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REGULATION OF GLUCOCORTICOID RECEPTOR EXPRESSION IN CULTURED FIBROBLASTS FROM A PATIENT WITH FAMILIAL GLUCOCORTICOID RESISTANCE

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Summary—The thermolabile glucocorticoid receptor (GR) in fibroblasts from a patient with familial glucocorticoid resistance (FGR) was characterized by solution hybridization, Northern blot analysis and Western immunoblotting using an hGR and cRNA probe and a GR specific monoclonal antibody. Specific DNA binding was measured by binding of cytosolic GR to mouse mammary tumour virus (MMTV) DNA. Northern blot analysis of total cellular RNA isolated from the fibroblasts showed hybridization of the hGR probe to 7.0 and 6.1 kb RNA species. Basal expression of hGR mRNA was 1.8 times higher in fibroblasts derived from the patient compared to control fibroblasts as assayed by solution hybridization. Even though nonsignificant, dexamethasone treatment maximally caused at 60% down-regulation of GR mRNA in normal fibroblasts after 12 h but only a 40% down-regulation in fibroblasts from the patient. In both cases, the initial mRNA values were restored after 72 h. No difference in GR mRNA stability was observed between fibroblasts from the patient and from controls. The induction of the glucocorticoid-regulated gene metallothionein IIA (MTIIA) by dexamethasone and cadmium sulphate was studied at different temperatures using a cRNA probe for human MTIIA. At elevated temperatures, cadmium sulphate but not dexamethasone increased MTIIA mRNA levels approximately three-fold in fibroblasts from the patient, whereas in normal fibroblasts regardless of temperature both cadmium sulphate and dexamethasone increased MTIIA mRNA levels approximately three- and two-fold, respectively. Cytosolic GR from FGR-fibroblasts showed an increased specific binding to MMTV DNA at 4°C. These data support our previous findings of a thermolabile GR, probably due to a defect intrinsic to the GR protein, in this patient with primary cortisol resistance and indicate a compensatory mechanism at the transcriptional level of GR expression. The data also indicate a receptor defect affecting specific DNA binding *in vitro*.

INTRODUCTION

Primary steroid hormone resistance due to abnormalities of steroid receptors has been reported for androgens, glucocorticoids, progesterone and mineralocorticoids [1]. In addition, the clinical syndrome caused by a defective vitamin D_3 receptor, referred to as type II vitamin D-resistant rickets [2], is analogous to androgen resistance in complete testicular feminization. Recently, several types of receptor abnormalities have been reported in familial glucocorticoid resistance (FGR) or "hypercortisolism without Cushing's syndrome". These abnormalities include decreased receptor affinity for cortisol, decreased binding of the glucocorticoid receptor (GR) complex to DNA, reduction in the number of GRs, and GR thermolability [3–6]. Similar GR abnormalities have been described in cultured lymphoma cells providing *in vitro* evidence for several types of resistance to glucocorticoids [7, 8].

The cloning of cDNA for hGR has made it possible to study the expression and regulation of the GR at the molecular level [9, 10]. Furthermore, the use of monoclonal antibodies against the human GR has made it possible to study

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Abbreviations: GR, glucocorticoid receptor; MMTV, mouse mammary tumor virus; FGR, familial glucocorticoid resistance; LTR, long terminal repeat; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; hsp 90, heat shock protein (90 kd); MTIIA, metallothionein IIA.

the receptor protein independently of ligand binding [11].

In the present paper, we report on an increased GR mRNA expression, an increased specific DNA binding of unactivated GR to mouse mammary tumour virus (MMTV) DNA, and a defect in induction of a glucocorticoid regulated gene at elevated temperatures in a patient with FGR previously shown to have a thermolabile GR [12].

MATERIALS AND METHODS

Cell culture methodology

Fibroblasts from a female patient previously shown to have a thermolabile GR [12] were maintained in MEM medium supplemented with 10% fetal calf serum (Gibco Laboratories), using previously described techniques [12]. Six fibroblast cell lines from age- and sex-matched healthy volunteers served as controls. Cells were cultivated in serum free medium for 8 h prior to addition of dexamethasone and cadmium sulphate to the medium as a 5 mM stock solution. Cell viability was determined by exclusion of 0.04% trypan blue before or after hormone or cadmium treatment. Greater than 95% viability was observed for all treatment conditions.

