Vol. 102, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 16, 1981 Pages 22-29

DEXAMETHASONE EFFECT ON THE GROWTH RATE, THE PLASMINOGEN ACTIVATOR, AND THE MMTV RNA DEPENDENT DNA POLYMERASE IN A MOUSE MAMMARY TUMOR CELL LINE

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Received June 30, 1981

SUMMARY

We have compared the effect of dexamethasone on the growth rate and the accumulation of two secreted proteins of an established cell line of mouse mammary carcinoma (GR). Whereas overall protein synthesis is not affected; the cell growth rate and both intracellular and secreted plasminogen activator are inhibited by dexamethasone treatment. In contrast the viral RNA dependent DNA polymerase secreted with viral particles is strongly stimulated by physiological concentrations of dexamethasone. These results, discussed with others, suggest that glucocorticoids regulate specifically but in opposite ways the synthesis of two secreted proteins.

Mouse mammary tumor cells have retained, like other cultured cells (hepatoma cells, lung cells), the capacity to respond to glucocorticoid hormones. Thus in mouse epithelial mammary cells, dexamethasone, a synthetic glucocorticoid, enhances the secretion of mouse mammary tumor viral MMTV particles (1,2). The hormone stimulates specifically the rate of MMTV RNA synthesis without affecting the overall RNA synthesis.

On the other hand, we know that numerous malignant cells transformed with oncogenic viruses contain, relative to their non malignant counterparts, an enhanced fibrinolytic activity, which is plasminogen dependent. We have previously shown that the GR mammary carcinoma cell

Abbreviations used: PA, Plasminogen activator; MMTV, Mouse mammary tumor virus; RT, reverse transcriptase; SDS, Sodium dodecyl sulfate.

0006-291X/81/170022-08$01.00/0

line expresses 2 forms of soluble intracellular plasminogen activator (PA) of molecular weight 45,000 and 68,000 daltons (3). Several investigations have shown that steroids inhibit PA activity. Thus, embryonic lung cells (4), mouse mammary carcinoma cells (5) and rat Hepatoma cells (6) have a reduced amount of PA in the presence of steroid and this effect is not correlated with a decreased cell growth rate. Conversely glucocorticoids have been shown to inhibit growth rate of established malignant cell lines (7) as well as normal cells lines.

We have analyzed the dexamethasone effect on the cell growth rate and on the intracellular PA activity of the GR mammary tumor cells. We have compared the inhibitory effect on PA with another secreted protein, the MMTV RNA dependent DNA polymerase (reverse transcriptase,(RT)).

MATERIAL AND METHODS

Cell culture: The GR cell line is currently subcultured in our laboratory since 1977 (gift of G. Tomkins Lab. San Francisco, California). It is derived from a spontaneous mammary epithelial carcinoma in GRS/A (GR) strain of mouse (8).

Cells are plated every 4 days at a seeding density of 300,000 cells/90/20 mm Falcon tissu culture dish in Dulbecco's modified Eagle medium with 10% horse serum, in an atmosphere of 94% air, 6%CO_2 at 37°C. In all experiments, cells seeded in the morning are allowed to attach overnight and the following day the medium removed and replaced by medium containing or not dexamethasone 10^{-7}M. Count of viable cells is done by trypan blue exclusion technic. For PA assays, medium with serum is removed and replaced by serum free medium with or without dexamethasone for 16 hours. In all experiments carried out, this time course have appeared to be non damageable for the treated or non treated GR cells.

PA assay. Extracts of GR cells and medium collecting are done as already described (3). PA is essentially found in the cellular fraction releasable by detergents meaning that it is mainly membrane-associated. For assays, every cell pellet is prepared with a ratio of 10^6 cells/10 µl of extraction buffer. Proteins determinations are done with Lowry method modified by Wang. (9).

a) Quantitative assay of PA is done in multiwell tissue culture dishes coated with ^{125}I-fibrogen (100,000 cpm per well) (10,11). Plasminogen and fibrinogen are purified following methods previously described (3). A unit of PA activity is defined as the activity which solubilizes 5% of initial radioactivity in an hour after correction for background using a substrate of 10 µg/cm^2; the specific activity is expressed in unit per 10^6 cells/hour unless otherwise mentioned.

b) Qualitative assay of PA is done one polyacrylamide SDS electrophoretic gel (12).

