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INTERFERON ENHANCES GUANYLATE CYCLASE ACTIVITY IN HUMAN LYMPHOMA CELLS

C. ROCHETTE-EGLY and M.G. TOVEY

Unité INSERM 61, Avenue Molière, 67200 Strasbourg, France and
Institut de Recherches Scientifiques sur le Cancer, BP 8,
94800 Villejuif, France.

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SUMMARY : Treatment of the human Burkitt lymphoma derived cell line Daudi with electrophoretically pure human interferon ∝ caused a rapid increase in the intracellular concentration of guanosine 3',5' cyclic-monophosphate (cyclic GMP). This increase was accompanied by an enhancement of guanylate cyclase activity in interferon-treated cells. No change in cyclic GMP phosphodiesterase was observed. However electrophoretically pure human interferon ∝ was without effect on the guanylate cyclase activity of cell-free lysates of Daudi cells. This strongly suggests that the increase in the intracellular concentration of cyclic GMP in interferon treated cells is due to an activation of guanylate cyclase which is mediated via an interaction of interferon with its specific cell surface receptor.

INTRODUCTION

Interferons are glycoproteins which exert multiple effects on cells including the establishment of an antiviral state, inhibition of cell multiplication, modulation of specialized cellular functions, and modification of the cell surface (1). In common with other biologically active substances such as peptide hormones and neurotransmitters (2), the initial step in interferon action is to bind with high affinity to a specific cell surface receptor (3, 4). Interferon does not appear to be internalized following binding to its cell surface receptor (4) which raises the possibility that some of the diverse effects of interferon on cells may be mediated by the induction of an intracellular messenger. We have recently shown that interferon induces a rapid and marked increase in the intracellular concentration of guanosine 3'-5'-cyclic monophosphate (cyclic GMP) in mouse leukemia L1210 cells (5-7) and mouse splenic lymphocytes (8). However, due to the high levels of endogenous phosphodiesterase in mouse L1210 cells we were unable to determine whether the observed increase in cyclic GMP in interferon treated cells was caused by an activation of guanylate cyclase or resulted from an inhibition of phosphodiesterase activity (7). We report herein that treatment of the human Burkitt lymphoma derived cell line Daudi, with electrophoretically pure human interferon ∝ which causes a rapid increase in the intracellular concentration of

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cyclic GMP, is accompanied by an enhancement of guanylate cyclase activity in interferon-treated cells.

MATERIALS AND METHODS

Interferon preparations

Partially purified human interferon α (specific activity 10^6 international units/mg protein) was prepared from peripheral blood leukocytes as previously described (9). Electrophoretically pure human interferon α (specific activity 2 x 10^8 international units/mg protein) was prepared from Namalwa cells and was purified by affinity chromatography as previously described (10). Partially purified mouse interferon (specific activity 2 x 10^7 international units/mg protein) was prepared from mouse C-243 cells as previously described (11). This material was further purified by affinity chromatography to give electrophoretically pure interferon with a specific activity of 0.5 to 1.0 x 10^9 international units/mg protein. Rat interferon of specific activity 10^6 Units/mg protein was prepared from rat fibroblasts as previously described (12). All dilutions of the various interferon preparations were prepared in distilled water containing 1 mg/ml of bovine serum albumin.

Chemicals

Alumina (W200-Neutral) was obtained from Woehlm Pharma (Eschwege, Germany) and Dowex 1 x 2 from Biorad Laboratories (Richmond, Calif., USA). [α-^{32}P] GTP was purchased from the Radiochemical Center (Amersham, Bucks, UK) and cyclic [8,5' - ^{3}H] GMP (36.9 Ci/mmole) from New England Nuclear Corporation (Boston, Mass., USA). Creatine kinase and phosphocreatine were from Boehringer (Mannheim, Germany).

Cell and Tissue treatment

Daudi cells were cultivated in static suspension in RPMI 1640 Medium (Flow Laboratories) supplemented with 10 % fetal calf serum (GIBCO, Grand Island, NY). At various times after treatment with interferon (10^4 Units/ml), one milliliter of cell suspension was centrifuged, (20,000 g for 30 minutes at 4°C) the pellet homogeneized in cold Buffer A (25 mM Tris-HCl pH 7.5 containing 1 mM dithiothreitol and 250 mM sucrose), and aliquots of the resulting homogenate were assayed for guanylate cyclase activity.

The livers used in the experiments were obtained from 200-250 g male Wistar Rats. After homogeneization in cold Buffer A, they were centrifuged at 37 000 xg at 4°C for 15 min. The supernatant was then assayed for guanylate cyclase activity in the absence or presence of various interferon preparations.

