ANTICANCER RESEARCH *10:* 1241-1246 (1990)

Response of Human Immunodeficiency Virus Long Terminal Repeat to Growth Factors and Hormones

D.A. SPANDIDOS[1,2], V. ZOUMPOURLIS[1], A KOTSINAS[1], C. TSIRIYOTIS[1] and C.E. SEKERIS[1]

[1]*Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., Athens 11635;*
[2]*Medical School, University of Crete, Heraklion, Greece*

Abstract. *We have employed a recombinant plasmid, pBHIV1, carrying the long terminal repeat (LTR) of the human immunodeficiency virus-1 (HIV-1) linked to the reporter chloramphenicol acetyl transferase* (cat) *gene and to the aminoglycoside phosphotransferase* (aph) *gene as a selectable marker. We have introduced pBHIV1 in rat 208F and human MRCSV40TGR fibroblasts and obtained stable geneticin resistant RFBHIV1-1 and SVTGHIV-1 transfectant cells respectively. Both RFBHIV1-1 and SVTGHIV1-1 cells express CAT activity from the HIV LTR promoter. The response to insulin, epidermal growth factor, hydrocortisone and dexamethasone was studied on the LTR regulated CAT activity in both cell lines. It was found that, at optimal concentrations, insulin, epidermal growth factor and hydrocortisone regulate positively the expression of CAT from the HIV LTR in rat RFBHIV1-1 but not in human SVTGHIV1-1 cells. On the other hand dexamethasone at 10^{-5}M stimulated CAT activity in both types of cells.*

Human immunodeficiency viruses are the cause of acquired immune deficiency syndrome (AIDS) (1,2). These viruses infect and destroy the T4 lymphocytes and establish chronic infections (3). Understanding the regulation of HIV gene expression is of paramount importance in order to prevent and eventually cure AIDS. The HIV LTR is a complex modular structure of protein binding sites through which cellular factors can regulate gene expression. Stimuli which are known to affect HIV LTR activity include several mitogens i.e. phytohemaglutinin (4, 5), phorbol esters (6, 8), ionomycin (4) and gene products i.e. *tat* (9, 10), or *ras* p21 (11).

The demonstration that several hormones enhance the production of HIV by mononuclear leukocytes infected *in vitro* and that hydrocortisone in particular facilitates the isolation of virus from peripheral blood mononuclear cells established in cell culture from AIDS patients (12) prompted us to examine further whether hormones and growth factors act through the virus LTR sequences. We find that both types of regulators can augment gene expression from the HIV LTR sequences in an *in vitro* system.

Correspondence to: Prof. D.A. Spandidos, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., Athens, 11635, Greece.

Key Words: HIV, AIDS, growth factors, hormones.

Materials and Methods

Recombinant plasmids and cell lines. Plasmid pBHIV1 carrying a 728 bp XhoI-HindIII DNA fragment containing the HIV-1 LTR sequences was constructed by inserting a 1.9 Kb BamHI fragment carrying the *aph* gene into the single BamHI site of plasmid pBC12 | HIV | CAT (13). Plasmid pBC12 | HIV | CAT was obtained from B.R. Cullen (13).

The spontaneously immortalized rat 208F and the SV40 immortalized human MRCSV40TGR fibroblasts were used as recipients to obtain the RFBHIV1-1 and SVTGHIV1-1 stable geneticin resistant transfectant cell lines with plasmid pBHIV1 (14). DNA transfections were carried out using the calcium phosphate technique (15) as modified (16).

Treatment of cells and CAT assays. Cells were plated at 1.5×10^6 / 75cm^2 flask in Ham's SF12 medium containing 10% FCS at 37° C. 24h later the medium was replaced with Ham's SF12 medium containing 0.5% FCS and left for another 24h at 37°C. Then the medium was changed with Ham's SF12 containing 0.5% FCS and the various concentrations of growth factor or hormone. Cells were harvested 24h later and tested for CAT activity as previously described (17).

