Hormonal regulation of hepatic enzymes involved in foreign compound metabolism

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This review in a series addresses the regulation of expression of hepatic cytochrome P4501A1 and other foreign compound metabolizing enzymes in rat liver, including glutathione S-transferase Ya_1 subunit, NAD(P)H:quinone oxidoreductase, aldehyde dehydrogenase class 3, and UDP-glucuronosyltransferase 1*6. All of these genes are regulated by the Ah receptor and in the case of P4501A1, there appears to be functional cooperativity between the glucocorticoid and Ah receptor in transcriptional activation of this gene. Biochemical and molecular biological studies demonstate that these genes are transcriptionally regulated by adrenal steroids, glucocorticoids, but not by the same molecular mechanism. In the case of *CYP1A1* and *UGT1*6*, glucocorticoids potentiate expression of these genes, while the *GSTYa1*, *QOR* and *ALDH3* are negatively regulated by glucocorticoids. Studies utilizing transient transfection of reporter gene constructs containing the 5'-flanking regions of these genes show that specific glucocorticoid responsive elements this region function in their regulation by adrenal steroids.

—Russell A. Prough, Coordinating Editor

ABSTRACT **The regulation of hepatic P450s has been the focus of numerous studies because of the importance of these proteins in endocrinology, oncology, and toxicology, as well as drug development. Considerable evidence exists demonstrating that many hepatic P450s are regulated by developmental, sex, or hormonal factors in addition to receptors that interact with foreign chemicals. The focus of work in our laboratory has been on the effects of steroid hormones, especially glucocorticoids, on expression of genes regulated by the *Ah* receptor. We have shown that most rat hepatic genes of the *Ah* receptor gene battery are regulated by glucocorticoids. We have used glucocorticoid-deficient animal models to demonstrate that these steroids do modulate the expression (basal and inducible) of these genes in vivo. Using cultured rat hepatocytes, we have demonstrated that polycyclic aromatic hydrocarbon (PAH) induction of cytochrome P4501A1, glutathione S-transferase Ya_1, and UDP-glucuronosyltransferase 1*6 are apparently potentiated two- to fourfold upon inclusion of glucocorticoids in the media to activate the glucocorticoid receptor and further, that the receptor antagonist RU 38486 reverses these phenomenon. NAD(P)H:quinone oxidoreductase and aldehyde dehydrogenase 3 gene expression were repressed 70–80% by glucocorticoids in cultured hepatocytes through a glucocorticoid receptor-mediated process as well. The effect of glucocorticoid concentration on PAH induction of glutathione S-transferase Ya_1 subunit for glucocorticoids was biphasic, but at physiological concentrations gene expression was repressed to ~20–40% of control. At supraphysiological concentrations, glucocorticoids alone induced expression two- to threefold and potentiated the PAH-inducible expression of the Ya_1 subunit gene. Subsequent work in our laboratory has focused on defining the molecular basis of this hormonal regulation, specifically elucidating responsive elements responsible for the action of the glucocorticoid receptor and the mechanisms by which some of these genes are positively regulated and others are negatively regulated.—Prough, R. A., Linder, M. W., Pinaire, J. A., Xiao, G.-H., Falkner, K. C. Hormonal regulation of hepatic enzymes involved in foreign compound metabolism. *FASEB J.* 10, 1369–1377 (1996)**

Key Words: Ah receptor · aldehyde dehydrogenase class 3 · cytochrome P4501A1 · glucocorticoid receptor · glutathione S-transferase · NAD(P)H: quinone oxidoreductase

DIVERSE AREAS OF BIOMEDICAL SCIENCES have focused on understanding the pathological effects of foreign chemicals and the mechanisms by which organisms protect themselves from these agents. This information is critical for understanding processes such as drug efficacy, drug

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[2]Abbreviations: ADX, adrenalectomized; *Ah*R, arylhydrocarbon receptor; *ALDH3*, aldehyde dehydrogenase class 3 gene; BA, 1,2-benzanthracene; CAT, chloramphenicol acetyltransferase; DEX, dexamethasone; EROD, 7-ethoxyresorufin *O*-deethylase; GC, glucocorticoids; GST, glutathione *S*-transferase; GR, glucocorticoid receptor; GRE, glucocorticoid-responsive element; 3-MC, 3-methylcholanthrene; PAH, polycyclic aromatic hydrocarbon; QOR, NAD(P)H:quinone oxidoreductase; RU 38486, 17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(prop-1-ynyl)-estra-4,9-dien-3-one; UGT1*6, UDP-glucuronosyltransferase 1*6.

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interaction, chemically induced toxicity and carcinogenicity, oxidative damage, and abnormalities in growth and development due to exposure to foreign chemicals. Central to these areas is the recognition that chemical induction processes control the expression of specific subclasses of genes of xenobiotic metabolizing enzymes and may underlie or ameliorate the processes of carcinogenesis, teratology, and toxicology. Based on this work, three chemical receptor systems have been clearly elucidated at the molecular level, while two others await resolution (**Table 1**).

