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The Nuclear Pregnane X Receptor: A Key Regulator of Xenobiotic Metabolism

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The nuclear pregnane X receptor (PXR; NR1I2) is an important component of the body's adaptive defense mechanism against toxic substances including foreign chemicals (xenobiotics). PXR is activated by a large number of endogenous and exogenous chemicals including steroids, antibiotics, antimycotics, bile acids, and the herbal antidepressant St. John's wort. Elucidation of the three-dimensional structure of the PXR ligand binding domain revealed that it has a large, spherical ligand binding cavity that allows it to interact with a wide range of hydrophobic chemicals. Thus, unlike other nuclear receptors that interact selectively with their physiological ligands, PXR serves as a generalized sensor of hydrophobic toxins. PXR binds as a heterodimer with the 9-*cis* retinoic acid receptor (NR2B) to DNA response elements in the regulatory regions of cytochrome P450 3A monooxygenase genes and a number of other genes involved in the metabolism and elimination of xenobiotics from the body. Although PXR evolved to protect the body, its activation by a variety of prescription drugs represents the molecular basis for an important class of harmful drug-drug interactions. Thus, assays that detect PXR activity will be useful in developing safer prescription drugs. (*Endocrine Reviews* 23: 687–702, 2002)

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Abbreviations: AF, Activation function; BXR, benzoate X receptor; CAR, constitutive androstane receptor; CARLA, coactivator receptor ligand assay; CYP, cytochrome P450 enzyme; DBD, DNA binding domain; DR, direct repeat; ER, everted repeat; ET-743, ecteinascidin 743; FXR, farnesoid X receptor; GR, glucocorticoid receptor; HNF-4, hepatocyte nuclear factor-4; LBD, ligand binding domain; LCA, lithocholic acid; *MDR1*, multidrug resistance protein 1; *MRP2*, multidrug resistance-associated protein 2; NR, nuclear receptor; PAR, pregnane-activated receptor; PCN, pregnenolone 16α-carbonitrile; PXR, pregnane X receptor; RXR, 9-*cis* retinoic acid receptor; SJW, St. John's wort; SPA, scintillation proximity assay; VDR, vitamin D receptor.

I. Introduction

EVERY DAY THE body must defend itself against myriad xenobiotics that are either ingested in the diet, inhaled, or otherwise absorbed. The cytochrome P450 enzymes (CYPs), which comprise a large family of heme-containing monooxygenases, represent an important constituent of the body's defense mechanism against foreign chemicals (1). Although some CYPs are highly selective in their interactions with substrates, others oxidize a wide variety of chemicals, including many xenobiotics. An important feature of the CYP family is that expression of some isoforms, notably CYP1A, CYP2B, CYP3A, and CYP4A subfamily members, can be dramatically increased by xenobiotics, thereby providing an adaptive, feedforward regulatory mechanism that amplifies the physiological response to xenobiotic challenge (2). Members of the CYP3A subfamily are noteworthy in that they are the most abundant CYPs in the human liver and intestine and display a broad substrate specificity (3–6). Expression of CYP3A family members can be strongly induced by a structurally diverse set of chemicals, many of which are CYP3A substrates (2–4, 6). Induction of CYP3A levels represents the basis for the actions of catatoxic steroids, which were first reported over 30 yr ago for their ability to induce hepatic monooxygenase activity and to confer resistance to toxic substances in rodents (7). However, because CYP3A isoforms are involved in the metabolism of more than 50% of all prescription medicines, the induction of their expression also represents a basis for a common class of potentially life-threatening drug-drug interactions in which one drug accelerates the metabolism of a second drug (8). Thus, elucidation of the mechanisms underlying *CYP3A* gene expression are important for the development of safer prescription medicines.

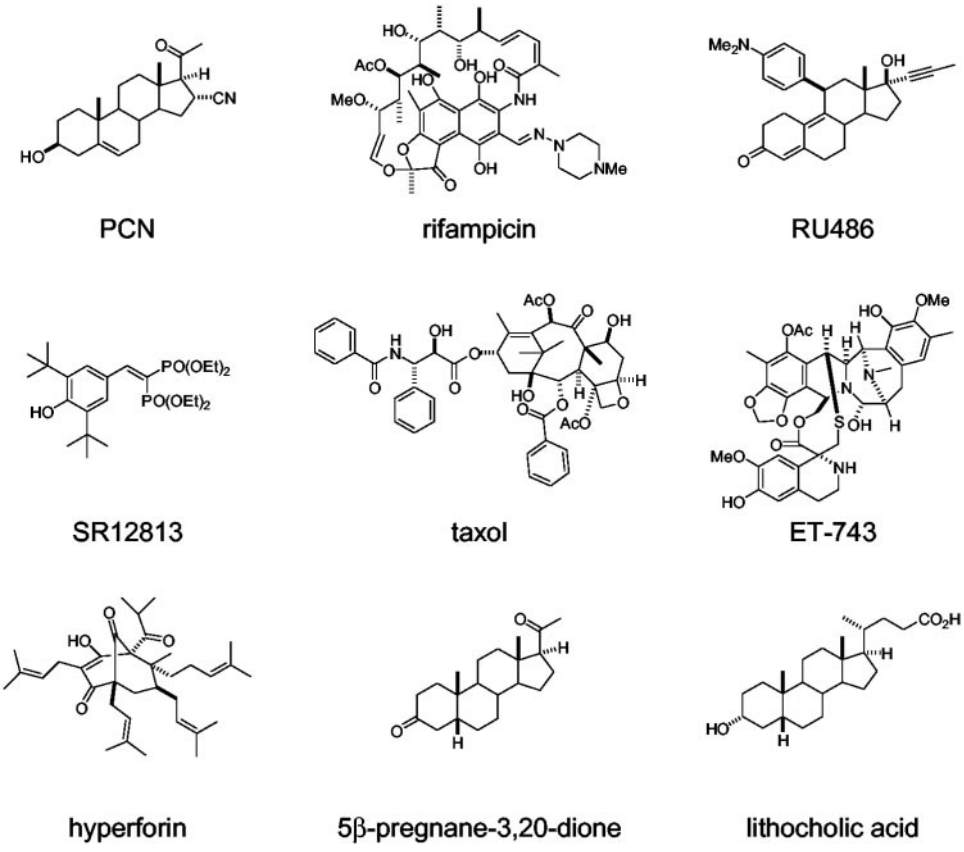

FIG. 1. PXR ligands. Chemical structures of xenobiotics and endogenous chemicals that regulate PXR activity. ET-743 is a PXR antagonist; all other chemicals are PXR agonists.

The identification and characterization of the pregnane X receptor (PXR; NR1I2)[1] was an important step forward in understanding the underpinnings of the body's xenobiotic defense system (9–12). PXR is a member of the nuclear receptor (NR) family of ligand-activated transcription factors that includes the steroid, retinoid, and thyroid hormone receptors as well as many orphan receptors for which physiological ligands have yet to be identified (13, 14). Several lines of evidence indicated that PXR regulated *CYP3A* gene expression: PXR is highly expressed in the liver and intestine; PXR binds as a heterodimer with the 9-*cis* retinoic acid receptor (RXR; NR2B) to previously characterized xenobiotic response elements in *CYP3A* gene promoters; and, importantly, PXR is activated by the spectrum of chemicals that are known to induce *CYP3A* gene expression. Experiments performed with transgenic mice have definitively established that PXR functions as a critical regulator of *CYP3A* expression *in vivo* (15, 16). Additional studies have shown that PXR regulates a large number of genes involved in different aspects of xenobiotic metabolism, including oxidation, conjugation, and transport. Recently, the elucidation of the three-dimensional structure of the PXR ligand binding domain (LBD) has provided important insights into the structural basis for the promiscuous ligand binding properties of this unusual NR (17). This review highlights the studies that have established PXR as a generalized xenobiotic sensor that protects the body from chemical challenge, but which also represents the basis for a number of common drug-drug interactions.

[1] This protein has been variously termed PXR, pregnane-activated receptor (PAR), or steroid and xenobiotic receptor (SXR). Work from laboratories using these various names will be cited throughout the text. In the interest of simplicity, we choose to adopt the name "PXR" throughout the manuscript.

