RAT INTERFERON INHIBITS STEROID-INDUCIBLE GLYCEROL 3-PHOSPHATE DEHYDROGENASE SYNTHESIS IN A RAT GLIAL CELL LINE

Dominique ILLINGER, Giliane COUPIN, Mary RICHARDS and Philippe POINDRON*
Université Louis Pasteur, U.E.R. des Sciences pharmaceutiques, Laboratoire de Virologie, 3 rue de l'Argonne, 67083 Strasbourg Cedex, France

Received 15 December 1975
Revised version received 15 February 1976

1. Introduction

The treatment of animal cells by an interferon (IF) from homologous species modifies the cellular translation apparatus and renders the cells incapable of translating viral mRNA. Several authors interested in studying this problem of discriminatory translation of mRNA in cells pretreated with interferon have utilized acellular protein synthesis. In certain systems the discrimination is total [1], in others it is partial [2,3]. One possible approach to this question would be to utilize hormonal induction of an enzyme in cell lines which have retained differentiated characteristics.

In a previous paper [4], we have shown that pretreatment of Hepatoma Tissue Culture (HTC) cells by rat and mouse IF inhibits the induction of tyrosine aminotransferase (TAT) by dexamethasone and that this inhibition depends on the concentration of IF. In these cells, the induced synthesis of TAT requires the neosynthesis of the corresponding mRNA [5].

We report there the effect of pretreating rat C_6 glial cells [6] with rat IF on the induction of cytoplasmic glycerol 3-phosphate dehydrogenase (GPDH) using hydrocortisone hemisuccinate (HCH) [7] as inducer.

2. Materials and methods

2.1. *Cells*

RGC_6 cells from Dr Ph. Benda, Collège de France, Paris, and C_6 cells (Dr Ciesielsky, Centre de Neurochimie, Strasbourg) were maintained in HAM F10 medium (Difco) containing 10% fetal calf serum (FCS). There was no difference in the inducibility of cells from the two sources. Cells were used between the 40th and 60th passages.

The rat embryo fibroblasts (REF) were prepared by trypsinizing rat embryos in the 17th or 18th day of gestation following the usual techniques. Repeated experiments show that the REF produce IF, remain sensitive to it and are capable of doing so until at least the 6th passage. In all cases the cells were counted with a Nageotte cytometer in the presence of trypan blue.

2.2. *Viruses*

Newcastle disease virus (NDV, local strain), used as inducer and vesicular stomatitis virus (VSV, Indiana serotype), used as challenge virus, were maintained and titrated as previously described [8].

2.3. *Production, assay and characterisation of IF*

IF was produced in 4–5 day old confluent REF (either primary culture or subculture, see above) using NDV as inducer. The culture fluid was harvested, centrifuged at 4000 *g* for 15 min at 4°C, and dialyzed for 5 days at 4°C against isotonic HCl–KCl buffer, pH 2. It was then neutralized by dialysis against phosphate buffered saline (PBS) and filtered through a 200 nm membrane (Sartorius). It was concentrated by pressure dialysis.

An inactive control preparation ('mock IF') was prepared under the same conditions using the culture fluid of REF not exposed to NDV.

* From whom reprints should be requested.

IF was titrated either by a plaque reduction test in REF culture using VSV as challenge virus, or by determining the VSV yield reduction in a single growth cycle in RGC_6 or C_6 cells [9]. One 'REF' unit of IF is defined as the quantity of IF which reduces by 50% the number of plaques formed by VSV in REF. One 'RGC_6' unit of IF is the quantity of IF which reduces by 50% the virus yield in RGC_6 cells, or C_6 cells. Preliminary experiments have shown that one 'REF' unit was equivalent to four 'RGC_6' units. Throughout this paper 'RGC_6' units, referred as to 'units', will be used, in order to correlate antiviral effects and other effects of IF on RGC_6 cells, or C_6 cells.

