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DIFFERENTIAL EFFECT OF INTERFERON ON ORNITHINE DECARBOXYLASE ACTIVATION IN QUIESCENT SWISS 3T3 CELLS

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SUMMARY

Ornithine decarboxylase activity was enhanced in quiescent Swiss 3T3 cells by the addition of either a combination of polypeptide hormones or L-asparagine and dibutyryl cyclic AMP. However, only the polypeptide hormones stimulated enzymatic activity was inhibited by interferon.

In addition to its well-known antiviral action, interferon (IF) induces in cells a variety of responses, including the inhibition of cell division and effects on cellular enzymes (1). IF inhibits the elevation of ornithine decarboxylase (EC4.1.1.17) level in quiescent mouse 3T3 cells which are stimulated to proliferate (2,3). Ornithine decarboxylase is a key enzyme catalyzing the initial step in the synthesis of polyamines, a group of cationic compounds intimately involved in cellular growth processes. The level of this enzyme is rapidly elevated in cells by a variety of stimuli such as serum, well-defined substances like polypeptide hormones, growth factors, amino acids, dexamethasone or cyclic AMP (cAMP) (4,5). The involvement of cAMP in the enhancement of the enzyme level in a variety of cell lines is well documented (5).

Quiescent Swiss 3T3 cells respond with a large increase in ornithine decarboxylase when stimulated to initiate DNA synthesis by the addition of fresh serum or a combination of three polypeptide hormones (epidermal growth factor (EGF), vasopression and insulin) (2,3). Addition of mouse IF at the time of stimulation of the cultures results in the inhibition of the enzyme activation, DNA synthesis and cell division (2,6). Recently, Costa and Nye (7) reported L-asparagine and dibutyrylcyclic AMP (dBcAMP) synergistically elevate the ornithine decarboxylase

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level in Chinese hamster ovary (CHO) cells. The kinetics of enzyme activation by the cyclic nucleotide and amino acid is similar to that observed with serum. They suggested that the enhancement of the enzyme by serum probably represents de novo synthesis while that produced by dBcAMP and asparagine results from an increase in the half-life of the enzyme. Therefore we determined whether dBcAMP and asparagine can elevate ornithine decarboxylase activity in quiescent Swiss 3T3 cells and, if so, whether IF can inhibit the stimulation of the enzyme. Our results showed that the enzyme can be stimulated in cells either by a combination of the three polypeptide hormones (EGF, insulin and vasopressin) or by dBcAMP and asparagine; however, interferon was inhibitory only in the former instance.

MATERIALS AND METHODS

Swiss 3T3 cells were propagated and grown to confluence in 33 mm or 100 mm Nunc dishes in Dulbecco's modified Eagle's medium with 10% calf serum (3). Such cultures were arrested in G_1/G_0 phase since, after exposure to ^{3}H-thymidine for 40 h, less than 1% of the nuclei was labelled. For stimulation of quiescent cultures they were washed with a 1:1 mixture of Dulbecco's medium and Waymouth medium and incubated with the same medium containing the appropriate agents as described recently (3). Unstimulated cultures received medium only. The cultures were harvested and assayed for ornithine decarboxylase activity as described earlier (3).

Mouse L cell interferon (specific activity of 2×10^5 reference units (U)/mg protein) was prepared by methods as described by Stewart (1). A mouse interferon preparation of 2×10^7 U/mg protein was provided generously by Dr. R.M. Friedman, National Institutes of Health, Bethesda, Maryland.

For determination of DNA synthesis, the cultures (33 mm dishes), after stimulation were incubated with (^{3}H)-thymidine (1 μCi/ml, 1 μM) and incorporation into acid-precipitable DNA was measured as described earlier (3). Cycloheximide, actinomycin-D, arginine-vasopressin, putrescine dichloride, L-ornithine hydrochloride, bovine insulin (26 IU/mg), L-asparagine, dBcAMP, colchicine, retinoic acid and cytochalasin B were purchased from Sigma Chemical Co. EGF was obtained from Collaborative Research, Waltham, Mass. Cholera toxin was purchased from Schwarz-Mann. DL-[1-^{14}C] ornithine hydrochloride (specific activity 49.9 mCi/mmole), methyl-^{3}H-thymidine (52.0 Ci/mM) and Protosol were obtained from New England Nuclear. Dulbecco's medium, Waymouth medium, calf serum, penicillin, streptomycin and trypsin were from Grand Island Biological Co. All other chemicals and reagents used were of reagent grade.

RESULTS AND DISCUSSION

Preliminary experiments indicated that the addition of a combination of the three polypeptide hormones (EGF, vasopressin and insulin) or a mixture of

dBcAMP and asparagine to quiescent cultures resulted in a gradual increase in ornithine decarboxylase activity, which reached a maximum within six hours post-stimulation. Thereafter, the enzymatic activity declined. Therefore the effect of IF on the enzyme activation was determined by comparing the level of activity in cultures stimulated for six hours with the appropriate stimuli in the presence or absence of IF.

