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SYNTHETIC DRUGS AND NATURAL PRODUCTS AS MODULATORS OF CONSTITUTIVE ANDROSTANE RECEPTOR (CAR) AND PREGNANE X RECEPTOR (PXR)

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Constitutive androstane receptor (CAR) and pregnane X receptor (PXR) are members of the nuclear receptor superfamily. These transcription factors are predominantly expressed in the liver, where they are activated by structurally diverse compounds, including many drugs and endogenous substances. CAR and PXR regulate the expression of a broad range of genes, which contribute to transcellular transport, bioactivation, and detoxification of numerous xenochemicals and endogenous substances. This article discusses the importance of these receptors for pharmacology and toxicology, emphasizing the role of individual drugs and natural products as agonists, indirect activators, inverse agonists, and antagonists of CAR and PXR.

Key Words: Constitutive androstane receptor; Cytochrome P450; Drug-metabolizing enzyme; Drug transporter; Nuclear Receptor; Pregnane X receptor.

INTRODUCTION

The constitutive androstane receptor (CAR), originally known as MB67 (Baes et al., 1994), and the pregnane X receptor (PXR) (Kliewer et al., 1998; Lehmann et al., 1998), also designated SXR (Blumberg et al., 1998) and PAR (Bertilsson et al., 1998), are transcription factors belonging to the nuclear receptor superfamily (Waxman, 1999). Based on the unified nomenclature for nuclear receptors, CAR is assigned the gene designation *NR1I3* and PXR is designated *NR1I2* (Giguere, 1999). CAR and PXR act as intracellular sensors for foreign chemicals ("xenosensors") (Willson and Kliewer, 2002) and certain endogenous lipophilic substances (Handschin and Meyer, 2005) in mammals. CAR and

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PXR may have evolved from a common ancestor related to chicken X receptor (CXR), which carries out analogous functions in the chicken (Handschin et al., 2000).

It is now widely recognized that CAR and PXR are major determinants in the regulation of a broad array of genes with diverse biological functions of fundamental importance. CAR and PXR regulate distinct, but overlapping sets of target genes (Maglich et al., 2002; Rosenfeld et al., 2003; Xie et al., 2000b); these include certain phase I cytochrome P450 (CYP) monooxygenase enzymes (e.g., *CYP2B* and *CYP3A*) (Waxman, 1999), phase II conjugation enzymes, such as UDP-glucuronosyltransferase *UGT1A1* and dehydroepiandrosterone sulfotransferase *SULT2A* (Maglich et al., 2002; Rosenfeld et al., 2003), and phase III transporters, such as P-glycoprotein and certain multidrug resistance-associated proteins and organic anion transporting polypeptides (Klassen and Slitt, 2005; Rosenfeld et al., 2003).

CAR and PXR have a major influence on the intracellular concentrations of many xenochemicals and endogenous substances as a consequence of their regulation of genes involved in the metabolism and disposition of these compounds. Therefore, drug exposure and other factors that influence the activity of these nuclear receptors may have a substantial impact on the metabolism and toxicity of many xenochemicals and endogenous substances. Indeed, many drug-drug interactions may now be understood in terms of the roles of CAR and PXR. Thus, treatment with a drug that activates CAR (Wei et al., 2000) or PXR (Xie et al., 2000a) may induce changes in the metabolism or transcellular transport of one or more other drugs that are administered concurrently. These CAR or PXR-mediated drug interactions can lead to reduced therapeutic efficacy. One example is the effectiveness of ethinylestradiol-containing oral contraceptives, which may be compromised as a result of induced metabolism in epilepsy patients taking the CAR and PXR activator phenobarbital (Crawford, 2002). The activation of CAR or PXR may also lead to drug-induced toxicity, as demonstrated in the case of phenobarbital, which induces the metabolism of acetaminophen along a CAR-regulated pathway leading to hepatotoxicity (Zhang et al., 2002). Similarly, the pregnenolone 16α-carbonitrile (PCN)-induced hepatotoxicity of acetaminophen requires PXR (Guo et al., 2004).

Receptor-mediated, drug-induced hepatotoxicity may be severe, and in some cases can even be fatal. For example, troglitazone, a clinically useful oral antidiabetic drug, was withdrawn from the market because of the development of severe liver toxicity in some patients. Troglitazone activates PXR (Jones et al., 2000) and induces the expression of CYP3A enzymes (Ramachandran et al., 1999), resulting in enhanced formation of a hepatotoxic quinone metabolite of the parent drug (Yamazaki et al., 1999). Drugs that activate CAR may also play a role in chemical carcinogenesis, as suggested by the finding that liver tumor promotion by phenobarbital occurs in wild-type mice, but not in CAR-deficient (CAR knockout) mice (Yamamoto et al., 2004), and by the hepatocarcinogenesis that occurs under conditions of chronic CAR activation in rats (Huang et al., 2005). CAR and PXR may also impact the toxicity of endogenous substances through the regulation of genes involved in the detoxification and disposition of bilirubin and bile acids, thereby preventing the accumulation and toxicity of these compounds (Huang et al., 2003; Saini et al., 2005; Wagner et al., 2005; Xie et al., 2001; Zhang et al., 2004).

Synthetic drugs, natural products, endogenous substances, and environmental toxicants are general classes of chemicals known to modulate CAR and PXR activity. This article presents an overview of the experimental findings that establish the roles of synthetic drugs and natural products as agonists, indirect activators, inverse agonists, or antagonists of CAR and PXR. The reader is referred to the many excellent recent review articles that have discussed the regulation of these receptors by endogenous substances, such as bilirubin, bile acids, and certain vitamins (Eloranta and Kullak-Ublick, 2005;

Goodwin and Moore, 2004; Handschin and Meyer, 2005; Makishima, 2005; Qatanani and Moore, 2005; Ruhl, 2005; Traber, 2004; Zhou et al., 2005), and the importance of environmental chemicals and pollutants (Hurst and Waxman, 2005; Kretschmer and Baldwin, 2005) as modulators of CAR and PXR activity.

