

Minireview

CYP3A REGULATION: FROM PHARMACOLOGY TO NUCLEAR RECEPTORS

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(Received October 27, 2000; accepted February 12, 2001)

This paper is available online at <http://dmd.aspetjournals.org>

ABSTRACT:

Among the human liver cytochrome P450s (P450s), a family of microsomal hemoproteins responsible for catalyzing the oxidative metabolism of clinically used drugs and environmental chemicals, attention has been focused on CYP3A, a form that is the most abundant and is inducible by many of its substrates. From early pharmacological studies that demonstrated induction of CYP3A by glucocorticoids and, paradoxically, by antiglucocorticoids, the existence of a nonclassical glucocorticoid receptor mechanism was inferred and prompted research that culminated in the identification of a unique member of the nuclear receptor family, the preg-

nane X receptor (PXR; NR1I2). It has become increasingly evident that PXR as well as other nuclear receptors mediate CYP3A induction in a unique and complex manner including inducibility by structurally diverse compounds and striking interspecies differences in induction profiles. Future understanding of the role of nuclear receptors in regulating expression of CYP3A and other genes of the P450 family offers an exciting promise of further defining the physiologic function and interindividual differences of CYP3A in health and disease.

Cytochrome P450s (P450¹) constitute a multigene family of hemoproteins responsible for the metabolism of numerous xenobiotics, including therapeutic drugs, environmental chemicals, and dietary constituents, as well as such endogenous compounds as steroids and bile acids (reviewed in Gonzalez et al., 1993). Early studies at both the pharmacological and biochemical levels recognized that this large repertoire of catalytic activities most likely represented multiple enzymes rather than a single isoform. This is clearly the case, with an estimated 50 or more individual P450 enzymes present in any given mammalian species. While some P450 enzymes (e.g., those involved in steroid biosynthesis) exhibit specific catalytic activities, many others display broad, but somewhat overlapping, substrate specificities (Nelson, 1999).

A unique feature of this large family of enzymes is that some members show increased expression upon xenobiotic challenge to the organism. Several decades have passed since the first inducible P450 (now classified as CYP2B) was identified in the liver of rats exposed

to phenobarbital (reviewed in Gonzalez et al., 1993). It was postulated that a receptor protein mediated the induction, but this conclusion was confirmed only recently (discussed below). Later, a second unique P450 (now classified as CYP1A), inducible by the arylhydrocarbon, 3-methylcholanthrene, was identified, and its regulation by a ligand-activated receptor described. In the early 1980s, several groups of investigators identified a third distinct form of P450 (now classified as CYP3A), one inducible by steroidal chemicals. These early investigations provided an understanding of many of the pharmacological, biochemical, and biophysical properties of P450 enzymes, including protein structure, substrate specificities, and enzyme kinetics. Through the application of molecular biology, investigators next began to uncover features of P450 gene structure and function that could account for the differences in pharmacologic events described in earlier studies. CYP3A was found to be a gene subfamily composed of multiple forms of CYP3A enzymes as characterized by immunochromatography, catalytic activities, and cDNA cloning and expression.

CYP3As, the liver microsomal enzymes responsible for the oxidative metabolism of numerous clinically used drugs, is known to be induced by a variety of compounds, including naturally occurring and synthetic glucocorticoids (e.g., dexamethasone, Dex), pregnane compounds (e.g., pregnenolone 16 α -carbonitrile, PCN), and macrolide antibiotics (e.g., rifampicin, RIF) (reviewed in Gonzalez et al., 1993). Multiple forms of CYP3A are present in rat and mouse liver and a single form in rabbit, while four CYP3A genes have been reported for humans (Gonzalez et al., 1993; Domanski et al., 2001). CYP3A cDNAs from various species have been used to probe for patterns of basal and inducible expression, for tissue distribution, and for interspecies differences in gene expression. More recently, techniques and reagents have been developed to isolate and characterize gene pro-

This work was supported by United States Public Health Service Grant ES05744.

¹ Abbreviations used are: P450, cytochrome P450; CAR, constitutive androstane receptor; COUP-TF, chicken ovalbumin upstream promoter transcription factor; CTZ, clotrimazole; Dex, dexamethasone; GC, glucocorticoids; GR, glucocorticoid receptor; GRE, glucocorticoid response element; HNF, hepatocyte nuclear factor; LBD, ligand binding domain; PB, phenobarbital; PBREM, PB response element module; PCN, pregnenolone 16 α -carbonitrile; PXR, pregnane X receptor; RIF, rifampicin; RXR, retinoid acid X receptor; SXR, steroid and xenobiotic receptor; XREM, xenobiotic responsive element module.

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TABLE 1

Response elements in the 5'-flanking region of the *CYP3A23* geneSequence and nomenclature of binding sites for the PXR and other nuclear receptors are given for *CYP3A23* and *CYP3A2* where noted.

