The mechanism of glucocorticoid resistance was studied in a rat hepatoma cell variant (6.10.2) which contains low levels of glucocorticoid receptor. These cells seem to have lost glucocorticoid-induced transcriptional responses as measured by the induction of expression of stably integrated mouse mammary tumor virus gene and the endogenous tyrosine aminotransferase gene as well as the transcriptional suppression of glucocorticoid receptor gene expression. However, characterization of the glucocorticoid resistance in 6.10.2 cells revealed that the receptor is indistinguishable from the wild-type receptor with respect to hormone binding and affinity for both nonspecific and specific DNA sequences. The levels of the receptor mRNA and the total immunoreactive protein found in 6.10.2 cells were about 20% of those found in wild-type cells. Further analysis of 6.10.2 cells demonstrated that the receptor was indeed biologically functional. First, treatment of 6.10.2 cells with 8-bromo-cAMP elevated the endogenous glucocorticoid receptor levels 2-fold and restored responsiveness to glucocorticoids. Second, pretreatment of the cells with cycloheximide also led to acquisition of cellular responsiveness to glucocorticoids. We propose that there exists a “threshold” level of glucocorticoid receptor which is required for responsiveness and that under normal culture conditions, the level of glucocorticoid receptor in 6.10.2 cells is below this threshold. However, glucocorticoid responsiveness can be restored by raising the glucocorticoid receptor level above the threshold with 8-bromo-cAMP or, alternatively, by removing the threshold barrier with cycloheximide.

The biological effects of glucocorticoids are mediated by a specific intracellular receptor protein. Once the glucocorticoid receptor has bound hormone, it undergoes a conformational change which enables the receptor to modulate the expression of a network of genes in a tissue-specific manner. In the presence of hormone, the glucocorticoid receptor binds to specific DNA sequences termed glucocorticoid response elements (GREs), which through an as yet unknown mechanism are able to induce or repress the transcription of target genes (for review see Yamamoto, 1985).

The cDNAs for the rat, mouse, and human glucocorticoid receptor have been isolated and sequenced (Miesfeld et al., 1986; Danielson et al., 1986; Hollenberg et al., 1985). Expression and functional analysis of the cDNAs have revealed that the glucocorticoid receptor consists of at least three separate domains: a COOH-terminal steroid-binding domain, a highly conserved central DNA-binding domain and an NH2-terminal domain which is essential for full trans-activating activity of the receptor (Danielson et al., 1987; Giguere et al., 1986; Hollenberg et al., 1987a; Miesfeld et al., 1987a; Rusconi and Yamamoto, 1987). Mutations in any of these domains could result in loss of glucocorticoid responsiveness.

Variants have been isolated from a number of cell lines and their glucocorticoid receptor have been characterized. Perhaps the best studied system is the S49 mouse lymphoma cell line (Northrop et al., 1985, 1986; Westphal et al., 1984). Three classes of mutants have been characterized to be defective in glucocorticoid receptor protein. The receptorless variant (r) exhibits a greatly reduced hormone binding capacity. However, its glucocorticoid receptor immunoreactivity and glucocorticoid receptor mRNA levels are not significantly reduced, suggesting that it may express a glucocorticoid receptor which is unable to bind hormone (Northrop et al., 1985). The nuclear transfer deficient variant (nt) expresses a receptor protein which binds hormone but has a reduced affinity for DNA caused by a single amino acid substitution within the steroid-binding domain. The second mRNA produces a receptor protein which binds hormone but has a reduced affinity for DNA caused by an as yet unknown mechanism which may express a mutant glucocorticoid receptor which is unable to bind hormone (Rabinow et al., 1987). Lastly, the increased nuclear transfer variant (nt+) has an increased affinity for nonspecific DNA due to the deletion of the NH2-terminal region (Miesfeld et al., 1987b).

