The involvement of calmodulin in interferon induction

Dalia Gurari-Rotman

Department of Virology, Weizmann Institute of Science, Rehovot 76100, Israel

Received 3 September 1982

Calmodulin *Interferon induction* *Trifluoperazine* *Superinduction* *Interferon synthesis, inhibition of* *Priming*

1. INTRODUCTION

Calmodulin has emerged as a protein playing a central role in the regulation of a wide range of cellular activities [1]. Calmodulin acts as an intercellular calcium-binding protein, binding Ca^{2+} when its concentration in the cell increases in response to a stimulus. The binding causes a shape change in the calmodulin molecule which may trigger the activation of several enzymes. This in turn will result in the biochemical reactions which are the response to the original stimulus. Several cellular processes [2,3] and the activation of several enzymes [4–6] have been established as calmodulin-dependent.

Calmodulin-dependent processes can be selectively inhibited by calcium chelators [7], by endogenous proteins found mainly in the brain [8], or by various psychotropic drugs [9]. Of the latter, trifluoperazine (a phenothiazine derivative) has been widely studied [10]. The drug has been shown to bind reversibly to calmodulin in the presence of Ca^{2+} and a clear relationship could be demonstrated between the binding and the ability of the drug to inhibit calmodulin-dependent activation of certain of the enzymes mentioned above.

In view of the central role of calmodulin in many cell processes, it seemed of interest to study the effect of this protein in the interferon induction system. This communication presents data on the role of calmodulin in interferon induction, using trifluoperazine as a specific inhibitor of calmodulin-dependent processes.

Direct correspondence to sabbatical address: zum Letzten Batzen, 4102 Binningen, Schlossgasse 11, Switzerland

2. MATERIALS AND METHODS

Human foreskin fibroblast cells FS-11, established in this laboratory, were cultured in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS: Gibco, New York), penicillin (100 units/ml), and streptomycin (100 μg/ml). For interferon production, cells were grown to confluency in 60 mm tissue culture plates (Nunc, Roskilde).

Superinduction was carried out essentially as in [11]; induction with poly(I):poly(C) (PL Biochemicals, Wisconsin) alone was carried out by incubation of cells with poly(I) : poly(C) (100 μg/ml) for 1.5 h followed by several washes with phosphate-buffered saline. In both procedures, priming [12] was carried out when indicated for 18 h using interferon (100 units/ml) produced in FS-11 cells and purified to a spec. act. $\sim 10^7$ units/mg. 'Production medium' into which cells secrete interferon after induction consisted of minimal essential medium supplemented with 0.1% human serum albumin. All experimental determinations were carried out in triplicate.

Assay for interferon activity was carried out using a cytopathic effect method [13] on FS-11 cells, with vesicular stomatitis virus as challenge virus. Interferon titres are expressed in International Reference Units, relative to the International Fibroblast Interferon Standard Go23-901-527, kindly supplied by the Infectious Diseases Branch, NIAID (Bethesda MD). Trifluoperazine was a gift

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

from Dr H. Munitz (Geha Hospital). It was included in the induction schedules at concentrations and for time periods indicated in section 3. Assays of calmodulin-dependent phosphodiesterase activity were done by a modification of the method in [14].

3. RESULTS AND DISCUSSION

When FS-11 cells were induced by poly(I): poly(C) (simple induction) after priming, no effect of trifluoperazine (20 μM) on interferon induction was detected (table 1).

The effect of varying concentrations of trifluoperazine on superinduction of human foreskin fibroblasts is shown in fig.1. The inhibitor was in contact with the cells for a total of 3.5 h, in the presence of poly(I) : poly(C) and cycloheximide (3 h), and subsequently with actinomycin D in addition (0.5 h). During this time period, trifluoperazine did not appear to have any toxic effect on the cells. This was judged by a lack of change both in morphology and in sensitivity to interferon in the cytopathic effect assay. Trifluoperazine showed inhibitory activity also when present only during the incubation of the cells with poly(I) : poly(C) and cycloheximide, but was removed (by washing of cells) prior to addition of actinomycin D. However, it was not active if added together with actinomycin D after poly(I) : poly(C) treatment had been initiated in its absence, or after removal of the actinomycin D at the end of the induction period (see table 2).

