SUPPRESSION OF CYTOCHROME P450 2C11 BY AROMATIC HYDROCARBONS: MECHANISTIC INSIGHTS FROM STUDIES OF THE 5’-FLANKING REGION OF THE CYP2C11 GENE

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ABSTRACT:

The aromatic hydrocarbon receptor (AHR) functions as a ligand-activated transcription factor that mediates responses to aromatic hydrocarbons (AHs). Induction of cytochrome P450 2A1 (CYP2A1) is the most fully characterized response and is mediated by binding of the activated AHR complex to dioxin-responsive elements (DREs) located in the 5’-flanking region of the gene. In contrast to CYP2A1 induction, several other genes including the rat male-specific constitutive hepatic CYP2C11 are suppressed by AHs. Our aim was to determine whether CYP2C11 suppression by AHs is mediated by the AHR via interaction with DRE-like sequences. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) suppressed CYP2C11 mRNA in primary rat hepatocytes without altering the mRNA half-life. We identified five regions in the CYP2C11 5’-flank containing the DRE invariant core; electrophoretic gel retardation assays showed that at least one of these DREs is a potential binding site for the AHR. To test the function of the CYP2C11-DREs, Hepa-1, BRL 5637, and HepG2 cells were transfected with reporter constructs containing regions of the CYP2C11 5’-flank and promoter. No decrease in luciferase activity was found following TCDD treatment. In primary rat hepatocytes, the luciferase reporter vectors were suppressed by interleukin-1β but not by TCDD. In vitro footprinting showed protein binding at several sites in the CYP2C11 5’-flank, but the pattern was not altered by in vivo 3-methylcholanthrene treatment. These studies imply that AHs down-regulate CYP2C11 by a negative transcriptional mechanism that is not simply due to AHR binding to an identified DRE-like sequence and that is distinct from that used by inflammatory cytokines.

Aromatic hydrocarbons (AHs) are ubiquitous environmental contaminants formed as byproducts of industrial chemical or combustion processes. This group includes halogenated AHs such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and polycyclic AHs such as 3-methylcholanthrene (MC). TCDD is the prototypic AH and is a well characterized potent toxicant in experimental animals. The toxic and biological effects of TCDD are mediated via the aromatic hydrocarbon receptor (AHR), which belongs to the family of basic-helix-loop-helix-PAS (PER-ARNT-SIM) proteins. The AHR acts as a ligand-activated transcription factor, and our understanding of its function derives mainly from studies of the transcriptional up-regulation of cytochrome P450 2A1 (CYP2A1) by TCDD (reviewed in Whillock, 1999). Briefly, the AHR binds ligand in the cytoplasm and is translocated to the nucleus. The activated DNA-binding form is a heterodimer, which consists of the AHR and the AHR nuclear translocator (ARNT). The complex binds specific DNA sequences known as dioxin-responsive elements (DREs), located in the 5’-flanking sequence of CYP2A1 and other target genes such as CYP1A2 and CYP1B1. The bound AHR complex causes a change in chromatin structure, making the promoter region more accessible to transcription factors and leading to an increase in the rate of transcription of the gene.

TCDD also down-regulates expression of several genes (reviewed in Safe, 1995) including the estrogen receptor, transforming growth factor-β2, pS2 (Gillesby et al., 1997), cathepsin D (Krishnan et al., 1995), and CYP2C11. The mechanisms of gene suppression are only partially understood.

CYP2C11 is the predominant cytochrome P450 enzyme expressed constitutively in the liver of adult male rats. Administration of AHs to adult male rats causes down-regulation of CYP2C11 at the catalytic activity, protein, and mRNA levels (Yeowell et al., 1987; Shimada et al., 1989; Jones and Riddick, 1996). This down-regulation is at least partly mediated at the transcriptional level (Lee and Riddick, 2000) and structure-activity relationships suggest a role for the AHR (Safa et al., 1997). Since most transcriptional changes mediated by the AHR occur via interactions with 5’-flanking DRE-like sequences, we have studied the 5’-flanking region of CYP2C11 to understand the mechanism by which AHs down-regulate the gene.

