MINIREVIEW

Molecular Mechanisms of Cytochrome P-450 Induction by Xenobiotics: An Expanded Role for Nuclear Hormone Receptors

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Drug exposures can lead to an increased expression of specific cytochrome P-450 proteins (P-450s) as well as of phase II drug-metabolizing enzymes that can greatly augment the metabolism and clearance of therapeutic drugs. Animal models have been used to estimate the potential of drugs to induce P-450s in humans, but differences in drug response among species hinder reliable extrapolation of observations in animals to humans. The past 2 years have witnessed a tremendous growth in our understanding of the mechanisms regulating the induction of drug metabolizing enzymes. These findings shed light on the basis for species differences in these inductive responses and will aid greatly in the development of in vitro methods to identify potential inducers of human drug-metabolizing enzymes.

Drug–drug interactions are especially apparent for P-450 3A enzymes. P-450 3A4 catalyzes the metabolism of numerous commonly used drugs and is the most abundantly expressed P-450 in human liver and small intestine (Guengerich, 1999). Primary cultures of human-derived cells and established cell lines have been instrumental in the identification of potential inducers of human P-450 enzymes. These studies indicate that a variety of structurally diverse compounds induce the expression of P-450 3A4. These include the synthetic glucocorticoid dexamethasone (DEX), the macrolide antibiotic rifampicin (RIF), clotrimazole, erythromycin, lovastatine, omeprazole, and phenobarbital (PB) (Maurel, 1996).

Divergent P-450 3A induction profiles are evident, however, for other species. For example, pregnenolone 16α-carbonitrile (PCN) induces rat P-450 3A23, but rabbit and human enzymes are not increased by PCN treatment. Rabbit and human P-450 3A orthologs are induced by RIF, but this compound is not an efficacious inducer of the rat P-450 enzyme (Wrighton et al., 1985; Kocarek et al., 1995). In the absence of specific information regarding the underlying molecular mechanisms mediating P-450 3A induction, the diversity of inducers and species differences suggested the potential for multiple regulatory pathways that might not be maintained in in vitro systems.

Recent breakthroughs have greatly extended our understanding of the underlying mechanisms regulating P-450 expression and should facilitate the identification of inducers of human drug-metabolizing enzymes. These studies indicate that two members of the nuclear hormone receptor (NHR) family of transcription factors, the pregnane X receptor (PXR) and the constitutively active receptor (CAR), mediate the induction of P-450s 3A and 2B by xenobiotics.

NHR signal transduction pathways were considered attractive mechanisms for the mediation of P-450 induction by xenobiotics, because these receptors are activated by lipophilic ligands with molecular properties similar to those of P-450 inducers. This potential was first realized with the observation that xenobiotics activate an orphan NHR, designated the peroxisome proliferator activated receptor α (PPARα) (Issemann and Green, 1990), and the demonstration that PPARα mediates the induction of P-450 4A enzymes by xenobiotics (Muerhoff et al., 1992). The basic aspects of PPARα action mirror common NHR mechanisms shared with other receptors that mediate P-450 induction. PXR, CAR, and PPARα are all members of the nuclear receptor superfamily of ligand-activated transcription factors that bind DNA response elements as homodimers or heterodimers with a related receptor of the retinoid x receptor (RXR) family.

ABBREVIATIONS: P-450, cytochrome P-450 protein; DEX, dexamethasone; RIF, rifampicin; PB, phenobarbital; PCN, pregnenolone 16α-carbonitrile; NHR, nuclear hormone receptor; PXR, pregnane X receptor; CAR, constitutively active receptor; PPAR, peroxisome proliferator activated receptor; RXR, retinoid x receptor; CYP, cytochrome P-450 gene; GR, glucocorticoid receptor; RU486, mifepristone; DR-n, direct repeat, n denotes the number of spacer nucleotides; ER-6, everted repeat, separated by 6 nucleotides; TCPOBOP, 1,4-bis[(3,5-dichloropyridyl)oxy]benzene; DBD, DNA-binding domain; CTE, carboxyl-terminal extension; COUPTF, chicken ovalbumin upstream promoter transcription factor; LBD, ligand-binding domain; SRC-1, steroid receptor co-activator protein; AF-2, activation function domain-2; ER, estrogen receptor.
PPAR form heterodimers with another NHR, the retinoid X receptor (RXR) and bind to cis-acting control elements upstream of CYP genes. In contrast to many NHRs, PPARs and PXR are activated by a structurally diverse spectrum of ligand-agonists.