II. Regulation of *CYP3A* by Xenobiotics

A. Induction of CYP3A by structurally diverse compounds

For more than 30 yr, it has been understood that certain catatoxic agents, including the synthetic C21 steroid (pregnane) pregnenolone 16α-carbonitrile (PCN; Fig. 1), exert their protective effects by inducing the expression of specific CYP isoforms (7, 18). The PCN-inducible CYP was purified and shown to be distinct from previously characterized isoforms (19). Isolation of the cDNA encoding this CYP, designated CYP3A23, established it as a member of a novel gene family (20, 21). Guzelian and co-workers demonstrated that, in addition to PCN, *CYP3A23* expression was also highly inducible by the potent glucocorticoid dexamethasone (22, 23). Notably, in primary cultures of rat hepatocytes, the dose-dependent increases in *CYP3A23* mRNA after dexamethasone treatment did not coincide with that of a typical glucocorticoid-responsive gene, tyrosine aminotransferase (24). Moreover, the dexamethasone-mediated increase in tyrosine aminotransferase expression was antagonized by PCN, whereas induction of *CYP3A23* was enhanced in the presence of both steroids (24, 25). Taken together, these studies dem-

onstrated that the molecular mechanism underlying activation of *CYP3A23* expression by dexamethasone was distinct from the classical glucocorticoid receptor (GR; NR3C1) signaling pathway.

It is now apparent that the rat *CYP3A23* and *CYP3A2* genes are inducible by an array of structurally dissimilar compounds, including steroids such as PCN, dexamethasone, betamethasone, hydrocortisone, α-methylprednisolone, mifepristone (RU486), dehydroepiandrosterone, and spironolactone; the antibiotic triacetyloleandomycin; antifungal drugs such as clotrimazole; polychlorinated biphenyls; the organochloride pesticides trans-nonachlor and γ-chlordane; the calcium channel antagonist nicardipine; the 11β-hydroxylase inhibitor metyrapone; and the barbiturate phenobarbital (23, 26–36).

In humans, CYP3A4 is the predominant CYP expressed in normal adult human liver, and it is reported to be involved in the oxidative metabolism of a plethora of compounds, including most prescription drugs. Induction of *CYP3A4* expression by xenobiotics is well documented (3, 4). As outlined above, this phenomenon underlies a number of clinically important drug interactions and, as such, has received considerable attention (8). Although CYP3A catalytic activity, mRNA, and immunoreactive protein have been shown to be elevated *in vivo* after treatment of patients with various drugs, including dexamethasone, triacetyloleandomycin, and rifampicin, most inducers of *CYP3A4* expression have been delineated using primary cultures of human hepatocytes (37–42). One of the most effective activators of *CYP3A4* expression, both *in vivo* and *in vitro*, is the macrocyclic antibiotic rifampicin (Fig. 1; Refs. 33, 39, 43, and 44). Like *CYP3A23*, *CYP3A4* expression is inducible by steroids, including dexamethasone, RU486 (Fig. 1), spironolactone, and cyproterone acetate (33, 37, 45, 46). Additionally, *CYP3A4* is inducible by the antifungal agent clotrimazole, phenobarbital, phenytoin, phenylbutazone, sulfadimidine, the proton pump inhibitors omeprazole and lansoprazole, and metyrapone (33, 39, 43, 47, 48).

Importantly, induction of *CYP3A* subfamily members exhibits a distinct species-specific pharmacology. For example, expression of the human *CYP3A4* and rabbit *CYP3A6* genes is strongly activated by rifampicin, whereas the rat *CYP3A23* and *CYP3A2* genes are poorly induced by this drug (27, 33, 49, 50). Conversely, PCN is an effective inducer of rat *CYP3A* genes but a weak inducer of the human *CYP3A4* and rabbit *CYP3A6* genes (27, 33). These data hinted that there were likely to be important species-specific differences in the receptor(s) that responded to xenobiotics and induced expression of CYP3A genes.

B. Xenobiotic response elements in CYP3A promoters

To delineate the xenobiotic response elements in the *CYP3A23* gene, Burger *et al.* (25) fused a 1.5-kb fragment of the 5′-flanking region to a heterologous thymidine kinase promoter and a reporter gene. Transient transfection of the resultant construct into primary cultures of rat hepatocytes revealed that this fragment was capable of conferring both dexamethasone- and PCN-responsiveness on reporter gene expression. Sequential deletion of this region resulted in the identification of a 164-bp fragment (bases −220 to −56, relative to the transcription initiation site) that mediated both the glucocorticoid and antiglucocorticoid effects on a reporter gene plasmid (25). In agreement with earlier studies that characterized induction of the *CYP3A23* gene, the dexamethasone-dependent activation of *CYP3A23*-reporter gene constructs was distinct from that of a GR-mediated response (23–25). A more detailed analysis of the responsive region led to the identification of three sites that were capable of forming complexes with nuclear proteins, namely site A (bases −110 to −91), site B (also known as DexRe-1, bases −136 to −118), and site C (DexRe-2, bases −169 to −144; Refs. 51 and 52). Each site contained binding motifs for members of the NR superfamily (53–59). Thus, site A was highly homologous to the consensus binding site for the orphan receptor hepatocyte nuclear factor-4 (HNF-4; Refs. 54 and 57); site B contained a direct repeat (DR) of the NR consensus binding site AGTTCA separated by three nucleotides (DR-3; Fig. 2); and site C contained an element that could be viewed as either an imperfect DR separated by four nucleotides (DR-4) or everted repeat (ER) separated by six nucleotides (ER-6). Nuclear extracts prepared from a rat hepatoma cell line (H4IIE) formed a common protein complex on sites B and C (54). Importantly, none of these sites were capable of directly interacting with GR (51, 52). Transient transfection of *CYP3A23*-reporter gene constructs into rat H4IIE hepatoma cells demonstrated that the integrity of all three sites was required for maximal dexamethasone responsiveness (51, 54). Similar studies performed in primary cultures of rat hepatocytes supported this multisite mechanism (52). Disruption of the NR half sites embedded in site B resulted in a complete loss of responsiveness (52, 54, 59). Moreover, the isolated site B was shown to be capable of supporting dexamethasone- and PCN-mediated induction of reporter gene expression in rat hepatocytes, indicating the importance of this element (52, 59). Although sites A and C appear to have little or no inherent ability to mediate the transcriptional activation of reporter genes by dexamethasone or PCN, they are clearly important for maximal induction of *CYP3A23* expression (51, 52, 54).

Xenobiotic response elements have also been characterized in the proximal promoter regions of the human *CYP3A4* and rabbit *CYP3A6* genes. When transfected into rat hepatocytes, chimeric *CYP3A23*- (bases −220 to −56), *CYP3A4*- (bases −179 to −35), and *CYP3A6*- (bases −220 to −56) reporter gene constructs were strongly activated by dexamethasone and PCN but not rifampicin. However, in primary cultures of rabbit hepatocytes, rifampicin and dexamethasone but not PCN transactivated the *CYP3A* reporter genes. This study elegantly demonstrated that interspecies differences in *CYP3A* induction were determined by cellular environment rather than the structure of the gene (60). The dexamethasone-, PCN-, and rifampicin-responsiveness was mapped to a conserved 18-bp motif spanning nucleotides −170 to −153 and −177 to −160 of *CYP3A4* and *CYP3A6*, respectively. This element contained two copies of a NR ER-6 motif (Fig. 2; Ref. 60).