In the context of the CYP2A1 gene, DRE sequences function as transcriptional enhancers; however, DRE-like sequences are also im-
plicated in gene suppression in the context of the pS2 (Gillesby et al., 1997), cathepsin D (Krishnan et al., 1995), human B lymphocyte cell surface marker CD19 (Masten and Shiverick, 1995), and c-fos (Duan et al., 1999) genes. In each case, the DRE-like sequence contains the four invariant core nucleotides required for AHR binding [5'-GCGT-GNN(A/T)NN(N/C)G-3'] (Yao and Denison, 1992) but contains one or more mismatches with the consensus sequence for functional enhancer activity [5'- (T/G)NGCGTG (A/C)(G/C)A-3'] (Lassaka et al., 1993). Binding of the AHR to these “inhibitory DREs” is thought to interfere with the binding of other positive trans-acting factors to nearby response elements, thus resulting in down-regulation of gene expression.

Our overall aim is to understand the molecular mechanisms by which AHRs down-regulate CYP2C11 expression, as a model for negative transcriptional regulation of gene expression by xenobiotics. In the present work, we showed that TCDD suppresses CYP2C11 mRNA in primary rat hepatocytes without altering the mRNA half-life. We identified five DRE-like sequences in the CYP2C11 5'-flank and showed that at least one of these is capable of binding the AHR with high affinity. In vitro DNase I footprinting was used to examine changes caused by AH treatment on protein binding in the 5'-flanking and promoter regions of the CYP2C11 gene. Luciferase reporter gene assays in transfected continuous cell lines and primary rat hepatocytes were used to study the functional role of the CYP2C11 5'-flanking region in the suppressive response to AHS. Our results suggest that AHSs down-regulate CYP2C11 by a transcriptional response that is not simply due to AHR binding to an identified DRE-like sequence.

Materials and Methods

Electrophoretic Gel Retardation Assay. Cytosol was prepared from confluent cultures of the mouse hepatoma cell line Hepa-1c1c7 (Hepa-1) by differential centrifugation. The following pairs of complementary DNA oligonucleotides were used, with the four invariant core DRE nucleotides underlined (differences from the binding consensus sequence are shown in lower case, and positions in the CYP2C11 gene are shown relative to the transcription start site, +1): the Cplai (WT)-DRE containing the functional AHR-binding site of DRE3 from the mouse Cplai 5'-flank (5'-GATCCGGATGATTGCAGAGAGCAC-3' and 5'-GATCTTCTTCCTCTACAGAACCTGC-3'); the MUT-DRE containing a mutation in one of the four invariant core nucleotides (bold) [5'-GATCCGGATGATTGCAGAGAGCAC-3' and 5'-GATCTTCTTCCTCTACAGAACCTGC-3']; CYP2C11-DRE1 (5'-GATCC-GATCTTGCTGGCAGGCAACCAATCC-3'); CYP2C11-DRE2 (5'-GATCC-AAGTACTCCATTCTCTC-CAAGGATGACGGAGACAT-3'); CYP2C11-DRE3 (5'-GATCC-GATCTGCGCTGTGGCAGGCAACCAATCC-3'); CYP2C11-DRE4 (5'-GATCC-GATCTGCGCTGTGGCAGGCAACCAATCC-3'); CYP2C11-DRE5 (5'-GATCC-GATCTGCGCTGTGGCAGGCAACCAATCC-3'). The PCR product was digested with NcoI and ligated into the pGL3-Basic vector digested with the same enzymes. The construct (−196–2C11)-pGL3 (containing −196 to +21) was generated using Sprague-Dawley rat genomic DNA as a template for PCR amplification of a fragment (−196 to +152) of the CYP2C11 gene. The PCR product was digested with MluI and NotI and ligated into the pGL3-Basic vector digested with the same enzymes. To generate 2C11-(pGL3-Promoter), pGEMs 2.3 kb was used as a template for PCR amplification of a fragment (−1935 to −101) of the CYP2C11 5'-flanking region. The PCR product was digested with MluI and BgII and ligated into pGL3-Promoter digested with the same enzymes.

