thematic review

Regulation of xenobiotic and bile acid metabolism by the nuclear pregnane X receptor

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Abstract The nuclear pregnane X receptor (PXR; NR1I2) is an integral component of the body's defense mechanism against chemical insult (chemoprotection). PXR is activated by a diverse array of lipophilic chemicals, including xenobiotics and endogenous substances, and regulates the expression of cytochromes P450, conjugating enzymes, and transporters involved in the metabolism and elimination of these potentially harmful chemicals from the body. Among the chemicals that bind and activate PXR is the toxic bile acid lithocholic acid; activation of PXR, in turn, protects against the severe liver damage caused by this bile acid. Thus, PXR serves as a physiological sensor of lithocholic acid and perhaps other bile acids and coordinately regulates genes involved in their detoxification. Interestingly, both the antibiotic rifampicin and the herbal antidepressant St. John's wort activate PXR and have anticholestatic properties, which suggests that more potent, selective PXR agonists may be useful in the treatment of biliary cholestasis or other diseases characterized by the accumulation of bile acids or other toxins in the liver.—Kliewer, S. A., and T. M. Willson. **Regulation of xenobiotic and bile acid metabolism by the nuclear pregnane X receptor. *J. Lipid Res.* 2002.** 43: **359–364.**

Supplementary key words nuclear receptor • cytochrome P450 • lithocholic acid • farnesoid X receptor • cholestasis • drug-drug interaction

The body must be constantly vigilant against attack by chemicals that are either derived from the environment (xenobiotics) or produced by the body itself under conditions of stress or disease. The cytochrome P450 (CYP) family of heme-containing monooxygenases represents a first line of defense in the body's armamentarium against a wide variety of lipophilic chemicals, including both xenobiotics and endogenous chemicals such as bile acids and other steroids (1). Members of the CYP3A subfamily are particularly important in this respect because they are the most abundant CYPs in the liver and intestine, and they metabolize a structurally diverse collection of compounds (2). Notably, CYP3A levels can be markedly induced by a variety of xenobiotics as well as by disease states that increase bile acid concentrations (2, 3). The induction of these enzymes provides a mechanism for amplifying the physiologic response to potentially toxic chemicals. However, the induction of CYP3A4 in human liver and intestine also represents the basis for an important class of drug-drug interactions, since many of the chemicals that induce its expression are prescription drugs, and CYP3A4 is responsible for the metabolism of >50% of drugs currently on the market (2). Thus, one drug can accelerate the metabolism of a second medicine, potentially resulting in adverse consequences to the patient.

Although the effects of xenobiotics on CYP3A expression were well established, the molecular basis for this induction had remained obscure. In this article, we highlight the critical role that the nuclear pregnane X receptor (PXR) plays in regulating the expression of *CYP3A* and other genes involved in the body's chemoprotective response to a broad spectrum of compounds, including xenobiotics and bile acids.

PXR: A PROMISCUOUS XENOBIOTIC RECEPTOR THAT REGULATES *CYP3A*

In 1998, a novel murine member of the steroid-retinoid-thyroid hormone receptor family of ligand-activated transcription factors was reported and named the PXR based on its activation by both natural and synthetic C21 steroids (pregnanes) (4). PXR had all the hallmark features of a nuclear receptor, including a highly conserved DNA binding domain of ~70 amino acids and a ligand-binding domain (LBD) of ~300 amino acids in the C-terminal portion of the protein. PXR orthologs have subsequently been cloned from the human, rabbit, and rat (**Fig. 1**) (5–10). The human PXR is alternately referred to as the pregnane activated

Abbreviations: CA, cholic acid; CDCA, chendeoxycholic acid; LBD, ligand-binding domain; LRH, liver receptor homolog; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; OATP, organic anion transport protein; PCN, pregnenolone 16α-carbonitrile; PXR, pregnane X receptor; RXR, 9-*cis* retinoic acid receptor; UDCA, ursodeoxycholic acid.

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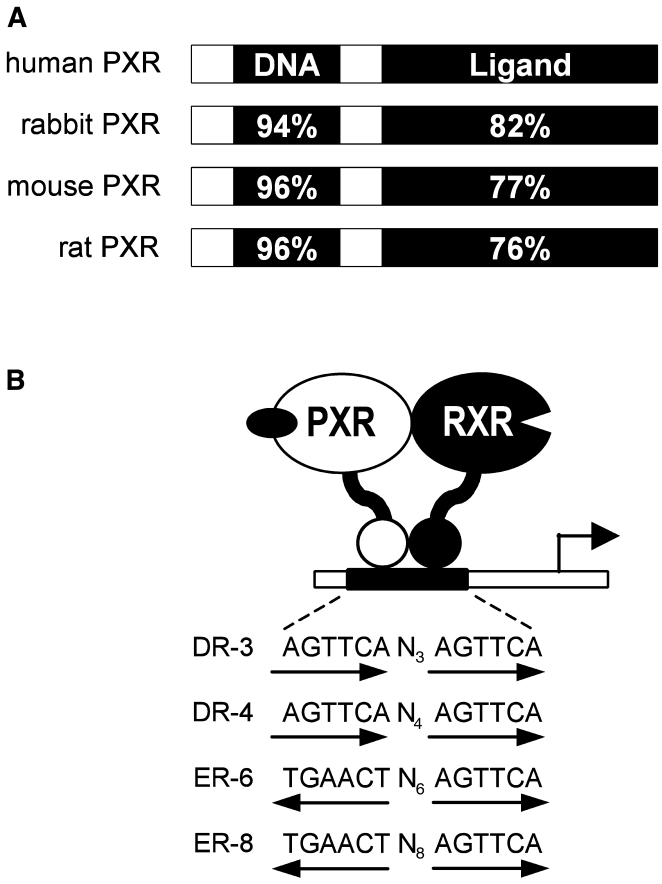

