Regulation of Aryl Hydrocarbon Receptor Expression and Function by Glucocorticoids in Mouse Hepatoma Cells

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ABSTRACT:

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates most biological responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related aromatic hydrocarbons. Although the role of the AHR in control of drug metabolism and endocrine disruption is partly understood, we know little about the regulation of the AHR itself by endocrine factors. Our work with hypophysectomized rats suggested that hepatic AHR protein level is positively regulated by pituitary-dependent factors. A current hypothesis is that adrenal glucocorticoids elevate AHR expression and enhance responsiveness to AHR agonists. Dexamethasone (DEX) at concentrations that activate the glucocorticoid receptor (GR) increased AHR mRNA, protein, and TCDD-binding by approximately 50% in Hepa-1 mouse hepatoma cells. This response was blocked by the GR antagonist 17β-hydroxy-11β-[4-dimethylamino phenyl]-17α-[1-propynyl]estra-4,9-dien-3-one (RU486), suggesting GR involvement. This small magnitude increase in AHR levels was functionally significant; pretreatment of Hepa-1 cells with DEX caused a 75% increase in the maximum induction of an AHR-activated luciferase reporter plasmid by TCDD. A luciferase reporter under control of the proximal 2.5 kilobases of the mouse Ahr promoter region and promoter was induced approximately 2.5-fold by DEX when cotransfected with a mouse GR expression plasmid. This is the first demonstration that glucocorticoids increase AHR levels in hepatoma cells via a GR-dependent transcriptional mechanism, suggesting a novel aspect of cross-talk between the AHR and the GR.

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates most biological responses to halogenated aromatic hydrocarbons (HAHs) such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polycyclic aromatic hydrocarbons (PAHs) such as 3-methylcholanthrene (MC). The most fully understood AHR-mediated biological response is the induction of cytochrome P450 genes belonging to the CYP1A subfamily. Binding of ligand to the cytoplasmic AHR complex triggers the translocation of the receptor into the nucleus, dimerization with the AHR nuclear translocator, and binding of the AHR·nuclear translocator heterodimer to dioxin-responsive elements (DREs) in regulatory regions of genes subject to transcriptional up-regulation such as CYP1A1 (Riddick et al., 1994). Although the role of the AHR in control of drug metabolism and endocrine disruption is partly understood (Safe, 1995), we know little about the regulation of the AHR itself by endocrine factors (Harper et al., 2006).

Our interest in endocrine control of AHR expression and function was stimulated by our observation that hypophysectomy results in a significant loss of hepatic AHR protein and TCDD-binding capacity in male rats without decreasing CYP1A1 induction in response to MC treatment (Timsit et al., 2002). This finding suggested that pituitary hormones and/or pituitary-dependent factors may act as positive regulators of hepatic AHR levels and aromatic hydrocarbon responsiveness. We are interested in identifying the pituitary-dependent factors that modulate hepatic AHR expression and function and determining the molecular mechanisms by which they act.

Our initial focus has been on adrenal glucocorticoids as potential candidates for the pituitary-dependent endocrine factors involved in modulation of AHR levels and activity. Several lines of evidence suggest that glucocorticoids can augment aromatic hydrocarbon responsiveness. Elevated levels of endogenous corticosterone (CORT) induced by stress result in enhanced induction of hepatic CYP1A1-catalyzed 7-ethoxyresorufin O-deethylation (EROD) in response to PAH exposure in rats (Konstandi et al., 2000). The in vivo induction of rat hepatic CYP1A1 and/or associated catalytic activities by MC is potentiated by the synthetic glucocorticoid dexamethasone (DEX) (Sherratt et al., 1989) and diminished by adenectomy (Nebert and Gelboin, 1969). Cell culture studies in PLHC-1 fish hepatocellular carcinoma cells (Celander et al., 1996) and H4IIE rat hepatoma cells (Lai et al., 2004) confirm that glucocorticoids enhance HAH- and/or PAH-dependent CYP1A induction. The ability of glucocorticoids to enhance the induction of CYP1A1 by PAHs is mediated, at least in part, by the presence of functional glucocorticoid-responsive elements.
(GREs) in the first intron of the rat CYP1A1 gene (Mathis et al., 1989). The enhancement of CYP1A inducibility by glucocorticoids may also be mediated in part by alterations in AHR protein levels. Treatment of H4IIIE rat hepatoma cells with DEX resulted in increased binding of TCDD to the cytosolic AHR (Wiebel and Cikryt, 1990). Treatment of pregnant mice with cortisol yielded offspring with elevated levels of AHR mRNA and protein in craniofacial tissue (Abbott et al., 1994).