CAR

CAR is primarily expressed in the liver (Baes et al., 1994; Savkur et al., 2003; Wei et al., 2002), although it is also detectable in certain extrahepatic tissues, including the small intestines (Lamba et al., 2004; Wei et al., 2002). Substantial interindividual variability characterizes CAR mRNA levels in human liver (Chang et al., 2003; Lamba et al., 2003), and this may relate to the fact that CAR expression can be influenced by exposure to drugs, such as dexamethasone (Pascussi et al., 2000b), and by pathophysiological conditions, such as the acute phase response (Beigneux et al., 2002) in response to infection, inflammation, or trauma.

CAR is active in the constitutive state, where it is localized in the cytoplasm in a complex with accessory proteins, including heat shock protein 90 (HSP90) and CAR cytoplasmic retention protein (CCRP) (Qatanani and Moore, 2005). CAR activity may be altered in one of several ways following exposure to a chemical. The classical mechanism of CAR activation involves direct binding of an *agonist* to the receptor's ligand binding domain, which leads to nuclear translocation and heterodimerization of CAR with another nuclear receptor family member, retinoid X receptor (RXR) α (Wan et al., 2000). CAR activation is also associated with recruitment of coactivators, such as steroid co-activator 1 (SRC-1) (Muangmoonchai et al., 2001), transcription factor Sp1 (Muangmoonchai et al., 2001), signal cointegrator-2 (ASC-2) (Choi et al., 2005), and peroxisome proliferator-activated receptor-binding protein (PBP) (Jia et al., 2005), and the dissociation of corepressors, such as nuclear receptor corepressor (NCoR) (Lempiainen et al., 2005). The CAR-RXRα-coactivator complex binds to specific DNA response elements found in the promoter or enhancer region of target genes leading to induction of gene expression (Goodwin and Moore, 2004). A well-studied agonist of mouse CAR is 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) (Tzameli et al., 2000), which is an environmental contaminant. An imidazole derivative, 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-[3,4-dichlorobenzyl)oxime (CITCO), is an example of an agonist of human CAR (Maglich et al., 2003).

CAR activity may also be modulated by *indirect activators* (Qatanani and Moore, 2005), which increase CAR activity by stimulating CAR nuclear translocation, but without binding directly to the ligand binding domain of the receptor. The detailed molecular mechanism of this indirect activation is not well-understood, but it is thought to involve AMP-activated protein kinase (Rencurel et al., 2005) or protein phosphatase 2A (Yoshinari et al., 2003). Bilirubin is an example of an indirect activator of CAR (Huang et al., 2003). CAR activity may also be modulated by *inverse agonists* (Tzameli and Moore, 2001), which bind to the receptor and induce the dissociation of coactivators, recruitment of corepressors, and a reduction in basal receptor transcriptional activity. Inverse agonists are distinguished from classical receptor antagonists insofar as the inverse agonists inhibit receptor activity in the absence of an agonist, whereas antagonists do not display intrinsic inhibitory activity in the absence of a bound agonist. The steroid metabolites androstanol (5α-androstan-3α-ol) and androstenol (5α-androst-16-en-3α-ol) are well-studied examples of CAR inverse agonists (Forman et al., 1998). CAR activity may also be decreased in other ways, as shown in studies with rexinoids (Kakizaki et al., 2002; Tzameli et al.,

2003), which are RXR agonists, such as the endogenous 9-cis-retinoic acid and various synthetic analogs. The mechanisms by which rexinoids affect CAR activity appears to be complex, and may involve reduced availability of RXR for heterodimerization with CAR (Kakizaki et al., 2002) or inhibition of ligand binding (Tzameli et al., 2003).

Many of the synthetic drugs (Table 1) and natural products (Table 2) that modulate CAR activity can be classified as agonists, indirect activators, or inverse agonists of this receptor. Overall, however, relatively few CAR modulators, especially agonists and indirect activators, have been identified. This may be related to the experimental challenges inherent in studying CAR function. For example, the high basal CAR activity in cell-based

Table 1 Synthetic drugs shown to modulate human and mouse CAR activity based on *in vitro* assays.

	Human CAR	Mouse CAR
Agonists		
Chlopromazine	No	Yes
Clotrimazole	No	Yes
Meclizine	No	Yes
Indirect Activators		
Acetaminophen	Yes[a]	Yes[b]
Phenobarbital[c]	Yes	Yes
Phenytoin	Yes	Yes
Other Activators (Mechanism Unknown)		
Atorvastatin[c]	Yes	Yes
Cerivastatin[c]	Yes	Yes
Clofibrate	Unknown	Yes
Fluvastatin[c]	Yes	Yes
Orphenadrine	Unknown	Yes
Pravastatin[d]	No	No
Simvastatin[c]	Yes	Yes
Inverse Agonists		
Clotrimazole	Yes	No
Meclizine	Yes	No

[a]Also shown *in vivo* in "humanized" mice expressing human CAR but not mouse CAR (see text).
[b]Also shown in CAR knockout mice (see text).
[c]Also activates rat CAR (see text).
[d]Does not activate rat CAR (see text).
See text for references.

Table 2 Natural products shown to modulate human and mouse CAR activity based on *in vitro* assays.