Preparation of RNA and TNA

Total nucleic acids (TNA) were prepared by digestion of homogenized cells with proteinase K in a sodium dodecyl sulphate (SDS)-containing buffer and subsequent extraction with phenol–chloroform (1:1) [13]. The concentration of nucleic acids in the TNA samples was measured spectrophotometrically at 260 nm and 280 nm. RNA was prepared by the guanidinium thiocyanate method combined with the $CsCl_2$ centrifugation procedure [14].

Hybridization analysis of RNA

RNA was analysed utilizing a solution hybridization protocol [13] and, in addition, Northern blots [16]. The hybridization analysis of RNA in solution was performed as described previously [15]. Oligonucleotide probes (50 bp) were synthesized and cloned into the Pst I/Hind III [human metallothionein IIA (hMTIIA), hGR] sites in the pGEM TM-I vector (Promega Biotech., SDS, Sweden). These plasmids were used for *in vitro* synthesis of cRNA using SP6 polymerase and the opposite mRNA strand using T7 RNA polymerase, according to Melton *et al.* [17]. The sequences of the hGR and the hMTIIA oligonucleotides used were the same as described previously [15]. In the case of β-actin measurements, results are expressed as a percentage of control value since the chicken derived probe that was used [18] may not form perfect hybrids with human RNA. The issue of the specificity of hybrid formation using short RNA probes has been addressed previously [15]. In Northern blot analysis, nylon membrane (Hybond, Amersham Corp., Sweden) to which RNA had been transferred, was prehybridized for 2 h at 55°C in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 5 mM phosphate buffer, pH 6.5, 5 mM EDTA, 0.1% SDS and 200 μg of salmon testis DNA. Hybridization was carried out for 48 h at 55°C in an identical solution containing 4–6 × 10 cpm/ml of ^{32}P-labelled cRNA hGR probe. After hybridization, the filter was washed serially with 0.1 × SSC and 0.1% SDS at 55°C to eliminate nonspecific binding of the probe to the filter and ribosomal RNA. Autoradiographs were obtained by exposure to Kodak XAR-5 film with an intensifying screen for 72 h at −70°C.

Measurement of RNA stability

The stability of hGR mRNA was measured by actinomycin D chase. The fibroblasts received either control medium or medium containing 1 μM dexamethasone for 48 h. On day 2 actinomycin D (5 μg/ml) was added to treated and control cells. TNA was isolated from the cells at the time of actinomycin D addition and at 0.5, 1, 3, 4, 6 and 12 h after addition, and solution hybridization was performed to determine mRNA levels as described above.

Immunoblotting

FGR and control fibroblasts were incubated with 10 μM dexamethasone and cytosol prepared after 0, 6, 12 and 24 h as described previously [12]. For preparation of cytosol a Tris–EDTA–glycerol buffer with 0.4 M NaCl was used. Following ammonium-sulphate precipitation the pellet was dissolved in the same buffer without NaCl and protein concentration determined according to Lowry [19]. 100 μg of protein from each preparation were separated on a 12% SDS–polyacrylamide gel and electroblotted onto nitrocellulose filter (Bio-Rad protocol, Richmond). Protein content on the nitrocellulose filters was also visualized with Pancean-red (Bio-Rad) and scanned (see below)

to ensure that equal amounts of total protein had been applied on the gel and evenly transferred to the nitrocellulose membrane. The GR protein bands were identified by the monoclonal anti-GR antibodies (no. 7; Ref. 20) and visualized by the Proto Blot Immunoblotting system (Promega Biotech.). The signal intensity of the filters was determined by measuring reflections using a Shimadzu Dual-Wavelength TLC scanner CS-930 (Kyoto, Japan) densitometer.

Measurement of specific DNA-binding

Binding of cytosolic FGR–GR and normal GR to isolated fragments from MMTV DNA was performed as described by Denis *et al.* [21]. Briefly, a 185 bp SstI–EcoRI fragment was isolated from the plasmid pLS 5′ 139 [22]. This fragment contains three individual GR binding sites within the 5′ long terminal repeat of MMTV as assessed by DNase I and methylation protection experiments [23]. As a nonspecific control fragment, a 277 bp Bam HI–Hind III fragment which does not contain any consensus sequence motifs for GR binding was isolated from pBR 322. Labelled fragments (5 ng) were incubated with cytosol previously exposed to the treatments indicated in Fig. 4, and subsequently supplemented with 10 mM sodium molybdate to prevent any further activation process. GR was immunoadsorbed on anti-GR monoclonal antibodies coupled to CNBr-activated Sepharose 4B [22, 24], and the co-precipitated DNA fragments analyzed on a 5% polyacrylamide gel and visualized by autoradiography.

