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# Interaction of cytosol fractions containing activated glucocorticoid-receptor complexes from rat liver and thymus with heterologous nuclei: effects on transcription

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Two rat liver cytosol fractions containing activated glucocorticoid-receptor complexes are able to stimulate the transcriptional activity of rat liver nuclei; the respective fractions from the cytosol of thymocytes inhibit the capacity of thymus nuclei for RNA synthesis. A similar inhibitory effect on thymus nuclei is exerted by the presence of rat liver cytosol fractions. Spot hybridization using a tyrosine aminotransferase (TAT) probe demonstrates that TAT gene expression is stimulated by the liver cytosol fractions acting on homologous nuclei whereas it is inhibited, in thymus nuclei, by the addition of thymus cytosol fractions. No effect on transcription is observed if the liver or thymus cytosol is heat activated in the presence of the glucocorticoid antagonist, cortexolone. Treatment of liver nuclei, previously subjected to the action of thymus cytosol fractions with the respective liver ones, restores transcriptional activity to control or higher levels. We conclude that rat thymocyte nuclei and cytosol contain transcriptional factors, which in the presence of the glucocorticoid-receptor complex, irrespective of its source, inhibit gene expression, whereas in the absence of such factors, the glucocorticoid-receptor complex positively regulates the respective genes.

Glucocorticoid receptor; Transcription; (Liver, Thymus, Nucleus)

## 1. INTRODUCTION

The phenotypic expression of glucocorticoid action varies according to the target cell involved. In the liver, a major effect is the stimulation of gluconeogenesis, involving induction of enzymes, such as tyrosine aminotransferase and tryptophan oxygenase, whereas in thymus these hormones lead to cell lysis and death [1,2]. The molecular basis for the differential response of target cells to the same hormone represents a central problem of current interest. Glucocorticoids exert their biological effects on target cells by binding with high affinity and specificity to a soluble receptor protein. This interaction triggers a structural change in the receptor molecule, resulting in the ability to recognize and to bind to transcriptional enhancer DNA elements of hormone-dependent genes, positively or negatively controlled by the hormone (review [11]). The fact that glucocorticoid receptors from rat liver and thymus display the same physico-chemical and immunological characteristics and have equal molecular masses [5,6], together with the recent finding that one copy of the glucocorticoid gene is present per haploid gene [3,4] constitute evidence in favour of the existence of solely a single glucorticoid receptor in all target cells. Recent findings demonstrating that glucocorticoid receptors exert their transcriptional control by cooperating with other trans-acting factors (NF-1, CACC, CAAT) [7] suggest that the target gene specificity of glucocorticoids is determined not solely by the receptor protein but also through its cooperation with other transcription factors.

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*Abbreviation:* TAT, tyrosine aminotransferase (EC 2.6.1.5)



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We have previously shown that isolated nuclei possess the ability to respond to addition of homologous cytosol fractions containing activated glucocorticoid-receptor complexes with increased (liver) or decreased (thymus) rates of RNA synthesis [8]. To explore the mechanism underlying the tissue specificity of glucocorticoids, we monitored the response of nuclear fractions to the presence of heterologous cytosol fractions.

## 2. EXPERIMENTAL

Cytosol and nuclei from rat liver and thymus were prepared from male Wistar rats (6–8 weeks old) that were adrenalectomized 6–7 days before being killed.

Fractionation of heat-activated cytosol labelled with [$^3$H]dexamethasone from rat liver or thymus through DEAE-A50 was performed as described [8]. Two fractions containing [$^3$H]dexamethasone-receptor complexes were obtained, one in the flow-through (DE-1) and the other eluting from the column at 150 mM $Cl^-$ (DE-2). With the exception of heat activation all other steps were performed at 0–4°C.

Translocation of the [$^3$H]dexamethasone-receptor complex to the nucleus was monitored by assaying $^3$H activity in aliquots of the nuclei, washed three times with buffer. Assuming that 1 mol hormone binds per mol receptor, the amount (in pmol) of receptor translocated could be determined.

The capacity for RNA synthesis of treated nuclei was evaluated in a standard reaction mixture containing the four nucleoside triphosphates one of which (UTP) was $^3$H-labelled. For dot-hybridization assays, UTP was labelled with $^{32}$P (200 $\mu$Ci per assay). The ion composition was 4.4 mM $MgCl_2$, 2.24 mM $MnCl_2$, 80 mM KCl, 6 mM NaF.

For dot-hybridization [$^{32}$P]RNA was hybridized to 6 $\mu$g linearized TAT cDNA containing the genomic fragments of 2.45, 1.05 and 0.9 kb, immobilized on nitrocellulose filters. For measuring non-specific hybridization, hybridization with pBR322 DNA was performed for each point.