Results

Effects of insulin and epidermal growth factor (EGF) on the HIV LTR. It has been shown previously that the HIV LTR contains the primary *cis*-acting regulatory sequences of the virus. It was therefore of interest to examine the effect of the growth factors and hormones on the transcriptional regulation of HIV LTR. Stable transfectants expressing the CAT gene driven by the HIV LTR were obtained. The effect of insulin on the LTR was tested as described in Materials and Methods. Autoradiographs of representative chromatograms from CAT assays are shown in Figure 1 for rat cells and in Figure 2 for human fibroblasts. The corresponding histograms representing the increase in CAT activity are shown in

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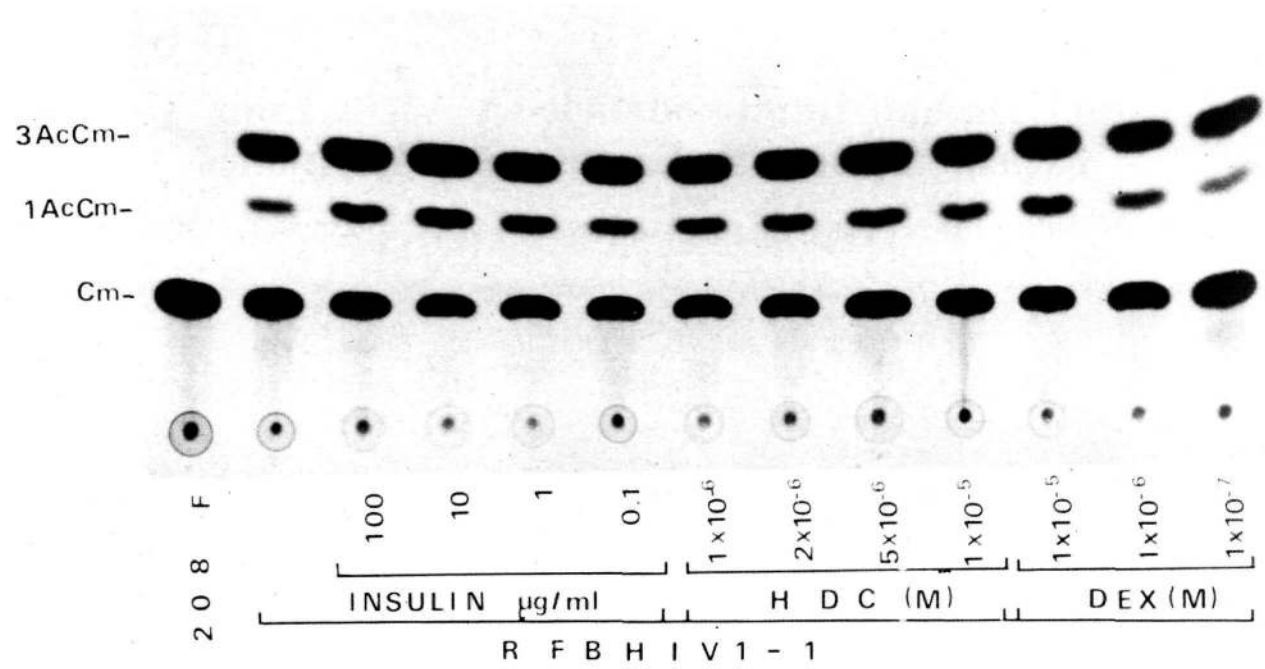

Figure 1. *Chromatogram of representative CAT assays with extracts from recipient and transfectant RFBHIV1-1 cells with and without treatment with insulin, HDC or DEX.*