Sequence	Site	Nuclear Receptor	Reference
→ TGAACT tca TGAACT ←	Fp1 Site-B DexRE-2 DR-3 6βA-B (<i>CYP3A2</i>)	PXR, COUP-TF	Quattrochi et al., 1995 Huss et al., 1996 Huss and Kasper, 1998 Miyata et al., 1995
← TTAACT caaagg AGGTCA →	Site-C DexRE-1 6βA-C (<i>CYP3A2</i>)	Unknown: candidates are PXR, COUP-TF	Huss and Kasper, 1998 Miyata et al., 1995
GTACCAAAGTCCAC GTACCAAAGTCCAG (<i>CYP3A2</i>)	Site-A 6βA-A	HNF-4	Huss and Kasper, 1998 Huss et al., 1999
CACGTG	Site-A	USF-1	Huss et al., 1999
GGCACA gca TGTAT	Footprint I (GRE)	GR	Pereira et al., 1998

motors and to develop a system to analyze promoters for inducer response elements. These genetic techniques, such as sensitive reporter gene vectors, techniques for transfecting DNA, and the establishment of primary cultures of hepatocytes as a cell model system for analysis of transfected DNA, have contributed greatly to understanding gene structure and regulation, including definition of the *CYP3A* induction process. These efforts have culminated in exciting recent recognition that nuclear receptors such as those characterized for steroid hormones are agents of *CYP3A* induction. Thus, the extensive data accumulated from the study of this group of physiologic ligand-activated transcription factors can now be extended to the drug-metabolizing system.

The purpose of this review is to evaluate the current concepts of *CYP3A* gene induction by xenobiotics, with special emphasis on the emerging role of nuclear receptors in this process. It now seems that there may be extensive cross talk among the nuclear receptor family members, as well as involvement of steroid receptors, such as the glucocorticoid receptor (GR), and other transcription factors. The fascinating question of "what regulates the regulators?" will also be discussed. Finally, we feel it prudent to discuss the implications of induction of *CYP3A* gene expression for disease states and risk assessment.

Inducibility of *CYP3A* Gene Expression

In humans, *CYP3A4* is believed to play the central role in drug metabolism since it is responsible for the metabolism of the largest number of currently used drugs (Watkins, 1994). Enhanced transcription of *CYP3A* occurs primarily in the liver and intestines in response to xenobiotic exposure. Agents known to activate *CYP3A* transcription include steroid hormones having either glucocorticoid or antigluco-corticoid activities, macrolide antibiotics, imidazole antifungal agents, and phenobarbital and phenobarbital-like agents such as polychlorinated biphenyls and organochlorine pesticides (reviewed in Gonzalez et al., 1993). This observation, coupled with the remarkable versatility of *CYP3A* catalytic activities, creates a potential for drug-drug interactions. Extrapolating data from studies utilizing rodents has proven to be an unreasonable approach for risk assessment because there is a remarkable species-specific induction profile characteristic of this family of P450s (Wrighton et al., 1985). For example, PCN, a strong inducer of *CYP3A* in the rat, does not induce *CYP3A6* in the rabbit, while RIF, a strong inducer of rabbit *CYP3A6*, is not an inducer in the rat. An understanding of the mechanism underlying this

phenomenon required cloning and characterization of the *CYP3A* promoters and identification of inducer response elements and the factors that interact with these promoter sequences.

Characterization of the *CYP3A* Promoter

Studies of the rank order of agonist potency and efficacy, of agonist-antagonist relationships, and of the time course of induction of *CYP3A* in cultured hepatocytes by various steroid hormones led to the conclusion that glucocorticoids (GC) induced *CYP3A* by a nonclassical GR mechanism probably involving a separate receptor or recognition system (Schuetz and Guzelian, 1984). Verifying the presence of a nonclassical glucocorticoid pathway by detailed molecular studies required the isolation of the cloned genomic DNA encoding the *CYP3A*² promoter (Burger et al., 1992). The first functional characterization of the rat *CYP3A* promoter involved transient transfections of various chimeric reporter gene constructs into primary cultures of rat hepatocytes (Burger et al., 1992). These investigators reported that the Dex/PCN response element resided within 164 bp of the start of transcription, and demonstrated that this region of the *CYP3A* gene maintained the same Dex responsiveness and synergy with PCN that was previously shown in the intact rat liver (Schuetz and Guzelian, 1984). These deletion studies confirmed earlier investigations concluding that the induction of *CYP3A* apparently utilized a nonclassical glucocorticoid receptor pathway (Schuetz and Guzelian, 1984), in that reporter gene activation occurred only at high doses of GC, and that RU486, a potent GR antagonist, induced *CYP3A* reporter gene activity, while it blocked GC induction of murine mammary tumor virus promoter-driven reporter activity. The lack of binding sites for the GR in the Dex responsive gene fragment further supported the hypothesis that the *CYP3A* gene is regulated by steroids through a mechanism that differs from the classical GR-mediated pathway (Schuetz and Guzelian, 1984).

