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STIMULATION BY CALCIUM CHLORIDE OF VIRUS-INDUCED INTERFERON FORMATION BY BLOOD, HAEMOPOIETIC ORGAN AND CONTINUOUS HUMAN CELLS

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Summary. — Calcium chloride stimulated virus-induced production of leukocyte interferon by human and animal blood and haemopoietic organ cells. $CaCl_2$ treatment of surviving cells (leukocytes, human and mouse bone marrow) and Namalva cells was the most effective when carried out simultaneously with adsorption of the virus-inducer or when $CaCl_2$ pretreatment was combined with its subsequent addition together with the virus-inducer. Optimal $CaCl_2$ concentrations were 5 mM for human bone marrow cells and 10 mM for human leukocytes and mouse bone marrow cells. $CaCl_2$ treatment was equivalent to priming in case of interferon induction in human leukocytes and, as distinct from priming, it considerably increased virus-induced interferon production by Namalva cells.

Key words: interferon production; stimulation by calcium chloride

Introduction

Interest in interferon has been increasing not only from the point of view of basic research but also from that of clinical applications. The latter has been based on the successful applications in treatment of several viral and oncological diseases. But great amounts of highly active preparations are required for more profound investigations on the therapeutic effects of interferon.

A stimulating effect of $CaCl_2$ on the production of interferon by human diploid fibroblast cells following induction by poly I: poly C was reported (Meager *et al.*, 1978). Therefore it was of interest to check whether $CaCl_2$ would stimulate the production of leukocyte interferon.

We are reporting data showing that virus-induced interferon production can be increased by $CaCl_2$ treatment of blood and haemopoietic organ cells as well as of continuous human lymphoblastoid cells.

Table 1. Effect of $CaCl_2$ treatment on interferon production

Inducer	Producer cells	Titres of interferon produced by cells			
		untreated	treated with $CaCl_2$ for		
			24 hr before induction	24 hr before induction and during adsorption	the adsorption period
Poly I:poly C	L	<8	ND	64	32
NDV	L	240	240	320	320
NDV	Bone marrow	800	ND	3200	1600
Sendai	Bone marrow	400	ND	1600	800
		64	128	512	128
NDV	J-96	<4	8	8	8
	J-41	<4	4	8	4

ND = not done.

Materials and Methods

Cells. Human and mouse bone marrow cells, leukocytes from blood donors, continuous human lymphoblastoid Namalva cells (Klein *et al.*, 1972), continuous J-96 and J-41 cells (Osgood and Brocke, 1955; Soloviev and Gulevich, 1966), and mouse L cells were used. Human bone marrow was obtained by punction from the thorax and hip bones of suddenly dead persons and mouse bone marrow was recovered by washing from isolated thigh bones. Continuous J-96, J-41 and L cells were grown in medium 199 supplemented with 10% bovine serum as monolayers. Namalva cells were cultivated as a stationary suspension culture in Eagle's medium with 10% foetal calf serum. Bone marrow, blood leukocyte and Namalva cells were used in suspensions containing 10^6-10^7 cells per ml. Monolayer cultures were treated without removing the cells from the glass.

Interferon induction. Cells were treated with Newcastle disease virus (NDV) in a dose of 5–10 plaque forming units (PFU) per ml. For treatment of continuous human and mouse cells, NDV previously irradiated with a dose of UV light decreasing NDV infectivity 10^4-10^5-fold was employed. The dose was calculated based on the titre of NDV before irradiation.

Calcium chloride treatment. $CaCl_2$ in concentrations from 5 to 10 mM was added to the cells either before virus induction or simultaneously with virus inducer (in most experiments) or for the time of interferon synthesis. The cells were incubated for 1 hr at 37 °C, after which the fluid phase was removed and the cells supplied with medium 199 containing 5 % bovine serum were incubated further at 37 °C for 20–24 hr.

Priming. One hundred units of human interferon were added to the cells for 2 hr. Thereafter the cells were treated with virus and CaCl as described above.