Transient Transfection of Continuous Cell Lines and Luciferase Assay. Cultures of Hepa-1, Buffal rat liver BRL 5637, and human hepatocellular carcinoma HepG2 cells were cotransfected with luciferase plasmids (3.5 μg for Hepa-1, 2.5 μg for BRL 5637, 4 μg for HepG2) and the pSVβ-galactosidase plasmid (2.5 μg for Hepa-1, 2 μg for BRL 5637, 2 μg for HepG2) with Superfect reagent QIAGEN (Valencia, CA) according to manufacturer’s instructions. Twenty-four hours after transfection, cells were treated with TCDD or DMSO vehicle for 24 h. Cell extract (50 μl) was mixed with 100 μl of luciferase assay reagent (Promega), and luciferase activity was measured with a TD-20/20 luminometer (Turner Designs, Inc., Sunnyvale, CA). β-Galactosidase activity of cell extracts was measured spectrophotometrically.

Luciferase Isoform, Culture, and Transfection. Male Fischer 344 rats (9–10 weeks of age; Harlan) were cared for in accordance with the principles of the Canadian Council on Animal Care. Hepatocytes were isolated by nonrecirculating in situ collagenase perfusion through the portal vein (Liddle et al., 1992; Safa et al., 1997). Cells were plated on 60-mm culture dishes coated with 0.2 ml of matrigel (7.5 mg/ml; Collaborative Biomedical Products, Bedford, MA). Medium was changed 4 h after plating and every 48 h thereafter.

Freshly isolated hepatocytes (12.5 × 10^6 cells/ml in PBS containing 5% fetal bovine serum) were electroporated with 60 μg of a luciferase plasmid and 10 μg of pRL-TK (pulse of 250 V; capacitance at 960 μF) and then seeded on matrigel-coated plates in 3 ml of Waymouth’s medium (containing 0.15 μM insulin and 100 units/ml penicillin G).

For transfection on day 4, 60,000 cells (2.8 × 10^6/ml/plate) were plated in 3 ml of the Waymouth’s medium on matrigel-coated dishes. On day 4, cells were transfected with luciferase plasmid (6 μg) using Transfast reagent (Promega) according to manufacturer’s instructions.

For reporter assays following treatment with TCDD or murine interleukin-1β (IL-1β; R & D Systems, Minneapolis, MN), cells were collected and extracted (100 μl) prepared in 1× passive lysis buffer (Promega) were used for dual luciferase measurements (Promega). Protein was determined by the method of Bradford (1976).

For RT-PCR studies, 3.5 × 10^6 cells/plate were cultured for the specified times, and total RNA was prepared using Tri-Reagent (Sigma-Aldrich, St. Louis, MO). RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo d(T) primer as described by Fran et al. (2001). The following primer pairs were used for mouse Cyp2c11, forward 5'-GTATCGCTGTCATCCATAC-3' and reverse 5'-GGAAATGGGGATAATGTG'-3' (Promega and Shapiro, 2000) α-tubulin, forward 5'-TGCTGGCATTTGCACTTCTGCTTGGCAGATCCACCGCAATATCC-3' and reverse 5'-CTCACCCTCACCCCTCACCCCATG-3'. PCR cycling parameters for the multiplex reactions were as follows: 94°C for 30 s, 56°C for 1 min, 72°C for 1 min; 21 cycles, input cDNA derived from 50 ng RNA. The 50-μl PCR reactions contained 1× PCR buffer, 0.4 μM α-tubulin primers, and 0.5 μM Cyp2c11 primers, 3 mM MgCl₂, 0.4 mM dNTPs, and 2.5 units Taq polymerase. PCR products were separated on a 6% polyacrylamide gel, stained with Vistra Green (Amersham Biosciences Inc, Piscataway, NJ), and quantitated by phosphorimaging analysis using ImageQuant v1.2 software (Molecular Imaging, Sunnyvale, CA).