Subsequent studies in primary nonproliferating adult rat hepatocytes (Quattrochi et al., 1995) and in a rat hepatoma cell line (Huss et al., 1996) led to the identification of the rat *CYP3A23* Dex/PCN response element. Although both groups of investigators mapped similar response elements, Quattrochi et al. (1995) identified a single

² The sequence of the 5'-noncoding region of the rat genomic clone used in previous studies referred to as *CYP3A1* is completely identical to that of the recently isolated *CYPRL33* (Komori and Oda, 1994). This gene, inducible by Dex, PCN, and phenobarbital, has been assigned the name *CYP3A23* (Nelson et al., 1996).

20-bp element (referred to as Fp1, Table 1) as conferring Dex or PCN inducibility, whereas Huss et al. (1996) reported inducible activity only when this same element (referred to as site B or DexRE-2) was linked to an additional upstream sequence (referred to as site C or DexRE-1, Table 1). The discrepancy likely lies in the use of primary hepatocyte cultures versus culture-adapted, continuously replicating hepatoma cell lines that might differ in the availability of transcription factors. Tests of patterns of binding of nuclear proteins to the *CYP3A23* Dex/PCN DNA response element gave identical results in the absence or presence of inducers, suggesting that induction occurred via a mechanism in which the unliganded protein factor was constitutively bound to DNA, much as has been reported for the retinoid acid X receptor (RXR) family of nuclear receptors (Evans, 1988). Indeed, the *CYP3A23* response element contained two copies of the half-site, AGG(T)TCA, which defines the consensus binding site for this family of nuclear receptors. Furthermore, the GR did not interact with the DNA at the *CYP3A23* response element. Patterns of nuclear protein binding to the rat *CYP3A23* promoter were similar to those reported by Miyata et al. (1995), who tested the rat *CYP3A2* gene. These authors identified three footprints of liver-specific binding proteins, one of which showed DNA sequence homology to the liver-enriched transcription factor HNF-4. Further characterization of the *CYP3A23* response element and its binding proteins revealed that "site A" was bound and activated by the nuclear receptor HNF-4, and that the chicken ovalbumin upstream promoter transcription factor (COUP-TF) could interact in vitro with "sites B and C", leading to the conclusion that the GC inducibility of *CYP3A* genes involves multiple binding sites for members of the nuclear receptor superfamily (Huss and Kasper, 1998; Quattrochi et al., 1998).

Species-Specific Induction of *CYP3A* Gene Expression

The early observation of interspecies differences in the induction of *CYP3A* led to studies that addressed whether these differences were due to gene structure or to an endogenous cellular factor. For example, the response element located in the rat *CYP3A23* promoter [Fp1 (Quattrochi et al., 1995) or DexRE-2 (Huss et al., 1996)] is composed of two consensus half-sites organized as direct repeats separated by three nucleotides. Subsequent cloning and sequencing of the promoters of the rabbit *CYP3A6* and human *CYP3A4*, *CYP3A5*, and *CYP3A7* genes identified distinct sequences related to the rat *CYP3A23* response element (Barwick et al., 1996; Pascucci et al., 1999).

These sequence comparisons could be interpreted in one of two ways: 1) the sequences of the response elements are closely related so that differences in species inducibility are conferred by the same transcriptional activator interacting at these DNA sites, or 2) the sequences are sufficiently different so that a transcriptional activator unique to each species interacts at these sites. To distinguish between these alternatives, Barwick et al. (1996) performed a suitably informative trans-species comparison study in which rat, rabbit, and human *CYP3A* response elements were transfected into primary cultures of rat or rabbit hepatocytes treated with one of three inducers, Dex, PCN, or RIF. They found that activation of the *CYP3A* response element-reporter gene acquired the induction characteristics of the cell type; for example, RIF was able to induce the rat *CYP3A23* response element when expressed in the rabbit hepatocyte cultures, and PCN induced the rabbit and human *CYP3A* response elements when expressed in rat cells. These studies clearly established the transcription factor, rather than the *CYP3A* promoter sequence, as the agent conferring species-specific induction on *CYP3A* gene transcription.