However, other lines of evidence do not support this positive influence of glucocorticoids on the AHR system and aromatic hydrocarbon responsiveness. Treatment of rat mammary fibroblasts with DEX resulted in a decrease in AHR protein levels (Brake et al., 1998). Adrenalectomy in male rats was reported to have no effect on the TCDD-binding capacity of the hepatic AHR as measured by isoelectric focusing (Carlstedt-Duke et al., 1979). Finally, adrenalectomized rats are highly sensitive to TCDD-induced lethality, and this toxic response can be ameliorated by CORT treatment (Gorski et al., 1988); however, modulation of lethality by adrenal steroids could occur via events downstream of the AHR. Although there are certainly effects of glucocorticoids on the AHR pathway, these effects appear to be complex and the molecular mechanisms and functional impacts remain poorly understood.

The goals of the present study were to determine whether glucocorticoids modulate AHR expression and function in Hepa-1 mouse hepatoma cells and to explore the molecular mechanisms involved. Hepa-1 cells express abundant levels of AHR protein and are highly responsive to TCDD and MC treatment (Riddick et al., 1994). These cells also possess a functional GR signaling pathway (Cuthill et al., 1987; Prokipcek and Okey, 1988), making them an excellent cell culture model to examine the influences of glucocorticoids on AHR expression and function.

Materials and Methods

Cell Culture. The Hepa-1c1c7 mouse hepatoma cell line was obtained from the American Type Culture Collection (Manassas, VA). Cells were grown as monolayer cultures in α-minimum essential medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and maintained in an atmosphere of 5% CO2 and 95% air at 37°C. According to technical data provided by Invitrogen, fetal bovine serum (Invitrogen, Carlsbad, CA) and maintained in an atmosphere of 5% CO2 and 95% air at 37°C. According to technical data provided by Invitrogen, our culture conditions resulted in cells being exposed to a combination of DEX (0.1, 1, and 10 μM), a 1.6 mM concentration of each 2′-deoxyguanosine 5′-triphosphate, and 1× PCR buffer containing 20 μM Tris-50 mM KCl-3 mM MgCl2. Reactions were allowed to proceed for 60 min at 37°C, followed by incubation at 70°C for 10 min.

PCR primers were synthesized by AGCT Corporation (Toronto, ON, Canada) or Integrated DNA Technologies, Inc. (Coralville, IA). The specificity of primers against the mouse genome was confirmed by BLAST search (www.ncbi.nlm.nih.gov/BLAST/) and by Primer-UniGene Selectivity analysis (Boutros and Okey, 2004). PCR primer sequences were as follows: mouse AHR, 5′-GGTGCCTGTTGATATCACCTGTG-3′ (forward primer) and 5′-TCTCCCCCTCCATTGCGATCGT-3′ (reverse primer) (Giannone et al., 1998); mouse β-actin, 5′-CTACAAGAGCTGCGTGTGG-3′ (forward primer) and 5′-TAGCTTCTTCGACGGA3A-3′ (reverse primer) (Giannone et al., 1998); and mouse tyrosine aminotransferase (TAT), 5′-GCAATCTCGTGGAGAATGTGCTG-3′ (forward primer) and 5′-TCTGAGGTCGCGTCTACT-3′ (reverse primer) (Sakuma et al., 2004).

All PCR assays began with a hot start phase, typically 3 or 5 min at 94°C and 95°C and ended with a final extension phase, typically 7 min at 72°C. Each 50-μl PCR sample contained input cDNA derived from 50 or 75 ng of RNA, 10 μl of 2×PCR buffer containing 1× PCR buffer containing 20 μM Tris-50 mM KCl-3 mM MgCl2. Cycling conditions were as follows: AHR and β-actin duplex reaction (95°C for 20 s, 58°C for 20 s, and 72°C for 40 s) for 19 to 21 cycles; TAT (94°C for 30 s, 53°C for 30 s and 72°C for 40 s) for 28 to 35 cycles. Amplified PCR products (AHR, 503 bp; β-actin, 450 bp; and TAT, 230 bp) were separated on 6% polyacrylamide gels, stained with Vistra Green (GE Healthcare Bio-Sciences), and quantitated by PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA) using IGLabGel software. Immunoblot quantitation was performed using IPLabGel software. Immunoblot quality control analyses were performed under conditions that yielded a linear relationship between the amount of cytosolic protein and immunoreactive signal intensity.