Fig. 1. A: Alignment of PXR orthologs. The similarity is expressed as percent amino acid identity in the DNA-binding (DNA) and ligand-binding (Ligand) domains. B: Pregnane X receptor (PXR) binds as a 9-*cis* retinoic acid receptor (RXR) heterodimer to xenobiotic response elements composed of two nuclear receptor half sites of the consensus sequence AGTTCA organized as either a direct repeat with a three or four nucleotide spacer (DR-3 and DR-4, respectively) or an everted repeat with a six or eight nucleotide spacer (ER-6 and ER-8, respectively).

receptor (6) or the steroid and xenobiotic receptor (7). The PXR orthologs share >90% amino acid identity in the DNA binding domain. Surprisingly, there is a marked divergence in the LBD across species. For example, human and mouse PXR share only 77% identity in their LBDs, which is much lower than most nuclear receptor orthologs (Fig. 1). These data suggested that the pharmacology of PXR was likely to vary between species.

It soon became apparent that PXR was an unusual nuclear receptor in that it was activated by a remarkably diverse collection of compounds, including both xenobiotics and natural steroids (4–10). Among the chemicals that activated PXR were the macrocyclic antibiotic rifampicin, the glucocorticoid dexamethasone, and the antiglucocorticoids pregnenolone 16α-carbonitrile (PCN) and RU486 (**Fig. 2**). All of these compounds are classic inducers of *CYP3A* expression, which provided the first hint that PXR regulated *CYP3A*. Over the past 4 years, a large body of evidence has accumulated linking PXR to the regulation of *CYP3A* gene expression. The evidence includes the following:

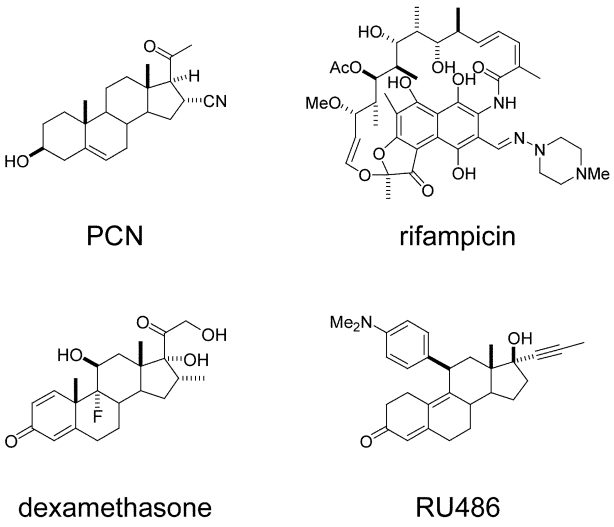

Fig. 2. Chemical structures of PXR agonists.

PXR and CYP3A are co-expressed in tissues

PXR is highly expressed in the liver and intestine of humans, rabbits, rats, and mice (4–10). These are the same tissues in which *CYP3A* genes are expressed and induced in response to xenobiotics.

PXR binds to xenobiotic response elements in CYP3A promoters

Studies from the Guzelian and Kaspar laboratories had identified response elements in human, rabbit, and rat *CYP3A* promoters that conferred responsiveness to xenobiotics, including PCN and rifampicin (11, 12). These elements contained two nuclear receptor half sites of the consensus sequence AGTTCA, organized as either a direct repeat with a three nucleotide spacer (DR-3) or an everted repeat with a spacer of six nucleotides (ER-6). PXR binds as a heterodimer with the 9-*cis* retinoic acid receptor (RXR; NR2B1) to both the DR-3 and ER-6 response elements (Fig. 1) and activates transcription efficiently through these response elements (4–7). The PXR/RXR heterodimer has also been shown to bind and to activate through DR-4 and ER-8 response elements located in the regulatory regions of CYP2B genes (13, 14) and the multidrug resistance-associated protein 2 (MRP2) gene (15). Thus, the PXR/RXR heterodimer is capable of activating transcription through a variety of response elements with distinct architectures.