In our preparations, IF was characterized (table 1) by its stability at pH 2 for five days, its relative thermostability for 1 h at 50°C, its specificity of action in rat cells, its small heterologous activity in mouse L cells, its inactivity in human KB cells, and its sensitivity to trypsin. IF was neither dialyzable nor sedimentable at 100 000 *g* for 2 h. Even after 48 h of contact with high concentrations of IF, neither homologous nor heterologous cells exhibited any toxic or cytopathic effects.

2.4. *Enzyme and protein assay*

The cell monolayers were gently rinsed with PBS and scrapped off the flask wall with a rubber policeman. The cells were suspended in PBS and centrifuged at 1500 *g* for 15 min at 4°C. They were then resuspended in a known volume of sodium phosphate buffer (0.01 M, pH 7.5) containing 5 mM β-mercaptoethanol (βME) and 1 mM EDTA, and disrupted by sonication (Ultrason, Annemasse, France). The cell debris was removed by centrifugation at 30 000 *g* for 1 h at 4°C. The supernatant was assayed for GPDH activity according to McGinnis and De Vellis [10] in a reaction volume of 0.5 ml, and for protein content according to Lowry et al. [11]. The results are expressed in specific activity, i.e. change in optical density per minute per milligram protein, (ΔE/min/mg), or in the per cent change relative to the specific activity of the enzyme in noninduced cells [12].

Table 1
Characterization of IF

	Titer (in U)
Control preparation	256
Trypsin treated preparation	< 8
Heat treated preparation	
(1 h 40°C)	256
(1 h 50°C)	256
(1 h 56°C)	< 8
Heterologous activity on L cells	32
Heterologous activity on KB cells	< 4

3. Results

3.1. *Effect of IF on RGC_6 cell growth (fig. 1)*

It is well known that IF exerts an inhibitory effect on cellular growth. Since antiviral effects and anticellular effects are probably linked [13,14], we chose to induce GPDH, in conditions where the anticellular effect of IF had a maximum expression.

At a concentration of 150 U/ml, IF exerted an inhibitory effect on RGC_6 cell growth. A minimum of 18 h of contact was required before this effect became detectable. After 54 h, there was a 26% inhibition of growth in IF-treated cells. Finally, whether IF-treated or not, cells began to detach from the flask wall 140 h after their seeding.

In order for the growth inhibition to be clearly visible there must be a minimum of 30 h contact with IF. This long delay indicated that the anticellular

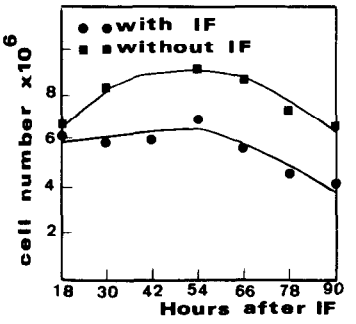

Fig.1. Growth curve of RGC_6 cells in the absence or presence of rat fibroblast IF (150 U/ml). Cell counting: cell monolayers were washed with PBS and resuspended in the sonicating buffer; an appropriate dilution of cell suspension was made immediately in PBS and counted as described in Materials and methods. The rapid passage of cells into a hypotonic buffer results in swelling that allows a better counting.

effect was not due to an acute toxicity of the preparations. This was supported by the fact that IF treated cells detached from the flask wall exactly at the same time as the nontreated cells.

We therefore induced GPDH after 18 h of contact with RGC_6 cells, without eliminating IF from the medium.

3.2. *Effect of pretreatment of RGC_6 cells with IF on the hormonal induction of GPDH (fig.2, table 2)*

Under our experimental conditions, the baseline activity of GPDH in glial cells ranged from 0.050 to 0.520 ΔE/min/mg (observed in the course of 8 experiments). The enzyme induction is visible 24 to 30 h after addition of steroid hormone. The induced activity is 5 to 8 times the baseline activity and is maintained at this level for 48 h. After this delay there is a new induction and the latterly induced activity can attain a level 20 times the baseline activity.

