HORMONAL PROTECTION OF INTERFERON-TREATED CELLS AGAINST DOUBLE-STRANDED RNA-INDUCED CYTOLYSIS

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1. Introduction

Interferon has been shown to affect several different cell functions. Part of its effect results in a decrease of virus multiplication in the cells. Other effects seem unrelated to the regulation of viral growth [1,2]. In some cases, the different effects of interferon on cells even appear to have opposing consequences. An example is the effect of interferon on cellular response to double-stranded (ds)RNA. Pretreatment of the cells by interferon (priming) increases significantly the ability of dsRNA to induce interferon synthesis [3], but on the other hand, interferon pretreated cells are also much more sensitive to the cytotoxic effect of dsRNA [4,5]. Interferon can also increase the cell damage, occurring during infection by some viruses, in spite of its ability to inhibit multiplications of these viruses [6–9]. This enhanced viral cytopathy might be related to an increased sensitivity of interferon-treated cells to the dsRNA produced during viral infection [10]. We have, therefore, searched for factors which could decrease the sensitivity of interferon-treated cells to the cytotoxicity of dsRNA without decreasing the antiviral action of interferon. This work shows that the cytotoxic effect is very sensitive to hormonal control.

2. Methods and materials

2.1. *Labeling of cells with ^{51}Cr and induction of lysis*

Confluent human foreskin fibroblasts of the strain FS11 (high interferon producer) were used. The cells were grown on 16 mm tissue culture dishes in Dulbecco's minimal essential medium with 10% fetal calf serum. Labeling was done by incubating for 24 h with sodium [^{51}Cr]chromate (10 μCi/ml). The label was then removed and the cells were further incubated for 2 days with interferon (500 U/ml) in growth medium which contained 10% fetal calf serum. Lysis was induced by rinsing the cells twice with serum-free medium, then incubating them, in the absence of serum, with a solution of poly(rI):(rC) (100 μg/ml) in growth medium. At various times after the addition of the dsRNA, samples were taken from the medium. Following the last sampling, the cells were detached by incubating in pancreatin solution (Gibco). The amount of ^{51}Cr in the suspended cells and in the samples taken from the growth medium during the incubation with the dsRNA was measured in a Packard γ-counter. The extent of ^{51}Cr released from the cells due to the lysis was expressed as % of the total label originally present in the culture. The total amount of the label was the same in interferon-treated and non-treated cultures. The experiments were run in duplicates; variation between the duplicates was 10–20%.

2.2. *Measurement of viral growth inhibition by interferon*

Confluent human foreskin fibroblasts were pretreated for 24 h with interferon and then infected with vesicular stomatitis virus (2 p.f.u./cell). After 12 h, samples of the growth medium were taken off the cells and frozen. The amount of viruses in these samples was later estimated by plaque-assay on mouse L-929 cells.

2.3. *Materials*

Sodium [^{51}Cr]chromate was obtained from the Radiochemical Center, Amersham; poly(rI):(rC) from P.L. Lab. Hydrocortisone succinate and dexamethasone phosphate from NV Organon Oss. Testosterone and progesterone from Ikapharm, Ramat Gan. Indomethacine from Asia, Israel. Methyl-isobutylxanthine from Alldrich. Cyclic AMP derivatives and 8-bromo AMP from Sigma. PGE_1 was kindly made available to us by Dr G. Pike of the Upjohn Co., Kalamazoo, MI. Interferon was induced in the FS11 fibroblasts by poly(rI):(rC) [24] and purified on carboxymethyl cellulose column to an activity of ~10^7 units/mg protein.

