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STEROID MEDIATED LYSIS OF LYMPHOBLASTS REQUIRES THE DNA BINDING REGION OF THE STEROID HORMONE RECEPTOR

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Summary—Glucocorticoids kill certain types of lymphoblasts, but the mechanisms are unknown. It is clear that sufficient numbers of functional glucocorticoid receptors are required to mediate lysis, but whether they do so through the classical model of steroid hormone activation and modulation of gene expression has not been established. In this report we have asked which region(s) of the steroid receptor are important for mediating lysis in leukemic T lymphoblasts. CEM-ICR 27 leukemic lymphoblasts, a clone of CEM cells which lack functional glucocorticoid receptors and therefore are neither lysed by dexamethasone nor capable of showing glutamine synthetase induction, were provided with steroid receptors by DNA transfections of various receptor gene constructs. We measured steroid mediated lysis, receptor number and induction of glutamine synthetase in the transfected cells. Our results provide evidence that the lysis mechanism in the ICR27 lymphoblasts is restored when functional receptor number is restored. The DNA binding region specifying high affinity for GRE sites is required. Lysis is mediated by any steroid that allows for activation of the receptor containing such a region. Our data support the view that steroid-mediated cell death occurs by a process requiring direct ineraction of steroid-receptor complexes with the genome.

INTRODUCTION

The mechanisms through which steroids induce and deinduce genes are becoming better understood, and central in the process are the steroid hormone receptors. These receptors clearly have been shown essential for gene induction. One of the earliest, compelling pieces of evidence for the critical nature of steroid hormone receptors in steroid action came from the lethal effect of glucocorticoids on certain lymphoblasts. Lymphoid cells selected for resistance to kill by glucocorticoid often have been found to contain altered glucocorticoid receptors [1]. It has been suggested that glucocorticoids kill lymphoid cells by inducing endonucleases, deinducing oncogenes, altering calcium influx, blocking cells in G1, deinducing lymphokine production, and a variety of other mechanisms (for review see [2]; [3–11]). However the actual mechanism by which glucocorticoids kill lymphoid cells still is unproven, and so is the exact nature of the role of their receptors in this process. All that is known is that the receptor is essential, and it would be premature to assume that cell kill is carried out by a mechanism directly comparable to the best-studied mechanism, enzyme induction.

Steroid hormone receptors belong to a super family of related genes which include the receptors for adrenal steroids, sex steroids, vitamin D3 and thyroid hormones [12]. Glucocorticoids follow the classical model of steroid hormone action, i.e. the steroid hormone binds to a cytosolic receptor, which then undergoes an "activation" or "transformation", translocates to the nucleus, and binds to specific *cis*-active regulatory sites (glucocorticoid response elements or GREs) on the DNA to alter gene expression either through induction or repression [13]. In recent years, several regions of the human glucocorticoid receptor important for gene induction have been mapped [14–16]. They demonstrate the domain structure common to all the steroid and thyroid hormone receptors. These regions include a highly conserved 66–68 amino acid DNA binding domain, a large carboxy-terminal hormone binding domain, and an amino-terminal "immunological" domain which includes a transcription-enhancing domain τ_1. The τ_1 region is also found in the progesterone receptor but not in the estrogen receptor [17, 18]. In addition there is in all these receptors a "hinge" region connecting the DNA binding and steroid binding regions. Whether this in fact serves as a flexible part of the protein is not clear, but some data suggests that it or nearby regions may be important for protein–protein binding [19]. In addition, recent evidence by several groups show that the exact relative positions of

hormone and DNA binding domains are not essential for hormone induced transcription activation [15, 20]. Webster *et al.* have suggested that the steroid hormone serves a dual role in that it "unmasks" the DNA binding domain and also induces a transcription activating domain located within the hormone binding domain itself [15]. These investigators showed transcription activation of Gal4 responsive elements only with the steroid hormone but not the anti-hormone. This data suggests that the particular steroid hormone used in an assay may be important in transcription activation of a particular gene. In addition to their transcriptional effects, glucocorticoids have been shown to modulate expression of genes at the post transcriptional, translational and post translational levels [21–24]. How steroids do so, and what topological parts of receptor are required (if any) for these effects are unknown.