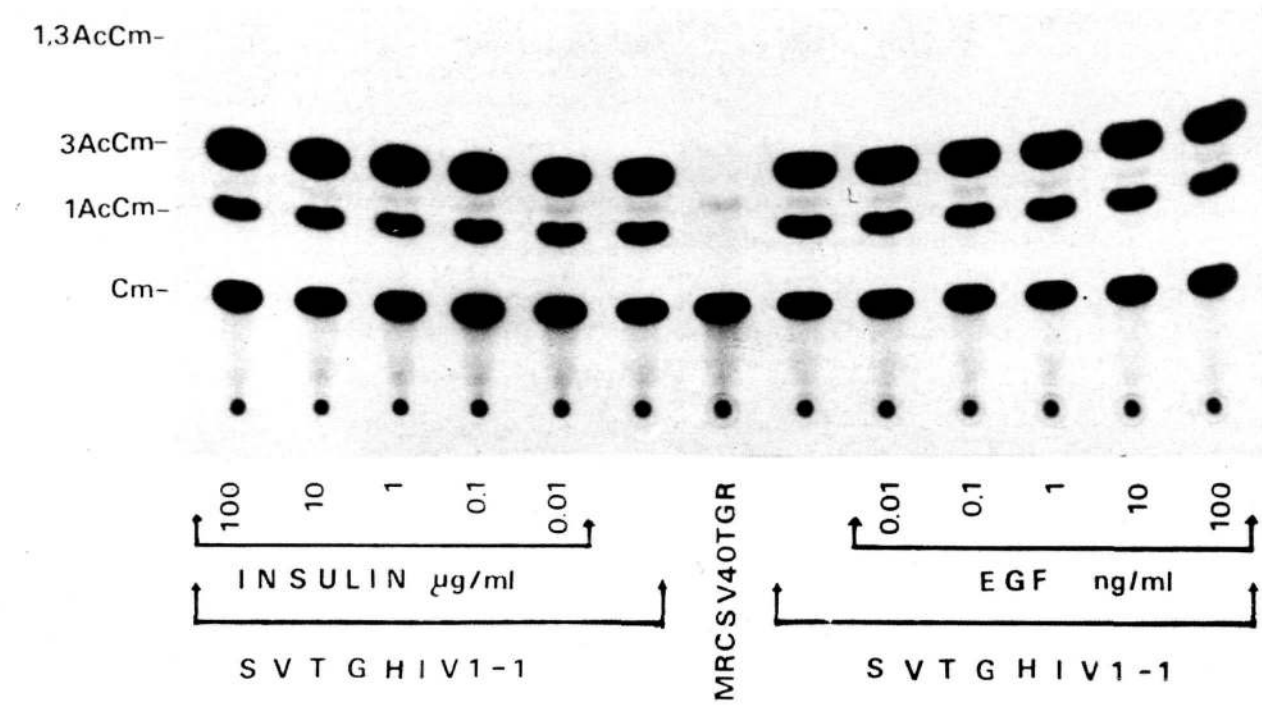

Figure 2. *Chromatogram of representative CAT assays with extracts from recipient and SVTGHIV1-1 cells with and without treatment with insulin or EGF.*

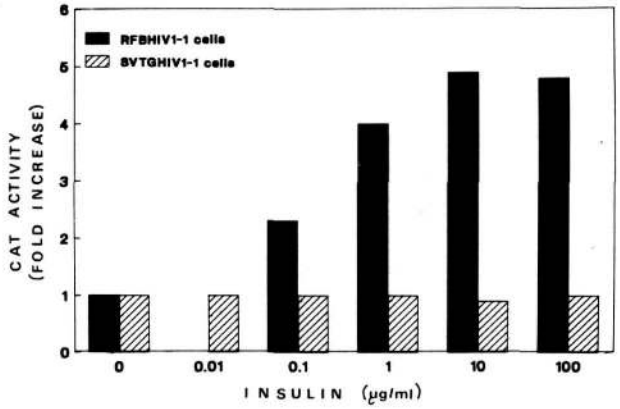

Figure 3. *Induction of CAT activity by insulin. RFBHIV1-1 and SVTGHIV1-1 cells were plated at 1.5×10⁶/75 cm² flask in Ham's SF12 medium containing 10% FCS at 37° C. 24 h later the medium was replaced with Ham's SF12 medium containing 0.5% FCS and left for another 24 h at 37°C. Then the medium was changed with Ham's SF12 containing 1% FCS and the various concentrations of insulin. Cells were harvested 24h later and tested for CAT activity as described in Materials and Methods. Relative values of CAT activity in RFBHIV1-1 and SVTGHIV1-1 cells were 13 and 29 pmole acetylated chloramphenicol /μg protein per hour incubation, respectively. Average from three experiments is given. Standard deviation was less than 10% of average values.*

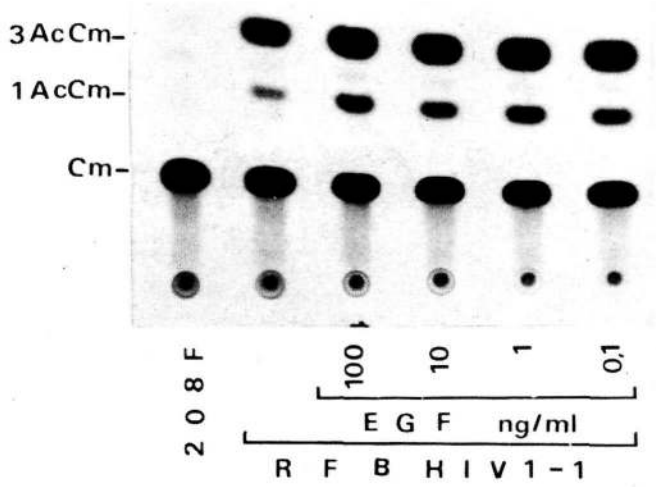

Figure 4. *Chromatogram of representative CAT assays with extracts from recipient and transfectant RFBHIV1-1 cells with and without treatment with EGF.*

Figure 3. Whereas at 1-100μg insulin/ml, a 4 to 5-fold stimulation of CAT activity was found in rat cells, in human cells insulin did not alter CAT activity from HIV LTR.