The degree of induction found differs notably

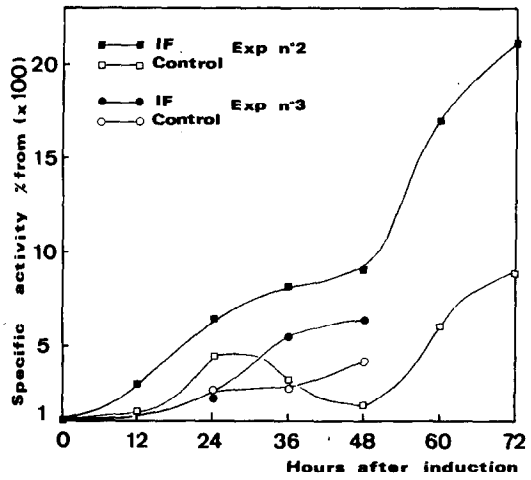

Fig.2. Kinetics of the GPDH induction in RGC_6 cells pretreated for 18 h with IF (150 U/ml), and not pretreated.

Table 2
Influence of IF pretreatment on the hormonal induction of GPDH in RGC_6 cells

	% ΔE/min/mg protein from base after addition of HCH[a] (time in hours)						
	0	12	24	36	48	60	72
Experiment no. 1							
Control	100 (0.526)[b]	–	314	–	568	–	–
Mock IF*	100 (0.214)	–	310	–	595	–	–
IF (75 U/ml)*	100 (0.474)	–	153	–	226	–	–
Experiment no. 2							
Control	100 (0.061)	300	649	818	922	1721	2137
IF (150 U/ml)*	100 (0.062)	119	445	330	185	603	897
Experiment no. 3							
Control	100 (0.204)	–	256	557	647	–	–
IF (150 U/ml)**	100 (0.162)	–	263	287	409	–	–

[a] Enzyme induction: 10^6 RGC_6 cells in 20 ml of HAM F 10 10% FCS were seeded in Falcon plastic flask (75 cm^2). 80 h latter, the medium was eliminated and replaced either with HAM 10 8% FCS in control flasks or with appropriate dilutions of IF or mock IF in HAM F 10 in the other flasks. In these latter cases, the concentration of FCS was 8% in some experiments (*), and in some others (since GPDH induction does not depend on FCS concentration [7]) FCS concentration was adjusted so as each flask contained exactly the same protein concentration in the culture medium, owing to the presence of IF preparation (**); pH was carefully adjusted to 7.3 and the cells were incubated for an additional period of 18 h prior to addition of HCH (final concentration 1 μg/ml). The time at which HCH is added is referred to as zero time. Experience has shown that if one took care to perform at least three assays per one cell extract, there was no need to use more than one flask per point. (Each value given in the table is the mean of three determinations).

[b] Specific activity of GPDH in ΔE/min/mg, taken as 100%.

from that of Davidson and Benda [12] and that of De Vellis et al. [7] but more closely resembles that of McGinnis and De Vellis [15]. On one hand this may be explained by the very low baseline levels we frequently found by comparison with those reported by other authors, perhaps because we used cells in later passages, and, on the other hand by the fact that we are using a modified GPDH assay [10] which includes βME and EDTA in the reaction mixture. βME is required for the correct functioning of rat muscle GPDH [16] perhaps because it maintains the integrity of the cysteine residue of the enzyme, and stabilizes it.

When the cells were pretreated for 18 h either with 'mock interferon' (table 2) or with low concentrations of interferon (2 U/ml) (data not shown), there was no inhibition of the GPDH induction.

When the cells were pretreated with stronger concentrations of IF (150 U/ml, 75 U/ml) (table 2, fig.2) we observed the beginning of an induction, but the induced level was not attained. In somes cases, after attaining a maximum, the synthesis decreased and returned almost to the baseline activity. On the other hand, the secondary induction was less strongly inhibited than the first the molecular basis of this secondary induction is not known [7]. Finally, increasing the amount of IF (300 U/ml) during the pretreatment did not change the response from that observed with 150 U/ml of IF (data not shown).

4. Discussion and conclusions

These results show that the effects of IF preparations on the induction of GPDH are analogous to those exerted on the induction of TAT [4]. Our observations need to be confirmed with purified rat IF because our preparations had a rather low specific activity (100 to 200 U/mg). Nevertheless it is very likely that the effects are actually due to IF because: (1) mock interferon even at high concentration did not exhibit any inhibitory effect on cell growth or on the GPDH induction, (2) the inhibitory effect on the enzyme induction depends on the IF concentration used during the pretreatment.