## 3. RESULTS AND DISCUSSION

To explore the possibility that the differential action of glucocorticoids on various target cells, i.e. positive transcriptional control on hepatocytes and negative on thymocytes, is exerted through the interaction of the glucocorticoid receptor with transcriptional factors, we followed the effects of liver or thymus cytosol fractions containing activated dexamethasone-receptor complexes, on transcription of isolated heterologous nuclei. On submitting cytosol to DEAE-Sephadex A-50 column chromatography, two fractions (DE-1 and DE-2), as described in [8], were isolated and shown by DNA-binding experiments (not shown) to contain activated dexamethasone-receptor complexes. The hormone-receptor complex contained in these fractions can: (i) translocate to nuclei, in a time-dependent fashion (table 1); (ii) saturate the nuclear acceptor sites (table 1); and (iii) modulate the capacity for RNA synthesis of nuclei (see below). As reported previously [8], rat liver nuclei respond with increased RNA synthesis to the addition of homologous cytosol fractions, whereas thymus nuclear transcription is inhibited by the homologous cytosol fractions. It should be noted that most of the RNA synthesis activity of the nuclear system is due to elongation and not initiation of transcription, as demonstrated by use of the rifampicin derivatives, AF05 and AF013; furthermore, it should be borne in mind that all three DNA-dependent RNA polymerases are active under the ionic conditions employed (35% of the total nuclear RNA synthesis activity is due to the action of polymerase II, as shown using $\alpha$-amanitin).

In order to decide whether the differential response of nuclei to the cytosol fractions added is due to tissue specificity of the glucocorticoid receptor or arises from other components within the confines of the nucleus, we have extended the previous experiments to encompass the effect of heterologous cytosol fractions on nuclear transcription. In these experiments we observed a

Table 1

Sequential translocation of thymus and liver glucocorticoid-receptor complexes to liver nuclei

| Nuclei in the presence | Nuclear translocated [$^3$H]dexamethasone (dpm/mg DNA) after incubation time (min) | | | |
|---|---|---|---|---|
| | 30 | 60 | 90 | 120 |
| Liver nuclei + thymus DE1 (step a) | 6600 | 8700 | 10500 | 10600 |
| Liver nuclei of step a after transcription assay (step b) | 1700 | 2000 | 3100 | 3200 |
| Liver nuclei of step b + liver DE1 (step c) | 7900 | 11800 | 13800 | 14800 |
| Liver nuclei + thymus DE2 (step a) | 2850 | 4140 | 5250 | 6000 |
| Liver nuclei of step a after transcription assay (step b) | 1250 | 1840 | 1730 | 2110 |
| Liver nuclei of step b + liver DE2 (step c) | 4800 | 6000 | 7100 | 8000 |