In parallel with these studies, others have been interested in whether any interplay exists between basal, chemical-dependent, and hormone-dependent regulation of these genes. Many of these enzymes also metabolize endogenous compounds, sterols, fatty acids, and fat-soluble vitamins, and their genes are also under developmental, sex-specific, and hormonal regulation. In this regard, the hemoproteins that catalyze steroid degradation have been most intensively studied and the area has been succinctly reviewed by Waxman (13). A major role for androgens and growth hormone has been established for *CYP*2A2 and 3A2 expression, as well as the unique observation of neonatal imprinting of *CYP*2C in immature male rats (14–17). In addition, other hormonal factors have been shown to be involved in the regulation of many hepatic P450s of the 2A, B, C, and E families. For example, Waxman and co-workers (14, 18, 19) have demonstrated the role of thyroid hormone in expression of *CYP*2B, *CYP*3A2, and NADPH:cytochrome P450 oxidoreductase. Other hormones or their analogs have been shown to affect expression of these genes including androgens, estrogens, glucocorticoids (GC),[2] insulin, and interleukins (20).

A key environmental health issue that led us to study the effects of glucocorticoids on the *Ah* gene battery was the observation that fetuses and children of mothers who smoke or are exposed to polycyclic aromatic compounds manifest more serious developmental or pathological problems during gestation and neonatal development than those of unexposed mothers (21). Based on the literature and our initial studies of glucocorticoid regulation in cell culture, we developed a hypothesis that there may be interplay between the hormonal and xenobiotic induction mechanisms that might account for some of the gender or developmental differences in expression of foreign compound metabolizing enzymes. These differences may affect the pathophysiological outcomes observed when mammals are exposed to foreign chemicals.

MODULATION OF EXPRESSION OF FOREIGN COMPOUND METABOLIZING ENZYMES BY ADRENAL STEROIDS

A long-standing interest of our laboratory has been the observation that glucocorticoids play a role in determining the levels of expression of genes regulated by the arylhydrocarbon receptor (*Ah*R). In the rat and human, there are characteristic patterns of the expression of the adrenal steroids, particularly glucocorticoids, during life. This is clearly seen from the data taken from Greengard (22, Fig. 1 therein). Circulating glucocorticoid levels are very low in the fetal rat in early gestation, and the fetal adrenal begins production of GC just before birth (day 18 of gestation). At birth, high levels of circulating GC are seen in the rat; by day 6 in the neonate, circulating levels decrease to nearly the lowest levels seen in ex vivo life. Circulating levels of GC in blood increase approaching adolescence and are relatively constant or decline slightly throughout the remainder of the life of the animal. One significant difference in the human is that the fetal human adrenal begins production of adrenal steroids at about the beginning of the second trimester of gestation (23). Therefore, one might expect to see differences in expression of the hepatic cytochromes P450 in fetal states between rodents and humans if adrenal steroids play a role in their regulation.

In collaboration with Simpson's group, we initiated studies to address whether glucocorticoids affect the induction of P4501A1 protein in cultured human and rat fetal hepatocytes (24–26). Our recent studies also demonstrated a synergism or potentiation of polycyclic aromatic hydrocarbon (PAH) -inducible enzyme activity associated with *CYP*1A1 by glucocorticoids in cultured adult rat hepatocytes using defined media lacking glucocorticoids (**Table 2**). That is, although GC alone have little or no effect on the basal expression of *CYP*1A1 in cultured rat hepatocytes, the presence of GC significantly enhanced PAH-dependent induction of the gene product

TABLE 1. *Mechanisms for induction of foreign compound metabolizing enzymes in rat liver by chemicals*

Chemical receptor system	Genes regulated	Citation
Arylhydrocarbon receptor	*CYP*1A1, *GST Ya1*, *QOR*, *UGT1*6*, *ALDH3*	(1, 2)
Antioxidant response element receptor	*GST Ya1*, *QOR*	(3, 4)
Peroxisome proliferator-activated receptor	*CYP*4A1, 4A2, 4A3, *P450 OR*, peroxisomal β-oxidation	(5–7)
Phenobarbital receptor?	*CYP*2B1/2, *P450 OR*, epoxide hydratase	(8, 9)
Nonclassical glucocorticoid receptor?	*CYP*3A, *CYP*2B, *GST Ya1*, *UGT1*6*	(10–12)

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TABLE 2. *Effect of dexamethasone on PAH induction of various members of the Ah gene battery in cultured adult rat hepatocytes*

Condition[a]	P4501A1[b]	GST Ya1[c]	UGT1*6[b]	QOR[c]	ALDH3[c]
Control	1.1 ± 0.1	88 ± 8	75 ± 6	99 ± 5	0.6 ± 0.2
DEX	1.0 ± 0.1	85 ± 1	112 ± 21	84 ± 1	1.0 ± 0.3
BA	64 ± 1.3	192 ± 28	570 ± 35	1130 ± 100	20.7 ± 0.9
BA + DEX	138 ± 11	305 ± 19	980 ± 230	610 ± 60	4.1 ± 0.3

[a]Adult hepatocytes were prepared as described by Xiao et al. (11) and treated with either DMSO, 50 μM 1,2-benzanthracene (BA), 1 μM dexamethasone (DEX), or a combination of these agents. The cell protein was harvested after 2–3 days and the enzyme activity measured for three pooled sets of cells. [b]The enzyme activity is expressed as pmol product produced/(min·mg cell protein) ±SD. [c]The enzyme activity is expressed as nmol product produced/(min·mg cell protein) ± SD.