Glucocorticoids effect numerous phenotypic changes in cells of the immune system, including lysis of some cells at certain stages of maturation. Whether or not the classical (transcriptional) mode of steroid hormone action is the mechanism behind the glucocorticoid mediated lysis is unknown; however, several possible pathways for this mechanism could include induction of an endonuclease resulting in nuclear fragmentation [3–5] or repression of growth promoting genes such as c-*myc* [6–8]. We have studied the effects of steroid hormones on human leukemic lymphoblasts using the receptor-containing (r^+) glucocorticoid sensitive, $T4^+$ (CD4+) CEM C7 parental cell line. Among several characteristics important for glucocorticoid mediated lysis of these cells is the expression of a sufficient number of functional glucocorticoid receptors [1]. From these cells Harmon and Thompson derived the receptor-deficient mutant clone, ICR 27 which is deficient in functional glucocorticoid receptors (r^-) [25, 40]. In the r^+ parent, glucocorticoids cause induction of glutamine synthetase (GS), inhibit growth by preventing passage of the cells from the G1 phase of the cell cycle, and eventually cause cell death. The wild-type cells contain about 14,000 glucocorticoid receptor sites per diploid cell. Upon treatment of the ICR 27 r^- mutants with glucocorticoids none of these events occur. Complementation studies in somatic cell hybrids show however that ICR 27 cells do respond if fused with r^+ cells lacking the lysis response [26]. By supplying ICR 27 r^- cells with intact receptor gene, we could find definitively whether the sole element they lacked for glucocorticoid-mediated lysis was functional receptor. If that were so, ICR 27 cells would represent an ideal test system in which to define the domains of the GR required to mediate lysis, and whether they coincided with or differed from those important in gene induction? In this report, we have transfected the ICR 27 r^- mutant with several steroid receptor expression vectors to explore these questions.

EXPERIMENTAL

Reagents

Dexamethasone was obtained from Sigma Chemicals, St Louis, Mo. 17β-Estradiol was obtained from Research Plus, Danville, N.J. Diethylstilbestrol for binding assays was obtained from Sigma chemical. These steroids were reconstituted in absolute ethanol immediately prior to use. The labeled hormones, [^{3}H]dexamethasone and [^{3}H]17β-estradiol were obtained from New England Nuclear and Amersham, Inc., respectively and used within 1 month of preparation and receipt. Leupeptin, used as a protease inhibitor, was obtained from Sigma Chemicals and stored at $-20°C$. Reagents for glutamine synthetase reactions were prepared fresh immediately prior to assay. L-Glutamine (Gibco Labs, N.Y.) was stored at $-20°C$ in stock concentrations at 2M. Manganese chloride and ferric cholride were obtained from Fischer Scientific; arsenic acid sodium salt was from Sigma Chemicals and hydroxylamine hydrochloride was from United States Biochemical Corporation and stored desiccated. Glutamine synthetase, α-glutamyl hydroxamate and adenosine diphosphate were obtained from Sigma Chemicals and stored at $-20°C$. Bovine serum albumin 98–99% pure from Sigma Chemicals was used as the protein standard for the Bradford protein determinations. The BSA was prepared analytically at 20 mg/ml in isotonic phosphate buffered saline (PBS) and stored in aliquots at $-20°C$.

Cell cultures and transfection assay

CEM C7 cells and their steroid-resistant subclone ICR 27 were kept in log growth in RPMI 1640 supplemented with 5% fetal calf serum by keeping the cultures at less than 10^6 cells/ml. ICR 27 cells were used for transfection as follows: ICR 27 cells in mid-log growth were washed 3 times in cold PBS which contained no magnesium or calcium. Viability of the cells after washes was determined to be >95%. The cells were reconstituted to 1×10^7 cells/ml in buffer and 0.8 ml was added to an electroporation cuvette (BIORAD) containing 15 μg of plasmid DNA or irrelevant lymphocyte DNA (generously donated by Dr Preston, Gadson). The cuvettes were placed on ice for approx. 15 min prior to electroporation and subsequently placed in a BIORAD gene pulser with a setting of 200 V and a capacitance extender setting of 500 μFD. The pulse time was approximately 10–14 ms. After the pulse, the cells were allowed to sit on ice for 5 min and placed in tissue culture dishes in 10 ml of appropriate medium for 24 h prior to asay. The ICR 27 cells receiving the ER-GR.cas plasmid DNA were placed in phenol free RPMI (Gibco Labs, Grand Island, NY) plus serum and all other cells were grown in RPMI 1640 containing Phenol Red and serum. The plasmid clones pRShGRα, 1422 and ER-GR.cas were as described [14, 32]. Plasmid DNA was transformed into *E.*

