0026-895X/98/030459-08$3.00/0

MOLECULAR PHARMACOLOGY, **53:**459–466 (1998).

Dexamethasone Regulation of the Rat 3α-Hydroxysteroid/Dihydrodiol Dehydrogenase Gene

YONG-TAI HOU,[1] HSUEH-KUNG LIN, and TREVOR M. PENNING

Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6084

Received August 25, 1997; Accepted December 8, 1997

This paper is available online at http://www.molpharm.org

ABSTRACT

Rat liver 3α-hydroxysteroid/dihydrodiol dehydrogenase (3α-HSD/DD), a member of the aldo-keto reductase superfamily, inactivates circulating steroid hormones and may contribute to the carcinogenicity of polycyclic aromatic hydrocarbons (PAHs) by oxidizing *trans*-dihydrodiols to reactive *o*-quinones with the concomitant generation of reactive oxygen species. The *3α-HSD/DD* gene has been cloned, and its 5′-flanking region contains a negative response element (NRE; −797 to −498 bp) that may repress constitutive expression by binding to Oct transcription factors. Upstream from the NRE are three distal imperfect glucocorticoid response elements (GRE1, GRE2, and GRE3); in addition, a proximal imperfect GRE (GRE4) is adjacent to an Oct binding site in the NRE. When rat hepatocytes were cultured on Matrigel and exposed to dexamethasone (Dex), steady state levels of 3α-HSD/DD mRNA were increased 4-fold in a dose-dependent manner, yielding an EC_{50} value of 10 nM. Time to maximal response was 24 hr, and the effect was blocked with the anti-glucocorticoid RU486. Measurement of the half-life of 3α-HSD/DD mRNA, with and without Dex treatment, indicated that the increase in steady state mRNA levels was not due to increased mRNA stability. By contrast, nuclear run-off experiments using nuclei obtained from Dex-stimulated hepatocytes indicated that Dex increased transcription of the rat *3α-HSD/DD* gene. Tandem repeats of the imperfect GRE1, GRE2, GRE3, and GRE4 were inserted into thymidine kinase-chloramphenicol acetyl-transferase vectors and cotransfected with the human glucocorticoid receptor into human hepatoma cells. On treatment with Dex, maximal *trans*-activation of the chloramphenicol acetyl-transferase reporter gene activity was mediated via the proximal GRE (GRE4). These data imply that GRE4 is a functional *cis*-element and that binding of the occupied glucocorticoid receptor to this element increases *3α-HSD/DD* gene transcription. A model is proposed for the positive and negative regulation of the rat *3α-HSD/DD* gene by the glucocorticoid receptor and Oct transcription factors, respectively.

Rat liver 3α-HSD/DD [3α-hydroxysteroid:NAD(P)$^+$ oxidoreductase-A face specific (EC 1.1.1.213)/dihydrodiol dehydrogenase,*trans*-1,2-dihydrobenzene-1,2-diol:dehydrogenase (EC 1.3.1.20); now referred to as AKR1C9 (Jez *et al*., 1997)] inactivates circulating androgens, progestins, and glucocorticoids (Tomkins, 1956; Hoff and Schriefers, 1973). It also oxidizes PAH *trans*-dihydrodiols to *o*-quinones (Smithgall *et al*., 1988), and in so-doing generates ROS (superoxide anion, hydrogen peroxide, and hydroxyl radical) and *o*-semiquinone anion radicals (Penning *et al*., 1996). This series of events may contribute to the carcinogenicity of the parent hydrocarbon. Cloning and expression of the 3α-HSD/DD cDNA indicate that this enzyme belongs to the AKR superfamily (Pawlowski *et al*., 1991; Pawlowski and Penning, 1994). This superfamily contains other HSDs, which share dihydrodiol dehydrogenase activity; these include human liver DD1 (20α-HSD), human liver DD2 (bile-acid binding protein with 3α-HSD activity), human liver DD4 (type 1 3α-HSD and chlordecone reductase) (Deyashiki *et al*., 1995b; Khanna *et al*., 1995; Hara *et al*., 1996), and murine liver 17β-HSD (Deyashiki *et al*., 1995a). Other AKRs involved in carcinogen metabolism include the aflatoxin aldehyde reductase that is induced by ethoxyquin (Ellis *et al*., 1993).

By identifying the factors that regulate *3α-HSD/DD* gene expression, clues can be obtained to how the inactivation of circulating steroid hormones and carcinogenicity of PAH can be controlled. We cloned the 5′-flanking region of the rat gene and conducted functional studies on its promoter (Lin and Penning, 1995). Salient features were that there was a weak basal promoter, an NRE that bound OTF, and a powerful

This work was supported by National Cancer Institute Grants CA39504 and CA55711 (T.M.P.).

[1] Current affiliation: Division of Gastroenterology, The University of Michigan Medical Center, Ann Arbor, MI 48109.

ABBREVIATIONS: AKR, aldo-keto reductase; ARE, androgen response element; CAT, chloramphenicol-acetyl transferase; Dex, dexamethasone; GRE, glucocorticoid response element; hGR, human glucocorticoid receptor; bp, base pair(s); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MVDP, mouse vas deferens protein; OTF, Oct transcription factor; PAH, polycyclic aromatic hydrocarbon; ROS, reactive oxygen species; SRU, steroid response unit; SRE, steroid response element; tk, thymidine kinase; SSC, standard saline citrate; SDS, sodium dodecyl sulfate.

distal enhancer that regulated the high constitutive expression of the gene. Interspersed through the promoter were a series of SREs, which were proposed to comprise an SRU.

Earlier studies from this laboratory have shown that estrogens may either directly or indirectly increase steady state levels of 3α-HSD/DD mRNA, enzyme protein, and enzyme activity in male rat liver and in rat liver from ovarectomized females (Hou *et al.*, 1994). Others have shown that Dex increases steady state levels of 3α-HSD/DD mRNA in rat hepatocytes maintained in culture (Straviz *et al.*, 1994). In these earlier studies, it was found that Dex increased 3α-HSD/DD mRNA stability but had no effect on gene transcription. Our analysis of the 5′-flanking region of the rat *3α-HSD/DD* gene has located one perfect half-palindromic GRE upstream from the NRE (GRE1). In addition, there are three imperfect GREs: two are upstream from the NRE (GRE2 and GRE3), and one is located within the NRE and adjacent to an OTF binding site (GRE4) (Fig. 1). In the current study, we readdress whether Dex increases *3α-HSD/DD* gene transcription in rat hepatocytes. Our findings provide evidence that Dex up-regulates transcription via binding to the GR, which in turn binds to the proximal GRE (GRE4) on the gene. We suggest that by increasing the transcription of the *3α-HSD/DD* gene, glucocorticoids may also regulate their own metabolism. These data provide direct evidence that the SRU on the 5′-flanking region of the rat *3α-HSD/DD* gene is functional and that steroid hormones may regulate the activation of PAH via transcription of this gene. A model is provided for the positive and negative regulation of the rat *3α-HSD/DD* gene by GR and OTF, respectively.

Materials and Methods

Chemicals and reagents. Adult male Sprague-Dawley rats (200 ± 20 g) were purchased from Charles River Breeding Laboratories (Wilmington, MA). The animals were allowed free access to food and water for 7 days before use. Dex and Dex-21-mesylate were obtained from Steraloids (Wilton, NH). RU486 was purchased from BIOMOL Research Laboratories (Plymouth Meeting, PA). Actinomycin-D was purchased from Sigma Chemical (St. Louis, MO) and fresh solutions were used. [^{3}H]Chloramphenicol (50 Ci/mmol), [α-^{32}P]dATP (3000 Ci/mmol), and [^{32}P]UTP (3,000 Ci/mmol) were purchased from DuPont-New England Nuclear (Boston, MA).