PXR is activated by CYP3A inducers in a species-specific manner

Nearly all of the chemicals that induce *CYP3A* expression activate PXR in reporter gene assays. In addition to rifampicin, dexamethasone, PCN, and RU486, chemicals that activate PXR include the anti-fungal drug clotrimazole (5), the 11β-hydroxylase inhibitor metyrapone (16, 17), the diabetes drug troglitazone (8), the HIV protease inhibitor ritonavir (18), the cancer drug Taxol (19), the herbal antidepressant St. John's wort (20, 21), and a variety of environmental pollutants such as bisphenol A, diethyl-

hexylphthalate, and nonylphenol (22, 23). PXR is also activated by a variety of naturally occurring steroids including progestins, estrogens, and corticosteroids (4, 6–8). PXR has a very large (>1100 Å^3), spherical ligand-binding cavity, which accounts for its promiscuous ligand-binding properties (24). Notably, there are marked differences in PXR activation profiles between species. For example, PCN is an efficacious activator of mouse and rat PXR, but has much less activity on the human and rabbit receptors (5, 7–9). Conversely, rifampicin activates the human and rabbit PXR but has virtually no activity on the mouse or rat receptors. The PXR activation profiles of these compounds correlate closely with their effects on *CYP3A* expression in hepatocytes derived from the different species (8, 25). These data provided strong pharmacologic evidence that PXR regulates *CYP3A* expression.

CYP3A is dysregulated in PXR-null mice

Two groups generated mice in which the *Pxr* gene was disrupted by homologous recombination (26, 27). The PXR-null mice developed and reproduced normally. However, *Cyp3a11* was not induced by PCN or dexamethasone in these animals (26, 27). Moreover, the PXR-null mice were hypersensitive to treatment with chemicals such as the sedatives tribromoethanol and zoxazolamine that are metabolized by CYP3A11 (26). These data demonstrated unequivocally that PXR serves as a master regulator of *CYP3A* expression.

We now know that PXR regulates an entire program of genes involved in the detoxification and elimination of xenobiotics from the body. Among the genes that are regulated by PXR in the liver and/or intestine are those encoding other phase I monooxygenases, including CYP2B6, CYP2B9, CYP2C8, CYP2C9, CYP2C19 (14, 19, 28, 29); genes encoding phase II enzymes involved in the conjugation of xenobiotics, including members of the glutathione-*S*-transferase (30), sulfotransferase (31–33), UDP-glucuronosyltransferase (34), and carboxylesterase families (35); and genes encoding proteins involved in the transport of xenobiotics such as multidrug resistance protein 1 (MDR1) (19, 36), MRP2 (15, 18, 37), and the organic anion transport protein 2 (OATP2) (15, 18, 27, 37). Thus, PXR regulates genes whose products are involved in all aspects of the metabolism of xenobiotics, including their oxidation, conjugation, and transport. Notably, many of these genes are also involved in the metabolism of bile acids and other steroids (see below).

Although PXR evolved as a chemoprotective receptor, its inadvertant activation by prescription drugs such as dexamethasone and rifampicin and the herb St. John's wort, represents the molecular basis for an important class of drug-drug interactions (5, 20, 21). Activation of PXR by one medication can increase the levels of CYP3A, MDR1, and other gene products, and enhance the metabolism of other medications, potentially resulting in adverse health consequences to the patient. For example, St. John's wort has been reported to interact with the immunosuppressant cyclosporin, the HIV protease inhibitor indinavir, the anticoagulant warfarin, and oral contraceptives (38). The availability of robust in vitro assays to detect PXR binding and activation now permits new drug candidates to be screened prospectively for PXR activity in order to minimize the potential for drug-drug interactions.

IMPLICATIONS OF PXR IN BILE ACID METABOLISM

Bile acids are essential for the absorption of dietary lipids and fat-soluble vitamins and are a means for eliminating excess cholesterol from the body. However, bile acids are detergents that can be extremely toxic if their levels become abnormally high. How are bile acid levels regulated? In 1999, three groups reported that the farnesoid X receptor (FXR; NR1H4), a member of the nuclear receptor family, serves as a bile acid receptor (39–41). Several bile acids and their taurine and glycine conjugates bind and activate FXR at physiological concentrations, including cholic acid and chenodeoxycholic acid, the primary bile acids in humans (Fig. 3) (39–41). FXR is also activated by the secondary bile acids deoxycholic acid and lithocholic acid and their conjugated derivatives. FXR stimulates the expression of genes involved in bile acid homeostasis, including the intestinal bile acid binding protein and the bile salt export pump (39, 42). 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 (39). FXR does not suppress *CYP7A1* expression directly. Rather, FXR induces the expression of short heterodimer protein (SHP), an atypical member of the nuclear receptor family that lacks a DNA-binding domain (43, 44). SHP, in turn, binds to and represses liver receptor homolog 1 (LRH-1), a nuclear receptor that activates *CYP7A1* expression. Thus, feedback regulation of bile acid synthesis involves a transcriptional cascade of three nuclear receptors. Mice lacking FXR do not induce *Shp* or repress *Cyp7a1* in response to bile acids and exhibit markedly elevated levels of bile acids in their serum (42). Interestingly, *Cyp3a11* expression is dramatically increased in the livers of the FXR-null mice (3).

