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EFFECT OF GLUCOCORTICOIDS ON PIT 1 INHIBITION OF SV40 ENHANCER ACTIVITY

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ABSRACT

Earlier studies had shown an effect of glucocorticoids receptor on Pit 1 activation of rPrl promoter activity Treacy. Therefore, the effect of glucocorticoids on Pit 1 inhibition of SV40 enhancer function was investigated in a series of co-transfection studies into glucocorticoids receptor deficient CV-1 cells. Under identical conditions co-transfection of CMV – Pit 1 activated transcription from prPrl (-1960-+38) – CAT; however, expression of GR in the presence of dexamethasone (10-6M) suppressed this activation. The effect of glucocorticoids on the inhibition of SV40 enhancer activity by Pit 1 was investigated. In co- transfection studies in CV-1 cells (Figure 4) it was shown that GR expression and the provision of glucocorticoids (dexamethasone 10-6M) blocked the ability of Pit 1 to inhibit SV40 enhancer activity. Under the same conditions the ability of Pit 1 to activate the rPrl promoter (-1960/+38) was inhibited.

INTRODUCTION

Earlier studies had shown an effect of glucocorticoids receptor on Pit 1 activation of rPrl promoter activity **Treacy *et al.*** [(1)]. Therefore, the effect of glucocorticoids on Pit 1 inhibition of SV40 enhancer function was investigated in a series of co-transfection studies into glucocorticoids receptor deficient CV-1 cells **(Figure 1)**, co-transfection of CMV - Pit 1 with pSV2 -CAT suppressed the level of

receptor gene expression markedly below control (lanes 1 & 2), additional transfection of pRSV – GR in the presence of dexamethasone reversed the effect of Pit 1 (lane 3). Under identical conditions co-transfection of CMV – Pit 1 activated transcription from prPrl (-1960-+38) – CAT; however, expression of GR in the presence of dexamethasone (10-6M) suppressed this activation.

MATERIALS & METHODS

An SV-40 enhancer fragment was isolated by restricting pSV2-CAT with Sph 1. It was gel purified and ligated into pRSV (enhancer – minus) – CAT (Sph1 restricted), transformed into E.coli (strain HB 101), followed by mini – preparation and selection of the plasmid, pR (-)-SV40E-CAT (Figure 2). It was large scale prepared and purified by tow successive bandings through CsCl – ethidium bromide gradients. Sambrook, J. (1989) the structure of pR (-) SV40-CAT and its restriction analysis with a range of different restriction enzymes **(Figure 3)**.

RESULTS

It was of interest to investigate the activity of the SV40 early gene enhacer/promoter system in various cells types. Studies on the SV40 enhacer/promoter have shown it to be active in a wide range of cells eg. C6, C127, CV-1 and Hela cells **Treacy *et al.*** [(4)]. the effect of glucocorticoids on the inhibition of SV40 enhancer activity by Pit 1 was investigated. Both **Camper *et al.*** [(2)] and **Adler *et al.*** [(1)] have shown that glucocorticoids suppress prolactin promoter activity **Adler *et al.*** [(1)] showed that the over expression of the glucocorticoids receptor repressed prolactin promoter function . In co- transfection studies in CV-1 cells **(Figure 4)** it was shown that GR expression and the provision of glucocorticoids (dexamethasone 10-6M) blocked the ability of Pit 1 to inhibit SV40 enhancer activity. Under the same conditions the ability of Pit 1 to activate the rPrl promoter (-1960/+38) was inhibited.

