Transcriptional Regulation of the Human CYP3A4 Gene by the Constitutive Androstane Receptor

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Received December 19, 2001; accepted May 2, 2002

ABSTRACT

Cytochrome P450 3A4 (CYP3A4), the predominant P450 expressed in adult human liver, is both constitutively expressed and transcriptionally activated by a variety of structurally diverse xenobiotics. In this study, we examined the role of the constitutive androstane receptor (CAR), a member of the steroid/retinoid/thyroid hormone receptor superfamily, in the transcriptional regulation of CYP3A4. Herein, we demonstrate that CAR is capable of trans-activating expression of the CYP3A4 gene, both in vitro and in vivo. Induction of CYP3A4 is dependent on cooperativity between elements within the promoter proximal region of the gene and the distal xenobiotic-responsive enhancer module. CAR responsiveness was shown to be primarily mediated by two high-affinity binding motifs located within the CYP3A4 gene 5′-flanking region, approximately 7720 and 150 bases upstream of the transcription initiation site. Importantly, the human CAR response elements also mediate trans-activation of CYP3A4 by the human pregnane X receptor, suggesting that interplay between these receptors is likely to be an important determinant of CYP3A4 expression.

The cytochromes P450 (P450) are a superfamily of heme-thiolate-containing proteins involved in the oxidative metabolism of a plethora of endogenous and exogenous compounds (Nelson et al., 1996). Cytochrome P450 3A4 (CYP3A4), the predominant P450 isoform constitutively expressed in adult human liver, is transcriptionally activated by a variety of structurally diverse compounds, including rifampicin, phenobarbital (PB), mifepristone, and clotrimazole (Maurel, 1996). Trans-Activation of P450 genes by xenobiotics increases the organism’s capacity to metabolize and ultimately excrete toxins and carcinogens (Denison and Whitlock, 1995; Waxman, 1999).

Until recently, the molecular mechanisms underlying the transcriptional activation of the CYP3A4 gene were poorly understood. A number of independent studies have demonstrated that the human pregnane X receptor (hPXR) is activated by compounds that are known CYP3A4 inducers, including drugs, steroids, and environmental chemicals (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998; Schuetz et al., 1998). Ligand-activated hPXR binds hormone response elements (HREs) in the 5′-flanking region of the CYP3A4 gene as a heterodimer with the 9-cis retinoic acid receptor-α (RXRα) (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998; Goodwin et al., 1999). Targeted disruption of PXR in mice results in selective loss of xenobiotic inducibility of the murine Cyp3a11 gene, but does not affect constitutive expression in liver or intestine (Xie et al., 2000a; Staudinger et al., 2001).

Although PXR-RXRα heterodimers bind an HRE in the promoter proximal region of CYP3A4 (prPXRE, bases −72 to −149) (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998), activation of the native CYP3A4 promoter is dependent upon the presence of a distal xenobiotic-responsive enhancer module (XREM, bases −7836 to −7208) (Goodwin et al., 1999). Cooperativity between promoter proximal and distal PXR-response elements is central to the PXR-mediated trans-activation of CYP3A4.

Recently, Negishi and coworkers demonstrated that induction of CYP2B genes by PB and a variety of structurally unrelated compounds collectively known as “PB-like” inducers (e.g., chlorpromazine, methoxychlor, and 1,1,1-trichloro-1,2-bis(o,p'-