Plasmids. Tandem repeats of the distal, proximal GREs and combinations of the distal GREs located on the 5′-flanking region of the rat *3α-HSD/DD* genes were synthesized (Table 1). The complementary strands also were synthesized and annealed to yield the ds-oligonucleotides. The synthesized oligonucleotides were designed to contain a *Sph*I linker at the 5′-end and a *Xba*I linker at the 3′-end for directional cloning. Double-stranded oligonucleotides containing a tandem repeat of a perfect GRE were obtained from Dr. Barry Komm (Women's Health Institute, Wyeth-Ayerst, Radnor, PA). These were inserted at the *Sph*I and *Xba*I sites 5′ upstream of the tk promoter in pBLCAT2. pRShGR (hGR driven by the constitutive Rous sarcoma virus promoter) and β-actin cDNA were obtained from American Type Culture Collection (nos. 67200 and 78818S, respectively; Rockville, MD). pSVβ-galactosidase was purchased from Promega (Madison, WI).

Hepatocyte culture. Adult male Sprague-Dawley (200 g) rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (90 mg/kg), and the hepatic portal vein was cannulated. Livers were perfused *in situ* with oxygenated Ca^{2+}-free buffer (10 mM HEPES, pH 7.4, containing 142 mM NaCl and 7 mM KCl at a flow rate of 20 ml/min) at 37°. The animal was killed by cutting the inferior vena cava, and the liver was attached to a mantle and perfused with 500 ml of Ca^{2+}-free perfusate at the same flow rate. The blanched liver was then reperfused by cycling 75 ml of the same buffer, containing 1.25 mM $CaCl_2$ and 0.16 mg/ml collagenase (Worthington-Biochemical, Freehold, NJ) for 15 min. The perfusion buffers were oxygenated by exchange through gas-permeable tubing. The whole-liver cell suspension was dispersed into the collagenase buffer at 37° for 1 min and filtered through nylon mesh (200 μm) (Failla and Cousins, 1978). Hepatocytes were harvested by centrifugation and washed twice in 35 ml of Hanks' balanced salt solution. All perfusates were supplemented with penicillin and streptomycin. Viability was determined by Trypan blue exclusion, and 4×10^6 cells were plated into 10-ml culture medium on 100-mm tissue culture dishes (Falcon, Franklin Lake, NJ) precoated with Matrigel (1:3 dilution with minimal essential medium with Earle's salt; Collaborative Biomedical Products, Bedford, MA). The culture medium consisted of Williams' E medium containing 0.3 mM ascorbic acid, 2.0

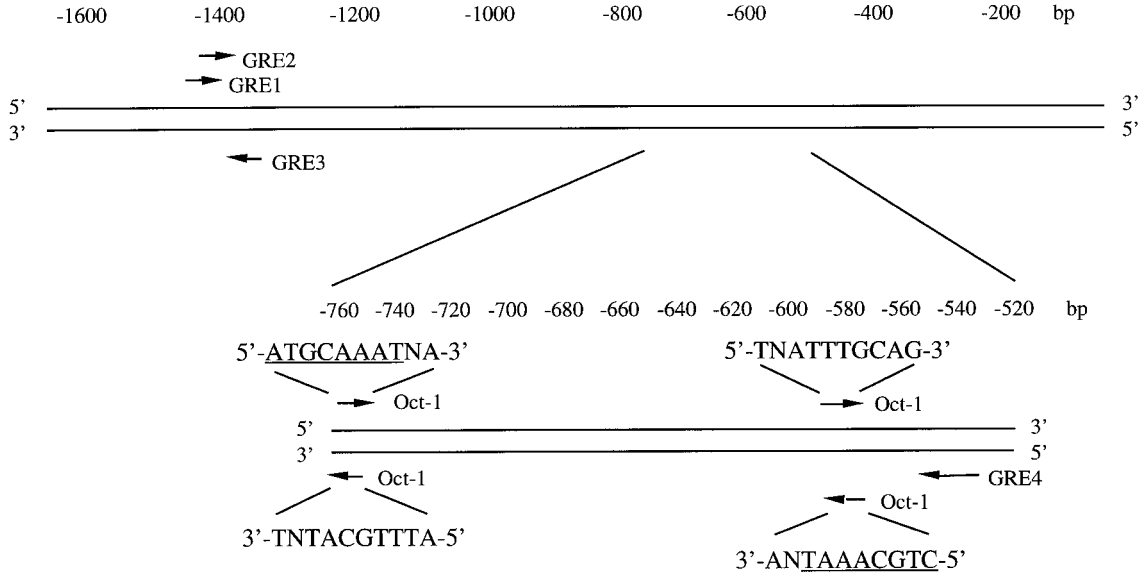

Fig. 1. Arrangement of OTF and GR binding sites in the promoter of rat *3α-HSD/DD* gene. The two OTF binding sites are located at −753 (+)-strand and −574 (−)-strand. GRE1 is located at −1417 (+)-strand, GRE2 is located at −1402 (+)-strand, GRE3 is located at −1364 (−)-strand, and GRE4 is located at −537 (−)-strand.

TABLE 1
Synthesized oligonucleotides corresponding to GREs in the 5′-flanking region of the rat *3α-HSD/DD* gene

GRE	Position	Strand	Sequence
GRE1	−1417	(+)	5′-AATCAA-NNN-TGTCCT-3′
GRE2	−1402	(+)	5′-ATACCA-NNN-TGATGT-3′
GRE3	−1364	(−)	5′-GGTTCA-NNN-TTTTCT-3′
GRE4	−537	(−)	5′-TTAACA-NNN-TGATTT-3′
The consensus GRE 5′-G/T G/T TAC A/C-NNN-TGT T/C CT-3′			

Underline indicates mismatches with the perfect GRE.

mM L-glutamine, 100 units/ml penicillin, 10 μg/ml streptomycin, 0.02 mM sodium selenite, and 8 μg/ml bovine insulin. The culture medium was replaced every 24 hr (Fig. 2).

Isolation of mRNA and dot-blot analysis. Total RNA was isolated according to the method of Lyttle and Komm (1984). RNA samples (10 or 20 μg) in 15× SSC buffer (1.5 M NaCl and 0.15 M sodium citrate, pH 7.0), containing formamide in diethylpyrocarbonate-treated water, were applied to a Nytran membrane (Schleicher & Schnell, Keene, NH) using a dot-blot manifold with vacuum aspiration. RNA was fixed using a UV cross-linker, prehybridized with 100 μg/ml sheared salmon sperm DNA, and then hybridized with a [α-^{32}P]dATP-labeled rat liver 3α-HSD/DD cDNA probe (containing +334–853 bp of the open-reading frame) prepared by random priming (Feinberg and Vogelstein, 1983). Hybridization was conducted at 42° overnight. The filters were washed twice in 0.1× SSC and 0.1% SDS at 60° for 45 min and exposed to X-ray film at −70°. The filters were stripped by boiling in 0.1× SSC and 0.1% SDS and reprobed with [α-^{32}P]dATP-labeled β-actin for normalization.

Preparation of hepatocyte nuclei. Seven plates of rat hepatocytes (3×10^6 cells/plate) were rinsed twice with ice-cold phosphate-buffered saline, and 2.0 ml of Matri-sperse (Collaborative Biomedical Products) was added to each plate. An identical number of plates that had been exposed to 1.0 μM Dex for 24 hr were treated in the same manner. Nuclei were isolated according to the method of Blobel and Potter (1966). Cells were harvested with a rubber policeman, incubated on ice for 30 min, and pelleted by centrifugation at 200 × *g*. The supernatant was removed, and the cells were resuspended for 5 min in 10 mM Tris·HCl, pH 7.4, containing 2 mM NaCl, 3 mM $MgCl_2$, 14 mM β-mercaptoethanol, 2 mM $CaCl_2$, 0.1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride. Nonidet P-40 (30%, 5 μl) was added to each tube, and the cells were homogenized in a Dounce homogenizer (25 strokes). The pellet was isolated by centrifugation and resuspended in glycerol storage buffer consisting of 50 mM Tris·HCl, pH 8.0, containing 40% glycerol, 5 mM $MgCl_2$, 1 mM KCl, and 0.1 mM EDTA. Nuclei were counted with a hemocytometer, and equal numbers of nuclei were stored in aliquots of 100 μl from control and Dex-treated cells.