It has been known for almost 30 years that PCN also suppresses cholesterol 7α-hydroxylase activity (45). Treatment of rodents with PCN results in a marked suppression of *CYP7A1* mRNA levels (46). Recently, Staudinger et al. demonstrated that PXR is required for this effect (27, 47). *Cyp7a1* was dysregulated in two respects in PXR-null animals. First, the basal expression of *Cyp7a1* was reduced ~2-fold in PXR-null mice relative to their wild type littermates. Second, suppression of *Cyp7a1* expression by PCN was abolished in the PXR-null mice. Thus, PXR regulates both the basal expression and repression of *Cyp7a1*. Interestingly, PXR did not regulate *Shp* expression. Thus, PXR represses *Cyp7a1* through a mechanism distinct from that of FXR. In addition to *Cyp7a1*, PXR also regulates other genes that have also been implicated in bile acid metabolism. These genes include *MRP2* (15) and *Oatp2* (27), which transport bile acids across hepatic canalicular and sinusoidal membranes, respectively (48); and, *CYP3A*,

which hydroxylates bile acids including lithocholic acid (LCA) (49).

Because PXR regulates a number of genes involved in bile acid metabolism, two groups tested whether bile acids activate PXR (27, 50). Unlike FXR, PXR was not activated efficiently by cholic acid, chenodeoxycholic acid, or their conjugated derivatives. However, both the mouse and human PXR were activated efficiently by the secondary bile acid LCA and its 3-keto metabolite (**Fig. 3**). LCA and 3-keto LCA bound to human PXR with IC_{50} values of ~10 μM (27). As expected, LCA treatment resulted in the induction of PXR target genes, including *Cyp3a11* and *Oatp2*, in the livers of wild type mice but not PXR-null animals (27, 50).

LCA is a highly toxic bile acid that, when administered to rodents, causes severe liver damage and biliary cholestasis, a disease state characterized by decreased bile flow and the accumulation of bile constituents in the liver and blood (51). Normally, LCA levels are low in mammals, but they can reach 5–10 μM in the livers of cholestatic patients and in rodent models of biliary cholestasis (52). These data suggest that pathophysiologic levels of LCA and/or its metabolites may activate PXR and regulate the expression of genes involved in the production, detoxification, and elimination of bile acids from the body. Consistent with this hypothesis, PXR-null mice treated with LCA had markedly higher LCA concentrations in their urine than their wild type littermates, indicating abnormal LCA metabolism (27). Moreover, it has long been known that treatment of rodents with the potent PXR agonist PCN blocks the severe hepatotoxicity and mortality caused by LCA treatment in rats (53). Two groups recently demonstrated that this hepatoprotection is dependent on PXR (27, 50). Co-administration of PCN severely reduced the liver damage caused by LCA in wild type mice as assessed by liver histology and serum levels of liver enzymes. No such hepatoprotection was detected in PXR-null mice treated with LCA. Mice expressing a constitutively active form of the human PXR were also protected against LCA toxicity (50). Thus, PXR can protect the body against pathophysiologic concentrations of toxic bile acids.

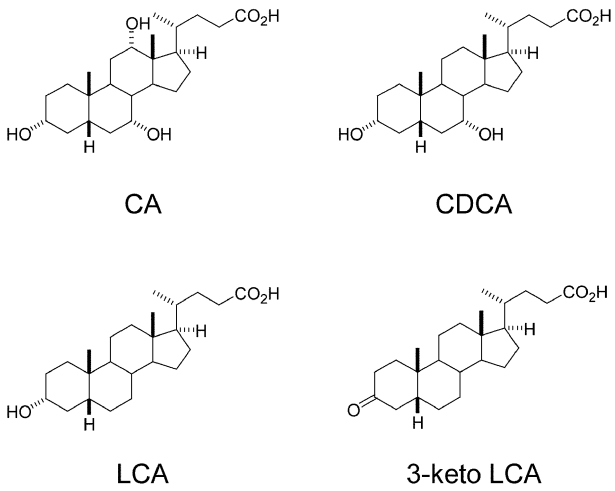

Fig. 3. Chemical structures of bile acids that activate PXR and/or farnesoid X receptor (FXR). FXR is activated efficiently by each of these bile acids. PXR is activated efficiently by lithocholic acid and 3-keto lithocholic acid. CA, cholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid.