DNase I Footprintting. Rats received a single i.p. injection of MC (40
The role of the AHR in mediating induction of CYP1A1 and other genes in response to AHs is well established. AHs also suppress several genes, but the mechanisms are not understood, and the role of the AHR remains controversial. In vivo administration of AHs to rats causes down-regulation of CYP2C11 via a transcriptional mechanism (Jones and Riddick, 1996; Lee and Riddick, 2000), and our structure-activity relationship study implicated the AHR in this response (Safa et al., 1997). To further our understanding of this down-regulation, we have now conducted molecular mechanistic investigations of the CYP2C11 5′-flanking region.

Primary rat hepatocytes cultured on matrigel for 5 to 7 days show relatively stable CYP2C11 expression (Liddle et al., 1992); AH treatment decreases CYP2C11 protein expression in this hepatocyte culture system (Safa et al., 1997). Using the same culture system, we found that TCDD caused an approximately 30% suppression of CYP2C11 mRNA expression (Fig. 1A). IL-1β, a well characterized suppressor of CYP2C11 (Iber et al., 2000), decreased CYP2C11 mRNA by approximately 70% (Fig. 1A). Following treatment of hepatocytes with the transcriptional inhibitor 5,6-dichlorobenzimidazole riboside (DRB), RT-PCR analysis showed that the half-life of CYP2C11 mRNA was 16 h, and this was not altered by TCDD treatment (Fig. 1B). In the same RT-PCR reactions, the half-life of α-tubulin mRNA was determined to be 9 h and also not affected by TCDD treatment (Fig. 1B); hence, α-tubulin was not used to normalize CYP2C11 levels. 18S rRNA levels were confirmed to be similar for all samples (data not shown).

Since our rat hepatocyte results are consistent with a transcriptional mechanism for CYP2C11 suppression by TCDD, we focused our mechanistic studies on the 5′-flanking region of the CYP2C11 gene. We identified five DRE-like sequences in the CYP2C11 5′-flank, which contain the four invariant core nucleotides and two or fewer mismatches with the AHR-binding consensus sequence (Yao and Denison, 1992). All of these sequences represent potential AHR-binding sites, but none are predicted to be functional enhancer elements, based on comparisons with the consensus sequence (Lusska, 1997)
Fig. 2. Competitive gel retardation analysis to determine relative abilities of CYP2C11 DRE-like sequences to compete for binding of the AHR to a 32P-labeled Cyp1a1 WT-DRE.

A, Hepa-1 cytosol was incubated for 2 h at room temperature with DMSO or 20 nM TCDD. Following incubation, cytosol was mixed with either no competitor oligonucleotide (lanes 1, 2), 10× or 100× molar excess of unlabeled Cyp1a1 WT-DRE (lanes 3, 4), or 10× or 100× molar excess of each of the unlabeled CYP2C11-DREs (lanes 5–14). 32P-labeled Cyp1a1 WT-DRE was then added, and DNA-protein interactions were visualized by gel retardation analysis. The arrow indicates the ligand-inducible interaction of the AHR complex with the labeled Cyp1a1 WT-DRE, and F indicates free Cyp1a1 WT-DRE probe. Similar results were obtained in at least one additional experiment with an independent batch of cytosol.

B, relative binding affinities of the CYP2C11 DRE-like sequences for the transformed Hepa-1 AHR. Competitive gel retardation assays were carried out as described above, except that a full range of concentrations for all unlabeled competitor oligonucleotides was investigated. TCDD-inducible binding of the AHR to the 32P-labeled Cyp1a1 WT-DRE was quantitated by phosphorimaging analysis. Specific binding is expressed as a percentage of the binding observed in the absence of a competitor oligonucleotide. Similar results were obtained in at least one additional experiment with an independent batch of cytosol.
Fig. 3. DNase I footprinting analysis of the CYP2C11 5′-flanking region with nuclear extracts from corn oil- and MC-treated rats.