Nuclear run-off assays. Nuclei (100 μl) were thawed on ice and mixed with an equal volume of 10 mM Tris·HCl, pH 8.0, containing 5 mM $MgCl_2$ and 300 mM KCl. The samples were transferred to 2-ml tubes containing an NTP cocktail (2 μl each of 100 mM ATP, CTP, and GTP plus 20 μl of [^{32}P]UTP and 0.5 μl of 1 M dithiothreitol). The transcription reaction was conducted at 30° for 30 min with agitation. The reaction was quenched by the addition of 16 μl of a mixture containing 2 μl of 100 mM $CaCl_2$, 10 μl of DNase-I (RNase free; 30 units/μl), and 4 μl of RNasin (20 units/μl), and incubated for an additional 10 min at 30°. After DNA digestion was complete, protein digestion was accomplished by the addition of 35 μl of a mixture containing 25 μl of 10 mM Tris·HCl, pH 7.4, 10 mM EDTA, 0.2% SDS, 5.0 μl of 10 mg/ml glycogen, and 2.4 μl of 10 mg/ml proteinase K, followed by incubation at 37° for 30 min. Total RNA was extracted as described previously.

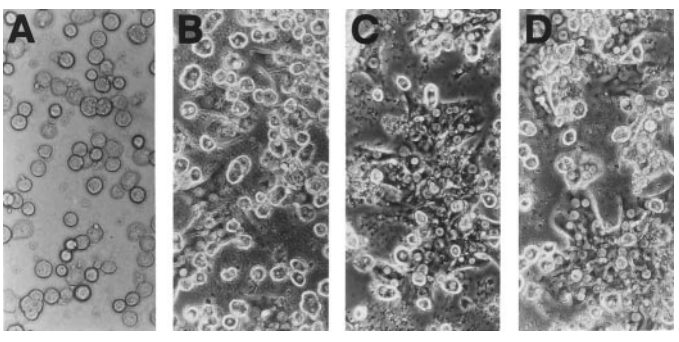

Fig. 2. Rat hepatocytes cultured on Matrigel. Hepatocytes were isolated from adult male Sprague-Dawley rats and cultured on plates coated with Matrigel (4×10^6 cells/100-mm plate) growth at 0, 24, 48, and 72 hr (A–D, respectively).

Plasmids (5 μg) containing 3α-HSD/DD (+1–853 bp of the open reading frame) (Pawlowski *et al*., 1991), β-actin, and empty vectors were linearized with appropriate restriction enzymes and denatured with 1 N NaOH, followed by neutralization with 6× SSC on ice. The plasmid DNA was spotted onto a membrane through a dot-blot manifold. The membrane was UV cross-linked and rinsed. After DNA was fixed, the blots were placed in Northern hybridization buffer at 45° for 6 hr. To each blot, an equal amount of radiolabeled RNA probe from the transcription assays (1×10^6 cpm/ml hybridization buffer) was added. Hybridization was conducted at 45° for 48 hr. The membranes were washed twice at room temperature for 15 min, once at 45° for 15 min, and once at 50° for 15 min and exposed to X-ray film for autoradiography.

Hepatoma cell culture and transfection. The human hepatoma cell line HepG2 (HB8065; American Type Culture Collection) was maintained in minimal essential medium with Earle's salt containing 10% heat-inactivated fetal bovine serum, 2.0 mM L-glutamine, 100 units/ml penicillin, and 10 μg/ml streptomycin at 37° in a humidified atmosphere with 5% CO_2. Cells were passaged every 4 days by seeding fresh plates with 3×10^6 cells.

All reporter gene plasmids, plasmids containing β-galactosidase, and plasmids containing the hGR were purified before transfection through cesium chloride gradients containing ethidium bromide. HepG2 cells were seeded at the concentration of 1.5×10^6 cells/60-mm tissue culture plate 24 hr before transfection. Culture media was replaced with 4.5 ml of phenol red-free minimal essential medium with Earle's salt plus 10% charcoal-dextran-treated fetal bovine serum (Hyclone, Logan, UT) at 4 hr before transfection. For each transfection, DNA-$CaCl_2$ solutions were prepared by mixing 37 μl of 2 M $CaCl_2$ and 9 μg of total plasmid DNA containing 5 μg of pGRE-tk-CAT constructs, 2 μg of pRShGR, and 2 μg of pSVβ-galactosidase in a final volume of 300 μl. DNA was precipitated by mixing with an equal volume of 2× HEPES-buffered saline (50 mM HEPES, pH 7.4, containing 280 mM NaCl and 1.5 mM Na_2HPO_4) with constant agitation. The precipitants were incubated at room temperature for 30 min before their addition to the cell culture medium. After 24 hr, the cells were exposed to 1 μM Dex in 0.2% dimethylsulfoxide.

CAT assays. Transfected HepG2 cells were rinsed twice with 1× Ca^{2+} and Mg^{2+}-free phosphate-buffered saline, pH 7.3, and lysed with 400 μl of 1× reporter lysis buffer (Promega) followed by incubation at room temperature for 15 min. The cells were harvested with a rubber policeman and transferred into 1.5-ml microcentrifuge tubes. For each β-galactosidase assay, a cell lysate (50 μl) was incubated in 60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 1 mM $MgCl_2$, and 50 mM β-mercaptoethanol, containing 0.67 mg/ml *o*-nitrophenyl-β-D-galactopyranoside as a substrate in a total of 300 μl at 37° for 2 hr. The reaction was terminated by the addition of 500 μl of 1 M sodium carbonate, and the absorbance of the *o*-nitrophenol anion was determined at 420 nm. This end-point assay was validated by showing that the absorbance was in the linear range with respect to time and amount of β-galactosidase. For CAT activity, the volumes of the cell lysates were adjusted to contain the same amount of β-galactosidase activity to normalize for transfection efficiency. This correction was <15% among the transfections. Lysates (80–100 μl) then were incubated in a final volume of 125 μl containing 5 pmol of chloramphenicol, 5 fmol of [^{3}H]chloramphenicol (0.25 μCi), and 50 μM *n*-butyryl

coenzyme A (Sigma). The reactions were performed at 37° for 2 hr and terminated by the addition of 300 μl of mixed xylenes, which were back-extracted twice with 100 μl of 0.25 M Tris·HCl, pH 8.0. Aliquots of the 200-μl xylene phases were counted in 5 ml of a toluene-based scintillant containing 4.0 g of 2,5-diphenyloxzaole plus 50 mg of *p*-bis-[2-(5-phenyloxazolyl)]benzene/liter of toluene. The radioactivity incorporated into monobutylated and dibutylated chloramphenicol was calculated for GRE constructs as the fold increase relative to that observed with the pBLCAT2 vector (negative control).

Results

Changes in 3α-HSD/DD mRNA in rat hepatocytes induced by Dex. Rat hepatocytes were cultured for 48 hr on Matrigel-coated plates and then exposed to increasing concentrations of Dex for an additional 24 hr. Dot-blot analysis of the total RNA using a randomly primed cDNA probe for rat liver 3α-HSD/DD revealed that over the first 48 hr in culture, untreated cells showed a 2–3-fold decrease in steady state 3α-HSD/DD mRNA levels, consistent with that observed in hepatocytes cultured on rat tail collagen (Straviz *et al.*, 1994). After steroid treatment, there was a dose-dependent increase in 3α-HSD/DD mRNA induced by Dex, whereas untreated cells showed no further change in 3α-HSD/DD mRNA levels. During the Dex response, there was no change in expression of β-actin mRNA, which was used as a control for an unregulated transcript (Fig. 3A). A dose-response curve was generated for Dex, and an EC_{50} value of 10 nM was obtained (Fig. 3B). To optimize this response, hepatocytes were treated with a maximal concentration of Dex (1.0 μM), and the time course for the increase in 3α-HSD/DD mRNA was monitored. It was found that the maximal response was obtained 16–24 hr after exposure to steroid hormone (Fig. 4).