ABBREVIATIONS: P450, cytochrome P450; PB, phenobarbital; hPXR, human pregnane X receptor; PXR, pregnane X receptor; HRE, hormone response element; RXRα, 9-cis retinoic acid receptor-α; prPXRE, proximal pregnane X receptor response element; XREM, xenobiotic-responsive enhancer module; CAR, constitutive androstane receptor; mCAR, murine constitutive androstane receptor; hCAR, human constitutive androstane receptor; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; PCR, polymerase chain reaction; EMSA, electrophoretic mobility shift assay; kb, kilobase; RT-PCR, reverse transcriptase-polymerase chain reaction; bp, base pair(s).
The PB-induced nuclear translocation is uncoupled by concomitant exposure to okadaic acid an inhibitor of phosphatases 1 and 2A, suggesting that dephosphorylation of mCAR is required for its nuclear compartmentalization (Kawamoto et al., 1999). Thus, the PB-induced dephosphorylation of CAR seems to be critical step in CYP2B induction (Kawamoto et al., 1999). In support of this observation, previous reports have documented the importance of phosphorylation status in CYP2B induction (Sidhu and Omiecinski, 1995; Nirodi et al., 1996; Honkakoski and Negishi, 1998). Importantly, nuclear translocation of mCAR in HepG2 cells is spontaneous and does not seem to be dependent on ligand binding and/or modification of phosphorylation status (Kawamoto et al., 1999; Sueyoshi et al., 1999). Thus, cDNA-directed expression of mCAR resulted in transcriptional activation of the endogenous CYP2B6 gene in the absence of inducer. Moreover, although mCAR ligands, namely, androstanol and androstenol, inhibited the trans-activational capacity of mCAR in HepG2 cells they did not block nuclear translocation of the protein (Sueyoshi et al., 1999). Targeted in vivo disruption of mCAR resulted in transcriptional activation of the endogenous P450s. The mechanisms by which PB and PB-like inducers exert a leucine-rich region near the C terminus of the CAR protein (Sueyoshi et al., 1999). Targeted in vivo disruption of mCAR conferred PB responsiveness on the endogenous CYP2B6 gene and heterologous reporter gene constructs containing the CYP2B6 PB-responsive enhancer module (Sueyoshi et al., 1999). In mammalian cell lines and yeast, mCAR is transcriptionally active in the absence of exogenous ligand (Forman et al., 1998; Kawamoto et al., 1999; Sueyoshi et al., 1999). The constitutive trans-activational capacity of mCAR is repressed by the steroids androstanol (5α-androstan-3α-ol) and androstenediol (5α-androst-16-en-3α-ol) (Forman et al., 1998).

In HepG2 cells, the ligand-dependent repression of mCAR is reversed by PB and PB-like inducers (Kawamoto et al., 1999; Sueyoshi et al., 1999). More recently, naturally occurring and xenobiotic mCAR and human CAR (hCAR) agonists were identified (Moore et al., 2000; Tzameli et al., 2000). In similarity to human and mouse PXR, hCAR and mCAR exhibited divergent activation profiles (Lehmann et al., 1998; Moore et al., 2000).

In the liver and primary cultures of hepatocytes, mCAR is sequestered in the cytoplasm and only translocates to the nucleus after exposure of the cell to PB or PB-like inducers. The PB-induced nuclear translocation is uncoupled by concomitant exposure to okadaic acid an inhibitor of phosphatases 1 and 2A, suggesting that dephosphorylation of mCAR is required for its nuclear compartmentalization (Kawamoto et al., 1999). Thus, the PB-induced dephosphorylation of CAR seems to be critical step in CYP2B induction (Kawamoto et al., 1999). In support of this observation, previous reports have documented the importance of phosphorylation status in CYP2B induction (Sidhu and Omiecinski, 1995; Nirodi et al., 1996; Honkakoski and Negishi, 1998). Importantly, nuclear translocation of mCAR in HepG2 cells is spontaneous and does not seem to be dependent on ligand binding and/or modification of phosphorylation status (Kawamoto et al., 1999; Sueyoshi et al., 1999). Thus, cDNA-directed expression of mCAR resulted in transcriptional activation of the endogenous CYP2B6 gene in the absence of inducer. Moreover, although mCAR ligands, namely, androstanol and androstenol, inhibited the trans-activational capacity of mCAR in HepG2 cells they did not block nuclear translocation of the protein (Sueyoshi et al., 1999). Targeted in vivo disruption of mCAR resulted in transcriptional activation of the endogenous CYP2B6 gene in the absence of inducer. Moreover, although mCAR ligands, namely, androstanol and androstenol, inhibited the trans-activational capacity of mCAR in HepG2 cells they did not block nuclear translocation of the protein (Sueyoshi et al., 1999). Targeted in vivo disruption of mCAR resulted in transcriptional activation of the endogenous CYP2B6 gene in the absence of inducer. Moreover, although mCAR ligands, namely, androstanol and androstenol, inhibited the trans-activational capacity of mCAR in HepG2 cells they did not block nuclear translocation of the protein (Sueyoshi et al., 1999). Targeted in vivo disruption of mCAR resulted in transcriptional activation of the endogenous CYP2B6 gene in the absence of inducer. Moreover, although mCAR ligands, namely, androstanol and androstenol, inhibited the trans-activational capacity of mCAR in HepG2 cells they did not block nuclear translocation of the protein (Sueyoshi et al., 1999). Targeted in vivo disruption of mCAR resulted in transcriptional activation of the endogenous CYP2B6 gene in the absence of inducer. Moreover, although mCAR ligands, namely, androstanol and androstenol, inhibited the trans-activational capacity of mCAR in HepG2 cells they did not block nuclear translocation of the protein (Sueyoshi et al., 1999).