The characterization of PXR orthologs from mouse (Kliwer et al., 1998), human (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998), rat, and rabbit (Savas et al., 1999) provided a simple mechanistic explanation for many documented species differences in inducer profiles. It is now clear that the differences in the ability of various agonists to activate each species' PXR parallel the relative effects of these compounds on P-450 3A induction in that species. This progress in understanding the mechanisms of P-450 induction reflects two independent and convergent lines of investigation. The first is the identification of the cis-acting control elements in P-450 genes that mediate these responses, and the second is the characterization of NHRs that recognize these response elements and that are activated by xenobiotics.

**Mapping of DNA Response Elements**

The first indication that the complex regulation of P-450 3A might reflect a common mechanism involving an NHR came from mapping cis-acting control elements in the proximal promoter regions of CYP3A genes. Rat P-450 3A23 is induced by both glucocorticoid receptor (GR) agonists such as DEX and by GR antagonists such as PCN and mifepristone (RU486) (Burger et al., 1992; Kocarek et al., 1995). Using deletion analysis and DNA footprinting, these responses were mapped to each of two sites that conform closely to NHR binding sequences (Table 1) and that are distinct from GR binding motifs (Quattrochi et al., 1995; Huss et al., 1996). One of these sites corresponds to a direct repeat of the hexamer, consensus binding site for NHRs, (A/G)(A/G)(G/T)TCA, separated by three nucleotides (DR-3), that is also demonstrated the presence of a PB-responsive enhancer element at 2400 to -2100 nucleotides upstream of the CYP2B1/2B2 translation start sites. Deletion analyses of the mouse Cyp2b10 promoter using in vitro transfection studies defined a 51-base pair PB-responsive segment. The activa-

<table>
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### TABLE 1
Nuclear receptor response elements in the 5'-flanking regions of P-450 genes

Uppercase sequences are related to the hexamer consensus binding site for nuclear receptors, (A/G)(A/G)(G/T)TCA. The repeated motifs are either direct repeats (DR, arrows pointing in one direction) or everted repeats (ER, arrows pointing away from each other) separated by different numbers of spacer nucleotides (lowercase letter nucleotides). A DR-4 element is also present within the ER-6 motif of the CYP3A4 upstream regulatory region (bold) and was shown to bind CAR/RXR heterodimers (Suyoshi et al., 1999). Genomic sequences originally designated as the cDNA CYP3A1 correspond more closely to the 5'-untranslated sequence of 3A23, and 3A23 mRNA is highly induced by DEX, whereas 3A1 and 3A2 mRNAs are not (Komori and Oda, 1994).
tion of a reporter construct containing this 51-bp enhancer element by several inducers paralleled the induction of P-450 2B10 mRNA by these compounds in the same cell system. The inducers included PB, chlorpromazine, metyrapone, methoxychlor, and 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) (Honkakoski et al., 1998a). Both the rat and mouse PB-responsive elements contain a nuclear factor 1 binding site, flanked by two sites, designated NR1 and NR2, that contain imperfect direct repeats of NHR consensus binding sites separated by four nucleotides (DR-4). However, the nuclear factor-1 binding site is not essential for the in vivo activation of the CYP2B2 transgene by PB (Ramsden et al., 1999). In contrast, mutations to either the NR1 or the NR2 site diminished the extent of PB induction, and disruption of both sites produced a loss of the response in cellular transfection assays (Honkakoski et al., 1998b). The effect of the disruption of the NR1 site was greater than the effect of alterations to the NR2 site. In addition, altering the spacing of the repeat or disrupting either of the hexameric NHR binding sites reduced enhancer activity (Honkakoski et al., 1998b). These results indicated that the PB response is mediated by the NR elements and that this response depends on the presence of the native hexameric repeats and their spacing.