The EC_{50} value obtained for Dex and the time to maximal response would be consistent with the increase in steady state levels of 3α-HSD/DD mRNA being mediated by Dex binding to the GR to cause a transcriptional response. The coadministration of RU486 (an anti-glucocorticoid) and Dex resulted in an attenuation of the Dex response (Fig. 5). Interestingly, Dex-mesylate, an irreversible ligand for the GR, also increased 3α-HSD/DD expression. These data suggest that occupancy of the GR leads to either an increase in *3α-HSD/DD* gene transcription or an increase in 3α-HSD/DD mRNA stability that may or may not be dependent on glucocorticoid-mediated gene transcription.

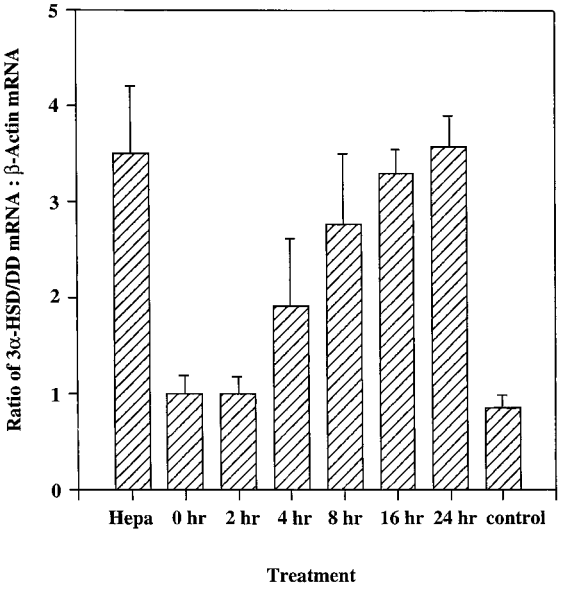

Fig. 4. Time-dependent increase in 3α-HSD/DD mRNA in rat hepatocytes induced by Dex. Rat hepatocytes (4×10^6 cells) were cultured on Matrigel-coated plates for 48 hr and then treated with 1.0 μM Dex for 24 hr. Total RNA was isolated at the times indicated after Dex treatment. 3α-HSD/DD mRNA was quantified by dot-blot analysis as described. Values are relative to the level of β-actin. Groups are *hepa*, hepatocytes at platting; 0 hr, hepatocytes after 48 hr in culture; 2–24 hr, hepatocytes after 48 hr in culture plus subsequent exposure to 10 μM Dex; and control, untreated hepatocytes after 72 hr in culture. The mean ± standard error is given from three different hepatocyte preparations.

Dex has no effect on rat 3α-HSD/DD mRNA stability. To determine whether Dex increased the stability of the 3α-HSD/DD mRNA in rat hepatocytes, the half-life of the mRNA was measured before and after steroid hormone treatment. In the control, hepatocytes were cultured for 48 hr, treated with an amount of Actinomycin-D (10 μg/ml) predetermined to block all [^{3}H]uridine incorporation into mRNA, and total RNA was isolated for dot-blot analysis at 5-hr intervals over the next 30 hr. This analysis revealed that the half-life for 3α-HSD/DD mRNA was 12 hr. In the Dex-treated cells, the steady state level of the 3α-HSD/DD mRNA was

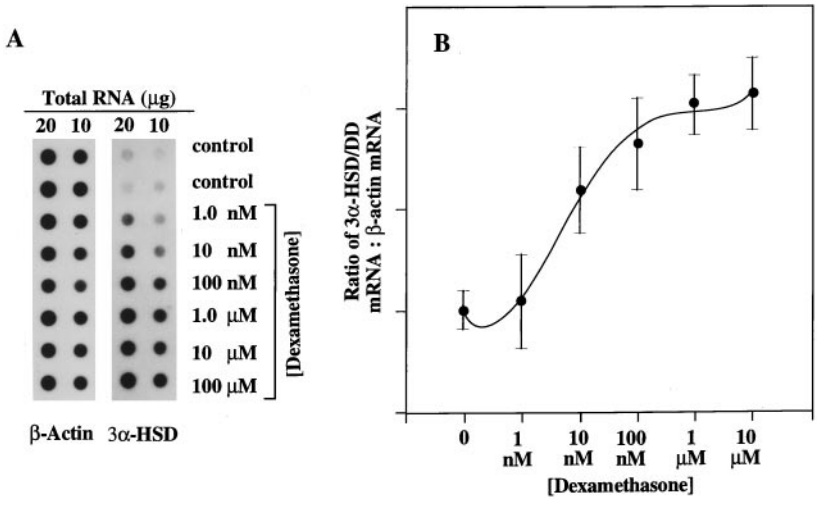

Fig. 3. Dose-dependent increase in 3α-HSD/DD mRNA in rat hepatocytes induced by Dex. Rat hepatocytes (4×10^6 cells) were cultured on Matrigel-coated plates for 48 hr and then treated with 0.2% DMSO (control) or increasing concentrations of Dex (1.0 nM to 10 μM). Total RNA was isolated, and 10 or 20 μg of the RNA was applied to Nytran membranes using a dot-blot manifold. Filters were hybridized to a [α-^{32}P]dATP random-primed 3α-HSD/DD cDNA probe (corresponding to +334–853 bp of the open-reading frame) at 42° for 18 hr followed by high temperature washes and autoradiography. A, Filters were stripped and reprobed with a control probe for β-actin. B, Experiments were performed on triplicate preparations of hepatocytes, and a dose-response curve was generated. *Error bar*, standard error.

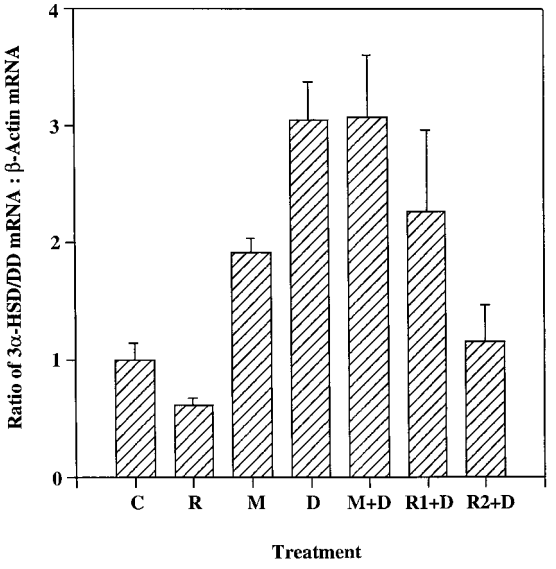

Fig. 5. The anti-glucocorticoid RU486 attenuates Dex-induced increases in 3α-HSD/DD mRNA in isolated rat hepatocytes. Rat hepatocytes (3×10^6 cells) were cultured on Matrigel-coated plates for 48 hr and treated with hormone in the presence or absence of RU486 for a subsequent 24-hr period. Total RNA was isolated and analyzed by dot-blot analysis as described. Values are relative to the level of β-actin. Treatment groups are no treatment (*C*), 1.0 μM RU486 (*R*), 1.0 μM Dex-mesylate (*M*), 1.0 μM Dex (*D*), 1.0 μM Dex plus 1.0 μM Dex-mesylate (*M+D*), 1.0 μM Dex plus 1.0 μM RU486 (*R1+D*), and 1.0 μM Dex plus 10 μM RU486 (*R2+D*). The mean ± standard error is given from three different hepatocyte preparations.

increased 3–4-fold, but there was no change in half-life of the mRNA after treatment with Actinomycin-D (Fig. 6).