Interferon assay. Interferon was determined in the samples after acid treatment (pH 2 for 5 days) based on inhibition of 100 CPD_{50} of vesicular stomatitis virus. Mouse interferon was titrated in mouse L cells and human interferon in skin-muscle diploid human cells. Titration of national standard human interferon (No. 5, Gamaleya Institute of Epidemiology and Microbiology) was included in each assay.

For details of the methods of bone marrow cell preparation, interferon induction and interferon titration see Soloviev *et al.* (1972, 1976).

Results

In the first experiments we investigated the effect of the time of addition of $CaCl_2$ (before induction, before and during induction, during induction) on virus-induced interferon synthesis in various mouse and human cell cultures (Table 1). $CaCl_2$ was added to a final concentration of 10–12 mM

Table 2. Dependence of NDV-induced interferon production on treatment of producer cells by different $CaCl_2$ concentrations

$CaCl_2$ concentration mM	Titres of interferon produced by human blood leukocytes	human bone marrow cells	mouse bone marrow cells
0	80	160	320
5	320	1280	640
10	480	640	1280
20	160	320	480
40	80	80	320

which, in experiments of Meager *et al.* (1978), proved to be optimal in enhancing the production of fibroblast interferon.

As shown in Table 1, treatment of L cells with 12 mM $CaCl_2$ during their interaction with the synthetic interferon inducer poly I: poly C markedly enhanced interferon synthesis (32—64 as compared with < 8 units/ml in the control). $CaCl_2$ added to L cells before and during adsorption of NDV practically had no effect on interferon production.

These results confirmed those by Meager *et al.* (1978) who observed a stimulating effect of $CaCl_2$ on fibroblast interferon production induced by poly I:poly C but not by viral inducers. Different results were obtained concerning the effect of $CaCl_2$ on virus-induced interferon synthesis by mouse bone marrow cells. In this case $CaCl_2$ enhanced virus-induced interferon synthesis 2- to 4-fold. The stimulating effect was the highest when $CaCl_2$ was added during virus adsorption or before and during adsorption.

$CaCl_2$ treatment of continuous cells of human origin before and during their interaction with viral inducer also enhanced interferon production. In this way we succeeded in demonstrating interferon production in J-96 and J-41 cells which are very poor interferon producers (in the given experiments these cells produced no interferon in response to NDV induction without $CaCl_2$ treatment).

Enhancement of virus-induced interferon production by blood leukocytes and human bone marrow nucleate cells depended on $CaCl_2$ concentration and

Table 3. Effects of $CaCl_2$ and priming on NDV-induced interferon production by Namalva cells

Priming	Pretreatment with $CaCl_2$ for 20 hr before induction with NDV	$CaCl_2$ treatment simultaneously with virus	Interferon titre
−	−	−	320
−	−	+	960
−	+	−	480
−	+	+	2560
+	−	−	320
+	−	+	1280
+	+	−	320
+	+	+	1280

Table 4. Effects of $CaCl_2$ and priming on interferon production by human peripheral blood leukocytes

Priming	$CaCl_2$ treatment simultaneously with virus	Interferon titre
−	−	300
−	+	1600
+	−	1200
+	+	1600

the cell type. The optimal concentrations of $CaCl_2$ proved to be 5 mM for treatment of human bone marrow cells and 10 mM for human blood leukocytes and mouse bone marrow cells (Table 2).

The method of pretreatment of producer cells by small doses of interferon has recently been introduced into practice to increase interferon synthesis (Mogensen and Cantell, 1977). Therefore we carried out comparative experiments on the efficiency of the two methods: interferon pretreatment and treatment of the cells with $CaCl_2$ (Tables 3 and 4). In Namalva cells (Table 3) we observed no positive effect of priming which is in accordance with the report by Zoon *et al.* (1978). $CaCl_2$ treatment of the cells, especially for 20 hr before induction and then during virus adsorption, markedly enhanced interferon synthesis by Namalva cells (interferon titres 960—2560 with $CaCl_2$ treatment as compared with 320 without $CaCl_2$ treatment). Combination of both methods — priming and $CaCl_2$ treatment — caused no further increase in interferon yields.

Cultivation of Namalva cells in medium containing 10 mM $CaCl_2$ for 24 hr had no effect on cell multiplication and their ability for interferon production.