The mutants described above have lost their ability to respond to glucocorticoids because of defects in the glucocorticoid receptor protein. However, not all nonresponsive cell lines have defective glucocorticoid receptors. There exist two S49 variants termed “deathless” which do not respond to glucocorticoids but in which the glucocorticoid receptor is indistinguishable from that of the wild type with regard to steroid-binding and DNA-binding properties (Yamamoto et al., 1976). Furthermore, one particular cell line which contains low levels of glucocorticoid receptor was found to be nonresponsive by one parameter but responsive by another (Rabinow et al., 1987). Therefore, not all of the variants which are nonresponsive and contain low amounts of glucocorticoid receptor may express a mutant receptor or be resistant to all of the effects of glucocorticoids.

One interesting aspect of glucocorticoid action is the mechanism determining how glucocorticoids and steroid hormones in general can regulate genes in a tissue-specific manner. Obviously, the presence of the steroid receptor itself is a
prerequisite for responsiveness in a given cell type. Moreover, it has been shown that in some model systems the cellular level of glucocorticoid receptor correlates relatively well with the response to glucocorticoids (Bourgeois and Newby, 1979; Bloom et al., 1980). Therefore, tissues with differing glucocorticoid receptor levels are likely to have different levels of responsiveness. However, tissue specificity cannot solely be explained by the presence or absence of glucocorticoid receptor or the level of receptor. It is therefore likely that additional factors are of importance.

We report here the characterization of the glucocorticoid receptor protein from an already described glucocorticoid nonresponsive hepatoma cell line, 6.10.2, which contains low levels of receptor (Miesfeld et al., 1986). It was previously shown that hormone treatment failed to induce endogenous tyrosine aminotransferase activity or stably integrated mouse mammary tumor virus (MMTV) mRNA levels. However, the defect could be complemented by expression of wild-type receptor cDNA (Miesfeld et al., 1986). We find that the receptor in 6.10.2 cells is indistinguishable from the wild-type protein with regard to affinity for ligand, size of protein and messenger RNA, and affinity for both nonspecific and specific DNA. We have previously shown that the levels of glucocorticoid receptor can be induced in wild-type hepatoma cells by 8-bromo-cAMP or forskolin resulting in an increased responsiveness to glucocorticoids (Dong et al., 1989). We show here that it is possible to overcome the nonresponsiveness in 6.10.2 cells when the levels of receptor are elevated by 8-bromo-cAMP. This proves that the cells still contain functional receptor. It was also possible to overcome nonresponsiveness in this cell line by treating the cells with cycloheximide, possibly indicating that a negative factor(s) might be involved in the control of cellular receptor levels and/or functions.

**MATERIALS AND METHODS**

**Cell Culture**—M1.19 and M1.54.762 (762) are cloned lines of stably MMTV-infected rat hepatoma tissue culture cells. Transcription of MMTV is induced in these cells (Ringo et al., 1977). NSM6.10 is a subclone of M1.19 selected for its low expression of the viral glycoprotein gp52 in the presence of dexamethasone by fluorescence-activated cell sorting (Grove et al., 1980) and has subsequently been shown to contain low levels of glucocorticoid receptor. 6.10.2 is an unselected subclone of NSM6.10 isolated in K. R. Yamamoto's laboratory (Miesfeld et al., 1986).

Cells were grown in monolayer and cultured in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 8% (v/v) heat-inactivated fetal calf serum (GIBCO) and benzylpenicillin/streptomycin (GIBCO). Serum steroids were stripped by dextran-coated charcoal precipitation (1 h, 37 °C).

**Preparation of Cytosol and Crude Cellular Extracts**—Either fresh or frozen cells were homogenized in ETG buffer (1 mM EDTA, 10 mM Tris-HCl, pH 7.5, 10% glycerol, and 2 mM dithiothreitol). The homogenates were centrifuged at 96,000 × g for 45 min at 4 °C, and the resulting supernatant was used as cytosol. For Western immunoblotting assays, crude cellular extracts were prepared as described above except that cells were homogenized in ETG plus 0.4 M NaCl.