DIRECT REPEAT (DR) 3

CYP3A23	TGAACT TCA TGAACT	<----- <-----
CYP3A2	TGAACT TTA TGAACT	<----- <-----
CYP3A2	TGACCT TCT TGAGCT	<----- <--x--
CYP3A4	TGAACT TGC TGACCC	<----- <-----

DIRECT REPEAT (DR) 4

CYP2B1 Cyp2b10	TGTACT TTCC TGACCT	<-x--- <-----
CYP2B6	TGTACT TTCC TGACCC	<-x--- <-----
CYP2B6	TGGACT TTCC TGAACC	<-x--- <-----
CYP2C9	CAAACT CTTC TGACCT	xx---- <-----
MDR1	TGAACT AACT TGACCT	<----- <-----

EVERTED REPEAT (ER) 6

CYP3A23	TTAACT CAAAGG AGGTCA	<x---- ----->
CYP3A4	TGAACT CAAAGG AGGTCA	<----- ----->
CYP3A5	TGAACT CAAAGG AGGTAA	<----- ----x>
CYP3A6	TGAACT CAGAGG AGGTCA	<----- ----->
CYP3A7	TTAACT CAATGG AGGTCA	<x---- ----->
MDR1	TGAGAT TAAACA AGTTCA	<--xx- ----->

EVERTED REPEAT (ER) 8

MRP2	TGAACT CTTAACCA AGTTCA	<----- ----->

FIG. 2. PXR response elements. PXR binds as a heterodimer with RXR to DR-3, DR-4, ER-6, and ER-8 elements in the regulatory regions of the indicated genes. The NR half sites and their orientations are indicated. Deviations from the consensus half-site sequence AG(G/T)TCA are indicated (x).

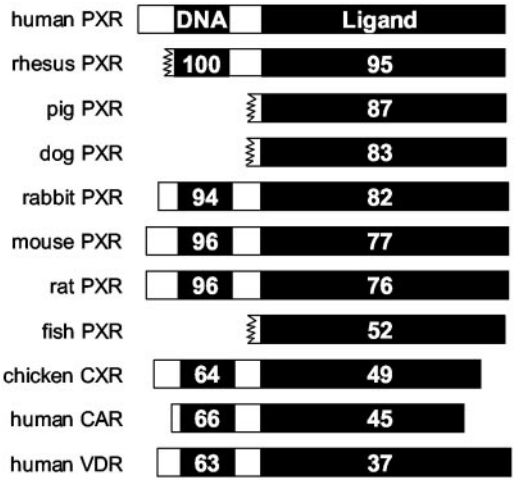

FIG. 3. Sequence comparison of PXR across species. Alignment of human PXR and other NR1I family members. The similarity is expressed as percentage amino acid identity in the DNA binding (DNA) and ligand binding (Ligand) domains. Only the LBDs of the pig, dog, and fish PXRs have been cloned, and the N-terminal domain of the rhesus PXR has not yet been cloned.

III. Cloning and Characterization of PXR

A. Cloning of PXR

NRs share a common domain structure that includes a highly variable N-terminal domain, a central DNA binding domain (DBD), and a C-terminal LBD (13, 14). The highly conserved DBD is approximately 70 amino acids in length and consists of two zinc fingers, each composed of four cysteine residues that chelate a zinc atom. The LBD is approximately 250 amino acids in length and folds to form a hydrophobic pocket into which the ligand binds. In addition to its ligand binding properties, the LBD also contains dimerization and transcriptional activation motifs, including the well characterized activation function-2 (AF-2) helix in the extreme C-terminal portion of the LBD (14, 61). Upon ligand binding, the AF-2 helix undergoes a conformational change that permits the receptor to interact with coactivator proteins and activate transcription. The N-terminal domain of NRs is highly variable both in terms of length and amino acid sequence. A second transcriptional activation function, termed the AF-1, has been characterized in the N-terminal domains of a number of the NRs (13, 14).

PXR belongs to the NR1I subfamily of NRs, a group that includes the mammalian vitamin D receptor (VDR; NR1I1) and constitutive androstane receptor (CAR; NR1I3) and the frog benzoate X receptors (BXRs; NR1I2; Fig. 3; Ref. 62). The two BXR subtypes are selectively activated by amino benzoates present in embryonic extracts (63–65). CAR is activated by phenobarbital and other xenobiotics and will be discussed in detail below. PXR was originally identified on the basis of its sequence homology with other NRs. In 1997, a mouse sequence first appeared in the Washington University Expressed Sequence Tag database that represented a fragment of a novel NR LBD. A cDNA encoding the full-length mouse protein was subsequently cloned, and the receptor was named PXR based upon its activation by various natural and synthetic pregnanes (9). Since then, the human, monkey, dog, rabbit, and rat PXR have been cloned as well as closely related receptors from chicken and fish, termed CXR and fish PXR, respectively (10–12, 65–69). The human

PXR was cloned by three groups and is alternately referred to as the pregnane-activated receptor (PAR; Ref. 11) or the steroid and xenobiotic receptor (SXR; Ref. 12). For the sake of consistency, we will refer to it as human PXR throughout this review.

The DBDs of the mammalian PXRs are highly conserved, sharing more than 95% amino acid identity (Fig. 3; Ref. 67). However, the LBDs of the PXRs are much more divergent across species than those of other NRs. For example, the human and rat PXR share only 76% amino acid identity in their LBD (Fig. 3), whereas most human and rodent NR orthologs share more than 90% amino acid identity. Because only one PXR gene is present in the human genome (70, 71), it appears likely that the PXRs identified to date are *bona fide* orthologs rather than paralogs.

Isoforms of NRs can arise from the same gene as a consequence of either alternate mRNA splicing or differential promoter usage. Isoforms of both mouse and human PXR have been described. A variant of mouse PXR, termed PXR.2, was cloned from liver cDNA and results from an in-frame splicing event that generates a protein lacking 41 amino acids in the N-terminal portion of the LBD (9). Mouse PXR.2 is still a functional receptor but responds to a much more limited set of compounds than PXR (9). A very similar variant that lacks 37 amino acids in the LBD has been described in humans (human PXR.2; Ref. 72). A second, relatively rare human PXR variant, termed hPAR-2, results in the addition of 39 amino acids to the N terminus of PXR (11). It is not known whether this N-terminal extension affects PXR activity.

The genomic structure of the human *PXR* gene was recently described (73, 74). The gene consists of 10 exons and 9 introns, and it spans approximately 30 kb of genomic DNA on chromosome 3q13–21. The first two exons are used as alternate 5′ ends of PXR transcripts, which accounts for the hPAR-2 isoform.

B. PXR expression pattern

PXR is highly expressed in the liver, small intestine, and colon in the human, rabbit, rat, and mouse (9–12, 67–69). Notably, these are the same tissues where *CYP3A* genes are most highly expressed and induced. In rodents, lower levels of PXR mRNA have also been detected in the kidney, stomach, lung, uterus, ovary, and placenta (9, 69, 75). In humans, PXR mRNA has been detected in both normal and neoplastic breast tissue (72).

The *PXR* promoter has not yet been characterized. However, dexamethasone increased *PXR* mRNA levels in primary cultures of human hepatocytes and rat H4IIE hepatoma cells (76, 77). This effect appears to be mediated through the GR because it required only nanomolar concentrations of dexamethasone and was blocked by the GR antagonist RU486. Induction of *PXR* mRNA levels may contribute to the strong stimulation of *CYP3A* gene expression by dexamethasone. Interestingly, *PXR* expression in the mouse liver and ovary was increased approximately 50-fold during pregnancy (75). These data suggest that *PXR* expression may be stimulated by other hormones and, furthermore, raise the intriguing possibility that PXR is involved in protecting the fetus and/or mother from either xenobiotics or high levels of endogenous steroids.

C. Activation of PXR by xenobiotics and natural steroids

Historically, compounds that activate NRs have been identified in cell-based reporter assays in which an expression plasmid encoding the receptor of interest is cointroduced into cells with a reporter gene plasmid (78). The receptor expression plasmid can encode either the full-length receptor or a chimera between the LBD of the receptor and a DBD of another protein such as the yeast transcription factor GAL4. The reporter gene plasmid contains binding sites for either the full-length receptor or the receptor chimera upstream of a gene encoding a reporter protein that can be readily quantitated such as chloramphenicol acetyltransferase or luciferase. The advantages of the chimera system are that it eliminates background caused by endogenous receptors present in the cells and it permits ligand screening without any prior knowledge of the DNA binding characteristics of the full-length receptor.

A variety of compounds have been shown to activate PXR in cell-based reporter assays (Fig. 1). Studies performed with the mouse PXR showed that it was activated efficiently by the classic *CYP3A* inducer PCN as well as by both glucocorticoids (*e.g.*, dexamethasone) and antiglucocorticoids (*e.g.*, RU486; Ref. 9). These data were the first to suggest that PXR might play a role in the regulation of *CYP3A*. Interestingly, the PXR.2 isoform, which lacks 43 amino acids in the LBD, displayed a much more restricted activation profile (9). To date, only the synthetic glucocorticoid dexamethasone-t-butyl acetate has been reported to activate mouse PXR.2 efficiently in cell-based reporter assays.

