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Cell Biology

Effect of interferon on concentrations of cyclic nucleotides in cultured cells

(chemostat culture/steady-state conditions/cell division)

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ABSTRACT **Constant intracellular concentrations of both adenosine 3′,5′-cyclic-monophosphate (cyclic AMP) and guanosine 3′,5′-cyclic-monophosphate (cyclic GMP) were obtained when mouse leukemia L1210 cells were cultivated under steady-state conditions in the chemostat. In this sensitive and controlled system addition of mouse interferon resulted in a rapid (5–10 min) increase in the intracellular concentration of cyclic GMP, which preceded by several hours an increase in the intracellular concentration of cyclic AMP. In contrast to the effect of interferon, addition of prostaglandin E_1 induced a rapid increase in the intracellular concentration of cyclic AMP without markedly affecting the intracellular concentration of cyclic GMP. It is suggested that the rapid effect of interferon on cyclic GMP plays a role in mediating some of the effects of interferon on cells.**

In addition to inhibiting viral multiplication, interferon exerts a number of effects on cells—such as inhibition of cell multiplication (1–3), enhancement of specialized cellular functions (3–9), and modification of the cell surface (3, 10–13). Interferon also exhibits a marked antitumor action (3) and can influence the immune system (3, 14). There are reports that interferon induces an increase in the levels of adenosine 3′,5′-cyclic-monophosphate (cyclic AMP) that precedes the establishment of the antiviral state (15, 16) or occurs concomitantly with an inhibition of cell multiplication (17). It is therefore tempting to speculate that activation of adenylate cyclase by interferon and the subsequent increase in the intracellular concentration of cyclic AMP is responsible in part for the diverse effects of interferon. However, these studies were undertaken in conventional batch culture in which the environment is continuously changing, rendering difficult the interpretation of changes in intracellular cyclic AMP concentration. These difficulties can be overcome by the use of chemostat culture, in which cell multiplication occurs at a constant rate and in a constant environment. The steady-state cell concentration is controlled by the concentration of glucose in the nutrient medium and cell growth rate is controlled by the rate of supply of glucose to the culture. We have recently described the establishment of steady-state cultures of animal cells in the chemostat and the application of this technique to the investigation of the effect of interferon on tumor cell multiplication (18–20). We report herein that treatment of steady-state chemostat cultures of mouse leukemia L1210 cells with interferon results in a rapid increase in the intracellular concentration of guanosine 3′,5′-cyclic-monophosphate (cyclic GMP). This effect precedes by several hours an increase in the concentration of cyclic AMP and inhibition of cell multiplication.

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MATERIALS AND METHODS

Chemostat Cultures. Mouse leukemia L1210 cells were cultivated under glucose limitation in a chemostat (300 ml working volume) in Eagle's minimal essential medium supplemented with 10% heat-inactivated horse serum as described (18). Samples (<1% of culture volume) were removed directly from the culture vessel via a sampling port.

The dilution rate D (day^{-1}) is defined as the quotient medium flow rate (ml/day)/culture volume (ml). In the steady state, $\mu = \ln 2/t_d = D$, in which μ is specific growth rate and t_d is doubling time of cells.

Cyclic Nucleotide Determinations. The intracellular concentrations of the cyclic nucleotides were determined in 1.0 ml of cell suspension after centrifugation (800 × *g*, 5 min) and extraction with 2 ml of 6% trichloroacetic acid at 0°C. The suspension was then centrifuged (2000 × *g*, 10 min) and the supernatant was extracted with ether and assayed for cyclic AMP content by using a protein binding assay as described (21) and for cyclic GMP content by using Boehringer Mannheim test kits. Recovery (70–80%) was monitored with 1 nCi (1 Ci = 3.7×10^{10} becquerels) of cyclic [^{3}H]AMP or cyclic [^{3}H]GMP. The acid-precipitable material was dissolved in 1.0 ml of 0.6 NaOH and assayed for protein content by the method of Lowry *et al.* (22). Results are expressed as the mean of four replicates.

[^{3}H]Thymidine Incorporation. Samples were removed directly from the chemostat and pulse labeled with 5 μCi of [^{3}H]thymidine (25 Ci/mmol; Commissariat à l'Energie Atomique, France) for 30 min at 37°C. Incorporation of [^{3}H]thymidine into cellular material was determined by measuring the radioactivity in the trichloroacetic acid-insoluble material retained on a Millipore filter, as described (19).