coli HB101 competent cells (Bethesda Research) Labs, Md.). Clones were tested for their resistance to ampicillin. Plasmids were amplified and prepared by cesium chloride density gradients are previously described [27]. The ER-GR.cas was verified for the presence of the ER-GR.cas cDNA sequence by the presence of a 1.8 kb band after digestion with Eco RI. The presence of the ShGR and 1422 inserts were verified by the presence of a single 6.72 kb band after digestion with Kpn 1. Plasmid DNA was stored in TE buffer [27] at 4°C prior to use.

Steroid lysis

The transfected cells were assayed for their sensitivity to steroids as follows: CEM C7 dexamethasone sensitive T cells, ICR 27 dexamethasone resistant mutants and the ICR 27 transfectants were cultured at 5×10^5 viable cells/ml in RPMI 1640 supplemented with 5% fetal calf serum (FCS) for the ShGR, mock and 1422 transfectants. Phenol free RPMI 1640 supplemented with 5% FCS was used for the ER-GR.cas transfectants. Dexamethasone or 17β-estradiol was reconstituted immediately prior to use in absolute ethanol and added to the 2 ml culture wells in 20 μl volumes at a final concentration of 10^{-6} M and 10^{-7} M, respectively. Mock treated wells received 20 μl ethanol vehicle. The cells are cultured in duplicate at 37°C, 5% CO_2 for 48 h. At the end of culture, an aliquot from each culture well was taken to determine the percent viability as measured by trypan blue dye exclusion according to standard procedures [28].

Dexamethasone binding assays

ICR 27 cells electroporated with 15 μg of glucocorticoid receptor plasmid DNA, ShGR or 1422, were allowed to rest for 24 h prior to assay. Transfected cells were pooled and washed 3 times in RPMI 1640. The cells were reconstituted to 1×10^{-7} cells/ml and 500 μl was placed in glass tubes. Radiolabeled [^{3}H]dexamethasone (New England Nuclear, specific activity 119 mCi/mg) was added to quadruplicate tubes in final concn of 10^{-7} M–10^{-9} M. Cold dexamethasone at 1000-fold excess was added in duplicate for each dilution of radiolabeled dexamethasone. The tubes were incubated with gentle rotation for 1 h at 37°C. After incubation, the supernatant fluid was removed and reserved from each tube for determination of total counts. The cells were rapidly washed 3 times in PBS and reconstituted in 500 μl for cell counts and determination of radioactivity using standard light scintillation techniques.

Estradiol binding

ICR 27 cells were electroporated with 15 μg/ 8×10^6 cells of ERGR plasmid DNA for each cuvette as described above. The cells were allowed to rest for 24 h and the transfected cells were pooled. The cells were washed in ice-cold Tris buffer (1.5 mM EDTA, 10 mM TRIS, 0.5 mM dithiothreitol, 10 mM sodium molybdate, pH 7.4) as described [29]. Briefly, the cells were disrupted by either sonication using a Branson sonicator or by freeze-thawing twice. The cell debris was removed by centrifugation at 800 *g* for 5 min. Cytosol was prepared by centrifugation of the supernatant fluid at 104,000 *g* for 1 h at 4°C. Free steroids were absorbed from the supernatant cytosol by vortexing and incubating with dextran coated charcoal for 15 min at 4°C followed by centrifugation at 1500 *g* for 15 min. The supernatant fluid was reserved and its protein content determined by the Bradford method [30] using BSA as the protein standard. Leupeptin (17 μIU/ml), a protease inhibitor, was added to cytosol containing 2 mg/ml protein. The binding assay was performed on ice in glass tubes as follows: 100 μl of cytosol was added to quadruplicate tubes. Radiolabeled [^{3}H]17β-estradiol (Amersham, specific activity 158 Ci/mmol) was added to quadruplicate tubes in 10 μl aliquots for a final dilution of 10^{-8}–10^{-12} M, and 1000-fold excess cold diethylstilbesterol (Sigma Chemicals) was added to duplicate tubes to determine specificity of binding. The reaction was allowed to proceed for 18 h at 4°C. Dextran-coated charcoal 500 μl was added to absorb all unbound steroid; the mixture was vortexed and centrifuged at 1500 *g* for 15 min. The supernatant fluid was removed and radioactivity monitored using standard liquid scintillation techniques.