3. Results

Interferon-treated human foreskin fibroblasts start to lyse a few hours after they are exposed to dsRNA. To quantitate the extent of cell lysis, we measured (fig.1) ^{51}Cr release from prelabeled fibroblasts [11,12]. In the absence of dsRNA there is a slow ^{51}Cr release not accompanied by visible lysis. Cell lysis was therefore estimated by the % of ^{51}Cr released in the presence of dsRNA minus the ^{51}Cr released in its absence (net ^{51}Cr released). Only cells pretreated with interferon show a high rate of lysis (fig.1) and continue to lyse

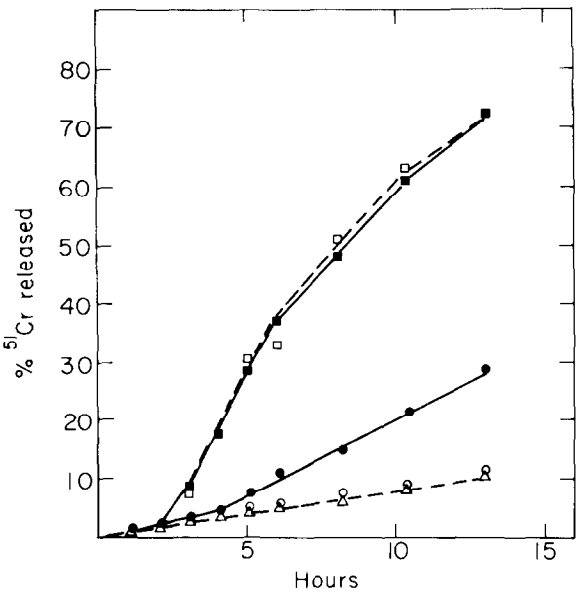

Fig.1. Kinetics of dsRNA-induced cytolysis of interferon-treated cells. Cytolysis was induced as in section 2 except that 2 h after its addition dsRNA was removed, cells were washed and dsRNA was added back to only part of the cultures. (■) Inteferon pretreated and continuous presence of dsRNA; (□) interferon pretreated but dsRNA for only 2 h; (●) not treated with interferon and continuous presence of dsRNA; (○) not treated with interferon and dsRNA for 2 h; (△) not treated with interferon and no dsRNA.

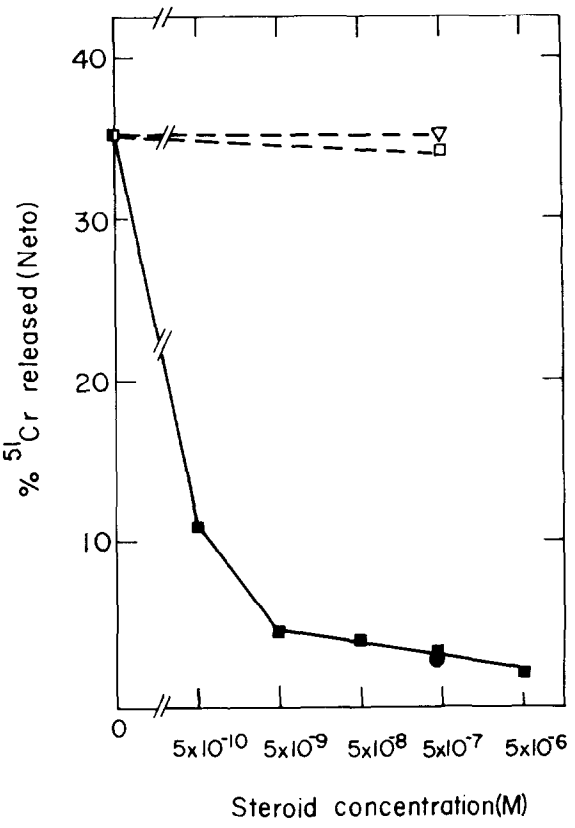

Fig.2. Inhibition by corticosteroids of the dsRNA-induced cytolysis. Lysis was induced as in section 2. The net ^{51}Cr released (see text) during 9.5 h continuous incubation with dsRNA is shown. Release without dsRNA (8%) was subtracted. Steroids were added to the cells 15 h before dsRNA and re-added together with the dsRNA. (■) dexamethasone phosphate; (●) hydrocortisone succinate; (□) testosterone; (▽) progesterone. (The latter two in 0.08% ethanol which had no effect on the lysis.)

even if dsRNA is removed after 2 h. Without interferon pretreatment, cell lysis is low and depends on continuous exposure to dsRNA (fig.1).