Additional studies revealed striking differences in the activation profiles of PXR across species. Whereas PCN was an effective activator of mouse and rat PXR, it had much less activity on the rabbit and human receptors (10, 12, 67, 68). Conversely, rifampicin activated human and rabbit PXR but had virtually no activity on the mouse or rat receptors (10, 12, 67, 68). The PXR activation profiles of these chemicals correlated closely with their *CYP3A* induction profiles in hepatocytes derived from these different species (33, 67). These data provided strong pharmacological evidence that PXR serves as a key regulator of *CYP3A* gene expression and that the species origin of PXR dictates the *CYP3A* induction profile.

The number of chemicals that are reported to activate PXR has grown rapidly and includes many drugs currently in use (Table 1). Among the xenobiotics that activate PXR from one or more species are the established *CYP3A* inducers metyrapone, clotrimazole, phenobarbital, spironolactone, and trans-nonachlor (10, 79–81). Other PXR activators include the calcium channel blocker nifedipine (82); the HIV protease inhibitor ritonavir (83); the anticancer drugs paclitaxel (Taxol; Ref. 84; Fig. 1), tamoxifen (85), and 4-hydroxytamoxifen (85); the antidiabetic agent troglitazone (67); the cholesterol-lowering drugs lovastatin and SR12813 (Fig. 1; Refs. 10 and 67); the sedative glutethimide (86); and the endocrine disruptors bisphenol A, diethylhexylphthalate, and nonylphenol (87, 88).

TABLE 1. Drugs that activate human PXR

Drug	Therapeutic Use	Reference(s)
Clotrimazole	Antimycotic	10, 11
Cyproterone acetate	Antiandrogen	10, 80
Dexamethasone	Anti-inflammatory	10
Glutethimide	Sedative	86
4-Hydroxytamoxifen	Anticancer	85
Lovastatin	Antihypercholesterolemic	10
Metyrapone	Diagnostic aid (pituitary function)	79, 154
Mifepristone (RU486)	Abortifacient	10, 11
Nifedipine	Antianginal, antihypertensive	11
Paclitaxel	Anticancer	84
Phenobarbital	Anticonvulsant, sedative	10
Rifampicin	Antibiotic	10, 12, 155
Ritonavir	HIV protease inhibitor	83
St. John's wort	Antidepressant	118, 119
Spironolactone	Antihypertensive	10
Tamoxifen	Anticancer	85
Troglitazone	Antidiabetic	67

In addition to these xenobiotics, PXR is also activated by a number of naturally occurring steroids. All PXR orthologs, from fish to human, are activated efficiently by the progesterone metabolite 5β-pregnane-3,20-dione (Fig. 1; Ref. 89). Other steroids also activate PXR, although the effects vary among species. Mouse PXR is activated by a variety of pregnanes, including pregnenolone and its 17α-hydroxylated derivative (9, 67). Rabbit PXR is efficaciously activated by progesterone and its 17α-hydroxylated derivative (67). Human PXR is activated efficiently by estradiol and to a lesser extent by corticosterone (12, 67). These results, together with the finding that PXR expression levels increase during pregnancy (75), raise the interesting possibility that PXR evolved in part to protect the body from high concentrations of endogenous steroids. PXR is also activated by natural bile acids as discussed below.

Recently, the first PXR antagonist was reported. Nanomolar concentrations of the marine-derived drug ecteinascidin 743 (ET-743; Fig. 1), a potent anticancer agent, blocked activation of human PXR by either SR12813 or paclitaxel in cell-based reporter assays (84). ET-743 also blocked the induction of the PXR target genes *CYP3A4* and multidrug resistance protein 1 (*MDR1*) in a human intestinal cell line (84).

D. Ligand binding properties of PXR

Do the structurally diverse chemicals that activate PXR do so by binding directly to the receptor? Two types of ligand binding assays have been developed for PXR. The first is a direct scintillation proximity assay (SPA) using [^{3}H]SR12813 as the radioligand (67). SR12813 (Fig. 1) is a cholesterol-lowering agent that efficiently induces *CYP3A* gene expression in human hepatocytes. SR12813 binds to human PXR with a K_d value of approximately 40 nM and is one of the most potent activators of human PXR identified to date (67). Notably, SR12813 is a much less potent activator of the rodent PXRs and therefore cannot be used in binding assays with the rodent receptors (67). In the SPA, human PXR is immobilized on a scintillant-containing bead and incubated with radioligand. When bound to the receptor, the radioligand is sufficiently close to activate the scintillant in the bead, an event that can be detected using a standard scintillation counter. The binding of nonradioactive ligands can be measured on the basis of their ability to compete with the radioligand for binding to PXR. SPA offers the advantage of being a true equilibrium assay in that it does not require separation of the bound from the free radioligand. Thus, it is readily adaptable to automated, high-throughput formats.

A second, less direct approach that has been used to determine whether compounds bind to PXR is the coactivator receptor ligand assay (CARLA; Ref. 90). CARLA exploits the fact that the binding of an agonist to a NR results in a conformational change that permits interactions with coactivator proteins such as steroid receptor coactivator 1. This interaction can be measured by the coprecipitation of radiolabeled coactivator with the receptor of interest. CARLA has been used to demonstrate that compounds bind directly to PXR (9, 10). Although CARLA is a labor-intensive technique, it has the distinct advantage of not requiring a high-affinity radioligand.

Studies employing SPA and CARLA have demonstrated that many of the diverse chemicals that activate PXR do so by binding directly to the receptor. Among the chemicals that have been shown to bind to human PXR are rifampicin, clotrimazole, phenobarbital, troglitazone, and ritonavir (9, 10, 67, 83). The K_i values for these interactions vary from low micromolar concentrations for most of the chemicals to millimolar concentrations for phenobarbital. The natural steroids 5β-pregnane-3,20-dione, corticosterone, and estradiol also bind directly to human PXR with K_i values in the low to mid micromolar range (67). As expected, PCN binds to rodent PXR but does not bind efficiently to the human ortholog (9, 67). Thus, PXR is capable of binding to a remarkably diverse collection of chemicals with molecular weights ranging from less than 250 kDa to more than 800 kDa. This promiscuity is unprecedented in the NR family.

E. DNA binding properties of PXR

NRs regulate the transcription of target genes by binding to specific DNA response elements. Members of the NR1 subfamily, which includes PXR, bind as obligate heterodimers with RXR to response elements composed of two

copies of the consensus NR binding motif AG(G/T)TCA (58). The relative orientation and spacing of the two half-site sequences dictate which RXR heterodimers can bind. For example, RXR heterodimers with the vitamin D, thyroid hormone, and RXRs preferentially bind to the two half sites organized as a DR with a spacer of three, four, or five nucleotides, respectively (58). PXR was originally shown to bind efficiently as an RXR heterodimer to DR-3 type xenobiotic response elements present in the proximal *CYP3A23* and *CYP3A2* gene promoters (Fig. 2; Refs. 9 and 79). The PXR/RXR heterodimer also binds to a DR-3 element in the *CYP3A4* enhancer (79) and an ER-6 element located in the proximal promoter of the *CYP3A4* gene (Fig. 2; Refs. 10–12). Reporter gene constructs containing multimerized copies of either the DR-3 or ER-6 response elements were activated by PXR in transient transfection assays. DR-3 and ER-6 response elements are conserved in other xenobiotic inducible CYPs including *CYP3A7* (Fig. 2; Refs. 60 and 91).