P4501A1. 1,2-Benzanthracene (BA) caused an 64-fold induction of 7-ethoxyresorufin *O*-deethylase (EROD) activity in the absence of added glucocorticoids, but the level of EROD activity was at least two- to threefold higher when the cells were treated with BA plus dexamethasone (Table 2). Further, experiments were performed to demonstrate whether this potentiation is observed when other PAH are used, including β-naphthoflavone (27), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 11), and 2,3,7,8-tetrachlorodibenzofuran (TCDF, 28). All ligands of the arylhydrocarbon receptor *AhR* tested displayed this same two- to threefold potentiation of PAH induction of P4501A1 by GC. In addition, the potentiation effect of glucocorticoids demonstrated the rank order of potency of GC that one would expect if a classical glucocorticoid receptor mechanism were functioning; i.e., only sterols of the glucocortocoid class caused this potentiation, with dexamethasone being more potent than cortisol or corticosterone (26, 27). Finally, antagonists of the action of the glucocorticoid receptor prevented this potentiation of PAH induction of P4501A1 by glucocorticoids (11, 25).

PAH induction of several other genes under regulatory control of the *Ah* receptor was also positively modulated by 1 μM concentrations of dexamethasone (DEX); namely, glutathione (GST) *S*-transferase Ya1 subunit and UDP-glucuronosyltransferase 1*6 (*UGT1*6*) genes (Table 2). Surprisingly, two other genes regulated by the *Ah*R receptor, NAD(P)H:quinone oxidoreductase (*QOR*) and aldehyde dehydrogenase 3 (*ALDH-3*), were negatively regulated by dexamethasone upon induction by 1,2-benzanthracene. These results demonstrate that both positive and negative regulation of PAH induction of these genes occurs in the presence of glucocorticoids; the differences in responses suggest that glucocorticoids must function by mechanisms that are not necessarily common for all of these genes.

When studying the concentration dependence for DEX potentiation of PAH induction of GST Ya1 and QOR in cultured adult rat hepatocytes using Northern analysis, we observed that expression of *CYP*1A1 displayed a relatively monotonic concentration response to DEX (25, 27). However, there was a pronounced biphasic response for GST Ya1 and QOR (**Fig. 1**). At concentrations below 1 × 10^{-7} M DEX, repression of basal expression of both *QOR* and *GST Ya1* specific mRNAs was observed, but GST Ya1 and QOR mRNA levels were increased at higher concentrations of GC (1 × 10^{-6} to 1 × 10^{-4} M) to levels higher than control (three- to fourfold). This biphasic effect was seen in both basal and PAH-induced expression of the *GST Ya1* gene (data not shown). The suppression of *QOR* expression at lower concentrations of GC were reversed by inclusion of 17β-hydroxy-11β-(4-dimethylaminophenyl)-17α-(prop-1-ynyl)-estra-4,9-dien-3-one (RU 38486), the potent glucocorticoid receptor (GR) antagonist, in the culture media suggesting that the effect at low concentration requires function of the glucocorticoid receptor (11). RU 38486 itself induced *GST Ya1* expression, suggesting a nonclassical GC response at the high concentration (10 μM) required for RU 38486 to function as an antagonist of GC potentiation of P4501A1 and QOR induction (data not shown). High concentrations of GC

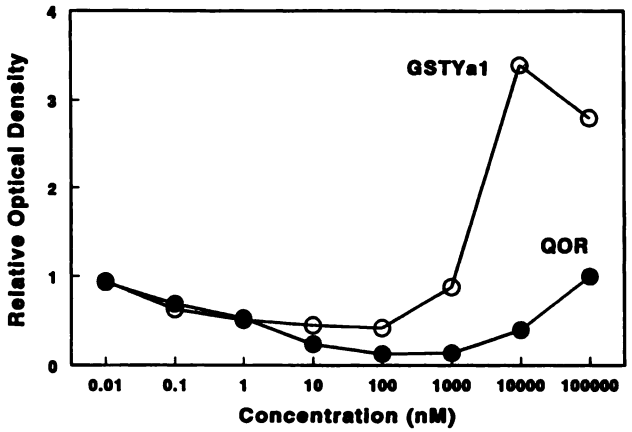

Figure 1. Effect of dexamethasone concentration on mRNA levels for glutathione *S*-transferase Ya1 and NAD(P)H:quinone oxidoreductase subunit genes in cultured adult rat hepatocytes treated with 1,2-benzanthracene. Cells were prepared and cultured as described by Xiao et al. (11). Various concentrations of dexamethasone were included in the media; cellular mRNA harvested 72 h after addition of BA. Northern analysis of mRNAs for GST Ya1 and QOR were performed; the optical densities of relevant bands on the autoradiograms corresponding to the hybridization complex were measured and normalized with the optical densities obtained from the autoradiograms relative to the loading control, β-actin.