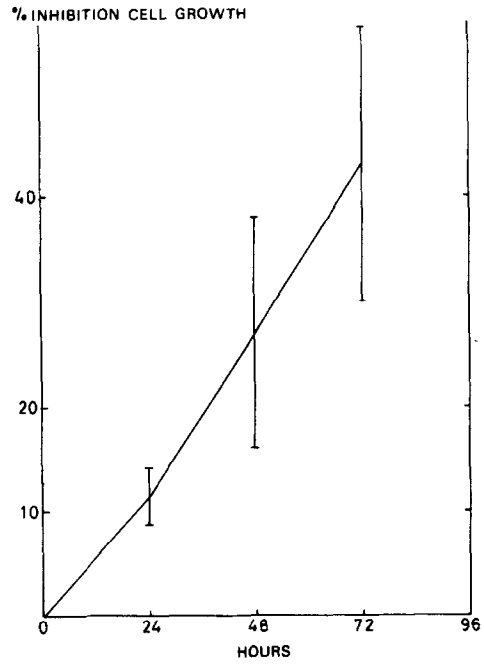

Figure 1. Effect of dexamethasone on cell growth rate

The curve is the result of 8 assays carried out as described in Methods. Cell growth is expressed in viable cells number and each assay was carried out with 10 tissue culture dishes (90 x20mm) and seeded with 3 x 10^5 cells per dish.

DNA polymerase, RNA dependent assay. The secretion of reverse transcriptase is assayed on 16 hour cell culture medium (complete medium or serum free-medium) following the method described by Dickson (13).

RESULTS

Effect of dexamethasone on cell growth rate

Figure 1. shows clearly an inhibitory effect of dexamethasone on exponentially growing GR cells which can reach a maximum of 50% in some experiments after 72 hours of hormone treatment. If dexamethasone is added on confluent cells no significant effect have been found after 24 or 48 hours of hormonal contact.

Effect of dexamethasone on total protein synthesis

By measuring the total protein concentration with the Lowry method modified by Wang (9) we do not observe any inhibition on the ove-

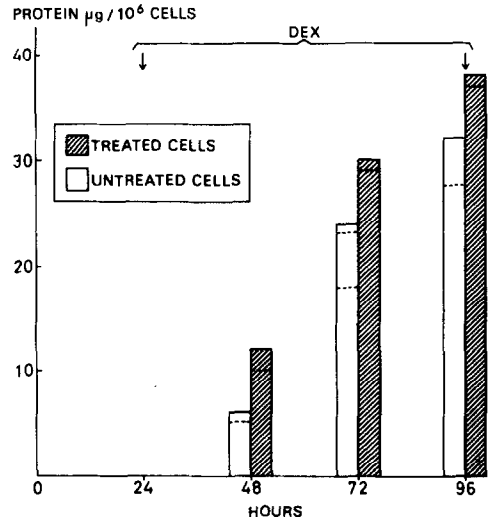

Figure 2. Effect of dexamethasone on the protein synthesis

Each block represents data of 3 assays done on a supernatant which has been prepared as described in Methods section; at time 0, the cells have been seeded at 3 x 10^5 cells per tissue culture dishes and after 24 hours dexamethasone was added. Medium with or without dexamethasone is routinely removed every 2 days.

rall protein synthesis but even a slight stimulation after 24,48 and 72 hours of treatment with desamethasone (figure 2). Analysis on two dimentional gel electrophoresis (14) of total proteins labelled for 4 hours with ^{35}S-methionine does not show any significant difference. (Results not shown).

Effect of dexamethasone on specific proteins

a) The intra and extracellular PA hase been measured as described in Material and Methods. In steady state condition, Figure 3 shows the inhibition of the accumulation of intracellular PA activity which appears to be linear and can reach 90% in some experiments after 72 hours of glucocorticoids treatment. The inhibition of PA activity does not seem to affect specifically one of the two active soluble forms of the enzyme (i.e, 45,000 and 68,000 daltons); it affects simultaneously both forms.

b) The reverse transcriptase associated with secreted MMTV viral

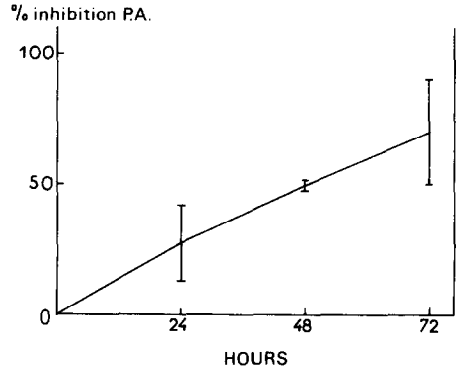

Figure 3. Effect of dexamethasone on the plasminogen activator activity.