NHR–DNA Interactions

The binding of NHRs to the hexameric NHR core binding sequences found in response elements is mediated by a highly conserved, autonomous DNA binding domain (DBD) located in the N-terminal portion of NHRs (Mangelsdorf et al., 1995). One of two zinc finger motifs in the DBD interacts directly with the hexameric sequence in the major groove of the DNA double helix. This is shown in Fig. 1 by the crystallographic structure of the RevErbα–DBD bound to a DR-2...

![Fig. 1. Two RevErbα–DBDs complexed to the Rev-DR-2 response element. A, the DNA response element is shown as a CPK model, and the RevErbα DBDs are shown as ribbon traces. The DNA strand in the 5' to 3' direction running from top to bottom is shown in light gray, whereas the complementary strand is dark gray. Each DBD of the homodimer is shown in rainbow colors tracing through the polypeptide from the N (blue) to the C terminus (orange). The coordination of four cysteine residues to a zinc atom (red spheres) leads to the formation of a loop and initiates a helix that together constitute a zinc finger motif. The N-terminal zinc finger (blue to green) of each DBD contacts the major groove of the DNA duplex at the sequence AGGTCA. The sequence of this zinc finger is highly conserved among NHRs. The CTE (orange) following the second zinc finger interacts with the minor groove at the sequence AACT upstream of the AGGTCA motif shown in C. The second, C-terminal zinc finger (light green to yellow) of the upstream DBD interacts with the CTE of the downstream DBD. When the homodimer was modeled on DR-3, -4, and -5 elements, the CTE of the downstream DBD produced steric clashes that make binding less favorable. The model was rendered from the Brookhaven Protein Data Bank coordinates 1AGY (Zhao et al., 1998). B, human RevErbα DBD protein sequence. Amino acid residues 123 to 216 encompassing the DBD of RevErbα have been used for co crystallization with DNA by Zhao and coworkers (1998). This portion of the protein contains eight cysteine residues (bold letters) that are conserved throughout NHRs. Each zinc atom coordinates with four cysteine residues in each zinc finger. The sequence of the N-terminal zinc finger (Zn1) is highly conserved, whereas the sequence of the second zinc finger (Zn2) and the CTE is more varied among NHRs. The sequence corresponding to these three regions is shown on separate lines. The reduced conservation in the second zinc finger affects the interactions between the DBDs and provides secondary interactions with the DNA binding sites. As a result, sequence differences contribute to the distinct preferences of each receptor for binding sites exhibiting different spacings and orientations of the AGGTCA binding site (Gronemeyer and Moras, 1995). C, Rev-DR-2 response element. The sequence of the double stranded oligonucleotide co crystallized with the DBD dimer is a direct repeat (bold letters) separated by two spacer nucleotides. The extended binding site (italics) upstream of the zinc finger binding site provides contacts for the CTE of RevErbα. This 5'-extension of the zinc finger-binding site is also recognized by PPARα, and the Rev-DR-2 can function as a response element for PPAR/RXR heterodimers (Hsu et al., 1998).
element. For some receptors, such as RevErbα (Zhao et al., 1998) and PPARs (Palmer et al., 1995), additional binding interactions occur between nucleotides in the minor groove immediately upstream of the direct repeat and a C-terminal extension (CTE) of the nuclear receptor DBD, as shown in Fig. 1.