The effect of EGF on HIV LTR was also tested. Similarly, CAT assays are shown in Figure 4 for the rat and Figure 2 for human fibroblasts. The corresponding histograms representing the increase in CAT activity are shown in Figure 5. Whereas at 0.1-100 ng/ml EGF a 2.3 to 3.8-fold increase in CAT activity was found in rat cells, EGF had no effect on the human cells.

The effect of hydrocortisone (HDC) and dexamethasone (DEX) on the HIV LTR. The effect of HDC was tested at concentrations varying from 1×10^{-5} to 1×10^{-6} M. Representative chromatograms from CAT assays are shown in Figure 1 and Figure 6 for rat and human cells respectively. The corresponding histograms representing the increase in CAT activity are shown in Figure 7. Whereas at 1×10^{-6} to 1×10^{-5}M HDC a 1.9 to 2.8-fold increase in CAT activity was found in rat cells, in human cells HDC has no effect.

The effect of DEX was also tested. At 10^{-5} M DEX a 3.6-fold increase in CAT activity was observed in rat (Figure 1 and 8) and a 2.4-fold increase in human (Figures 6 and 7) cells, respectively.

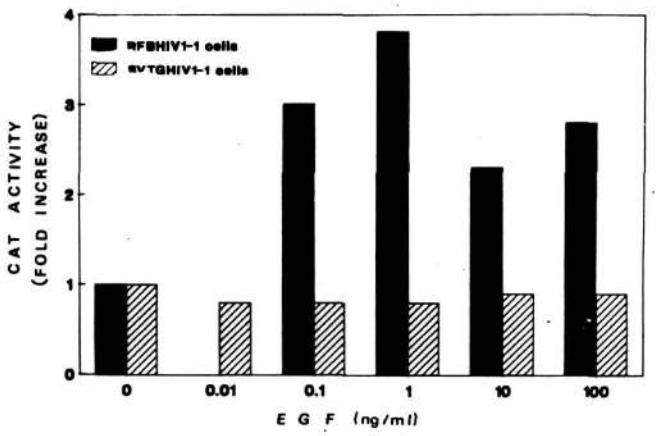

Figure 5. *Induction of CAT activity by EGF. CAT values were obtained as described in Figure 3.*

Discussion

Several regulatory elements have been described on the HIV-1 LTR. Among them are included a trans-acting responsive element (9, 10), an enhancer region including binding sites for NF-kB and Sp1 transcripsion factors (18, 19), a phorbol ester inducible element (5) and a negative regulatory element (10).

Glucocorticosteroids are known to have a wide range of effects including the modulation of expression of some cellular genes (20-24).

Previous studies have suggested a role for corticosteroids and possible gonadal steroids in the modulation of virus expression and/or release and have suggested that the capacity of these and other compounds to induce virus replication