Glutamine synthetase assay

ICR 27 parents, CEM C7 cells, as well as pooled transfectants were washed 3 times in RPMI 1640 and reconstituted at 4×10^6 cells/ml in 10 ml RPMI containing 0.5 mM L-glutamine. The transfectants were treated with 10^{-6} M dexamethasone, 10^{-7} M estradiol or ethanol alone for 24 h at 37°C, 5% CO_2. After incubation the cells were harvested, washed 2 times in PBS and resuspended in 300 μl of cold 25 mM sodium citrate buffer containing 0.15 M NaCl, pH 6.4. The cells were disrupted by sonication or by rapid freeze thawing twice. The cellular debris was removed by centrifugation at 800 *g* for 10 min. The supernatant fluid was removed and assayed for glutamine synthetase activity as previously described with some modifications [31]. The reaction mixture was as follows: 100 μl of 260 mM L-Glutamine, 20 μl of a mixture of 100 mM $MNCl_2$, 250 mM hydroxylamine hydrochloride, and 1 mM ADP, 20 μl of 420 mM Arsenic acid and either 50 μl of cell extract + 50 μl buffer or 50 μl of glutamine synthetase standard (Sigma Chemicals) + 50 μl of assay buffer. All of the above reagents were prepared in the citrate buffer. The reaction mixture was mixed well and incubated on a shaker platform at 37°C for 40 min. The reaction was stopped with 200 μl of stop reagent (0.2 N tricholoacetic acid, 0.7 N HCl, 0.37 M $FeCl_2$–$6H_2O$ in citrate buffer). The mixtures were centrifuged at 1500 *g* for 10 min. The supernatant fluid was removed and the A_{500} was read. A standard curve was plotted from the results with the glutamine synthetase

standard or from measured amounts of α-glutamyl hydroxamate. Protein determinations were made from the remaining supernatant fluids using the Bradford method [30] and BSA as the standard protein.

RESULTS

Figure 1 shows the experimental protocol and the properties provided by the cDNA insertions present in each eukaryotic expression vector. Three were chosen for this initial study, to test specific questions of receptor function. First, pRShGR contains ShGR, the complete coding sequence of wild type human glucocorticoid receptor; therefore the receptor it codes can bind to GREs as well as to the ligand dexamethasone [14]. Expression of pRShGR in r^- transfected cells would therefore test whether the lytic effect of glucocorticoids can occur in those cells developing sufficient levels of GR, and whether their sole defect is lack of GR. The need for the DNA binding domain of the GR for cell lysis was tested by 1422, an insertional mutant of the ShGR in positions 422–424. This mutant has been shown to bind dex but not to enhance transcription from a GRE-containing promoter [14]. The ER-GR.cas (ERGR) hybrid gene contains the entire cDNA coding region for the estrogen receptor with the exception of the section coding for the domain that binds to the estrogen response element in DNA. That section has been replaced by the gene segment of the glucocorticoid receptor coding for the domain that binds the significantly different glucocorticoid response element [32]. Therefore this receptor binds GREs and estrogens but not EREs or glucocorticoids. It has been shown to convert a glucocorticoid-inducible gene to estrogen-inducible in transfected cells [32]. Therefore, this construct tests the importance of the specific steroid in mediating lysis mechanisms. The ICR 27 (r^-) T leukemic lymphoblasts were transiently transfected with the expression vectors by electroporation as described in methods and previously [33, 34].

