Antiviral activity of phorbol myristate acetate and possible relationships with interferon action

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Signal-induced turnover of membrane phospholipids represents a fundamental transducing mechanism that induces a signal cascade resulting in mobilization of calcium, activation of protein kinase C by diacylglycerol, release of arachidonic acid and stimulation of cyclic GMP production. In this pathway tumor-promoting phorbol esters such as phorbol myristate acetate (PMA) may substitute for diacylglycerol. The interferon-like antiviral effect of PMA described here suggests that the inositol phospholipid-diacylglycerol-protein kinase C signal-transducing mechanism may be involved in interferon action.

Antiviral activity *Phorbol ester* *Second messenger* *Interferon action* *Phosphatidylinositol turnover*

1. INTRODUCTION

Interferons exert a wide range of effects on cells including establishment of an antiviral state, inhibition of cell division, modulation of specialized cellular functions and modification of the cell surface [1]. In common with other biologically active substances the initial step in interferon (IFN) action is its binding to cell surface receptors [2]. It has been reported that following binding IFN appears to be internalized; experimental data suggest, however, that internalization and degradation of IFNs are not responsible for their antiviral action [3]. This observation raises the possibility that IFN effects may be mediated by activation of intracellular messengers.

Most tissues seem to possess only two major classes of receptors to control cellular functions. One class triggers the production of cyclic AMP, while the other initiates a cascade reaction resulting in the mobilization of Ca, activation of protein kinase C, release of arachidonic acid (for the synthesis of prostaglandins, thromboxanes and leukotrienes) and stimulation of guanilate cyclase to form cyclic GMP [4,5].

Both of these receptor-controlled systems are obvious candidates for the transmission of an IFN signal. Although cAMP is known to influence a number of processes affected by IFN [2], this system does not appear to be responsible for all IFN effects.

It was reported recently that protein kinase C plays a very important role in signal transduction for a variety of biologically active substances [4]. When cells are stimulated protein kinase C is transiently activated by diacylglycerol produced in the cell membrane during the signal-induced turnover of inositol phospholipids. In this respect tumor-promoting phorbol esters are able to substitute diacylglycerol and there is growing evidence that the cellular receptor for the phorbol ester is protein kinase C.

We hypothesized that if IFN utilizes the inositol phospholipid-diacylglycerol-protein kinase C pathway then PMA should have an effect on cells similar to that of IFN. For this reason experiments were performed to determine whether PMA induces an interferon-like antiviral state of cells.

2. MATERIALS AND METHODS

2 mM PMA (4-phorbol 12-myristate 13-acetate,

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Sigma) in 0.2 ml portions was stored at −20°C in dimethyl sulfoxide and diluted with medium before use.

Hu-IFNα was obtained from Klára Ódony (Egyt Pharmacochemical Works, Budapest).

UAC cell line was obtained from Dr V. Sorrentino (University of Rome, Italy). This is a human amniotic cell line widely used for IFN assay and known as a high producer of IFNβ [6].

Vesicular stomatitis virus (VSV), Indiana strain, was used for challenge at a dose of 50 TCD_{50}/100 μl.

UAC cells were grown in Microtest TC plates (Falcon). Samples containing 1.5×10^4 cells/well in Parker's 199 medium with 2% fetal calf serum were incubated at 37°C in a 5% CO_2 atmosphere before IFN and/or PMA treatment.

Antiviral activity was tested by the cytopathic inhibition method [7].

3. RESULTS AND DISCUSSION

Table 1 shows the anticytopathic effect of both IFN and PMA. 10^4 units of Hu-IFNα totally inhibited the cytopathic effect of VSV, and the same result was obtained with 20 nM PMA, a dose which is optimal for tumor promotion. It is worth mentioning that 1 nM PMA also proved to have some anticytopathic effect and that the presence of PMA during VSV infection was not necessary for the antiviral effect; a 3 h pretreatment of cells with PMA 1 day before virus infection resulted in a similar anticytopathic effect.

Table 1

Anticytopathic effect of different doses of Hu-IFNα and PMA

Treatment of UAC cells before virus challenge by	Induced resistance to VSV infection[a]
None	−
1 nM PMA	+
5 nM PMA	+ +
20 nM PMA	+ + +
200 IU IFN	+ +
10000 IU IFN	+ + +
1 nM PMA + 200 IU IFN	+ + +
5 nM PMA + 200 IU IFN	+ + +

[a] (+ + +) Total inhibition, (+ +) 50% inhibition, (+) 10–25% inhibition, (−) no inhibition, of the cytopathic effect

Table 1 also shows that after giving suboptimal doses of IFN and PMA simultaneously, a synergistic effect could be observed.

As PMA treatment proved to have a per se effect on cell morphology (some cells became spherical), in further experiments anticytopathic effect of PMA was expressed in such a way that PMA-treated but not virus-infected cells were used as controls. The data are shown in fig.1. It can be seen that 20 nM PMA proved to be an optimal dose; higher doses are less effective presumably as a consequence of their toxic effect.

