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EFFECT OF RAT (BETA)-INTERFERON ON INTRACELLULAR LEVELS OF HYDROLASES IN RAT EMBRYONAL FIBROBLASTS (WISTAR STRAIN)

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Summary. — The effect of rat beta-interferon (IFN) on the intracellular level of thiole and acid proteases and alkaline phosphatases was investigated. When nontransformed rat embryonal fibroblasts (REF) (Wistar strain) were treated with homologous IFN, a time independent decrease of hydrolase enzyme levels was observed. IFN treatment of transformed cells lead to a time dependent decrease of thiole proteases within 90 min. The values for acid proteases remained unchanged after the short term IFN treatment.

Key words: beta interferon; transformed and nontransformed rat embryonal fibroblasts (Wistar strain); intracellular hydrolases; thiole proteases; acid proteases; alkaline phosphatases; inhibition

Introduction

In search for the possible mechanisms of the anticellular activity of IFN, the growth inhibition was found to be parallel with the intracellular enzymatic changes in different model systems (Koono *et al.*, 1974; Strauli, 1980; Korbelik *et al.*, 1984). All three types of IFN's (alpha, beta, gamma) showed a substantially stronger inhibition of proteolytic enzymes in transformed as compared to normal cells (Schauer *et al.*, 1983).

The described experiments were performed on rat embryonal fibroblasts (Wistar strain). It was found, that heterologous IFN's (human alpha and gamma) decreased the values of neutral proteases in nontransformed cells and have different effects on cathepsins B and H. When nontr ansformed cells were analysed an increase of the values was obtained in comparison to transformed cells, showing decreased specific enzyme activities. Following the effect of homologous IFN (rat beta), a decrease of neutral protease and cathepsin B and H activities could be observed. It was found that the reduction of neutral proteases was stronger in nontransformed than in transformed cells, where a stronger inhibition for cathepsin B and H was detected.

To assess the so-called short term changes occurring within 15, 30, 45, 60, 90 and 190 min, experiments were started to find out the morphological and growth characteristics changes. During these experiments (Filipič *et al.*,

1984*c*) after binding to the cell surface IFN acted relatively fast; the changes connected with the anticellular activity differed, probably depending on the phase of the cell cycle, and on the phase of cell transformation. It seems, that the majority of changes connected with anticellular activity were triggered within first 60—180 min.

Considering these facts, we performed experiments to detect the influence of homologous (rat beta) IFN on the intracellular levels of some hydrolases of nontransformed and transformed rat embryonal fibroblasts (Wistar strain). The changes of specific activities were analysed in cells after a short term treatment of 15—180 min.

Materials and Methods

Cells. Rat embryonal fibroblasts (Wistar strain) and its nontransformed counterparts (Filipič *et al.*, 1982, 1984*a*) were used. The cells were cultivated in plastic Petri dishes (Nunc) using Eagle's medium supplemented with 10% newborn calf serum (Flow). The cultivation was carried out in the CO_2 atmosphere at 37 °C.

IFN used in these experiments was poly I : C-induced rat beta showing specific activity of $10^8 - 10^9$ units/mg protein. The method of its preparation and purification was described in detail elsewhere (Filipič *et al.*, 1984*b*). For the cell treatment 1000 units/ml were used (mock in control).

Cell treatment. Trypsinized cells with Eagle's medium and serum were seeded into plastic Petri dishes for 60 min. Thereafter, the IFN (mock in control) was added for 15, 30, 45, 60, 90 and 180 min. After incubation the cells were detached using trypsin. The suspension obtained was then centrifuged (to sediment the cells) and washed twice with medium containing no serum. Thereafter, 2 ml of distilled water was added to the sediment suspension obtained was homogenized by the freezing-thawing method (3—4 times). The next step was the centrifugation (1200 rev/min; 10 min) to sediment the nuclei. The supernatant was used for protein determination (Lowry *et al.*, 1961) and for enzyme analyses.

Enzyme analyses. The following enzyme analyses were performed:

Thiole proteases were measured using the following method (Suhar *et al.*, 1981): to 50 µl of the sample 200 µl of PBS (phosphate buffered saline, pH 6.8) and 2.5 mmol/l of the cysteine were added. After incubation for 5 min at 37 °C, 25 µl of BANA (benzoil-arginin-2-naphtyl amide) was added, and incubation continued for 60 min at 37 °C. The reaction was stopped using the parachlor-mercuribenzoate and Fasgarnet (1 : 1, 1 ml). The extinction was measured at 520 nm.