In addition to the DR-3 and ER-6 elements, the PXR/RXR heterodimer also binds to DR-4 and DR-5 type response elements (12). Functional DR-4 response elements have been identified in the promoter regions of several genes regulated by PXR, including various *CYP2B* family members (92, 93) and the *MDR1* (Fig. 2; Ref. 94). Recently, the PXR/RXR heterodimer was shown to bind to an ER-8 response element located in the 5′ flanking sequence of the multidrug resistance-associated protein 2 (*MRP2*) gene (Fig. 3; Ref. 95). Reporter gene constructs containing multimerized copies of this element were activated efficiently by PXR. Thus, the PXR/RXR complex is capable of binding to a variety of xenobiotic response elements with remarkably different architectures, including DR-3, DR-4, ER-6, and ER-8 motifs. Notably, the PXR/RXR complex is nonpermissive in that it is not activated by chemicals that bind to the RXR component of the heterodimer (67). However, micromolar concentrations of several RXR agonists such as 9-*cis* retinoic acid and LG100268 can activate the PXR/RXR heterodimer (67). This effect appears to be mediated through the PXR subunit of the heterodimer, because both 9-*cis* retinoic acid and LG100268 can bind directly to PXR at these micromolar concentrations (67).

F. PXR polymorphisms

The level of *CYP3A4* mRNA in the liver can vary by more than 50-fold from person to person. These differences may account for much of the interindividual variability in the metabolism of drugs (96). A recent study suggests that much of this difference is due to genetic variation (97). However, only a few polymorphisms have been described in either the CYP3A4 protein or the *CYP3A4* promoter, and these changes generally do not appear to have dramatic effects on either CYP3A4 expression or function (96). Do polymorphisms in PXR contribute to differences in drug metabolism? Two recent studies identified approximately 40 single nucleotide polymorphisms in the human *PXR* gene, including seven missense mutations leading to variant PXR proteins in Caucasian, African-American, and/or African populations (73, 74). Four of these (R122Q, V140M, D163G, and A370T) displayed altered basal and/or rifampicin-induced activation in cell-based reporter gene assays (73, 74). R122Q, which alters an amino acid in the DNA binding domain of PXR and decreases its DNA binding activity, resulted in a slight attenuation in PXR transcriptional activity in response to rifampicin. V140M, D163G, and A370T all change residues in the LBD of PXR. D163G had reduced basal and rifampicin-induced activity relative to wild-type PXR when tested on a reporter gene construct containing the proximal promoter and distal enhancer of *CYP3A4*. In contrast, V140M and A370T exhibited modest increases in basal activity in this same assay. The allele frequency of each of these missense polymorphisms was less than 2%, suggesting that they are unlikely to account for much of the interindividual differences observed in drug metabolism (73, 74). Interestingly, three of the silent *PXR* polymorphisms that do not result in amino acid changes correlated with changes in the expression of *CYP3A4* (74). However, the locations of these polymorphisms do not suggest obvious mechanisms to account for altered PXR activity. Further studies will be required to determine whether these or other *PXR* polymorphisms represent reliable predictors of CYP3A4 activity *in vivo*.

IV. Function of PXR *in Vivo*

A. PXR-null mice

Genetic evidence that PXR regulates xenobiotic metabolism by modulating *CYP3A* and other genes was recently provided by studies using mice lacking a functional *Pxr* gene (15, 16). These mice developed and reproduced normally and did not display any overt phenotypic changes. Extensive serum analysis did not reveal any significant differences in a number of parameters, including cholesterol, triglyceride, glucose, or liver enzyme levels. Thus, PXR does not appear to be essential for normal development or adult physiology under standard laboratory conditions. However, the PXR-null mice did not respond normally to xenobiotic treatment. PCN causes hepatomegaly in mice through the induction of cellular hypertrophy and hyperplasia. These effects were absent in PXR-null mice (98). Moreover, as predicted, the PXR-null mice did not regulate *Cyp3a11* expression normally in response to xenobiotic treatment. Neither PCN nor dexamethasone induced *Cyp3a11* in the liver or intestine in PXR-null mice (15, 16). These changes were also seen at the level of enzymatic activity. In PXR-null mice, PCN treatment did not increase the level of testosterone 6β-hydroxylation, nor did it decrease the duration of paralysis by the muscle relaxant zoxazolamine, both measures of *Cyp3a11* activity (98). Moreover, mice expressing a constitutively active form of PXR (VP16-human PXR) were resistant to zoxazolamine-induced paralysis and tribromoethanol-induced anesthesia (15). Thus, PXR serves as a key regulator of *Cyp3a11* expression *in vivo*. The PXR-null mice have also been used to establish conclusively that PXR regulates a number of other genes, including those encoding other P450 enzymes, conjugating enzymes, and transporters (16, 92, 99).

Notably, one group found that the basal level of *Cyp3a11* expression was elevated approximately 4-fold in the livers of PXR-null mice relative to their wild-type counterparts (16). Although the molecular basis for this change is not yet

known, there are at least two possibilities. First, the absence of PXR could allow other NRs that are constitutively active, such as CAR, access to the xenobiotic response elements in the *Cyp3a11* promoter. Alternately, PXR may interact with corepressor proteins such as nuclear receptor corepressor and silencing mediator of retinoid and thyroid receptor (SMRT) and actively repress *Cyp3a11* in the absence of ligand. The absence of PXR would then result in an increase in the basal expression of *Cyp3a11*. Indeed, there is evidence that PXR interacts with SMRT and that this interaction is disrupted by PXR agonists (84).

B. Humanized PXR mice

To further investigate the differences in PXR pharmacology across species, Xie *et al.* (15) expressed human PXR in the livers of PXR-null mice. As expected, *Cyp3a11* was not induced by PCN in these transgenic animals but was efficiently induced by the human-specific inducer rifampicin. Thus, the species origin of PXR determines the induction profile of *CYP3A*. As discussed above, Xie *et al.* (15) also expressed a constitutively active form of human PXR in the livers of PXR-null mice. In addition to metabolizing zoxazolamine and triethanolamine more rapidly than their wild-type counterparts, the VP16-hPXR transgenic mice exhibited growth retardation, hepatomegaly, and liver toxicity, suggesting that sustained activation of PXR may be deleterious (15).

C. PXR target genes

The list of genes regulated by PXR is growing rapidly. In the liver, PXR-selective ligands have been shown to stimulate the expression of genes involved in the oxidation (phase I), conjugation (phase II), and transport (phase III) of xenobiotics. In addition to *CYP3A* family members, phase I genes regulated by PXR in various species include *CYP2B6*, *Cyp2b9*, *CYP2C8*, *CYP2C9*, and *CYP2C19* (84, 92, 93, 100, 101). Phase II genes that are up-regulated by PXR ligands include members of the glutathione-S-transferase (102), sulfotransferase (103–105), UDP-glucoronosyltransferase (106), and carboxylesterase (107) families. Among the hepatic transporters, PXR has been shown to stimulate the expression of *Oatp2* (16) and *MRP2* (83, 108, 109). OATP2 is a basolateral transporter that mediates the transmembrane movement of numerous xenobiotics destined for biliary excretion (110, 111). MRP2 plays a key role in determining the rate of bile flow and is involved in the transport of conjugated anions, including xenobiotics, bilirubin, and bile acids, across the canalicular membrane (110, 111). Natural mutations in MRP2 cause Dubin-Johnson syndrome/hyperbilirubinemia II, a disorder characterized by impaired transfer of anionic conjugates into bile (110, 111). In the intestine, PXR has been shown to stimulate the expression of *MDR1* (84, 94), which encodes an ATP-dependent efflux pump that transports a wide variety of xenobiotics, including many widely used prescription drugs. In sum, PXR coordinately regulates a large number of genes in the liver and intestine that are involved in all aspects of the detoxification and elimination of xenobiotics from the body.

D. PXR as a basis for drug interactions

The induction of *CYP3A4* expression represents the basis for an important class of drug-drug interactions in which one drug accelerates the metabolism of a second medicine. It is now evident that most of the prescription drugs that induce *CYP3A4* do so through activation of PXR. Notably, PXR has also been implicated in the interaction between the herbal remedy St. John's wort (SJW) and prescription drugs. SJW, which is derived from the flowering plant *Hypericum perforatum*, is widely used as an over-the-counter treatment for a variety of indications including depression and inflammation. In patients, SJW induced *CYP3A4* expression in the intestine (112) and enhanced the metabolism of oral contraceptives, the immunosuppressant cyclosporin, the HIV protease inhibitor indinavir, the anticoagulant warfarin, and the cardiotonic digoxin (113–117). Each of these drugs is metabolized by CYP3A4 and/or CYP2C8, which suggested the involvement of PXR. Indeed, extracts prepared from commercial sources of SJW were shown to activate PXR in cell-based reporter assays (118, 119). Analysis of the different chemical constituents of SJW revealed that nearly all of the PXR activity resided in a single compound, hyperforin (Fig. 1; Refs. 118 and 119). Interestingly, there is evidence that hyperforin is the ingredient in SJW responsible for its antidepressant activity (120), raising the possibility that PXR is the target for this pharmacological activity. Hyperforin bound to human PXR with an IC_{50} value of approximately 20 nM and induced *CYP3A4* expression in primary cultures of human hepatocytes (118). These results provide a molecular basis for the reported interactions between SJW and various prescription drugs and, furthermore, indicate that SJW is likely to interact with many more drugs than was previously realized.