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cause QOR mRNA levels to return to control levels. These results suggest that like P4502B and 3A, inclusion of high concentrations of glucocorticoids in the culture media resulted in a nonclassical induction of GST Ya_1 and QOR, but not P4501A1.

PROOF THAT GLUCOCORTICOIDS REGULATE PAH INDUCTION OF THESE GENES IN VIVO

Experiments with intact animals were performed to establish whether glucocorticoid regulation of PAH induction occurs in vivo. Two models were used that took advantage of either surgical adrenalectomy or the natural variations in the levels of circulating levels of glucocorticoids observed during the life of the rodent. In the first model (29), we obtained adrenalectomized (ADX) rats (150–200 g) and maintained them on standard chow and 0.9% saline in their drinking water. Four groups of animals were used; sham injection with corn oil or injection with either 10 mg/kg dexamethasone (DEX) in corn oil, 10 mg/kg 3-methylcholanthrene (3-MC) in corn oil, or both DEX and 3-MC in corn oil. Maximal induction by 3-MC was observed between 24 and 48 h. As seen in **Fig. 2**, we measured the levels of EROD activity in the livers of sham-operated and ADX animals. As expected in the sham and ADX animals, tyrosine aminotransferase activity, reflective of a known GC-responsive gene, was increased four- to sevenfold in livers of rats treated 2 days with DEX or BA + DEX (data not shown). DEX alone had little or no effect on EROD activity in livers of sham-operated or ADX rats, but 3-MC increased the levels of EROD activity by 20- to 30-fold, as expected. However, ADX rats made replete in GC by administration of DEX demonstrated an approximately threefold greater level of EROD activity relative to animals treated with 3-MC alone. A less profound increase was seen in sham animals (1.9-fold increase over levels seen in 3-MC treated animals), suggesting that in these animals, circulating levels of glucocorticoids may not be sufficiently high to saturate the GC potentiation phenomenon. Western immunoblotting confirmed that these increases in EROD activity were the result of increased levels of P4501A1 protein (data not shown; 29).

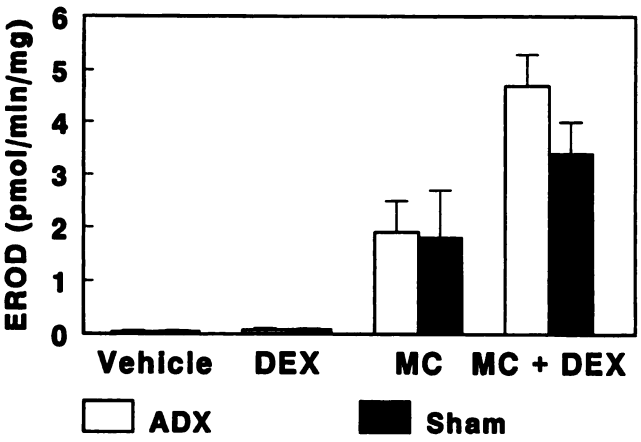

Figure 2. The effect of adrenalectomy on PAH induction of liver microsomal ethoxyresorufin *O*-deethylase activity. Male Sprague-Dawley rats (150–200 g) were obtained that had either been adrenalectomized (ADX) or sham operated. The animals were maintained on saline drinking water and injected daily with vehicle (corn oil) or the chemical shown. The enzyme activities of livers obtained at 48 h treatment are expressed as nmol product/(min·mg protein) ±SD and were taken from the work of Sherratt et al. (29).

In the second model, we used the natural variations in the levels of circulating GC observed during development to test whether higher levels of glucocorticoids would enhance the inductive process for CYP1A1 and other genes regulated by the *Ah* receptor (30). The mRNA levels for rat hepatic P4501A1 were 10- to 15-fold lower in neonatal animals than in adolescent rats after induction by MC (**Fig. 3**). However, administration of DEX (25 mg/kg body wt) caused a further 10-fold induction of P4501A1 mRNA levels in neonatal rats (where circulating GC levels were low), whereas there was no further increase in hepatic CYP1A1 mRNA levels in the adolescent rat. These results were nearly identical to the fold increases seen in P4501A1 protein levels and nuclear run-on transcriptional assays (30). Taken together, these two animal models demonstrate that adrenalectomy or low circulating concentrations of GC result in diminished levels of induction of P4501A1 by the PAH, but readministration of a synthetic glucocorticoid to the GC-deficient animal caused the levels of P4501A1 enzyme activity, protein, and mRNA to be as high as those seen in the normal animal. Further, the studies in neonatal animals demonstrate that during periods when circulating glucocorticoids are low, maximal inducibility is not attained unless additional GC is administered. However, maximal levels of induced P4501A1 protein were not significantly affected when GC-sufficient conditions existed during adolescence, suggesting that the circulating levels of GC in the adolescent rat are sufficient to largely saturate the response to glucocorticoids and possibly to ligand-activation of GR. However, circulating levels of GC in the adult (Fig. 2) or neonatal (Fig. 3) rat are not sufficient to saturate the response.