AHR Radioligand Binding by Sucrose Density Gradient Analysis. Hepa-1 cytosol (0.5 ml, ~2 mg of protein/ml) was incubated with 10 nM [3H]TCDD (26.2–26.7 Ci/mmol at the time of use; Chemsys, Lenexa, KS) in the absence or presence of a 100-fold molar excess of nonradioactive 2,3,7,8-tetrachlorodibenzo-p-dioxin (Dr. Stephen Safe, Texas A&M University, College Station, TX) for 75 min at 4°C. TCDD and 2,3,7,8-tetrachlorodibenzo-p-dioxin were added to cytosol in DMSO in 5-μl volumes. After incubation, unbound radioligand was removed by treating samples with dextran-coated charcoal (1 mg/ml cytosolic protein), and samples were analyzed by sucrose density gradient centrifugation as described previously (Riddick et al., 1994). Gradients were fractionated using an ISCO model 640 Fractoration (Instrumentation Specialties Co., Lincoln, NE), and radioactivity in each fraction was measured by liquid scintillation spectrometry.

Electrophoretic Mobility Shift Assay. The following complementary synthetic DNA oligonucleotides were synthesized and purified by Integrated DNA Technologies, Inc.: 5′-GATCCTGCTTCTCTCACGAATCCGG-3′ and 5′-GATCGGATTGTCGATGAGAAGACCA-3′. The core nucleotides of a well characterized DRE from the mouse Cyp1a1 5′-flanking region of these oligonucleotides were annealed and radiolabeled with...
Diagnostics), and 32P-labeled DRE probe (forward primer) and 5′-CACCGCTGAAGGGTCGCTCCACCATG-TCGGTCTC-3′ (reverse primer). Each primer contained a XhoI restriction site at the 5′-end to facilitate cloning of the PCR product into the Xhol site of the promoterless pGL3-Basic plasmid. The identity of the mAHR-pGL3 reporter plasmid was confirmed by restriction analysis and DNA sequencing.

**Transient Transfection and Luciferase Assay.** For all transfection studies, Hepa-1 cells were seeded in 12-well plates and cultured for approximately 24 h to 50% confluence, followed by the manipulations described below.

To assess induction of pGudluc1.1 activity by TCDD and MC, cells were initially exposed to vehicle (0.1% DMSO) or DEX (0.1 µM) for 24 h. After a change of medium, cells were cotransfected with pGudluc1.1 (1.4 µg) and pRL-TK (0.1 µg) using SuperFect reagent (QIAGEN, Valencia, CA). Immediately after transfection, cells were treated with vehicle (0.1% DMSO), TCDD (1 µM–1 nM), or MC (Sigma-Aldrich) (0.1 nM–1 µM). Cells were harvested after a treatment period of 24 h, cell extracts were prepared in 1× passive lysis buffer, and dual luciferase measurements (Promega) were performed using a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). Firefly luciferase activity was normalized to Renilla luciferase activity.

To assess the response of mAHR-pGL3 to histone deacetylase inhibitors, cells were transfected with pGL3-Basic (1.5 µg) or mAHR-pGL3 (1.5 µg) using SuperFect reagent. After culturing for 24 h, cells were treated with vehicle (0.1% DMSO or ethanol), n-butyrate (Sigma-Aldrich) (0.1–5 mM), or trichostatin A (TSA) (0.5–50 nM; Sigma-Aldrich). Cells were harvested after a treatment period of 24 h, and firefly luciferase activity was normalized to cellular protein concentration as determined by the method of Bradford (1976). The effects of n-butyrate and TSA on endogenous levels of AHR mRNA and protein were assessed according to the RT-PCR and immunoblot procedures described above.

