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TWO INDUCERS OF CELL DIFFERENTIATION ENHANCE THE 2'5' OLIGOADENYLATE SYNTHETASE ACTIVITY IN MSV TRANSFORMED CELLS.

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SUMMARY - The interferon induced 2'5' oligoadenylate synthetase activity can be increased upon treatment of Moloney Sarcoma virus transformed cells with two inducers of cell differentiation : sodium n-butyrate and dimethyl sulfoxide. This effect does not seem to be the consequence of the inhibition of cell growth by butyrate since the basic level of the enzyme stayed the same in control cells whether growth was inhibited by the absence of serum in the medium or not. It did not seem either to be due to the induction of IFN by these compounds since we could not detect any antiviral activity in the supernatant of the treated cells. Treatment by interferon of the butyrate pretreated cells results in a higher enzyme activity and a higher antiviral state than in non-pretreated cells.

INTRODUCTION

The enzyme 2'5' oligoadenylate synthetase was first discovered in interferon (IFN) treated cells (for review see ref. (1)). It catalyzes the synthesis of a series of oligoadenylates characterized by a 2'5' phosphodiester linkage with the general structure pppA2'(p5'A)$_n$, $n \geq 1$ (2-4). 2'5' oligoadenylates are potent inhibitors of protein synthesis in cell free systems and also when artificially introduced into intact cells (5, 6) where they activate an endogeneous endonuclease which degrades mRNA. The presence of 2'5' oligoadenylate synthetase has since been detected in all mammalian cells tested (7). It has been suggested that high levels of this enzyme may be present in differentiating cells where mRNA degradation may play a part in preparing the cell for specialized functions (8).

Abbreviations : Interferon : IFN ; dimethyl sulfoxide : DMSO ; Moloney sarcoma virus : MSV ; encephalomyocarditis virus : EMV ; polyinosinic acid : poly (I) ; polycytidilic acid : poly (C) ; multiplicity of infection : m.o.i.

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In order to produce evidence supporting this hypothesis, we have investigated whether the oligonucleotide synthetase activity in cells could be increased by chemicals known to induce cell differentiation.

Sodium n-butyrate induces various reversible changes in mammalian cells in culture (9, 10, 11, 12, 13) and is a potent inducer of differentiation of cultured erythroleukaemic cells (Friend cells) into resting, hemoglobin containing cells (14). The mechanism of these effects is not known.

We report that butyrate and dimethyl sulfoxide (DMSO) which is also an effective inducer of Friend (15) and neuroblastoma cell differentiation (16) are both inducers of the 2'5' oligoadenylate synthetase activity in MSV transformed cells.

MATERIALS AND METHODS

Materials

The sources of the following chemicals are : sodium n-butyrate from Merck, Darmstadt, G.F.R. ; dimethyl sulfoxide from British Drug House, Poole, England ; $\alpha[^{32}p]$ATP from Amersham, England ; poly (I) . poly (C) agarose and murine interferon (10^6 units/mg protein) from Institut Choay, Montrouge, France ; medium from Laboratoires Eurobio, Paris ; calf serum from Flow Laboratories S.A., Asnières, France.

Cells

The effect of sodium n-butyrate and DMSO was tested on MSV transformed cells, originally from Dr. Chany (U.43 INSERM, Hôpital Saint Vincent de Paul, Paris). This cell line was obtained by transformation of murine BALB/c cells with murine sarcoma virus, strain Moloney (17). Interferon titration and virus titration were done using murine L 929 cells. Both cell lines were routinely propagated in Eagle Minimum Essential Medium containing non-essential amino acids and supplemented with 10% (vol/vol) new born calf serum.

Preparation of cell extracts and determination of protein concentration

The cells were washed in buffer containing 140 mM NaCl, 35 mM Hepes pH 7.5, 3 mM $MgCl_2$ and lysed in buffer (18) containing 10 mM Hepes pH 7.6, 10 mM KCl, 2 mM $Mg(OAc)_2$, 7 mM 2-mercaptoethanol, 0.5% NP40, for 5 minutes in ice. The cell lysate was then centrifuged for 6 minutes in an Eppendorf centrifuge. The enzyme present in the supernatant was then bound to poly (I). poly (C) agarose beads. Protein concentration was determined according to Spector (19).

