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INHIBITION BY GLUCOCORTICOIDS OF PIT 1 INDUCED ACTIVATION OF TRANSCRIPTION FROM RPR1 PROMOTER FRAGMENTS IN NON PITUITARY CELLS

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ABSRACT

Bovine and rat prolactin gene promoter function by glucocorticoids. It was of interest, therefore to examine the possible inhibition by glucocorticoids of pit l induced activation of transcription from the 3'- rPrl promoter mutant generated for this study. The studies were carried out by co-transfection into glucocorticoids receptor (GR) deficient CV-1 cells both pin1 and (GR) expression vectors and the reporter CAT plasmids with upstream rPrl promoter fragments. The effect of glucocorticoids was examined by the Addition of dexamethasone (10-6 M) to the cultures immediately after transfection. In these studies glucocorticoids receptor (GR) deficient CV-l cells were used and the (GR) was expressed from an expression vector parker et al. (1994). However, in the case of prolactin promoter fragments (eg, - 1960/+ 38, -44/ -423, -190/-423 and – 75 /+ 38) decreased in pitl induced DAT gene expression was seen when the (GR) was expressed but it was evident even in the absence of glucocorticoids.

INTRODUCTION

Comper *et al.* [2] and **Adler *et al.*** [1] clearly demonstrated the inhibition. Bovine and rat prolactin gene promoter function by glucocorticoids. It was of interest, therefore to examine the possible inhibition by glucocorticoids of pit l induced activation of transcription from the 3'- rPrl promoter mutant generated for this study.

As illustrated in **(Figure1)** the studies were carried out by co-transfection into glucocorticoids receptor (GR) deficient CV-1 cells both pin1 and (GR) expression vectors and the reporter CAT plasmids with upstream rPrl promoter fragments. Treacy et al. (1988).

The effect of glucocorticoids was examined by the Addition of dexamethasone (10-6 M) to the cultures immediately after transfection. **(Figure 2)** shoes an inhibitory effect of expression of glucocorticoids receptor on pit 1 activation. Of prPrl (-1960/+38)-CAT, pS (-) p (-423/-44)-CAT on transfection into CV-1 cells Schuster et al. (1988). However the effect is seen UN the presence and absence of dexameha – some (10-6M).

This may reflect an excessive over production of (GR) from the expression vector **(Figure 3)** again shows the effect on prPrl (-1960/+38) – CAT but low induction of pS (-) – (-75/+38)-CAT expression allowed no conclusion to be drawn in the case of pS (-) p (-75/+38)-CAT **Eljaafry *et al.*** [3].

MATERIAL & METHODS

A small set of 3, -deletion mutants of the rat prolactin promoter were generated using the exonuclease Bal 31. They had 5, -border at -423 (transcription start site +1).

DNA sequencing analysis showed the deletion mutant to have 3, -borders at (-44, -120, --190, -230, -960 and -1960) for characterization, they were introduced into pRSV _ Grand pCMV_pit1 (glucocorticoids receptor) expression vectors. The resulting plasmids were then transfected into CV-l cells.

The transcriptional activity of the prolactin promoter fragments was assayed by measuring the chloramphenicol acetyltransferase (CAT).

RESULTS

In **(Figures l-3)** results of a series of preliminary studies on the effects of glucocorticoids on pitl activated expression from the rPrl promoter fragments is shown. **Comper *et al.*** [2] and **Adler *et al.*** [1] had shown that glucocorticoids suppress transcription from mammalian prolactin promoters.

In these studies glucocorticoids receptor (GR) deficient CV-l cells were used and the (GR) was expressed from an expression vector **Parker *et al.*** [5].

However, in the case of prolactin promoter fragments (eg, -1960/+ 38, -44/ -423, -190/-423 and – 75 /+ 38) decreased in pitl induced DAT gene expression was seen when the (GR) was expressed but it was evident even in the absence of glucocorticoids. **Maniaatis *et al.*** [3]. Whether this reflects the high level of (GR) expression.

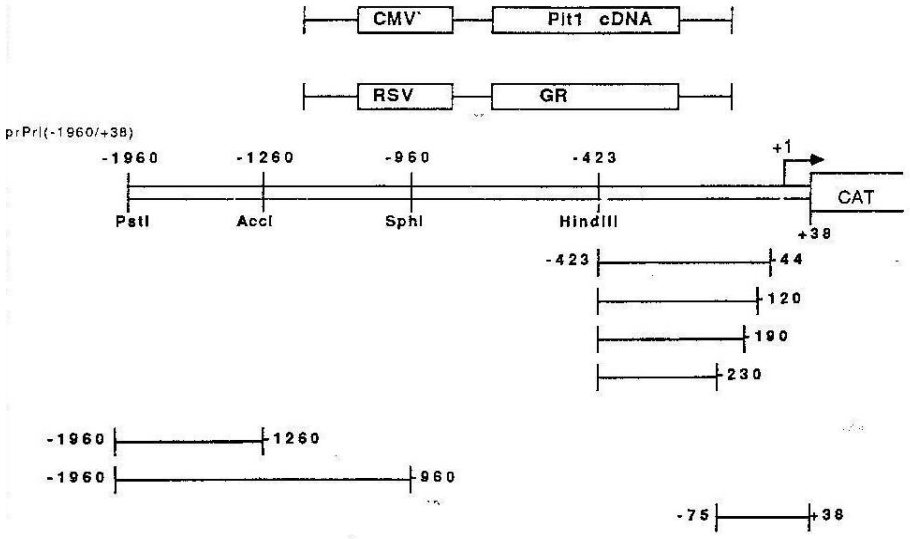

Fig. 1: Schematic representation of the pCMV-Pit1 and pRSV-GR (glucocorticoid receptor) expression vectors and the reporter plasmid prPrl(-1960/+38)-CAT and the rPrl promoter fragments tested in pS(-)P(Δ)-CAT constructs.