**Nuclear Isolation**—Nuclear extracts were prepared by suspension of the cells in 0.5 M potassium phosphate. Proteins were then eluted from the resin with 200 μl of 0.5 M potassium phosphate buffer, pH 7.8. Specific binding was quantitated by Scatchard analysis (Scatchard, 1949).

**Western Immunoblotting**—Cytosol extracts were concentrated by ammonium sulfate precipitation (0.25 g of solid ammonium sulfate/0.5 ml protein). Protein concentration was determined according to Bradford et al. (1976). The identification of glucocorticoid receptor by Western immunoblotting using a monoclonal anti-glucocorticoid receptor antibody was carried out as described (Dong et al., 1988) except that sheep anti-mouse 125I immunoglobulin (Amersham Corp.) was used as the second antibody. Filters were autoradiographed and the immunoreactive bands were quantitated by gamma-counting.

**Isolation of RNA and Blot-Hybridization**—Total cellular RNA was isolated according to Chomzynski et al. (1987). RNA blots and hybridization with radiolabeled probes were carried out as described (Dong et al., 1988). The autoradiograms of RNA blots were analyzed densitometrically with a Shimadzu Dual-wave-length TLC scanner, cs-990 (Kyoto, Japan). Alternatively, the specific bands were excised and radioactivity determined by scintillation counting.

**Assay of Tyrosine Aminotransferase Activity**—The assay was carried out according to Diamondstone (1966).

**DNA-Cellulose Chromatography**—DNA-cellulose (4 mg of double-stranded DNA/g of resin) was obtained from Sigma. A 2 ml DNA-cellulose column was prepared in ETG buffer. Cytosols were labeled with 100 nM [5,6,7,3H]triiodoacetyl-2-deoxyuridine (Du Pont-New England Nuclear) at 4 °C for 1 h. Receptor activation was carried out by heat treatment at 25 °C for 30 min. 1 ml of cytosol was applied to the column, washed with ETG buffer, and eluted with a linear (0-0.5 M NaCl)-gradient in ETG buffer.

**Gel Retardation Assay**—Cytosol was labeled with 100 nM dexamethasone and concentrated by ammonium sulfate precipitation. Protein was resuspended in ETG buffer at a concentration of 10-20 mg/ml. 0.2 ng (20,000 cpm) of 32P-labeled GRE (see below) probe and 20 μg of cytosolic protein were incubated in a 20 μl binding reaction. The binding buffer was the same as described (Hagood et al., 1989). When indicated, various amounts of radiolabeled GRE or non-specific competitor DNA (see below) were included in the reaction mixture. After 15 min at 25 °C, samples were loaded onto a 4% polyacrylamide gel and electrophoresed. The gels were dried and autoradiographed. Specific bands were excised and quantitated by liquid scintillation counting.

The synthetic oligonucleotide GRE was from the promoter region of the rat tyrosine aminotransferase gene (Strahlé et al., 1987) and the nonspecific competitor DNA (NS) was a synthetic oligonucleotide encompassing randomly arrayed nucleotides. The upper strand sequences of both GRE and NS are shown below:

**GRE**: 5'-GAGCCCTAGAGGATCGTGATCGTATGCACATTGC-3' NS: 5'-GGATCCCCGTCTCTAGATTGACCTATCTGGAG-3'

**RESULTS**

**Regulation of Glucocorticoid Receptor by Dexamethasone in 6.10.2 Cells**—Classification of 6.10.2 cells as glucocorticoid unresponsive was based on the observation that treatment of the cells with dexamethasone failed to induce transcription of several target genes, e.g., those of MMTV, tyrosine aminotransferase, and H-1 acid glycoprotein (Miesfeld et al., 1956, 1987a; Vanderbilt et al., 1987). Glucocorticoids not only stimulate but also suppress gene expression. Since the mechanism of action of the glucocorticoid receptor complex to either activate or inhibit gene expression is still poorly understood, we considered it important to examine 6.10.2 cells with regard to negative regulation of genes by glucocorticoids. An example of a gene which is transcriptionally down-regulated by glucocorticoids is the glucocorticoid receptor gene itself (Dong et al., 1988).

