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Differential Dynamics of Receptor Down-regulation and Tyrosine Aminotransferase Induction Following Glucocorticoid Treatment

Debra C. DuBois,[1]* Zhi-Xin Xu,[2] Lorraine McKay,[1] Richard R. Almon,[1] Nancy Pyszcznski[2] and William J. Jusko[2]

[1]Department of Biological Sciences and [2]Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14260, U.S.A.

Autoregulation of glucocorticoid receptor (GR) concentration *in vivo* may be an important determinant of steroid sensitivity. The dynamics of GR regulation were assessed and compared to regulation of tyrosine aminotransferase (TAT) expression in liver tissue taken from rats treated with a single 50 mg/kg i.v. dose of methylprednisolone. Plasma methylprednisolone concentrations were determined by HPLC analysis. Receptor and TAT message levels were determined by quantitative Northern hybridization. Methylprednisolone plasma kinetics showed a half-life of 0.6 h. Receptor occupancy occurred rapidly and cytosolic GR reappeared over 2–12 h. TAT activity rose between 2 and 6 h and then dissipated. Reduction in receptor mRNA levels occurred very rapidly, being detectable by 30 min following steroid administration. A down-regulated steady-state in GR message expression was reached by 2 h post-injection, and was maintained throughout the 18 h examined in this study. Comparison of methylprednisolone kinetics demonstrated that down-regulation was maintained long after drug was eliminated. In contrast, TAT message induction occurred with a sharp peak; maximal induction occurred between 5–6 h and return to baseline at approx. 8–10 h post-induction. This study shows that unlike TAT induction, GR message repression *in vivo* does not require continual presence of hormone.

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INTRODUCTION

Adrenal corticosteroids are both a major regulatory factor controlling systemic gluconeogenesis and an important component of long-term stress responses in mammals. In addition, synthetic glucocorticoids are widely employed as clinical immunosuppressive and anti-inflammatory agents. Therefore, ascertaining the dynamics of corticosteroid responsiveness *in vivo* is important to understanding both normal physiology and the treatment of a variety of pathophysiological states.

Many of the effects of corticosteroids are mediated through regulation of gene expression at the level of transcription. Corticosteroids function as tissue specific *trans*-acting transcription regulators, inducing or repressing a small subset of genes within a given target. The generally accepted model of steroid action [reviewed in 1, 2] involves binding of hormone to cytoplasmic receptor, translocation of the hormone–receptor complex to the nucleus, and interaction of the complex with specific regulatory sites within the DNA termed "hormone response elements". Interaction of the hormone–receptor complex with nuclear regulatory elements alters the rate of transcription of genes functionally associated with those elements, leading to changes in tissue levels of those mRNAs. Therefore, changes in the levels of specific message populations in a given target tissue is an important cellular component of glucocorticoid action.

*Correspondence to D. C. DuBois.
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Abbreviations: MPL, methylprednisolone; TAT, tyrosine aminotransferase (L-tyrosine 12-oxogluterate aminotransferase, EC 2.6.1.5); GR, glucocorticoid receptor; SSPE, 0.15 M NaCl, 0.01 M sodium phosphate, 1 mM EDTA; SDS, sodium dodecyl sulfate; Denhardt's solution, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin.

The consensus of experimental evidence indicates that cytoplasmic receptor concentration is a major factor governing steroid responsiveness. In steroid sensitive cell culture models [3–5], transfected cell studies [6, 7], *in vivo* animal studies [8], and clinical studies [9] a correlation between cellular receptor concentration and magnitude of response has been noted. As with virtually all hormone systems studied to date, glucocorticoid receptor number is not static, but fluctuates with changing physiological circumstances. A major factor determining receptor concentration is exposure to the hormone itself. Autoregulation of glucocorticoid receptor has been documented in a variety of systems both at the protein and mRNA levels [reviewed in 1, 3, 10].

