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RELATIONSHIP BETWEEN THE CELLULAR EXPRESSION OF THE ANTIVIRAL AND ANTICELLULAR ACTIVITIES OF INTERFERON

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This study suggests that the expression of the antiviral and anticellular activities of interferon in rat cells treated with homologous interferon are closely linked After addition and subsequent removal of interferon, the two activities appeared and disappeared simultaneously Both activities were equally influenced by the exposure time and by the temperature during exposure to interferon Pretreatment of the cells with cholera toxin inhibited both activities to the same extent at low interferon levels

rat interferon antiviral anticellular kinetics

INTRODUCTION

Interferon preparations have multiple effects on cells [8]. From studies with pure interferon, it is clear that most of the effects reside in the same protein [9,13]. The most thoroughly studied effects of interferon are the induction of the antiviral state and the inhibition of cell growth. Much is known about the mechanism of action of the antiviral activity of interferon. The first step is binding of interferon to cell surface receptors. The cell membrane interaction seems to be biphasic: when cells are treated with interferon at 4°C, the binding of interferon is reversible. At 37°C, interferon binds irreversibly to the cell membrane [4] and a metabolic pathway resulting in the inhibition of viral replication is activated, probably by induction of 2′,5′-oligonucleotides [18]. It is not yet clear whether the inhibition of cell growth by interferon follows the same pathway. Both activities in human cells seem to be regulated by chromosome 21 [1,10]. Degré [3] reported that cholera toxin inhibited both the anticellular and antiviral activities, suggesting that the same receptor site is involved in both activities. Fuse and Kuwata [6] reported that cholera toxin inhibited only the antiviral and not the anticellular effect, indicating a different pathway. Similar conflicting results were also reported for the effect of ouabain [2, 14]. Kimchi et al. [12] reported that, like the antiviral activity, the anticellular effect involves the induction of 2′,5′-oligo(A)synthetase. Hovanessian and Wood [11] recently showed that treatment of cells with 2′,5′-oligonucleotides resulted in inhibition of cell growth as well as induction of the antiviral state.

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The present study was undertaken to relate the antiviral and anticellular activities of rat interferon by comparing the kinetics of the two effects. We also studied the effect of incubation temperature and of cholera toxin on both effects.

MATERIALS AND METHODS

The origin of the Ratec cells was as previously described [15]. Cells were cultivated in minimal essential medium (Dulbecco's modification: DMEM), supplemented with antibiotics and 10% heat-inactivated foetal calf serum (FCS) at 37°C in a CO_2 incubator with a humidified atmosphere. Cholera toxin was purchased from Sigma and stored at 4°C. Rat interferon was induced in Ratec cells with Newcastle disease virus (NDV), semi-purified and concentrated as described earlier [15]. Unless stated otherwise, the specific activity of the interferon preparations was about 10^6 units · mg^{-1} protein. Control preparations were prepared from supernatant of Ratec cells harvested immediately after challenge with NDV. The activity of the interferon preparations was assayed by a semi-micromethod employing Ratec cells and vesicular stomatitis virus (VSV) as described earlier [15,17]. Alternatively, the antiviral activity was measured by a yield reduction assay in a single cycle of VSV infection. Cell growth inhibition was determined by measuring tritiated deoxythymidine ([^{3}H]TdR) incorporation as described by Fuse and Kuwata [5].