PXR and Its Role in Species-Specific Induction of *CYP3A*

PXR and the *CYP3A* Response Element. Identification of the Dex/PCN response element of the *CYP3A* genes provided the initial firm evidence for involvement of a nuclear receptor in *CYP3A* induction. Cloning of PXR (NR1I2), a PCN-activated nuclear receptor in mouse liver, by Kliewer's group at Glaxo Wellcome (Kliewer et al., 1998) confirmed this conclusion. Kliewer's group and others demonstrated that PXR, named the pregnane X receptor because of its strong activation by pregnane compounds, seemed to mediate *CYP3A* induction not only in mice, but through its homologous counterparts in rat, rabbit, and humans, as well (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998; Zhang et al., 1999; Savas et al., 2000). The human PXR receptor is also referred to as steroid and xenobiotic-sensing receptor (SXR) (Blumberg et al., 1998) or as the pregnane-activated receptor (Bertilsson et al., 1998). Because there is no generally accepted nomenclature for the human receptor, for the purposes of this review we have chosen to use the term "human PXR". Mutational analysis of the previously identified *CYP3A* response elements established the PXR binding site of the rodent *CYP3A* promoter/enhancer as a direct repeat, DR-3 (TGAACn3TGAAC) (Kliewer et al., 1998), and the rabbit and human *CYP3A* response elements as everted repeats (TGAACn6AGGTCA) (ER6; Lehmann et al., 1998) or inverted repeats (IR6; Blumberg et al., 1998). The PXR forms a heterodimer with RXR, a requirement for binding and activation. The heterodimer formed between PXR and RXR can interact with either the DR-3 or ER6/IR6 elements in *CYP3A* genes.

The human *CYP3A4* gene appears to have not only the ER6 response element, located in the proximal promoter (Barwick et al., 1996), but also a second, distal xenobiotic responsive element module (referred to as XREM), located ~7.7 kb upstream of the transcriptional start site (Goodwin et al., 1999). XREM contains a PXR binding site composed of an imperfect DR-3 motif. In transient cotransfections of human PXR expression vector and chimeric reporter constructs in HepG2 cells, XREM was required for induction of *CYP3A4* by numerous drugs (Goodwin et al., 1999). However, these investigators were unable to demonstrate *CYP3A4* reporter gene inducibility with constructions containing the proximal promoter alone. In contrast, chimeric *CYP3A4* reporter gene constructions containing only a single copy of the *CYP3A4* ER6, whether linked to a viral promoter (Barwick et al., 1996) or to the *CYP3A4* cellular promoter (~ -1 kb) (Xie et al., 2000a), are fully sufficient to confer inducible reporter gene expression in cultures of adult rat hepatocytes. Thus, the relevance to the living animal of the results obtained from such molecular transfer experiments may critically depend on the choice of cellular hosts.

A comparison of the PXR sequences from different mammalian species indicates that the PXR proteins share less than 80% amino acid identity in their ligand binding domains (LBD), while the DNA binding domain is ~95% similar (Jones et al., 2000). This striking difference in LBD sequence across species is unusual for members of the nuclear receptor family which share greater than 90% amino acid identity in both LBD and DNA binding domains (Mangelsdorf et al., 1995). The recently identified constitutive androstane receptor (CAR), also seems to be an exception to the rule (Moore et al., 2000). Based upon their tissue distribution, DNA binding activity, and ability to mediate *CYP3A* induction, it has been suggested that PXR is an ortholog rather than closely related members of a subfamily of nuclear receptors (Bertilsson et al., 1998; Jones et al., 2000). The differences in LBD among species are assumed to be responsible for the selectivity in ligand binding, and thus the striking differences in the induction profiles among species.

TABLE 2

Compounds that activate the PXR: a comparison of species and cultured cells

Activation profiles were generated by transient cotransfections of the PXR and various response elements. Information listed in the table is intended as a general guide to compounds that activate the PXR, and should not be considered quantitative.