If receptor availability governs steroid responsiveness, then there are several unanswered questions that are important to understanding steroid sensitivity *in vivo*, as well as for optimizing the design of clinical dose regimens. The first is ascertaining the dynamics of autoregulation of receptor gene transcription. The second is understanding the co-ordinate regulation of receptor and non-receptor target gene expression. The third is understanding the relationship between the dynamics of message expression and the expression of corresponding protein. We have used quantitative Northern hybridization procedures developed in our laboratory [11] to examine the dynamics (temporal pattern and magnitude) of receptor mRNA and TAT mRNA expression in liver tissue taken from a population of animals treated with a single dose of methylprednisolone. In addition, we also examined the expression of receptor protein as reflected by equilibrium binding analyses and TAT protein as reflected by enzymatic activities concurrently with GR and TAT message expression. TAT was chosen as a non-receptor target gene in this study because its induction by glucocorticoids in liver is well characterized [e.g. 1, 12, 13]. The pharmacokinetics and pharmacodynamics of methylprednisolone have been partly assessed in the rat and a dose of 50 mg/kg has been shown to produce a moderate TAT response *in vivo*. This study demonstrates that regulation of TAT message and TAT enzymatic activity as compared to receptor message and ligand binding occur with distinctly different temporal patterns in the same tissue. Furthermore, this study demonstrates that receptor regulation *in vivo* occurs extremely rapidly and lasts long after the removal of hormone from the system.

MATERIALS AND METHODS

Animal manipulations

Male adrenalectomized Wistar rats were purchased from Hilltop Lab Animal Inc. (Scottsdale, PA). Animals were housed in a 12 h light–dark, constant temperature environment (22°C) with free access to rat chow (Agway RMH 1000) and a 0.9% NaCl drinking solution. Animals were acclimatized to this environment for at least 1 week. 1 day prior to study, rats were subjected to right external jugular vein cannulation under light ether anesthesia. Cannula patency was maintained with sterile saline. Food was removed 14 h before each experiment. MPL as the sodium succinate salt (Upjohn Co., Kalamazoo, MI) was reconstituted with the supplied diluent. Rats received a single injection of either MPL at a dose of 50 mg/kg or an equal volume of saline (control) via the cannula over 30 s. At various times (0.5–18 h) after MPL administration, rats were sacrificed; generally, 2 animals were sacrificed at each time point. Blood was drained from the abdominal aorta into a heparinized syringe. Blood was immediately centrifuged, plasma was harvested, and frozen at −20°C until assay. After blood drainage, the liver was rapidly excised. A portion of the liver was rapidly frozen in liquid nitrogen and stored at −80°C until RNA preparation. The remainder of the liver was used immediately for receptor measurements and measurement of TAT enzymatic activity.

Quantitative Northern hybridization

Procedures for the quantitation of Northern hybridization data have been described previously [11]. In brief, those procedures use an externally added cRNA pseudomessage standard in conjunction with cRNA concentration curves for both the standard and the experimental message of interest. These procedures allow for conversion of hybridization signals into moles of message per gram of tissue. Hybridization signals were quantitated using a Betascope (Betagen Corp.) Triplicate determinations of hybridization signals were performed for each animal, and co-efficients of variance were in all cases less than 12%. Details of RNA preparations, external standards, *in vitro* transcription procedures, and TAT hybridizations are as previously reported. For GCR hybridization studies, a 1.0 kb clone of the mouse glucocorticoid receptor (generously supplied by Dr Gordon Ringold) was employed [14]. A 777 bp piece of that cDNA was first subcloned into pGEM3Z in the Pst1 and HindIII sites, in order that both unlabeled sense cRNA standards as well as radiolabeled antisense riboprobe could be prepared by *in vitro* transcription. In preparing riboprobe, ^{32}P-GTP was used as the labeled nucleotide. Hybridizations were carried out overnight at 65°C in a solution of 5 × SSPE, 1% SDS, 2 × Denhardt's reagent 50% formamide, 100 μg/ml salmon sperm DNA. Figure 1 presents an autoradiogram of 1 of the 9 GCR hybridization matrices used in this study. It should be pointed out that this autoradiogram is presented only as an illustration of these hybridizations; hybridization signals (CPMs) were quantitated directly from hybridization matrices using a Betascope.

Cytosolic glucocorticoid receptor

Hepatic cytosolic GCR was determined using full isothermal association curves and analyzed by

Scatchard analyses as previously described [15]. Briefly, liver was homogenized and then centrifuged to obtain a clear supernatant. Cytosol was incubated at 4°C for 18 h with [^{3}H]dexamethasone (40 Ci/mmol, Amersham, Arlington Heights, IL) in the presence and absence of excess unlabeled dexamethasone. Bound and free steroid were separated by charcoal adsorption. Aliquots were counted, and total binding (D_T) and non-specific binding (D_{NS}) of dexamethasone were determined. Based on data obtained for total bound, non-specific bound, and free dexamethasone (D_f), glucocorticoid receptor density (B_{max}) was estimated using PCNONLIN (SCI Software Inc., Lexington, KY) by solving equations 1 and 2 simultaneously:

$$D_T = D_{NS} + \frac{D_f * B_{max}}{D_f + K_D} \quad (1)$$

$$D_{NS} = K_{NS} * D_f \quad (2)$$

where K_D is the equilibrium dissociation constant, and K_{NS} is the non-specific binding constant.