Interferon Preparations. Interferon was prepared from suspension cultures of mouse sarcoma C243-3 cells inoculated with Newcastle disease virus. The methods of production, partial purification, and assay of the interferon have been described (23). The interferon used in this study had a specific activity of 2×10^7 reference units/mg of protein. Mock interferon was prepared and purified in a manner identical to that for interferon (23), with the exception that the virus inducer was added just prior to harvesting the culture supernatant.

Experimental Plan. Because of the long duration of chemostat experiments it was feasible to use only a single interferon concentration throughout this study. On the basis of previous experience (19, 20) we chose 6400 units/ml, which corresponds to between 1 and 2 units of interferon per 10^3 cells in the chemostat. Interferon was introduced into the chemostat by injection into the culture vessel and by simultaneous addition to the inflowing medium to maintain a constant concentration for the duration of each experiment (2–4 days).

Abbreviation: PGE_1, prostaglandin E_1.

RESULTS

Concentrations of Cyclic Nucleotides during the Steady State. A period of adjustment ranging from approximately 100 to 450 hr of continuous operation of the chemostat was necessary for the establishment of steady-state cultures of L1210 cells. Steady states were characterized by a constant content of DNA, RNA, and protein per cell, a constant percentage of cells labeled by autoradiography, and constant rates of incorporation of [^{3}H]thymidine, [^{3}H]uridine, and ^{14}C-labeled amino acids into cellular acid-precipitable material (18). Individual steady states have been maintained for up to 600 hr of continuous operation of the chemostat (18).

Constant intracellular concentrations of cyclic AMP and cyclic GMP were not obtained until some time after the establishment of a constant cell concentration. However, once a steady state became established, the intracellular concentrations of both cyclic AMP and cyclic GMP remained constant, so that the standard deviation of the mean concentrations of cyclic AMP and cyclic GMP were the same as the standard deviation of replicates of individual points (Fig. 1).

Effect of Interferon on the Steady-State Intracellular Concentrations of Cyclic GMP and Cyclic AMP. In three experiments a 2- to 4-fold increase in the intracellular concentration of cyclic GMP was observed 5–10 min after the addition of interferon. The concentration of cyclic GMP then returned to its previous steady-state value between 20 and 50 min later (Fig. 2). No increase in the intracellular concentration of cyclic AMP was observed during the first 5 hr after contact with interferon. A slight increase (35–64%) in the concentration of cyclic AMP was observed at 6 and 9 hr in one experiment,

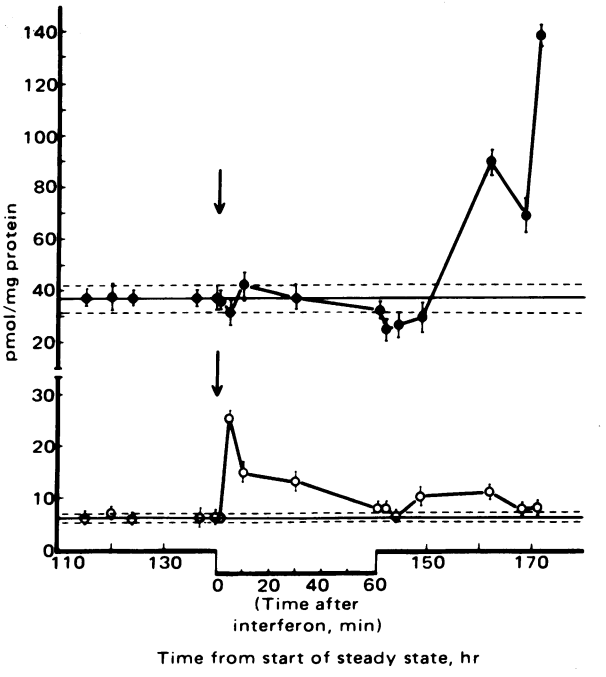

FIG. 2. Effect of interferon on the steady-state intracellular concentrations of cyclic AMP (●) and cyclic GMP (○). Dilution rate 0.5 day^{-1} (t_d 33.2 hr). Initial glucose concentration 1.0 mg/ml. The horizontal solid lines with broken lines above and below represent steady-state mean value with SD. The error bars represent the SDs of the replicates for a particular point. ↓, Time of addition of interferon. Note expanded time scale for 1 hr after addition.