A, RT-PCR analysis of CYP1A1 mRNA and the internal control β-actin, using RNA from livers used in footprinting reactions. B to E, DNase I (0.01–0.25 units) and rat liver nuclear extracts (20–30 μg protein) were used. G + A/C + T indicate ladders generated by Maxam-Gilbert sequencing of the labeled probe, − indicates no extract, V indicates extract from corn oil-treated rat, and MC indicates extract from MC-treated rat. Vertical lines denote important footprinted regions. Arrows show sites of nuclear extract-induced DNase I hypersensitivity. B, radiolabeled lower strand of sequence from +30 to −185; probe A (numbering is relative to transcription start site). C, radiolabeled upper strand of sequence from −405 to −268; probe A. D, radiolabeled upper strand of sequence from −1625 to −1504; probe B. E, radiolabeled upper strand of sequence from −1280 to −1133; probe C. Similar results were obtained in at least one additional experiment with an independent set of nuclear extract preparations.

GRE, glucocorticoid response element; STAT5, signal transducer and activator of transcription 5.

A, 3-MC

CYP1A1

β-Actin

B

C

D

E

G+T

V

MC

G+A

V

MC

G+A

V

MC

G+A

V

MC

G+A

V

MC

G+A

V

MC

GRE

-142

HNF-1 like

HNF-1 like

HNF-3 like

NF-1 like

-30

TATA box

+1

+24

A/T

-405

-1618

SIL

400

E-box

-1150

STAT5

HNF3

SIL

1200

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et al., 1993) for Cyp1a1 up-regulation. We hypothesized that one or more of these elements may function as "inhibitory DREs" in the down-regulation of CYP2C11. Electrophoretic gel retardation analysis indicated that incubation of in vitro transformed Hepa-1 cytosol with a \(^{32}\)P-labeled DRE (Cyp1a1 WT-DRE) resulted in formation of a TCDD-inducible band representing the interaction of the transformed cytosolic AHR with the DRE sequence (Fig. 2A). Competitive gel retardation analysis using 10- and 100-fold molar excess unlabeled CYP2C11 DRE-like sequences showed that CYP2C11-DRE3 displayed the greatest ability to bind the AHR complex (Fig. 2A). An identical pattern of competition was obtained when rat liver cytosol was used as the source of AHR (data not shown). To determine the relative affinities of the CYP2C11-DRE sequences for the activated AHR complex, we performed competition studies using increasing amounts (0.1- to 1000-fold excess) of unlabeled CYP2C11-DREs to compete with the labeled Cyp1a1 WT-DRE for binding the activated AHR (Fig. 2B). This confirmed that CYP2C11-DRE3 was an effective competitor with an IC\(_{50}\) value in the nanomolar range and only a 5-fold lower affinity for the AHR complex than the Cyp1a1 WT-DRE. The other CYP2C11-DRE sequences showed at least 50-fold lower affinity than the Cyp1a1 WT-DRE, and were similar in affinity to the MUT-DRE, which contains a mutation in one of the four invariant core nucleotides. Additionally, we showed that radiolabeled CYP2C11-DRE3 can directly interact with the AHR complex (data not shown); this interaction shows DRE sequence specificity.

FIG. 4. Effects of TCDD and IL-1\(\beta\) on CYP2C11-luciferase reporter activity in transiently transfected primary hepatocytes.

A and B, hepatocytes were electroporated with luciferase and Renilla constructs. After 4 h, cells were treated with 10 nM TCDD (A) or 7.5 ng/ml IL-1\(\beta\) (B) and incubated for 24 h. Luciferase activity was normalized to Renilla activity. C and D, after 4 days in culture on matrigel, hepatocytes were transfected with luciferase vectors using Transfast. After 2 h, cells were treated with 10 nM TCDD (C) or 7.5 ng/ml IL-1\(\beta\) (D) and incubated for 24 h. Luciferase activity was normalized to protein content. All data are expressed in arbitrary normalized luciferase units as mean ± S.D. of triplicate determinations in a single experiment. *, significantly different (p ≤ 0.05) from DMSO or PBS/BSA control; **, significantly different (p ≤ 0.01) from DMSO control; ***, significantly different (p ≤ 0.001) from PBS/BSA control, based on Student’s t test. Similar results were obtained in at least one additional independent transfection experiment. BSA, bovine serum albumin.
SUPPRESSION OF CYP2C11 BY AROMATIC HYDROCARBONS