Tyrosine aminotransferase enzyme activity

Hepatic TAT activity was determined by the method of Diamondstone [16]. Absorbance was recorded at 331 nm using a Perkin-Elmer double-beam spectrophotometer. Tissue protein content was measured using the method of Lowry *et al.* [17]. TAT activity was expressed as the change in absorbance (ΔA) per minute per milligram of hepatic protein.

Plasma hormone assay

Plasma samples were thawed at room temperature. Aliquots (0.2–2.0 ml) were transferred into Pyrex glass culture tubes (Corning Glass Works, Corning, NY). Plasma samples were extracted with methylene chloride. The organic layer was washed with 0.1 N sodium hydroxide followed by distilled water. Plasma concentrations of MPL (C_{MPL}) were determined by high performance liquid chromatography [18]. The calculated limit of quantitation was 8.0 ng/ml and coefficients of variance were less than 10% (inter-day and intra-day). Plasma MPL concentration versus time (t)

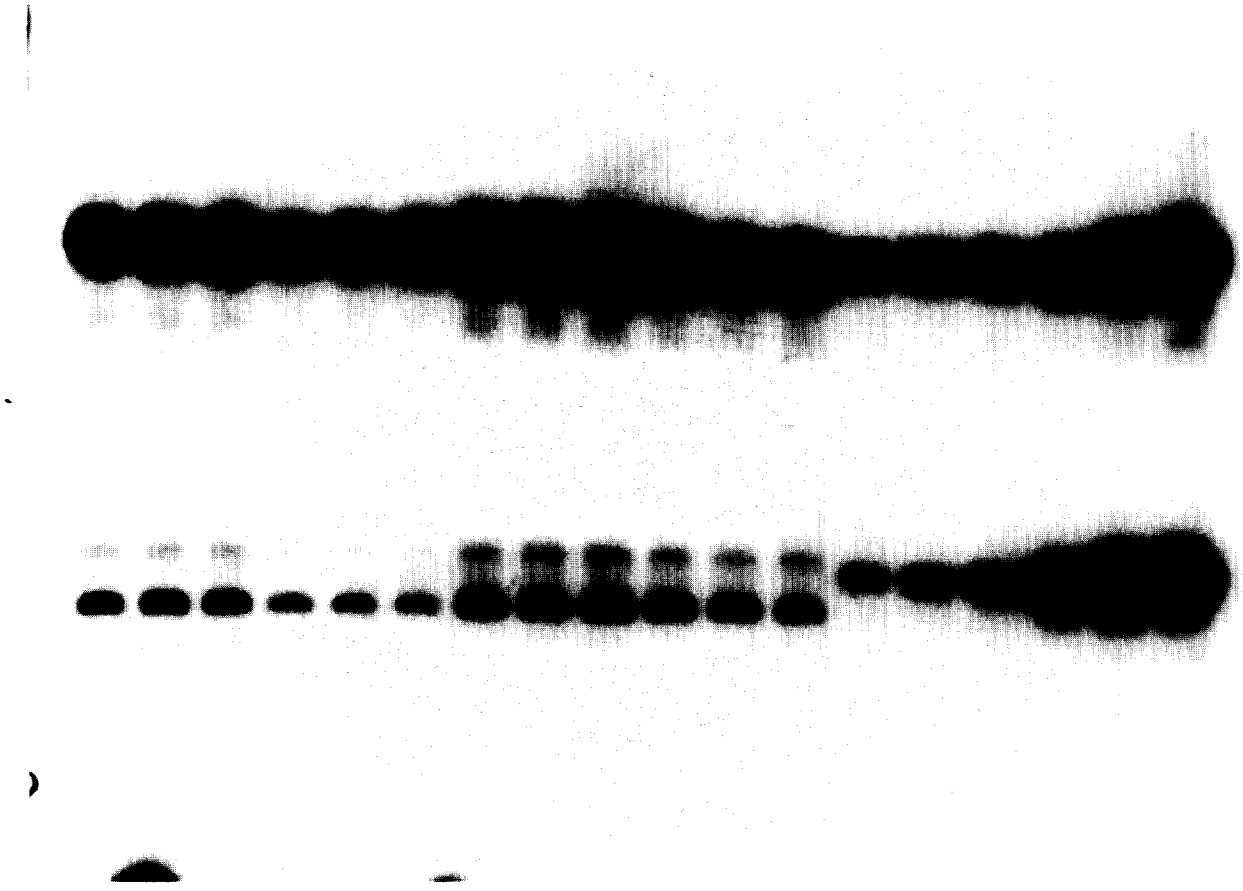

Fig. 1. Autoradiogram of a representative matrix used for quantitative Northern hybridization of GCR mRNA. The 12 lanes on the left contain triplicate determinations of 4 different liver samples. The remaining lanes contain cRNA standards of known concentrations. The top signals reflect hybridization to the GRG-1 probe (external standard), bottom signals reflect hybridization to the GCR probe.