Dex increases the rat *3α-HSD/DD* gene transcription. To determine whether Dex mediates its effects via increased transcription of the *3α-HSD/DD* gene, nuclei were isolated from control hepatocytes and hepatocytes treated with 1.0 μM Dex for 24 hr. New transcripts were labeled with [^{32}P]UTP and extracted from the isolated nuclei. The RNA pool was quantified for 3α-HSD/DD transcripts on blots containing immobilized linearized plasmids containing the open-reading frame for 3α-HSD/DD and β-actin. These nuclear run-off assays showed that the ratio of 3α-HSD/DD to β-actin mRNA increased 3-fold in the Dex-treated nuclei, indicating this steroid increases transcription of the *3α-HSD/DD* gene (Fig. 7).

Function of the GREs on the 5′-flanking region of the rat *3α-HSD/DD* gene. Cloning of the 5′-flanking region of the rat *3α-HSD/DD* gene indicates it contains one perfect half-palindromic GRE (GRE1) and three imperfect GREs (GRE2, GRE3, and GRE4) (Table 1). GRE1, GRE2, and GRE3 are located upstream from the basal promoter and an NRE that binds OTF (Lin and Penning, 1995). The remaining GRE (GRE4) is located proximally to the basal promoter and is adjacent to an Oct site in the NRE (Fig. 1). To test the functionality of these GREs, oligonucleotides corresponding to tandem repeats of the four GREs were synthesized and subcloned upstream from the reporter gene CAT linked to the tk promoter. These constructs were used in heterologous cotransfection studies in which the p(GRE)$_2$-tk-CAT constructs were cotransfected with plasmids containing the cDNA for the hGR into HepG2 cells. Dex was unable to induce CAT activity in HepG2 cells cotransfected with the control vector (ptk-CAT or pBLCAT2) plus the hGR (Fig. 8). CAT activity was not stimulated by Dex in cells transfected with a plasmid containing a perfect GRE linked to tk-CAT (p(GRE)$_2$-tk-CAT) alone. These data are consistent with the low levels of GR present in HepG2 cells (Lui *et al*., 1993). In a complete system, in which HepG2 cells were cotransfected with p(GRE)$_2$-tk-CAT plus hGR and stimulated with 1 μM Dex, CAT activity was elevated 26-fold. When HepG2 cells were cotransfected with plasmids containing tandem repeats of either the single distal GREs (GRE1, GRE2, or GRE3) or the single proximal GRE (GRE4) linked to tk-CAT plus the hGR, no increase in CAT activity was observed. However, after Dex treatment, there was a 5- and 8-fold increase in CAT activity from constructs containing tandem repeats of GRE2 and GRE4, respectively. To determine whether the distal GREs act synergistically, HepG2 cells also were cotransfected with consecutive GREs [e.g., p3α-(GRE1, GRE2)-tk-CAT, p3α-(GRE2, GRE3)-tk-CAT, and p3α-(GRE1, GRE2, GRE3)-tk-CAT] plus the hGR and stimulated with Dex. In this instance, no increase in CAT activity was observed over basal levels. These data indicate that in a complete system containing a tandem repeat of either the imperfect distal GRE (GRE2) or the imperfect proximal GRE (GRE4), hGR and Dex will increase *trans*-activation of the reporter gene. The results for the combination of GREs suggest that the effects of the distal GRE may be attenuated by adjacent elements. Thus, the proximal GRE (GRE4) may play the predominant role in *trans*-activation by glucocorticoids. These data indicate that GRE4, residing in the 5′-flanking region of the rat *3α-HSD/DD* gene, is a functional *cis*-acting element provided it is presented with a liganded hGR. This increase in reporter gene activity further supports that Dex elevates 3α-HSD/DD expression at the level of gene transcription.

Discussion

3α-HSD activity was originally detected in rat liver because of its ability to metabolize dihydroglucocorticoids to tetrahydroglucocorticoids (Tomkins, 1956). Our findings clearly show that glucocorticoids increase the transcription of the rat *3α-HSD/DD* gene. This effect is mediated by glucocorticoids binding to the occupied GR, which can mediate its effects at both a distal and proximal GRE on the 5′-flanking region of the rat 3α-*HSD/DD* gene. The greatest effects were mediated via binding to the proximal GRE. The ability of glucocorticoids to up-regulate 3α-HSD expression implies that these steroid hormones can regulate their own metabolism by increasing levels of an enzyme that will convert dihydroglucocorticoids to tetrahydroglucocorticoids. It will be of interest to determine whether 5β-reductase, which precedes 3α-HSD in steroid hormone metabolism, is regulated by the same mechanism, because the rat and human 5β-reductases also are members of the AKR gene superfamily (Kondo *et al*., 1994; Onishi *et al*., 1994).

Our results, which support *trans*-activation of the *3α-HSD/DD* gene via a GRE/GR, complex differ from those of Straviz *et al*. (1994), which demonstrated that Dex increased 3α-HSD/DD expression in hepatocytes by increasing the stability of the mRNA. Our data show that Dex has no effect on 3α-HSD/DD mRNA half-life. Several reasons may exist for these differences. In the studies of Straviz *et al*., rat-tail

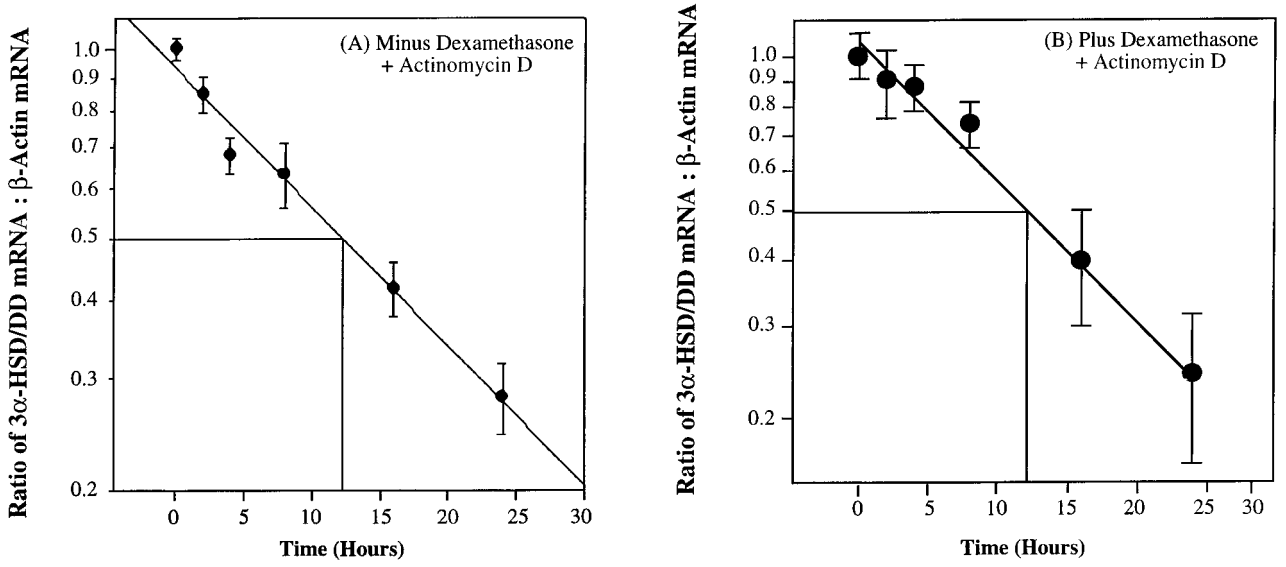

Fig. 6. Measurement of the half-life of 3α-HSD/DD mRNA in hepatocytes in the presence and absence of Dex. Rat hepatocytes (3×10^6 cells) were cultured on Matrigel-coated plates for 48 hr and then treated with actinomycin D (10 μg/ml). A, Total RNA was isolated at the times indicated, and 3α-HSD/DD mRNA was quantified relative to β-actin by dot-blot analysis. B, In identical experiments, rat hepatocytes were treated with 1.0 μM Dex at 48 hr and then treated with actinomycin D (10 μg/ml) at 72 hr, and the 3α-HSD/DD mRNA was quantified. The mean ± standard error is given from three different hepatocyte preparations.

collagen was used as a matrix for hepatocyte culture, and not Matrigel. Under the rat-tail collagen culture conditions, the half-life of the mRNA in untreated cells was 4–6 hr, whereas under the Matrigel culture conditions, the half-life of the mRNA was 12 hr. Thus, under our study conditions, the half-life of the 3α-HSD/DD mRNA in untreated cells is the same as that observed in Dex-treated hepatocytes grown on rat-tail collagen, suggesting that stabilization of the mRNA has been optimized. By contrast, our results, which show a 3–4-fold increase in steady state 3α-HSD/DD mRNA, can be accounted for by a comparable increase in transcription rate measured by nuclear run-off and a 3–6 fold increase in *trans*-activation of CAT reporter gene activity driven by the proximal GRE (GRE-4). Our results show that Dex increases *3α-HSD/DD* gene expression solely at the level of transcription and make intuitive sense based on the presence of multiple imperfect GREs on the 5′-flanking region of this gene.