**Experimental Procedures**

**Materials.** The mammalian expression vector pSG5 and SuperScript II reverse transcriptase were from Stratagene (La Jolla, CA). PCR primers, TaqMan probes, and PCR master mix were sourced from Applied Biosystems (Foster City, CA). FuGENE 6 transfection reagent was provided by Roche Applied Science (Castle Hill, NSW, Australia).

**Reporter Gene Constructs and Expression Vectors.** Preparation of the chimeric CYP3A4-luciferase reporter gene constructs, including the constructs containing mutated HREs, was described in detail previously (Goodwin et al., 1999). The structure of the p3A4-362 and p3A4-362(7836/7208ins) is shown in Fig. 1A. Site-directed mutagenesis of the prPXR in the CYP3A4 promoter (as shown in Fig. 3) was designed to disrupt both the ER-6 motif as well as an overlapping imperfect DR-4 motif (AACTCAaaggAGGTCA). The hCAR and hRXRα expression vectors pSG5-hCAR and pSG5-hRXRα, respectively, were generously provided by Dr. Steven A. Kliewer (GlaxoSmithKline Research, Research Triangle Park, NC).

**Transient Transfection of Mammalian Cells.** The human hepatoblastoma cell line HepG2 was obtained from the American Type Culture Collection (Manassas, VA) and maintained in antibiotic-free Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum. Cells (1 × 10^6) were inoculated into 24-well plates 24 h before transfection with FuGENE 6 (Roche Applied Science) according to the manufacturer’s instructions. Typically, cells were transfected with 80 ng of luciferase reporter gene construct, 30 ng of β-galactosidase control vector (pCMVβ), and 0 to 100 ng of receptor expression vector (adjusted to 100 ng with pSG5). Subsequently, cells were cultured for 48 h in fresh medium supplemented with 10% charcoal-stripped serum. Luciferase activities were determined on cell lysates using a commercially available system (Promega, Madison, WI). β-Galactosidase assays were performed as described previously (Foster et al., 1988).

**Electrophoretic Mobility Shift Assay.** EMSA of putative hCAR-hRXRα-binding motifs was performed using in vitro transcribed/translated hCAR and hRXRα exactly as described previously (Goodwin et al., 1999).

**Quantitation of CYP3A4 and PXR mRNA Levels.** CYP3A4 and hPXR mRNA levels were examined by real-time reverse transcriptase-polymerase chain reaction (RT-PCR). HepG2 cells were transfected in 60-mm diameter culture dishes, as described above, with the pSG5-hCAR expression vector (0–1600 ng adjusted to 1600 ng with pSG5). The cells were cultured for a further 48 h before extraction of RNA using a commercially available reagent (TRIzol; Invitrogen, Carlsbad, CA). cDNA was synthesized from 5 μg of total RNA using random hexamers and Superscript II reverse transcriptase according to the manufacturer’s instructions. An aliquot of each cDNA synthesis reaction (1 μl) was subjected to PCR amplification using a Prism 7700 real-time PCR platform (Applied Biosystems). Primers and TaqMan probes were as follows: CYP3A4 151 to 323 bp, forward primer TGGTCCTCACCATAAGGGCTTTTGT, reverse primer AAAGGCGCTCCGGTTCAGGTA, probe 210 to 234 bp FAM-AGTGCGGGCTTTTATGATCGTCAACAGC-TAMRA; and hPXR...
DNA sequences for eukaryotic translational start and stop signals, simian virus 40 transcriptional termination and polyadenylation signals, and an intron. Mice carrying the CYP3A4/lacZ transgene was created by microinjection of the DNA constructs into the pronuclei of zygotes harvested from FVB/N strain mice. Microinjection and manipulation of embryos were carried out by standard techniques (Hogan et al., 1994). Stable transgenic mouse lines were established by breeding from transgenic founders identified by Southern analysis and the line used for the present studies was termed CYP3A4-13kb-9/4. This transgenic model will be described in detail elsewhere (G. R. Robertson, B. Goodwin, J. Field, C. Liddle, in preparation). Mice (n = 3/group) were treated with 3 mg/kg TCPOBOP or vehicle alone (corn oil) daily for 3 days by intraperitoneal injection as described previously (Wei et al., 2000) before sacrifice on day 4. β-Galactosidase activity was visualized in cut sections of liver by staining with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