To assess the response of mAHR-pGL3 to DEX, cells were cotransfected with pGL3-Basic (0.7 µg), pGRE-luc (0.7 µg), or mAHR-pGL3 (0.7 µg) and

![Graph A](image1.png)  
**A** Effect of DEX treatment on AHR mRNA levels in Hepa-1 cells. A, Vistra Green-stained polyacrylamide gel depicting relative AHR and β-actin mRNA levels is shown in the top, and the corresponding image in the bottom shows the relative AHR / ß-actin mRNA levels for each sample, included as a positive control for GR activation. Representative results are shown for a single RNA isolation of three independent experiments conducted. B, semiquantitative image analysis of AHR mRNA levels. RT-PCR data are expressed as a percentage of the mean of the vehicle control and represent the mean ± S.D. of determinations from three independent RNA isolations.

![Graph B](image2.png)  
**B** Reporter Gene Constructs. A, AHR (~95 kDa) and β-actin (450 bp) sequences: 5′-AACACTGGAGTTAGACTCTCTCCCTAATCAGACACT-3′ (forward primer) and 5′-CCACCGCTGAAGGGTCGCTCCACCATG-TCGGTCTC-3′ (reverse primer). Each primer contained a XhoI restriction site at the 5′-end to facilitate cloning of the PCR product into the Xhol site of the promoterless pGL3-Basic plasmid.

- **AHR (503 bp)**  
- **β-actin (450 bp)**  
- **TAT (230 bp)**

![Graph C](image3.png)  
**C** Immunoblot analysis of cytosolic AHR protein levels in Hepa-1 cells. A, immunoblot analysis of cytosolic protein (10 µg) using polyclonal antibody directed against mouse AHR. Representative results are shown for a single cytosol isolation of three independent experiments conducted. B, semiquantitative image analysis of AHR protein levels. Immunoblot data are expressed as a percentage of the mean of the vehicle control and represent the mean ± S.D. of determinations from three independent cytosol isolations.

- **AHR (~ 95 kDa)**  
- **[**AHR**]**  
- **[**β-actin**]**  
- **[**TAT**]**

![Graph D](image4.png)  
**D** Transient Transfection and Luciferase Assay. For all transfection studies, Hepa-1 cells were seeded in 12-well plates and cultured for approximately 24 h to 50% confluence, followed by the manipulations described below.

- **mAHR-pGL3**  
- **pGL3-Basic**  
- **pGRE-luc**  
- **vehicle control**
pRL-TK (0.1 µg) using SuperFect reagent. At the same time, cells were also cotransfected with 2.0 µg of a mouse GR expression plasmid, pSVmGR (Dr. John A. Cidlowski, National Institute of Environmental Health Sciences, Research Triangle Park, NC) (Webster et al., 1997), or the empty vector, pSV2 (American Type Culture Collection). After culturing for 24 h, cells were treated with vehicle (0.1% DMSO) or DEX (1 nM–1 µM). Cells were harvested after a treatment period of 24 h, and firefly luciferase activity was normalized to Renilla luciferase activity. Levels of GR protein were assessed according to the immunoblot procedure described above.

Statistical Analysis. Data are presented as means ± S.D. of the indicated number of determinations. For experiments assessing the effects of DEX on AHR mRNA, protein, TCDD binding, and DRE binding, data were analyzed using a repeated-measures design one-way ANOVA followed by a post hoc Newman-Keuls test. For all transfection experiments, data were analyzed initially using a randomized design two-way ANOVA to identify significant influences of the two independent variables in a given experiment (variable 1 = DEX pretreatment or plasmid identity or GR cotransfection; variable 2 = chemical concentration). If a significant effect of variable 1 was identified, a Student’s t test was performed at each chemical concentration. If a significant effect of variable 2 was identified, a randomized design one-way ANOVA followed by post hoc Newman-Keuls test was performed to identify the chemical concentrations producing effects that differed from the vehicle control. In all cases, a result was considered to be statistically significant if p < 0.05.