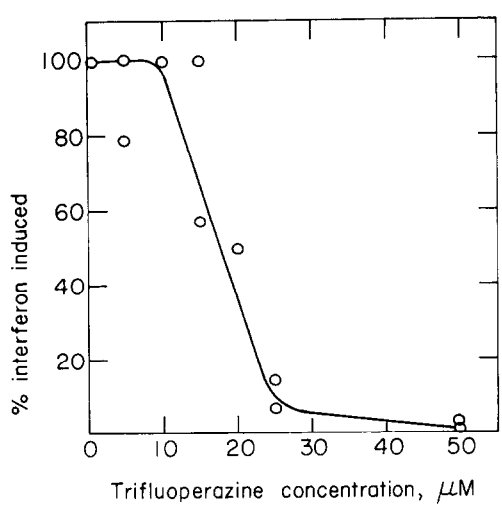

Table 1

Effect of calmodulin inhibition on induction and superinduction of interferon in human fibroblast cells

Treatment of cells[a]			Interferon titre (% activity)[b]
Priming	Induction	Production	
√	Poly(I):poly(C) alone	√	100
√	Poly(I):poly(C) alone[c]	√	125
√	Poly(I):poly(C) alone	√[c]	100
√	Superinduction	√	100
√[c]	Superinduction	√	45
√	Superinduction[c]	√	50
√	Superinduction	√[c]	55
√	Superinduction	√	100
–[c]	Superinduction	√	5
–	Superinduction[c]	√	5

[a] For induction protocol see section 2

[b] Interferon titres are given as % of control values, induced or superinduced as in section 2; these control values were in the range of 1000 U/ml for induction and 8000 U/ml for superinduction

[c] Trifluoperazine addition at 20 μM

After treatment of the cells with trifluoperazine followed by superinduction, their calmodulin content was estimated by measurement of calmodulin-dependent phosphodiesterase activity, as in section 2. A good correlation was found between the interferon titre obtained on superinduction and the residual calmodulin activity of the cells after trifluoperazine treatment.

Superinduction of FS-11 cells in the absence of priming in these experiments gave rise to titres 5–10-fold lower than those obtained with priming. However, trifluoperazine inhibited interferon production also under these conditions, when included during the incubation period.

The possible involvement of calmodulin in the priming and production stages of interferon synthesis was also tested by including trifluoperazine at these stages of the induction scheme. In cells primed and treated concomitantly with trifluoperazine, and then induced with poly(I) : poly(C) alone, the trifluoperazine treatment reduced interferon induction to 25% of control values, whereas, inclusion of the inhibitor only during the production period did not affect the titres observed. In the case of superinduction, carried out after priming in

Table 2

Effect of trifluoperazine on various stages of interferon superinduction in human fibroblast cells

Treatment of cells[a]		[Trifluoperazine]	Interferon titre
Superinduction	Production	(μM)	(% activity)[b]
(poly(I):poly(C) + CHX) + (act D)	√	–	100
(poly(I):poly(C) + CHX)[c] + (act D)[c]	√	20	50
(poly(I):poly(C) + CHX)[c] + (act D)	√	20	50
(poly(I):poly(C) + CHX) + (act D)[c]	√	20	100
(poly(I):poly(C) + CHX) + (act D)	√[d]	20	100
(poly(I):poly(C) + CHX)[c] + (act D)[c]	√	40	16
(poly(I):poly(C) + CHX)[c] + (act D)	√	40	6
(poly(I):poly(C) + CHX) + (act D)[c]	√	40	> 100

[a] For induction protocol, see section 2; all cells were primed in the absence of trifluoperazine prior to induction; CHX, cycloheximide; act D, actinomycin D

[b] Interferon titres are given as % of control cultures, superinduced as in section 2; these control values were in the range of 8000 U/ml

[c] Trifluoperazine addition

[d] Trifluoperazine addition; during first hour only

the presence of trifluoperazine, a concentration-dependent inhibition of interferon synthesis was again observed. Since the priming period was ~ 18 h, higher concentrations (50 μM range) of trifluoperazine could not be utilized in priming since they were found to be toxic to the cells over these time periods. This applies also to concentrations studied during the period of interferon production. This toxicity was observed microscopically. However, at ≤ 25 μM trifluoperazine, where no toxic effect was apparent, inhibitory effects both in priming and in production were observed, which paralleled those found during the superinduction period itself.