	Human CAR	Mouse CAR
Agonist		
Artemisinin	Yes	Unknown
Indirect Activator		
6,7-dimethylesculetin	Yes	Unknown
Inverse Agonists		
cis-guggulsterone	Unknown	Yes*
trans-guggulsteone	Unknown	Yes*

*Depends on the ratio of PXR and CAR (see text).
See text for references.

transfection assays (Kawamoto et al., 1999) hampers the identification of CAR activators (Faucette et al., 2004). This issue may be addressed by carrying out receptor trans-activation studies in the presence of an inverse agonist, such as androstanol or androstenol, in order to reduce the high basal activity of the receptor (Burk et al., 2005). Of note, CAR inverse agonists may exhibit species specificity. For example, androstanol and androstenol are efficacious inverse agonists of mouse CAR, but not human CAR (Makinen et al., 2002; Moore et al., 2000b). Another issue is that CAR spontaneously translocates to the nucleus in many cell lines, including HepG2 cells (Kawamoto et al., 1999). Accordingly, CAR nuclear translocation is best studied using primary hepatocyte cultures.

Synthetic Drugs as Agonists of CAR

Meclizine. This histamine-1 receptor blocker is available as an over-the-counter antiemetic drug. Meclizine is an agonist of mouse CAR, based on the findings that 1) it trans-activates mouse CAR in an *in vitro* cell-based report assay (EC_{50} ~25 nM), and 2) this effect is blocked by the CAR inverse agonist androstanol (Huang et al., 2004b). Meclizine stimulates recruitment of the coactivator SRC-1 to mouse CAR, consistent with its acting as an agonist. *In vivo*, meclizine induces the expression of CAR target genes (e.g., *Cyp2b10*) in wild-type mice, but not in CAR knockout mice (Huang et al., 2004b). In contrast to the agonist activity of meclizine on mouse CAR, this drug is not an agonist of human CAR (Huang et al., 2004b). The species differences may be explained by the low (~71%) amino acid sequence identity in the ligand binding domains of human CAR and mouse CAR (Choi et al., 1997). Meclizine is an inverse agonist of human CAR, as discussed below.

Chlorpromazine. This antipsychotic drug is a phenobarbital-type inducer that has been shown to stimulate the activity of the phenobarbital-responsive enhancer module (PBREM) present in the human *CYP2B6* (Sueyoshi et al., 1999) and the mouse *Cyp2b10* genes (Honkakoski et al., 1998a). Chlorpromazine also increases Cyp2b10 mRNA levels in mouse liver (Wei et al., 2002). The induction of *Cyp2b10* expression by chlorpromazine is dependent on CAR, as indicated by the absence of induction in CAR knockout mice treated with this drug (Wei et al., 2002). Consistent with this finding, chlorpromazine activates mouse CAR in cell-based reporter assays (Honkakoski et al., 2001; Makinen et al., 2003). Chlorpromazine is considered an agonist of mouse CAR based on the ability of the drug to stimulate the recruitment of a coactivator (i.e., SRC-1) (Makinen et al., 2003). The effect of this drug on human CAR activity has not been reported.

Clotrimazole. Conflicting data exist as to whether this antifungal agent activates mouse CAR. An earlier study reported no effect of clotrimazole in an *in vitro* cell-based reporter assay (Moore et al., 2000b). In contrast, a later study showed that clotrimazole increases mouse CAR activity, and the effect is enhanced by the mouse CAR inverse agonist androstenol (Makinen et al., 2003). The same study demonstrated that clotrimazole stimulates the recruitment of SRC-1 (Makinen et al., 2003). Thus, clotrimazole appears to be an agonist of mouse CAR. However, as discussed below, clotrimazole is an inverse agonist of human CAR (Lempiainen et al., 2005; Moore et al., 2000b).

Synthetic Drugs as Indirect Activators of CAR

Phenobarbital. This barbiturate is one of the first drugs shown to induce a broad range of enzymes active in xenochemical and steroid metabolism (Conney, 1967), most

notably those in the *CYP2B* gene subfamily (Waxman and Azaroff, 1992). Phenobarbital is an efficacious inducer of human and rodent CYP2B enzymes. This induction is mediated by CAR, which binds to the PBREM of *CYP2B* genes (Honkakoski et al., 1998b; Muangmoonchai et al., 2001; Sueyoshi et al., 1999; Yoshinari et al., 2001). The inductive response does not occur in CAR knockout mice (Wei et al., 2000). In contrast to certain other CAR modulators, where the effect is species-dependent (Moore et al., 2000b), phenobarbital activates the CAR protein of multiple species, including human (Sueyoshi et al., 1999), mouse (Honkakoski et al., 1998b), and rat (Muangmoonchai et al., 2001; Yoshinari et al., 2001). Phenobarbital stimulates translocation of CAR to the nucleus (Kawamoto et al., 1999) and the recruitment of coactivators (Min et al., 2002; Muangmoonchai et al., 2001; Yoshinari et al., 2003). However, in contrast to a classical agonist, phenobarbital does not bind to the receptor (Huang et al., 2003; Moore et al., 2000b). Thus, phenobarbital is an indirect activator of CAR, similar to bilirubin (Huang et al., 2003).

Phenytoin. This antiepileptic drug is involved in many drug-drug interactions, which may relate to the induction of drug-metabolizing enzymes, most notably CYP2B6 and CYP3A4, as demonstrated in primary cultures of human hepatocytes (Faucette et al., 2004). Phenytoin activates human CAR (Wang et al., 2004) and mouse CAR (Jackson et al., 2004), but does not activate human PXR at concentrations where it induces the expression of CYP2B6 and CYP3A4 (Faucette et al., 2004). Phenytoin is thus a rare example of a compound that activates CAR but not PXR. Phenytoin induces CAR nuclear translocation and/or activates *CYP2B6* reporter gene activity, as shown in experiments with mouse liver (Jackson et al., 2004), primary cultures of human hepatocytes, and CAR knockout mice injected with a human CAR expression plasmid (Wang et al., 2004). However, phenytoin shows little or no binding to CAR (Wang et al., 2004) and is thus an indirect activator of CAR, similar to phenobarbital.

