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Inositol phospholipid turnover and protein kinase C translocation are stimulated by poly(I) · poly(C) in human amnion cells (UAC)

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Polyinosinic-polycytidylic acid, a potent inducer of interferon (IFN) production and activator of some IFN-induced enzymes, inhibits [^{3}H]uridine incorporation into the RNA of vesicular stomatitis virus even in the absence of IFN synthesis, transiently triggers the breakdown of inositol phospholipids and activates the translocation of protein kinase C. Since IFNs also have similar activities these results suggest that IFN induction and IFN function are realised through common biochemical pathways.

Poly (I) · poly(C); Antiviral activity; Phosphatidylinositol turnover; Protein kinase C; Interferon induction; Interferon function

1. INTRODUCTION

A variety of growth factors including interleukin-1, interleukin-2, colony-stimulating factor, epidermal growth factor, fibroblast growth factor and platelet derived growth factor (PDGF) stimulate expression of interferon (IFN) genes [1]. PDGF activates membrane phosphatidylinositol hydrolysis through a receptor-mediated interaction [2,3]. In addition, IFNs also stimulate the turnover of membrane phosphatidylinositol and transiently activate diacylglycerol and inositol trisphophate release [4,5]. As it was reported that polyinosinic:polycytidylic acid (poly(I) · poly(C)) and PDGF activate the same genes in 3T3 cells [6], it is thus reasonable to suppose that the effects of poly(I) · poly(C) and PDGF are realised by common mechanisms.

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On the basis of these data we postulate that (i) poly(I) · poly(C) per se has IFN-like effects, (ii) activates inositol phospholipid breakdown and (iii) induces protein kinase C (PK-C) activity.

2. MATERIALS AND METHODS

UAC monolayer cell cultures grown in Microtest TC plates (Falcon) were infected with 50–100 TCD_{50}/50 μl vesicular stomatitis virus (VSV), Indiana strain, and actinomycin D in a final concentration of 5 μg/ml was added. To allow virus absorption poly(I) · poly(C) in different concentrations together with 40 kBq [^{3}H]uridine in 50 μl medium was given to each well after 1 h incubation. After a 4 h incubation period the radioactivity incorporated was measured as described in detail elsewhere [7].

Suspension cultures of UAC cells (10^6 cells/ml) were incubated with 40 kBq/ml *myo*-[2-^{3}H]-inositol (Amersham). After 16 h the cells were washed three times in Dulbecco's phosphate buf-

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fered saline (PBS). Samples containing 5×10^6 cells in 1.0 ml PBS were treated with 50 μl/ml poly(I)·poly(C) for the indicated intervals. This treatment was terminated by adding 4.0 ml of chloroform/methanol/HCl (2:1:0.0075) containing 50 μg/ml 2,6-di-*t*-butyl-4-methylphenol (BHT). The chloroform phase was evaporated under N_2 and taken up in 100 μl chloroform. Aliquots with authentic phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-diphosphate (PIP_2) (Sigma) were spotted onto precoated HP TCL plates (Merck) and developed using chloroform/methanol/3.3 M NH_4OH (43:38:12) as solvent. Visualisation took place under UV light after spraying the plates with 0.05% 8-anilino-1-naphthalenesulphonic acid in methanol. The spots were then transferred to vials and counted in a toluene-based cocktail.

Water-soluble metabolites were separated by anion-exchange chromatography according to Berridge et al. [8].

Phospholipid-dependent PK-C activity was measured in the cytosol and detergent-solubilized membrane fraction according to Kikkawa et al. [9].