We initially determined the percent viable cells after the transfectants were treated with either 10^{-6} M dexamethasone, 10^{-7} M estradiol or ethanol vehicle. Figure 2A shows that ethanol treated transfected cells and parents are more than 90% viable after 48 h incubation. We next determined whether the introduction of an intact glucocorticoid receptor,

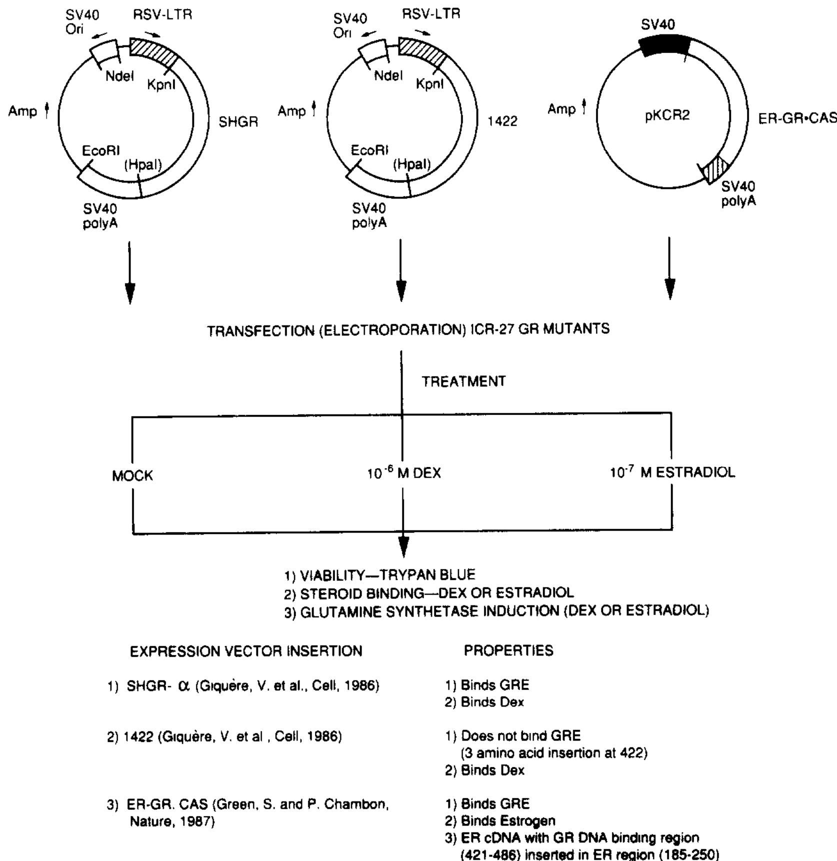

Fig. 1. Experimental protocol and properties of expression vectors used in transfecting ICR/27 glucocorticoid resistant mutants.

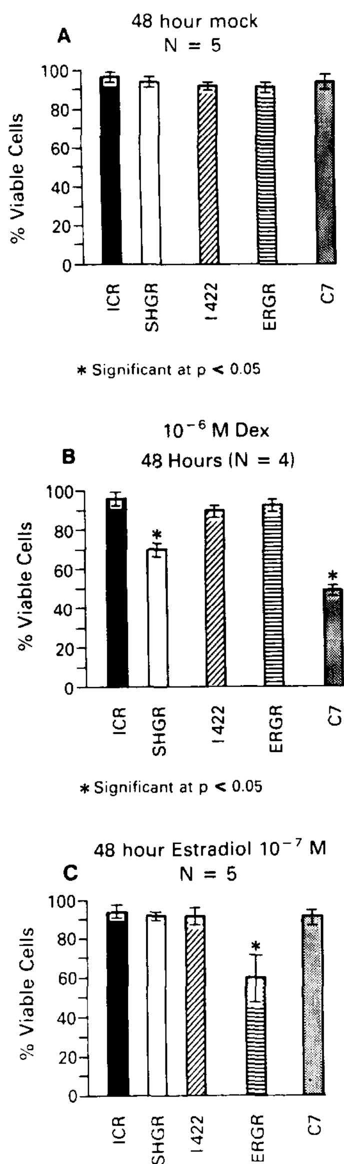

Fig. 2. Treatment of ICR27 mutants, transfected cells and CEM C7 parent cells with steroids. Numbers are expressed as the average of several experiments with standard deviation determined between experiments. Viable cell percentage was visualized and determined at 10 × magnification. The statistical analysis was performed using the Students *t*-test. (A) represents viability studies of transfected cells treated with ethanol. (B) represents transfected cells treated with dexamethasone. (C) represents transfected cells treated with estradiol. ICR27 and CEM C7 cells are mock transfected cells, ShGR are transfectants which received plasmid DNA containing ShGR, 1422 are transfectants which received plasmid DNA containing the 1422 insertional mutant, ERGR are transfectants which received plasmid DNA containing the 1422 insertional mutant, ERGR are transfectants which received plasmid DNA containing ER-GR.cas hybrid receptor.