Cell Type	PXR Species	Compounds That Activate:			Response Element	Reference
		Most Efficacious	Moderate	Weak/None		
CV-1	Mouse	Cyproterone-acetate PCN RU486 5 β -Pregnane-3,20-dione 17-OH-Pregnenolone	Dexamethasone Spironolactone Pregnenolone Progesterone 17-OH-progesterone	Clotrimazole Lovastatin Phenobarbital Rifampicin Trans-nonachlor Troglitazone Corticosterone Cortisone 17 β -Estradiol	CYP3A1 (DR3) ₂ -tk-CAT GAL4 constructs (ER6) ₃ -tkCAT	Blumberg et al., 1998 Kliwer et al., 1998 Lehmann et al., 1998 Jones et al., 2000
LLC-PK1 pig kidney epithelial cells	Mouse	Cyproterone-acetate RU486 5 β -Pregnane-3,20-dione	Chlordane nonplanar PCBs Spironolactone Pregnenolone Progesterone	Trans-nonachlor	(CYP3A23) ₂ -tk-CAT	Schuetz et al., 1998
CV-1	Rat	Cyproterone-acetate PCN RU486 5 β -Pregnane-3,20-dione	Dexamethasone trans-nonachlor Spironolactone Progesterone 17-OH-progesterone	Rifampicin Troglitazone Phenobarbital Clotrimazole Corticosterone Cortisone 17 β -Estradiol Pregnenolone 17-OH-pregnenolone	(ER6) ₃ -tkCAT (DR3) ₃ -tk CAT	Jones et al., 2000 Savas et al., 2000
Primary rat hepatocytes	Rat	Nifedipine PCN RU486		Clotrimazole Phenobarbital Rifampicin Cortisol Corticosterone 17 β -Estradiol	CYP3A23 or CYP3A4 promoters—luciferase	Xie et al., 2000a
CV-1	Rabbit	Dexamethasone Rifampicin 17-OH-progesterone	Clotrimazole Cyproterone-acetate PCN Phenobarbital RU486 Spironolactone Trans-nonachlor Troglitazone 5 β -Pregnane-3,20-dione Progesterone	Pregnenolone 17-OH-pregnenolone Corticosterone Cortisone 17 β -Estradiol	CYP3A1 (DR3) ₂ -tk-CAT CYP3A23-(DR3) ₂ -TK Luc	Jones et al., 2000 Savas et al., 2000
CV-1	Human	Clotrimazole Rifampicin 5 β -Pregnane-3,20-dione	Lovastatin Nifedipine Phenobarbital RU486 Trans-nonachlor Troglitazone Corticosterone 17 β -Estradiol Pregnenolone Progesterone	Dexamethasone Cyproterone-acetate PCN Spironolactone Aldosterone Cortisone 17-OH-pregnenolone 17-OH-progesterone	(ER6) ₃ tk-CAT CYP3A1 (DR3) ₂ -tk-CAT	Blumberg et al., 1998 Lehmann et al., 1998 Jones et al., 2000
Caco-2	Human	Clotrimazole Nifedipine Rifampicin 5 β -Pregnane-3,20-dione	RU486 Cortisol 17 β -Estradiol	Dexamethasone PCN Aldosterone Pregnenolone Progesterone 17-OH-pregnenolone 17-OH-progesterone	GAL4 constructs or CYP3A4 IR-6 luciferase	Bertilsson et al., 1998
Primary rat hepatocytes	Human	Rifampicin Clotrimazole	Nifedipine Phenobarbital RU486 17 β -Estradiol Pregnenolone	PCN Progesterone	CYP3A23 or CYP3A4 promoters—luciferase	Xie et al., 2000a

PXR Ligands. Following the cloning of the PXR from various species, numerous studies identified compounds capable of activating the receptor in a species-specific manner (see Table 2 for examples). The majority of these studies used a cell-based assay system in which CV-1 cells, or other non-liver-derived cells, were transiently transfected with a PXR expression vector and a synthetic oligomer defining the DR-3 or ER6 linked to a viral promoter-driven reporter construct (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998; Schuetz et al., 1998; Jones et al., 2000; Moore et al., 2000; Savas et al., 2000). For the most part, drugs known to induce CYP3A *in vivo* were also able to activate the PXR in a species-specific manner. Jones et al. (2000) performed comprehensive comparisons of the PXR from human, rabbit, rat, and mouse using reporter constructs containing two copies of the *CYP3A23* DR-3 response element transfected into CV-1 cells. In general, activation of PXR from different species has been in good agreement with the reported induction of CYP3A expression in primary cultures of hepatocytes from these same species. For example, human and rabbit PXR were both efficiently activated by RIF, the antimycotic clotrimazole (CTZ), trans-nonachlor, and phenobarbital (PB); however, rabbit PXR was more sensitive than human PXR to activation by Dex, PCN, cyproterone acetate, and spironolactone. These studies demonstrated that there are clear differences in the activation profiles between human and rabbit receptors. To gain further support for the observations that structurally diverse compounds are ligands of the PXR, Jones et al. (2000) developed a competition radioligand binding assay using [³H]SR12813, a bisphosphonate ester shown to be a potent activator of both the human and rabbit PXR. These experiments showed good agreement between those compounds able to displace [³H]SR12813 from the PXR and those able to activate PXR in transfection assays.

PXR from each species were also activated by naturally occurring steroids. Although each displayed a distinct activation profile, in each case the most efficacious activator was a pregnane (Jones et al., 2000). 5 β -Pregnane-3,20-dione was the most efficacious activator of human (Bertilsson et al., 1998; Jones et al., 2000), rat, and mouse PXR, while 17-OH-progesterone was the most potent activator of rabbit PXR (Jones et al., 2000). However, these pregnane compounds activated only at superphysiological concentrations, leaving unanswered the question of the identity of the natural ligand for PXR. Based on the findings that PXR is activated by pregnenolone and its metabolites at low micromolar concentrations, it has been suggested that the natural ligand of PXR is probably a pregnane, and that the PXR defines a novel endocrine signaling pathway (Kliewer et al., 1998). Alternatively, the PXR has been described as a steroid and xenobiotic "sensing" receptor, responding to a broad range of compounds that humans are exposed to in the environment, as well as to circulating steroids (Blumberg et al., 1998). These postulates are not mutually exclusive.