From the results presented, it is apparent that calmodulin is involved in various stages of the interferon synthetic process in cultured fibroblast cells. A clear-cut distinction between 'induction' and 'superinduction' is observed (see table 1) and within the superinduction process, inhibition of interferon synthesis by the calmodulin inhibitor exerts its action at the early stages of the action of poly(I) : poly(C). It will be of interest to study the possible involvement of synthesis of IFN mRNA or of its regulation. In [15], treatment of the human fibroblast cell line MG63 with Ca^{2+} led to enhanced production of interferon in these cells on induction with poly(I) : poly(C). This effect was not additive to the increased titres obtained on superinduction of these cells. The mechanisms by which Ca^{2+} acts were not elucidated but were shown not to result from inhibition of RNA synthesis. Other observations on stimulation of interferon induction by Ca^{2+} have also been made (Kobayashi, unpublished). In this system the strongest stimulation was obtained if Ca^{2+} were added 1 h after addition of poly(I) : poly(C) and the possibility of stabilization of the inducer must be considered.

The calmodulin-inhibitor effect on interferon synthesis is observed both in cells which have undergone priming prior to superinduction or in unprimed cells, but the effect is more marked in unprimed cells (although the interferon titres obtained are quantitatively lower). The parallel comparison between primed and unprimed cells induced by poly(I) : poly(C) alone could not be made since interferon production in FS-11 cells in the absence of priming was too low (20–30 units/ml) to enable significant conclusions to be drawn.

ACKNOWLEDGEMENTS

I would like to thank Professor Michel Revel and Dr Avner Rotman for stimulating discussions and encouragement.

REFERENCES

[1] Cheung, W.Y. (1980) Science 207, 19–27.
[2] Marcum, M.J., Dedman, J.R., Brinkley, B.R. and Means, A.R. (1978) Proc. Natl. Acad. Sci. USA 75, 3771–3775.
[3] Shulman, H. and Greengard, P. (1978) Proc. Natl. Acad. Sci. USA 75, 5432–5436.
[4] Brostrom, C.O., Huang, Y.C., Breckenridge, B.M. and Wolff, D.T. (1975) Proc. Natl. Acad. Sci. USA 72, 64–68.
[5] Yagi, K., Yazawa, M., Kakiuchi, S., Oshima, M. and Venishi, K. (1978) J. Biol. Chem. 253, 1338–1340.
[6] Jarret, H.W. and Penniston, J.T. (1977) Biochem. Biophys. Res. Commun. 77, 1210–1216.
[7] Wolff, D.J. and Brostrom, C.O. (1974) Arch. Biochem. Biophys. 163, 349–358.
[8] Larsen, F.L., Raess, B.V., Hinds, T.R. and Vincenzi, F.F. (1978) J. Supramol. Struct. 9, 269–274.
[9] Weiss, B., Fertel, R., Figlin, R. and Uzunov, P. (1974) Mol. Pharmacol. 10, 615–625.
[10] Levin, R.M. and Weiss, B. (1976) Mol. Pharmacol. 12, 581–589.
[11] Havell, E. and Vilcek, J. (1972) Antimicrob. Agents Chemother. 2, 476–484.
[12] Stewart, W.W. II, Gosser, L.B. and Lockart, R.Z. jr (1972) J. Gen. Virol. 15, 85–87.
[13] Bucknall, R.A. (1970) Symp. Ser. Immunobiol. Stand. 14, 97–100.
[14] Thompson, W.J., Terasaki, W.L., Epstein, P.M. and Strada, S.J. (1979) Adv. Cyclic Nucl. Res. 10, 69–92.
[15] Meager, A., Graves, H.F. and Bradshaw, T.K. (1978) FEBS Lett. 87, 303–307.