Statistical analysis

A P-value <0.05 was considered statistically significant. The patient's results are presented as the mean $\pm$ SD. When measurements of biochemical variables of the patient were compared to those of normal subjects, the normal means and variances were used to calculate the probability that patient's measurements were not different from those observed in normal subjects.

RESULTS

Size of GR mRNA

Total mRNA derived from FGR-fibroblasts was denatured and examined by agarose gel electrophoresis and hybridization with a ^{32}P-labelled hGR cRNA probe. As demonstrated in

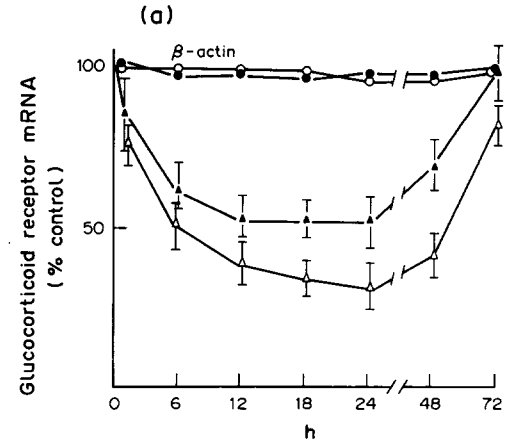

Fig. 1a. Time course of dexamethasone-induced GR mRNA down-regulation. FGR (▲) and control (△) fibroblasts were incubated with dexamethasone (1 μM) for the time points indicated. Total cellular TNA was extracted, and solution hybridization carried out. The values obtained were then expressed as a percent of untreated controls. The data shown represents, in the case of FGR fibroblasts, the mean of four experiments and, in the case of the controls, the mean of three experiments from six control cell lines. β-actin mRNA determinations were included and represent the mean of four experiments after dexamethasone treatment of FGR (○) and control (●) fibroblasts.

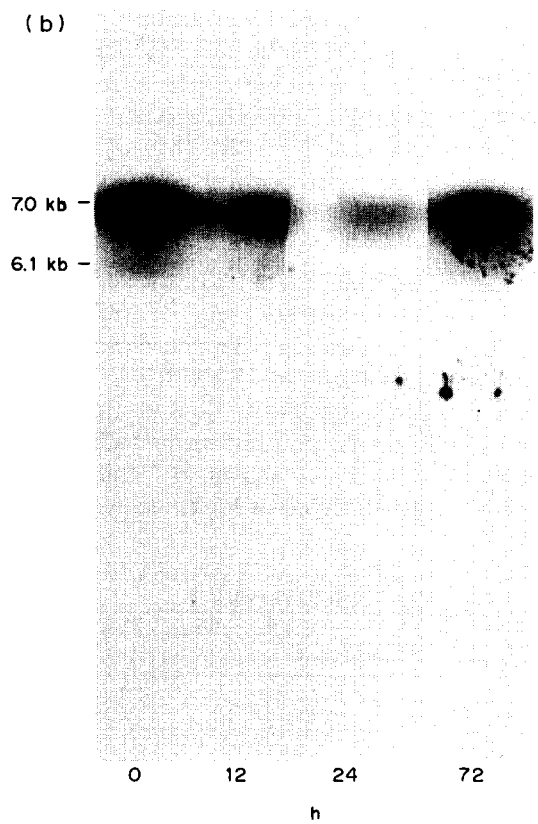

Fig. 1b. Northern blot analysis of GR mRNA down-regulation. FGR fibroblasts were incubated with dexamethasone (1 μM) for the time points indicated. 20 μg of total RNA isolated from FGR fibroblasts was fractionated on a formaldehyde-agarose gel and transferred to nylon filters for hybridization with a cRNA probe for the human GR (see Materials and Methods).