MOLECULAR MECHANISMS TO ACCOUNT FOR GLUCOCORTICOID ACTION IN REGULATING PAH INDUCTION OF THE *Ah* GENE BATTERY

To establish the mechanism by which glucocorticoids secondarily regulate the induction of these genes by PAH, we used information in the GenBank nucleic acid databank to identify any putative glucocorticoid responsive elements in the genes of interest. In the case of the rat *CYP*1A1, *GSTYa*$_1$, *QOR*, and aldehyde dehydrogenase class 3 gene (*ALDH3*) genes, significant sequence data are available for the 5′ flanking regions of these genes and some information exists for their intronic sequences. Initially, we looked for canonical glucocorticoid-respon-

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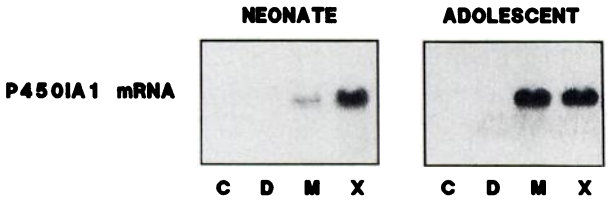

Figure 3. Northern analysis of hepatic cytochrome P4501A1 mRNA levels in adolescent and neonatal male rats. Total hepatic RNA (20 μg) from adolescent or neonatal rats was resolved in a denaturing agarose gel and transferred to a Zeta-probe membrane. The level of P450 mRNA was determined by hybridization with a [^{32}P]-radiolabeled 653 bp Pst I fragment of plasmid pA8 and visualized by autoradiography. Equivalent loading of samples was assessed by ethidium bromide staining. The relative abundance of the P4501A1 mRNA in each of treatment group was measured using a BioRad model 620 videodensitometer. C, control (corn oil treatment); D, dexamethasone treatment (10 mg/kg body wt daily for 4 days); M, 3-methylcholanthrene treatment (10 mg/kg body wt daily for 4 days); or X, combined treatment with dexamethasone and 3-methylcholanthrene.

sive elements (GRE half-site: TGT-C/T-CT) in these genes to study whether they may be under direct control of the ligand-activated glucocorticoid receptor.

Although the rat *CYP*1A1 gene does not contain any easily discernable GREs in its 5′ flanking region, Hines et al. (31) sequenced the first intron of that gene, which is 2451 bp in length, and there are three GREs within a 367 basepair fragment (XbaI/Sau3a) of the intron. The first exon of the *CYP*1A1 gene is noncoding, with the translation initiation site being located in exon II. The intronic GRE sequences are conserved between the human, murine, and rat *CYP*1A1. In collaboration with Pickett and Rushmore (3), we found similar responsive elements in the glutathione *S*-transferase Ya_1 gene. At position -1606 in the 5′ flanking region, there is a near perfect consensus palindromic GRE and four other GRE half-sites (bp positions -1537, -1524, -1361, -1063, -546). Similarly, we evaluated the rat aldehyde dehydrogenase 3 gene (32, 33) and have observed a palindromic GRE at position -1166 bp in the 5′ flanking region of the gene. Similar attempts to identify GREs in the known sequence of *QOR* gene have not demonstrated existence of these elements, but little intronic sequence information for *QOR* introns have been obtained to date.

The presence of putative GREs in the 5′ flanking or intronic sequences for some of these genes directed us to establish whether these elements can function to bind GR and serve as enhancers in their own right. Many genes contain GRE-like sequences, but the literature on GR regulation of genes has demonstrated that in order for genes to be regulated solely by GR itself, they must have multiple GREs, including at least one palindromic GRE. For example, the tyrosine aminotransferase gene contains 3 GREs, two of which are palindromic. Jantzen et al. (34) have demonstrated that the two palindromic GREs are minimally required to allow GR-dependent regulation of the tyrosine aminotransferase gene. Other mechanisms of gene expression have been demonstrated, including regulation of specific transcription factors whose expression occurs later in time than activation of the GR (35) or interaction with other transcription factors, such as *fos/jun*, which bind to AP1 sites on genes (36). Therefore, our studies have focused on whether direct interaction of the GR with the GREs can occur and whether these GREs are functionally active as enhancers.