It is very likely that the inhibitory effect of IF on enzyme induction is due to a specific effect on GPDH synthesis since the relative proportion of GPDH in IF-pretreated induced cells is much smaller than in the nontreated induced cells. If the effect was due to an inhibition of total protein synthesis, such a relative difference would not be seen.

The inhibition of induction is also independent of protein concentration in the medium whether of serum origin or IF preparations origin.

These results allow us, if not to generalize, at least to extend the conclusions previously stated on the inhibition of translation of cellular in RNA [4], since the hormonal induction of GPDH is inhibited in C_6 and RGC_6 cells pretreated with actinomycin D and acetoxycycloheximide. The induced synthesis is not potentiated by norepinephrine, but depends on the neosynthesis of mRNA [15].

An interesting but irregularly seen effect of the IF pretreatment of C_6 or RGC_6 cells on the hormonal induction of GPDH is the return towards the baseline rate of synthesis after the start of induction. Such an effect was observed during the induction of TAT in IF-pretreated HTC cells. In this case the post-transcriptional control of enzyme synthesis exerted by the continuous presence of the hormone is no longer exerted in the presence of IF.

These problems are presently under study in our laboratory.

Acknowledgments

We thank Drs Benda and Cieselsky who kindly supplied RGC_6 and C_6 cells, and Dr J. P. Beck for the useful discussion and suggestions he has made.

This work was realized with the aid of the Centre National de la Recherche Scientifique (ATP N° 1873) and the Fondation pour la Recherche Médicale Française.

References

[1] Samuel, C. E. and Joklik, W. K. (1974) Virology 58, 476–491.
[2] Falcoff, E., Falcoff, R., Lebleu, B. and Revel, M. (1973) J. Virol. 12, 421–430.
[3] Gupta, S. L., Graziadei, W. D. III, Weideli, H., Sopori, M. L. and Lengyel, P. (1974) Virology 57, 49–63.
[4] Beck, G., Poindron, P., Illinger, D., Beck, J. P., Ebel, J. P. and Falcoff, R. (1974) FEBS Lett. 48, 297–300.

[5] Beck, G., Beck, J. P. and Tomkins (1973) Colloque INSERM, Synthèse normale et pathologique des protéines chez les animaux supérieurs, pp. 29–44, INSERM, Paris.
[6] Benda, P., Lightbody, J., Sato, G., Levine, L. and Sweet, W. (1968) Science 161, 370–371.
[7] De Vellis, J., Inglish, D., Cole, R. and Molson, J. (1970) Influence of hormones on the nervous system, Proceedings of the International Society of Psychoneuroendocrinology (Ford, D. H., ed.) pp. 25–39. Karger, Basel.
[8] German, A., Quero, A. M. and Poindron, P. (1971) Ann. Inst. Pasteur 121, 207–221.
[9] Billau, A. and Buckler, C. E. (1970) Symposia series in immunobiological standardization no. 14, International Symposium on Interferon and Interferon inducers, London 1969, pp. 37–44, Karger, Basel and New York.
[10] Mc Ginnis, J. F. and De Vellis, J. (1974) Biochim. Biophys. Acta 364, 17–27.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Davidson, R. L. and Benda, P. (1970) Proc. Natl. Acad. Sci. US 67, 1870–1877.
[13] Gresser, I., Brouty-Boye, D., Thomas, M. T. and Macieira Coelho, A. (1970) Proc. Natl. Acad. Sci. US 66, 1052–1058.
[14] Lindahl Magnusson, P., Leary, P. and Gresser, I. (1971) Proc. Soc. Exp. Biol. Med. 138, 1044–1050.
[15] Mc Ginnis, J. F. and De Vellis, J. (1974) Nature 250, 422–424.
[16] Fondy, T. P., Lesin, L., Sollohue, S. J. and Ross, G. R. (1968) J. Biol. Chem. 243, 3148–3160.