Recently, studies in primary cultures of rat hepatocytes have shed light on differences between rat and human receptors. Xie et al. (2000a) used primary cultures of rat hepatocytes to critically test whether the PXR is indeed the postulated cellular factor that confers species specificity to the induction of CYP3A. The human PXR was transiently expressed in primary cultures of rat hepatocytes, and the effects of a panel of steroid and nonsteroid inducers on expression of cotransfected cellular promoters of rat *CYP3A23* or human *CYP3A4* linked to the firefly luciferase gene were examined. In control rat cells without expressed human PXR, *CYP3A23* was strongly induced, as expected, by PCN, nifedipine, or RU486, whereas RIF, CTZ, PB, corticosterone, cortisol, 17 β -estradiol, progesterone, pregnenolone, and cortisone produced little or no induction. In contrast, cotransfection of human PXR resulted in significant induction of *CYP3A23*

reporter by drugs known to be active in humans, including RIF, CTZ, PB, 17 β -estradiol, and pregnenolone. Furthermore, the induction of *CYP3A23* by nifedipine and RU486 increased significantly with added human PXR, indicating that these drugs activate both rat and human receptors, while the induction by PCN remained unchanged in the presence of human PXR, indicating that PCN specifically activated the rat receptor. These trans-species transfection assays clearly demonstrated that transfection of human PXR is sufficient to convert the induction response characteristics of hepatocyte from rat to human.

In Vivo Role of the PXR. Transgenic mice have been developed to establish the role of the PXR *in vivo* (Xie et al., 2000a). Targeted disruption of the mouse PXR gene eliminated the induction of *CYP3A* by PCN. PXR-null transgenic mice harboring the human PXR gene, when challenged with drugs known to induce human *CYP3A* such as RIF and CTZ, displayed induced *CYP3A* mRNA in the liver. Transgenic mice expressing a constitutively active human PXR were shown to develop sustained *CYP3A* expression, resulting in enhanced protection against challenges of xenobiotic toxicants (Xie et al., 2000a). This "humanized" rodent model system has potential applications for drug and toxicity screening.

The discovery of PXR represents an important advance both in identifying the transcriptional mediator of *CYP3A* induction and identifying the agent responsible for the striking species differences in the induction profiles. The ability of a single transcription factor to determine species differences in response to xenobiotics is in itself without precedent. The differences in the activation profiles among the PXRs clearly highlight the need to develop an appropriate model for drug screening and discovery.

Mechanisms: PXR, CAR, GR, and Others

The mechanism by which numerous structurally diverse compounds induce *CYP3A* expression can be for the most part explained by the PXR. However, several questions remain unanswered. The nuclear receptors COUP-TF and HNF-4 have been implicated as factors interacting with sequences, including those at the Dex/PCN response element, of the rat *CYP3A23* promoter. What role they play in the responsiveness of *CYP3A* to inducers remains to be determined. For example, do these proteins interact directly with the PXR to produce a highly active transcriptional complex? One of the most pressing questions is whether the GR plays a role in *CYP3A* induction. Dex, an efficacious inducer of *CYP3A* in rodents and humans, seems to be a relatively weak activator of the PXR, suggesting that the GR may be involved in the induction of *CYP3A* in these species. It now seems that another nuclear receptor is involved in the regulation of drug-metabolizing enzymes. The CAR has been shown to mediate induction of both *CYP3A* and *CYP2B*. And finally, how does the wealth of information gathered over the past several years explain the well established observation of the synergistic effects of GCs and PCN on *CYP3A* expression?

Factors that Modulate the PXR-Mediated Induction. Recent work has revealed the existence of other proteins that appear to modulate the induction of *CYP3A* expression. Several transcription factors have been identified that interact with the *CYP3A23* promoter and seem to play a role in modulating the expression of *CYP3A23* (Huss and Kasper, 1998; Huss et al., 1999; Ogino et al., 1999). Among these are members of the nuclear receptor family, COUP-TF and HNF-4. A postulated mechanism is that COUP-TF competes with the PXR for binding to the DR-3 response element of the *CYP3A23* gene or that binding of COUP-TF to the upstream imperfect ER6 (DexRE-1, Table 1) may repress HNF-4-mediated basal expression (Huss and Kasper, 1998). It could be reasoned that if such interactions

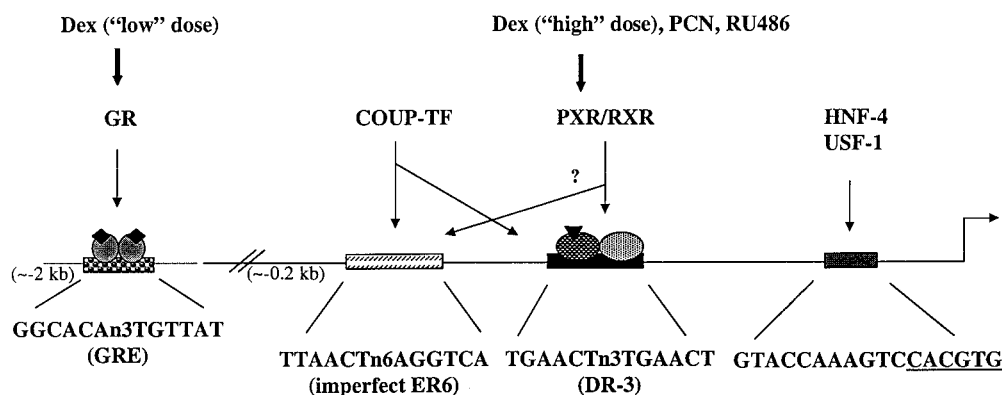


FIG. 1. Model of *CYP3A23* regulation.