Results

Treatment of Hepa-1 cells with DEX, a potent synthetic glucocorticoid, resulted in a 68% increase in the level of AHR mRNA (Fig. 1). Concentrations of DEX (0.1 and 1 µM) that were effective in increasing AHR mRNA levels were also able to activate the GR, as demonstrated by pronounced induction of TAT mRNA (Fig. 1A). The same concentrations of DEX also increased the levels of AHR protein (Fig. 2) and specific [3H]TCDD binding (Fig. 3), and the maximal increases observed in these parameters were 45 and 48%, respectively. A 35% increase in specific [3H]TCDD binding ([DEX] µM : 0.001 0.01 0.1 1) was also produced after exposure of Hepa-1 cells to 1 and 10 µM concentrations of CORT, the major endogenous rodent glucocorticoid (data not shown). CORT produced similar changes in AHR mRNA and protein levels, but these responses did not achieve statistical significance (data not shown).

The role of the GR in the induction of AHR expression by DEX was tested using the GR antagonist RU486. At a concentration of 1 µM, RU486 blocked the GR-mediated induction of TAT mRNA.
caused by DEX (0.1 μM) without causing TAT induction on its own (Fig. 4). DEX alone at a concentration of 0.1 μM increased AHR mRNA, protein, and [3H]TCDD binding by 26, 33, and 35%, respectively, and these small magnitude increases were not observed when Hepa-1 cells were cotreated with DEX and RU486 (Fig. 4).

To examine whether small changes in the AHR protein level caused by DEX resulted in functionally important alterations in the AHR signaling pathway, we first determined the ability of TCDD to transform the cytosolic AHR in vitro to a DRE-binding form. By electrophoretic mobility shift assay, the amount of AHR · DRE binding elicited by a maximally effective TCDD concentration did not differ for cytosol isolated from Hepa-1 cells that were previously treated in culture with vehicle or DEX at concentrations of 0.01, 0.1, or 1 μM (Fig. 5).

As a second indicator of AHR function, we examined the ability of TCDD and MC to cause AHR-dependent induction of a luciferase reporter plasmid containing four DREs from the Cyp1a1 5′-flank (pGudluc1.1). In the absence of DEX pretreatment, the maximal inductions of pGudluc1.1 luciferase activity by TCDD and MC were 151- and 118-fold, respectively (Fig. 6). After DEX pretreatment, the inductions of pGudluc1.1 luciferase activity by TCDD and MC were 150- and 115-fold, respectively (Fig. 7). The marked effects of histone deacetylase inhibitors on mAHR-pGL3 luciferase activity were accompanied by only small magnitude increases (<45%) in endogenous AHR mRNA (Fig. 7B) and protein (Fig. 7C) levels. Our initial characterization of the mAHR-pGL3 construct revealed no significant response to the following chemicals in Hepa-1 cells in comparison with the promoterless pGL3-Basic construct (data not shown): TCDD (1 pM–10 nM), MC (0.1 nM–1 μM), phenobarbital (10 μM–1 mM), hydroxyprogesterone (0.1–1 mM), testosterone (0.1–10 μM), cyclic AMP (0.01–2 mM), retinoic acid (0.1–100 nM), phorbol 12-myristate 13-acetate (1–500 nM), and 5-aza-2′-deoxycytidine (0.01–10 μM).

The final goal of this study was to determine whether mAHR-pGL3 luciferase activity is induced by DEX in a GR-dependent manner. Transfection of Hepa-1 cells with a mouse GR expression vector (pSVmGR) elevated cytosolic GR protein levels by approximately 2.4-fold in comparison with cells transfected with the empty vector (pSV2) (Fig. 8A). The promoterless pGL3-Basic construct showed a 12% induction in response to DEX in the absence of exogenous GR, and this induction was augmented to 55% in the presence of exogenous GR (Fig. 8B). The pGRE-luc construct contains consensus GREs from the TAT gene and was used as a positive control for GR activation. This reporter plasmid showed a 4.5-fold induction in response to DEX in the absence of exogenous GR, and this induction was augmented to 17.7-fold in the presence of exogenous GR (Fig. 8B). The mAHR-pGL3 construct showed a 29% induction in response to DEX in the absence of exogenous GR, and this induction was augmented to 2.5-fold in the presence of exogenous GR (Fig. 8B).

**Discussion**

Many factors affect the levels of circulating glucocorticoids, including circadian rhythms, developmental stage, stress, exposure to exogenous steroid therapeutic agents, and diseases of glucocorticoid deficiency and excess. We are interested in the molecular mechanisms by which glucocorticoids modulate the expression of the hepatic AHR and the functional responsiveness to HAHs and PAHs, high-priority environmental toxicants.