RESULTS

Kinetics of the antiviral and anticellular effects of interferon

The time necessary for the antiviral and anticellular effects to become evident after contact with interferon is shown in Fig. 1. Cell cultures were incubated with 200 units · ml^{-1} of interferon. At regular time intervals, triplicate cultures were used for assays of antiviral and growth inhibitory effects. It can be seen that the two activities developed with similar kinetics. Both activities were manifest in cells 2 h after contact with interferon and both required 6–8 h to develop fully. Table 1 shows an experiment in which the cultures were exposed to interferon (200 units · ml^{-1}) for 20 h, washed and reincubated for various time intervals, before assaying antiviral and growth inhibitory effects. The results indicated that, when interferon was removed, the two activities disappeared from the cells at comparable speed. Both activities were still maximally expressed 24 h after removal of interferon, and both started to diminish after 48 h. After 120 h, virtually no antiviral or anticellular effect could be demonstrated. The antiviral and anticellular activities were also closely linked, when the influence of exposure time on both was determined. Table 2 shows the results of an experiment in which cultures were treated with interferon for different time periods, washed and reincubated for a fixed number of hours, after which the antiviral and growth inhibitory effects were measured. Five min exposure was sufficient to induce both activities and a 6 h contact period was necessary for both to be fully expressed.

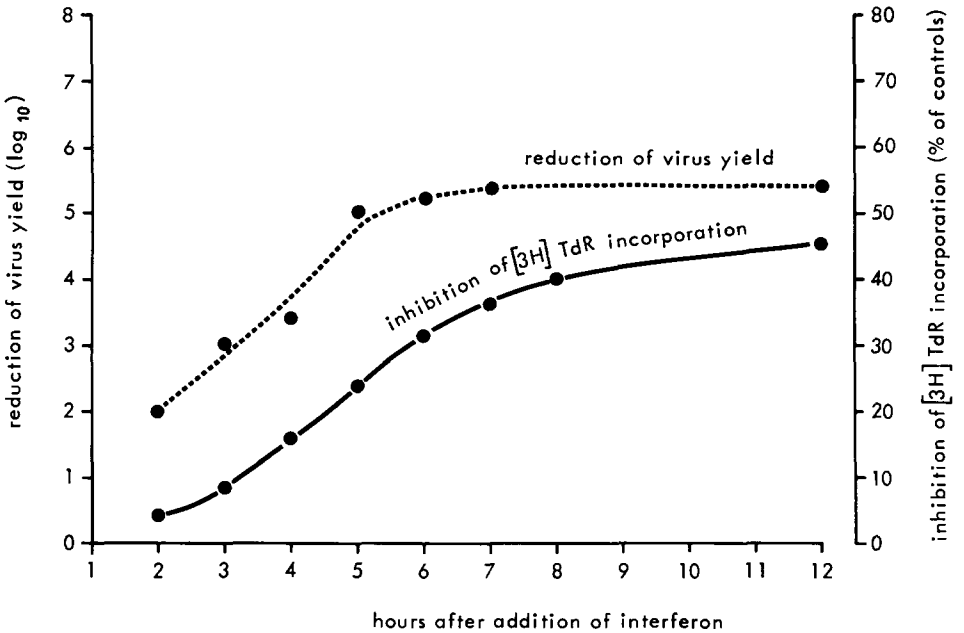

Fig 1. Kinetics of the development of the antiviral and anticellular effects of interferon. Ratec cells were treated with 200 units rat interferon · ml^{-1}. Antiviral as well as anticellular activity were measured at the indicated time points. ●—●, Inhibition of [^{3}H]TdR incorporation (anticellular effect). ●---●, Inhibition of virus yield.

Incubation of cells at 4°C during exposure to interferon was found to reduce the expression of both the antiviral and the anticellular effects of interferon to a comparable extent. This was shown by the results of an experiment in which cell cultures were incubated with 200 units · ml^{-1} of interferon for 6 h at 37°C or at 4°C. After removal

TABLE 1

Kinetics of disappearance of the antiviral and anticellular effects of interferon after its removal from cells

Time (h) after removal of interferon	Anticellular activity (% inhibition)	Antiviral activity (log_{10} yield reduction)
0	73	7.9
24	77	8.1
48	56	5.9
120	0	0.5

Ratec cells were treated with 200 units of rat interferon · ml^{-1} for 20 h. The interferon was then removed, the cells replenished with fresh medium, and the antiviral effects determined as well as the growth inhibition were measured at the indicated time intervals

TABLE 2

Influence of length of exposure time on the development of the antiviral and anticellular effects of interferon