PXR AND HUMAN CHOLESTASIS

Primary biliary cirrhosis and other human cholestatic liver diseases are characterized by elevated serum levels of liver enzymes, severe itching (pruritus), lethargy, jaundice, and, ultimately, liver failure. There are currently no effective long-term treatments for these chronic liver diseases short of liver transplant. Several lines of evidence suggest that the chemoprotective actions of PXR agonists may be useful in the treatment of human cholestatic liver disease. Patients suffering from cholestasis have elevated urinary levels of 6-hydroxylated bile acids, including the LCA metabolite hyodeoxycholic acid, which are generated by CYP3A4 (49, 54). Thus, 6-hydroxylation appears to be 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 cholestasis and, in certain cases, has been reported to induce remission of cholestasis (55–57). Similarly, the herbal PXR agonist St. John's wort has been used for centuries as a tonic for liver disorders, including cholestasis (58). Finally, the bile acid ursodeoxycholic acid (UDCA), which is the primary drug 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 reporter gene assays (3). Although the molecular underpinnings of the anticholestatic effects of rifampicin, St. John's wort, and UDCA are not well understood, the discovery that all of these chemicals activate PXR suggests that their therapeutic actions may be mediated at least in part through activation of this nuclear receptor. These results raise the intriguing possibility that more potent PXR ligands may prove to be efficacious drugs for the treatment of biliary cholestasis.

SUMMARY

During the past several years, the study of orphan nuclear receptors has provided important insights into the mechanisms underlying bile acid homeostasis. We now know that at least two nuclear receptors are activated by bile acids and impact bile acid homeostasis. The first of these, FXR, binds to several bile acids and their conjugated derivatives, including cholic acid and chenodeoxycholic acid, the primary bile acids in man, and regulates the expression of genes involved in their biosynthesis and transport. As such, FXR represents the body's rheostat for setting bile acid homeostasis (**Fig. 4**). The second bile acid receptor, PXR, is activated by a remarkable diversity of endogenous and exogenous chemicals, and represents an important component of the body's chemoprotection mechanism. Among the many compounds that activate PXR is the

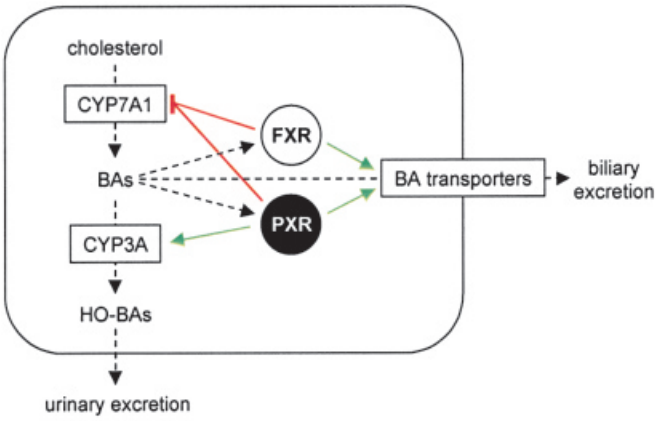

Fig. 4. PXR and FXR are bile acid receptors. Bile acids (BAs) bind and activate PXR and FXR in the liver, resulting in a suppression of *CYP7A1* expression and bile acid synthesis, and the stimulation of bile acid transporter expression and the excretion of bile acids in the bile. PXR also stimulates *CYP3A* expression and the formation of hydroxylated bile acids, which are excreted from the body in the urine. Genes that are upregulated by PXR or FXR are indicated by green arrows; repression of *CYP7A1* expression by PXR and FXR is indicated by red lines. The actions of bile acids on PXR and FXR and the pathways for the elimination of bile acids are indicated with dotted lines.

toxic bile acid LCA. Because PXR regulates genes involved in bile acid metabolism and excretion, this nuclear receptor represents a second line of defense against the accumulation of toxic bile acids in the liver (Fig. 4). These findings provide a molecular explanation for the long-standing observation that PCN protects against the severe hepatotoxicity caused by LCA in rodents (53). Although PXR and FXR are activated by distinct sets of bile acids, they are both activated by LCA. These data, together with the finding that PXR and FXR regulate distinct but overlapping sets of target genes involved in bile acid metabolism, suggest that these two nuclear receptors may cooperate in eliminating LCA and other bile acids from the body when their concentrations reach pathophysiologic concentrations. Moreover, these findings raise the exciting possibility that potent PXR activators, either alone or together with FXR agonists, may have utility in the treatment of biliary cholestasis and other diseases in which bile acids or other toxins accumulate in the liver.