Table 1. GR mRNA and MTIIA mRNA expression in fibroblasts from a patient with FGR

	Temperature	amol hGR mRNA/μg TNA		
FGR patient	35°C	67.2 ± 5.5* ($n = 3$)[a]		
	40°C	55.0 ± 5.0* ($n = 3$)		
Controls ($n = 6$)[b]	35°C	37.5 ± 9.5		
	40°C	29.5 ± 8.0		
FGR-patient ($n = 3$)[a]		*amol hMTIIA mRNA/μg TNA*		
		−dex	*+dex*	*cadmium*
	15°C	40.3 ± 2.9	68.4 ± 3.2 (1.7)[c]	113.2 ± 8.4 (2.8)[c]
	25°C	43.1 ± 8.5	70.1 ± 5.5 (1.6)[c]	137.1 ± 9.3 (3.2)[c]
	35°C	44.1 ± 5.0	50.0 ± 6.5* (1.1)[c]	135.8 ± 5.3 (3.1)[c]
	40°C	40.5 ± 5.5	37.3 ± 4.5* —	120.5 ± 5.0 (3.0)[c]
Controls ($n = 6$)[b]	15°C	39.4 ± 4.8	72.0 ± 3.0 (1.8)[c]	117.2 ± 8.8 (3.0)[c]
	25°C	44.3 ± 3.5	78.4 ± 2.1 (1.8)[c]	123.1 ± 7.6 (2.8)[c]
	35°C	43.5 ± 9.1	74.2 ± 6.8 (1.7)[c]	120.5 ± 8.5 (2.8)[c]
	40°C	42.1 ± 4.4	71.6 ± 3.8 (1.7)[c]	133.3 ± 9.0 (3.2)[c]

[a] Results from 3 separate experiments presented as mean ± SD. [b] Results from analysis of GR mRNA in fibroblasts from 6 healthy volunteers presented as mean ± SD. [c] Fold induction as compared to −dex.
*Significantly different compared to controls.

Fig. 1b, two distinct mRNAs of approx. 7.0 and 6.1 kb were identified. The same mRNA transcripts have previously been identified in fibroblasts from healthy volunteers [15]. The use of alternative polyadenylation sites gives rise to these two receptor mRNA species, both of which are recognized by the cRNA probe [9].

Quantitation and regulation of hGR in FGR and control fibroblasts

The relative amounts of hGR mRNA in FGR and control fibroblasts were determined by solution hybridization. Trichloroacetic acid (TCA)-precipitation of RNA/RNA hybrids demonstrated a significant 1.8-fold increase in basal hGR mRNA expression in FGR fibroblasts ($P < 0.01$). As shown in Table 1, this increase was preserved at higher temperatures.

Table 2. Dose–response data for hGR mRNA determined by solution hybridization

	amol GR mRNA/μg TNA	
Conc dex (M)	FGR patient ($n = 3$)[a]	Controls ($n = 6$)[b]
—	72.0 ± 5.0	40.0 ± 3.1
10^{-10}	65.0 ± 3.5	35.0 ± 2.4
10^{-9}	52.5 ± 3.0	26.3 ± 2.1
10^{-8}	36.5 ± 2.1	22.1 ± 4.0
10^{-7}	33.4 ± 2.1	15.3 ± 3.1
10^{-6}	28.1 ± 4.5	14.3 ± 2.1
10^{-5}	30.5 ± 4.0	18.1 ± 3.5
10^{-4}	35.5 ± 3.5	15.3 ± 2.9

Fibroblasts were incubated with indicated concentrations of dexamethasone for 12 h and solution hybridization carried out as described in Materials and Methods. (Conc = concentration.)
[a] Results from 3 separate experiments presented as mean ± SD. [b] Results from analysis of GR mRNA in fibroblasts from 6 healthy volunteers presented as mean ± SD.

In both FGR and normal fibroblasts down-regulation of GR mRNA was half maximal in the presence of 1 nM dexamethasone and maximal at 1 μM (Table 2). In control fibroblasts, GR mRNA levels were maximally decreased to 35–40% of control levels after 12 h of incubation with dexamethasone (1 μM), whereas in FGR fibroblasts mRNA levels were maximally decreased to 55–60% of control levels after 12 h (Fig. 1a). These differences were not statistically significant. In both cases, GR mRNA levels were restored after 72 h. As demonstrated in Fig. 1a, dexamethasone treatment did not influence the expression of β-actin mRNA. The decrease in GR mRNA could not be blocked by 10 μg/ml cycloheximide, suggesting that dexamethasone acts directly on the expression of GR mRNA with no need for protein synthesis (data not shown).

