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Interferon (IFN)-like antiviral effect is induced by unspecific cross-linking of cell surface receptors

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Treatment of human amniotic cells (UAC) with Cytodex 1 (DEAE-dextran) results in the development of an antiviral state of the cells, as proven by studying (i) the cytopathic effect and (ii) [^{3}H]uridine incorporation into the RNA of vesicular stomatitis virus (VSV) after VSV infection. The same treatment transiently triggers the breakdown of inositol phospholipids and activates the translocation of protein kinase C (PKC). On the basis of these data it can be suggested that cross-linking of cell surface receptors by a solid carrier bearing covalently bound positive charges may result in IFN-like effects.

Cytodex 1; Antiviral activity; Interferon function; Phosphatidylinositol turnover; Protein kinase C

1. INTRODUCTION

It is still an open problem whether entry of IFN into the cells is necessary for its action or binding to the membrane receptor is satisfactory in this respect. Experiments with matrix-bound IFN demonstrated that surface-bound IFNs induce an antiviral effect [1,2]. Although release of IFN from the carrier cannot be completely excluded in such experiments, on the basis of these and several other experimental data surface action of IFN was presumed [3]. In addition it was demonstrated that IFNs stimulate the turnover of membrane phosphatidylinositol and activate diacylglycerol and inositol trisphosphate release [4,5].

There is evidence that cross-linking of membrane IgM on resting B cells results in the hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate [6,7]. One may presume, therefore, that cross-linking of membrane surface receptors by an insoluble matrix (Cytodex 1) carrying covalently bound positive charges will also produce IFN-like effects.

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2. MATERIALS AND METHODS

UAC cells (a human amniotic cell line obtained from V. Sorrentino, University of Rome, Italy) were grown in monolayer cultures using Parker's 199 medium or suspension cultures using Eagle's medium, both containing 10% fetal calf serum. Cytodex 1 microcarrier of tissue cultures was developed by Pharmacia (Uppsala). Hu-IFNα (spec. act. 1.23×10^7 IU/mg protein) was manufactured by EGIS Pharmacochemical Works (Budapest).

The Indiana strain of vesicular stomatitis virus (VSV) was plaque purified and passaged at low multiplicities in UAC cells.

To study the putative antiviral effect of Cytodex 1, suspensions of UAC cells (10^6 cells/ml) were incubated overnight. After centrifuging, the cells were suspended in Eagle MEM (10^7 cells/ml) containing 2% fetal calf serum and VSV to obtain infection at a multiplicity of 1 PFU/cell. The virus was allowed to adsorb for 60 min. Four samples were then separated and tenfold diluted by Eagle's medium. Two of the samples were treated with 5 mg/ml Cytodex 1 (a dose found to be optimal in preliminary experiments) and two served as control. After 22 h incubation in magnetic spinner vessels the samples were centrifuged at $2000 \times g$ for 10 min and the virus titres of the supernatants determined by the cytopathic effect on UAC cells grown

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in Microtest TC plates (Falcon) and expressed in 50% tissue culture infectious doses ($TCDI_{50}$).

Effect of Cytodex 1 on VSV-RNA replication in UAC cells was tested by measuring [^{3}H]uridine incorporation into the viral RNA in the presence of 5 μg/ml actinomycin D as described in detail elsewhere [8]. The effect of Cytodex 1 on the turnover of membrane phosphatidylinositol was studied as described previously [9].

Phospholipid-dependent protein kinase C (PKC) activity was measured in the cytosol and in the detergent-solubilized membrane fraction according to Kikkawa et al. [10] as described in [9].

3. RESULTS AND DISCUSSION

Fig.1 shows that in the supernatants of VSV-infected UAC cells treated with Cytodex 1 significantly lower virus titers could be demonstrated. In separate experiments we have found that adding of Cytodex to virus-containing suspensions does not reduce the virus titers as compared to control suspensions (not shown).

Since it was reported recently that IFN treatment inhibits replication of VSV-RNA [11], it was reasonable to investigate the effect of Cytodex 1 on the synthesis of VSV-RNA.

Fig.2 shows that, similar to IFN, Cytodex 1 also inhibits [^{3}H]uridine incorporation into the VSV-RNA. This effect could be observed when addition of Cytodex 1 to the cells was performed simultaneously with virus infection, and when Cytodex 1 was given 16 h prior to virus infection. To exclude the possibility that Cytodex 1, as a weak anion exchanger, directly decreases the infecting capacity of the virus, virus suspensions were incubated with Cytodex 1 and after elimination of Cytodex 1 UAC cells were infected with these viruses. We can state that this treatment did not alter the infecting ability of the viruses (not shown).