Glucocorticoids potentiate HAH- and/or PAH-dependent CYP1A induction (Nebert and Gelboin, 1969; Sherratt et al., 1989; Celder et al., 1996; Konstandi et al., 2000; Lai et al., 2004). Induction of other AHR target genes is augmented or diminished by glucocorticoids, suggesting multiple molecular mechanisms (Linder et al., 1999). Potentiation of CYP1A1 induction by glucocorticoids is GR-mediated and involves binding of the GR to multiple GREs, initially identified in the first intron of the rat CYP1A1 gene (Mathis et al., 1989). Conserved GREs are found in the first intron of the CYP1A1 gene from rat, mouse, and human (Linder et al., 1999). However, a dramatic species difference in the CYP1A1 response exists: DEX potentiates the induction of rat CYP1A1 by MC at the transcriptional level via a GR · GRE interaction in the first intron, whereas DEX inhibits the induction of human CYP1A1 by MC at the protein level via a GR-independent mechanism (Monostory et al., 2005).

Our study focused on a distinct mechanism that may contribute to potentiated induction of AHR target genes by glucocorticoids: alteration of AHR expression and function. The first major finding of our
study is that DEX increases AHR mRNA, protein, and TCDD binding by approximately 50% in Hepa-1 cells. While the present study was nearing completion, two highly relevant reports appeared in the literature (Sonneveld et al., 2007; Dvořák et al., 2008). Dvořák et al. (2008) found that DEX decreases AHR mRNA levels in HepG2 human hepatoma cells without affecting AHR protein levels. DEX decreased the induction of an AHR-activated luciferase reporter by TCDD, and TCDD-induced EROD activity was also inhibited. It
remains unclear how these effects are produced in the absence of a decrease in AHR protein levels. Sonneveld et al. (2007) found that DEX acts via the GR to augment the induction by TCDD of AHR-activated luciferase reporters, EROD activity, and several endogenous AHR target genes in rodent cells (rat H4IIE and mouse Hepa-1) but not human cells (HepG2 and T47D breast carcinoma cells). DEX increased AHR mRNA levels in rat H4IIE cells but not in human cells. Together with our current results, these reports solidify the importance of species difference in AHR · GR interactions; glucocorticoids have a positive impact on AHR expression and function in rodent hepatoma cells but a negative impact on AHR expression and function in human hepatoma cells.

The magnitude of the increase in AHR levels elicited by DEX in our study was relatively small, in the 50% range. Hepa-1 cells express very high basal levels of AHR protein, with concentrations that are at least 1 order of magnitude higher than rodent liver levels (Timsit et al., 2002). An upper ceiling may be placed on the glucocorticoid response in Hepa-1 cells by the high basal AHR levels. Also, background levels of cortisol, a lower affinity GR agonist, in the culture medium may have limited the DEX response to a small degree.

The second major finding of our study is that the increase in AHR levels caused by glucocorticoids is mediated by the GR via a transcriptional mechanism. Our evidence for GR involvement in AHR induction by glucocorticoids includes the following: 1) AHR induction occurred at DEX concentrations effective in elevating TAT mRNA, a prototypical GR target; 2) AHR induction by DEX was blocked by RU486, a GR antagonist; and 3) induction of the mAHR-pGL3 luciferase reporter by DEX was augmented in the presence of a GRE interaction; however, it is important to consider other potential indirect mechanisms. We suspect that the pregnane X receptor (PXR) does not play a significant role in the induction of AHR by DEX. Although supramicromolar concentrations of DEX activate murine PXR (Kliewer et al., 1998), we used submicromolar concentrations of DEX that selectively activate the GR. RU486 is a murine PXR agonist at high micromolar concentrations (Kliewer et al., 1998), whereas the concentration of RU486 used in the present study (1 μM) was previously shown to have minimal effect on murine PXR activation (Kliewer et al., 1998) and a clear ability to inhibit DEX-induced TAT activity in rat hepatoma cells (Gagne et al., 1985). Submicromolar concentrations of DEX act via the GR to stimulate
expression of PXR via a transcriptional mechanism (Pascussi et al., 2000). Although PXR may be a potential player in AHR induction by DEX, any role would seem to be minor compared with that for the GR.