The cells were treated with 0.5 μM dexamethasone for 24 h. The levels of glucocorticoid receptor protein and mRNA were analyzed by immunoblotting and RNA blot hybridization techniques, respectively. As shown in Fig. 1, treatment of 6.10.2 cells with dexamethasone had no effect on the level of...
of glucocorticoid receptor mRNA. However, dexamethasone caused a significant decrease in the level of glucocorticoid receptor protein (approximately 65% decrease). The decrease of the glucocorticoid receptor protein is probably due to decreased receptor stability (Dong et al., 1988). The above result indicates that also a negatively regulated gene is non-responsive in 6.10.2 cells.

Characterization of Glucocorticoid Receptor in 6.10.2 Cells—A functionally active glucocorticoid receptor is a prerequisite for glucocorticoid-induced modulation of gene transcription. It is known that under normal culture conditions 6.10.2 cells contain a lower glucocorticoid binding activity as compared with wild-type cells (Miesfeld et al., 1986). However, it is not known whether the low glucocorticoid receptor concentration in itself or a mutational defect in the glucocorticoid receptor protein is the cause for the resistant phenotype in 6.10.2 cells. We carried out experiments to characterize glucocorticoid receptor in 6.10.2 cells and compared the data with wild-type receptor in glucocorticoid-sensitive 762 cells.

Fig. 2 shows the autoradiograms of glucocorticoid receptor mRNA and protein from both 762 and 6.10.2 cells. On the Northern blot, two transcripts, 7 and 5 kilobases, characteristic of the rat glucocorticoid receptor, were detected in both cell lines. The amount of glucocorticoid receptor mRNA expressed in 6.10.2 cells was around 10-20% of the level detected in 762 cells as measured by the densitometric scanning of the autoradiograms. On the Western immunoblot, a monoclonal anti-glucocorticoid receptor antibody reacted with a 94-kDa component in both cell lines. Quantitation of the 125I-labeled immunoreactive bands revealed that 6.10.2 cells contained about 20% immunoreactive glucocorticoid receptor as compared with 762 cells.

To determine the hormone binding activity, cytosol preparations of cells were incubated with [3H]dexamethasone in the presence or absence of an excess of unlabeled hormone. The maximum number of binding sites (Bmax) and the dissociation constant (Kd) were calculated according to Scatchard (1949). It was found that Bmax of 6.10.2 cells was about 14-20% of that found in 762 cells; i.e., 21-30 vs 145-150 fmol/mg protein (Table I). However, the Kd values of the receptor-ligand complex were found to be similar in both cell lines (0.7-1.6 nM in 6.10.2 cells versus 1-3 nM in 762 cells, Table I).

Affinity of the glucocorticoid receptor for nonspecific DNA was assessed by DNA-cellulose chromatography. Cytosols prepared from both 762 and 6.10.2 cells were labeled with [3H]triamcinolone acetonide, heat activated, and analyzed on DNA-cellulose columns. Proteins were eluted with a linear salt gradient. The glucocorticoid receptor from 6.10.2 cells was eluted from the column at 185 mM NaCl which was virtually identical to the salt concentration required to elute the glucocorticoid receptor from 762 cells (180 mM, Table I).