**Statistics.** Multiple comparisons were performed by factorial analysis of variance. Post hoc comparisons between categories were accomplished using the Bonferroni/Dunn test.

**Results**

The ability of hCAR to trans-activate the CYP3A4 5′-flanking region was examined by transient transfection of HepG2 cells. As shown in Fig. 1B, inset, the native CYP3A4 proximal promoter (bases −362 to +53) did not confer hCAR responsiveness on luciferase reporter gene expression. In contrast, the p3A4-362(7836/7208)ins) construct, which contains the XREM region (bases −7836 to −7208) in addition to the proximal promoter, was enhanced in a dose-dependent manner by cDNA-directed expression of hCAR (Fig. 1B). Maximal induction of reporter gene expression (approximately 8- to 10-fold) was observed with 25 to 100 ng of pSG5-hCAR (Fig. 1B). Subsequent cotransfection experiments were performed with 25 ng of pSG5-hCAR.

The interaction between hCAR-hRXRα heterodimers and putative nuclear receptor-binding motifs located in the XREM and proximal promoter regions of CYP3A4 was examined by EMSA using in vitro-translated hCAR and hRXRα (Fig. 2). In keeping with previous reports (Sueyoshi et al., 1999), an everted repeat with a six-base spacer (ER-6) within the proximal promoter known to bind hPXR-hRXRα heterodimers (prPXRE) complexed hCAR-hRXRα. Additionally, a direct repeat with a three-base spacer (DR-3) within the XREM (dNR1) bound hCAR-hRXRα heterodimers (prPXRE) complexed hCAR-hRXRα. Importantly, the affinity of dNR2 and dNR3 for hCAR-hRXRα was substantially lower than either dNR1 or prPXRE (Fig. 2, B and C; data not shown).

The relative affinity of prPXRE, dNR1, and dNR2 for hCAR-hRXRα was examined by competition binding studies. A 5-fold molar excess of unlabeled dNR1 effectively competed with 32P-labeled prPXRE for hCAR-hRXRα (Fig. 2C). Indeed, these competition-binding studies indicated that the affinity of dNR1 for hCAR-hRXRα was approximately 4- to 5-fold higher than that of prPXRE. Thus, CAR-RXRα heterodimers exhibit higher affinity for DR-3 than ER-6 elements. In support of this observation, the intensity of the band-shift resulting from complexation of hCAR-hRXRα with dNR1 was significantly stronger than that seen when prPXRE was used

**Fig. 1.** trans-ACTivation of the chimeric CYP3A4-luciferase reporter gene constructs by hCAR. A, structure of the p3A4-362(7836/7208)ins) and p3A4-362 constructs. The high-affinity hPXR binding motifs in the promoter proximal and XREM region (bases −7836 to −7208) of CYP3A4, prPXRE, and dNR1, respectively, and their positions relative to the transcription initiation site are shown. The nuclear receptor half-sites are delineated by horizontal arrows. B, p3A4-362(7836/7208)ins) construct (80 ng) was transiently transfected into HepG2 cells in the presence of increasing amounts of hCAR, as described under Experimental Procedures. The total amount of expression vector was adjusted to 100 ng by the addition of pSG5-hCAR. The effect of exogenous hCAR expression (25 ng of pSG5-hCAR) on the activity of p3A4-362 is also shown (inset). Luciferase values were normalized to β-galactosidase. Data are mean ± S.D. of four individual transfections from a single representative experiment performed on two separate occasions. **, p < 0.01; ***, p < 0.001, relative to zero hCAR control.