Pretreatment of the interferon-treated cells with corticosteroids prevented the cytolysis by dsRNA. Significant protection against the cytolysis was seen with as low as 5×10^{-10} M dexamethasone or hydrocortisone (fig.2). In contrast, testosterone and progesterone, which do not have anti-inflammatory action, did not show any protection (fig.2).

It was shown [13] that dsRNA stimulates prostaglandin E synthesis in human foreskin fibroblasts. Similarly to the cell lysis shown in fig.1, this effect of dsRNA is strongly potentiated by interferon pretreatment (D. W., R. Ben-Dori, unpublished data) and prevented by corticosteroids [13]. Prostaglandin E is, however, not the cause of cell lysis; on the contrary, addition of prostaglandin E inhibited the dsRNA-induced lysis of interferon-treated cells (table 1). Moreover, indomethacine which inhibits the conversion of arachidonic acid to prostaglandin, increased the cell lysis. Prostaglandin E increases the level of cyclic AMP in fibroblasts by stimulating their adenylate cyclase [14,15] and indeed protection against cytolysis was also seen with several other agents that elevate cAMP in the cells. These include (table 1):

Table 1
Prevention of cell lysis by agents that elevate cyclic AMP

Expt	Addition		Net ^{51}Cr[a] released (%)
1.	None		22
	PGE_1	10 μg/ml	3
	Indomethacin	1 μg/ml	41
	Isoproterenol	10^{-5} M	8
	Methyl-isobutylxanthine	100 μg/ml	2
	8-bromo-cyclic AMP	1 mM	1
2.	None		46
	8-bromo-cyclic cAMP	1 mM	3
	8-bromo AMP	1 mM	49
	Dibutyryl cyclic AMP	1 mM	3
	Sodium butyrate	1 mM	50

[a] ^{51}Cr release in the absence of dsRNA of 9% in expt 1 and 8% in expt 2 was subtracted. In the absence of dsRNA there was no stimulation of cytolysis by indomethacin

Cytolysis was induced by incubation of interferon-treated cells for 8.5 h with poly(rI):(rC) as in fig.1. Reagents mentioned above were added 5 min before the addition of dsRNA

(i) Isoproterenol which stimulates the adenylate cyclase through β-adrenergic receptors on these cells [16];
(ii) The cyclic nucleotide phosphodiesterase-inhibitor methylisobutylxanthine;
(iii) The cAMP derivatives 8-bromo-cAMP and dibutyryl cAMP.

We verified that 8-bromo-AMP or butyric acid alone were inactive (table 1).

The inhibition by cAMP of the dsRNA-induced cytolysis in interferon-treated cells was rapid and could be induced even after the beginning of the incubation with dsRNA (table 2). Surprisingly, preincubation of cells with 8-bromo-cAMP for 15 h reduced its inhibitory effect on cytolysis (table 2), suggesting that this protective effect of cAMP is subject to 'desensitization'. In addition to corticosteroids and cAMP, calf serum protected the interferon-treated cells against cytolysis induced by dsRNA. The protection by serum was again rapid (table 2). In contrast, protection by corticosteroids, even at high concentrations, required prolonged pretreatment (table 2). The serum factor(s) that protect interferon-treated cells against dsRNA-induced cytolysis are not, therefore, corticosteroids; it is also unlikely that serum protection is related to cAMP since serum decreases the cAMP level in fibroblasts [17]. This was recently confirmed by purifica-

Table 2
Time course of the protection against the cytolysis

Addition	Time (h) of addition relative to dsRNA	Net ^{51}Cr released
None		31
8-bromo cyclic AMP (1 mM)	−15	13
	− 1	0
	+ 2	3
Serum 10%	0	3
	+ 2	1
Dexamethasone (5×10^{-7} M)	−15	0
	− 1	15
	0	15
	+ 2	20

Cytolysis was induced by incubation of interferon-treated cells for 8 h with poly(rI):(rC) as in fig.1. ^{51}Cr released in the absence of dsRNA (11%) was subtracted

Table 3
Antiviral effect of interferon in the presence of dexamethasone and of cyclic AMP derivatives