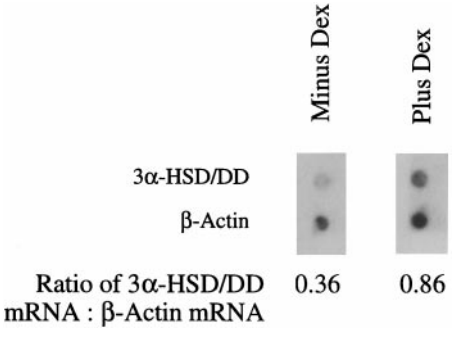

Fig. 7. Effect of Dex on the transcription of the *3α-HSD/DD* gene measured by nuclear run-off assay. Nuclei were harvested from seven plates of hepatocytes after 72 hr in culture or seven plates of hepatocytes that were treated from 48–72 hr with 1.0 μM Dex. Each plate contained 3×10^6 cells, and nuclei were harvested as described. Aliquots containing the same number of nuclei from both groups were used in transcription assays containing [^{32}P]UTP and NTPs and incubated at 37°. The newly synthesized [^{32}P]RNA was extracted after 30 min, and equal amounts of radioactivity (1×10^6 cpm/ml) were applied to a dot-blot to which plasmids containing β-actin, 3α-HSD/DD, or empty vectors had been immobilized previously. Plasmids (5 μg) were linearized, denatured with 1 N NaOH, and neutralized before application to the Nytran membrane. Hybridization was performed at 45°, and membranes were washed and exposed to X-ray film for 7 days. No hybridization to the empty vectors was observed. Hybridization to the linearized plasmids containing coding sequences for either β-actin of 3α-HSD/DD are shown with and without Dex treatment.

It is not uncommon for the GR to bind to imperfect sites. Most GREs recognized by GR are imperfect palindromes separated by 3 bp (Beato, 1989). A cluster of imperfect GREs in the rat *3α-HSD/DD* gene promoter is similar to that described for the mouse mammary tumor virus promoter (Scheidereit *et al.*, 1983) and the rat tyrosine amino-transferase gene (Jantzen *et al.*, 1987). On forming homodimers, the occupied GR binds these imperfect palindromic GREs (Jantzen *et al.*, 1987; Schmid *et al.*, 1989). By binding to several imperfect GREs, the GR may work cooperatively to increase gene transcription. Because increased CAT activity was not observed in constructs containing consecutive distal GREs found in the *3α-HSD/DD* gene promoter, these data suggest that these elements either function independently of one another or that the most important *cis*-element is the proximal GRE (GRE4).

GRE4 is located proximally to the basal promoter but lies within the NRE adjacent to an Oct site. Both the GRE and the Oct site are located on the (−)-strand, raising the possibility that there may be interaction between the GR and OTF to positively and negatively regulate the expression of the rat *3α-HSD/DD* gene, respectively. Several mechanisms have been reported by which these transcription factors interact. First, the progesterone and glucocorticoid receptors when bound to their SREs facilitate binding of OTF to octamer motifs in the MMTV promoter. In this example, the factors work cooperatively to increase gene transcription (Brüggemeier *et al.*, 1991). Second, glucocorticoid repression of the mouse gonadotropin-releasing hormone gene is achieved by the tethering of the GR to a negative GRE by virtue of a direct or indirect association with DNA-bound Oct-1 (Chan-

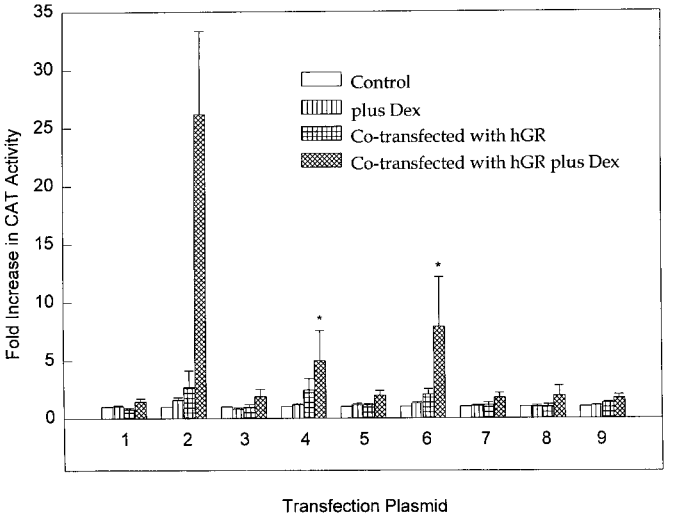

Fig. 8. Effect of GREs and human GR on the transcription of the *3α-HSD/DD* gene measured by reporter gene assays. Four GREs (GRE1, GRE2, GRE3, and GRE4) identified on the 5′-flanking region of the rat *3α-HSD/DD* gene were synthesized as either 2× tandem repeats or consecutive GREs. These GREs were subcloned upstream of a CAT reporter gene construct containing the tk promoter to yield p3α-$(GRE1)_2$-tk-CAT, p3α-$(GRE2)_2$-tk-CAT, p3α$(GRE3)_2$-tk-CAT, p3α-$(GRE4)_2$-tk-CAT, p3α-(GRE1, GRE2)-tk-CAT, p3α-(GRE2, GRE3)-tk-CAT, and p3α-(GRE1, GRE2, GRE3)-tk-CAT. The CAT constructs (5 μg) were cotransfected with human GR (2 μg) into HepG2 cells seeded 24 hr earlier at the concentration of 1.5×10^6 cells/60-mm tissue culture plates using a $CaPO_4$ precipitation procedure. Dex (1 μM) was added at 24 hr after transfection, and cells were harvested after 48 hr after treatment. Cell lysates were incubated with *n*-butyl coenzyme A and [^{3}H]chloramphenicol at 37° for 2 hr. The reactions were terminated by the addition of 300 μl of mixed xylenes and analyzed by liquid scintillation counting. Data have been normalized to β-galactosidase activity cotransfected as 2 μg of pSV-β-galactosidase. The transfection plasmids are ptk-CAT (*1*), $p(GRE)_2$-tk-CAT (*2*), p3α-(GRE1)-tk-CAT (*3*), p3α-(GRE2)-tk-CAT (*4*), p3α(GRE3)-tk-CAT (*5*), p3α-(GRE4)-tk-CAT (*6*), p3α-(GRE1, GRE2)-tk-CAT (*7*), p3α-(GRE2, GRE3)-tk-CAT (−1402 to −1364 bp) (*8*), and p3α-(GRE1, GRE2, GRE3)-tk-CAT (−1417 to −1364 bp) (*9*). The mean ± standard error is given from six separate transfections. ∗, Significance differences were determined using a paired *t* test that rejected the null hypothesis at a value of $p < 0.05$.

dran *et al*., 1996). Third, the lymphocyte-specific transcription factor Oct-2A is inhibited in the presence of liganded GR when there is no GRE present (Wieland *et al*., 1991). Thus, when OTF and GR bind in *cis*, their actions have up to now been synergistic. In the case of the rat *3α-HSD/DD* gene, we hypothesize that when these factors bind in *cis*, their actions may be antagonistic, so the positive effect of GR is negated by OTF. The fact that the *3α-HSD/DD* gene is regulated by a functional GRE suggests that the gene may be under the control of multiple steroid hormones because it contains many potential SREs that may comprise a SRU. This report provides the first evidence that functional SREs reside in the SRU.