The basal level of enzymatic activity in quiescent cells was low (Table 1). The addition of asparagine or dBcAMP to cells increased the level of the enzyme approximately three-fold. When present together they synergistically increased the enzymatic activity. However, the level obtained was lower than that observed using a combination of the three polypeptide hormones. IF appeared to enhance rather than inhibit ornithine decarboxylase activity in cells incubated with asparagine or dBcAMP. Although IF markedly suppressed activation of the enzyme in cells stimulated with polypeptide hormones (by 65% over control), no significant inhibition (15% over control) was observed in cells incubated with a combination of asparagine and dBcAMP. Even when quiescent cells were pretreated with IF for 2 hours prior to stimulation, there was no enhancement of the observed inhibition (results not presented). DNA synthesis stimulated by a combination of the three polypeptide hormones was inhibited by IF as previously reported (3). Asparagine alone was weakly mitogenic and the observed stimulation of DNA synthesis was inhibited by IF. dBcAMP, alone or in combination with asparagine, did not augment DNA synthesis.

Cholera toxin can stimulate DNA synthesis and cell division in 3T3 cells (8). Since the level of cAMP is elevated in the cells by the toxin (8) we determined whether elevation of ornithine decarboxylase activity occurs also. Table 1 shows that although the toxin, when used alone was not mitogenic, increased the enzyme level. IF enhanced rather than inhibited the toxin induced enzyme activity. When added with asparagine or insulin, cholera toxin synergistically stimulated DNA synthesis and ornithine decarboxylase activity. Again, no significant inhibition (18% over control) was observed in the stimulation of the enzyme although DNA synthesis was inhibited.

Table 1. Effect of interferon on appearance of ornithine decarboxylase and on DNA synthesis initiated in quiescent 3T3 cells by various agents.

Agent	ornithine decarboxylase activity (nmoles/hr/mg protein) at 6 hr post-stimulation		(^{3}H)-TDR incorporation CPM x 10^{-4}/dish	
	Control	Interferon treated	Control	Interferon treated
None	0.7±0.20	ND	0.13	ND
L-asparagine	2.4±0.80	3.2±1.2	1.40	0.20
dBcAMP	1.8±0.70	2.5±1.3	0.20	0.13
Insulin	2.6±0.10	1.3±0.5	0.60	0.24
Cholera toxin	3.7±0.40	5.7±2.0	0.23	0.20
L-asparagine + dBcAMP	9.4±2.3	8.1±1.9	1.40	0.40
L-asparagine + Cholera toxin	17.7±7.6	20.6±6.0	3.40	1.0
Cholera toxin + Insulin	16.0±4.9	13.2±3.5	4.9	1.6
EGF + Insulin + Vasopression	15.5±1.3	5.5±1.3	5.8	3.6

Quiescent cultures of 3T3 cells were prepared (2,3). Two cultures, in 100 mm dishes, were used for enzyme determination while DNA synthesis was done in 30 mm dishes. The quiescent cultures were washed with a 1:1 mixture of Dulbecco's medium and Waymouth medium and then incubated with the same medium containing the various agents. Unstimulated cultures received medium only. The concentration of the agents were as follows: - L-asparagine 10 mM, N^6, O^2 - dibutyryl adenosine 3'-5' cyclic monophosphoric acid-sodium salt (dBcAMP) 1.0 mM, bovine insulin (26 international units/mg) 1.0 µg/ml, cholera toxin, 100 ng/ml, EGF 2.5 ng/ml, vasopressin 10 ng/ml. Interferon at 1000 U/ml (sp. act $2x10^7$ U/mg protein) was added to cultures at the time of stimulation. Six hours after stimulation the cultures were harvested and enzyme activity contained in cell extracts was determined (3). The values presented are the mean ± SEM of six independent experiments. Determination of DNA synthesis was performed by adding 1 µCi ^{3}H-TdR (1 µM) to the cultures. ^{3}H-thymidine incorporated into acid insoluble DNA was determined after 40 hr of incubation (3). ND = not done.

The preceding results indicated that the effect of IF on ornithine decarboxylase activation in quiescent 3T3 cells depended on the type of agent used for stimulation. Two possible explanations may be entertained to explain the observed differential effect of IF on enzyme activation (i) the presence of dBcAMP or asparagine may reverse the action of IF or (ii) the enzyme stimulation by dBcAMP or asparagine may involve an IF insensitive pathway. The former can be

Table 2. Inhibitory effect of interferon on the appearance of ornithine decarboxylase initiated in quiescent 3T3 cells by polypeptide hormones.

Stimulant	Enzyme activity (nmoles/hr/mg protein) at 6 hr post-stimulation	
	Control	Interferon treated
None	0.25	ND
EGF + Insulin + Vasopressin }	24.0	5.4
L-asparagine + dBcAMP }	12.0	13.8
EGF + Insulin + Vasopressin + L-asparagine + dBcAMP }	34.0	20.0

Quiescent 3T3 cells (100 mm dishes) were stimulated with the medium containing various mitogens at the concentrations as indicated in Table 1. Unstimulated cultures received medium only. Interferon, 1000 U/ml (sp. act $2x10^7$ U/mg protein) was added to cultures at the time of stimulation. Six hours after stimulation the cultures were harvested and assayed for enzymatic activity. ND = not done.

ruled out based on the results shown in Table 2. When a combination of hormones, dBcAMP and asparagine was used as the stimulant, an additive response in enzyme activation was observed in the control cultures. The level of the enzyme found in the IF treated cultures under the conditions approximated the level expected if IF inhibited only the enzymatic activity stimulated by the hormones.