data were described by:

$$C_{MPL} = C_1 * e^{-\lambda_1 * t} + C_2 * e^{-\lambda_2 * t}$$

The intercept coefficients (C_1 and C_2) and slopes (λ_1 and λ_2) were estimated by least-squares fitting using the PCNONLIN program.

RESULTS

Figure 2 presents the plasma MPL concentrations versus time after administration of MPL in this group of animals. The decline was biexponential with a terminal half-life of 0.58 h. By 8 h, measurable hormone was completely cleared from the system. The fitted coefficients were $C_1 = 48.37\ \mu g/ml$, $\lambda_1 = 7.83\ h^{-1}$, $C_2 = 13.23\ \mu g/ml$, and $\lambda_2 = 1.20\ h^{-1}$.

Figure 3(A and B) shows the amount of GCR message as determined by Northern hybridization and receptor number as determined by ligand binding in liver tissue from these animals versus time after administration of MPL. At 30 min post-injection (the earliest time point examined in this study), a measurable decline in GCR message was observed, and by 2 h had declined to a new baseline whose average value (2–18 h animals) was 42% of controls (controls = 9.95 ± 1.53; injected = 4.17 ± 0.99 fmol/g tissue). GCR message remained repressed at this level throughout the 18 h examined in this study. In contrast, ligand binding had dropped to essentially zero at the 30 min time point, from a base-line of 43 nM measured in control tissues. After approx. 2–3 h, measurable receptor binding began to rise, reaching a new steady-state of 58% baseline value at 8–10 h post-injection.

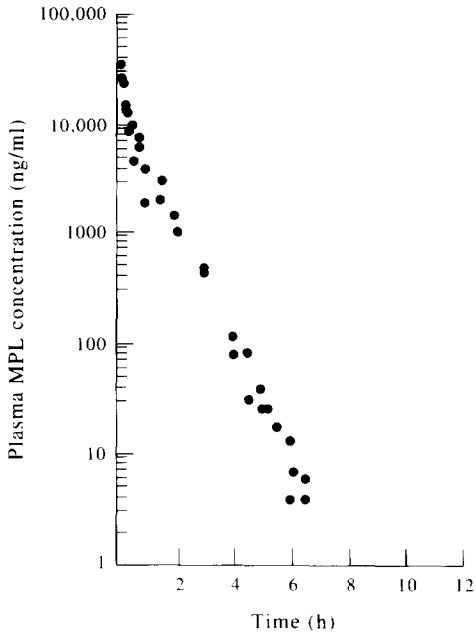

Fig. 2. Plasma methylprednisolone concentrations determined from HPLC analysis as a function of time following administration of a 50 mg/kg i.v. dose to a group of rats.

Figure 3(C) presents the dynamics of TAT mRNA expression and Fig. 3(D) the TAT enzymatic activities in liver tissue taken from this same animal population. Following a 2–3 h lag period, TAT mRNA concentrations began to rise, reaching a peak at 5.5 h post-injection. TAT message concentration thereafter declined and reached baseline by 8–10 h after injection. The pattern of TAT enzyme activity was very similar to the pattern of TAT mRNA expression, except that peak TAT activities were found at 6.5 h post-injection, with a return to baseline at approx. 12 h.

DISCUSSION

This study involved treatment of an animal population with a single bolus i.v. dose of the glucocorticoid, methylprednisolone (50 mg/kg). This and other related synthetic glucocorticoids are used clinically for immunosuppressive/anti-inflammatory therapy. Intravenous administration through jugular vein cannulae was employed because this method allows for rapid and controlled hormone delivery. Figure 2 presents the kinetics of plasma MPL in the blood of these animals; the pharmacokinetic parameters calculated from these data are comparable to data previously reported for this dose and type of rats [19]. Plasma hormone levels persist at concentrations that are above the K_D value of methylprednisolone for about 8 h. Since it has been shown that tissue levels of MPL closely parallel blood levels, the results suggest that by 8 h tissue MPL is also below K_D concentration.