To study the binding properties of the glucocorticoid receptor to a specific DNA sequence, we utilized a gel retardation assay. In this assay, a synthetic oligonucleotide containing a functional GRE sequence derived from the tyrosine aminotransferase promoter was used as the probe for specific glucocorticoid receptor-DNA interaction. Cytosolic glucocorticoid receptor labeled with dexamethasone was heat activated and incubated with 32P-GRE probe in the presence or absence of unlabeled competitor DNA. As shown in Fig. 3A, three retarded bands (1, 2, and 3) were formed when cytosols from both 762 and 6.10.2 cells were analyzed. Only formation of complex 1 was competed for by an excess of unlabeled GRE. However, nonspecific DNA did not compete for the formation of this complex indicating that complex 1 was the result of a specific interaction between glucocorticoid receptor and GRE. Lending support to this contention, complex 1 was further retarded in mobility in the native polyacrylamide gels upon incubation with monoclonal anti-glucocorticoid receptor antibodies (Nemoto et al., 1990). Fig. 3B summarizes the data of competition experiments. Competition with increasing amounts of unlabeled GRE showed that the glucocorticoid receptor from both 762 and 6.10.2 cells bound to GRE with very similar affinity.

Recovery of Cellular Glucocorticoid Sensitivity Following
FIG. 3. Specific glucocorticoid receptor-DNA interaction studied by gel retardation assay. Dexamethasone-labeled cytosolic proteins were heat treated and incubated with \(^{32}P\)-labeled GRE probe with or without various molar ratio excess of radio-inert competitor DNAs. The samples were subsequently electrophoresed through a non-denaturing polyacrylamide gel. A, autoradiograms of the gels. NS, nonspecific competitor DNA; C, sample without cytosolic protein; F, free probe; 1–3, retarded protein-DNA complexes. B, quantification of radioactivity retained in complex 1. The data were summarized from four separate experiments and presented as molar excess competitor DNA versus the amount of complex 1 and expressed as a percentage of complex 1 in the absence of competitor. The radioactivity in the samples with 100 molar excess GRE was used as the background value and subtracted from each sample.

**Treatment with 8-Bromo-cAMP**—Physicochemical characterization of glucocorticoid receptor from 6.10.2 cells did not reveal any major defects in important functional properties such as hormone and DNA binding, although minor mutations in the receptor could still not be excluded. However, the concentration of glucocorticoid receptor in 6.10.2 cells is low which possibly might determine glucocorticoid insensitivity. Thus, if the endogenous receptor level was increased, it might be possible to override the glucocorticoid unresponsiveness of 6.10.2 cells. Recently, we have shown that CAMP treatment of rat hepatoma cells (both H4IIE and 762) increases both the cellular amount of glucocorticoid receptor protein and glucocorticoid responsiveness (Dong et al., 1989). Thus, 6.10.2 cells were treated with 50 \(\mu\)M 8-bromo-cAMP in the presence or absence of 0.5 \(\mu\)M dexamethasone for 18 h and analyzed for the expression of glucocorticoid receptor, MMTV, and tyrosine aminotransferase. In the case of expression of the glucocorticoid receptor mRNA, dexamethasone alone had no effect (Fig. 4, lane 2; compare with Fig. 1). Treatment of the cells with 8-bromo-cAMP induced the glucocorticoid receptor mRNA level about 2-fold (lane 3 in Fig. 4) consistent with the result obtained in wild-type cells (Dong et al., 1989). This increase in glucocorticoid receptor mRNA level by 8-bromo-cAMP was reflected in a similar degree of increase in the glucocorticoid receptor protein level (data not shown). Treatment of cells with 8-bromo-cAMP and dexamethasone restored negative gene regulation since glucocorticoid receptor mRNA was now down-regulated (compare lanes 3 and 4 in Fig. 4). In the case of the expression of MMTV mRNA and the enzyme activity of tyrosine aminotransferase, neither dexamethasone nor 8-bromo-cAMP alone had any effect (Fig. 4, lanes 2 and 3). However, combined treatment of cells with both agents significantly induced the level of MMTV mRNA as well as the activity of tyrosine aminotransferase (Fig. 4, lane 4). The above results demonstrate that the 2-fold increase in the endogenous glucocorticoid receptor concentration was correlated with acquisition of a hormone-sensitive phenotype in 6.10.2 cells.