REFERENCES

1. Nelson, D. R., L. Koymans, T. Kamataki, J. J. Stegeman, R. Feyereisen, D. J. Waxman, M. R. Waterman, O. Gotoh, M. J. Coon, R. W. Estabrook, I. C. Gunsalus, and D. W. Nebert. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics.* **6:** 1–42.
2. Maurel, P. 1996 The CYP3A family. *In* Cytochromes P450: metabolic and toxicological aspects. C. Ioannides, editor. CRC Press, Inc., Boca Raton, FL. 241–270.
3. Schuetz, E. G., S. Strom, K. Yasuda, V. Lecureur, M. Assem, C. Brimer, J. Lamba, R. B. Kim, V. Ramachandran, B. J. Komoroski, R. Venktaramanan, H. Cai, C. J. Sinal, F. J. Gonzalez, and J. D. Schuetz. 2001. Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters and cytochrome P450. *J. Biol. Chem.* **276:** 39411–39418.
4. Kliewer, S. A., J. T. Moore, L. Wade, J. L. Staudinger, M. A. Watson, S. A. Jones, D. D. McKee, B. B. Oliver, T. M. Willson, R. H. Zetterstrom, T. Perlmann, and J. M. Lehmann. 1998. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell.* **92:** 73–82.
5. Lehmann, J. M., D. D. McKee, M. A. Watson, T. M. Willson, J. T. Moore, and S. A. Kliewer. 1998. The human orphan nuclear receptor PXR is activated by compounds that regulate *CYP3A4* gene expression and cause drug interactions. *J. Clin. Invest.* **102:** 1016–1023.
6. Bertilsson, G., J. Heidrich, K. Svensson, M. Asman, L. Jendeberg, M. Sydow-Backman, R. Ohlsson, H. Postlind, P. Blomquist, and A. Berkenstam. 1998. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Natl. Acad. Sci. USA.* **95:** 12208–12213.
7. Blumberg, B., W. Sabbagh, Jr., H. Juguilon, J. Bolado, Jr., C. M. van Meter, E. S. Ong, and R. M. Evans. 1998. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev.* **12:** 3195–3205.
8. Jones, S. A., L. B. Moore, J. L. Shenk, G. B. Wisely, G. A. Hamilton, D. D. McKee, N. C. O. Tomkinson, E. L. LeCluyse, M. H. Lambert, T. M. Willson, S. A. Kliewer, and J. T. Moore. 2000. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol. Endocrinol.* **14:** 27–39.
9. Savas, U., M. R. Wester, K. J. Griffin, and E. F. Johnson. 2000. Rabbit pregnane X receptor is activated by rifampicin. *Drug Metab. Dispos.* **28:** 529–537.
10. Zhang, H., E. LeCulyse, L. Liu, M. Hu, L. Matoney, W. Zhu, and B. Yan. 1999. Rat pregnane X receptor: molecular cloning, tissue distribution, and xenobiotic regulation. *Arch. Biochem. Biophys.* **368:** 14–22.
11. Barwick, J. L., L. C. Quattrochi, A. S. Mills, C. Potenza, R. H. Tukey, and P. S. Guzelian. 1996. Trans-species gene transfer for analysis of glucocorticoid-inducible transcriptional activation of transiently expressed human CYP3A4 and rabbit CYP3A6 in primary cultures of adult rat and rabbit hepatocytes. *Mol. Pharmacol.* **50:** 10–16.
12. Huss, J. M., S. I. Wang, A. Astrom, P. McQuiddy, and C. B. Kasper. 1996. Dexamethasone responsiveness of a major glucocorticoid-inducible CYP3A gene is mediated by elements unrelated to a glucocorticoid receptor binding motif. *Proc. Natl. Acad. Sci. USA.* **93:** 4666–4670.
13. Xie, W., J. L. Barwick, C. M. Simon, A. M. Pierce, S. Safe, B. Blumberg, P. S. Guzelian, and R. M. Evans. 2000. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev.* **14:** 3014–3023.
14. Goodwin, B., L. B. Moore, C. M. Stoltz, D. D. McKee, and S. A. Kliewer. 2001. Regulation of the human *CYP2B6* gene by the nuclear pregnane X receptor. *Mol. Pharmacol.* **60:** 427–431.
15. Kast, H. R., B. Goodwin, P. T. Tarr, S. A. Jones, A. M. Anisfeld, C. M. Stoltz, P. Tontonoz, S. A. Kliewer, T. M. Willson, and P. A. Edwards. 2002. Regulation of multidrug resistance-associated protein 2 (MRP2;ABCC2) by the nuclear receptors PXR, FXR, and CAR. *J. Biol. Chem.* In press.
16. Goodwin, B., E. Hodgson, and C. Liddle. 1999. The orphan human pregnane X receptor mediates the transcriptional activation of *CYP3A4* by rifampicin through a distal enhancer module. *Mol. Pharmacol.* **56:** 1329–1339.
17. Wright, M. C. 1999. The cytochrome P450 3A4 inducer metyrapone is an activator of the human pregnane X receptor. *Biochem. Soc. Trans.* **27:** 387–391.
18. Dussault, I., M. Lin, K. Hollister, E. H. Wang, T. W. Synold, and B. M. Forman. 2001. Peptide mimetic HIV protease inhibitors are ligands for the orphan receptor SXR. *J. Biol. Chem.* **276:** 33309–33312.
19. Synold, T. W., I. Dussault, and B. M. Forman. 2001. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat. Med.* **7:** 584–590.
20. Moore, L. B., B. Goodwin, S. A. Jones, G. B. Wisely, C. J. Serabjit-Singh, T. M. Willson, J. L. Collins, and S. A. Kliewer. 2000. St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc. Natl. Acad. Sci. USA.* **97:** 7500–7502.
21. Wentworth, J. M., M. Agostini, J. Love, J. W. Schwabe, and V. K. Chatterjee. 2000. St John's wort, a herbal antidepressant, activates the steroid X receptor. *J. Endocrinol.* **166:** R11–16.
22. Masuyama, H., Y. Hiramatsu, M. Kunitomi, T. Kudo, and P. N. MacDonald. 2000. Endocrine disrupting chemicals, phthalic acid and