The DNA binding of PPAR, PXR, and CAR requires the dimerization of each receptor with the nuclear receptor RXR. Each receptor in the dimer contacts one of the NRH binding sites of the repeat forming the enhancer (Fig. 1). Differences in the spacing and orientation of the NRH binding sites in the repeat contribute to NRH specificity and reduce competition for binding by other receptors (Gronemeyer and Moras, 1995). The cis-acting elements found in the promotors of CYP genes that are recognized by PPAR, PXR, and CAR are presented in Table 1. PPARs has been shown to preferentially target DR-1 response elements that include a conserved 5′-extension such as the enhancers found in the promotors of CYP4A1 (Aldridge et al., 1995) and CYP4A6 (Muerhoff et al., 1992; Palmer et al., 1995). In contrast, in vitro translated PXR from mouse (Kliever et al., 1998), human (Bertilsson et al., 1998; Blumberg et al., 1998), or rabbit (M.-H. Hsu and E. F. Johnson, unpublished observation) display a preference for binding to DR-3, DR-4, and ER-6 elements in electrophoretic mobility shift assays. These response elements are found in the promotors of the CYP3A23, -3A4, -3A7, and -3A6 genes (Table 1). This conservation of binding specificity reflects a high degree of DBD homology (93–94% protein sequence identity) across species, which generally is seen for NRH paralogs. CAR recognizes an imperfect DR-4 element that overlaps the ER-6 elements seen in the CYP3A promotors as well as the DR-4 elements that have been identified in CYP2B enhancer regions (Sueyoshi et al., 1999). CAR can also bind to DR-5 elements and mediate transcriptional activation through these elements (Baes et al., 1994).

Although features of DNA response elements provide a means for targeting specific receptors to the genes that they regulate, competition with other NRHs for the same binding site can occur. Competitive binding has been reported for the DR-3 response element of CYP3A23 between PXR and the abundant nuclear receptor chicken ovalbumin upstream promoter transcription factor (COUPTF) (Huss and Kasper, 1998; Ogino et al., 1999). The consequence of binding site competition by different nuclear receptors on transcriptional activation depends on the relative expression level and response element affinity of the receptors involved. In cells cotransfected with PPARα and either COUPTF1 (Miyata et al., 1993) or COUPTF II (Palmer et al., 1994), the response to peroxisome proliferators is suppressed, indicating that COUPTFIs can also compete with PPARα for binding and affect activation of PPARα responsive genes. The RevErbα DR-2 element shown in Fig. 1 also binds PPARα, and RevErbα antagonizes PPARα transcriptional activation mediated by the DR-2 element (Hsu et al., 1998).

Ligand-Dependent trans-Activation

The ligand-dependent activation of transcription by nuclear receptors is mediated through the ligand-binding domain (LBD) located at the C-terminal portion of NRHs (Mangelsdorf et al., 1995). Ligand-dependent transcriptional activation is associated with the recruitment of coactivator proteins and, in some cases, with the release of corepressor proteins. Various coactivators have been identified and exhibit differential affinities for different nuclear receptors. Ligand-induced conformation changes in the LBD alter the position of the C-terminal helix of NRHs, where the activation function domain-2 (AF-2) resides (Fig. 2). This change in position facilitates the recruitment of coactivators. The interaction of coactivators such as the steroid receptor coactivator-1 (SRC-1) with NRH LBDs involves a conserved LXXLL motif that occurs in multiple copies in coactivator proteins (Glass et al., 1997). The crystallographic structure of PPARγ complexed with a single domain of SRC-1 indicates that the helix containing the LXXLL motif is sequestered between the C-terminal end of helix 12 and the N-terminal end of helix 3 of PPARγ (Fig. 2, bidirectional arrow) in an orientation that allows the LXXLL motif to interact with the LBD (Nolte et al., 1998). PCN-dependent binding of SRC-1 to PXR has also been demonstrated (Kliever et al., 1998).