ShGR, could mediate lysis of the ICR 27 mutants. Figure 2B shows the results of various transfectants after treatment with dexamethasone for 48 h. The results show that a significant percentage of those transfected cells receiving the intact receptor, ShGR,

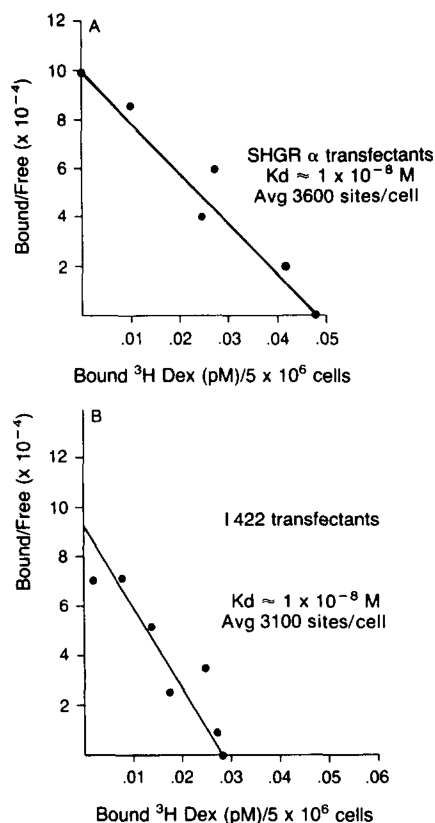

Fig. 3. Scatchard analysis of glucocorticoid binding assay with transfected cells. Results are expressed as average of 3 experiments. (A) Scatchard analysis of ShGR transfectants. (B) Scatchard analysis of 1422 transfectants.

were lysed by treatment with dexamethasone. (Since these initial, statistically significant results, we have repeated this experiment 7 times and found 15–30% kill each time in the cells that received ShGR and then were exposed to dexamethasone-data not shown.) The transfectants with a GR mutated in the DNA binding region 1422 continued to be insensitive to dexamethasone.

In order to verify that the lysis was in fact being mediated by the presence of functional glucocorticoid receptors, we performed radioligand binding assays for dexamethasone binding. Figure 3 is a verification that glucocorticoid receptors are now present in the cells. Those cells receiving the ShGR receptor showed an average K_d of 1×10^{-8} M with an average in all experiments of approximately 3600 sites per cell in the entire population, as compared to < 1000 sites/cell in the untransfected ICR 27 parent population. Indeed, binding in the untransfected cells was so low that under our assay conditions no clear-cut, reproducible data adequate for Scatchard analysis could be obtained, and the < 1000 sites per cell is estimated from those concentrations of ligand at which some competable binding was seen. Figure 3b shows that the 1422-containing transfectants bind dexamethasone with a K_d of approximately 1×10^{-8} M and an average in all experiments of 3100 sites per cell. These

studies strongly suggest that lack of ability of glucocorticoids to mediate lysis of the ICR 27 mutants is solely due to the lack of expression of sufficient numbers of functional glucocorticoid receptors and not an inherent resistance to lysis in these cells. We interpret the kill of only part of the transfected cells to be the consequence of only a fraction of the transfectants achieving sufficient plasmid or GR concentrations to respond. In several pilot experiments, we have found radioautography of cells transfected with labelled DNA, about $20 \pm 10\%$ of cells retain significant label at the time of the tests done herein (data not shown).

In a similar sequence of experiments, we asked whether the ER-GR.cas hybrid gene was able to mediate lysis in the ICR 27 mutants. Figure 2C shows the results of 10^{-7} M estradiol treatment on similar transfected cells. A significant reduction in the number of viable cells was seen in ICR 27 r-mutants receiving the ER-GR.cas expression vector when they were exposed to estradiol, an effect which was not seen in the mock treated transfectants. In contrast, there was no significant change in viability of the transfectants receiving receptor genes not coding for an estrogen binding site and treated with estradiol.