Autoregulation of glucocorticoid receptor has been noted in a variety of systems studied [reviewed in 3, 10]. Decreased receptor protein associated with increased hormone concentration has been measured both by immunologic techniques [e.g. 20, 21] and by binding studies [e.g. 22, 23]. In addition, a relative decrease in GCR mRNA content following hormone treatment has been reported both in cell culture systems [e.g. 21, 24–28] and in tissue taken from hormone treated animals [21, 29]. In cell culture systems, variations in the kinetics of relative receptor mRNA changes have been noted. Some reports have observed a monophasic decrease in message expression, with variations in time to a new steady-state ranging from 2 to 48 h of hormone treatment [25–28]. Others have reported a biphasic change in message expression, with an initial relative increase in message at 6 h followed by a decrease at 24–48 h [21, 24]. Differences in the reported dynamics of GR mRNA expression in culture probably reflect inherent differences in the cell systems employed. Therefore, cell culture results may or may not be similar to *in vivo* regulation. However, two reports of GCR message repression in tissue taken from adrenalectomized rats treated with glucocorticoids have previously been published. Kalyinyak *et al.* [29] found a 40% relative decrease in GCR message expression in liver 6 h after i.p. dexamethasone treatment (7 mg/kg).

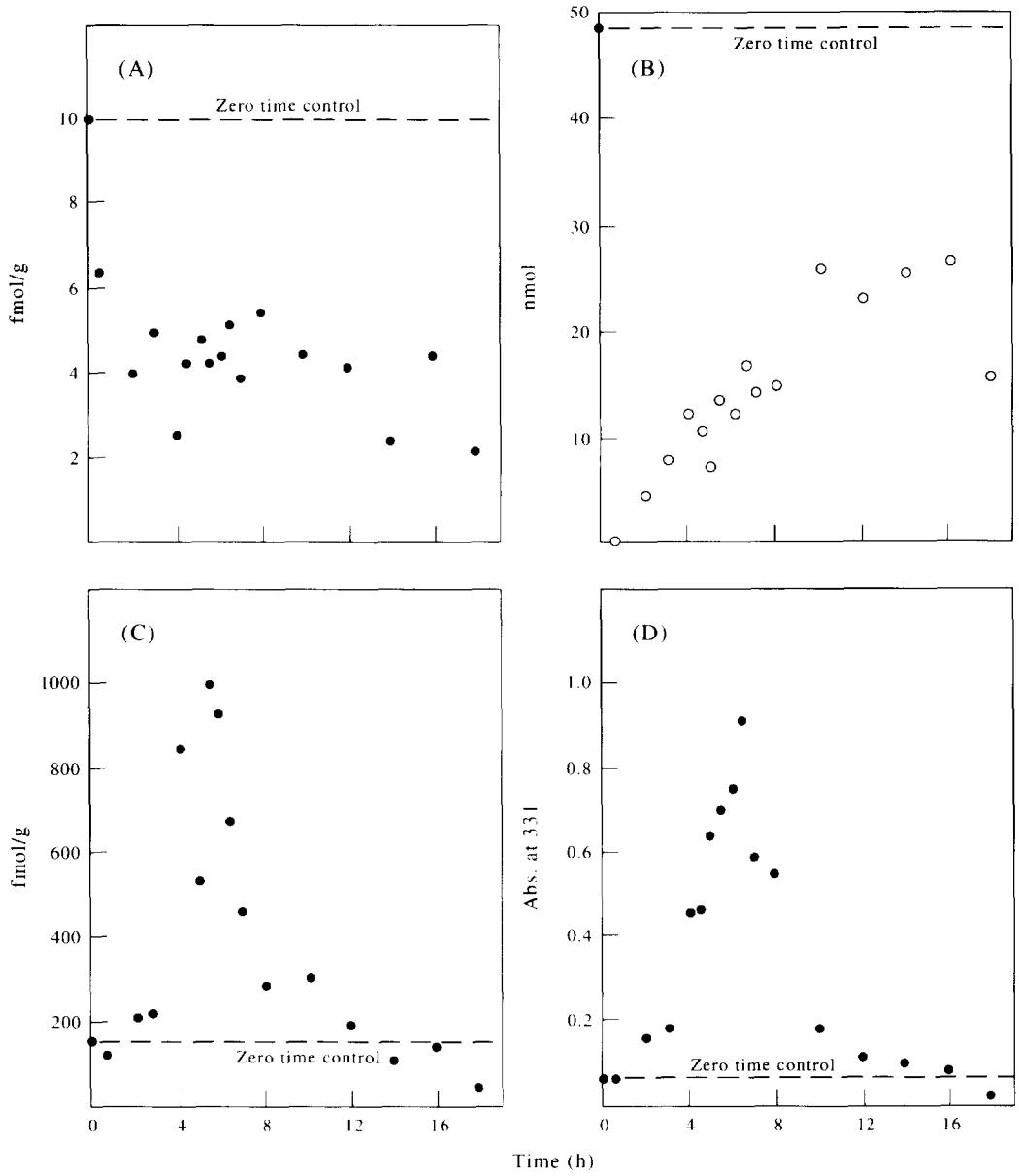

Fig. 3. Average message and protein levels in liver tissue as a function of time following administration of a 50 mg/kg dose of methylprednisolone to a group of rats. (A) GR mRNA as determined by quantitatiave Northern hybridization; (B) GR as determined by ligand binding; (C) TAT mRNA as determined by quantitative Northern hybridization; (D) TAT activity determined spectrophotometrically.

In that report, a semi-quantitative analysis was performed using scanning densitometry of relative hybridization signals obtained from control and treated samples. Dong *et al.* [21] presented qualitative data on hybridization signals in liver tissues taken from animals at 4, 8, 18, 24, and 48 h after a single i.p. dose of dexamethasone (4 mg/kg). In that report, an autoradiogram showed a visible decrease in GCR hybridization signal at 4 h, a greater decrease at 8 h, disappearance of the signal at 18 and 24 h, and reappearance of signal at 48 h. However, neither study included analysis of plasma drug levels or was as comprehensive as the present investigation.

We present here a quantitative analysis of the dynamics of GCR message expression in liver tissue taken from a series of animals following a single bolus i.v. injection of the synthetic glucocorticoid methylprednisolone [Fig. 3(A)]. Somewhat surprisingly, down-regulation of receptor message occurred extremely rapidly in this study; even more surprising was the fact that down-regulation was maintained long after hormone levels reached below concentrations active in mass action driven receptor mediated reactions. By 30 min following hormone injection a decrease in message expression was evident, and expression reached a new steady state level by 2 h. The more rapid changes in message expression observed here as compared to that observed by Dong *et al.* [21] may be attributable to differences in hormone dose and/or delivery. As illustrated by Fig. 2, hormone delivery to tissue by our