We made use of our novel mAHR-pGL3 reporter construct to demonstrate for the first time that DEX increases Ahr promoter activity in a GR-dependent manner. The GRE consensus sequence is defined as 5′-GGTACANNNTGTCT-3′ (Lu et al., 2006), with the italicized positions known to tolerate mismatches. An imperfect GRE half-site of uncharacterized function (5′-TGATCT-3′) located at positions −1009 to −1004 of the mouse Ahr gene was noted previously (Mimura et al., 1994; FitzGerald et al., 1996; Garrison and Denison, 2000). Our bioinformatic analysis identified four potential GREs (with three or fewer mismatches from the consensus) within the cloned region of the mouse Ahr 5′-flank contained in the mAHR-pGL3 construct: positions −2432 to −2418, −1073 to −1059, −712 to −698, and −237 to −223. The GRE sequence located at positions −2432 to −2418 (5′-GCCACATAGTGTGCCT-3′) is particularly attractive as the two mismatches from the consensus are located in the italicized positions known to tolerate substitution. We do not yet know if these potential GRE sequences mediate the induction of the mAHR-pGL3 luciferase activity by DEX in Hepa-1 cells, but these sites are strong candidates for further study.

We and others (FitzGerald et al., 1996; Garrison and Denison, 2000) found that luciferase reporters driven by the mouse Ahr proximal 5′-flank and promoter are refractory to modulation by a wide range of chemicals. Of note, our mAHR-pGL3 construct showed no induction by TCDD or MC although there are putative DREs containing the conserved core (5′-GCGTG-3′) at positions +54 to +58, +89 to +93, and +92 to +96. Although the mechanism is uncertain, we confirmed that two histone deacetylase inhibitors caused strong induction of mAHR-pGL3 luciferase activity in Hepa-1 cells (Garrison and Denison, 2000; Garrison et al., 2000).

The third major finding of our study is that a small increase in AHR levels caused by glucocorticoids has a functionally significant impact on aromatic hydrocarbon responsiveness. Pretreatment of Hepa-1 cells with DEX for 24 h increased AHR levels by approximately 50%, and the subsequent induction of an AHR-activated luciferase reporter (pGudLuc1.1) by TCDD and MC was significantly augmented. The pGudLuc1.1 reporter construct is devoid of functional GREs and, importantly, basal pGudLuc1.1 luciferase activity was not elevated in Hepa-1 cells pretreated for 24 h with DEX relative to vehicle control. Thus, the enhanced responsiveness to TCDD and MC exposure can be attributed to a cellular effect of DEX during the 24-h pretreatment period and not to any direct effect of DEX on the reporter construct. The increase in AHR levels produced by DEX is a likely mechanistic explanation.

It is puzzling how DEX produces an increase in AHR levels and an enhanced transcriptional response to ligands without an apparent change in AHR · DRE binding. Differences in analytical sensitivity in experimental endpoints may be important, but we also note that cells
contain distinct pools of AHR proteins that bind ligand but differ in transformation to DNA-binding status (Denison, 1992).

Do changes in AHR levels have a significant impact on the responsiveness of cells to ligands? As addressed in detail in our recent review (Harper et al., 2006), both receptor theory and experimental manipulations demonstrate that changes in AHR levels affect the CYP1A1 induction response. Our findings support the idea that relatively small increases in AHR protein levels result in significant augmentation of cellular responsiveness to HAHs and PAHs.

In conclusion, glucocorticoids increase AHR levels in mouse hepatoma cells via a GR-dependent transcriptional mechanism. This small increase in AHR levels is associated with an augmented transcriptional response to HAHs and PAHs. This work reveals that glucocorticoid potentiation of CYP1A1 induction is mediated not only by the presence of GREs in the first intron of the CYP1A1 gene but also by increasing AHR expression at the transcriptional level. The present work is important and novel as it provides the first direct evidence for the transcriptional induction of AHR expression by DEX. Alterations in glucocorticoid levels have the potential to modulate the responsiveness of organisms to the toxic and/or adaptive effects of aromatic hydrocarbons. Future studies will address additional details of the molecular mechanisms involved, and the in vivo relevance of this aspect of cross-talk between the AHR and the GR will be examined in adrenalectomized rodent models with glucocorticoid replacement.

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Reference


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