To verify that receptors with estrogen binding sites were actually being expressed in the ICR 27 ER-GR.cas transfectants, we performed estradiol binding assays on cytosol extracts of transfectants and ICR 27 r-parents. Figure 4 shows a representative binding assay. The cells contain classic ER binding sites, with a K_d of approximately 1.2×10^{-10} M and a B_{max} of approximately 4.2 fmol/mg protein. This K_d is consistent with the 4×10^{-10} M K_d previously reported for the transfected intact estrogen receptor [32]. The slightly higher K_d for the ER-GR.cas, if significant, may be explained by the altered DNA binding region, which may cause a slight conformational change in the ligand binding region and thereby a change in affinity for the steroid.

Because Thompson and coworkers had shown alterations in the expression of several genes in leukemic cells after treatment with glucocorticoids, we analyzed the induction of one of those, glutamine synthetase, in the various transfectants after dexamethasone treatment [31, 35]. Table 1 confirms that the glucocorticoid sensitive CEM C7 parent is induced by dexamethasone to express increased GS activity, whereas the glucocorticoid resistant clone ICR 27 as expected, does not express induction of the enzymatic activity. Transfectants receiving ShGR increased their GS activity after treatment with dexamethasone but not estradiol. The transfectants receiving 1422, the GR DNA binding site mutant, were found to bind dexamethasone, but they did not show increased glutamine synthetase activity after treatment with dexamethasone. This suggests that specific DNA binding of a steroid receptor to the endogenous nuclear GRE is necessary for induction of this endogenous gene. We were unable to induce glutamine synthetase activity in the ER-GR.cas transfectants by treatment with either glucocorticoid or estradiol, suggesting that there is a dissociation of the mechanism of lysis and GS induction at the nuclear level. Since the ER-GR.cas construct lacks the τ_1 region of the GR, perhaps it is important for inducing GS activity. We are currently analyzing the ability of dexamethasone to induce GS activity in cells transfected with various τ_1 mutants of the glucocorticoid receptor to try to determine whether the lack of GS induction is due to conformational alterations brought about by the hybrid receptor or a lack of transcriptional factor interaction with the receptor. Use of these τ_1 mutants will allow for finer mapping of the GS induction by the glucocorticoid receptor as well as address the regions necessary for lysis.

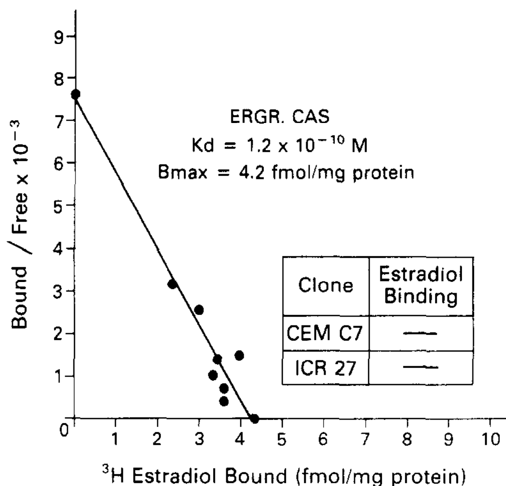

Fig. 4. Scatchard analysis of cytosolic estradiol binding to ERGR transfectants. Results are representative of 3 experiments.

Table 1. Glutamine synthetase induction in parental clones and transfectants

Clone or transfectant	Dex binding	Estradiol binding	Lysis: Dex sensitive	Lysis: Estradiol sensitive	GS activity*: Basal	GS activity*: Dex induced	GS activity*: Estradiol induced
CEM C7	+	–	+	–	1.25 ± 0.4	4.6 ± 0.9	1.8 ± 0.5
ICR 27	–	–	–	–	1.9 ± 0.6	1.6 ± 0.1	1.5 ± 0.9
SHGR	+	–	+	–	1.6 ± 0.1	4.2 ± 0.1	1.3 ± 0.8
1422	+	–	–	–	1.4 ± 0.4	1.8 ± 0.1	ND
ER-GR. CAS	–	+	–	+	1.25 ± 0.2	1.22 ± 0.3	1.3 ± 0.2

*μmol min^{-1} mg^{-1} $\times 10^2$. GS activity was determined from standard curves of glutamine synthetase or α-glutamyl hydroxamate. The values read were corrected for time and protein content/sample. ND, not determined. Previous experiments in our laboratory have shown CEM C7 cells to lack estradiol binding sites (E. B. Thompson, unpublished results). Results are representative of three experiments.