The discovery that PXR is responsible for many drug-drug interactions and at least one herb-drug interaction has important pharmaceutical ramifications. Historically, the identification of compounds that induce *CYP3A4* gene expression was performed during the latter stages of the drug discovery process using primary cultures of human hepatocytes, because studies in animals were not necessarily predictive of induction potential in humans. These assays were time consuming and required human liver tissue, which is not readily available and varies considerably in quality and induction potential from donor to donor. The knowledge that PXR is a critical regulator of *CYP3A4* gene expression together with the availability of robust, high throughput cell-based and scintillation proximity binding assays for PXR permits the rapid identification of chemicals that will induce *CYP3A4* expression at the earliest stages of drug discovery. Entire libraries of drug candidates can now be screened for PXR activity. Those drug candidates that test positive can be replaced with chemicals that have similar therapeutic efficacy but lack PXR activity. For example, the antidiabetic drugs troglitazone, pioglitazone, and rosiglitazone are effective PPARγ agonists, but only troglitazone is also a potent PXR agonist (67). In humans, troglitazone interacted with other drugs and was associated with a rare hepatotoxicity that led to its withdrawal from the market (121). Pioglitazone and rosiglitazone do not activate PXR and have not been linked

to these adverse events. A second example is provided by the cancer drug paclitaxel and its taxane analog docetaxel. Both drugs have similar antineoplastic activity, yet paclitaxel is an efficacious PXR activator, whereas docetaxel is not (84). This difference is likely to account for the superior pharmacokinetic properties of docetaxel. PXR assays also provide a rapid and economical means to test herbal preparations and other over-the-counter dietary supplements for their potential to induce *CYP3A4* expression. The knowledge that PXR is the molecular basis for common drug-drug and herb-drug interactions should aid in the development of safer medicines.

V. PXR and Bile Acid Metabolism

Bile acids, which are produced by the liver, are essential for the elimination of excess cholesterol from the body and the solubilization, absorption, and transport of dietary lipids in the intestine. Bile acid homeostasis is tightly regulated because bile acids are detergents that can be extremely toxic if their levels become elevated. An important insight into the regulation of bile acid homeostasis was provided by the discovery that the farnesoid X receptor (FXR; NR1H4), a member of the NR family, is a bile acid receptor (122–124). Several bile acids and/or their taurine and glycine conjugates bind and activate FXR at physiological concentrations, including cholic acid and chenodeoxycholic acid, the principal bile acids in humans. FXR stimulates the expression of genes involved in bile acid homeostasis, including the intestinal bile acid binding protein and the bile salt export pump (125, 126). Activation of FXR also represses the expression of CYP7A1, which catalyzes the rate-limiting step in the classical pathway for the conversion of cholesterol to bile acids (127, 128). Thus, FXR mediates both feedforward and feedback regulation of bile acid homeostasis.

It has been known for many years that treatment of rodents with PCN also results in a marked suppression of hepatic cholesterol 7α-hydroxylase activity (129). More recently, it was shown that this repression occurred at the level of *CYP7A1* mRNA (130). Studies in PXR-null mice demonstrated that *Cyp7a1* was dysregulated in two respects (16). First, the basal expression of *Cyp7a1* was reduced approximately 50% in PXR-null mice relative to their wild-type littermates. Second, PCN-induced suppression of *Cyp7a1* was abolished in the PXR-null mice. These data established roles for PXR in both the basal expression and repression of *Cyp7a1*. Interestingly, many of the other PXR target genes that are involved in xenobiotic metabolism also participate in bile acid metabolism. For example, CYP3A4 hydroxylates bile acids, and MRP2 and OATP2 transport bile acids across hepatic canalicular and sinusoidal membranes, respectively.

Because PXR coordinately regulates several genes involved in bile acid metabolism, a series of bile acids were tested for their ability to bind and activate PXR in cell-based reporter assays and SPA. Notably, the secondary bile acid lithocholic acid (LCA; Fig. 1), which is formed in the intestine by the bacterial 7α-hydroxylation of chenodeoxycholic acid, and its 3-keto metabolite were both efficacious activators of the mouse and human PXR (16, 131). LCA and 3-keto LCA bound to human PXR in an SPA with IC_{50} values of approximately 10 μM. As predicted, LCA treatment resulted in the induction of PXR target genes, including *Cyp3a11* and *Oatp2*, in the livers of wild-type mice but not in PXR-null animals (16).

LCA is a particularly toxic bile acid that causes cholestasis, a disease state characterized by the impairment or cessation of bile flow and the accumulation of bile acids and other biliary toxins in the liver and serum (132). The most serious forms of the disease can progress to complete liver failure and must be treated by liver transplant. Normally, LCA levels are low in healthy mammals. However, LCA concentrations of 5–10 μM have been reported in the livers of cholestatic patients and in rodent models of biliary cholestasis (133). Taken together, these findings suggest that pathophysiological levels of LCA and/or its metabolites activate PXR and turn on the expression of a program of genes involved in the detoxification and removal of these bile acids from the body. A prediction of this model is that potent PXR agonists may be useful for the treatment of cholestatic liver disease. Indeed, it has been known for nearly 30 yr that treatment of rodents with PCN blocks the severe hepatotoxicity and mortality caused by LCA treatment in rats (134). Two groups recently demonstrated that this hepatoprotective effect of PCN is dependent on PXR (16, 131). Coadministration of PCN severely reduced liver damage in wild-type mice treated with LCA as assessed by liver histology and measurement of serum concentrations of liver enzymes. No such hepatoprotection from LCA toxicity was detected in PXR-null mice. Mice expressing the constitutively active form of the human PXR were similarly protected against LCA toxicity (131). Thus, PXR can protect the body against pathophysiological concentrations of toxic bile acids.

Several lines of evidence suggest that these findings may have implications in the treatment of human cholestatic liver disease. Patients suffering from cholestasis have been reported to have elevated levels of 6-hydroxylated bile acids, including the LCA metabolite hyodeoxycholic acid, which are generated by CYP3A4 (135, 136). These findings suggest that increased 6-hydroxylation is a relevant mechanism for reducing the levels of toxic bile acids in humans. Interestingly, the PXR ligand rifampicin has been used successfully in the treatment of pruritus caused by intrahepatic cholestasis and, in certain cases, has been reported to induce remission of cholestasis (137–139). Similarly, the herb SJW promotes bile flow and has been used to treat a variety of hepatic disorders, including cholestasis (140, 141). Finally, ursodeoxycholic acid, which is used clinically for the treatment of cholestasis, was recently shown to induce *CYP3A4* expression in primary cultures of human hepatocytes and to activate PXR in cell-based reporter assays (142). Although the molecular bases for the anticholestatic effects of rifampicin, SJW, and ursodeoxycholic acid have remained obscure, the findings that they all activate PXR suggest that their anticholestatic effects may be mediated in part through activation of this NR. These results raise the intriguing possibility that more potent PXR ligands may prove to be efficacious drugs for the treatment of cholestasis.