Guanylate cyclase assay

Guanylate cyclase activity was performed essentially as described by Goridis et al (13). The reaction mixture contained in a final volume of 0.1 ml, 60 mM Tris-HCl pH 7.5, 8 mM $MnCl_2$, 5 mM cyclic GMP, a GTP regenerating system (15 mM phosphocreatine, 75 μg creatine kinase), 0.5 mM 3-isobutyl - 1 methylxanthine (IBMX) and the enzyme preparation (0.15 - 0.50 mg protein). The reaction was initiated by adding 0.2 mM [α-^{32}P] GTP (approximately 1 x 10^6 cpm) and 100 000 cpm of (^{3}H) cyclic GMP (to estimate recovery in the subsequent steps). After a 10 min incubation at 37°C, the reaction was terminated by adding unlabelled GTP (500 nmol) in 4 mM EDTA. After boiling and cooling in an ice-bath, the [^{32}P] cyclic GMP formed was isolated by sequential chromatography on neutral alumina and Dowex 1 x 2 (formate form) columns as described by Goridis et al (13). All results were corrected for recovery of cyclic (^{3}H) GMP (70-80 %) and expressed as picomoles of cyclic GMP formed per minute and per 10^6 cells (for Daudi cells) or per mg protein (for rat liver supernatant).

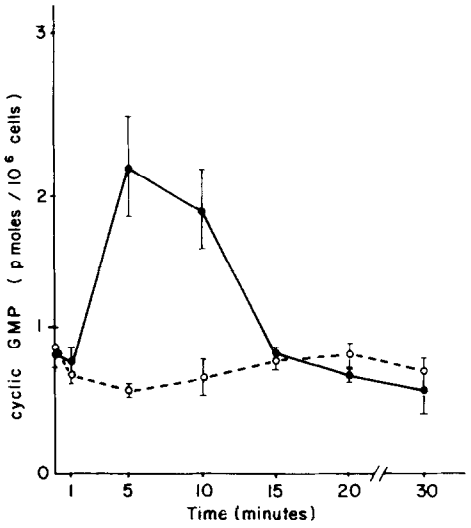

Figure 1 : The effect of interferon on the intracellular concentration of cyclic GMP in human lymphoblastoid cells. Daudi cells (2.3 x 10^6 cells/ml) were treated with either 10^4 Units/ml of electrophoretically pure human interferon α (specific activity 2 x 10^8 Units/ml protein) in phosphate buffered saline (PBS) or with an equal volume of PBS alone. Samples (1.0 ml) of cell suspension were then taken at the times indicated, and assayed for cyclic GMP content as previously described (7). Treated with interferon (●) or treated with PBS (o).

Other methods

Cyclic GMP content was determined using a radioimmune assay (Amersham, UK) as previously described (7). Cyclic GMP - dependent phosphodiesterase activity was determined by chromatography on Acriflavin - Sephadex as previously described (14).

RESULTS AND DISCUSSION

A rapid increase in the intracellular concentration of cyclic GMP was observed 1 to 5 minutes after treatment of mouse leukemia L1210 cells with electrophoretically pure mouse interferon. However, due to the high level of endogenous cyclic GMP phosphodiesterase activity in these cells we were unable to determine the effect of interferon on guanylate cyclase activity (7) even in the presence of high concentrations of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine IBMX (15). We therefore investigated the effect of human interferon on the intracellular concentration of cyclic GMP in the highly interferon sensitive human Burkitt lymphoma derived cell line Daudi. The response observed in Daudi cells was very similar to that seen in mouse L1210 cells with a rapid and transient increase in the intracellular concentration of cyclic GMP occurring 5 minutes after treatment of Daudi cells with electrophoretically pure human interferon (Figure 1).

When extracts of interferon treated Daudi cells were assayed for guanylate cyclase activity, a marked increase in the activity of the enzyme was

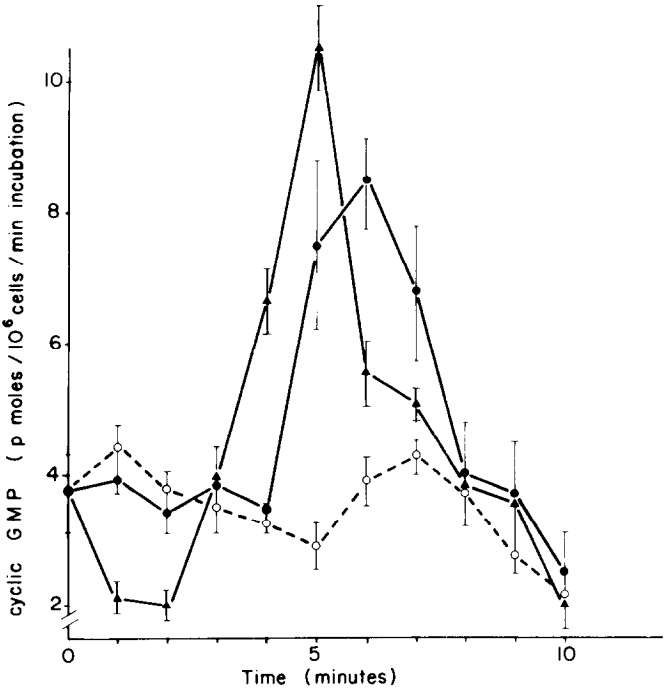

Figure 2 : Guanylate cyclase activity of Daudi cells after incubation of intact cells with electrophoretically pure human interferon ∝ .

▲ Treated with 10^4 Units/ml of electrophoretically pure interferon ∝ .