Although this paradigm of regulation is evident for the activation of PXR and PPARs by xenobiotics, the role of ligand-dependent activation of CAR is less clear. The initial characterization of human CAR, designated MB67 (Baes et al., 1994), and mouse CAR (Choi et al., 1997) indicated that neither require the addition of exogenous ligands for activation of reporter gene expression. Similarly, CAR exhibits strong positive trans-activation of reporter constructs containing the Cyp2b10 NR1 element that is not increased further by the addition of PB (Honkakoski et al., 1998b).

Recently, androstenol and androstanol were shown to inhibit CAR binding to SRC-1 and the capacity of CAR to activate transcription (Forman et al., 1998). It seems likely that these androstane metabolites bind to CAR and invoke conformational changes that result in the dissociation of coactivators such as SRC-1. This effect could be similar to the changes observed for the estrogen receptor (ER)–LBD by the binding of the antagonist 4-hydroxy-tamoxifen (Brzozowski et al., 1997) that displaces helix 12 and disrupts the SRC-1 binding site (Fig. 2).

In contrast to the effect of cotransfected CAR on reporter genes, the heterologous expression of CAR in human HepG2 cells leads to PB induction of the endogenous CYP2B6 gene and provides the most direct evidence that CAR mediates PB induction. The basal expression of P-450 2B6 is undetectable in HepG2 cells. However, after transfection with a CAR expression plasmid, P-450 2B6 mRNA is readily detectable in untreated cells. Treatment of the transfected cells with androstenol represses P-450 2B6 mRNA levels, whereas cotreatment with androstenol and the potent inducer TCPOBOP increased P-450 2B6 mRNA levels (Sueyoshi et al., 1999). These results indicate a role for CAR in the regulation of P-450 2B6 induction and could reflect a mechanism for trans-activation that involves displacement of androstenol by PB and reactivation of coactivator binding.

PB has not yet been shown to bind to CAR, and alternative regulatory mechanisms are possible. As indicated earlier, CAR is likely to act directly on CYP2B genes as it binds to the NR-1 and the NR-2 elements found in PB-responsive P-450 genes. However, PB may modulate the extent of DNA binding activity by CAR in nuclei. Negishi and coworkers (Honkasoski et al., 1998b) observed that the binding of mouse liver nuclear proteins to the Cyp2b10-NR1 site increases after PB treatment. Fractionation and immunoblotting of these proteins indicated that CAR was increased in the nucleus after
PB treatment, suggesting that PB could control CYP2b10 transcription by regulating the nuclear accumulation of CAR.

**Ligand Diversity**

In contrast to the steroid hormone receptors, the NHRs that are involved in xenobiotic-induced P-450 expression exhibit a more structurally diverse spectrum of agonists and lower ligand-binding affinities. The ligand-binding cavities of the PPARγ and PPARα LBDs exhibit volumes that are approximately 3-fold larger than those seen for the progesterone receptor, the ER (Fig. 2), or the retinoic acid receptor (Nolte et al., 1998; Oberfield et al., 1999). The larger volume is likely to underlie the capacity of PPARγ to bind structurally diverse ligands and display a wider range of ligand-binding affinities. Determination of the structure for PPARγ with a partial agonist highlighted the potential for differences in the ability of bound ligands to provoke conformational changes favoring coactivator binding, which can impact the transcriptional competence of the bound receptor complex. Based on the diversity and relatively large size of the PXR agonists, it seems possible that PXR also could exhibit a relatively large ligand-binding cavity and display similar variations in ligand-binding orientation and transcriptional competence. In addition, the much greater sequence variation of PXR and CAR LBDs among species, compared with PPAR orthologs, suggests that such differences could make significant contributions to species-specific responses in PXR and CAR mediated pathways.