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as a probe (Fig. 2B). In comparison, a 200-fold molar excess of unlabeled dNR2 failed to effectively compete with prPXRE for hCAR-hRXRα binding (Fig. 2C).

The relative contribution of the two high-affinity hCAR-hRXRα-binding motifs, dNR1 and prPXRE, to the hCAR-mediated trans-activation of p3A4-362(7836/7208ins) was examined by site-directed mutagenesis. Cotransfection of pSG5-hCAR and p3A4-362(7836/7208ins) resulted in an 8- to 9-fold induction in reporter gene activity (Fig. 3). Mutation of dNR1 in the context of this construct resulted in a 56% reduction in hCAR responsiveness. Thus, cotransfection of pSG5-hCAR and the p3A4-362(7836/7208ins) construct harboring a mutated dNR1 site resulted in a 4-fold increase in luciferase expression. Similarly, mutation of prPXRE decreased hCAR-mediated trans-activation by approximately 45% (5-fold induction). Although the prPXRE in the context of the p3A4-362 construct (Fig. 1B, inset) has no inherent ability to confer hCAR inducibility on the luciferase reporter gene, this element seems to cooperatively interact with elements within the XREM region. This functional cooperativity was further investigated by linking the XREM region to a minimal thymidine kinase promoter (−105 to +52 bp). The presence of the heterologous promoter completely abrogated hCAR-mediated expression (data not shown), in contrast to the partial loss of expression seen when the prPXRE alone was mutated. Mutation of both dNR1 and prPXRE removed approximately 85% of the wild-type hCAR responsiveness. The residual hCAR inducibility (1.5-fold) of this construct is most probably mediated by the low-affinity hCAR-hRXRα-binding motifs described above, namely, dNR2 and dNR3. These data are summarized in Fig. 3.

In HepG2 cells, exogenously expressed mCAR is known to spontaneously translocate to the nucleus. In this system, nuclear compartmentalization of the receptor and trans-ac-

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Table 1: Oligonucleotide Sequences

<table>
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<tr>
<th>Probe</th>
<th>Sequence</th>
<th>Position</th>
</tr>
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<tbody>
<tr>
<td>prPXRE</td>
<td>ata TGAACCTaag GAAGGTCATgtg</td>
<td>-172 to -149</td>
</tr>
<tr>
<td>dNR1</td>
<td>gaa TGAACCTlgc TGACCTagt</td>
<td>-7736 to -7716</td>
</tr>
<tr>
<td>dNR2</td>
<td>cct TGAATCATatg GGTCTAAGct</td>
<td>-7696 to -7669</td>
</tr>
<tr>
<td>dNR3</td>
<td>ata TATTGTtat TGAACCTatc</td>
<td>-7290 to -7270</td>
</tr>
</tbody>
</table>

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Fig. 2. EMSA of putative hCAR-hRXRα-binding elements. The ability of potential hCAR-response elements to bind hCAR-hRXRα heterodimers was investigated using EMSA as described under Experimental Procedures. A, oligonucleotides used as probes. Putative nuclear receptor half-sites (uppercase) and the position relative to the CYP3A4 transcription initiation site are shown. B, EMSA using radiolabeled prPXRE, dNR1, dNR3, and dNR2. Incubations received 1 μl of in vitro-translated hCAR, hRXRα, or both as indicated. C, hCAR competition binding study. The ability of dNR1 and dNR2 to compete with prPXRE for binding of hCAR-hRXRα heterodimers was investigated by EMSA. Each lane contains 50 fmol of 32P-labeled prPXRE and in vitro-translated hCAR and hRXRα (0.5 μl each), as indicated. Unlabeled competitor oligonucleotides were added to the binding reaction 5-, 25-, 75-, and 200-fold molar excess.
tivation of target genes are ligand-independent. Therefore, we examined the ability of hCAR to regulate expression of the endogenous CYP3A4 gene in HepG2 cells, a cell line that has been reported not to express significant amounts of CYP3A4 mRNA or protein. Using the sensitive technique of real-time RT-PCR CYP3A4-specific transcripts were routinely detected in HepG2 cells. Transient transfection of HepG2 cells with the hCAR expression vector pSG5-hCAR resulted in a dose-dependent increase in CYP3A4 mRNA levels (Fig. 4A). To exclude the possibility that the hCAR-induced increase in CYP3A4 mRNA was secondary to induction of hPXR expression, the abundance of hPXR mRNA was also determined by real-time RT-PCR. No increase in hPXR expression was observed (Fig. 4B).