Acetaminophen. This over-the-counter analgesic and antipyretic agent has been the subject of numerous studies to determine the mechanism by which it induces liver toxicity. A major mechanistic insight was provided by the finding that CAR knockout mice are resistant to the hepatotoxic effects of 500 or 800 mg/kg acetaminophen, as indicated by the absence of an increase in serum alanine aminotransferase (ALT) (Zhang et al., 2002), a biochemical marker for liver toxicity. Analysis of serum ALT levels and histological evaluation of liver sections supports a role for CAR in acetaminophen-induced liver toxicity. Thus: 1) pretreatment with the CAR activators phenobarbital and TCPOBOP (Honkakoski et al., 1998b; Tzameli et al., 2000) exacerbates the liver toxicity of acetaminophen in wild-type mice, but not in CAR knockout mice; and 2) the CAR inverse agonist androstanol blocks the liver toxicity of acetaminophen in wild-type, CAR-expressing mice. Consistent with these findings: 1) meclizine, an inverse agonist of human CAR, blocks acetaminophen-induced liver toxicity in mice that express human CAR (Huang et al., 2004b); and 2) coactivators, such as ASC-2 and PBP, are required for acetaminophen to induce liver toxicity in wild-type mice (Choi et al., 2005; Jia et al., 2005). Thus, CAR is likely to mediate the hepatotoxic effects of acetaminophen. Acetaminophen is not an agonist of CAR, but may be an indirect activator, similar to phenobarbital (Zhang et al., 2002).

Other Synthetic Drugs as Activators of CAR

The 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors simvastatin, fluvastatin, atorvastatin, and cerivastatin activate human, mouse, and rat CAR activities in cell-based

reporter gene assays, whereas pravastatin has no effect (Kobayashi et al., 2005). The anticholinergic drug orphenadrine (Murray et al., 2003) and the antihyperlipidemic drug clofibrate (Makinen et al., 2003) increase mouse CAR activity; however, their mechanism of action remains to be elucidated.

Natural Products as Activators of CAR

6,7-Dimethylesculetin. Several naturally occurring compounds, either individually or as a mixture in herbal extracts, have been reported to activate CAR. Yin Zhi Huang, which is a traditional Chinese medicine containing extracts from the plants *Artemisia capillaris*, *Gardenia jasminoides Ellis*, *Rheum officinale Baill*, and *Scutellaria baicalensis Georgi*, is used to treat neonatal jaundice (Chen and Guan, 1985) because of its ability to increase the clearance of bilirubin (Yin et al., 1991). This increase in clearance is mediated by CAR, as is evident from the decrease in total serum bilirubin that follows treatment of wild-type mice, but not CAR knockout mice, with Yin Zhi Huang, and by the reduction in bilirubin levels seen in mice expressing human CAR (Huang et al., 2004a). Consistent with these findings, Yin Zhi Huang increases hepatic expression of CAR target genes, including *Cyp2b10* and *UGT1A1*. 6,7-dimethylesculetin (scoparone), a coumarin derivative present in Yin Zhi Huang, does not stimulate coactivator recruitment (Huang et al., 2004a), suggesting that it is not an agonist. However, 6,7-dimethylesculetin stimulates nuclear translocation of CAR and also increases liver expression of CAR target genes, such as *Cyp2b10* in primary hepatocyte cultures from mice expressing human CAR (Huang et al., 2004a). Thus, 6,7-dimethylesculetin may be an indirect activator of human CAR.

Artemisinin. This antimalarial drug is a sesquiterpene lactone endoperoxide extracted from the plant *Artemisia annua* (Klayman, 1985). Artemisinin induces human and mouse CAR-mediated transcriptional activity in a reporter gene assay (Burk et al., 2005). The extent of activation is weak (< 2-fold), but it can be enhanced when basal CAR activity is inhibited by incubation with the inverse agonist androstenol. Artemisinin stimulates recruitment of the coactivator DRIP205 (vitamin D receptor interacting protein 205) in mammalian two-hybrid assays and induces target gene expression (i.e., *CYP2B6*, *CYP3A4*, and *MDR1*) in cultured LS174T cells at a concentration of 100 μM (Burk et al., 2005). However, the peak plasma concentrations of artemisinin are < 2 μM (Svensson et al., 1998), suggesting that this drug may exhibit minimal CAR agonist activity in patients *in vivo*.

Synthetic Drugs as Inverse Agonists of CAR

Clotrimazole. This drug acts as an agonist of mouse CAR (Makinen et al., 2003), as noted previously, but is an inverse agonist of human CAR (Lempiainen et al., 2005; Maglich et al., 2003; Moore et al., 2000b). Clotrimazole reduces basal CAR activity *in vitro* with an IC_{50} of ~0.7 μM (Moore et al., 2000b). This drug inhibits recruitment of the coactivator SRC-1 by human CAR with IC_{50} values of 58 nM (Maglich et al., 2003) and 100 nM (Moore et al., 2000b) as determined using a fluorescence resonance energy transfer ligand-sensing assay.