Expression and regulation of MTIIA mRNA in FGR and control fibroblasts

hMTIIA mRNA is known to be increased in human fibroblasts both by glucocorticoids and heavy metals [25]. Time course studies of dexamethasone and $CdSO_4$ action of MTIIA mRNA levels in human fibroblasts have previously shown a maximal effect at 6 and 6–8 h after onset of treatment, respectively [15]. Based on these data, FGR and control fibroblasts were treated with either dexamethasone (1 μM) or $CdSO_4$ (1 μM) for 6 h prior to TNA

Table 3. Dose–response data for MTIIA mRNA determined by solution hybridization

amol MTIIA mRNA/μg TNA					
Conc dex (M)	FGR patient ($n = 3$)[a]	Controls ($n = 6$)[b]	Conc $CdSO_4$ (M)	FGR patient ($n = 3$)[a]	Controls ($n = 6$)[b]
—	43.1 ± 8.5	44.3 ± 3.5	—	—	—
10^{-10}	48.3 ± 3.1	50.0 ± 3.1	10^{-10}	55.5 ± 3.4	62.3 ± 3.9
10^{-9}	52.4 ± 4.1	56.3 ± 2.5	10^{-9}	90.4 ± 6.7	85.3 ± 6.3
10^{-8}	58.2 ± 4.1	69.3 ± 4.0	10^{-8}	104.3 ± 8.1	99.3 ± 5.5
10^{-7}	70.1 ± 5.5	78.4 ± 2.1	10^{-7}	120.4 ± 4.1	112.4 ± 4.8
10^{-6}	58.3 ± 2.1	74.5 ± 3.1	10^{-6}	132.1 ± 8.3	123.1 ± 7.6
10^{-5}	50.1 ± 5.5	68.4 ± 2.1	10^{-5}	90.3 ± 4.1	102.1 ± 3.5
10^{-4}	55.4 ± 4.2	63.3 ± 3.9	10^{-4}	50.2 ± 3.5	63.4 ± 4.1

Fibroblasts were incubated and solution hybridization carried out as described in Materials and Methods. (Conc = concentration).
[a]Results from 3 separate experiments presented as mean ± SD. [b]Results from analysis of MTIIA mRNA in fibroblasts from 6 healthy volunteers presented as mean ± SD.

preparation. At 25°C, dexamethasone and $CdSO_4$ both caused a dose-related increase in MTIIA mRNA with a maximal inductive effect of dexamethasone at a concentration of 0.1 μM and $CdSO_4$ at a concentration of 0.1 μM (Table 3). Similar dose-related increase in MTIIA mRNA expression was observed at 35 and 40°C (data not shown).

At elevated temperatures, 35 and 40°C, no differences in basal MTIIA mRNA expression were observed between FGR and control fibroblasts. However, in contrast to control fibroblasts in which dexamethasone caused at 1.7-fold induction of MTIIA mRNA regardless of temperature (15, 25, 35 and 40°C), dexamethasone did not induce MTIIA mRNA in FGR fibroblasts at elevated temperatures (35 and 40°C) ($P < 0.001$) (Table 1). At lower temperatures (15 and 25°C) dexamethasone induced MTIIA mRNA similarly in FGR and control fibroblasts. This indicates the presence of a defective (temperature sensitive) GR protein in FGR fibroblasts, since it is known that the induction of MTIIA mRNA by dexamethasone requires a functional GR [25]. Regardless of temperatures applied, $CdSO_4$ (1 μM) caused a 2.8–3.2-fold induction of MTIIA mRNA both in FGR and control fibroblasts.

Effect of dexamethasone on GR mRNA stability

In order to investigate whether glucocorticoids down-regulate GR mRNA steady-state levels by decreasing mRNA half-life differently in FGR fibroblasts compared to control fibroblasts, studies using the RNA polymerase inhibitor actinomycin D were carried out. FGR and control fibroblasts were preincubated with dexamethasone (1 μM) for 48 h prior to the addition of actinomycin D. The receptor half-life was 6–7 h in both FGR and control fibroblasts. Dexamethasone treatment had no effect on the GR mRNA half-life of FGR or control cells (data not shown).

Effect of dexamethasone on GR protein levels

Incubation of FGR and control fibroblasts with dexamethasone (1 μM) for various time-periods indicated no differences in down-regulation of GR protein as assessed by Western immunoblotting (Figs 2a and b). As seen from the nitrocellulose filters and scanning data, down-regulation of GR was maximal after approx. 12 h with no further effects on the protein after 24 h. According to scanning data, approx. 35% of base line GR levels remained after 12 h and 30–35% after 24 h (Figs 2a and b).