Acid proteases (*cathepsin D*) were measured by the method of Anson (1939) as follows: to the 50 µl of the sample, 150 µl of the distilled water and 1 ml of 2% haemoglobin (Hb) were added. After incubation for 1 hr, 1 ml of 0.3 mol/l TCA (trichloroacetic acid) was added, and the mixture filtered. Two ml of filtrate was then used for each measurement. To measure the enzyme activity 4 ml of the folin reagent and 1.2 ml of 0.5 mol/l NaOH were added. The extinction was determined at 750 nm.

Alkaline phosphatases were measured according to Chou (1979): to the 50 µl of the substrate (p-nitrophenyl phosphate) 1 ml of distilled water was added. After 5 min preincubation, the cell supernatant (100 µl) was added. Thereafter, the incubation was continued for 20 min, in the presence of PBS pH 9.0. The extinction was measured at 550 nm.

Results

Studying the effect of rat beta-IFN on the intracellular thiole proteases a time independent decrease of enzyme level could be seen in nontransformed cells (Fig. 1). A different picture was observed in IFN-treated transformed cells, where thiole proteases reached the maximum activity after 90 min. Later on a slight decrease could be observed. Analysis of the intracellular

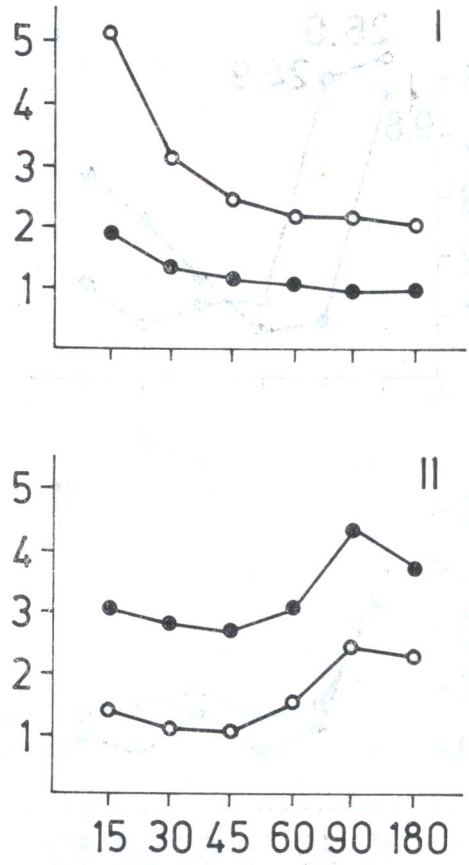

Fig. 1.
Effect of short term rat beta-IFN treatment of the intracellular level of thiole protease (specific activity)
Nontransformed (I) and transformed (II) rat embryo fibroblasts (Wistar strain) were treated with 1000 units/ml of rat beta-IFN for 15—180 min (The enzyme level was determined as described in Materials and Methods).
○———○ Nontreated cells (control)/ /mock treated
●———● IFN-treated cells
Abscissa: time (min); ordinate: NA = = nM naphtyl amine/mg/min.

levels of acid proteases showed the following (Fig. 2): in IFN-treated transformed cells protease activity remained unchanged, while in nontransformed cells, after an initial decrease, the enzyme activity culminated by 90 min, the maximum being reached within 180 min. In the case of alkaline phosphatase (Fig. 3), the kinetics of enzyme levels seemed similar in both nontreated as well as IFN-treated transformed cells, but quantitative differences could be seen. In nontransformed cells, the enzyme level decreased in untreated cells, while in IFN-treated ones a time independent decrease was seen throughout.