**The Effect of Cycloheximide on Glucocorticoid-mediated Cellular Responses**—It was shown in our previous studies that wild-type rat hepatoma cells treated with the protein synthesis inhibitor cycloheximide, exhibited a higher steady-state level of the glucocorticoid receptor mRNA as compared to non-treated cells (Okret et al., 1986). Given this background, 6.10.2 cells were treated with 1.5 \(\mu\)g/ml cycloheximide in the presence or absence of 0.5 \(\mu\)M dexamethasone for 18 h. Cellular levels of the glucocorticoid receptor mRNA were analyzed by RNA blot hybridization. For comparison, the level of MMTV mRNA was analyzed simultaneously. Cycloheximide increased the glucocorticoid receptor mRNA level about 4.4-fold (Fig. 5, lane C). The effect of cycloheximide on the mRNA level of MMTV was not as pronounced as for gluco-
Glucocorticoid receptor mRNA, but a significant 2-fold increase was obtained (Fig. 5, lane C). Dexamethasone alone had no effect on either glucocorticoid receptor mRNA or MMTV mRNA (Fig. 5, lane B). Surprisingly, in the presence of cycloheximide, both the expression of glucocorticoid receptor and MMTV mRNAs were modulated by dexamethasone; the glucocorticoid receptor mRNA level was down-regulated by 60% (compare lanes C and D in Fig. 5) and the MMTV mRNA was increased by a factor of about 2.5-fold (compare lanes C and D in Fig. 5). The above results demonstrate that 6.10.2 cells regained certain hormone-regulated responses after cycloheximide treatment.

**DISCUSSION**

Many glucocorticoid-resistant clones have been isolated from different wild-type cell lines through mutagenesis or hormone selection. Most of these clones show alterations in receptor function either due to defects in receptor quantity or quality (for a review see Gehring, 1988). Thus, the receptor seems to be the primary target for somatic mutations leading to glucocorticoid resistance.

6.10.2 cells are an unselected subclone of MSN6.10 which was isolated as a hormone nonresponsive derivative of M1.19 (Grove et al., 1989; Miesfeld et al., 1986). Both clones, MSN6.10 and 6.10.2, contain reduced levels of glucocorticoid receptor protein and mRNA, and the hormonal induction of tyrosine aminotransferase and MMTV gene expression is impaired (Miesfeld et al., 1986; Vanderhart et al., 1987; Northrop et al., 1986). To explore the molecular basis of glucocorticoid resistance in 6.10.2 cells, we have investigated several biochemical and physical properties of the receptor as well as its functional activities.

Based on the data of steroid binding and DNA binding (both specific and nonspecific) properties, immunoreactivity, and electrophoretic mobility, 6.10.2 cells appeared to contain glucocorticoid receptor with no obvious physical abnormalities, although the receptor was present in considerably lower concentrations as compared with wild-type cells (about 20% as abundant as in 762 cells; cf. Figs. 2 and 3 and Table I). Furthermore, glucocorticoid receptor mRNA levels were proportionally reduced along with glucocorticoid receptor protein levels in 6.10.2 cells indicating that the lesion causing the reduced glucocorticoid receptor protein level might be due to a reduction in transcription of the glucocorticoid receptor gene or stability of the glucocorticoid receptor mRNA.

In many cultured cells and tissues, autologous feedback regulation of glucocorticoid receptor gene expression is a common feature of the cells for control of cellular glucocorticoid sensitivity (Shirwany et al., 1986; Dong et al., 1989). Treatment of 6.10.2 cells with dexamethasone failed to down-regulate glucocorticoid receptor mRNA levels. However, the glucocorticoid receptor protein level was significantly decreased (about 65%, Fig. 1) by dexamethasone. We and others have previously shown that glucocorticoid-modulated glucocorticoid receptor expression occurs via at least two mechanisms; a decrease in the transcriptional rate of the glucocorticoid receptor gene and an increase in the turn-over rate of the glucocorticoid receptor protein (McIntyre et al., 1985; Dong et al., 1988; Rosewicz et al., 1988). We now show that these two mechanisms are not linked to each other, i.e. down-regulation of glucocorticoid receptor protein could occur in the absence of a decrease in the glucocorticoid receptor mRNA. The resistance of 6.10.2 cells to glucocorticoid-mediated down-regulation of glucocorticoid receptor mRNA together with the cellular insensitivity to hormonal induction of MMTV mRNA and tyrosine aminotransferase activity (Figs. 4 and 5) demonstrate that both positively and negatively controlled genes are nonresponsive in 6.10.2 cells.