Binding of GR to MMTV DNA

The DNA-binding properties of cytosolic FGR and control GR were investigated by the binding of different molecular states of GR to the LTR-region of MMTV DNA. Interaction of the FGR–GR with MMTV DNA occurred both in the presence of hormone at 4°C and in the presence of hormone after exposure to elevated temperature, 25°C (Fig. 3, lanes E and G). Control GR interacted with MMTV DNA only in the presence of hormone and after exposure to elevated temperature (lanes F and H). These results indicate an increased specific DNA-binding of the unactivated (=unexposed to elevated temperature) FGR–GR. Heat-treated, unliganded FGR and control GR did not bind to MMTV DNA (Fig. 3, lanes C and D). Even when FGR and control GR were labelled following heat-treatment no binding was observed (data not shown). No immunoprecipitation of GR–MMTV–DNA complexes occurred when the samples were incubated with ascites from Sp 2/0 cells, which served as the control [22, 24] (Fig. 3, lanes I and J).

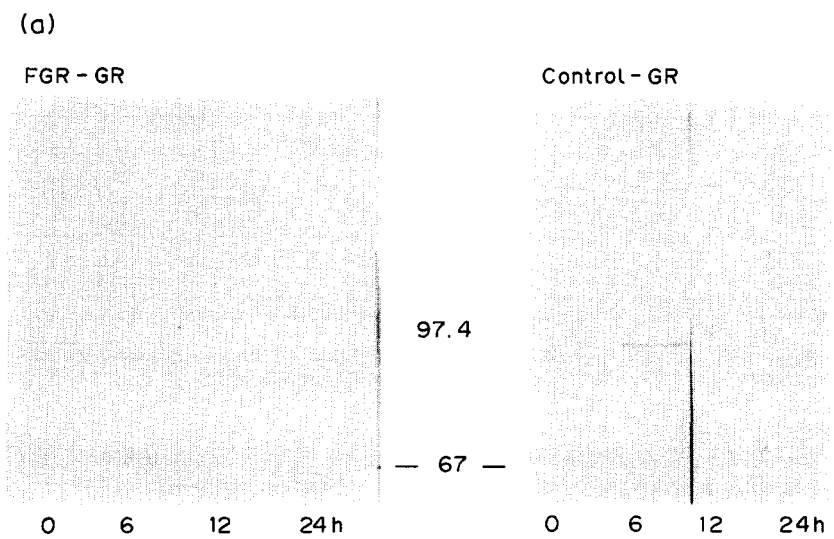

Fig. 2a. Western blot analysis of cytosolic PCR and control GR separated by SDS–PAGE. Fibroblasts were treated with 1 μM dexamethasone for the time indicated. Cytosol was prepared and subjected to Western blot analysis as described in Materials and Methods. Each lane contained 100 μg total cellular protein. Standard proteins: M_r 97,000 (phosphorylase b) and M_r 67,000 (bovine serum albumin).

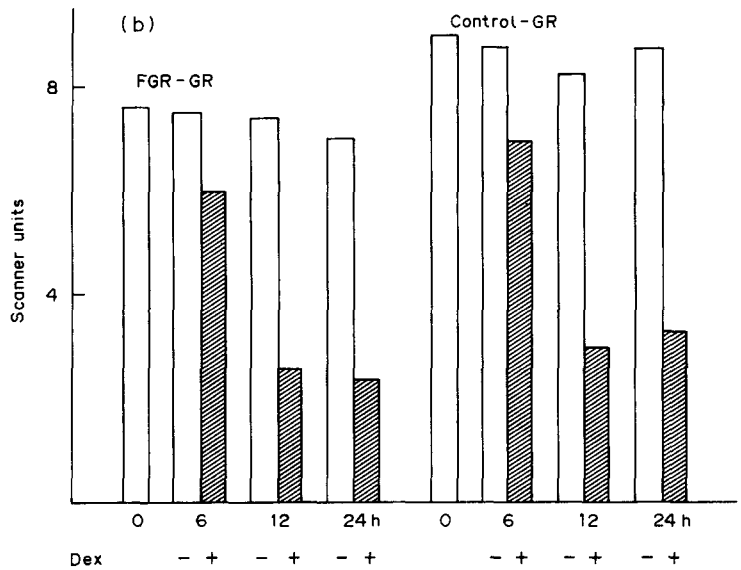

Fig. 2b. Densitometric scans of nitrocellulose membranes. Results from densitometric scans of nitrocellulose filters shown in Fig. 2a. The data are presented as scanner units from densitometric peak areas of two separate experiments. Densitometric scans of Western blot analysis of cytosolic FGR and control GR not treated with dexamethasone are included (dex-).