DISCUSSION

We have developed an efficient protocol for transfection of human leukemic lymphoblasts with DNA, using it to explore the functions of the human GR gene. We show that the transfected genes for receptors produce measureable levels of classic, appropriate ligand binding sites in the recipient cells. These levels are clearly and significantly higher than the borderline quantity of binding sites otherwise measureable in these r-cells. Since only a portion of the cells are successfully transfected (*ca* 20%) it would seem that on average, we achieve a level of receptor content per cell similar to or slightly in excess of that measured in wild-type, glucocorticoid sensitive CEM C7 cells. Our results provide strong evidence for our conclusion that functional glucocorticoid receptors alone restore glucocorticoid mediated lysis and endogenous to the ICR 27 r-mutant, indicating that lack of proper GR is the sole relevant lesion in these cells. With that fact established, we therefore can use these cells to define the functional regions of the receptor gene necessary for various responses in these cells. The receptor gene has been extensively mapped for those regions requisite for induction of transcription; however much of this work has been carried out by using promoter regions carrying glucocorticoid response elements attached to reporter genes as a way of measuring receptor function. Both receptor and promoter response elements often have been expressed at high levels, to achieve readily measureable effects. Less has been done measuring the inductive response of endogenous genes to transfected receptor constructs. Some receptor mapping work has been carried out on steroid-mediated deinductive responses, much of this has been much more limited (see [36] for example).

As to cell lysis, despite decades of work and numerous theories, it is still unclear whether the way glucocorticoids bring this about is due to the upregulation of lethal gene product(s), the down regulation of essential product(s), or some combination of the two. Taken together with the facts that steroids not only act at the transcriptional level but in some cases at least, post-transcriptionally, and that the mechanism by which receptors are involved in the latter is quite unknown, it seems relevant to explore receptor topology *vis à vis* cell kill. What we report here are our initial results in this area. We find that the DNA binding region of the GR specifies cell lysis. Steroid occupancy of receptor is required to set off the process, but the molecule works with a heterologous ligand when the glucocorticoid DNA binding region is set into an estrogen receptor gene, replacing the DNA binding site of the ER. In addition, our preliminary evidence suggests that the lysis mechanism in these cells may be reversible (data not shown). We also found that induction of an endogenous gene, glutamine synthetase, is not mediated by this construct, effective in cell lysis. Only intact GR, and not a GR mutated in the DNA binding site, would induce this product. This suggests that portions of the GR outside the DNA binding regions are important for induction of at least some endogenous genes. This is consistent with the findings that the τ_1 region from the amino terminal portion of the GR is a significant factor in maximizing gene induction [16, 37–39]. Our results also suggest that the exact processes by which glucocorticoids evoke induction of certain genes and the events leading to cell lysis may not be the same. While GS may function in the cell growth pathway, it is not a requirement for all mechanisms of cell lysis. This is evidenced by the fact that we see no GS induction in the ER-GR.cas transfectants, but cell killing is still inducible with the "right" steroid, estradiol in this case. The fact that the portion of the receptor amino-terminal to the DNA binding region seems important for enzyme induction but not for lysis would be consistent with the receptor acting as a gene repressor to cause lysis, since several other studies emphasize that the amino-terminal portion of the receptor is less important for repression [36, 38]. We also note that although it appears that only some of the ICR 27 cells take up and retain enough ShGR DNA to show cell lysis, the fold and level of GS induction equals that of wild-type CEM C7 cells. It is as if the transfected cells GR is greater in quality or higher in concentration per cell than that of wild-type. This latter seems reasonable, since pRShGRα was designed to overproduce the GR, and since extent of induced response has been shown to be proportional to GR concentration [41]. An induced response (GS) can reflect such a relationship; a finite response (cell death) cannot. Once the endpoint of death is reached, additional signal is irrelevant. Thus it might be expected that only some cells are effectively transfected, but those that are may produce considerable GR. Further study is necessary to verify these concepts.

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