nonylphenol, activate pregnane X receptor-mediated transcription. *Mol. Endocrinol.* **14:** 421–428.
23. Takeshita, A., N. Koibuchi, J. Oka, M. Taguchi, Y. Shishiba, and Y. Ozawa. 2001. Bisphenol-A, an environmental estrogen, activates the human orphan nuclear receptor, steroid and xenobiotic receptor-mediated transcription. *Eur. J. Endocrinol.* **145:** 513–517.
24. Watkins, R. E., G. B. Wisely, L. B. Moore, J. L. Collins, M. H. Lambert, S. P. Williams, T. M. Willson, S. A. Kliewer, and M. R. Redinbo. 2001. The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. *Science.* **292:** 2329–2333.
25. Kocarek, T. A., E. G. Schuetz, S. C. Strom, R. A. Fisher, and P. S. Guzelian. 1995. Comparative analysis of cytochrome P4503A induction in primary cultures of rat, rabbit, and human hepatocytes. *Drug Metab. Dispos.* **23:** 415–421.
26. Xie, W., J. L. Barwick, M. Downes, B. Blumberg, C. M. Simon, M. C. Nelson, B. A. Neuschwander-Tetri, E. M. Brunt, P. S. Guzelian, and R. M. Evans. 2000. Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature.* **406:** 435–439.
27. Staudinger, J. L., B. Goodwin, S. A. Jones, D. Hawkins-Brown, K. I. MacKenzie, A. LaTour, Y. Liu, C. D. Klaassen, K. K. Brown, J. Reinhard, T. M. Willson, B. H. Koller, and S. A. Kliewer. 2001. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc. Natl. Acad. Sci. USA.* **98:** 3369–3374.
28. Sueyoshi, T., and M. Negishi. 2001. Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu. Rev. Pharmacol. Toxicol.* **41:** 123–143.
29. Gerbal-Chaloin, S., J. M. Pascussi, L. Pichard-Garcia, M. Daujat, F. Waechter, J. M. Fabre, N. Carrere, and P. Maurel. 2001. Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab. Dispos.* **29:** 242–251.
30. Falkner, K. C., J. A. Pinaire, G. H. Xiao, T. E. Geoghegan, and R. A. Prough. 2001. Regulation of the rat glutathione S-transferase A2 gene by glucocorticoids: involvement of both the glucocorticoid and pregnane X receptors. *Mol. Pharmacol.* **60:** 611–619.
31. Liu, L., and C. D. Klaassen. 1996. Regulation of hepatic sulfotransferases by steroidal chemicals in rats. *Drug Metab. Dispos.* **24:** 854–858.
32. Dunn II, T., B. A. Gleason, D. P. Hartley, and C. D. Klaassen. 1999. Postnatal ontogeny and hormonal regulation of sulfotransferase SULT1B1 in male and female rats. *J. Pharmacol. Exp. Ther.* **290:** 319–324.
33. Runge-Morris, M., W. Wu, and T. A. Kocarek. 1999. Regulation of rat hepatic hydroxysteroid sulfotransferase (SULT2–40/41) gene expression by glucocorticoids: evidence for a dual mechanism of transcriptional control. *Mol. Pharmacol.* **56:** 1198–1206.
34. Madhu, C., and C. D. Klaassen. 1991. Protective effect of pregnenolone-16α-carbonitrile on acetaminophen-induced hepatotoxicity in hamsters. *Toxicol. Appl. Pharmacol.* **109:** 305–313.
35. Hosokawa, M., K. Hattori, and T. Satoh. 1993. Differential responses of rat hepatic microsomal carboxylesterase isozymes to glucocorticoids and pregnenolone 16α-carbonitrile. *Biochem. Pharmacol.* **45:** 2317–2322.
36. Geick, A., M. Eichelbaum, and O. Burk. 2001. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J. Biol. Chem.* **276:** 14581–14587.
37. Fromm, M. F., H. M. Kauffmann, P. Fritz, O. Burk, H. K. Kroemer, R. W. Warzok, M. Eichelbaum, W. Siegmund, and D. Schrenk. 2000. The effect of rifampin treatment on intestinal expression of human MRP transporters. *Am. J. Pathol.* **157:** 1575–1580.
38. Ernst, E. 1999. Second thoughts about safety of St John's wort. *Lancet.* **354:** 2014–2016.
39. Makishima, M., A. Y. Okamoto, J. J. Repa, H. Tu, R. M. Learned, A. Luk, M. V. Hull, K. D. Lustig, D. J. Mangelsdorf, and B. Shan. 1999. Identification of a nuclear receptor for bile acids. *Science.* **284:** 1362–1365.