DISCUSSION

We have previously described a patient with increased secretion of cortisol due to FGR, associated thermolability of the GR protein and with fatigue as the only clinical feature [12]. In more severe forms of the disease, hypertension and hypokalemic alkalosis are present owing to increased secretion of the sodium-retaining glucocorticoids, corticosterone and deoxycorticosterone [6]. In our patient, presenting with a less severe resistance to cortisol, the thermolability of GR was reflected in a loss of specifically bound ligand and lack of dexamethasone-induced decrease in thymidine incorporation *in vitro* at elevated temperatures. This paper describes a more detailed analysis of this patient's FGR syndrome. We have used GR mRNA as an indirect assessment of the regulation of GR. The regulation of steroid receptors is of significance since target tissue sensitivity to hormonal signals has been demonstrated to correlate to receptor number [27, 28]. Furthermore, a recent study by Dong *et al.* [28],

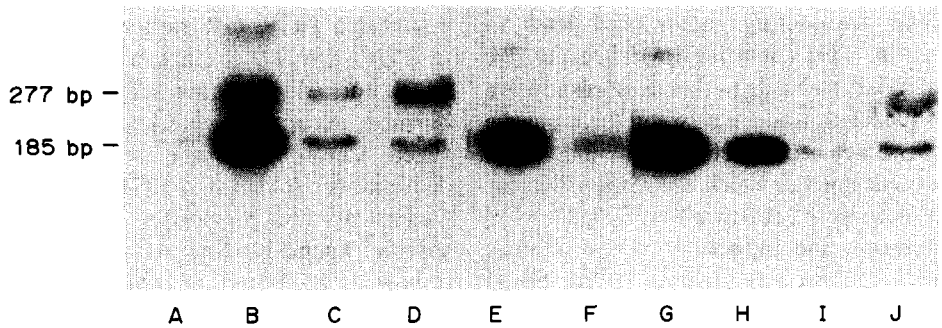

Fig. 3. Binding of GR to MMTV DNA. A 185 bp ^{32}P-labelled fragment isolated from the plasmid pLS5 139 [21] and a 277 bp ^{32}P-labelled fragment isolated from pBR 322, were incubated with GR and anti-GR monoclonal antibodies as described in Materials and Methods. Treatments of the GR in crude cytosol from FGR and control fibroblasts were carried out according to the following protocol. (i) immunoprecipitated control GR in the absence of an antibody (lane A); (ii) input mixture of the two DNA fragments used (lane B); (iii) FGR and control GR exposed to elevated temperature (25°C, 30 min) in the absence of dexamethasone (lanes C and D, respectively); (iv) labelling of FGR–GR with [^{3}H]-dexamethasone (1 μM, 4°C, 1 h) (lane E); (v) labelling of control GR with [^{3}H]-dexamethasone (1 μM, 4°C, 1 h) (lane F); (vi) labelling of FGR–GR with [^{3}H]-dexamethasone (1 μM, 4°C, 1 h) followed by exposure to elevated temperature (25°C, 30 min) (lane G); (vii) labelling of control GR with [^{3}H]-dexamethasone (1 μM, 4°C, 1 h) followed by exposure to elevated temperature (25°C, 30 min) (lane H); and (viii) immunoprecipitated FGR and control antibodies Sp2/0 (lanes I and J, respectively).

demonstrated the presence of down-regulation of GR in the intact animal and showed that the decrease of GR was accompanied by reduction of target organ response to glucocorticoids.

We have previously shown that human skin fibroblasts normally contain approx. 40 amol GR mRNA per μg TNA [15]. Similar values have been found in most other human cell types with the exception of peripheral lymphocytes which only express 10–15 amol GR mRNA per μg TNA [15]. As indicated in this study, basal GR mRNA expression in FGR fibroblasts was significantly increased. This difference may reflect a compensatory mechanism caused by the GR thermolability. In contrast to our study, Linder and Thompson [29] reported lowered levels of GR mRNA and consequently no compensatory up-regulation of GR mRNA levels in cells from two patients with abnormal glucocorticoid-binding affinities and lowered number of GR-binding sites. These contradictory results show that autoregulation of GR by its cognate ligand is complex, and apparently, glucocorticoids regulate GR at both the transcriptional and post-transcriptional level [28, 30].