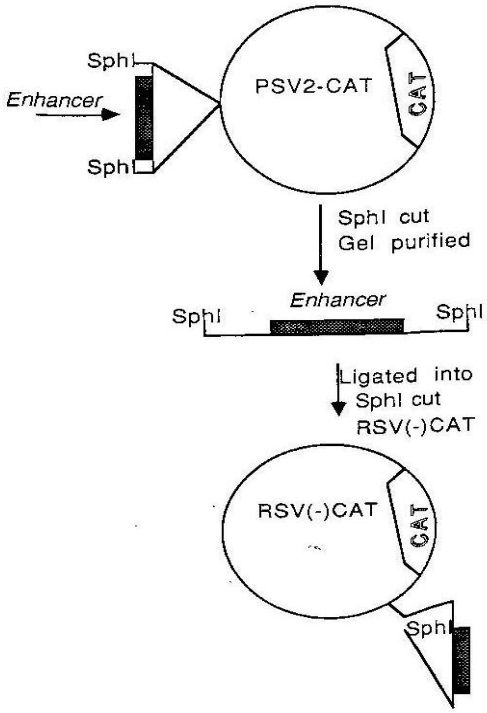
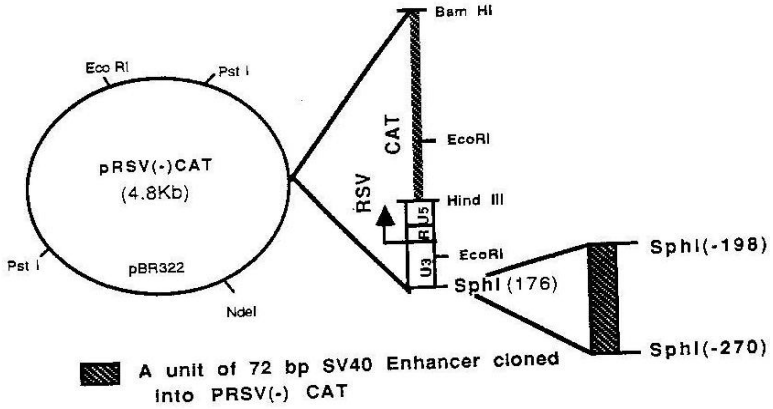


Fig Subcloning strategy used to insert a unit of the 72 bp SV40 enhance into pRSV(enhancer-minus)$_{SphI}$ CAT construct.



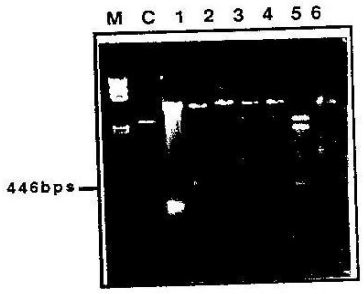


Fig 2 Plasmid map and restriction analysis of pRSV(enhncer-minus)/SV40-enhancer)CAT.
Analysis shows:**Lane1:** pRSV(enhancer-minus)CAT, cut SphI(4,803bps) **lane2:**pRSV(SV40-E)CAT, cut HindIII/NdeI,(4,429;446bps), **lane3:** HindIII cut(4,875), **lane4:** BamHI cut(4,875bps), **lane5:** EcoRI cut(2,331; 2,100;339bps), **lane6:** PstI cut(3,917,958bps), **laneC:** unrestricted plasmid. M:corresponds to HindIII cut phage λ DNA.