The anticytopathic effect of PMA observed proves its potent in vitro antiviral activity.

Referring to the mechanism of the antiviral action of phorbol esters, at least 4 different possibilities arise: (i) PMA has some kind of direct antimetabolic effect on virus synthesis, (ii) phorbol esters act as IFN inducers, (iii) the effect is due to changed virus adsorption, (iv) it acts through the activation of protein kinase C.

(i) Although phorbol esters are not metabolic inhibitors, in a cloned macrophage-like cell line PMA-induced resistance to VSV has been described [8]. Here it was concluded that part of this

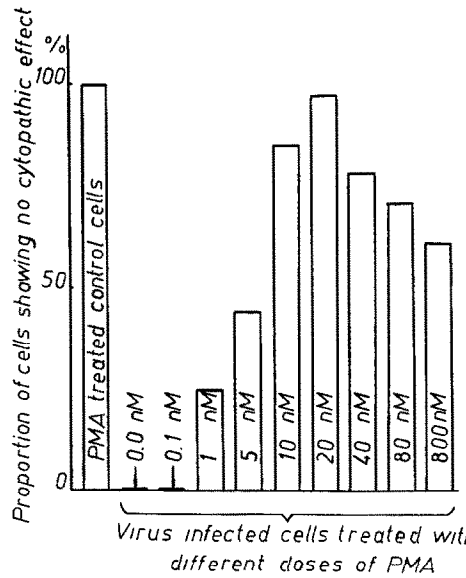

Fig.1. Anticytopathic effect of different doses of PMA. PMA-treated cells were used as controls in the cytopathic inhibition assay described in section 2.

resistance was due to the H_2O_2 induced. In our case this mechanism is unlikely, since UAC cells did not produce H_2O_2 as a result of PMA treatment. (ii) IFN induction can be also rejected as a possibility, as a very short PMA treatment (15 min) before viral infection proved to be satisfactory in producing an antiviral effect; IFN induction and production are known to require a much longer period. It is worth mentioning that tumor-promoting phorbol esters have been reported to act as enhancers or inhibitors of IFN induction, but no IFN-inducing capacity has been observed [9,10]. (iii) The fact that PMA added to the cells 30 min after virus infection also proved to be potent in the cytopathic inhibition assay excludes the putative role of altered virus infection in the phenomenon observed. (iv) The PMA-induced antiviral state of cells strongly supports our hypothesis, according to which IFN also acts through the inositol phospholipid-diacylglycerol-protein kinase C pathway.

The antiviral effect of PMA described is not the only one suggesting its interferon-like properties. PMA and other biologically active phorbol esters have a variety of effects – including stimulation of synthesis of macromolecules, modulation of the metabolism of polyamines and cyclic nucleotides, stimulation of prostaglandin synthesis – similar to those of IFNs [11]. There are also reports of immunomodulatory effects of PMA [12,13]. There is, however, one important difference between the effects of the two substances: phorbol esters stimulate cell proliferation whereas IFNs generally do not. This discrepancy appears to be due to the ability of IFNs to induce the cAMP signal-transducing system which acts negatively and may block other positive signals [4]. Our experimental results are thus consistent with reported data and support the idea that the inositol phospholipid-diacylglycerol signal-transducing pathway is also involved in IFN action.

REFERENCES

[1] Taylor-Papadimitriou, J. (1980) in: Interferon 2 (Gresser, I. ed.) pp.13–46, Academic Press, London.
[2] Tovey, M.G. (1982) in: Interferon 4 (Gresser, I. ed.) pp.23–46, Academic Press, London.
[3] Branca, A.A., Faltynek, C.R., D'Alessandro, S.B. and Baglioni, C. (1982) J. Biol. Chem. 257, 13291–13296.
[4] Nishizuka, Y. (1984) Nature 308, 693–698.
[5] Berridge, M.J. (1984) Biochem. J. 220, 345–360.
[6] Sorrentino, V., Di Francesco, P., Soria, M. and Rossi, G.B. (1982) J. Gen. Virol. 63, 509–511.
[7] Finter, N.B. (1973) in: Interferons and Interferon Inducers (Finter, N.B. ed.) pp.135–169, Elsevier, Amsterdam, New York.
[8] Rager-Zisman, B., Kunkel, M., Tanaka, Y. and Bloom, B.R. (1982) Infect. Immun. 36, 1229–1237.
[9] Yip, Y.K., Pang, R.H.L., Oppenheim, J.D., Nachbar, M.S., Henriksen, D., Zerebeckyj-Eckhardt, I. and Vilcek, J. (1981) Infect. Immun. 34, 131–139.
[10] Frankfort, H.M. and Vilcek, J. (1982) Arch. Virol. 73, 295–309.
[11] Shoyab, M. and Todaro, G.J. (1980) Nature 288, 451–455.
[12] Johnson, H.M. and Torres, B.A. (1982) Infect. Immun. 36, 911–917.
[13] Fuller-Farrar, J., Hilfiker, H.L., Farrar, W.L. and Farrar, J.J. (1981) Cell. Immunol. 58, 156–164.