Assay for 2'5' oligoadenylate synthetase and chromatography of $pppA(pA)_n$ (20)

75 µl of cell extract was added to 15 µl of poly (I) . poly (C) agarose. After 15 minutes incubation at room temperature, the agarose was washed twice with 500 µl of buffer D containing : 20 mM Tris-HCl pH 8, 5 mM $Mg(OAc)_2$, 1 mM dithiothreitol, 25 mM KCl and 10% glycerol. To the pellet of agarose was added 10 µl of a mixture of 7 mM $\alpha[^{32}p]$ATP, 25 mM $Mg(OAc)_2$, 12 µg/ml poly (I) . poly (C), 0.25 mg/ml bovine serum albumin in buffer D. After 2 hours of incu-

bation at 37°c, the reaction was stopped by addition of 20 µl 50 mM EDTA. 6 µl of the incubation mixture were withdrawn, and spotted on a PEI cellulose plate which was developed in 2 M Tris-HCl, pH 8.6 to separate 2'5' oligoadenylates from ATP. The radioactive spots were localized on the chromatogram using X Ray film, cut out, and then radioactivity was determined by Cerenkof counting. From this the percentage conversion of ATP into oligoadenylates was calculated as a measure of 2'5' oligoadenylate synthetase activity.

Determination of the antiviral state

The MSV cells treated with butyrate, or IFN, or both, were infected with EMV at a multiplicity of 1. After 1 hour at 37°C, the medium containing non-adsorbed virus was removed and the cells were incubated in fresh medium for 18 hours at 37°C. The cultures were then frozen at - 80°C, thawed and the viral yield was determined by plaque assay.

Titration of interferon in cell supernatant

Monolayers of L 929 cells were treated with MSV cells supernatant or a known IFN preparation for 24 hours at 37°C. The cells were then infected with encephalomyocarditis virus (EMV) (m.o.i. = 1) and further incubated at 37°C for 18 hours.

IFN titers were defined as the reciprocal of the IFN preparation or cell supernatant dilution necessary to reduce by 50% the cytopathic effect of EMV on L 929 cells.

RESULTS AND DISCUSSION

1) Effect of Na-butyrate on 2'5' oligoadenylate synthetase activity in MSV transformed cells.

In order to test if butyrate, like IFN, can increase 2'5' oligoadenylate synthetase activity, MSV transformed cells were treated with 1 mM sodium butyrate in growth medium supplemented with 10% calf serum. The level of the enzyme activity was measured daily in one batch of cells. We observed (Fig. 1) an approximately linear increase with time of the 2'5' oligoadenylate synthetase activity in butyrate treated cells while the enzyme activity stayed the same in control cells.

2) Effect of serum on the induction by Na butyrate of 2'5' oligoadenylate synthetase activity in MSV transformed cells.

It has been shown that the activity of the synthetase increased in cells when they grew from the subconfluent to confluent state (7) and from fast

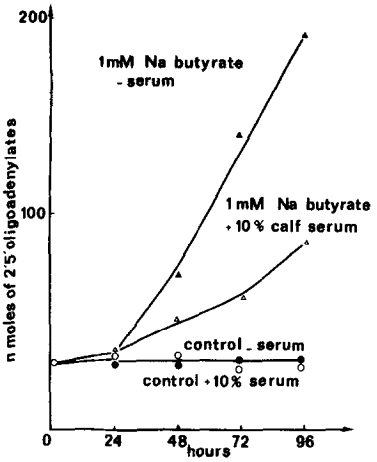

FIGURE 1. Increase of 2'5' oligoadenylate synthetase activity in MSV transformed cells treated with Na-butyrate. Effect of serum. MSV cells were seeded (7 10^5/ dish,35 mm diameter) in MEM supplemented with 10% calf serum in the absence (●●) or in the presence of 1 mM Na n butyrate (▲Δ). After every 24 hours incubation, fresh medium was added to the cells containing : MEM + 1 mM Na butyrate (▲), MEM supplemented with 10% calf serum + Na butyrate (Δ) ; for the control cells : MEM alone (●), MEM supplemented with 10% calf serum (○). The 2'5' oligoadenylate synthetase activity was determined as described at the times inducated and expressed as in Table 1.

growing to quiescent serum starved cells (21). In order to test if the increased level of the enzyme activity was the consequence of the butyrate induced resting state we have compared the basal level of the enzyme activity and its inducibility by butyrate in 10% serum stimulated and in serum starved cells (Fig. 1). The basic level of the enzyme stayed the same in control cells over a 3 day period whether serum was present in the medium or not. On the contrary in the absence of serum the sodium butyrate induced enzyme activity is about four times higher than in the presence of 10% calf serum.