VI. PXR Cross-Talk with CAR

The NR most closely related to PXR is CAR. These two receptors share approximately 70% and 50% amino acid identity in their DBDs and LBDs, respectively (Fig. 3; Ref. 12). CAR was originally described as MB67, a liver-enriched orphan NR that strongly activated reporter gene activity in cell-based assays in a ligand-independent fashion (143). The subsequent discovery that the testosterone metabolites androstanol and androstenol bind directly to MB67 and suppress its basal activity led to it being renamed the CAR (144). More recently, biochemical and genetic studies have demonstrated that CAR is responsible for many of the well characterized biological effects of the xenobiotic phenobarbital. Treatment of rodents with phenobarbital causes dramatic hypertrophy and hyperplasia of the liver and a marked induction of *CYP2B* gene expression. These effects are completely absent in mice in which the *Car* gene has been disrupted by homologous recombination (145). Experiments performed in hepatocytes show that CAR is localized predominantly in the cytoplasm in its inactive state (146, 147). Phenobarbital activates CAR by promoting its translocation into the nucleus. Notably, phenobarbital does not activate CAR by binding directly to its LBD (89). Rather, phenobarbital appears to modulate CAR activity through an indirect mechanism involving phosphorylation, because the phosphatase inhibitor okadaic acid blocks its effects (147).

CAR and PXR were originally shown to regulate *CYP2B* and *CYP3A* genes, respectively (9, 79, 146). CAR regulates *CYP2B* genes by binding to two imperfect DR-4 motifs within a conserved phenobarbital responsive element module (148). Because these xenobiotic response elements are different from the DR-3 and ER-6 elements located upstream of the *CYP3A* genes, the CAR and PXR signaling pathways appeared to be distinct. However, several groups have now demonstrated that PXR can bind to the DR-4 elements in the phenobarbital responsive element module and regulate *CYP2B* genes in cell-based reporter assays (92, 93, 149). Moreover, transgenic mice expressing the constitutively active VP16-human PXR have elevated expression of *Cyp2b10*, demonstrating that this regulation occurs *in vivo* (92). Conversely, CAR binds to the DR-3 and ER-6 xenobiotic response elements that were originally characterized as PXR/RXR binding sites and regulates *CYP3A* expression (92, 93, 145, 150, 151). Recent studies indicate that the overlap in PXR and CAR target genes extends well beyond the *CYP2B* and *CYP3A* genes. In the liver, CAR and PXR coregulate members of the *CYP2C*, glutathione-S-transferase, sulfotransferase, and UDP-glucoronosyltransferase families and the canalicular *MRP2* transporter (95, 99–101). These findings suggest a functional redundancy in the CAR and PXR signaling pathways (Fig. 4). Nevertheless, there are differences in the degree to which specific genes are activated by either CAR or PXR agonists (99), which undoubtedly contributes to the distinct pharmacologies of these xenobiotics.

Is CAR a generalized xenobiotic sensor like PXR? PXR and CAR are regulated by several of the same chemicals, including xenobiotics and natural steroids (89). However, studies in which CAR and PXR were tested against collections of xenobiotics and natural steroids in cell-based reporter assays revealed that CAR is much less promiscuous in its interactions with chemicals than PXR (65, 89). Molecular modeling of the CAR LBD based on the PXR crystal structures suggests that CAR is likely to have a much smaller ligand binding pocket than PXR (see below). Nevertheless, CAR may be activated indirectly by a variety of chemicals that promote its translocation into the nucleus. Thus, CAR and PXR may play complementary roles in sensing potentially harmful chemicals that either alter the phosphorylation status of the cell or enter the nucleus.

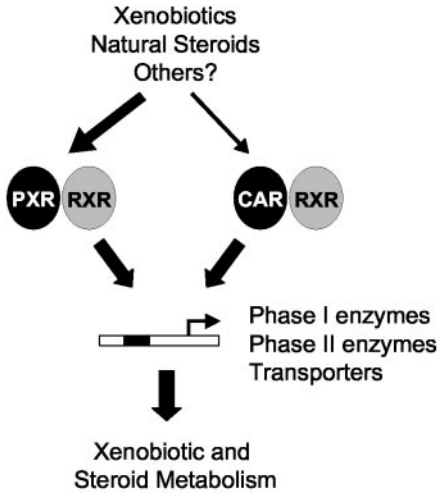

FIG. 4. Cross-talk between the PXR and CAR signaling pathways. Both PXR and CAR are modulated by xenobiotics and natural steroids and bind as RXR heterodimers to response elements in the regulatory regions of genes involved in xenobiotic and steroid metabolism, including genes encoding phase I and phase II enzymes and transporters.

VII. Structure of the PXR Ligand Binding Domain

To function as a xenobiotic sensor, PXR has evolved the ability to recognize a wide variety of toxic substances. Despite this promiscuity, striking species differences are seen in the activation profiles between PXR orthologs. The recent determination of the three-dimensional structure of the human PXR LBD by x-ray crystallography has shed light on the molecular basis of these properties (17). The LBD displays the signature fold of the NR superfamily, comprising a three-layer sandwich of α-helices and a short region of β-strands (Fig. 5; Ref. 61). Notably, there are several unique features of the PXR LBD that diverge from the architecture of other NRs. Helix 2 in PXR is replaced by a four-residue turn and a pseudohelical segment that forms the floor of the ligand binding pocket. Adjacent to this region, PXR has a β-sheet structure comprising five strands, rather than the usual three seen in other NRs. Two of these β-strands (β1 and β1′; Fig. 5) are responsible for unwinding helix 6 into a flexible loop, which may provide the ligand binding pocket with the potential to expand and contract to accommodate ligands of various sizes. These features combine to give PXR a spherical ligand binding pocket that has a volume of more than 1150 $Å^3$ (Fig. 5). Twenty of the 28 amino acids lining the pocket

are hydrophobic, with the remaining eight residues (four polar and four charged) evenly spaced around the pocket.

The cocrystal structure of PXR with SR12813 revealed that a single drug molecule was bound in the pocket in three distinct orientations (17). Each molecule was equally represented in the total electron density with 19 of the 28 residues lining the pocket contacted by the ligand. Remarkably, the receptor employed different hydrogen bonds and van der Waals interactions to bind each orientation of the ligand. These properties may allow PXR to recognize a wide range of xenobiotics, because the large spherical pocket does not require ligands to satisfy a single shape or arrangement of hydrogen bonding interactions. The species specificity of PXR activation can be explained, in part, by variations in the sequence of the amino acids lining the binding pocket. The mouse and human PXR LBDs have five nonconservative changes in these residues (Fig. 6). Mutation of four of the variant amino acids in mouse PXR to the corresponding human sequence gave a hybrid receptor that showed a human-like activation profile (17). The fish receptor, a distant PXR ortholog that is activated by pregnanes but not by bile acids, has 10 nonconservative changes, 5 of which are residues mutated in the mouse receptor (Fig. 6; Ref. 65). The evolution of these changes in specific residues lining the PXR binding pocket may have been driven by the need to recognize different xenobiotics in each species.

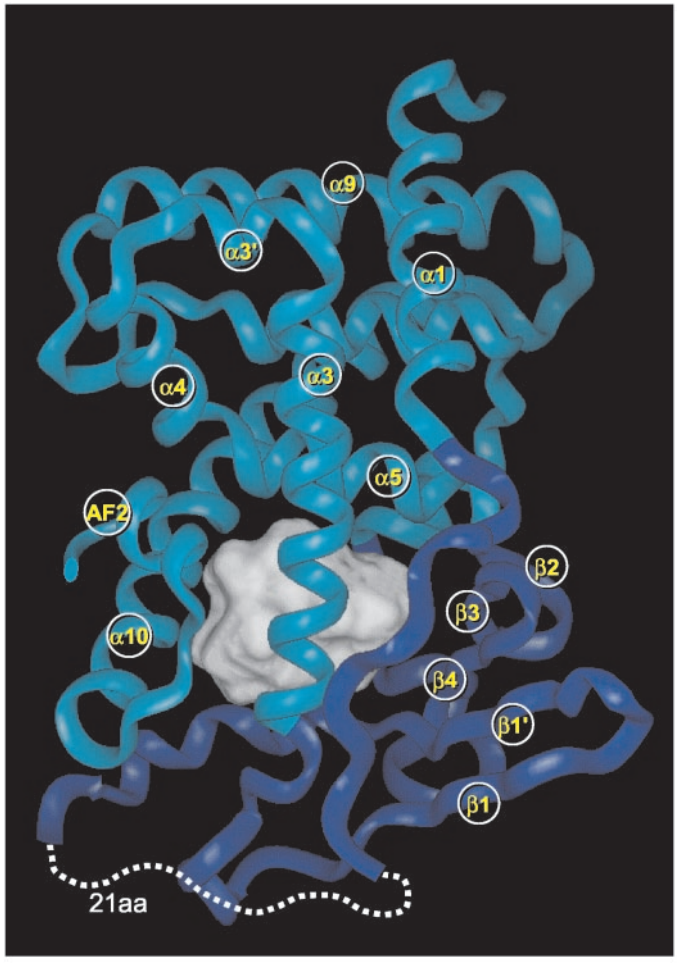

FIG. 5. Structure of the PXR LBD. The three-dimensional structure of the human PXR LBD is presented as a *ribbon diagram*. The α-helices and β-sheets are numbered, and the AF-2 helix is indicated. The helix 1–helix 3 insert, which is unique to PXR among all the NRs, is indicated in *dark blue*. The large (1150 Å^3) solvent-accessible ligand binding pocket is outlined in *white*. The 21 amino acids that were absent from the electron density in the crystal structure are indicated by a *dotted line*.