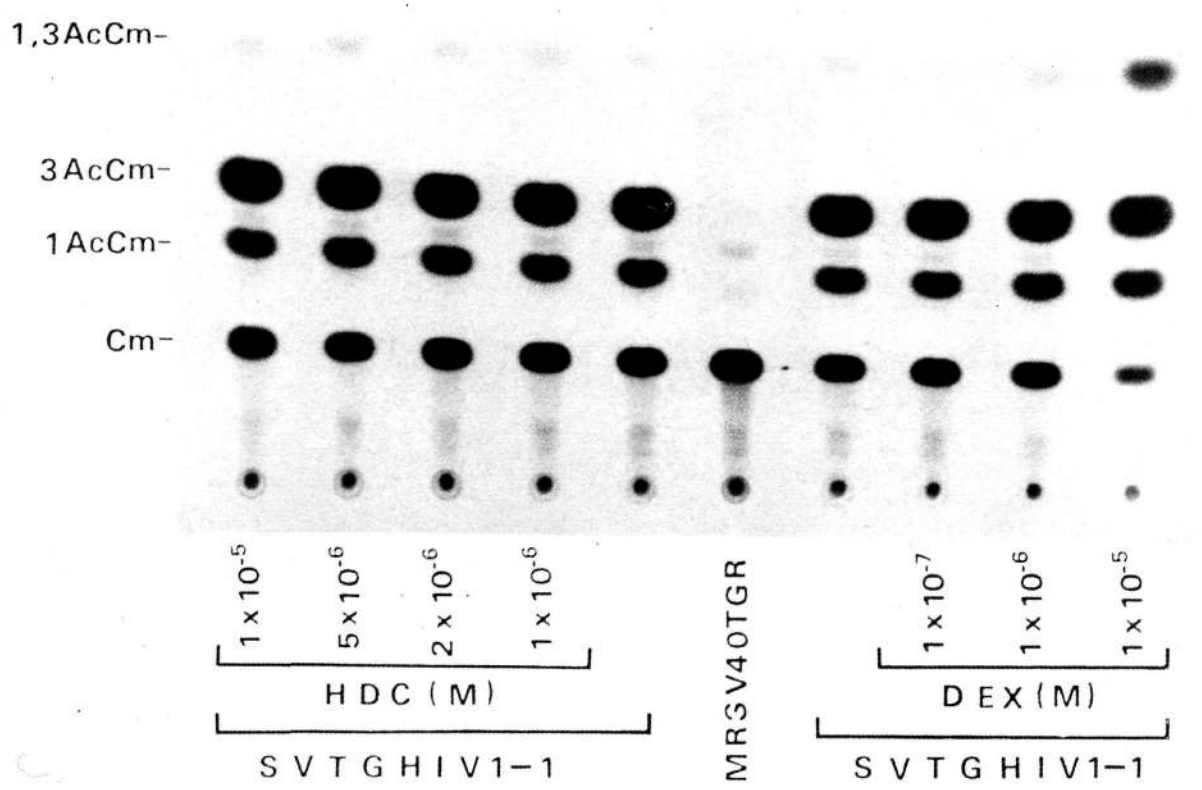

Figure 6. *Chromatogram of representative CAT assay with extracts from recipient and SVTGHIV1-1 cells with and without treatment with HDC or DEX.*

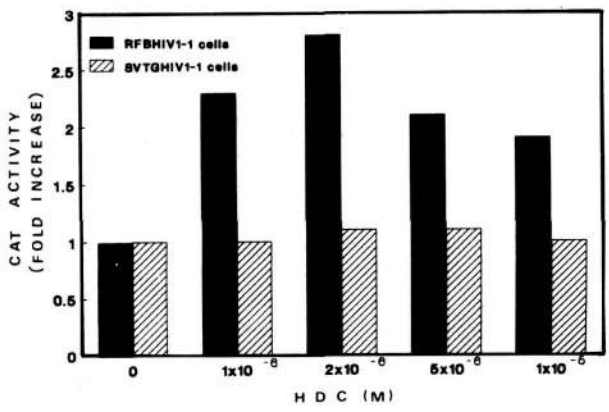

Figure 7. *Induction of CAT activity by HDC. CAT values were obtained as described in Figure 3.*

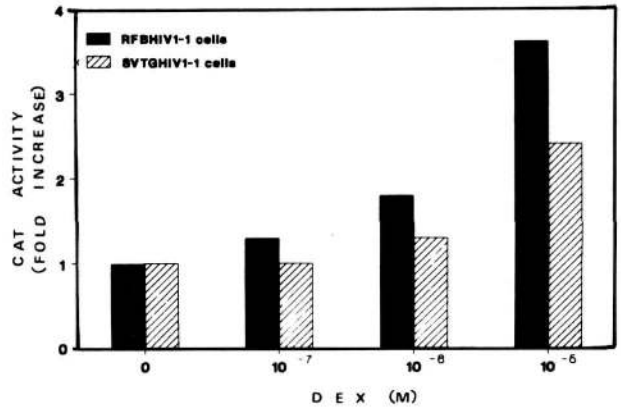

Figure 8. *Induction of CAT activity by DEX. CAT values were obtained as described in Figure 3.*

should be considered prior to their possible clinical use (12).

In the present study we have investigated the effect of growth factors, i.e. insulin and EGF, and hormones, i.e. HDC and DEX, on the transcriptional activity of HIV LTR. We found that the response was cell-type dependent Although in rat fibroblasts the HIV LTR responded strongly to all the above factors, in human fibroblasts the LTR responded only to DEX.

The mechanism of action of these various factors on the HIV LTR is yet unknown. However, it may be postulated that they operate through the modification of DNA binding proteins which interact with the HIV LTR.

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Received July 20, 1990
Accepted August 3, 1990