procedures was extremely rapid and the drug would be presented to the liver as a high bolus concentration. Following an i.p. injection drug introduction to the liver would be presumably slower and more diffuse. Furthermore, a comparison of Figs 2 and 3 demonstrates that down-regulation is maintained long after hormone has cleared from the system, since no recovery from down-regulation is seen throughout the 18 h studied.

The dynamics of TAT message expression [Fig. 3(C)] differ substantially from receptor message expression. Initial changes in TAT message expression occur several hours after changes in receptor message first become apparent (i.e. an increase in TAT message levels are first measurable 2–3 h after hormone injection). Also, in contrast to changes in receptor message expression, TAT mRNA levels reach a peak at 5–6 h post-injection and then decline. These data substantially agree with a previous report which measured relative changes in TAT mRNA expression in rat liver following time after hormone treatment. Chesnokov *et al.* [30] measured peak TAT message concentrations that represented a 7-fold increase relative to controls at 4 h following hormone administration (hydrocortisone, 50 mg/kg i.p.). Our results found a peak TAT message content of 1000 fmol/g tissue at 5.5 h post-injection, with baseline values of 160 fmol, representing a 6-fold induction of message expression.

The profile of TAT activity [Fig. 3(D)] exhibits similarities to the profile of TAT mRNA expression. Both exhibit a relatively sharp increase followed by an equally sharp decrease in expression, with the TAT activity curve being shifted to the right by approx. 1 h. In contrast, GCR activity as measured by cytosolic binding exhibited a distinctly different profile from that of GCR message expression. Cytosolic receptor as measured by ligand binding virtually disappeared at early times following hormone administration [Fig. 3(B)]. Since these studies entailed equilibrium binding and since cytosol fractions were charcoal absorbed to remove free steroid prior to the addition of ligand, it is unlikely that the presence of high concentrations of drug at these early time points interfered with the measurement of receptor number. Although altered half-lives of receptor protein have been reported in cell culture following glucocorticoid treatment [reviewed in 3, 10], it is unlikely that this could account for the virtual disappearance of binding activity at early times after MPL administration in our study. More likely, the absence of detectable cytosolic binding may reflect nuclear localization of receptor at these early time points. In any case, a new steady-state in binding activity is reached by about 10 h after injection; like receptor message expression, cytosolic binding remained reduced throughout the 18 h examined here, long after hormone had cleared the system.