The effect of glucocorticoids on the regulation of GR mRNA was studied in FGR and control fibroblasts at different times after dexamethasone treatment. Of particular interest is our finding that the FGR fibroblasts demonstrated a 40% (as opposed to 60% in control fibroblasts) decrease in GR mRNA. These differences are not statistically significant but indicate that although the FGR fibroblasts have higher basal GR mRNA levels and the patient shows high endogenous levels of cortisol, the regulation of the receptor mRNA is similar (although less efficient) when compared to that found in control fibroblasts. Furthermore, dexamethasone treatment down-regulated FGR and control GR protein to 30–40% of control, which is in accordance with previously presented data on GR autoregulation [28, 30]. Thus, glucocorticoid treatment of cells *in vitro* or *in vivo* can not completely inhibit the endogenous GR expression. The results presented in this study suggest that there may be alterations in autoregulation of receptor gene expression in the affected patient. However, the changes detected by measurements of GR mRNA accumulation are indirect evidence for changes in gene transcription and therefore difficult to evaluate. The data imply that larger differences would be detected if rates rather than accumulation of receptor gene transcription were measured. Such analyses are not yet available.

Activated GR complexes bind to specific sites on DNA termed glucocorticoid responsive elements (GREs) [31]. The GRE within the human MTIIA gene is located in the 5′-flanking DNA and has been shown to function independently of the gene's promoter [25]. Glucocorticoids, as well as heavy metal ions, increase the levels of MTIIA gene transcripts and this gene serves as a useful model to understand gene expression in cases of glucocorticoid resistance. It is assumed that heavy metal ions bind to an

intracellular transacting factor and lead to its activation. This (putative) metal-responsive factor (MRF) then binds to the metal responsive elements (MREs), and because of their unique location, contact other factors that bind to the adjacent *cis*-elements, which are responsible for the basal activity of the promoter [25, 32].

Furthermore, the induction of the MTIIA gene by glucocorticoids requires a functional GR protein [25]. Thus, any biochemical defect intrinsic to the receptor protein affecting specific DNA-binding and interaction with GREs, will influence transcription of glucocorticoid regulated genes. In this study, $CdSO_4$, but not dexamethasone, induced MTIIA mRNA levels in FGR fibroblasts at elevated temperatures indicating a structural abnormality in the receptor molecule making it temperature labile. From our study it is thus concluded that the induction of MTIIA mRNA by dexamethasone at elevated temperatures is a relevant indicator of GR thermolability.

The ability of FGR and control GR to bind to GREs was also used in studies of GR-binding to MMTV DNA. The 5′ long terminal repeat of MMTV is well defined with regard to GR-binding sites (for a review, see [33]). *In vitro*, it has been shown that the GR is associated with the 90 kd heat shock protein (hsp 90) when it is not bound to its ligand and before it is activated [21, 24, 34, 35]. In addition, it has been shown that hsp 90 interacts with the steroid-binding domain of the GR [21]. The activation process of steroid hormone receptors is poorly understood but it has been shown that there is a requirement of hormone for thermal conversion of the GR to a DNA-binding state. Thus, ligand-binding may induce a conformational change within the steroid-binding domain of the receptor leading to a dissociation of the hsp 90–GR complex and an increased specific DNA-binding activity of GR. Based on these data and considerations, a model has been proposed in which steroid receptor function is repressed by association of the receptor with hsp 90 [24].

In this study, liganded FGR–GR, in contrast to liganded control–GR, was shown to bind specifically to MMTV DNA in the absence of heat-treatment. According to the model presented above, these results indicate an altered interaction between hsp 90 and FGR–GR consequently affecting specific DNA-binding and the inducibility of GR-regulated genes. It is more likely that such an alteration is caused by a mutated FGR–GR rather than a mutation in FGR–hsp 90, in that a defective hsp 90, which normally also interacts with other steroid hormone receptors, would cause a more generalized steroid hormone receptor dependent disturbance. A mutated FGR–GR is also supported by the previously described loss of specific ligand-binding at elevated temperatures [12]. It should be pointed out that the putative GR-defect in this patient did not seem to alter binding to DNA-cellulose as compared to controls [12].

In summary, this case of FGR is associated with a defect in the GR protein affecting the binding of GR to MMTV DNA as well as the specific ligand-binding and the induction of the GR-regulated gene MTIIA at elevated temperatures. Studies are currently in progress to further characterize this defect.

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