Length of exposure time (min)	Antiviral activity (log_{10} yield reduction)	Anticellular activity (% of inhibition)
5	2 0	17
15	2 1	18
30	2.5	15
60	3 1	23
120	4 0	31
180	5 4	30
240	8 2	37
300	8 3	37
360	8 3	44

Ratec cells were treated with 200 units rat interferon · ml^{-1} for the time intervals indicated The interferon was then removed and the antiviral effect determined after 21 h further incubation The anticellular effect was determined 18 h after the start of interferon treatment

of interferon, the cells were reincubated at 37°C for 24 h and tested for both cell growth inhibitory and antiviral effects. Incubation at 4°C reduced the cell growth inhibitory effect from 50 to 16 (% inhibition of DNA synthesis) and the antiviral effect from 5.3 to 2.1 (log_{10} virus yield reduction).

Finally, we tested whether pretreatment of the cells to cholera toxin could be able to discriminate between the two activities of interferon on cells. Cell cultures were incubated for 3.5 h with cholera toxin at different concentrations, as indicated in Table 3. Interferon was then added to the medium (2 or 200 units · ml^{-1}) and the cultures were further incubated for 16 h. Cell growth inhibition and viral yield reduction was then measured. It can be seen that, with the lower concentration of interferon, both activities of interferon were inhibited in parallel. With the higher concentration neither activity was influenced by pretreatment of the cells with cholera toxin.

DISCUSSION

This study seems to indicate that there is a close link between the development of the antiviral state and the inhibition of cell growth by interferon. The receptor sites for both activities on the cell membrane are likely to be identical as both activities were equally affected by pretreatment with cholera toxin, exposure time with interferon, and temperature during exposure with interferon. The existence of identical or closely related receptor sites for both activities is not a surprising concept, as it is now well established that both activities are caused by the same protein, but it does not exclude that different pathways are followed for both effects after interaction with the receptor. However,

TABLE 3

The effect of cholera toxin on the antiviral and anticellular activities of interferon

Cholera toxin ($\mu g \cdot ml^{-1}$)	Antiviral activity (log_{10} yield reduction)	Anticellular activity (% inhibition)
2 units interferon $\cdot ml^{-1}$		
0	2 7	64
0.1	1.9	36
1.0	1.7	41
10.0	1 6	25
200 units interferon $\cdot ml^{-1}$		
0	4.9	80
0.1	5.2	78
1.0	5 6	77
10 0	5 7	76

Ratec cells were incubated for 16 h in medium containing cholera toxin (0–16 h) and interferon (3 5–16 h) At the end of the incubation period both antiviral and cell growth inhibitory effects were measured

the similar kinetics of development of both effects after interferon treatment and the similar kinetics after removal of interferon indicate that both activities are the result of the same intracellular processes.

The effect of exposure time and temperature during exposure, and the kinetics after addition and removal of interferon are comparable to earlier studies on the *antiviral* effect of interferon [10, 16]. The effect of cholera toxin found by us is in agreement with that reported by Degré [3]. Fuse and Kuwata [6], however, reported that cholera toxin inhibited the antiviral activity of interferon, while the anticellular effect remained unaffected. This could be due to the particular cell/interferon system which they chose for their study. It should, however, be stressed that cholera toxin can have varied effects on cells. It elevates the intracellular cyclic AMP level and cyclic AMP has been reported to influence interferon activity [7]. Therefore, our results and those of others are no conclusive evidence that a receptor site is involved in the interaction between interferon and cells. However, aside from the question whether an identical receptor is involved, both the antiviral and anticellular effects of interferon are equally affected by cholera toxin, indicating a close relation between these two effects.

From our results, it could well be argued that the antiviral and the anticellular activities are expressions of the same intracellular events, as suggested by Kimchi et al. [12] and Hovanessian and Wood [11]. However, the question how close both activities are linked can only be answered when the exact molecular mechanisms involved in those activities will be elucidated.

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