Effect of TCDD on luciferase reporter gene activity in transiently transfected Hepa-1, BRL 5637, and HepG2 cells

All data are expressed in arbitrary luciferase units (normalized for β-galactosidase activity) as mean ± S.D. of triplicate determinations in a single transfection experiment. Similar results were obtained in at least one additional independent transfection experiment.

<table>
<thead>
<tr>
<th>Vector</th>
<th>Time (h)</th>
<th>Hepa-1 Cells</th>
<th>BRL 5637 Cells</th>
<th>HepG2 Cells</th>
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<tbody>
<tr>
<td></td>
<td>DMSO 10 nM TCDD</td>
<td>DMSO 10 nM TCDD</td>
<td>DMSO 1 nM TCDD</td>
<td></td>
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<tr>
<td>pGL3-Basic</td>
<td>24</td>
<td>571 ± 37</td>
<td>932 ± 37***</td>
<td>12 ± 1</td>
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<td>pGL3-Promoter</td>
<td>24</td>
<td>N.D.</td>
<td>N.D.</td>
<td>493 ± 25</td>
</tr>
<tr>
<td>pGL3-Control</td>
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<td>42568 ± 2619</td>
<td>55245 ± 1808**</td>
<td>210147 ± 77997</td>
</tr>
<tr>
<td>(−2390–2C11)−pGL3</td>
<td>24</td>
<td>382 ± 43</td>
<td>559 ± 31**</td>
<td>34 ± 5</td>
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<tr>
<td>(−196–2C11)−pGL3</td>
<td>24</td>
<td>356 ± 57</td>
<td>347 ± 29**</td>
<td>75 ± 9</td>
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<tr>
<td>2C11-(pGL3-Promoter)</td>
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<td>4817 ± 354</td>
<td>7340 ± 20***</td>
<td>637 ± 75</td>
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<td>pGudluc1.1</td>
<td>24</td>
<td>892 ± 142</td>
<td>22660 ± 4434**</td>
<td>9384 ± 301</td>
</tr>
</tbody>
</table>

N.D., not determined.

* Significantly different (p ≤ 0.05) from DMSO control; ** significantly different (p ≤ 0.01) from DMSO control; *** significantly different (p ≤ 0.001) from DMSO control; based on Student’s t-test.

and antibodies were used to confirm that AHR and ARNT are components of this complex. These data suggest that a potential binding site for the AHR exists in the 5′-flanking region of the CYP2C11 gene.

To further study the role of the 5′-flanking sequence of CYP2C11 in suppression by AHS, we did Dnase I footprinting studies. The in vivo MC treatment regimen causes an approximately 50% decrease in the rate of CYP2C11 transcription within 6 h (Lee and Riddick, 2000). RT-PCR analysis showed that hepatic CYP1A1 mRNA was induced by this treatment, suggesting that the AHR had been activated (Fig. 3A). In the CYP2C11 proximal promoter region (Fig. 3B), we observed footprints at the translation start site (ATG), the transcription start site (+1), the TATA box, the NF-1 like, the HNF-3 like, and the HNF-1 like consensus sites, and at the glucocorticoid response element (Fig. 3B), confirming some of the previous findings of Sundseth et al. (1992). The transcription start site contains an NF-κB consensus site that has been implicated in suppression of CYP2C11 by IL-1β (Iber et al., 2000). Further upstream, we observed footprints at the Silencer 400 (Fig. 3C) and 1200 sites (Fig. 3D) [characterized by Strom et al. (1994)], the HPF-1 site (Fig. 3C), the signal transducer and activator of transcription 5 binding site and its adjacent HNF3 site (Fig. 3E) [implicated in growth hormone control, characterized by Park and Waxman (2001)], an E-box element (Fig. 3D), and the CYP2C11-DRE3 that we characterized in the gel retardation assays (Fig. 3D). In all regions examined, there was no difference in protein binding pattern between the nuclear extracts prepared from vehicle- and MC-treated rats. The presence of a footprint at the CYP2C11-DRE3 site with both vehicle- and MC-treated samples is reminiscent of rabbit CYP1A1 DREs, which bind the upstream stimulatory factor-1 in the presence or absence AH treatment (Takahashi and Kamataki, 2001). It will be necessary to study binding of nuclear proteins to the CYP2C11 gene in its nucleosomal configuration.