As it was reported that IFNs stimulate the turnover of membrane phosphatidylinositol and transiently activate diacylglycerol and inositol

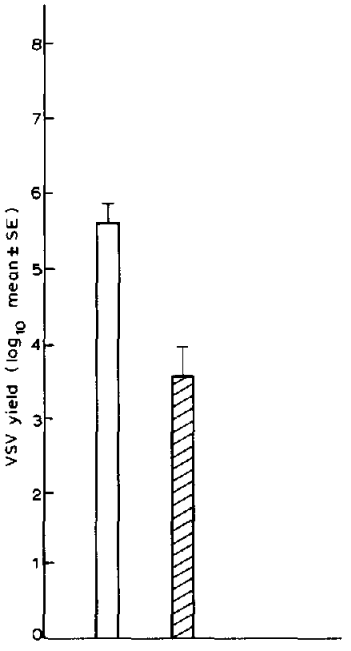

Fig.1. Effect of Cytodex 1 on virus multiplication in VSV-infected UAC cells. The results are presented as mean of VSV yield/0.1 ml $\log_{10}$ ± SE of three experiments measured by the cytopathic effect. (Details are given in section 2.) (□) Control, VSV-infected but not treated cells; (■) VSV-infected cells treated with 5 mg/ml Cytodex 1.

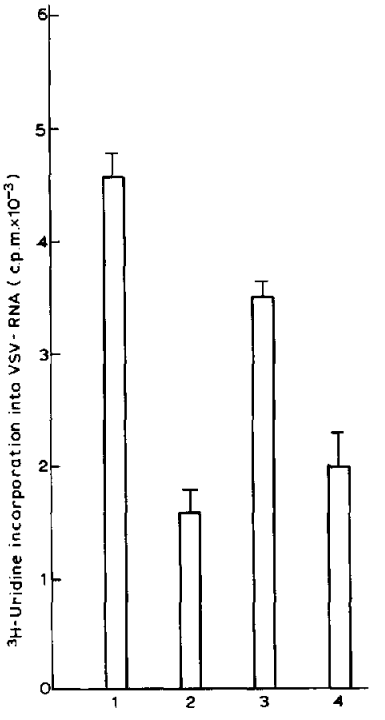

Fig.2. Effect of Cytodex 1 on VSV-RNA synthesis in UAC cells. Bars: 1, VSV-infected, actinomycin D-treated UAC cells; 2, cells treated with 1000 IU/ml Hu-IFNα for 16 h before virus infection; 3, cells treated with Cytodex 1 for 16 h before virus infection; 4, cells treated with Cytodex 1 simultaneously with virus infection. Each point represents the mean value ± SD of [^{3}H]uridine incorporation into the viral RNA (n = 6).

trisphosphate release, it was important to determine whether the observed inhibition of virus replication was due to the activation of this signal transduction mechanism.

Fig.3 shows that after Cytodex 1 treatment the amounts of [^{3}H]phosphatidylinositol 4-phosphate decrease in the membrane-phospholipid fraction of UAC cells, while the amounts of [^{3}H]phosphatidylinositol 4,5-phosphate significantly increase. The activity incorporated into the phosphatidylinositol fraction does not change considerably.

Stimulation of membrane phosphatidylinositol turnover could also be demonstrated by measuring the amounts of water-soluble [^{3}H]inositol phosphates (fig.4).

It is known that several extracellular signals able to increase the breakdown of inositol phospholipids activate the association of PKC to the cell membrane [13,14]. Fig.5 demonstrates that Cytodex 1 also induces PKC transposition.