EXAMINATION OF THE INTRONIC GREs OF THE RAT *CYP*1A1 GENE

Double-stranded oligonucleotides were generated that corresponded to each of the three GREs (GREI, GREII, and GREIII) found in the first intron of the *CYP*1A1 gene (31). Electrophoretic mobility shift assays were subsequently performed to demonstrate whether DNA-protein complexes could be observed using either rat hepatic nuclear extract or partially purified preparations of human glucocorticoid receptor (**Fig. 4**). Using a *Baculla virus*-expressed human GR (≥70% pure), the second intronic

Figure 4. Electrophoretic mobility shift analysis of intronic GREs of *CYP*1A1 with human glucocorticoid receptor. Radiolabeled *CYP*1A1 GREII oligonucleotide, lanes 1 through 5, was incubated with 500 ng of partially purified human glucocorticoid receptor (hGR) in the absence or presence of 100-fold excess of nonradiolabeled oligonucleotide competitors, MMTV, GREII and XRE. The protein-DNA complexes were resolved on 4% polyacrylamide gels in Tris-acetate buffer and transferred to filter papers, dried, and exposed to X-ray film.

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GRE of *CYP*1A1 (GREII) formed DNA-protein complexes (Fig. 4). Specificity of the DNA-protein complexes was demonstrated by performing the assays in the presence of nonradiolabeled GREII or the well-characterized palindromic GRE found within the LTR of the mouse mammary tumor virus (37). *CYP*1A1 intronic GREI and III also competed for DNA-protein complex formation (data not shown). An oligonucleotide that contains the sequences corresponding to the proximal xenobiotic response element sequence of the murine *CYP*1A1 gene served as a negative control for these experiments; it had little or no effect on DNA-protein complex formation by GREII. Subsequent experiments were performed with rat hepatic nuclear extracts which formed an DNA-protein complex whose migration was retarded to the same extent as the complexes formed using rat nuclear extract (data not shown). DNA-rat nuclear protein complex formation was also competed for by the MMTV GRE-containing oligonucleotide, but not by the *CYP*1A1 XRE-containing oligonucleotide.

To test whether the interaction of GR with the putative glucocorticoid responsive elements of the *CYP*1A1 intron, chimeric reporter gene constructs were prepared by ligation of the 384 bp Xba1 fragment containing the three GRE half-sites into the chloramphenicol acetyltransferase (CAT) expression vector pBLCAT2, which contains an SV-40 promoter. This construct was named pBLCAT1A1. Human hepatoma (HepG2) cells were used to test the ability of various glucocorticoid-responsive elements from the MMTV long-terminal repeats and *CYP*1A1 intron I. The expression plasmid for the human GR was cotransfected, because we could not obtain effective glucocorticoid responsiveness of these cells alone.

Cells transfected with the plasmid containing the intronic GREs of *CYP*1A1 as a promotor, pBLCAT1A1, expressed only basal CAT activity in the absence or presence of 1 μM dexamethasone at the level observed for the parent vector pBLCAT2 (data not shown). The negative control vector pBLCAT2, which contains no GRE sequences, was also transfected in a separate experiment and its basal expression was also shown not to be affected by dexamethasone. When cells cotransfected with plasmids expressing human GR and pMMTV-CAT, which contains the palindromic GRE and three GRE half-sites from the mouse mammary tumor virus long-terminal repeats, were cultured in the presence of DEX, there was a 14-fold induction of CAT expression with the MMTV-GRE CAT construct. Based on these experiments we conclude that the putative glucocorticoid half-site response element sequences identified within the first intron of the rat *CYP*1A1 gene, especially *CYP*1A1-GREII, are able to form a DNA-protein complex with GR, but do not function alone as a glucocorticoid receptor-dependent enhancers as observed with pMMTV-CAT.

Mathis et al. (26) demonstrated that a CAT construct containing 1 kb of 5′ flanking region, exon I, intron I, and a small portion of exon II of the *CYP*1A1 gene was PAH inducible in cultured HepG2 cells. This construct was responsive to inclusion of DEX in the culture media, demonstrating that we could recapitulate the GC potentiation of PAH induction of P4501A1 using CAT constructs containing portions of the 5′ flanking region, exon I, and intron I of *CYP*1A1 gene. When Mathis (26) prepared a construct with the XbaI fragment deleted (which contains the three intronic GREs), GC responsiveness was lost. These results demonstrate that GC potentiation of PAH induction of expression of *CYP*1A1 is apparently dependent on the intronic GRE half-site sequences, but the construct, like the native gene, is apparently unaffected by glucocorticoids alone. Normal expression of *CYP*1A1 is very low for the native gene or reporter constructs, making reliable measurement of the effect of GC on basal expression impossible.

EXAMINATION OF THE GREs OF THE RAT *GSTYa*$_1$ GENE

As an approach to study the putative function of the palindromic and half-site GREs in the 5′ flanking region of the rat *GST Ya*$_1$ gene, we used a CAT construct obtained from Rushmore et al. (3) containing more than 1600 bp of 5′ flanking region (1.6YaCAT); the expression of this reporter gene was strikingly negatively regulated over 60% (**Fig. 5*A***). We subsequently prepared a second construct (MUT1.6YaCAT), which contained a mutated palindromic GRE (AGAACA changed to AGCACG), that was shown to be unaffected by inclusion of dexamethasone in the media (Fig. 5*A*). As expected for a GR-related process, RU38486, the glucocorticoid antagonist, blunted this negative regulation of the full-length *GST Ya*$_1$ CAT construct (data not shown), strongly suggesting that this phenomenon is due to action of the ligand-activated GR binding to its consensus sequence.