Meclizine. This drug also acts as an agonist of mouse CAR, but is an inverse agonist of human CAR (Huang et al., 2004b), decreasing basal transcriptional activity by ~50% at a concentration of 20 μM in a cell-based reporter gene assay. When compared to clotrimazole (Maglich et al., 2003; Moore et al., 2000b), meclizine is a less potent inverse

agonist of human CAR, although both drugs appear to have similar efficacy (Huang et al., 2004b). *In vitro*, meclizine also decreases the interaction between human CAR and the coactivator SRC-1. In humanized CAR mice treated with hepatotoxic doses of acetaminophen, meclizine (100 mg/kg) prevented liver toxicity, as assessed by serum ALT levels and histological evaluation of liver sections (Huang et al., 2004b). However, meclizine may not act as an inverse agonist of CAR in humans *in vivo*, given that its peak plasma concentration, ~0.2 μM (Fouda et al., 1978), is 100-fold lower than the IC_{50} value of 20 μM reported for *in vitro* inverse agonist activity (Huang et al., 2004b).

Other drugs. Temazepam and thioridazine have been reported to reduce human CAR-mediated transcriptional activity in a cell-based reporter gene assay (Kobayashi et al., 2005). Additional studies will be required to determine whether these drugs are inverse agonists of human CAR.

Natural Products as Inverse Agonists of CAR

Guggulsterone. This naturally occurring ketosteroid exists as both a *cis* form (also known as *E*-guggulsterone or *cis*-4,17(20)-pregnadiene-3,16-dione) and a *trans* form (also referred to as *Z*-guggulsterone or *trans*-4,17(20)-pregnadiene-3,16-dione) (Verma et al., 1998). These ketosteroids are active ingredients in gugulipid, a herbal medicine derived from the *Commiphora mukul* tree and used to treat rheumatoid arthritis, inflammation, and hyperlipidemia. The *cis*- and *trans*-stereoisomers of guggulsterone both inhibit the basal transcriptional activity of mouse CAR (Ding and Staudinger, 2005b); an IC_{50} value of 900 nM was reported for *cis*-guggulsterone (IC_{50} was not determined for the *trans*-stereoisomer). *cis*- and *trans*-guggulsterone are both able to dissociate the coactivator SRC-1 from mouse CAR, as determined in a mammalian two-hybrid assay, consistent with their serving as inverse agonists. However, *cis*- and *trans*-guggulsterone do not decrease, but, rather, increase expression of the CAR target gene *Cyp2b10* in cultured hepatocytes from wild-type mice. This increase is apparently due to the agonistic effects of these compounds on mouse PXR. Thus, suppression of hepatocyte Cyp2b10 mRNA levels by *cis*- and *trans*-guggulsterone can occur, but this suppression is only evident in PXR knockout mice. The same paradoxical finding was obtained with the established CAR inverse agonist androstanol (Ding and Staudinger, 2005b). These findings exemplify how the relative abundance of CAR and PXR *in vivo* may serve as an important determinant of whether the inverse agonist activity for CAR will predominate over the agonist activity for PXR in animals or humans treated with such dual action compounds. This illustrates another level of complexity of the nuclear receptor network.

PXR

PXR mRNA and protein are expressed predominantly in the liver and small intestines and, to a lesser extent, in other tissues, such as the kidney (Miki et al., 2005). Substantial interindividual variability exists in PXR mRNA expression in human liver (Chang et al., 2003; Gardner-Stephen et al., 2004). This variablility may, in part, reflect the fact that PXR expression can be increased by exposure to drugs, such as dexamethasone (Pascussi et al., 2000a) and clofibrate (Ma et al., 2005), and can be decreased by chronic exposure to alcohol (Wang et al., 2005) and in pathophysiological states, such as acute phase response (Beigneux et al., 2002) and inflammatory bowel disease (Langmann et al., 2004). PXR expression is controlled by several transcription factors, including the liver-enriched transcription factor

HNF4α (Kamiya et al., 2003; Li et al., 2000; Tirona et al., 2003) and small heterodimer partner (SHP; NR0B2) (Ourlin et al., 2003), which play important roles in the liver-specificity of PXR expression.

PXR transcriptional activity is increased following ligand binding. Ligand-bound PXR forms a heterodimer with a second nuclear receptor, RXRα (Kliewer et al., 1998). The resulting PXR-RXRα heterodimer binds to DNA response elements in the promoter or enhancer regions of PXR target genes and recruits coactivator proteins, such as SRC-1 (Watkins et al., 2003a), which contribute to the stimulation of gene transcription (Carnahan and Redinbo, 2005). PXR was initially thought to reside in the nucleus, however, it is now apparent that PXR is localized to the cytoplasm prior to ligand binding in the form of a complex with HSP90 and CCRP (Squires et al., 2004), and that ligand binding induces nuclear translocation, similar to that which characterizes certain other nuclear receptors. For example, treatment of mice with the PXR ligand PCN induces nuclear accumulation of PXR in the liver (Kawana et al., 2003; Squires et al., 2004). PXR may also regulate gene expression in the absence of ligand binding, as suggested by the finding that a nuclear receptor corepressor, SMRT, interacts with PXR. However, in the presence of a PXR agonist, such as rifampin, the PXR-SMRT complex dissociates and allows for the binding of a coactivator (e.g., RAC3) to PXR (Johnson et al., 2006).

Many synthetic drugs (Table 3) and natural products (Table 4) have been identified as regulators of PXR activity. Some of these compounds are agonists of PXR, whereas in

Table 3 Synthetic drugs shown to modulate human and mouse PXR activity based on *in vitro* assays.