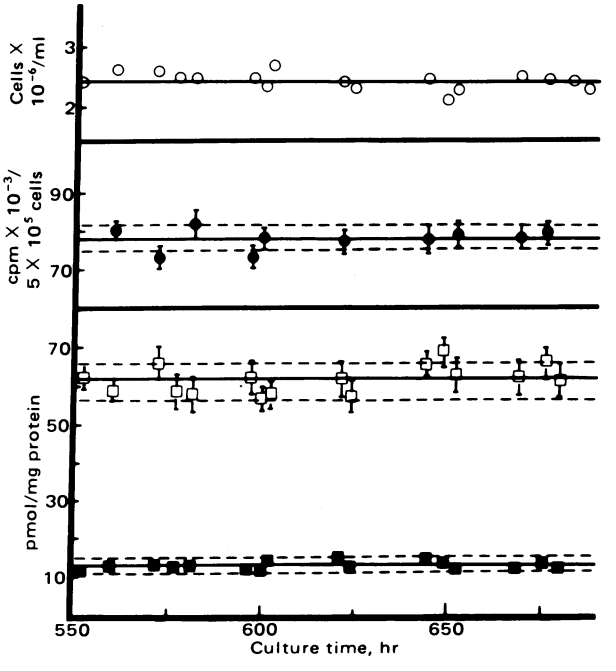

FIG. 1. A chemostat culture of L1210 cells under steady-state conditions at a dilution rate of 0.5 day^{-1} (t_d 33.2 hr). ○, Cell concentration (steady-state mean ± SD: 2.4 ± 0.16 × 10^6 cells per ml). ●, Incorporation of [^{3}H]thymidine into acid-precipitable material (steady-state mean ± SD: 77,860 ± 3113 cpm/5 × 10^5 cells). □, Intracellular concentration of cyclic AMP (steady-state mean ± SD: 61.8 ± 5.0 pmol/mg protein). ■, Intracellular concentration of cyclic GMP (steady-state mean ± SD: 12.7 ± 1.2 pmol/mg protein). Solid lines with broken lines above and below represent steady-state mean value with SD. The error bars represent the SDs of the replicates for a particular point.

and at 9 hr only in a second experiment, whereas in a third experiment no increase in the concentration of cyclic AMP occurred until 24 hr after interferon. In all three experiments the first pronounced increase (2 to 3.8 fold) in the concentration of cyclic AMP occurred 24 hr after the addition of interferon (Fig. 2) concomitantly with the first significant inhibition of cell multiplication. Mock interferon had no effect on the steady-state concentrations of either cyclic AMP or cyclic GMP. The increase in the concentration of cyclic GMP in the chemostat after addition of interferon was followed 2 hr later by an inhibition of [^{3}H]thymidine incorporation (30–50%)—that is, 22 hr prior to an inhibition of cell multiplication (19). [We have previously shown that the inhibition of thymidine incorporation in the chemostat results from an inhibition of thymidine transport rather than from an inhibition of DNA synthesis (24), and it may reflect an effect of interferon on the cell surface.]

Effect of Prostaglandin E_1 on the Steady-State Intracellular Concentrations of Cyclic AMP and Cyclic GMP. The effect of prostaglandin E_1 (PGE_1) on the steady-state concentration of cyclic AMP was compared to that of interferon, in order to determine the sensitivity of chemostat cultures to substances that are known to enhance cyclic AMP levels. Treatment of chemostat cultures of L1210 cells with PGE_1 resulted in a marked inhibition (45–64%) of [^{3}H]thymidine incorporation after 2 hr, followed by an inhibition of cell multiplication at 24 hr (data not shown). These effects were quite comparable to those observed with interferon. However, as previously reported (25), PGE_1 caused a marked rapid increase (5 and 10 min) in the steady-state concentration of cyclic AMP without exerting a pronounced effect on cyclic GMP (Fig. 3).

