INTERFERON SUPPRESSES GLUTAMINE SYNTHETASE INDUCTION IN CHICK EMBRYONIC NEURAL RETINA

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SUMMARY: Induction of glutamine synthetase in cultured embryonic chick neural retina cells was suppressed by the addition of chick interferon preparation. The interferon-induced suppression was observed in both spontaneous and hydrocortisone-stimulated elicitation of the enzyme activity during the *in vitro* incubation. Levels of lactate dehydrogenase, acetylcholine esterase and of total cellular protein synthesis were unaffected by the interferon preparation.

INTRODUCTION: In addition to its well documented antiviral activity, interferon has been suggested to exert various mutually unrelated effects on cells, i.e., inhibition of cell division (1), enhancement or suppression of particular cell functions (2-4).

This report deals with an inhibitory effect of interferon on the synthesis of a specific enzyme protein, GS, in the neural retina tissue of chick embryo. Level of GS activity in neural retina was shown to be closely related with the functional differentation of the tissue (5,6). Addition of hydrocortisone to the cultured retina cells induces a precocious increase of the enzyme activity which is due to *de novo* synthesis of the enzyme (7,8). Therefore, it seemed worthwhile to examine an action of interferon in relation to the regulation of gene expression in this embryonic differentation system.

MATERIALS AND METHODS

Organ culture of retina: A whole excised neural retina from 12-day-old chick embryo was transferred in a 30 ml flask and cultured in 3 ml per retina of Eagle's minimum essential medium containing 20 % fetal bovine serum, penicillin and streptomycin (200 units/ml, 200 μg/ml, respectively). Incubation was carried out at 38 C under humidified atmosphere containing 5 % CO_2 with gyratory shaking of approximately 70 cycles/min.

Abbreviations: GS, glutamine synthetase (EC 6.3.1.2)
LDH, lactate dehydrogenase (EC 1.1.1.27)
AChE, acetylcholine esterase (EC 3.1.1.7)
HA, hemagglutinin
PFU, plaque forming units

Interferon was added to the medium in 0.01M phosphate buffered saline. To induce retinal GS, the tissue was incubated with the fresh culture medium containing 2 μg/ml of hydrocortisone.

Enzyme assays: Tissue samples to be determined for the enzyme activity were first homogenized with sonic oscillation (Kontes, Microsonicator) for 15 sec in cold 0.01M phosphate buffer (pH 7.1) followed by addition of Nonidet P-40 to a final concentration of 0.1 %. GS was assayed according to the method of Kirk (9) and expressed by specific activity calculated as μmoles of glutamylhydroxamate formed per hr per mg of tissue protein. LDH and AChE were determined by the method of Reeves et al. (10) and Leuzinger (11), respectively. Each enzyme unit was one μmole of NADH oxidized or acetylcholine hydrolysed per min. Protein was measured by the method of Lowry et al. (12).

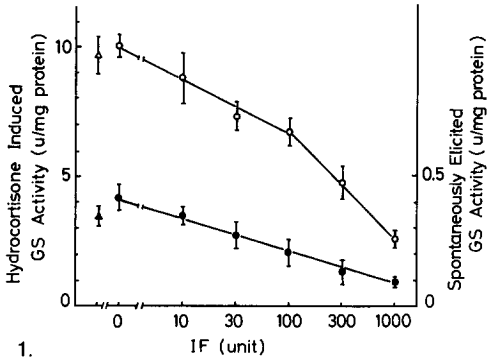
Protein synthesis: Incorporation of [^{3}H]leucine (58 Ci/mmole, Radiochemical Centre, Amersham) into TCA-insoluble material was determined as follows. Retina cultures in replicate flasks were labelled by replacing the culture medium with 2 ml of Eagle's minimum essential medium containing 4 μCi/2 ml of labelled leucine in place of cold leucine. Incorporation was stopped at 30 min by addition of SDS to a final concentration of 0.5 % followed by a sonication for 20 sec. After addition of distilled water to solubilize the tissue, a portion of the lysate was precipitated with 5 % TCA and was collected on a glassfiber paper (Whatman, GF/C), washed four times with 5 % TCA, dried and counted in scintillation fluid.

Preparation of interferon and assay of its antiviral activity: Monolayer cultures of chick embryo cells made from 11-day-old eggs were 'aged' for more than 6 days in Eagle's medium containing 2 % calf serum and induced for interferon by addition of UV-irradiated Newcastle disease virus at multiplicity of about 3 PFU before irradiation/cell. The culture fluid was harvested at 24 hrs. The interferon was partially purified by zinc acetate precipitation according to the method described by Lampson et al. (13). Mock interferon was obtained from uninduced chick cell cultures in the same manner as interferon preparation. Neural retina tissue was incubated overnight with serial a half $\log_{10}$-fold dilutions of interferon and then challenged with mouse encephalomyelitis virus, GD-7 strain, at a multiplicity of approximately 10 PFU/cell and the HA yields were determined at 20 hrs post infection. Specific activities of the partially purified interferon preparations were neighbourhood of 20,000 units/mg protein/ml. One unit was defined as the concentration that reduces the HA yield of GD-7 virus by $\log_{10}0.5$. Interferon titers obtained by chick retina cells were about one-third of those determined by chick embryonic cells and Sindbis virus system (14).