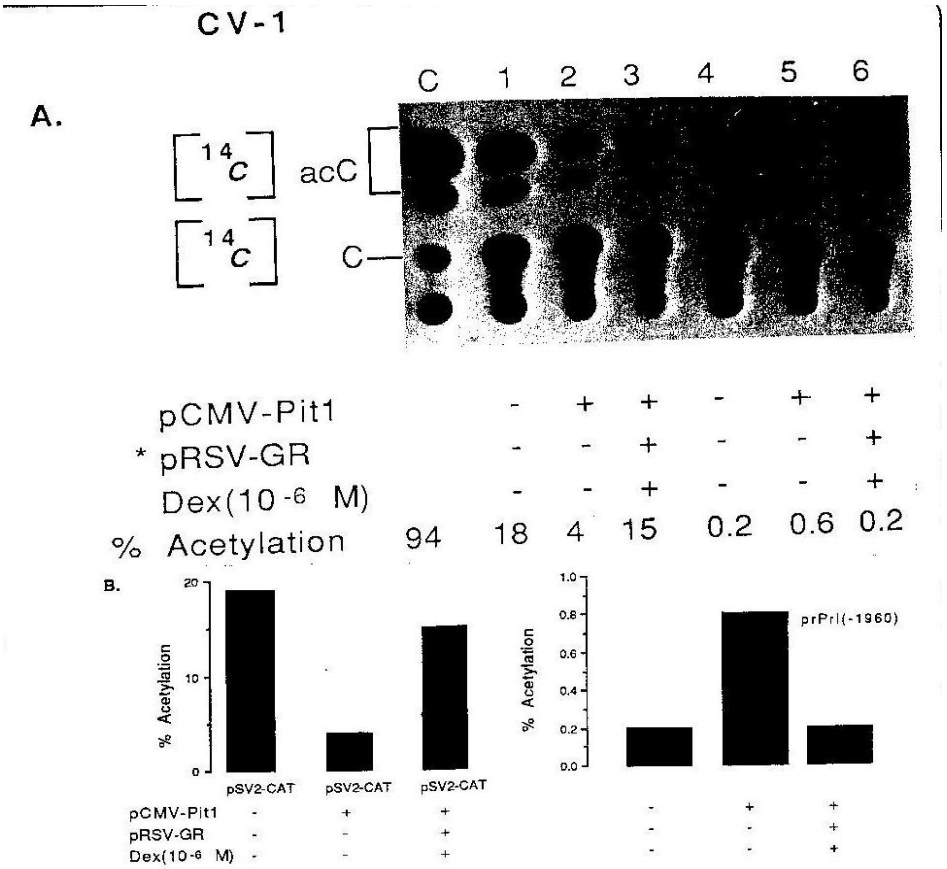


Fig 3: Effect of glucocorticoids on the SV40-enhancer after co-transfection with pCMV-Pit1 into non pituitary cells.

(A) Shown is an autoradiograph of a representative CAT enzyme assay indicating the effect of pCMV-Pit1 activity on co-transfection into CV-1 cells with pRSV-GR in the presence and absence of dexamethasone (10^{-6} M) : **Lanes1-3:** pSV2-CAT **lanes4-6:** prPrl(-1960/+38)-CAT, **laneC:** represents where pure CAT enzyme was incubated in place of cell extract. Values for CAT activity are presented as % acetylation of [**14 C**]-chloramphenicol.

(B) Shown is the mean CAT activity from 3 independent experiments presented as % acetylation of [**14 C**]-chloramphenicol observed in CV-1 cells co-transfected with pSV2-CAT and pCMV-Pit1 in the presence and absence of dexamethasone (10^{-6} M). The effect on prPrl(-1969/+38)-CAT is shown for comparison.

* Glucocorticoid-Receptor expression vector.

DISCUSSION

It was of interest to investigate the activity of the SV40 early gene enhacer/promoter system in various cells types. Studies on the SV40 enhacer/promoter have shown it to be active in a wide range of cells eg. C6, C127, CV-1 and Hela cells **Treacy *et al.*** [4]. The effect of glucocorticoids on the inhibition of SV40 enhancer activity by Pit 1 was investigated. Both **Camper *et al.*** [2] and **Adler *et al.*** [1] have shown that glucocorticoids suppress prolactin promoter activity **Adler *et al.*** [1] showed that the over expression of the glucocorticoids receptor repressed prolactin promoter function. In co- transfection studies in CV-1 cells **(Figure 4)** it was shown that GR expression and the provision of glucocorticoids (dexamethasone 10-6M) blocked the ability of Pit 1 to inhibit SV40 enhancer activity. Under the same conditions the ability of Pit 1 to activate the rPrl promoter (-1960/+38) was inhibited.

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