Pretreatment	Virus yield (log p.f.u./10^6 cells)	
	No interferon	5 U/ml interferon
–	12.1	3.9
Dexamethasone (5×10^{-7} M)	12.0	4.0
8-bromo cyclic AMP (1 mM)	12.7	3.9
Dibutyryl-cyclic AMP (1 mM)	13.1	4.0

Confluent human foreskin fibroblasts were pretreated for 24 h with interferon, then infected with vesicular stomatitis virus (2 p.f.u./cell). After 12 h, samples of the growth medium were taken off the cells and frozen. The amount of viruses in these samples was later estimated by plaque-assay on mouse L-929 cells. Dexamethasone was added 12 h and cyclic AMP derivatives 2 h before the infection

tion of the protective factor from serum (unpublished data).

Effects of hormones and cAMP on the antiviral effects of interferon were reported [18,19]. To verify that corticosteroids or cAMP do not have a general inhibitory effect on interferon functions in our system, we tested again these agents on the antiviral function of interferon. As shown in table 3, dexamethasone, 8-bromo-cAMP and dibutyrylcyclic AMP, in concentrations in which they fully protected interferon-treated cells from the cytolysis induced by dsRNA, did not decrease the inhibition by interferon of vesicular stomatitis virus multiplication.

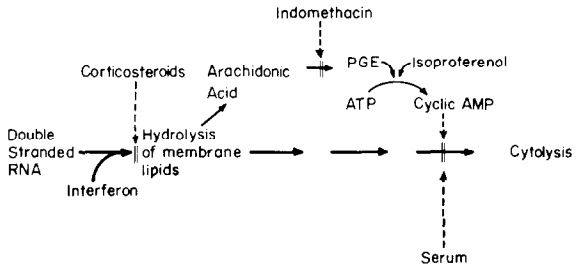

Fig.3. Model for the relation between induction of cytolysis and induction of prostaglandin synthesis by dsRNA. Broken arrows denote inhibitory effects. See text for other details.

4. Discussion

The mechanism by which dsRNA can cause cell lysis in interferon-treated cultures is not known. Several dsRNA-dependent enzymes are induced by interferon [20], and interferon enhances the ability of dsRNA to induce new interferon (priming) [3]. A relation between these phenomena is possible, but is far from clear. The one effect of dsRNA that, at present, may be related to cytolysis, is the induction of prostaglandin formation in fibroblasts. Both are inhibited by corticosteroids [13] and strongly potentiated by pretreatment with interferon (unpublished observations). A possible model for the relation between the induction of prostaglandin and cytolysis is shown in fig.3. It is suggested that an increased lipid hydrolysis in the cellular membrane leads both to cell lysis and, as was shown for several agents [21–23], to release of arachidonic acid and to prostaglandin synthesis. Corticosteroids inhibit prostaglandin synthesis by preventing membrane lipid hydrolysis [21–23], and this could explain their anti-cytolytic activity. Indomethacin, does not prevent lipid hydrolysis but inhibits the conversion of arachidonic acid to prostaglandin [21–23]. Inhibition by indomethacin of prostaglandin formation in the course of cytolysis, would prevent the elevation of cellular cAMP that the prostaglandins produce and, in this way, increase the rate of cytolysis. Prostaglandin synthesis, in this model, would be a feedback inhibitor of cell lysis by dsRNA in interferon-treated cells.

Interferon increases not only the sensitivity of cells to the cytotoxicity of dsRNA but also cell sensitivity

to cytolysis by some viruses [6–8] and also by killer lymphocytes [25]. Cell multiplication [26] and motility (J. Werenne, M. R., D. W., unpublished observations) are inhibited by interferon. The damage caused by these various 'anticellular' effects of interferon seem, in some cases, to overweigh its protective 'antiviral' effects [9]. The fact that corticosteroids, cAMP and serum, can counteract the increased sensitivity of the interferon-treated cells to dsRNA without decreasing the antiviral effect, raises the possibility that many anticellular effects of interferon could be specifically reduced by hormones. Aspirin and indomethacin may, on the other hand, potentiate these anticellular effects. The hormonal control of interferon and of dsRNA actions should, therefore, be carefully examined. Such studies may clarify the mechanisms involved and improve the practical use of the interferon and of its inducers.

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