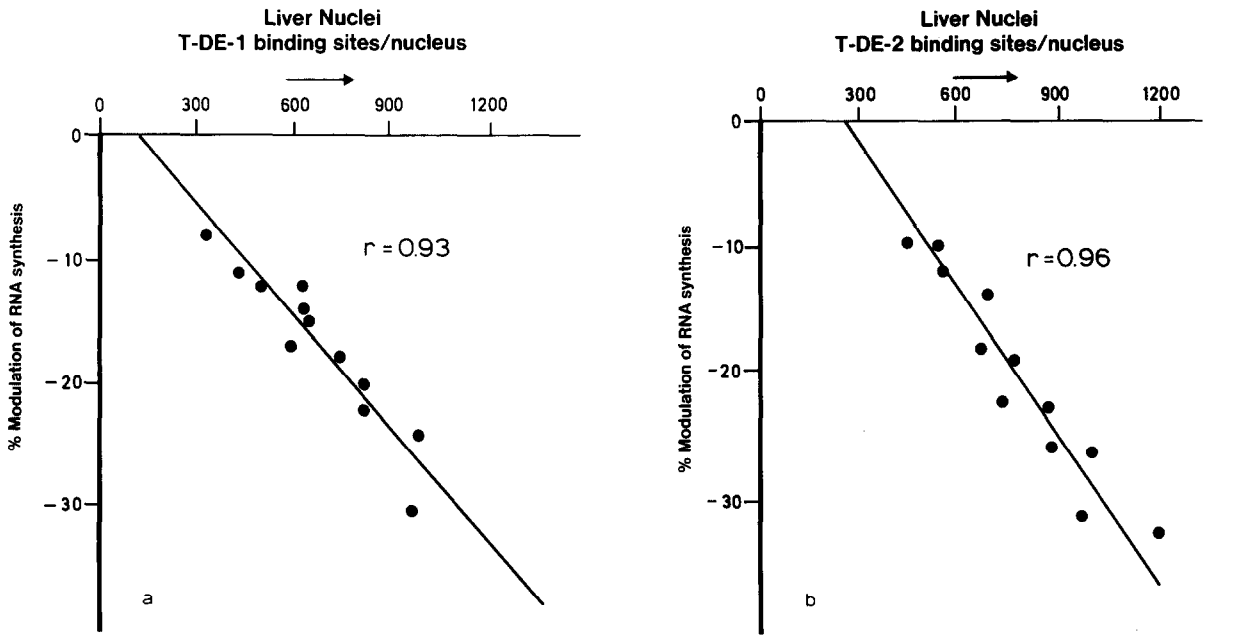


Fig.1. (a,b) Correlation between nuclear translocation (binding sites per nucleus) of thymus DE-1 (a) and DE-2 (b) and RNA synthesis in liver nuclei in vitro. Data are represented as % difference of RNA synthesis of DE-fraction-treated vs control nuclei. Each point represents the mean value of triplicate assays of three experiments.

25–30% inhibitory effect on transcription of these heterologous fractions, independent of the source of nuclei (figs 1a,b, 2a,b). The relationship between bound glucocorticoid-receptor complex and transcriptional activity is linear. To elicit these effects on transcription, an activated glucocorticoid-receptor complex is necessary, since fractions corresponding to DE-1 and DE-2, but obtained from cytosol preincubated with the glucocorticoid antagonist, cortexolone, have no effect on thymus or liver nuclear transcription [8]. TAT mRNA is one of the mRNA species induced by glucocorticoids in target cells. We monitored the amount of TAT gene transcripts in liver nuclei after treatment with liver and thymus DE fractions and observed (table 2) a 28% increase in RNA hybridizing with the TAT cDNA probe in the presence of liver DE fractions, whereas in the presence of the thymus fractions, hybridization was decreased to an approximately similar extent. We should add that nuclease activity was monitored in these experiments and found to be constant and not to be related to the observed differences in rates of RNA synthesis. The finding that transcription is modulated similarly by fractions DE-1 and DE-2 can be ascribed to the fact that both of these fractions contain glucocorticoid receptor possessing the same DNA- and hormone-binding domain and to the possible existence of multiple transcription factors distributed in the various chromatographic fractions obtained. In a further series of experiments, we investigated whether the observed effects on transcription of the various cytosol fractions are reversible. Liver nuclei were pretreated with thymus DE fractions and then incubated under conditions of active transcription. This resulted in gradual release of the bound receptor (table 1). Subsequent addition of liver DE frac-

Table 2

Effect of DE fractions on tyrosine aminotransferase gene expression in liver nuclei

| Nuclei in the presence of | Level of hybridization (%) |
|---|---|
| L-DE-1 | +28 |
| L-DE-2 | +25 |
| T-DE-1 | −25 |
| T-DE-2 | −27 |

6 μg genomic TAT subclones hybridized with [$^{32}$P]RNA from treated nuclei; 3 μg pBR322 was used as control