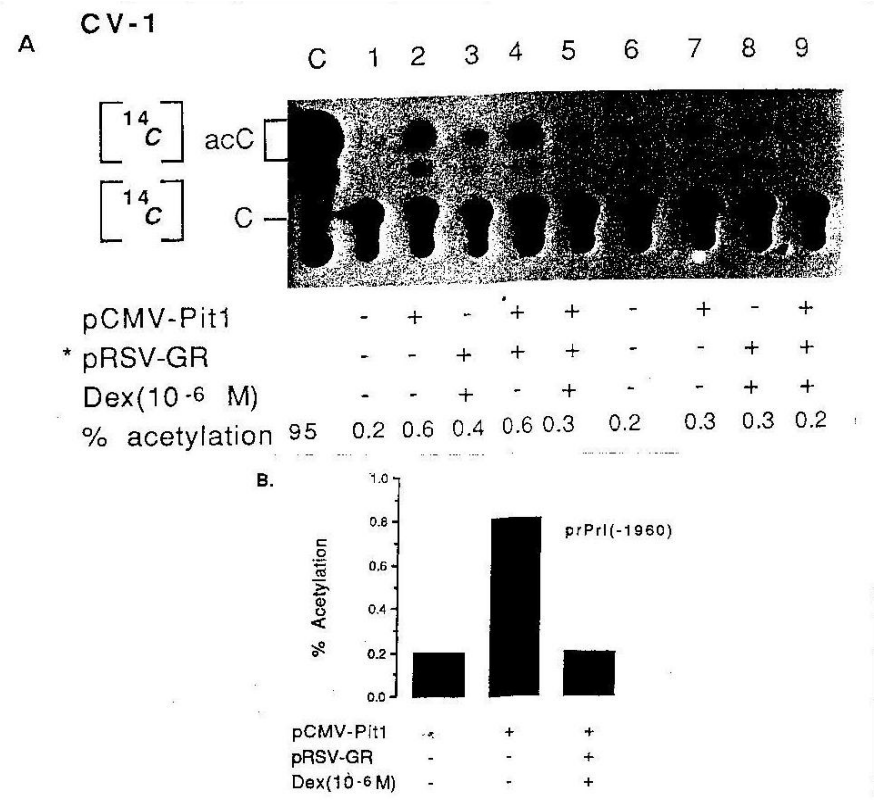

Fig3C: Effect of glucocorticoids on Pit1 transcription activity from the 5'-rPrl-promoter deletion fragments after co-transfeciton with pCMV-Pit1 into non pituitary cells.

A). Shown is an autoradiograph of a representative CAT enzyme assay indicating the effect on Pit1 activity on co-transfection into CV-1 cells with pRSV-GR in the presence and absence of dexamethasone (10^{-6} M) : **Lanes1-5:** prPrl(-1960/+38)-CAT, **lanes6-9:** prPrl(-75/+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 expriments presented as % acetylation of [^{14}C]-chloramphenicol observed in CV-1 cells co-transfected with prPrl(-1960/+38)-CAT and pCMV-Pit1 in the presence and absence of pRSV-GR and dexamethason (10^{-6} M).

* Glucocorticoid-Receptor expression vector.

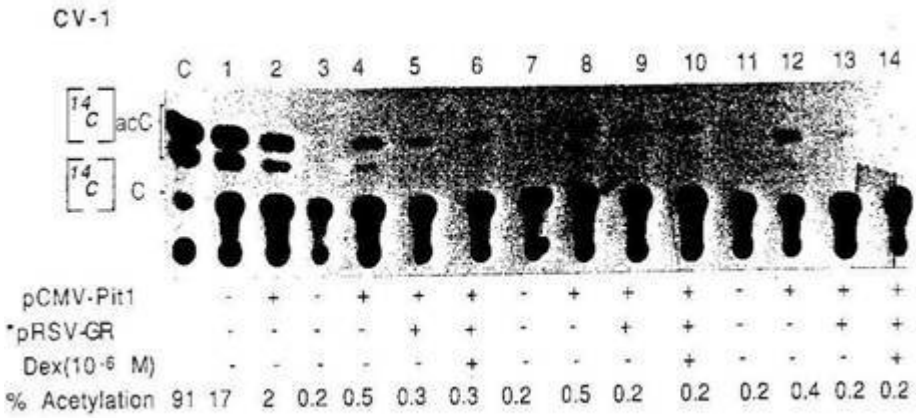

Fig 2. Effect of glucocorticoids on Pit1 transcription from the 3'-rPrl-promoter deletion fragments after co-transfection with pCMV-Pit1 into non pituitary cells.

Shown is an autoradiograph of a representative CAT enzyme assay indicating the effect on Pit1 activity on co-transfection into CV-1 cells with pRSV-GR in the presence and absence of dexamethasone (10^{-6} M) : **Lanes1,2:** pSV2-CAT, **lanes3-6:** prPrl(-1966/+38)-CAT, **lanes7-10:** pS(-)P(-423/-44)-CAT, **lanes 11-14:**pS(-}P(-423/-190)-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.

* Glucocorticoid-Receptor expression vector.

DISCUSSION

In **(Figures l-3)** results of a series of preliminaey studies on the effects of glucocorticoids on pitl activated expression from the rPrl promoter fragments is shown. **Comper *et al.*** [2] and **Adler *et al.*** [1] had shown that glucocorticoids suppress transcription from mammalian prolactin promoters. In these studies glucocorticoids receptor (GR) deficient CV-l cells were used and the (GR) was expressed from an expression vector **Parker *et al.*** [5].

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