Regulation of other members of the AKR superfamily by steroids has been reported. Both the MVDP, which is highly homologous to human aldose reductase, and the human aldose reductase genes contain consensus sequences for an ARE on their 5′-flanking regions. Reporter gene (CAT) constructs containing the ARE were cotransfected into HepG2 cells with either the androgen receptor or the progesterone receptor. CAT activity was elevated in response to 5α-dihydrotestosterone or progesterone stimulation (Ruepp *et al*., 1996). In addition, because the ARE and GRE share a similar 15-bp motif, the ability was tested of the MVDP AREs to respond to glucocorticoid stimulation. When a human mammary carcinoma cell line (T47D) was cotransfected with an MVDP-ARE-CAT reporter gene construct plus the hGR, Dex stimulation increased CAT activity 9.6-fold. Mutagenesis of the ARE completely abrogated Dex-elevated CAT activity (Fabre *et al*., 1995). The presence of functional SREs on the 5′-flanking regions of several AKR genes suggests members of this superfamily can be regulated by steroid hormones.

As well as metabolizing glucocorticoids, the rat *3α-HSD/DD* gene has been implicated in the metabolism and activation of PAHs. Thus, by oxidizing PAH *trans*-dihydrodiols (proximate carcinogens) to *o*-quinones, there is concomitant generation of ROS and *o*-semiquinone radicals. Once generated, the *o*-quinones enter into futile redox cycles to generate ROS multiple times. This mechanism of free radical amplification may contribute to the carcinogenicity of the parent PAH. CYP1A1 is a major enzyme involved in the activation of PAH, and it converts *trans*-dihydrodiols to diol-epoxides, which alkylate and mutate DNA. Analysis of the promoter for the rat *CYP1A1* gene indicates that like the rat *3α-HSD/DD* gene, it contains an NRE that binds OTF (Bhat *et al*., 1996; Sterling and Bresnick, 1996). Thus, OTF may be repressors of both the rat *3α-HSD/DD* and rat *CYP1A1*

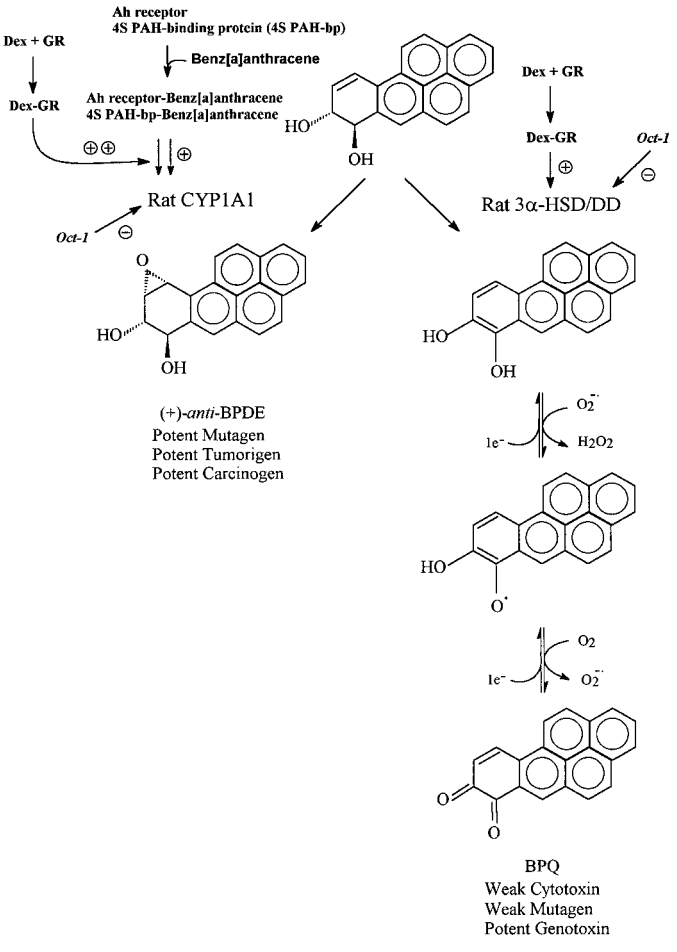

Fig. 9. Transcriptional control of PAH activation. Dexamethsone and benz[*a*]anthracene (PAH) can act synergistically to regulate expression of the *CYP1A1* gene by binding to their receptors (e.g., glucocorticoid receptor, Ah receptor, and 4S PAH binding protein).

genes and block the formation of reactive PAH *o*-quinones and PAH diol-epoxides, respectively (Fig. 9). In addition, Dex will act synergistically with benz[*a*]anthracene to increase expression of the rat *CYP1A1* gene in fetal hepatocytes (Mathis *et al*., 1986). This is achieved by benz[*a*]anthracene *trans*-activation of the Ah receptor and the 4S PAH binding protein and by Dex *trans*-activation of the GR (Mathis *et al*., 1986, 1989; Bhat and Bresnick, 1997). These liganded factors bind to their respective *cis*-elements on the *CYP1A1* gene. In the case of the occupied GR, it binds to intron-1 of this gene (Mathis *et al*., 1989). Thus, the formation of activated PAH metabolites by different enzymes may be regulated by common repressors (e.g., Oct-1) and common coinducers (e.g., liganded GR) at the level of gene expression. It should be noted that the human *CYP1A1* gene also has an NRE but the repressor in this instance is NF-Y and not Oct-1 (Boucher *et al*., 1995). Other CYPs are regulated by glucocorticoids. In this regard, rat CYPIIIA1 is induced by steroid antagonists (pregnenolone-16α-acetonitrile) and by glucocorticoids (Ciuzedium, 1988). There are three *P-450III* genes in human that show a >90% sequence identity, and one of these is P-450$_{NF}$ (Gonzalez, 1992). Importantly, P-450$_{NF}$ seems to be a major enzyme involved in the activation of (±)-*trans*-7,8-dihydroxy-7,8-dihydro-benzo[*a*]pyrene in human liver (Shimada *et al*., 1989). On this basis, it will be of interest to determine whether human P-450$_{NF}$ also is regulated by glucocorticoids and OTF.

References

Beato M (1989) Gene regulation by steroid hormones. *Cell* ***56:***335–344.

Bhat R and Bresnick E (1997) Glycine *N*-methyltransferase is an example of functional diversity: role as a polycyclic aromatic hydrocarbon-binding receptor. *J Biol Chem* **272:**21221–21226.

Bhat R, Weaver JA, Sterling KM, and Bresnick E (1996) Nuclear transcription factor Oct-1 binds to the 5′-upstream region of *CYP*1A1 and negatively regulates its expression. *Int J Biochem Cell Biol* **28:**217–227.

Blobel G and Potter VR (1966) Nuclei from rat liver: isolation method that combines purity with high yield. *Science (Washington DC)* **154:**1662–1665.

Boucher PD, Ruch RJ, and Hines RN (1993) Specific nuclear protein binding to a negative regulatory element on the human *CYP1A1* gene. *J Biol Chem* **268:**17384–17391.

Brüggemeier U, Kalff M, Franke S, Scheidereit C, and Beato M (1991) Ubiquitous transcription factor OTF-1 mediates induction of the MMTV promoter through synergistic interaction with hormone receptors. *Cell* **64:**565–572.

Chandran UR, Attardi B, Friedman R, Zheng Z-W, Roberts JL, and DeFranco DB (1996) Glucocorticoid repression of the mouse gonadotropin-releasing hormone gene is mediated by promoter elements that are recognized by heteromeric complexes containing glucocorticoid receptor. *J Biol Chem* **271:**20412–20420.

Ciuzedium PS (1988) Regulation of the glucocorticoid-inducible cytochrome P-450, in *Microsones and Drug Oxidations* (Miners JO, Birkett DJ, Drew R, and McManns M, eds) pp 148–155, Tayler and Grancis, London.