To establish whether the palindromic GREs function independently as other reported GREs, we prepared constructs containing the minimal promoter of the *GST Ya*$_1$ gene linked with multiple copies (21 mers) of the palindromic GRE of this gene. When three copies of the palindromic GRE were included in the construct (Fig. 5*B*), the reporter gene was positively regulated five- to sixfold by the glucocorticoid DEX in HepG2 cells cotransfected with a plasmid expressing the human GR. These results clearly demonstrate that when only the palindromic GRE in multiple copy is included in the reporter gene, it functions exactly like other such GRE-containing constructs. However, in the context of the native 5′ flanking region, the palindromic GRE apparently serves as a negative regulatory element. Studies are in progress to define other *cis*-acting elements that must mediate the negative regulation of this gene.

EXAMINATION OF THE GREs OF THE RAT *ALDH3* GENE

In a similar manner, we have prepared several CAT constructs of the 5′ flanking region of the *ALDH3* gene, one of which contains a mutated GRE instead of the native

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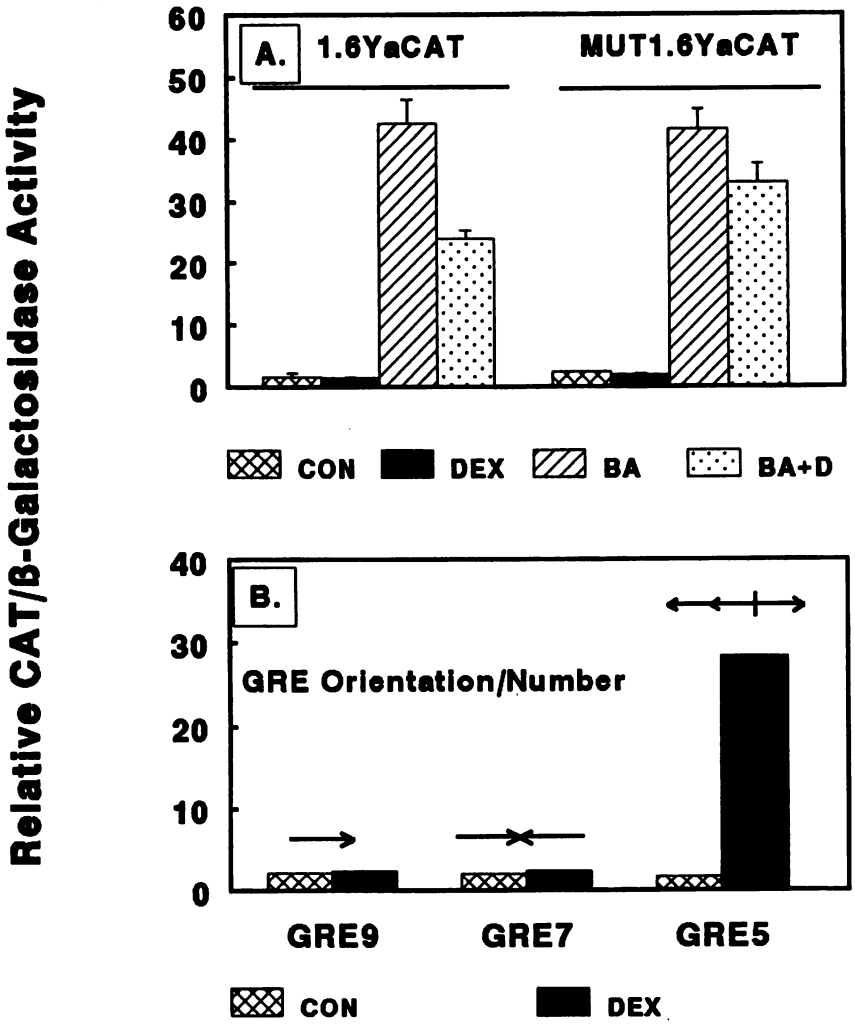

Figure 5. Effect of glucocorticoids on chloramphenicol acetyltransferase reporter constructs containing portions of the 5′ flanking region of the rat glutathione *S*-transferase Ya_1 subunit gene. Constructs were prepared containing either 1.6 kb of the 5′ flanking region of the GST Ya_1 subunit gene or various numbers of 21mer oligonucleotides identical to the palindromic GRE of the GST Ya_1 5′ flanking region. The calcium phosphate method was used to transfect 10 μg quantities of plasmid DNA into HepG2 cells using standard culture techniques. After changing media, the cells were cultured for 24 h and cellular protein was isolated to measure CAT and β-galactosidase activity. The activity (% conversion) for the constructs was normalized to β-galactosidase cotransfected activity. *A)* Effect of conservative mutation of the palindromic GRE at position -1606 bp in the 5′ flanking region of the GST Ya_1 subunit gene. *B)* Effect of number of palindromic GREs on glucocorticoid responsiveness (arrow denotes the orientation and number of the palindromic GREs relative to the normal sequence)