The agonist concentrations needed to activate PXR in cellular assays are generally >1 μM. This is similar to the concentrations that are required to induce P-450 3A in cultured hepatocytes. The molecular diversity and the low potency of PXR agonists resemble the peroxisome proliferators that are ligands for PPARs in that they also exhibit a broad molecular diversity and similar potencies in cell-based assays (Forman et al., 1997; Kliwer et al., 1997). Relatively high concentrations of PB-like inducers also are required for reversing the inactivation of CAR by androstanone metabolites. It is not clear whether the constitutive activity of CAR results from a bound high-affinity ligand. However, several relatively high-affinity ligands for PPARs have been identified that exhibit binding constants between 10 and 100 nM. These include synthetic agonists such as BRL-49563 for PPARγ and naturally occurring compounds such as 8(S)-hydroxyeicosatetraenoic acid, which is the most potent natural agonist identified thus far for PPARs, with a binding constant of 90 nM (Forman et al., 1997). It is possible that higher-affinity ligands also will be identified for PXR.

**Species Differences as a Result of NHR Action**

The differential activation profiles generated by PXR from human (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998), mouse (Kliwer et al., 1998; Lehmann et al., 1998), rat, and rabbit (Savas et al., 1999) are summarized in Table 2 for compounds that have been investigated in all four species. These results correspond generally to the differences noted earlier in animal studies or cultured hepatocytes (Wrighton et al., 1985; Kocarek et al., 1995). RIF is an efficacious activator of human and rabbit, but not of rat, PXR. However, mouse PXR is poorly activated by RIF (Lehmann et al., 1998), although P-450 3A is induced in mice by RIF (Wrighton et al., 1985). PCN activates rat and mouse PXR, but not human and rabbit PXR, whereas RU486 activates human, mouse, and rat PXR, but not rabbit PXR. Thus,

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**Fig. 2.** Crystallographic structures of the PPARγ and the ER LBDs. Ribbon diagrams depict the structures of the PPARγ-LBD complexed to the weak agonist GW0072, the ER-LBD liganded with the agonist 17β-estradiol (estradiol), and the ER-LBD with the bound antagonist 4OH-tamoxifen. The ligands are rendered as CPK atoms. The LBDs exhibit a similar overall topology evident for other NHR LBDs. The PPARγ-LBD complex is shown with CPK atoms. The LBDs exhibit volumes that are approximately 3-fold larger than those seen for the progesterone receptor, the ER (Fig. 2), or the retinoic acid receptor (Nolte et al., 1998; Oberfield et al., 1999). The larger volume is likely to underlie the capacity of PPARγ to bind structurally diverse ligands and display a wider range of ligand-binding affinities. Determination of the structure for PPARγ with a partial agonist highlighted the potential for differences in the ability of bound ligands to provoke conformational changes favoring coactivator binding, which can impact the transcriptional competence of the bound receptor complex. Based on the diversity and relatively large size of the PXR agonists, it seems possible that PXR also could exhibit a relatively large ligand-binding cavity and display similar variations in ligand-binding orientation and transcriptional competence. In addition, the much greater sequence variation of PXR and CAR LBDs among species, compared with PPAR orthologs, suggests that such differences could make significant contributions to species-specific responses in PXR and CAR mediated pathways.

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there are significant parallels among the species differences in the induction of P-450 3A enzymes and the properties of each species’ PXR.

Other species differences are also evident. Clotrimazole activates human PXR, but not mouse PXR. In addition, the progesterone metabolite 5β-pregnane-3,20-dione activates human, mouse, and rat PXR, but not rabbit PXR. Rabbit, rat, and mouse PXR are activated by DEX. Although DEX also induces P-450 3A4 in human hepatocytes (Strom et al., 1996), there are discrepancies regarding the efficacy of this compound to activate human PXR. Two laboratories did not observe an activation of human PXR by DEX (Bertilsson et al., 1998; Blumberg et al., 1998), although another laboratory reported that DEX activated human PXR (Lehmann et al., 1998). The origin of this discrepancy is unclear.