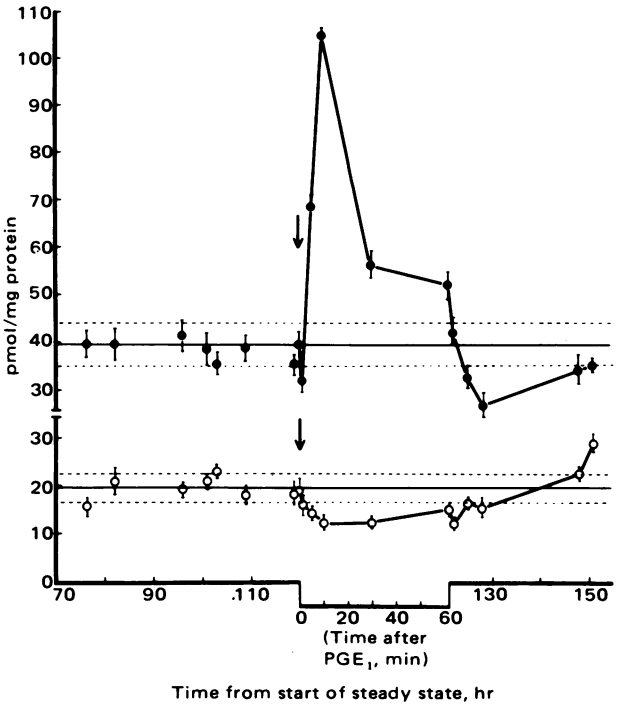

FIG. 3. Effect of PGE_1 on the steady-state intracellular concentrations of cyclic AMP (●) and cyclic GMP (○). Dilution rate 1.0 day^{-1} (t_d 16.6 hr). Initial glucose concentration 1.0 mg/ml. Horizontal lines and error bars have same meanings as in preceding figures. PGE_1 (Sigma) was introduced directly into the culture vessel at the time indicated by ↓ and was added continuously to maintain a final concentration of 10 μM for the duration of the experiment.

DISCUSSION

Both cell density and cell growth rate are thought to influence and in turn be influenced by the intracellular concentrations of cyclic GMP and cyclic AMP (26–28), and the concentrations of both cyclic nucleotides have been found to change as cells multiply in batch culture (26–28). We have shown, however, that when mouse leukemia L1210 cells are cultivated under steady-state conditions in the chemostat the intracellular concentrations of both cyclic nucleotides remain constant.

The use of this sensitive and controlled system has enabled us to demonstrate that mouse interferon induces a rapid and marked increase in the intracellular concentration of cyclic GMP several hours prior to an effect on the concentration of cyclic AMP. This increase in the concentration of cyclic GMP, occurring 5–10 min after the addition of interferon, is the earliest effect of interferon on cells described to date.

A number of the effects of interferon on cells that occur within a few hours suggest an action of interferon on the cell surface (3, 14). The transitory elevation of cyclic GMP in interferon-treated cells may also reflect an interaction of interferon with the cell surface and may play a role in the development of some of the effects of interferon on cells. Thus, interferon has been reported to stimulate a number of processes that are also influenced by agents that increase cyclic GMP levels; such effects include enhancement of lysosomal enzyme release (29, 30), cytotoxicity of sensitized T lymphocytes (31, 32), benz[*a*]anthracene-induced synthesis of aryl hydrocarbon hydroxylase (5, 33), interferon production (7, 34), histamine release (6, 35), and the excitability of neurons (27, 36).

Interferon is not unique in affecting the concentration of cyclic GMP. Similar rapid yet transitory effects on the intracellular concentration of cyclic GMP have been observed in cells treated with mitogens (27, 37), transfer factor (38), cholinergic agents (27), and imidazole (27); these effects are thought to reflect a "membrane to nuclear signal" (37). Although the increase in the concentration of cyclic GMP is associated with a stimulation of cell division in lymphocytes treated with mitogens (27), some substances such as carbamoylcholine and imidazole increase the concentration of cyclic GMP without stimulating cell division (27).

It has been suggested that interferon activates adenylate cyclase and that the resultant increased levels of cyclic AMP mediate the inhibition of DNA synthesis and cell division (17). However, our results show that a significant effect of interferon on cyclic AMP levels was usually observed at 24 hr [in contrast to PGE_1, which induced a rapid increase in cyclic AMP (Fig. 3)]. Because these increased levels of cyclic AMP were associated temporally with an inhibition of cell multiplication in interferon-treated cultures, it seems likely that the changes in the intracellular concentrations of cyclic AMP were a consequence rather than a cause of the inhibition of cell multiplication.

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