	Human PXR	Mouse PXR
Agonists		
BK8644	Yes	No
Clotrimazole	Yes	Yes (but weak)
Dexamethasone	Yes	Yes
Isradipine	Yes	Unknown
Nicardipine	Yes	No
Nifedipine	Yes	No
Paclitaxel	Yes	Conflicting data
Phenobarbital	Yes	Yes
Rifampicin	Yes	No
Ritonavir	Yes	No
Other Activators		
Avasimibe	Yes	Unknown
Bosentan	Yes	Unknown
Carbamazepine	Yes	Unknown
Efavirenz	Yes	Unknown
Etoposide	Yes	Unknown
4-hydroxytamoxifen	Yes	Unknown
Lovastatin	Yes	Unknown
Mevastatin	Yes	Unknown
Spironolactone	Yes	Unknown
Tamoxifen	Yes	Unknown
Topiramate	Yes	Unknown
Topotecan	Yes	Unknown
Antagonist		
Ketoconazole	Yes	Unknown

See text for references.

Table 4 Natural products shown to modulate human and mouse PXR activity based on *in vitro* assays.

	Human PXR	Mouse PXR
Agonist		
Artemisinin	Yes	No
Forskolin	Yes	Yes
cis-guggulsterone	Yes	Yes
trans-guggulsteone	Yes	Yes
Hyperforin	Yes	Yes
Other Activators		
Desmethoxyyangonin	Yes	Unknown
Dihydromethysticin	Yes	Unknown
Kava extract	Yes	Unknown
Antagonist		
Trabectedin (ET-743)	Yes	Unknown

See text for references.

other cases, the mechanism of activation has yet to be elucidated. Receptor antagonists, which have no intrinsic activity, but simply block the activity of an agonist, have also been identified for PXR. In contrast to CAR, it is not clear whether PXR has substantial basal activity (Takeshita et al., 2002). As discussed below, artemisinin inhibits basal mouse PXR-mediated transcriptional activity *in vitro* (Burk et al., 2005), but whether this represents inverse agonist activity remains to be investigated. Interestingly, the mouse CAR inverse agonist androstenol does not inhibit the basal activity of mouse PXR (Ding and Staudinger, 2005b).

Synthetic Drugs as Agonists of PXR

Rifampicin. This antituberculosis drug (also known as rifampin) was one of the first xenochemicals shown to activate human PXR, as demonstrated in cell-based reporter gene assays (Bertilsson et al., 1998; Blumberg et al., 1998; Lehmann et al., 1998). Rifampicin also activates PXR from pig, rabbit, dog, and rhesus monkey, but not the corresponding mouse or rat PXR (Blumberg et al., 1998; Jones et al., 2000; Moore et al., 2000b). This finding illustrates the species specificity of PXR activation, which is a reflection of the fact that the amino acid sequences of the rat and mouse PXR ligand-binding domains are only 76–77% identical to the corresponding human PXR sequence (Moore et al., 2002). The species differences in PXR activation by rifampicin reflect the known species differences in liver in the induction of *CYP3A*, which is a PXR target gene; rifampicin induces the expression of human and rabbit *CYP3A* genes, but not rat or mouse *CYP3A* genes (Gibson et al., 2002). Rifampicin activates human PXR at relatively low concentrations; i.e., EC_{50} values of 0.71 μM (Moore et al., 2000b) or 1.8 μM (Lemaire et al., 2004), which is >10-fold lower than the EC_{50} (20 μM) reported for activation of PXR by the endogenous steroid, 5β-pregnane-3,20-dione (Lemaire et al., 2004). Rifampicin binds to PXR (Jones et al., 2000) and stimulates coactivator recruitment, as determined in an *in vitro* coactivator-receptor ligand assay (Lehmann et al., 1998), consistent with it serving as a receptor agonist. In contrast to human PXR, human CAR is not activated by rifampicin (Moore et al., 2000b). This illustrates a lack of coordinate activation of PXR and CAR by xenochemicals, which is consistent with the relatively low (~45%) amino acid sequence identity between the ligand-binding domains of human PXR and human CAR (Moore et al., 2002).

Phenobarbital. This barbiturate activates PXR by a receptor binding mechanism (Jones et al., 2000; Moore et al., 2000b), in contrast to the receptor binding-independent mechanism for CAR activation discussed previously. Phenobarbital activates PXR from various species, including human, pig, rabbit, dog, and rhesus monkey (Moore et al., 2002), but has little or no effect on rat or mouse PXR activity (Jones et al., 2000; Moore et al., 2000b; Smirlis et al., 2001; Wei et al., 2002). Although phenobarbital is as efficacious as rifampicin in activating human PXR, it is considerably less potent (EC_{50} values: 370 ± 11 μM for phenobarbital vs. 1.8 ± 0.2 μM for rifampicin) (Lemaire et al., 2004). The differences in the potency of PXR activation by these drugs are reflected in the EC_{50} values for induction of the PXR gene *CYP3A4* by phenobarbital (142 ± 36 μM) and rifampicin (0.37 ± 0.1 μM) in primary human hepatocyte culture (Faucette et al., 2004).

Clotrimazole. As mentioned previously, this drug is an agonist of mouse CAR, but is an inverse agonist of human CAR (Lempiainen et al., 2005; Moore et al., 2000b). By comparison, clotrimazole activates by up to 10-fold human PXR (Bertilsson et al., 1998; Lehmann et al., 1998) and its dog, pig, rabbit, and rhesus monkey orthologs (Moore et al., 2002), but is a weak activator (2 to 3-fold) of rat and mouse PXR (Bertilsson et al., 1998; Jones et al., 2000). EC_{50} values ranging from 0.8–2.5 μM have been reported for the activation of human PXR by clotrimazole (Bertilsson et al., 1998; Lemaire et al., 2004; Moore et al., 2000b). These values are similar to the EC_{50} of 1.8 μM for human PXR activation by rifampicin (Lemaire et al., 2004). Clotrimazole (10 μM) binds to human PXR (Jones et al., 2000) and stimulates coactivator recruitment (Lehmann et al., 1998). Receptor binding and co-activator recruitment are not seen with mouse PXR (Lehmann et al., 1998), in agreement with the species differences reported in the transactivation assays (Bertilsson et al., 1998; Jones et al., 2000).