40. Parks, D. J., S. G. Blanchard, R. K. Bledsoe, G. Chandra, T. G. Consler, S. A. Kliewer, J. B. Stimmel, T. M. Willson, A. M. Zavacki, D. D. Moore, and J. M. Lehmann. 1999. Bile acids: natural ligands for an orphan nuclear receptor. *Science.* **284:** 1365–1368.
41. Wang, H., J. Chen, K. Hollister, L. C. Sowers, and B. M. Forman. 1999. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol. Cell.* **3:** 543–553.
42. Sinal, C. J., M. Tohkin, M. Miyata, J. M. Ward, G. Lambert, and F. J. Gonzalez. 2000. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell.* **102:** 731–744.
43. Goodwin, B., S. A. Jones, R. R. Price, M. A. Watson, D. D. McKee, L. B. Moore, C. Galardi, J. G. Wilson, M. C. Lewis, M. E. Roth, P. R. Maloney, T. M. Willson, and S. A. Kliewer. 2000. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol. Cell.* **6:** 517–526.
44. Lu, T. T., M. Makishama, J. J. Repa, K. Schoonjans, T. A. Kerr, J. Auwerx, and D. J. Mangelsdorf. 2000. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol. Cell.* **6:** 507–515.
45. Einarsson, K., and J. A. Gustafsson. 1973. Effects of a potent catatoxic steroid, 16-cyanopregnenolone, on microsomal metabolism of steroid hormones, sterols and bile acids in rats. *Eur. J. Biochem.* **32:** 197–206.
46. Li, Y. C., D. P. Wang, and J. Y. Chiang. 1990. Regulation of cholesterol 7α-hydroxylase in the liver. Cloning, sequencing, and regulation of cholesterol 7α-hydroxylase mRNA. *J. Biol. Chem.* **265:** 12012–12019.
47. Staudinger, J., Y. Liu, A. Madan, S. Habeebu, and C. D. Klaassen. 2001. Coordinate regulation of xenobiotic and bile acid homeostasis by pregnane X receptor. *Drug Metab. Dispos.* **29:** 1467–1472.
48. Kullak-Ublick, G. A., B. Stieger, B. Hagenbuch, and P. J. Meier. 2000. Hepatic transport of bile salts. *Semin. Liver Dis.* **20:** 273–292.
49. Araya, Z., and K. Wikvall. 1999. 6α-hydroxylation of taurochenodeoxycholic acid and lithocholic acid by CYP3A4 in human liver microsomes. *Biochim. Biophys. Acta.* **1:** 47–54.
50. Xie, W., A. Radominska-Pandya, Y. Shi, C. M. Simon, M. C. Nelson, E. S. Ong, D. J. Waxman, and R. M. Evans. 2001. An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc. Natl. Acad. Sci. USA.* **98:** 3375–3380.
51. Javitt, N. B. 1966. Cholestasis in rats induced by taurolithocholate. *Nature.* **210:** 1262–1263.
52. Setchell, K. D., C. M. Rodrigues, C. Clerici, A. Solinas, A. Morelli, C. Gartung, and J. Boyer. 1997. Bile acid concentrations in human and rat liver tissue and in hepatocyte nuclei. *Gastroenterology.* **112:** 226–235.
53. Selye, H. 1972. Prevention by catatoxic steroids of lithocholic acid-induced biliary concrements in the rat. *Proc. Soc. Exp. Biol. Med.* **141:** 555–558.
54. Bremmelgaard, A., and J. Sjovall. 1979. Bile acid profiles in urine of patients with liver diseases. *Eur. J. Clin. Invest.* **9:** 341–348.
55. Cancado, E. L., R. M. Leitao, F. J. Carrilho, and A. A. Laudanna. 1998. Unexpected clinical remission of cholestasis after rifampicin therapy in patients with normal or slightly increased levels of gamma-glutamyl transpeptidase. *Am. J. Gastroenterol.* **93:** 1510–1517.
56. Bachs, L., A. Pares, M. Elena, C. Piera, and J. Rodes. 1989. Comparison of rifampicin with phenobarbitone for treatment of pruritus in biliary cirrhosis. *Lancet.* **1:** 574–576.
57. Gillespie, D. A., and C. R. Vickers. 1993. Pruritus and cholestasis: therapeutic options. *J. Gastroenterol. Hepatol.* **8:** 168–173.
58. Chevallier, A. 1996. The Encyclopedia of Medicinal Plants. Dorling-Kindersley, New York.