To provide additional evidence for a functional role for CAR in CYP3A4 regulation, mice bearing a transgene consisting of the CYP3A4 5′-flanking region (−13 kb to +53 bp) linked to a β-galactosidase reporter gene were treated with the mCAR-specific ligand TCPOBOP or vehicle (n = 3/group), as described under Experimental Procedures. In mice receiving vehicle alone reporter gene expression was restricted to a small number of hepatocytes immediately adjacent to central veins or larger hepatic veins. In contrast, mice treated with TCPOBOP exhibited a striking induction of hepatic reporter gene expression, extending outward from central veins, such that approximately one-third of all hepatocytes exhibited positive staining for β-galactosidase (Fig. 5). Identical results were obtained for all three animals within each group.

**Discussion**

The recognition that nuclear receptors capable of recognizing a range of lipophilic xenobiotic and endobiotic ligands can in turn regulate metabolizing and transporting genes has provided a new paradigm to explain how an organism is able to mount an adaptive response to potentially toxic compounds. The most extensively studied receptor in this respect is the PXR, although it is clear that there is substantial overlap between the ligand specificities of PXR and CAR (Moore et al., 2000). CYP3A4 represents a major pathway for clearance of both xenobiotics and endobiotics, and it is clear that PXR is a major mediator of transcriptional induction of this enzyme. To date, however, it has been uncertain whether CAR is also able to trans-activate this enzyme in response to xenobiotic challenge.

In the present study we have shown that hCAR is capable of interacting with the regulatory 5′-flanking region of the CYP3A4 gene. Recent reports suggested that the prPXRE of CYP3A4, an inverted repeat of the AG(G/T)CACA hexamer separated by six nucleotides (ER-6), was capable of conferring CAR responsiveness on a heterologous thymidine kinase promoter (Sueyoshi et al., 1999; Tzameli et al., 2000). However, in the present study this element, in the context of the native CYP3A4 promoter (bases −362 to +53), did not confer hCAR responsiveness in transient transfection studies performed in HepG2 cells. Similar observations have been made for the hPXR-mediated trans-activation of CYP3A4-reporter gene constructs (Goodwin et al., 1999). The ability of hCAR-RXRα and hPXR-RXRα heterodimers to interact with common nuclear receptor-binding motifs (Sueyoshi et al., 1999; Tzameli et al., 2000) suggested that the PXR-responsive XREM region of CYP3A4 (bases −7836 to −7208) may also be capable of mediating trans-activation of CYP3A4 by hCAR. Indeed, when the XREM region was linked to the proximal promoter, a CAR-dependent increase in reporter gene expression was observed.

To further understand the nature of the interaction between hCAR and CYP3A4, EMSA was performed on putative response elements within the proximal promoter and XREM. As described previously (Sueyoshi et al., 1999), the prPXRE complexed hCAR-hRXRα heterodimers. Additionally, there was a high-affinity site within the XREM, denoted as dNR1, that also bound hCAR-hRXRα. Importantly, the DNA binding profile of hCAR-hRXRα delineated in this study was highly homologous to that of hPXR-hRXRα (Goodwin et al., 1999). The importance of these DNA motifs in CAR-directed gene expression was confirmed by site-directed mutagenesis. Importantly, mutagenesis experiments revealed cooperation between dNR1 and the prPXRE, mirroring what we have observed previously for the PXR (Goodwin et al., 1999). This
is despite our finding that the prPXRE alone lacks the ability to mediate CAR-directed gene expression. To further examine this, the CYP3A4 proximal promoter was replaced with a heterologous minimal thymidine kinase promoter. Surprisingly, all CAR-mediated transcription was lost, despite the presence of the XREM. This demonstrates that there is a functional dependence of the XREM on the native promoter that is independent of the prPXRE.