The reason for a higher inducibility of the enzyme by butyrate in serum free than in serum containing medium is not clear ; this could be due to the presence of antagonizing factors such as hormones or binding proteins in the serum.

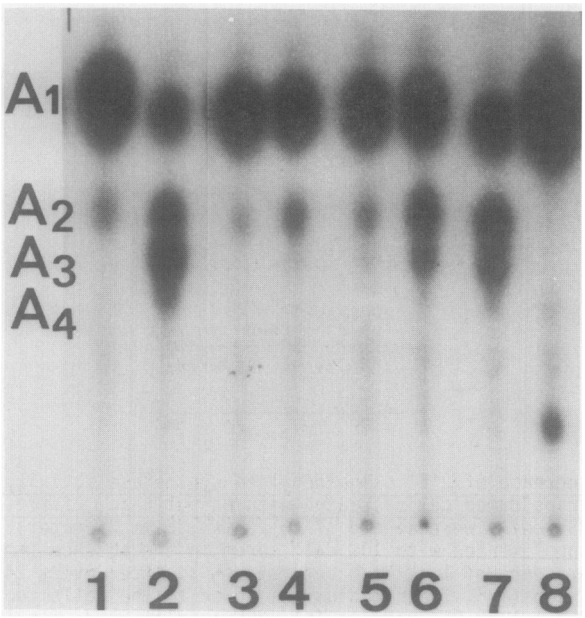

FIGURE 2. 2'5' oligoadenylates synthesis with enzymes induced by butyrate or by interferon.
Incubation conditions and chromatographic analysis of the reaction products are described in methods. Samples from untreated cells were spotted on lanes 1) 24 hours in the presence of serum, 3) 24 hours in the absence of serum, 5) 48 hours in the absence of serum. Samples from treated cells were lanes 2) 24 hours IFN (250 units/ml) treated cells in serum ; 4) 6) 7) butyrate treated cells in the absence of serum for 24, 48 and 72 hours respectively, lane 8) $\alpha[^{32}P]$ ATP. A_1, A_2, A_3, A_4 stand for ATP, 2'5' pppApA, pppApApA and pppApApApA.

3) Is interferon present in the supernatant after butyrate treatment ?

Subsequent experiments were done to see whether the induction of the enzyme activity was due to the induction of IFN by butyrate in MSV transformed cells. The supernatant of control and butyrate treated cells was kept every day, treated 24 hours at pH 2, centrifuged and assayed for IFN activity on L 929 cells in parallel with different concentrations of a murine IFN preparation of known titer. We could not detect any antiviral activity in these supernatants. The oligonucleotide synthetase activity observed after butyrate treatment is considerably higher than that induced by the smallest quantity of interferon (4 reference units/ml) that we can detect

(data not shown). However we cannot exclude the possibility of a rapid inactivation of the induced IFN which prevents us from detecting it.

4) Similarity between the butyrate and IFN induced enzymes.

Further experiments were performed in order to establish the similarity between the butyrate and IFN induced 2'5' oligoadenylate synthetase. The enzyme activity was strictly dependent on the presence of double stranded RNA as previously shown for the IFN induced enzyme. The analysis by chromatography on PEI cellulose of the products synthesized by the two enzymes revealed a similar pattern (Fig. 2). The predominant compounds synthesized by both enzymes were the dimer and the trimer.

5) Increase by Na-butyrate of 2'5' oligoadenylate synthetase and antiviral state in MSV transformed cells treated with interferon.

