 1977; Stanwick et al. 1978; Tovey et al. 1979). However, although interferon similarly potentiated cAMP levels in 3T3 cells, it was unable to do so in the SV40-transformed 3T3 cell line. This may possibly be related to the decreased sensitivity of SV 3T3 cells to the antiviral action of interferon compared to L and 3T3 cells. The results showing the potentiation of the antiviral effect of interferon in L cells by epinephrine and MIX is again similar to that reported by others (Friedman & Pastan, 1969; Allen et al. 1974; Kost & Hayes, 1974; Weber & Stewart, 1975). In SV 3T3 cells, however, the elevation of cAMP levels by MIX and epinephrine inhibited rather than potentiated interferon action. In 3T3 cells, epinephrine and MIX treatment had no effect on the activity of interferon and, although these compounds did not elevate cAMP levels, the yield of virus was substantially increased following MIX and epinephrine treatment. Soltysiak & Maassab (1977) similarly found that dibutyryl cAMP increased the growth of influenza in primary kidney cells but not in other cell lines. That the particular virus may also determine the outcome of such experiments was shown in their study when db cAMP had no effect on two other viruses in the kidney cell system. Similarly, Stanwick & Nahmias (1979) reported that elevated cAMP levels stimulated the growth of cytomegalovirus in human cells but not that of HSV type 1 in the same system.

The results of this study, utilizing a single virus in three mouse-derived cell lines, emphasize not only the variability of cAMP as a regulator of virus growth and potentiator of interferon antiviral activity but also the variability of the response of these cell lines to substances potentiating cAMP levels.

This research was supported by a grant (MT-1515) from the Medical Research Council of Canada.
REFERENCES


(Received 27 November 1979)