Northern hybridization techniques allow for determination of steady state levels of specific mRNA populations. Therefore, these techniques are unable to mechanistically distinguish between changes in transcription rate versus changes in message stability as contributory factors to changes in message population levels. In the case of receptor message, conflicting evidence exists from cell culture studies as to whether or not glucocorticoids induce a destabilization in receptor message [reviewed in 3, 10]. However, when receptor stability was assessed at the inception of down-regulation as opposed to after a new steady-state has been achieved, Vedeckis *et al.* [31] found evidence for hormone-induced message destabilization. The rapidity of the drop in receptor message levels observed in our study is not inconsistent with receptor destabilization as a contributory factor in message down-regulation. Furthermore, evidence obtained primarily from cell culture studies [reviewed in 1, 2] indicates that modulation of transcriptional rates of at least non-receptor target genes is dependent on hormone presence. The dynamics of TAT message induction in our study are consistent with this evidence, since peak message induction occurred at around 5.5 h, a time at which measured hormone levels (10 ng/ml) approximate MPL K_D values (7.5 ng/ml). In contrast, the extraordinarily long lasting suppression of steady state GR message levels demonstrates that either transcriptional repression of the receptor gene does not require continued presence of hormone, or that mechanisms other than transcriptional repression are involved in GR message autoregulation.

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REFERENCES

1. Gustafsson J., Carlstedt-Duke J., Poellinger L., Okret S., Wikstrom A., Bronnegard M., Gillner M., Dong W., Kjell F., Cintra A., Harfstrand A. and Agnati L.: Biochemistry, molecular biology, and physiology of the glucocorticoid receptor. *Endocrine Rev.* **8** (1987) 185–234.
2. Miesfeld R. L.: Molecular genetics of corticosteroid action. *Am. Rev. Respir. Dis.* **141** (1990) S11–S17.
3. Oakley R. H. and Cidlowski J. A.: Homologous down regulation of the glucocorticoid receptor: the molecular machinery. *Crit. Rev. Eukarytotic Gene Exp.* **3** (1993) 63–88.
4. Bourgeois S. and Newby R. F.: Correlation between glucocorticoid receptor and cytolytic response of murine lymphoid, cell lines. *Cancer Res.* **39** (1979) 4749–4753.
5. Dong W., Aronsson Y., Gustafsson J-A. and Okret S.: The mechanism of cAMP-induced glucocorticoid receptor expression: correlation to cellular glucocorticoid response. *J. Biol. Chem.* **264** (1989) 13,679–13,683.
6. Bellingham D. L., Sar M. and Cidlowski J. A.: Ligand-dependent down-regulation of stably transfected human glucocorticoid receptors is associated with the loss of functional glucocorticoid responsiveness. *Molec. Endocr.* **6** (1992) 2090–2102.
7. Vanderbilt J. N., Miesfield R., Maler B. A. and Yamamoto K. R.: Intracellular receptor concentration limits glucocorticoid-dependent enhancer activity. *Molec. Endocr.* **1** (1987) 68–74.