palindromic GRE. As seen in **Fig. 6**, DEX strikingly represses the expression of the wild type CAT construct in the same manner as that seen with the native gene in Table 1 (11). Conservative mutation of the palindromic GRE (AGGACA changed to ACGGCA) in the 5′ flanking region of the ALDH3 CAT construct significantly ameliorated the negative regulation of this construct, demonstrating that this palindromic GRE must function to alter the expression of the reporter gene (Fig. 6). In addition, the GR antagonist RU-38486 blunts this negative induction with transfected reporter genes in HepG2 cells (data not shown). The *ALDH3* gene is poorly expressed in cultured rat hepatocytes or in vivo in the absence of added PAH, but we have noted repression of the basal expression of the *ALDH3* reporter gene by DEX in the absence of PAH using HepG2 cells, which suggests that the action of glucocorticoids may be independent of action of the *Ah*R. Low basal expression of the *ALDH3* gene most likely is due to the presence of tissue-specific regulation, as this gene is expressed in high level in corneal tissue or in liver and other tissues when animals are treated with PAH (38). Our results are clearly in concord with

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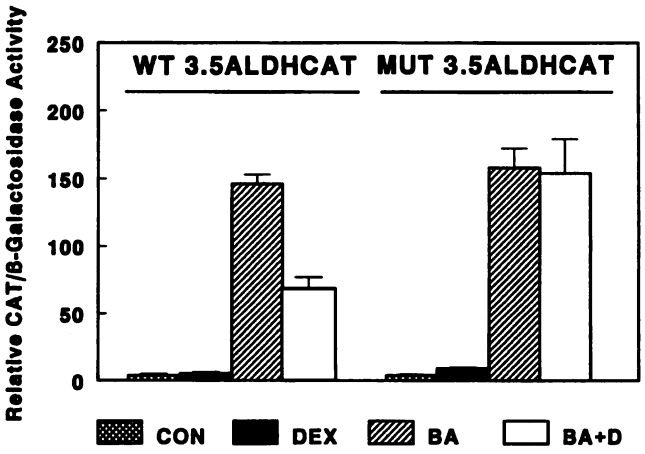

Figure 6. Effect of glucocorticoids on wild-type and GRE-mutated chloramphenicol acetyltransferase reporter constructs containing the 5′ flanking region of the rat aldehyde dehydrogenase 3 gene. Constructs were prepared containing ~3.5 kb of the 5′ flanking region of the rat aldehyde dehydrogenase 3 gene and the plasmids were transfected as described in Fig. 5. The activity (% conversion) for the constructs was normalized to cotransfected β-galactosidase activity.

the effects noted on regulation of the native gene in cultured rat hepatocytes (Table 1; ref 11): that glucocorticoids decrease the expression of the *ALDH3* gene.

CONCLUSION

The studies we have performed indicate that at least three genes of the *Ah* gene battery are under *direct* control of glucocorticoid receptor. In the case of rat *CYP*1A1 gene, there is almost no expression of this gene in cultured cells or animals not treated with PAH; therefore, it is difficult to ascertain whether there is any effect of glucocorticoids on basal expression of the gene. It is clear that the *CYP*1A1 intronic GREs do bind GR, but they do not serve as enhancers in their own right. However, deletion of these intronic GRE half-sites does result in loss of GC potentiation of PAH induction of the requisite reporter gene. Both basal and PAH-inducible expression of the rat *GST* Ya_1 and *ALDH3* genes are negatively regulated at concentrations of glucocorticoids required to maximally activate the GR (≤1 μM DEX). For the GST Ya_1 gene, high concentrations of glucocorticoids (≥1 μM DEX) also serve to potentiate the induction of the gene by PAH, a response also seen for *CYP*1A1 and *UGT1*6* in cultured rat hepatocytes. The palindromic GRE from the 5′ flanking region of the *GST* Ya_1 functions as an enhancer when present in multiple copies in reporter gene constructs, demonstrating that they not only bind GR, but also function independently as enhancer elements as reported for other GREs from other GC-regulated genes.

Our studies suggest that glucocorticoids clearly regulate the genes of the *Ah* gene battery and may play a role in the basal expression of these genes throughout development. As shown by Abrahamovitz and Listkowski (39), there are natural variations in expression of the rat *GST* Ya_1 gene during development that may reflect, in part, the variations in circulating levels of blood GC in the fetal, neonatal, and adult rat (22). Other regulatory mechanisms may also regulate these genes, such as protein phosphorylation through protein kinase A or C, leukotrienes, etc., and may have pronounced roles in the expression of these genes throughout the life of mammals. A major question to address is why palindromic GREs, which respond positively to GC when isolated from the gene, act as negative regulatory elements in the native gene. Clearly, other *cis*- and *trans*-acting factors must function in these genes. A second question is the mechanism by which the *glutathione* S-*transferase* Ya_1 and *NAD(P)H:quinone oxidoreductase* genes are positively modulated by GC in neonatal liver and negatively in adolescent or adult rat liver (30). Our laboratory is attempting to define the molecular mechanism for these phenomena. FJ

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