The model is described in the text. Low dose Dex is 10^{-7} M or less, while high dose is 10^{-5} M. The underlined sequence, CACGTG, is the putative binding site for upstream stimulatory factor-1.

occur in vivo, then modulation of the induction response would depend on relative amounts of each transcription factor, as well as their affinities for the DNA binding sites. In addition to COUP-TF, an unidentified protein referred to as the "B" protein also may interact with the upstream imperfect ER6, and it was suggested that this protein functions as an accessory factor in cooperation with PXR/RXR and HNF-4 (Huss and Kasper, 1998). The identities of all of the proteins interacting at this upstream element remain unknown, although the possibility exists for the PXR itself to also interact weakly with the upstream imperfect ER6. It may be concluded that binding of PXR to the DR-3 of the *CYP3A23* response element is necessary but insufficient for optimal *CYP3A23* expression (Quattrochi et al., 1995; Huss et al., 1996). Other factors may interact with other sites in the *CYP3A* gene or with the PXR at this DR-3 site through protein-protein interactions with the PXR. A summary of the currently postulated relationships of nuclear receptors to the regulation of the rat *CYP3A23* promoter is illustrated in Fig. 1.

Role of the Glucocorticoid Receptor in *CYP3A* Induction. An important unanswered question is whether the GR plays a role in the regulated expression of *CYP3A*. Some lines of evidence suggest that induction of *CYP3A* by GCs can also occur through a pathway distinct from the PXR. For example, there is a functional glucocorticoid response element (GRE) in a region of the rat *CYP3A23* gene ~2 kb upstream of the PXR/RXR binding site (Fig. 1) (Pereira et al., 1998). In addition, the human *CYP3A5* gene promoter contains two GRE half-sites, separated by 160 bp, that confer GC responsiveness to reporter genes (Schuetz et al., 1996). Conversely, studies in a GR-null mouse model suggest the opposite. Schuetz et al. (2000), using mice containing a targeted disruption of the GR to test its role on induction of *CYP3A*, found that *CYP3A* induction by GC, RU486, or RIF can occur in the absence of the GR. However, since the concentrations of Dex used in these studies, 50 mg/kg, may have activated the PXR, it is not possible to discern whether the induction of *CYP3A* in the wild-type or null mice is mediated through the PXR. Moreover, inasmuch as dose response data were apparently not obtained, this study does not exclude the possibility of involvement of the GR to augment the induction process. In fact, recent studies demonstrating the ability of Dex to increase PXR levels in hepatoma cell lines and primary hepatocytes (Huss and Kasper, 2000; Pascucci et al., 2000a) lend support to the concept that the GR plays a role in the induction process (discussed below). Finally, it should be noted that the mouse *CYP3A* promoters have not been characterized as extensively as the rat or human promoters. Thus, the possibility that the GR plays a role

in the induction of rat *CYP3A23* and/or human *CYP3A4* cannot be excluded. Indeed, cotransfected GR was shown to enhance the GC induction of a *CYP3A4* reporter gene construct in transfected HepG2 cells (El-Sankary et al., 2000).

Cross Talk: PXR and CAR. Early studies of *CYP3A* induction profiles established that the induction of *CYP3A* and *CYP2B* by PB occurred through distinct mechanisms (Kocarek et al., 1990). The cloning of both PXR and CAR has provided insight into this divergent regulatory mechanism. CAR binds DNA as a heterodimer with RXR and activates gene transcription in a constitutive manner; however, CAR-mediated transcriptional activation can be inhibited by androstane metabolites (reviewed in Honkakoski and Negishi, 2000). The CAR/RXR heterodimer interacts with the PB response element module (PBREM) located in *CYP2B* promoters of rat, mouse, and human genes, and mediates PB-inducible gene transcription. The PBREM contains a nuclear factor 1 binding site flanked by two nuclear receptor binding sites composed of imperfect direct repeats of half-sites spaced by four nucleotides (DR-4 motif). The mechanism by which PB derepresses CAR is presently unknown, but may involve direct ligand binding. Phenobarbital is also a fairly strong activator of the human PXR, but it has little or no effect on the rodent PXR (Jones et al., 2000; Xie et al., 2000a). Each PB-activated receptor can then interact with its own recognition site (i.e., PXR for the DR-3/ER6 in *CYP3A* promoters, and CAR for the PBREM in *CYP2B* promoters). However, CAR was shown to bind to and activate a reporter gene through the *CYP3A4* ER6 response element, thus establishing the possibility for cross talk between CAR and PXR (reviewed in Honkakoski and Negishi, 2000).