Ritonavir. This protease inhibitor and anti-HIV drug activates human PXR with an EC_{50} of 2 μM in an *in vitro* cell-based reporter assay, but has little or no effect on mouse PXR activity (Dussault et al., 2001). Ritonavir does not activate many of the other nuclear receptors, including mouse CAR. Several other protease inhibitors, such as saquinavir, nelfanavir, and indinavir, do not activate human or mouse PXR when tested at 10 μM. Ritonavir binds to human PXR, as shown in a scintillation proximity assay, and stimulates the interaction between human PXR and its coactivators DRIP205, TRAP220, SRC-1, ACTR, and GRIP, as demonstrated in a mammalian two-hybrid assay. Ritonavir (3 μM) induces mRNA expression of PXR target genes (i.e., *CYP3A4*, *CYP2C8*, *MRP2*, and *MDR1*) in primary cultures of human hepatocytes and in cultured LS180 human colon adenocarcinoma cells. However, ritonavir does not increase CYP3A-mediated drug metabolism in humans *in vivo*, as indicated by the ratio of plasma 1-hydroxymidazolam and midazolam (Fellay et al., 2005). Rather, it inhibits CYP3A catalytic activity, which is the basis for dual protease inhibitor regimens that include ritonavir to inhibit the metabolism of other CYP3A4-inactivated protease inhibitor substrates, such as lopinavir (King et al., 2004). Thus, while ritonavir activates human PXR in cultured cell models, it does not have this effect in humans *in vivo*.

Paclitaxel. This taxane antimicrotubule agent is used clinically as an anticancer drug. Paclitaxel activates human PXR in cell-based reporter assays (EC_{50} = 5 μM) (Synold et al., 2001). However, metabolites of paclitaxel (i.e., 6α-OH-paclitaxel and 3′-*p*-OH-paclitaxel) and docetaxel, another taxane anticancer drug, do not activate human PXR (Synold et al., 2001). Conflicting data exist as to whether paclitaxel activates mouse PXR (Nallani et al., 2003b; Synold et al., 2001), but according to one study, it does not activate several other nuclear receptors, including mouse CAR (Synold et al., 2001). Paclitaxel acts as an agonist. It stimulates the interaction of human PXR with the coactivators SRC-1, PBP, and GRIP, dissociates the corepressors SMRT and NcoR from human PXR,

and induces the expression of PXR target genes, including *CYP3A4* and *MDR1* in LS180 human colon adenocarcinoma cells (Synold et al., 2001) and in primary cultures of human hepatocytes (Kostrubsky et al., 1998). It is not known whether paclitaxel activates PXR *in vivo*, but if it does, the consequences might be limited given the frequency in which this drug is administered clinically (e.g., a single dose once in three weeks).

Calcium channel blockers. Nifedipine, a calcium channel blocker, activates human PXR with an EC_{50} value of 4.3 μM (Bertilsson et al., 1998). This drug also activates rabbit, pig, dog, and rhesus monkey PXR (Moore et al., 2002). Structure activity studies with a series of calcium channel blockers and rifampicin revealed the following rank order for activation of human PXR: rifampicin ~ nifedipine > isradipine > BK8644 > nicardipine (Drocourt et al., 2001). These drugs act as agonists of human PXR, based on their ability to enhance the interaction between human PXR and the coactivator SRC-1. These compounds also activate the expression of the PXR target genes *CYP3A4*, *CYP2C9*, and *CYP2B6* in primary cultures of human hepatocytes; however, the potential contribution of human CAR to these responses is not known.

Dexamethasone. This steroid activates human and mouse PXR at relatively high concentrations (e.g., 50–250 μM) (Pascussi et al., 2001; Yueh et al., 2005). At these non-physiological and supra-pharmacological concentrations, dexamethasone acts as a PXR agonist, binding to the receptor (Pascussi et al., 2001) and inducing PXR target gene expression (Pascussi et al., 2001; Yueh et al., 2005). By contrast, dexamethasone activates the glucorticoid receptor in the low nanomolar concentration range (Pascussi et al., 2001). Dexamethasone does not influence CAR transcriptional activity when tested at 20 μM (Faucette et al., 2004).

Other Synthetic Drugs as Activators of PXR

Cell-based reporter gene assays have shown that human PXR activity can be increased by many other drugs, including avasimibe (Sahi et al., 2003), bosentan (van Giersbergen et al., 2002), carbamazepine (El-Sankary et al., 2001; Luo et al., 2002), efavirenz (Hariparsad et al., 2004), etoposide (Schuetz et al., 2002), lovastatin (Lehmann et al., 1998), mevastatin (Raucy et al., 2002), spironolactone (El-Sankary et al., 2001), tamoxifen and its metabolite, 4-hydroxytamoxifen (Desai et al., 2002), topiramate (Nallani et al., 2003a), and topotecan (Schuetz et al., 2002). However, the mechanism by which these drugs activate PXR remains to be investigated.