Discussion

The presented experiments were conducted on spontaneously transformed rat embryonal fibroblasts (Wistar strain) and on their nontransformed counterpart. The cells used throughout were in the phase A (primary culture)

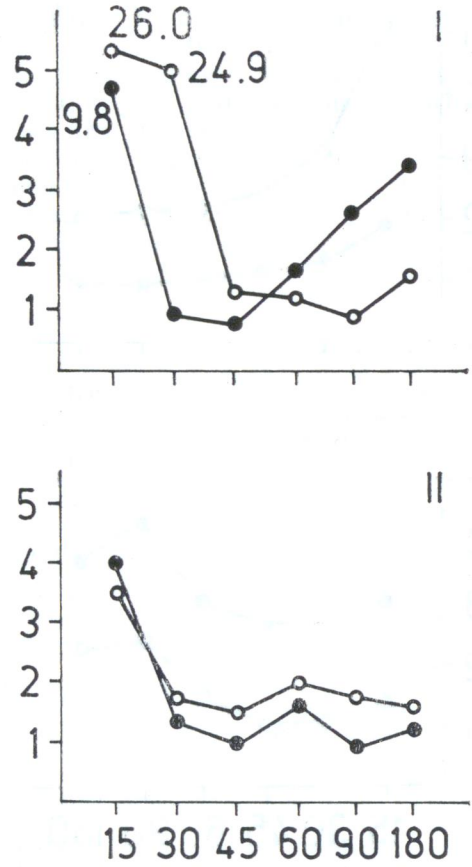

Fig. 2.
Effect of short term rat beta-IFN treatment on the intracellular level of acid proteinase (specific activity)
Abscissa: time (min); ordinate: tyrosine nM/min/mg. For other explanations see Fig. 1.

and phase E (permanent cell line) (Filipič *et al.*, 1984*a*). This is of importance from the standpoint of so-called basic levels of different hydrolases and of the differences in sensitivity to the IFN action as well. It has to be pointed out, that both morphological and growth characteristics were studied in parallel in the same system.

Generally we found no differences in cell morphology, but the effects on transformed cells were observed earlier than on nontransformed cells. The first morphological changes were noticed after 15—30 min. In contrast to this, in our study of IFN effect on the actine organisation (Filipič *et al.*, 1985), the nontransformed cells were found to be more sensitive than transformed cells. Having all this in mind, we attempted to find out whether effects of IFN listed above (morphology, growth characteristics, nuclear blebs, actine organization) were parallelled by changes in enzyme levels (thiole, acid proteases and alkaline phosphatases). In nontransformed cells the decrease

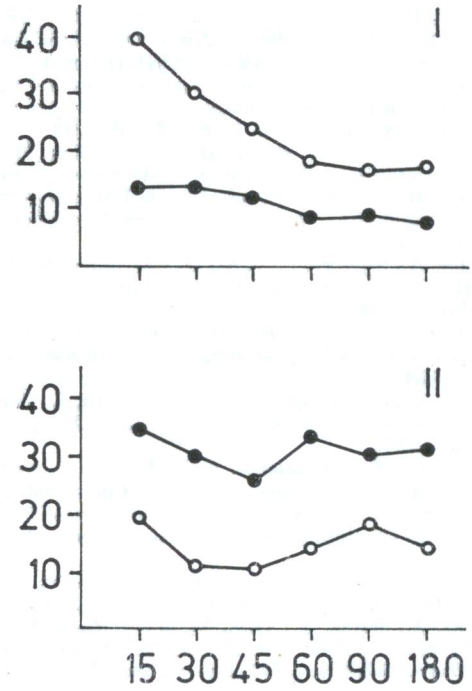

Fig. 3.
Effect of short term rat beta-IFN treatment of the intracellular level of alkaline phosphatase (specific activity) Abscissa: time (min); ordinate: I.U. = International units/mg
For further explanations see Fig. 1.

of thiole protease and alkaline phosphatase levels was time independent in contrast to transformed cells, where time-dependent changes were observed (thiole proteases reached maximum after 90 min, alkaline phosphatases by 180 min). The values for the acid proteases (specific activities) remained unchanged after the short term IFN treatment.

We conclude that the enzyme changes during the spontaneous transformation and concomitant sensitivity to IFN show the "trend" to a time dependent differentiation. The mechanism of relatively greater sensitivity of transformed cells to IFN remains unknown, even if it seems possible that the regulatory role of different intracellular hydrolases (proteases, alkaline phosphatases) and in connection to this also the role of proteases and their inhibitors should be taken into account (Bohley *et al.*, 1984).

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