Recent studies have demonstrated that PXR and CAR have the potential to cross-regulate *CYP3A* gene expression by two independent mechanisms: 1) sharing of some ligands and 2) binding of PXR and CAR to each other's DNA response elements. Moore et al. (2000) compared PXR versus CAR for activation by their respective ligands. They found dual activation of receptors by a subset of compounds. For example, androstanol deactivated (i.e., repressed) mouse CAR, and human CAR to a lesser extent, while CTZ deactivated only human CAR. Both of these compounds activated PXR. In cotransfections of human XREM-*CYP3A4* reporter constructions [containing the proximal ER6 and the upstream XREM, described by Goodwin et al. (1999)], the negative effects of androstanol and CTZ on human CAR were found to be insufficient to overcome their positive effects on human PXR. These studies led to the conclusion that the human PXR is the dominant regulator of *CYP3A4* expression. Studies in primary

cultures of rat hepatocytes, using natural *CYP3A* and *CYP2B* promoters, demonstrated that CAR could regulate *CYP3A* reporter gene activity, but only in response to its own ligands (i.e., the human PXR activator RIF had little effect on CAR activity). Likewise, cotransfection of human PXR resulted in induction of *CYP2B* reporter gene activity in response to RIF and RU486, two activators of the human PXR (Xie et al., 2000b). Studies comparing CAR/RXR with PXR/RXR binding to each other's response element established that PXR and CAR exhibited similar binding affinity toward the response element of the human *CYP3A4* gene. In contrast, while PXR could interact with the nuclear receptor-1 of the PBRE of *CYP2B*, this element showed preference for CAR (Xie et al., 2000b). The most convincing evidence was obtained when this same group of investigators demonstrated that even in PXR-null mice, the PXR ligands CTZ and PB were efficacious inducers of *CYP3A*. Thus, cross-regulation of P450 enzymes by distinct nuclear receptors can be viewed as a versatile adaptive mechanism by which numerous structurally diverse compounds induce enzymes that in turn catalyze their metabolism and elimination. Future study of xenoregulation in CAR/PXR double knockout mice would provide additional insight for this proposed cross talk and might disclose the presence of other response modifiers.

Synergy: Who Are the Players? A final question concerns the identity of the factors mediating synergy. The GR has been implicated in playing a role in the previously reported synergistic interaction between PCN and Dex for induction of *CYP3A* (Schuetz and Guzelian, 1984). An open possibility is that the GR could interact with the PXR promoter transcription complex through protein-protein interactions. Another suggestion is that the GRE found upstream of the PXR/RXR binding site of the rat *CYP3A1* gene might play a role in the synergistic effects of Dex and PCN on *CYP3A* expression (Pereira et al., 1998). Recently, it was shown that Dex treatment of rat hepatoma cells and primary cultures of human hepatocytes increased levels of both PXR and RXR (Huss and Kasper, 2000; Pascussi et al., 2000a). It was concluded from these studies that the effect of Dex on PXR mRNA accumulation was most likely through direct activation of the GR. Therefore, another possibility for the reported synergy is that physiological levels of GC may activate the GR and increase the production of both PXR and RXR proteins, which in turn can further function as transcriptional regulators of *CYP3A*. This idea would require the levels of both nuclear receptors to be rate limiting in the hepatocyte, and evidence suggests that this may be the case in vivo, at least for the PXR (Zhang et al., 1999). Cloning of the PXR and RXR promoters and the identification of functional GREs within the promoters would lend support for this theory.

Future

The cloning of the PXR and its involvement in *CYP3A* induction represents a critical advance in the field of drug metabolism. Its demonstrated interaction with structurally diverse chemicals provides an explanation for much of the pharmacology of this P450 gene subfamily. As with many of the nuclear receptors, the PXR likely will be found to play a role in human physiology, outside that of drug metabolism. If so, this will surely lead to studies directed at certain disease states and risk assessment. For example, Pascussi et al. (2000b) demonstrated that interleukin-6 negatively regulates both PXR and CAR, suggesting that this provides direct evidence for the molecular mechanism underlying one of the earliest observations: that xenobiotic and drug metabolism is markedly impaired during inflammation and infections. Furthermore, endocrine functions may be altered by the interaction of endocrine-disrupting chemicals, such as

phthalic acid and nonylphenol, recently shown to activate the mouse PXR (Masuyama et al., 2000). It seems likely that *CYP3A* plays one or more essential roles in body homeostasis, since to date this enzyme system has been found in every human liver examined. However, there is no comparable database on the possibility that *CYP3A* induction itself could be polymorphic. Considering the growing evidence that *CYP3A* induction may be more complex than initially believed, involving not only the PXR, which itself may be under inducible regulatory control, but also other nuclear receptors and response-modifying proteins, a fruitful area of future research will be exploring human variation in *CYP3A* induction.

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