To determine the functional relevance of the interaction between CAR and CYP3A4, we used two entirely different models. First, we sought to determine whether hCAR was capable of regulating the endogenous CYP3A4 gene in HepG2 cells. In this cell line exogenously expressed mCAR is known to spontaneously translocate to the nucleus and activate expression of the PB-inducible CYP2B6 gene (Sueyoshi et al., 1999). In this system, nuclear compartmentalization of the receptor and trans-activation of the target gene are inducer-independent (Kawamoto et al., 1999; Sueyoshi et al., 1999). Transient transfection of hCAR resulted in a significant increase in endogenous CYP3A4 mRNA expression. Moreover, this was not mediated by an indirect effect of hCAR on endogenous PXR expression. These data demonstrate that endogenous CYP3A4 gene in HepG2 cells is sensitive to hCAR-mediated regulation. Second, we performed a functional in vivo experiment. We were able to take advantage of the observation that the PB-like inducer TCPOBOP is a selective ligand for mCAR and does not bind to or activate mPXR (Moore et al., 2000). There is a lack of a similarly selective ligand for hCAR, making experiments performed in human models, such as primary human hepatocytes, difficult to interpret. Thus, mice carrying a CYP3A4 regulatory transgene provide a useful system to determine the ability of CAR to trans-activate CYP3A4. The finding that TCPOBOP was able to remarkably induce hepatic expression of the transgene provides strong evidence in favor of a functional role for CAR in CYP3A4 regulation.

In the mouse, induction of Cyp2b gene expression by a range of xenobiotics, including PB, is mediated by CAR. Targeted disruption of the CAR gene completely abrogates Cyp2b10 induction by PB and TCPOBOP (Wei et al., 2000). In contrast, disruption of the murine PXR gene does not affect PB induction of Cyp3a11, indicating that in the mouse CAR is capable of mediating the inductive response of CYP3A genes to PB as well (Staudinger et al., 2001). Given that hCAR is capable of trans-activating the human CYP2B6 gene in response to PB and PB-like inducers (Sueyoshi et al., 1999), it would be reasonable to assume that CYP3A4 and CYP2B6 would be coordinately regulated on exposure of the hepatocyte to compounds that activate hCAR. Induction of both CYP3A4 and CYP2B6 expression by PB is well documented (Pichard et al., 1990; Schuetz et al., 1993; Kocarek et al., 1995; Chang et al., 1997; Gervot et al., 1999; Sueyoshi et al., 1999). Additionally, the few bone fide CYP2B6 inducers identified to date, including TCPOBOP (Smith et al., 1993) and cyclophosphamide (Chang et al., 1997; Gervot et al., 1999), also up-regulate CYP3A4 expression. Evidently, induction of multiple P450 genes upon exposure to potentially toxic or carcinogenic compounds increases the probability that the organism can successfully metabolize and ultimately excrete xenobiotics.

Goodwin et al. (2001) have shown that both hPXR and hCAR can mediate the PB induction of the CYP2B6 gene in primary human hepatocytes, although hCAR predominates in this respect. It seems likely that the conformation of the HREs in the target genes is the main determinant as to which nuclear receptor predominates. In CYP2B6 there are two adjacent DR-4 motifs, separated by only 16 base pairs (Sueyoshi et al., 1999). In contrast, in the CYP3A4 gene the two predominant PXR/CAR binding motifs are separated by in excess of 7.5 kilobase pairs, an arrangement that seems to favor PXR-mediated induction over CAR (Goodwin et al., 1999, 2001).

In summary, CAR was shown to directly regulate the transcriptional activity of the CYP3A4 gene, both in vitro and in
vivo. trans-Activation of CYP3A4 by CAR was mediated by nuclear receptor-binding motifs located in the distal XREM and promoter proximal regions of the gene. These elements are capable of binding both hCAR-hRXRα and hPXR-hRXRα heterodimers with high affinity. The convergence of hCAR- and hPXR-mediated signaling pathways at common response elements in the CYP3A4 gene clearly demonstrates that cross talk between these two nuclear receptors is probably an important factor in the regulation of this gene. Furthermore, the ability of CAR and PXR to regulate the same gene suggests that these proteins are integral parts of common homeostatic pathways.

References

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