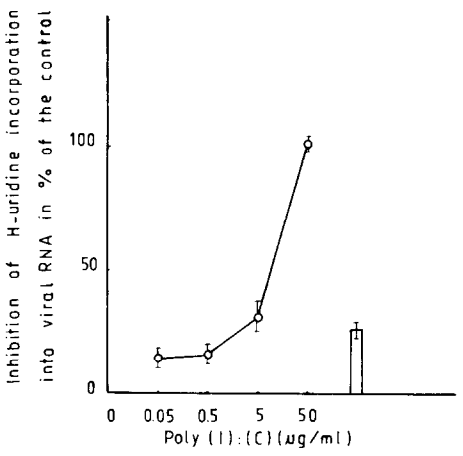

Fig.1. Effect of poly(I)·poly(C) on VSV-RNA replication in UAC cells. UAC monolayer cultures grown in Microtest TC plates (Falcon) were infected with 50–100 TCD_{50}/50 μl VSV, Indiana strain, and actinomycin D in a final concentration of 5 μg/ml was added. To allow virus absorption, poly(I)·poly(C) in different concentrations together with 40 kBq [^{3}H]uridine in 50 μl medium was given to each well after 1 h incubation. Then after a 4 h incubation period the radioactivity incorporated was measured. Each point represents the mean inhibition ± SD of isotope uptake into the viral RNA in % of the control values (n = 6). The column shows the inhibitory effect of 500 U/ml IFN-α (EGIS, Pharmacochemical Works, Budapest).

3. RESULTS AND DISCUSSION

(i) Pretreatment of UAC cells with poly(I)·poly(C) inhibited VSV-RNA synthesis and this effect proved to be dose dependent (not shown). In other experiments the antiviral activity of poly(I)·poly(C) was followed by measuring viral RNA synthesis in the presence of actinomycin D (5 μg/ml) and poly(I)·poly(C) was given only after virus infection. In this condition synthesis of host mRNAs (among those that of IFN) are inhibited. Fig.1 shows that 50 μg/ml poly(I)·poly(C), even under this experimental condition, completely inhibits viral RNA synthesis. In simultaneous experiments 500 U/ml IFN-α had only a modest effect.

(ii) Suspension cultures of UAC cells were incubated overnight with *myo*-[2-^{3}H]inositol and then treated with 50 μg/ml poly(I)·poly(C). Fig.2 demonstrates that after poly(I)·poly(C) treatment the amounts of [^{3}H]phosphatidylinositol 4-phosphate and [^{3}H]phosphatidylinositol 4,5-diphosphate increased within 60 s and later decreased to below the control value.

Similar results were observed by measuring water-soluble inositol 1-phosphate (fig.3). Fig.3 also shows that a relatively high dose of Li^+ (12 mM), a potent inhibitor of *myo*-inositol-1-phosphatase [10], added together with poly(I)·poly(C) does not alter this effect.

(iii) Stimulation of phosphatidylinositol turnover provoked by a wide variety of extracellular signals such as neurotransmitters, hormones, growth factors and many other biologically active substances appears to be an activator of PK-C [11]. This enzyme is a key factor in signal transduction. It has been shown that phorbol myristate acetate (PMA), interleukin-2 and interleukin-3 produce a rapid and transient redistribution of PK-C from the cytosol to the plasma membrane [12–14]. Fig.4 shows that poly(I)·poly(C) also induces PK-C transposition. Within a few minutes of

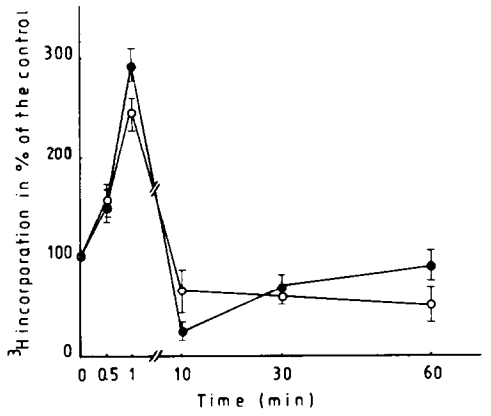

Fig.2. Changes of membrane phosphatidylinositol phosphates in UAC cells treated with poly(I)·poly(C) for different time intervals. Phosphatidylinositol phosphates (PIP, PIP_2) were measured as described in detail in section 2. Counts (n = 4) were corrected for quenching and counting efficiency. (○—○) PIP; (●—●) PIP_2.

adding poly(I)·poly(C) to the cells, the cytosolic PK-C activity began to fall rapidly, then slowly increased. The membrane-bound enzyme activity shows an opposite effect.