Deyashiki Y, Ohshima K, Nakanishi M, Sato K, Matsuura K, and Hara A (1995a) Molecular cloning and characterization of mouse estradiol 17β-dehydrogenase (A-specific), a member of the aldo-keto reductase family. *J Biol Chem* **270:**10461–10467.

Deyashiki Y, Tamada Y, Miyabe Y, Nakanishi M, Matsuura K, and Hara A (1995b) Expression and kinetic properties of a recombinant 3α-hydroxysteroid/dihydrodiol dehydrogenase isoenzyme of human liver. *J Biochem* **118:**285–290.

Ellis EM, Judah DJ, Neal G, and Hayes JD (1993) An ethoxyquin-inducible aldehyde reductase from rat liver that metabolizes aflatoxin B1 defines a sub-family of aldo-keto reductases. *Proc Natl Acad Sci USA* **90:**10350–10354.

Fabre S, Darne C, Beyssiere G, and Jean C (1995) Characterization of the promoter of the gene for a mouse vas deferens protein related to the aldo-keto reductase superfamily: effect of steroid hormones and phorbol esters. *J Steroid Biochem Mol Biol* **55:**315–325.

Failla ML and Cousins RJ (1978) Zinc uptake by isolated rat liver parenchymal cell. *Biochim Biophys Acta* **538:**435–444.

Feinberg AP and Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* **132:**6–13.

Gonzalez FJ (1992) Molecular genetics of the P450 superfamily, in *Pharmacogenetics of Drug Metabolism* (Kalow W, ed) pp 413–452, Pergamon Press, New York.

Hara A, Matsuura K, Tamada Y, Sato K, Miyabe Y, Deyashiki Y, and Ishda N (1996) Relationship of human liver dihydrodiol dehydrogenases to hepatic bile-acid binding protein and an oxidoreductase of human colon cells. *Biochem J* **313:**373–376.

Hoff H-G and Schriefers H (1973) Sexuell differenzierte und sexuell undifferenzierte 3α- and 3β-hydroxysteroid dehydrogenase-aktivaten und ihre intra zellulore lokalisation in der rattenleber. *Hoppe-Seyler's Z Physiol Chem* **354:**507–513.

Hou Y-T, Xia W, Pawlowski JE, and Penning TM (1994) Rat dihydrodiol dehydrogenase: complexity of gene structure and tissue specific and sexually dimorphic gene expression. *Cancer Res* **54:**247–255.

Jantzen HM, Strahle U, Gloss B, Stewart F, Schmid W, Boshart M, Miksicek R, Schutz G (1987) Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. *Cell* **49:**29–38.

Jez JM, Flynn TA, and Penning TM (1997) A new nomenclature for the aldo-keto reductase superfamily. *Biochem Pharmacol* **54:**639–647.

Khanna M, Qin K-N, Wang RW, and Cheng K-C (1995) Substrate specificity, gene structure and tissue-specific distribution of multiple human 3α-hydroxysteroid dehydrogenases. *J Biol Chem* **270:**20162–20168.

Kondo K, Kai M, Setoguchi Y, Eggertsen G, Sjoblom P, Setoguchi T, Okuda K, and Bhorkhem I (1994) Cloning and expression of cDNA of human Δ^4-3-oxosteroid-5β-reductase and substrate specificity of the expressed enzyme. *Eur J Biochem* **219:**357–363.

Lin H-K and Penning TM (1995) Cloning, sequencing and functional analysis of the 5′-flanking region of the rat 3α-hydroxysteroid/dihydrodiol dehydrogenase gene. *Cancer Res* **55:**4105–4113.

Lui W-Y, P'eng F-K, Chang Y-F, Chang T-J, Tsai T-F, Hsu M-L, Su T-S, Tsay S-H, Wu C-W, Liu T-Y, and Chi C-W (1993) Analysis of glucocorticoid receptors in human hepatocellular carcinoma and HepG2 cells. *Hepatology* **18:**1167–1173.

Lyttle CR and Komm BS (1984) Steroidal regulation of rat uterine in vitro mRNA translation products. *J Steroid Biochem* **21:**571–577.

Mathis JM, Houser WH., Bresnick E, Cidlowski JA, Hines RN, Prough RA, and Simpson ER (1989) Glucocorticoid regulation of the rat cytochrome P450c (P4501A1) gene: receptor binding within intron I. *Arch Biochem Biophys* **269:**93–105.

Mathis JM, Prough RA, Hines RN, Bresnick E, and Simpson ER (1986) Regulation of cytochrome *P*-450*c* by glucocorticoids and polycyclic aromatic hydrocarbons in cultured fetal rat hepatocytes. *Arch Biochem Biophys* **246:**439–448.

Onishi Y, Noshiro M, Shimosato T, and Okuda K (1994) Molecular cloning and sequence analysis of cDNA encoding Δ^4-3-ketosteroid-5β-reductase of rat liver. *FEBS Lett* **283:**215–218.

Pawlowski JE, Huizinga M, and Penning TM (1991) Cloning and sequencing of the cDNA or rat liver 3α-hydroxysteroid/dihydrodiol dehydrogenase. *J Biol Chem* **266:**8820–8825.

Pawlowski JE and Penning TM (1994) Overexpression and mutagenesis of the cDNA for rat liver 3α-hydroxysteroid/dihydrodiol dehydrogenase: role of cysteines and tyrosines in catalysis. *J Biol Chem* **269:**13502–13510.

Penning TM, Ohnishi ST, Ohnishi T, and Harvey RG (1996) Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon *trans*-dihydrodiols catalyzed by dihydrodiol dehydrogenase. *Chem Res Toxicol* **9:**84–92.

Ruepp B, Bohren KM, and Gabbay KH (1996) Characterization of an element resembling an androgen response element (ARE) in the human aldose reductase promoter, in *Enzymology and Molecular Biology of Carbonyl Metabolism 6* (Weiner H, Lindahl R, Crabb DW, and Flynn TG, eds) pp 515–519, Plenum Publishing, New York.

Scheidereit C, Geisse S, Westphal HM, and Beato M (1983) The glucocorticoid receptor binds to defined nucleotide sequences near the promoter of mouse mammary tumor virus. *Nature (Lond)* **304:**749–752.

Schmid W, Strahle U, Schutz G, Schmitt J, Stunnenberg H (1989) Glucocorticoid receptor binds cooperatively to adjacent recognition sites. *EMBO J* **8:**2257–2263.

Shimada T, Martin MV, Pruess-Schwartz D, Marnett LJ, and Guengerich FP (1989) Roles of individual human cytochrome P-450 enzymes in the bioactivation of benzo(a)pyrene, 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. *Cancer Res* **49:**6304–6312.

Smithgall TE, Harvey RG, and Penning TM (1988) Spectroscopic identification of *ortho*-quinones as the products of polycyclic aromatic *trans*-dihydrodiol oxidation catalyzed by dihydrodiol dehydrogenase. *J Biol Chem* **263:**1814–1820.

Sterling K and Bresnick E (1996) Oct-1 transcription factor is a negative regulator of rat *CYP1A1* expression via an octamer sequence in its negative regulatory element. *Mol Pharmacol* **49:**329–337.

Straviz RT, Vlahevic ZR, Pandak M, Stolz A, and Hylemon PB (1994) Regulation of rat hepatic 3α-hydroxysteroid dehydrogenase *in vivo* and in primary cultures of rat hepatocytes. *J Lipid Res* **35:**239–247.

Tomkins GM (1956) A mammalian liver 3α-hydroxysteroid dehydrogenase. *J Biol Chem* **218:**437–447.

Wieland S, Dobbeling U, and Rusconi S (1991) Interference and synergism of glucocorticoid receptor and octamer factors. *EMBO J* **10:**2513–2521.

Send reprint requests to: Dr. Trevor M. Penning, Dept. of Pharmacology, 130c John Morgan Building, 3620 Hamilton Walk, Philadelphia, PA 19104-6084. E-mail: penning@pharm.med.upenn.edu