As distinct from Namalva cells, priming of leukocytes markedly enhanced interferon synthesis (titre 1200 as compared with 300 in the control, see

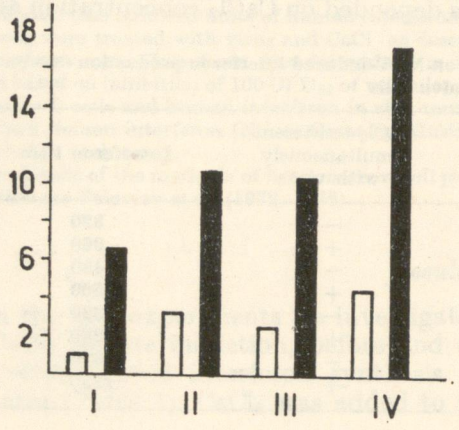

Fig. 1.
Mean titres of interferon produced by human and animal cells with (black columns) and without (emptycolumns) $CaCl_2$ stimulation
Ordinate: interferon units per ml $\times 10^{-2}$
Abscissa: I — human peripheral blood cells; II — human bone marrow cells; III — Namalva cells; IV — mouse bone marrow cells

Table 4). Similar results were obtained by treating human leukocytes with $CaCl_2$ simultaneously with virus. Combination of priming with $CaCl_2$ treatment resulted in no further increase in interferon production.

Our experiments thus showed that treatment by calcium ions of producer cells was suitable for increasing the production of virus-induced leukocyte interferon. Such treatment resulted on the average in a 3- to 5-fold increase in interferon yield from human blood leukocytes, human and mouse bone marrow cells and the lymphoblastoid Namalva cells (Fig. 1).

Discussion

Meager *et al.* (1978) reported recently that $CaCl_2$ stimulates the production of human and animal fibroblast interferon induced by polyI:poly C.

Our experiments clearly demonstrated a stimulating effect of $CaCl_2$ on virus-induced interferon production by human and animal blood and haemopoietic organ cells. Especially interesting was the possibility of increasing interferon production by the lymphoblastoid Namalva cells under conditions of $CaCl_2$ treatment. Priming, i.e. pretreatment of cells with interferon before viral induction, had no effect on interferon formation by these cells, which is in accordance with the report by Zoon *et al.* (1978). A comparison of two methods of stimulation of interferon formation (priming and $CaCl_2$ treatment of the cells) showed their equivalence in treatment of human blood leukocytes. This makes possible to substitute priming, widely employed in the production of leukocyte interferon.

In studiyng the conditions of $CaCl_2$ treatment, we found that its optimal dosage varied for animal and human cells and even for different cells of the same species.

The mechanism of stimulated interferon formation by $CaCl_2$ remains obscure. It is known that $CaCl_2$ in concentrations up to 12 mM did not inhibit cellular protein and RNA synthesis (Meager *et al.*, 1978). Moreover, in the same concentrations it did not affect the multiplication of Namalva cells. It has been suggested that the phenomenon could be explained by $CaCl_2$ enhancing the binding of poly I:poly C with specific sites of interferon induction (Booth and Borden, 1978) or that the effect is due to lowered degradation of poly I:poly C (Meager *et al.*, 1978). But taking into account that the mechanism of stimulation of interferon production by $CaCl_2$ is active on induction not only with poly I:poly C but also with virus, another possibility can be suggested. Falcoff *et al.* (1976) showed that interferon, like other substances in the cell, is not freely located in it, but occurs in membrane vesicles. Consequently, accumulation of interferon in extracellular medium depends not only on its synthesis, but also on its release into the medium. The mechanism initiating the coalescence of the vesicle and cell membrane and release of the contents into extracellular environment, has been called "association stimulus — secretion" and recently connected with an increase in Ca^{2+} ions in the medium (Setir, 1977). It is very probable that the observed effect of $CaCl_2$ enhances interferon release in this way.

Our results are of practical importance indicating a novel and efficient approach, easily realizable under production conditions, to increase the production of human leukocyte interferon. Moreover, of great interest is the $CaCl_2$-induced interferon production by Namalva cells which at present are considered to offer the possibility of obtaining great amounts of interferon.

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