The results of this study show that cross-linking

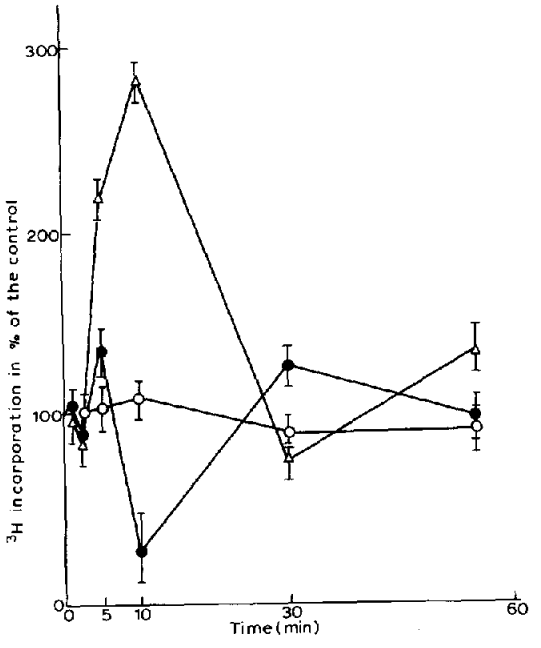

Fig.3. Changes of membrane phosphatidylinositol phosphates in UAC cells treated with 5 mg/ml Cytodex 1 for different intervals. Phosphatidylinositol phosphates (PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-phosphate) were measured as described in detail in [9]. Counts (n = 3) were corrected for quenching and counting efficiency. (○) PI, (●) PIP, (△) PIP_2.

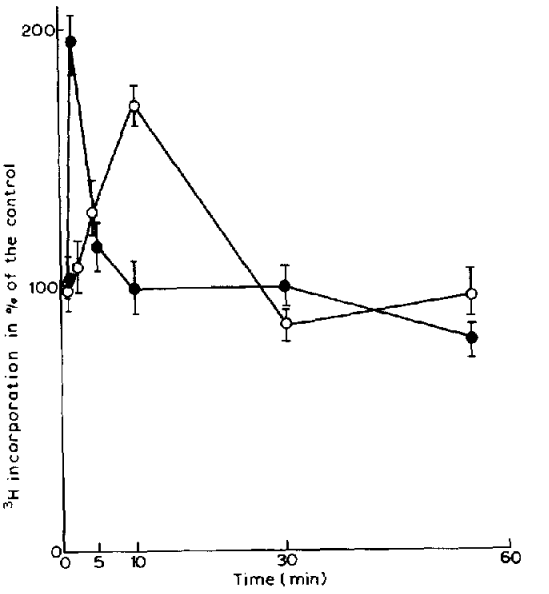

Fig.4. Changes of inositol 1-phosphate (○) and inositol trisphosphate (●) in UAC cells treated with 5 mg/ml Cytodex 1 for different intervals. Water-soluble metabolites were separated by anion-exchange chromatography according to Berridge et al. [11]. Counts (n = 3) are given in % of control values.

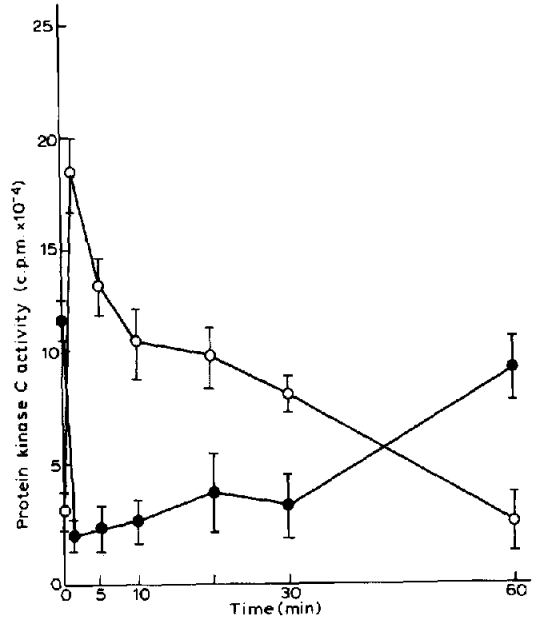

Fig.5. Effect of Cytodex 1 treatment of UAC cells on cytosolic (●) and particulate (○) PKC activity in function of time. Mean values of three experiments. Details of PKC assay are given in [9]. PKC activity was defined as that seen in the presence of $CaCl_2$ and lipids minus that seen in the presence of EGTA. PKC activity is expressed as cpm of ^{32}P incorporated/2 min per total volume of each cytosolic and membrane preparation.

of cell surface receptors with Cytodex 1 (a solid matrix carrying covalently bound positive charges) activates the signal transduction mechanism in question triggered generally by specific biological substances. Thus, cross-linking of cell surface receptors by non-specific substances may result in an antiviral state of cells similar to that induced by IFNs.

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