Miesfeld et al. (1986) have shown that the mutant phenotype was functionally complemented by expression of cloned glucocorticoid receptor cDNA in 6.10.2 cells indicating that the machinery for glucocorticoid responsiveness is still functional. However, this experiment does not elucidate the functional activity of the endogenous glucocorticoid receptor present in 6.10.2 cells. In the present study, cellular synthesis of the endogenous glucocorticoid receptor in these cells was
increased 2-fold by treatment with 8-bromo-cAMP. This minor increase in the glucocorticoid receptor level was sufficient to activate the glucocorticoid responsiveness in 6.10.2 cells as measured by induction of MMTV mRNA and tyrosine aminotransferase activity as well as the suppression of glucocorticoid receptor mRNA levels by dexamethasone (Fig. 4). The magnitude of the induction of MMTV mRNA and tyrosine aminotransferase activity was not as pronounced as in wild-type hepatoma cells (Dong et al., 1989). However, the cAMP-induced glucocorticoid receptor levels were still below the glucocorticoid receptor levels in wild-type cells. In any case, these results demonstrate that the endogenous glucocorticoid receptor in 6.10.2 cells is indeed biologically active.

An interesting question is why the residual amount of glucocorticoid receptor present in 6.10.2 cells is not enough to induce a detectable, albeit low, glucocorticoid response. A threshold level of glucocorticoid receptor required to support the growth inhibition and cytolytic response in mutant lymphoma cells has been established to be in excess of 25% of the wild-type levels in WEHI 17 cells (Haet-Minkowski et al., 1992). Other glucocorticoid responses might also require such a threshold level of receptor. Our data suggest that the residual glucocorticoid receptor number maintained in 6.10.2 cells is probably below the threshold level required for glucocorticoid-induced transcriptional responses.

The increased basal level expression of both the MMTV and glucocorticoid receptor mRNA after cycloheximide treatment (Fig. 5) might indicate the existence of a labile protein(s) which suppresses the constitutive activity of both promoters or, alternatively destabilizes both messenger RNAs. Cis-acting negative regulatory elements have been described in several steroid hormone-regulated genes. A cis-acting negative regulatory element acting on the MMTV promoter and suppressing the GRE enhancer activity has been found in mouse mammary epithelial cells (Langer et al., 1988). Other glucocorticoid responses might also require such a threshold level of receptor. Our data suggest that the residual glucocorticoid receptor number maintained in 6.10.2 cells is probably below the threshold level required for glucocorticoid-induced transcriptional responses. Thus, it seems likely that the balance between cellular receptor and labile repressor(s) levels determines the sensitivity of target cells to glucocorticoid treatment. Changes in concentration or activity of any of these factors in relation to each other may change cellular glucocorticoid sensitivity. Experiments identifying these putative factors in 6.10.2 cells may help us to further characterize the molecular basis of glucocorticoid regulation of gene expression and glucocorticoid resistance.

Acknowledgments—We are very grateful to Dr. Lorenz Poellinger for his valuable suggestions and help in preparation of this manuscript. We thank Dr. Carol Cairns for methodological advice and Cecilia Tommas for skillful technical assistance.

REFERENCES

A glucocorticoid-resistant rat hepatoma cell variant contains functional glucocorticoid receptor.

Y Dong, W Cairns, S Okret and J A Gustafsson


Access the most updated version of this article at http://www.jbc.org/content/265/13/7526

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/13/7526.full.html#ref-list-1