8. Yi-Li Y., Jin-Xing T. and Ren-Bao X.: Down-regulation of glucocorticoid receptor and its relationship to the induction of rat liver tyrosine aminotransferase. *J. Steroid Biochem.* **32** (1989) 99–104.
9. Bloomfield C. D., Smith K. A., Peterson B. A. and Munck A.: Glucocorticoid receptors in adult acute lymphoblastic leukemia. *Cancer Res.* **41** (1981) 4857–4860.
10. Burnstein K. L. and Cidlowski J. A.: The down side of glucocorticoid receptor regulation. *Molec. Cell. Endocr.* **83** (1992) C1–C8.
11. DuBois D. C., Almon R. R. and Jusko W. J.: Molar quantification of specific messenger ribonucleic acid expression in Northern hybridization using cRNA standards. *Anal. Biochem.* **210** (1993) 140–144.
12. Nichols J. M., Lee K-L. and Kenney F. T.: Changes in hepatic levels of tyrosine aminotransferase messenger RNA during induction by hydrocortisone. *J. Biol. Chem.* **253** (1978) 4009–4015.
13. Schmid E., Schmid W., Jantzen M., Mayer D., Jastorff B. and Schutz G.: Transcription activation of the tyrosine aminotransferase gene by glucocorticoids and cAMP in primary hepatocytes. *Eur. J. Biochem.* **165** (1987) 499–506.
14. Northrop J. P., Danielsen M. and Ringold G. M.: Analysis of glucocorticoid unresponsive cell variants using a mouse glucocorticoid receptor complementary DNA clone. *J. Biol. Chem.* **261** (1986) 11,064–11,070.
15. Boudinot F. D., D'Ambrosio R. and Jusko W. J.: Receptor-mediated pharmacodynamics of prednisolone in the rat. *J. Pharmacokin. Biopharm.* **14** (1986) 469–493.
16. Diamondstone T. I.: Assay of tyrosine aminotransferase activity by conversion of *p*-hydroxyphenylpyruvate to *p*-hydroxybenzaldehyde. *Anal. Biochem.* **16** (1966) 395–401.
17. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193** (1951) 265–272.
18. Ebling W. F., Szefler S. J. and Jusko W. J.: Methylprednisolone disposition in rabbits: analysis, prodrug conversion, reversible metabolism and comparison with man. *Drug Metab. Dispos.* **13** (1985) 296–304.
19. Haughey D. B. and Jusko W. J.: Receptor-mediated methylprednisolone pharmacodynamics in rats: steroid-induced receptor down-regulation. *J. Pharmacokin. Biopharm.* **19** (1992) 333–355.
20. Okret S., Wikstrom A-C., Wrange O., Andersson B. and Gustafsson J-A.: Monoclonal antibodies against the rat liver glucocorticoid receptor. *Proc. Natn. Acad. Sci.* **81** (1984) 1609–1613.
21. Dong Y., Poellinger L., Gustafsson J-A. and Okret S.: Regulation of glucocorticoid receptor expression: evidence for transcriptional and posttranslational mechanisms. *Molec. Endocr.* **2** (1988) 1256–1264.
22. Svec F. and Rudis M.: Glucocorticoids regulate the glucocorticoid receptor in the AtT-20 cell. *J. Biol. Chem.* **256** (1981) 5984–5987.
23. Cidlowski J. A. and Cidlowski N. B.: Glucocorticoid receptors and the cell cycle; evidence that the accumulation of glucocorticoid receptors during the S phase is dependent on ribonucleic acid and protein synthesis. *Endocrinology* **110** (1982) 1653–1662.
24. Okret S., Poellinger L., Dong Y. and Gustafsson J-A.: Down-regulation of glucocorticoid receptor mRNA by glucocorticoid hormones and recognition by the receptor of a specific binding sequence within a receptor cDNA. *Proc. Natn. Acad. Sci.* **83** (1986) 5899–5903.
25. Rosewicz S., McDonald A. R., Maddux B. A., Goldfine I. D., Miesfeld R. and Logsdon C. D.: Mechanism of glucocorticoid receptor down-regulation by glucocorticoids. *J. Biol. Chem.* **263** (1988) 2581–2584.
26. Hoeck W., Rusconi S. and Groner B.: Down-regulation and phosphorylation of glucocorticoid receptors in cultured cells. *J. Biol. Chem.* **264** (1989) 14,396–14,402.
27. Burnstein K. L., Bellingham D. L., Jewell C. M., Powell-Oliver F. E. and Cidlowski J. A.: Autoregulation of glucocorticoid receptor gene expression. *Steroids* **56** (1991) 52–58.
28. Alksnis M., Barkhem T., Stromstedt P-E., Ahola H., Kutoh E., Gustafsson J-A., Poellinger L. and Nilsson S.: High level expression of functional full length and truncated glucocorticoid receptor in Chinese hamster ovary cells. *J. Biol. Chem.* **266** (1991) 10,078–10,085.
29. Kalinyak J. E., Dorin R. I., Hoffman A. R. and Perlman A. J.: Tissue-specific regulation of glucocorticoid receptor mRNA by dexamethasone. *J. Biol. Chem.* **262** (1987) 10,441–10,444.
30. Chesnokov V. N., Gadzhiev V. G., Leontyeva G. I., Zelenin S. M. and Mertvetsov N. P.: Hydrocortisone regulation and expression of tyrosine aminotransferase gene in various tissues of rat. *Biomed. Biochim. Acta* **49** (1991) 1177–1186.
31. Vedeckis W. V., Ali M. and Allen H. R.: Regulation of glucocorticoid receptor protein and mRNA levels. *Cancer Res. (Suppl.)* **49** (1989) 2295–2301.