RESULTS

When neural retina was incubated in vitro for 39 hrs, an approximately 3-fold increase of GS activity was observed (Fig.1). While, the addition of hydrocortisone in incubation medium resulted in some 35-fold increase of GS activity. As seen in Fig.1, presence of interferon at the concentration higher than 30 units suppressed spontaneous or the hormone-induced development of the enzyme activity depending on its concentration.

On the other hand, the levels of LDH and AChE remained unchanged after the hormonal treatment and were unaffected by interferon (Fig.2). Level of protein synthesis as determined by incorporation of [^{3}H]leucine was not significantly affected by the hormonal treatment or by addition



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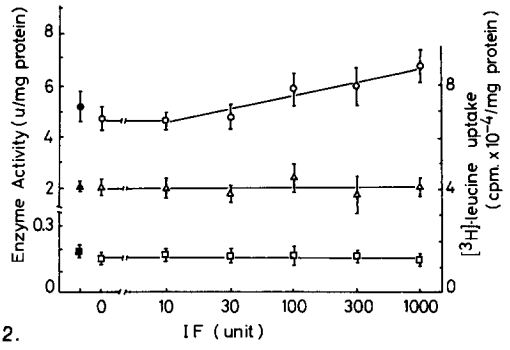

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Fig. 1. Effect of interferon on the GS activity of embryonic chick neural retina. Retinas were incubated for 15 hrs in the presence of various concentrations of interferon. Subsequently, the culture medium was replaced with the fresh one with or without 2 μg/ml of hydrocortisone. After a 24-hrs of induction period for GS, the tissues were harvested and the enzyme activities were determined as described in Methods. Each value represents the mean ± S.E. of 6 flasks subtracted the enzyme level at the initiation of the culture (0.27 u/mg protein). ●—●, without hydrocortisone; O—O, with hydrocortisone; triangles: treated with mock interferon of which protein concentration was approximately equal to that of 1,000 u of interferon preparation. ▲ , without hydrocortisone; △, with hydrocortisone. Four independent experiments yielded essentially the same results.

Fig. 2. Effect of interferon on the retinal enzyme activities and protein synthesis. O, LDH; □, AChE; △ , [^{3}H]leucine incorporation. Each value represents the mean ± S.E. of 6 flasks. Filled symbols indicate the value without interferon and hydrocortisone.

of interferon throughout the culture period of 39 hrs (Fig.2).

A portion (3-5 %) of antiviral activity of added interferon was found in the enzyme preparation (retinal homogenate). However, addition of 1,000 units of interferon in the assay mixture for GS did not reduce nor enhance GS activity.

To test whether the establishment of interferon-stimulated antiviral state is sensitive to the presence of hydrocortisone or not, following experiments (data not shown) were performed. Immediately after the initiation of the culture, retinas received serial dilutions of interferon or mock interferon with or without 2, 5, 10 and 20 μg/ml of hydrocortisone. At 15 hrs, these cultures were infected with GD-7 virus (Methods). The levels of antiviral state in the hormone-treated retinas (as determined by interferon titer obtained) were equal to those of control cultures which received interferon alone.

DISCUSSION

The hydrocortisone-induced increase of GS activity was shown to be a precocious synthesis and accumulation of the enzyme (7,8), which is normally scheduled *in ovo* later by 5-6 days. Evidences presented here, therefore, seem to indicate that interferon suppresses one of those processes leading to the differentiation of chick retina. As other parameters of the cultured retina, i.e., the levels of 1) LDH and 2) AChE, 3) rate of protein synthesis, 4) total protein content of retina (approximately 2.5 mg per piece of retina), and 5) the total cell numbers per culture (approximately 10^8, counted by hemocytometer after tryptic dispersion) were unaffected by the presence of either interferon or hydrocortisone, the suppression of GS in terms of specific activity was neither due to a cytolytic or cytocidal events, nor due to a proliferation of the cells. Considering the fact that interferon did not interfere with the assay for GS in retinal homogenate, the inhibitory effect of interferon preparation reported herein seemed to be an event specific to GS induction.

Although the results with 2 μg/ml of hydrocortisone were reported in this communication, it has been demonstrated that the concentration of the hormone as low as 0.01 μg/ml induced maximal development of GS. Furthermore, as expression of antiviral activity of interferon in cultured retina was not affected by the hormone, it seems unlikely that the observed suppression of GS level by interferon is related to a competition between interferon and hydrocortisone in binding to cellular receptors. In addition, spontaneous increase of GS level by 3-fold during *in vitro* cultivation was also sensitive to the suppressive effect of interferon (Fig.1).

Besides its antiviral activity, interferon has been shown to exhibit various effects on animals and cultured cells (1-4). Findings reported here appear to add another complexity to the biological role of interferon. However, considering the present status that interferon preparation free from contaminants of cellular proteinous material is not available, further analysises are required to ascribe these biological activities to interferon molecule *per se*.

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