In accord with the possible role of 2'5' oligoadenylates in the inhibition of viral replication, we have observed (Fig. 3) a concomittant increase in the level of the 2'5' oligoadenylate synthetase and the appearance of a slight antiviral state in cells treated with butyrate (1 mM) for 24 hours (Time 0 in the figure). When IFN was added to the butyrate pretreated cells the level of the enzyme activity and the inhibition of viral multiplication were higher than in the non-pretreated cells.

In particular, at a very low concentration of IFN (10 units/ml) where the inhibition of viral multiplication is scarcely detected pretreatment of cells with butyrate enhances significantly the antiviral action of IFN in MSV transformed cells.

An enhancement of antiviral and antitumor action of IFN by butyrate has already been established (22).

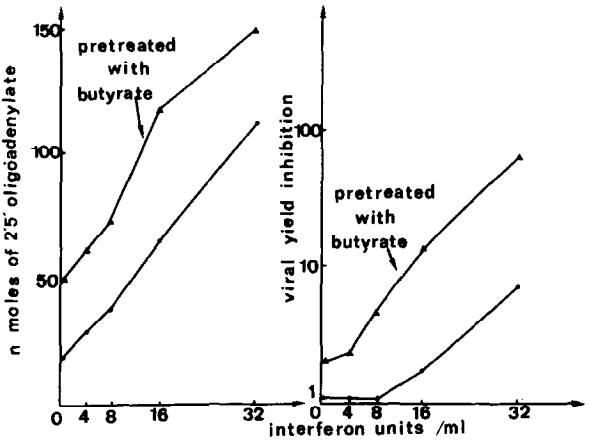

FIGURE 3. Increase by Na n-butyrate of 2'5' oligoadenylate synthetase and antiviral state in MSV transformed cells treated with interferon. MSV cells were seeded (7 10^5/dish 35 mm diameter in MEM supplemented with 10% calf serum. They were then treated (▲) or not (●) with 1 mM Na n-butyrate for 24 hours in the absence of serum and then with increasing concentrations of interferon for another 24 hours.
At this time, the 2'5' oligoadenylate synthetase activity and antiviral state were determined as described in Materials and Methods. The viral yield inhibition is expressed as the ratio of virus yield in control cells to that in cells treated by butyrate, interferon or butyrate + interferon.

6) Effect of DMSO on 2'5' oligoadenylate synthetase activity in MSV transformed cells.

We have also investigated the effect of DMSO, another inducer of cell differentiation, on MSV transformed cells with regard to its capacity to enhance the 2'5' oligoadenylate synthetase activity. The enzyme activity measured after a 48 hours treatment of the cells with the chemical, was increased at least 4 fold as compared to the control cells (Table 1). Again no IFN activity could be detected in the supernatant of the treated cells.

Recently, Krishnan and Baglioni have reported that the 2'5' oligoadenylate synthetic activity of Daudi and Raji lymphoblastoid cells increases upon treatment with glucocorticoids (23). Here also, this effect does not appear to be mediated by the synthesis and secretion of IFN.

TABLE 1. Increase of 2'5' oligoadenylate synthetase activity in MSV transformed cells treated with DMSO 1 %.
The cells were treated as described in the legend of Fig. 1 except that DMSO 1 % was added instead of sodium- n-butyrate. The 2'5' oligoadenylate synthetase activity was measured after 48 hours of treatment. The enzyme activity is expressed as nonomoles AMP incorporated into 2'5' oligoadenylates in 2 hours per mg protein.

EXPERIMENT	I	II	III
Control cells	27,8	34,5	45,5
1 % DMSO treated cells	170	177	148,7

It is striking that all these 2'5' oligoadenylate synthetase inducing compounds : glucocorticoids (23, 24), butyrate (25), dimethyl sulfoxide (25) and IFN (26) have all been reported to inhibit DNA synthesis and to enhance IFN production upon viral infection.

The similarity between the effect of these compounds suggests that there could be a common step in their mechanism of action, such as the 2'5' oligoadenylate synthetase system.

Moreover, recent experiments showing that the enzyme can catalyze 2' adenylation of NAD and tRNA (27) and the formation of hetero oligonucleotides (28) different from the 2'5' oligoadenylate, emphasize its putative wider significance in regulating cell metabolism.

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