● Treated with 10 mM sodium azide.

observed 5 minutes after the addition of electrophoretically pure interferon ∝, guanylate cyclase activity then declined to previous levels 8 minutes post interferon treatment (Figure 2). The peak of guanylate cyclase activity corresponded almost exactly to the kinetics of cyclic GMP production in the same experiment. No change in cyclic GMP phosphodiesterase activity was observed during the first 10 minutes following treatment of Daudi cells with interferon (data not shown). The stimulation of guanylate cyclase activity was obtained with electrophoretically pure interferon preparations strongly suggesting that the observed effects were indeed due to interferon.

The increase in guanylate cyclase activity in interferon treated cells closely resembled that induced by 10 mM sodium azide (Figure 2) a non physiologic activator of guanylate cyclase which is capable of activating the enzyme in acellular in vitro systems (15). It was therefore in interest to investigate the effect of interferon on the guanylate cyclase activity of cell free lysats of Daudi cells. Neither partially purified nor electrophoretically pure human interferon ∝ had any effect on guanylate cyclase activity in this system (data not shown) strongly suggesting that the activation of guanylate cyclase in interferon treated cells is mediated via interaction of

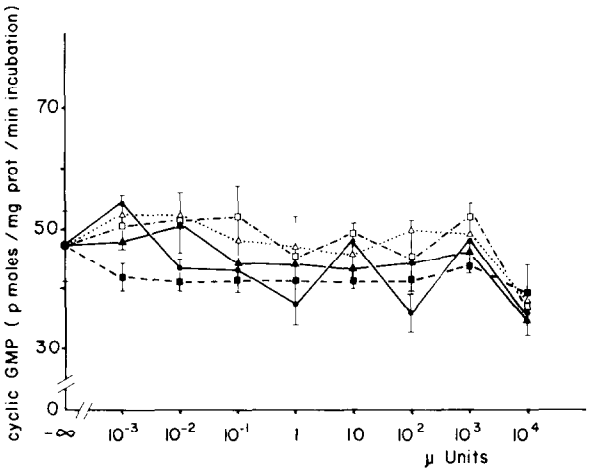

Figure 3 : Guanylate cyclase activity of a cell free extract of rat liver after incubation with increasing concentrations of :

● — ● partially purified human leucocyte interferon
▲ — ▲ rat interferon
■ -- ■ electrophoretically pure human interferon ∝
△ .+. △ electrophoretically pure mouse interferon
□ .+. □ partially purified mouse interferon

interferon with its specific cell surface receptor. However, partially purified human interferon has recently been reported to enhance guanylate cyclase activity in cell-free extracts of rat liver (16). We therefore tested the effect of various types of interferons, including both partially purified and electrophoretically pure human interferon ∝ , on guanylate cyclase activity of the soluble fraction of a rat liver homogenate. In contrast to a previous report (16) neither partially purified nor electrophoretically pure human interferon ∝ at doses ranging from 0.001 to 10^4 μ units had any significant effect on guanylate cyclase activity in this system (Figure 3) eventhough guanylate cyclase activity was enhanced 3 to 6 fold by treatment with sodium azide, hydroxylamine, or sodium nitroprusside (Table 1). Both rat and mouse interferons (partially purified and electrophoretically pure preparations) were similarly without effect. Negative results were also obtained when the extraction and assay procedures described in reference 16 were used instead of the slightly different procedures described in the materials and methods.

We have shown that human interferon ∝ stimulates guanylate cyclase activity in Daudi cells, strongly suggesting that the increase in the intracellular concentration of cyclic GMP in interferon treated cells is due to an activation of guanylate cyclase. However, interferon does not appear to act directly on guanylate cyclase, since interferon had no effect on the activity of the enzyme in cell-free extracts of Daudi cells, but may rather act via an interaction with its specific cell surface receptor. We have recently shown

TABLE 1

Activation of rat liver guanylate cyclase by sodium azide, hydroxylamine and sodium nitroprusside

TREATMENT	GUANYLATE CYCLASE (pmol Cyclic GMP formed/min/mg protein)
none	33.86 ± 3.44
NaN_3 (1 mM)	210.53 ± 17.99
NH_2OH (1 mM)	106.55 ± 0.68
Sodium nitroprusside (1 mM)	95.98 ± 1.78

The 37 000 g supernatant was incubated at 37°C for 10 min. With the various effectors and guanylate cyclase activity was measured as indicated in Materials and Methods.

that interferon had no effect on the intracellular concentration of cyclic GMP in a clone of interferon resistant mouse L1210 cells which lack a specific cell surface receptor for interferon (7). These results are in accord with recent evidence suggesting that interferon is not internalized following binding to its cell surface receptor (4).

Although the pathway leading from the initial interaction of interferon with its cell surface receptor to the activation of guanylate cyclase remains unknown, we have shown that in common with increases in cyclic GMP induced by certain hormones and neurotransmitters (15,17), the increase in cyclic GMP in interferon treated cells is calcium dependent and is abrogated by treatment with the cyclo-oxygenase inhibitors aspirin and indomethacin. Studies are currently underway in an attempt to elucidate the events leading to the activation of guanylate cyclase in interferon treated cells.

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