 Luciferase reporter constructs were used in transient transfection studies to assess functional aspects of CYP2C11 transcriptional regulation. Our initial transfection experiments were performed in liver-derived cell lines of mouse, rat, and human origin. In transfected Hepa-1, BRL 5637, and HepG2 cells (Table 1), no decrease in luciferase activity was found with any vector tested following a 24-h treatment with TCDD. The pGudluc1.1 vector (a positive control for AHR activation) was strongly induced by TCDD in Hepa-1 (25-fold), BRL 5637 (13-fold), and HepG2 (214-fold) cells. In Hepa-1 and HepG2 cells, all constructs, regardless of whether sections of the CYP2C11 5′-flank were present, showed a small (approximately 2-fold) but significant increase in luciferase activity in response to TCDD. A similar but less pronounced trend was observed in BRL 5637 cells. The small inductive response to TCDD with all pGL3-based vectors may be attributed to a nonspecific transcriptional effect or a potentially functional DRE we have identified in the ampicillin resistance gene; however, this requires further investigation with suitably modified neutral vectors. Additionally, TCDD was unable to suppress luciferase activity in HepG2 cells stably transfected with the (−2390–2C11)−pGL3 construct (data not shown).

To conduct transfections in a cellular system that maintains CYP2C11 expression and in which the endogenous CYP2C11 gene is suppressed by AHS, we used primary rat hepatocytes cultured on matrigel. Following electroporation, hepatocytes were treated with TCDD or IL-1β 4 h after plating and harvested 20 h later. TCDD did not cause suppression of any CYP2C11-reporter construct but did cause an 11-fold increase in pGudluc1.1 activity (Fig. 4A). At this point-time, IL-1β caused a weak suppression of (−196–2C11)−pGL3, but not of the longer CYP2C11 reporter constructs (Fig. 4B). At the time of these reporter studies, the hepatocytes have not recovered expression of endogenous CYP2C11 (Liddle et al., 1992). To study our reporter constructs under conditions that maintain endogenous CYP2C11 expression, we developed a method to transfect hepatocytes after 4 days in culture on matrigel. Treatment with TCDD did not cause suppression of luciferase activity from the reporter constructs (Fig. 4C). In fact, there was significant induction of luciferase activity from the (−196–2C11)−pGL3 construct and an insignificant increase from the other CYP2C11-reporter constructs. The control plasmid pGL3-Basic showed a 3-fold induction and the pGudluc1.1 plasmid showed a 15-fold induction. Under the same conditions, IL-1β caused marked suppression of all CYP2C11-reporter constructs; the pGL3-Basic construct was also suppressed by IL-1β to a lesser degree (Fig. 4D).

In summary, the present study showed that the 5′-flanking region of the CYP2C11 gene contains a potentially important binding site for the AHR complex. However, under conditions in primary hepatocytes in which the endogenous gene is suppressed by TCDD and the CYP2C11-reporter constructs are suppressed by IL-1β, TCDD is unable to cause suppression of CYP2C11-reporter constructs containing up to −2390 bp of the 5′-flank. This implies that AHS down-regulate CYP2C11 by a negative transcriptional mechanism that is not simply due to AHR binding to an identified DRE-like sequence and that is distinct from that used by inflammatory cytokines.

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References