As described above, three naturally occurring variants of human PXR were recently identified within the LBD (73). V140M and D163G lie at the N-terminal and C-terminal ends of helix 1, respectively, and A370T is in the middle of helix 9. Although none of these mutations directly impinges on the ligand binding pocket, they showed some effect on the transcriptional activity of the receptor (73). The A370T mutant had slightly enhanced basal activity in the absence of exogenous ligands. Ala^{370} forms part of the dimerization interface with RXR, and mutation to Thr would increase the size and polarity of this residue. Thus, the PXR A370T mutant may show altered heterodimerization with RXR, although this has not been examined. The V140M mutant also showed slightly enhanced basal activity; however, the structural basis for this difference remains unknown because this residue lies outside of the domain examined by x-ray crystallography. In contrast to the V140M and A370T mutants, the D163G mutant was almost devoid of basal activity. Asp^{163} initiates the β-turn leading into the helix 1–helix 3 insert. Mutation of the residue to Gly would be expected to destabilize this region of the receptor. Interestingly, the D163G mutant was activated on an ER-6 response element by rifampicin but not corticosterone (73), indicating that disruption of the helix 1–helix 3 insert has differential effects on the activities of these two PXR ligands.

The region between helix 1 and helix 3, which comprises approximately 50 amino acids, plays a critical role in shaping the properties of the PXR ligand binding pocket (17). This insert forms the floor of the binding pocket and encompasses two of the five β-strands (17). Sequence alignment reveals that the CAR lacks this insert, whereas the corresponding region in VDR is unstructured and does not contribute to the binding pocket (152, 153). All of the PXR orthologs have a sequence element in the helix 1–helix 3 insert described as a capping segment that results in the unwinding of helix 6 and builds additional volume and flexibility into the binding

	206	208	209	211	240	243	244	246	247	251	281	284	285	288	299	306	308	321	323	324	327	408	410	411	414	420	425	429
human	L	S	L	V	L	M	A	M	S	F	F	C	Q	F	W	Y	L	E	M	L	H	H	R	L	I	F	M	F
mouse	R	P	M	I	L	L	A	V	S	F	F	C	I	F	W	Y	F	D	L	M	H	Q	Q	L	I	F	M	F
zebra fish	W	F	N	T	L	F	T	L	T	I	F	I	L	F	W	Y	M	D	M	M	H	Y	Q	V	I	E	W	I

FIG. 6. Residues lining the PXR ligand binding pocket. The residues lining the human PXR ligand binding pocket are shown aligned with corresponding residues in PXR from other species. Numbers are derived from the human PXR sequence. Residues whose charge or polarity are not conserved are *boxed*.

pocket (65). Because CAR, BXR, and VDR lack the capping segment, their binding pockets may be unable to accommodate ligands that vary considerably in size. The naturally occurring PXR.2 variant, which deletes the helix 1–helix 3 insert through alternate splicing of exon 5 (74), does not show promiscuous activation by xenobiotics (9). A phylogenetic analysis of the NR1I subfamily suggests that CAR and BXR may have evolved from an ancestral receptor through deletion of an exon that removed a segment of the helix 1–helix 3 insert (89). Thus, it appears that only PXR has all of the structural features consistent with a role as a promiscuous xenobiotic receptor.

VIII. Summary and Perspectives

In this review, we have highlighted the role that PXR plays in protecting the body against chemical insult. PXR was originally shown to regulate *CYP3A* genes, but we now know that this receptor regulates an entire program of genes in the liver and intestine that are involved in the metabolism and elimination of potentially toxic chemicals from the body. Importantly, PXR is activated by a remarkably diverse set of chemicals, including both xenobiotics and substances made by the body such as bile acids and other steroids. Thus, in contrast to almost all other NRs, which are specialized to recognize discrete physiological ligands, PXR has evolved to function as a broad substrate chemical sensor. Although PXR is a promiscuous receptor, there are marked differences in PXR activation profiles between species. These differences may reflect differences in diets or exposure to other chemicals across species. Alternatively, the PXR activation profiles may be driven by differences in the production of endogenous chemicals such as bile acids.

Can the biological actions of PXR be exploited to treat human disease? Hans Selye (7) first proposed many years ago that catatoxic steroids, which we now know act through PXR, might prove useful in the detoxification of chemicals ranging from xenobiotics to stress-induced hormones to bile acids. Indeed, in the case of bile acids and biliary cholestasis, there is already evidence that this might be the case. In rodents, PXR ligands protect against the severe liver damage induced by LCA. In humans, the PXR ligand rifampicin has been used in the treatment of biliary cholestasis, although the mechanism underlying this effect remained unknown. Moreover, SJW has been used for many years as an anticholestatic agent for the treatment of liver disease (140, 141). Although speculative, the knowledge that both of these agents activate PXR raises the intriguing possibility that drugs optimized against human PXR may prove efficacious in the treatment of cholestasis. It remains to be determined whether PXR ligands will have a broader role in treating diseases in which toxins accumulate in the liver, intestine, or other tissues as originally postulated by Selye.

Although PXR evolved to fulfill a protective role, its promiscuity has become a major liability in an era in which patients are routinely treated with multiple medications. PXR is activated by a variety of prescription drugs and at least one widely used medicinal herb. It is now established that activation of PXR by these medicines represents the molecular basis for a common class of potentially life-threatening drug interactions, in which one drug accelerates the metabolism of another. Fortunately, it should now be possible to exploit our knowledge of PXR to minimize the chances of this type of drug interaction. High throughput binding and activation assays can be used prospectively to test new drug candidates as well as currently approved drugs for their activity on PXR. Moreover, these assays can also be extended to herbal remedies and other over-the-counter dietary supplements. Finally, a detailed understanding of the three-dimensional structure of the PXR LBD may make it possible in the future to determine whether a chemical will bind to PXR through *in silico* modeling, ideally before the molecule has even been synthesized. Thus, compounds that are likely to activate PXR can be removed at the early stages of the drug discovery process and replaced with safer molecules.

Although a tremendous amount has been learned about the biological functions of PXR, a number of outstanding questions remain. What other biological pathways are regulated by PXR? For example, PXR activation is known to cause hepatomegaly in the livers of rodents. Does PXR regulate genes involved in the cell cycle? What genes and biological processes are regulated by PXR in tissues where it is expressed at low levels such as the lung and kidney? Some of the most fascinating questions about PXR relate to its genetic variation. A number of PXR polymorphisms have now been described, including several that affect PXR transcriptional activity *in vitro*. Do these polymorphisms contribute to the tremendous interindividual differences in drug metabolism? If so, can this knowledge be used to tailor drug regimens to individual patients so as to maximize therapeutic impact and minimize adverse events? Along similar lines, does genetic variation in PXR contribute to human disease? Because PXR plays a central role in the detoxification of many different substances, polymorphisms that decrease its activity might be expected to increase exposure to a wide variety of dietary constituents and environmental pollutants and, ultimately, contribute to diseases as diverse as liver cirrhosis and cancer. Thus, a field that was born in the 1960s with the discovery of steroids that had unusual pharmacological activities in rodents has grown up to have tremendous implications in both the development and treatment of human disease.

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