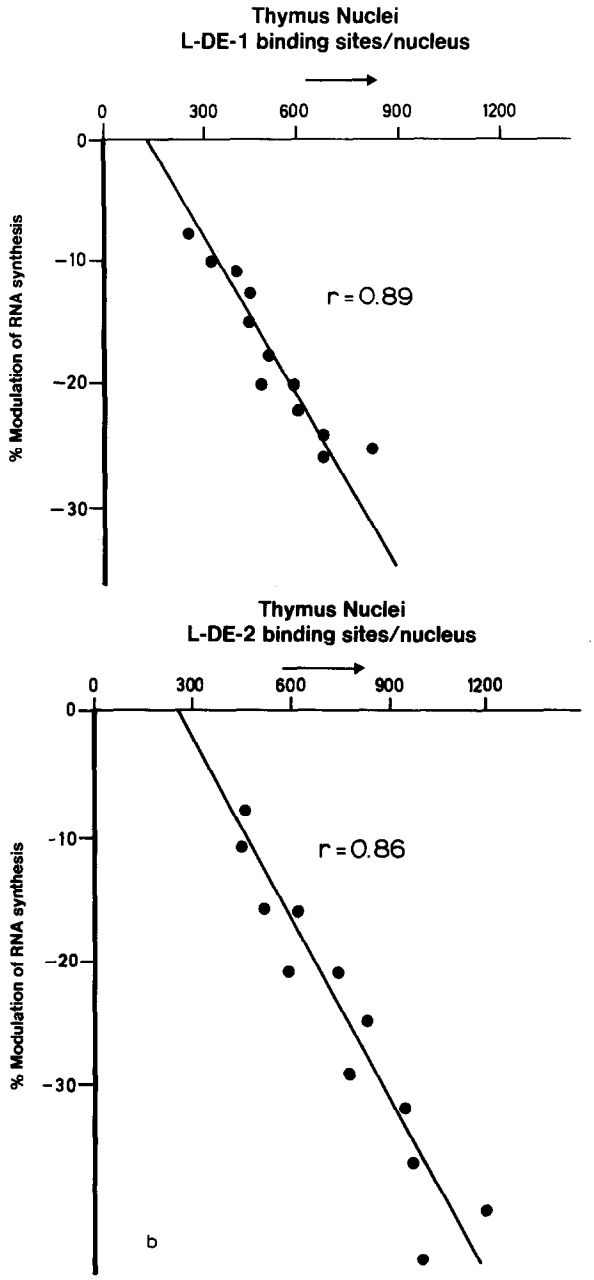


Fig.2. (a,b) Correlation between nuclear translocation of thymus DE-1 (a) and DE-2 (b) and RNA synthesis in thymus nuclei in vitro. Experimental details as in fig.1.

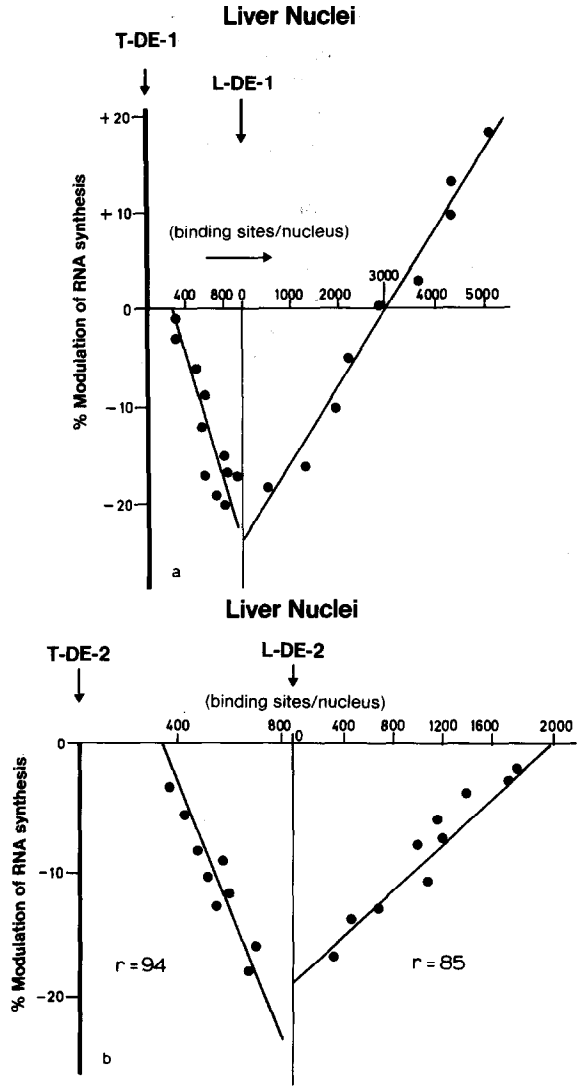


Fig.3. (a,b) Effects of liver DE fractions on RNA synthesis of liver pretreated with thymus DE fractions. Liver nuclei were incubated with thymus DE-1 (a) or DE-2 (b) for varoius periods, followed by liver DE-1 (a) and liver DE-2 (b). Translocation of glucocorticoid receptor complex and transcriptional activity were measured as described in the text. Data are expressed as % difference in RNA synthesis of treated vs control nuclei.

tions led to binding of the liver glucocorticoid receptor to acceptor sites and to restoration of transcriptional activity, either to control levels (incubation in the presence of DE-2) or to a level of approx. 20% over control (incubation with DE-1) (fig.3a,b). We ascribe the inhibition of transcription observed on addition of liver DE fractions to thymus nuclei and of thymus DE fractions to liver nuclei, to the presence in thymus nuclei and thymus cytosol of tissue-specific factors that can affect this process negatively only when acting

cooperatively with the glucocorticoid-receptor complex. These factors would originally have been mainly localized in nuclei, but very probably would have entered the cytoplasm during preparation. In the absence of such specific inhibitory molecules (i.e. in the liver system), the glucocorticoid receptor, in cooperation with other nuclear transcription factors, as shown recently [10], positively regulates gene expression. We are currently attempting to isolate such putative tissue-specific factors which, in conjunction with the glucocorticoid receptor, play a major role in glucocorticoid-regulated tissue-specific expression, and to explore their interaction with the receptor at the level of the gene.

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