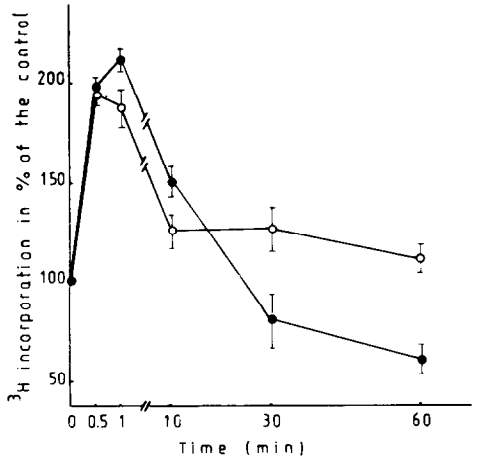

Fig.3. Changes of inositol 1-phosphate in UAC cells treated with poly(I)·poly(C) for different time intervals. UAC cells were labelled and treated with poly(I)·poly(C) (○) and poly(I)·poly(C) + LiCl (12 mM) (●) as described in section 2. Counts (n = 4) are given in % of control values.

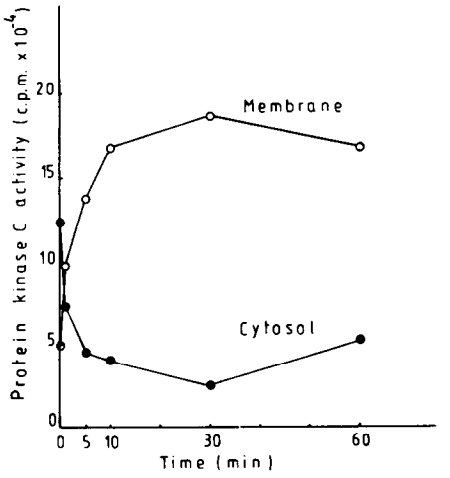

Fig.4. Time course of the effect of poly(I)·poly(C) treatment of UAC cells on cytosolic (●) and particulate (○) PK-C. Suspension cultures of UAC cells were washed with PBS and then treated with 50 μg/ml poly(I)·poly(C). Samples containing 2 × 10^7 cells were homogenized by sonication for 3 × 20 s at 0°C in 0.5 ml of 20 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulphonyl fluoride buffer. The supernatant was saved (cytosolic fraction). The pellet was resuspended by brief sonication and solubilized (on ice for 1 h) in 0.5 ml homogenization buffer containing 1% Triton X-100. This suspension was centrifuged at 48000 × g for 30 min and the supernatant (particulate fraction) was retained. The reaction mixture contained, in a total volume of 80 μl, 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 0.2 mg/ml histone (Sigma, type III-S), 15 μl enzyme preparation (6 × 10^5 cells) and either 1 mM EGTA or 1 mM $CaCl_2$, 50 μg/ml phosphatidylserine, 2 μg/ml diolein. The reaction was started by addition of [γ-^{32}P]ATP (40 kBq, 200 TBq/mmol) and was stopped after 2 min by spotting aliquots (26 μl) of the reaction mixture onto phosphocellulose paper in ice-cold 10% trichloroacetic acid. The filter papers were then washed in 5% trichloroacetic acid (four changes in washing solution). The papers were then transferred sequentially through methanol and acetone before drying and radioactivity was determined by scintillation counting. PK-C activity was defined as that seen in the presence of $CaCl_2$ and lipids minus that seen in the presence of EGTA. PK-C activity is expressed as cpm of ^{32}P incorporated per 2 min per total volume of each cytosolic and membrane preparation.

A considerable number of substances (specific antigens, mitogens, lymphokines, etc.) having a stimulating effect on phosphatidylinositol turn-

over [15,16] proved to be inducers of different kinds of IFNs [17–22]. In addition, compounds that activate PK-C, for example PMA or interleukin-2, also induce the expression of IFN-γ genes [21,22]. A per se antiviral effect of PMA was also demonstrated [23].

All these findings, together with our present results, comprise strong evidence that a major pathway of induction and mode of action of IFNs are realised through a common signal transduction mechanism of the cells. This hypothesis helps to explain the growth factor-like effect of IFN [24,25], which is hardly compatible with its known antiproliferative effect and is in accordance with the finding that IFN-pretreated cells are much more sensitive to the cytotoxic effect of poly(I)·poly(C) than control cells [26].

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