Several agents can inhibit the activation of ornithine decarboxylase in cells. These include non-specific agents like actinomycin D or cycloheximide which are general inhibitors of RNA and protein synthesis, respectively. Other specific agents include putrescine (9), cytochalasin B, colchicine (10), or retinoic acid (11). We determined whether the enzyme activated by the hormones and other stimuli differed in their sensitivity to these inhibitors. The results in Table 3 show that of the agents tested, only IF displayed a differential effect.

The present results clearly show that IF displays a discriminatory effect on the activation of ornithine decarboxylase in 3T3 cells by various stimuli. At present it is not clear how this effect is brought about. The differential effect of IF can be explained best by assuming that more than one pathway exists in 3T3

Table 3. Effect of some inhibitors on the appearance of ornithine decarboxylase in quiescent 3T3 cells stimulated with a combination of polypeptide hormones or dBcAMP and asparagine.

Inhibitor	Enzyme activity (nmoles/hr/mg protein) in cells incubated with EGF + Insulin + Vasopressin	Enzyme activity (nmoles/hr/mg protein) in cells incubated with L-asparagine + dBcAMP
None	14.8	9.0
Actinomycin D	0.01	0.20
Cycloheximide	0.001	0.001
Putrescine	1.00	0.40
Retinoic acid	5.50	5.70
Cytochalasin B	1.30	2.80
Colchicine	3.40	3.50
Interferon	3.80	8.40

Quiescent 3T3 cells were stimulated with medium containing either a combination of EGF, insulin and vasopressin or L-asparagine and dBcAMP. The concentrations of the various agents used are indicated in Table 1. The inhibitors were added to cultures at the time of stimulation and the concentrations used were as follows: - actinomyin D 1.0 µg/ml, cycloheximide 10.0 µg/ml, putrescine 1 mM, retinoic acid 1 µg/ml, cytochalasin B 10 µg/ml, colchicine 2 µm and interferon, 1000 U/ml, (sp. act 2.0×10^7 U/mg protein). Six hours after stimulation the cultures were harvested for enzyme assay.

cells for the activation of the enzyme. Based on studies concerning the antiviral activity, it is currently thought that IF acts by blocking transcription and/or translation of viral messages. If an analogous situation holds true concerning IF's effect on cellular enzymes then one may conclude that the processes involved in enzyme activation by polypeptide hormones differ from those initiated by dBcAMP and asparagine. In addition to a transcriptional/translation control other mechanisms regulated by cAMP and amino acids may exist for enzyme activation in 3T3 cells. Evidence for such a mode of regulation has been proposed recently. Costa and Nye (7) reported that in CHO cells activation of the enzyme by serum was inhibited by actinomycin D or cycloheximide. However, the cAMP induced enhancement of enzymatic activity was only partially sensitive to the above inhibitors. Similarly Clark and Greenspan (12) provide intriguing evidence for

a cytoplasmic control of ornithine decarboxylase in 3T3 cells. They reported that the enzyme can be activated in intact cells as well as cytoplasts. Interestingly, actinomycin D blocked the activation of the enzyme in both instances. The inhibitory effect of the antibiotic in cytoplasts was attributed to effects other than the inhibition of transcription. In light of the above findings and more specifically on the effect of actinomycin D (Table 3) it is difficult to determine from the present data whether de novo synthesis of RNA is required for enzyme activation in 3T3 cells. Regardless of the exact mechanism involved, our data clearly show that IF can discriminate between the processes initiated by the polypeptide hormones and cAMP in 3T3 cells for enzyme activation. Thus, IF may prove to be a useful inhibitor for studying the regulation of polyamine enzymes in intact cells.

It has been reported that pretreatment of cells with cholera toxin can abolish IF's antiviral activity (13). Additionally, IF can inhibit toxin binding to mouse cell plasma membranes (14). Therefore, it could be argued that the toxin can reverse IF action and this may explain why the toxin stimulated enzyme activation is not inhibited. Such an explanation appears unlikely since DNA synthesis initiated by the toxin and insulin or asparagine was inhibited by IF (Table 1).

Finally, the present results provide strong evidence to indicate that the inhibitory effect of IF on DNA synthesis is not dependent on its inhibition of ornithine decarboxylase activation. Concomitant inhibition of DNA synthesis and of activation of the enzyme was observed only when the polypeptide hormones were used. Our results indicate that a poor correlation exists between the activation of ornithine decarboxylase and subsequent DNA synthesis in quiescent 3T3 cells stimulated to proliferate. This conclusion is consistent with the previous report by Clark and Duffy (15) using diverse growth factors, enzymes and hormones to stimulate the enzyme and DNA synthesis in 3T3 cells.

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