Species differences in PXR trans-activation profiles probably reflect an unusual degree of LBD divergence. Nuclear receptor orthologs generally share >90% protein sequence identity in their LBDs among mammalian species. For example, human and mouse PPARα orthologs show 92% protein sequence identity in their LBDs. This close sequence relationship is generally reflected in similar agonist profiles for orthologs from different species. However, different paralogs that have arisen by gene duplication and subsequent divergence usually exhibit 60 to 70% protein sequence identity in their LBDs, and this reduced conservation results in different agonist profiles for each, as exemplified by PPARα and PPARγ. Pair-wise comparisons of amino acid sequences of PXR LBDs among rabbit, human, and rat or mouse indicate that they exhibit from 77 to 82% identity (Savas et al., 1999). In contrast, the rat and mouse PXR LBD sequences are 97% identical (Savas et al., 1999; Zhang et al., 1999), suggesting a much greater likelihood for similar activation profiles between mice and rats. There is no evidence for PXR paralogs in any species. This, together with the higher degree of sequence divergence among PXR LBDs and the distinct agonist profiles, suggests that the evolutionary selective pressure is more relaxed for PXR orthologs than that seen for orthologs of other NHRs, including PPARs. This also appears to be the case for CAR. The mouse and human forms of CAR display low sequence identity (72%) between their LBDs, and this increases the likelihood for divergent ligand-binding profiles across species. [A unified nomenclature system was recently proposed for NHRs based on protein sequence conservation in the DNA and ligand-binding domains (Nuclear Receptors Nomenclature Committee, 1999). A receptor family is given an arabic numeral, followed by a capital letter denoting related proteins in the group, followed by an arabic numeral for individual paralogs. Accordingly, peroxisome proliferator activated receptor α (PPARα) is designated NR1C1, human and mouse PXR represent orthologous genes and are called NR1I2. Human CAR (or MB67) and mouse CAR are considered different paralogs and are designated as NR1I3 and NR1I4.

In contrast, PPAR orthologs are highly homologous and display similar ligand-activation profiles among mammalian species. PPARα has been shown to mediate the toxicity resulting from peroxisome proliferator exposure in susceptible species (Lee et al., 1995). The elevated expression of PPARα in mouse liver, compared with the levels found in nonresponsive species such as guinea pigs (Bell et al., 1998) and humans (Palmer et al., 1998), could contribute to the pathologic consequences of exposure observed in mice. High expression levels of PPARα could diminish competition with other NHRs for regulatory elements or could result in the misappropriation of marginal response elements by PPARα. Nonproductive RNA splicing was shown to contribute to the low expression levels of wild-type functional receptor in humans (Palmer et al., 1998).

Conclusions

The mechanisms governing xenobiotic modulation of P-450 expression and species differences in these responses are relevant considerations in risk assessment and for the minimization of adverse drug effects. It is clear that NHRs, including PPAR, PXR, and probably CAR, play an important role in the regulation of P-450s by xenobiotics and contribute to species variations in xenobiotic-induced drug responses and xenobiotic toxicity. PXR exhibits striking differences among species in agonist specificity. The activation profile produced by each PXR ortholog is similar to the activation profile of P-450 3A target genes in the corresponding species. Rodent, rabbit, and human PXR exhibit an unusually low degree of sequence conservation in their LBDs, and the differential induction profiles obtained in these species probably reflect this sequence divergence. A similar degree of sequence divergence is evident for the LBD of CAR. Such differences definitely confound the problems associated with the extrapolation of animal experimental data to humans and undermine the usefulness of animals for predictive assessment of human responses. This reinforces the use of human-derived systems such as human cell lines that contain the necessary regulatory pathways or primary human hepatocytes to study the regulation of P-450 expression. Certainly, the use of assays based on the human receptors provides an attractive means for testing the efficacy and potency of chemicals to induce human P-450 enzymes.

Table 2

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<td>–</td>
<td>–</td>
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<tr>
<td>Clofibrate, 10 μM</td>
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References