This curve represents 8 assays done on the supernatant of cell cultures by measuring the solubilization of ^{125}I-fibrinogen into ^{125}I-fibrin; the specific activity of PA is expressed in unit/10^6 cells per hour (see text). One unit is usually around 5000 cpm of ^{125}I-fibrinogen solubilized.

particles have been measured (Figure 4). Control untreated cells show a slight decreased of this activity possibly related to the establishment of the exponential growth phase. Reverse transcriptase secreted from treated cells is 4 fold enhanced after 48 hours of treatment. This maximal stimulation can change (± 10%) following different experiments but it always appeared after 48 hours of contact with dexamethasone.

DISCUSSION

In this paper we have demonstrated two effects of dexamethasone on mouse mammary tumor cells in culture i) an inhibitory effect on the cell growth rate, ii) a specific inhibitory effect on the intracellular and extracellular PA.

In contrast to other cell lines described (4,5,6) the growth of GR cells plated at low density is strongly reduced by dexamethasone. Moreover, this effect does not induce morphological changes since both treated and untreated cultures form dome like structures after reaching confluence. Using different mammary cell lines Dickson (13) and Mcgrath (15,16)

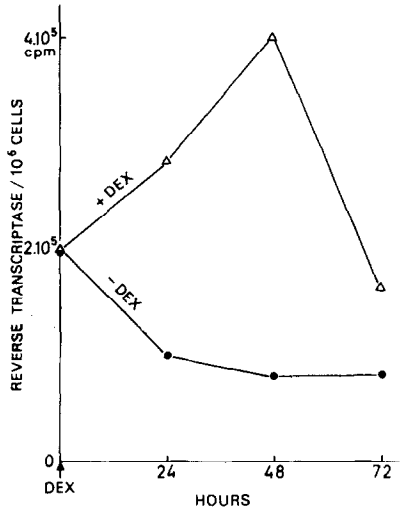

Figure 4. Effect of dexamethasone on the reverse transcriptase activity associated with viral particles secreted in the culture medium.

Reverse transcriptase was measured on the virus particles pellet obtained by centrifugation at 100,000g of 10 ml of culture medium. (^{3}H)TTP (specific activity: 25 Ci/mM) incorporation was measured as described by Dickson (13).

show that the absence of dome like structures in cells plated a low density is accompanied by a lower level of MMTV particle production. Our results do not show any cell density dependent effect on the amount of MMTV particles. This discrepency could be due either to the different mouse strains used (C_3H and GR) or to the different cell material: primary mouse mammary tumor cells culture on one hand, cloned epithelial mammary cell line one the other hand.

In parallel with this effect we were able to show an inhibition of the PA activity by physiological concentrations of dexamethasone. The inability to detect any difference in the rate of overall protein synthesis between hormonally stimulated and untreated cultures would indicate that this effect is specific and not a result of general metabolic inhibition.

An effect related to dexamethasone induced membrane conforma-

tional change is unlikely since the extent of inhibition is similar on the intracellular and the secreted PA. Moreover dexamethasone which stimulates MMTV RNA synthesis (17,18) and concomitately the production of MMTV particles does not decrease the virus secretion in the culture fluid. A 5 fold increase in the amount of MMTV synthesis, measured either by RNA hybridation with MMTV cDNA (results not shown) or by virus associated DNA polymerase activity, is detectable 48 hours after exposure of cells to dexamethasone.

Steroid hormones have been shown to exert their effect through cytoplasmic receptor mechanisms (19). In mammary tumor GR cells there are high affinity cytoplasmic glucocorticoid receptors which translocate to the nucleus and bind to specific nuclear sites (20). Consequently there is strong circumstantial evidence for the control of the rate of MMTV DNA synthesis through a receptor acting on the integrated viral genome. Dexamethasone could act in an opposite way on the PA synthesis through or independently of a decreased cell growth rate. Experiments measuring the concentration of PA molecules with monoclonal antibodies against PA will confirm this interpretation.

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