Natural Products as Agonists of PXR

St. John's wort and hyperforin. St. John's wort is a herbal remedy that is widely used as an antidepressant. Long-term use of St. John's wort may lead to interactions involving drugs that serve as substrates of CYP3A and the transporter P-glycoprotein (Dresser et al., 2003). Insight into the molecular mechanism of these effects of St. John's wort was provided by the finding that various extracts of St. John's wort, in particular the bioactive compound hyperforin, activate human and mouse PXR in cell-based reporter assays (Moore et al., 2000a; Wentworth et al., 2000). Hyperforin also induces human and mouse CYP3A expression (Cantoni et al., 2003; Komoroski et al., 2004). This compound (7.5-fold activation at 1.9 μM) is as efficacious as rifampicin (7.5-fold at 20 μM) (Wentworth et al., 2000) in activating human PXR, but is more potent (EC_{50} = 32 nM) than either rifampicin (EC_{50} = 463 nM) or SR12813 (EC_{50} = 127 nM) (Watkins et al., 2003b). Hyperforin binds to PXR as an agonist; it competes with ^{3}H-SR12813 for

binding to human PXR (EC_{50} = 27 nM) (Moore et al., 2000a); and it stimulates the interaction between human PXR and the coactivator SRC-1 (Wentworth et al., 2000). Direct binding of hyperforin to the ligand-binding domain of PXR has been visualized by x-ray crystallography (Watkins et al., 2003b).

Guggulsterones. The plant sterols *Z*-guggulsterone and *E*-guggulsterone, constituents of gugulipid, activate human and mouse PXR (Brobst et al., 2004; Ding and Staudinger, 2005b). *Z*-guggulsterone activates mouse PXR and human PXR with EC_{50} values of 1.4 μM and 2.4 μM, respectively (Brobst et al., 2004). Both compounds stimulate the recruitment of SRC-1 to mouse and human PXR and induce PXR target gene *(CYP3A)* expression. *E*- and *Z*-guggulsterone appear to function as CAR inverse agonists when the PXR/CAR ratio is low, but they act as PXR agonists when the ratio is high (Ding and Staudinger, 2005b).

Forskolin. This diterpene is derived from the plant *Coleus forskohlii* and is used in traditional medicine to treat various conditions, including cardiovascular disorders (Seamon et al., 1981). Forskolin is a modulator of protein kinase A signaling through its activation of adenyl cyclase. Forskolin is an agonist of human PXR (Ding and Staudinger, 2005a): 1) it activates human PXR (EC_{50} = 0.4 μM) and mouse PXR (EC_{50} = 0.9 μM) in cell-based reporter gene assays (interestingly, it does not activate mouse CAR); 2) it binds to the ligand-binding domain of human and mouse PXR; 3) it decreases the interactions between human PXR and the corepressor protein NcoR1; 4) it stimulates interactions between human PXR and its coactivator proteins (i.e., SRC-1, SRC-2, and PBP); and 5) it induces PXR target gene expression in hepatocytes isolated from wild-type mice, but not those from PXR knockout mice.

Artemisinin. As discussed previously, this naturally occurring sesquiterpene lactone endoperoxide stimulates the transcriptional activity of human and mouse CAR. Artemisinin also activates human PXR (EC_{50} = 34 μM), but not mouse PXR (Burk et al., 2005). This compound binds to the ligand-binding domain of human PXR, reduces the interaction between human PXR and a corepressor (i.e., SMRT), increases the interaction between this receptor and a coactivator (i.e., DRIP205), and induces PXR-mediated gene expression of *CYP2B6*, *CYP3A4*, and *MDR1* in LS174 cells and primary cultures of human hepatocytes. Thus, artemisinin is an agonist of human PXR.

In contrast to the activation of human PXR by artemisinin, this compound has been shown to decrease the basal transcriptional activity of mouse PXR by ~70% in a reporter gene assay (Burk et al., 2005). However, further investigation is needed to determine whether this represents inverse agonist activity by artemisinin.

Other Natural Products as Activators of PXR

Kava extract (100 μg/ml) activates human PXR (~12-fold) (Raucy, 2003), as determined in cell-based reporter gene assays. The kavalactones, desmethoxyyangonin and dihydromethysticin (50 μM), also activate human PXR in addition to rat PXR, but the magnitude of activation is only ~2-fold (Ma et al., 2004). It remains to be shown whether these compounds are PXR agonists.

Synthetic Drugs as Antagonists of PXR

Ketoconazole. This imidazole antifungal drug is a well-established inhibitor of CYP3A4 catalytic activity (Thummel and Wilkinson, 1998). Ketoconazole (10 μM) attenuates

human PXR activation by the agonist, corticosterone, as assessed at a clinically relevant concentration (Huang et al., 1986) in an *in vitro* reporter gene assay performed with HepG2 cells (Takeshita et al., 2002). Ketoconazole does not inhibit basal PXR activity in the absence of an agonist, consistent with the action of a receptor antagonist. Ketoconazole (5 μM) also blocks coactivator recruitment by the PXR agonists rifampicin and corticosterone. Collectively, these *in vitro* findings suggest that ketoconazole acts as an antagonist of human PXR.

Natural Products as Antagonists of PXR

Trabectedin. This naturally occurring tetrahydroisoquinolone alkaloid (formerly known as ecteinascidin-743 or ET-743 (Beumer et al., 2005), is derived from the marine ascidian, *Ecteinascidia turbinata*, and has relatively potent (IC_{50} = 1–100 nM) *in vitro* antiproliferative activity against various human tumor cell lines (Izbicka et al., 1998). Consistent with the actions of a receptor antagonist, trabectedin has no effect on basal human PXR activity, but it blocks (IC_{50} value = 3 nM) human PXR activation by the agonists paclitaxel and SR12813, as determined in reporter gene assays conducted in CV-1 cells (Synold et al., 2001). As a result, trabectedin prevents agonist-mediated induction of PXR target genes (i.e., *CYP3A4* and *MDR1*).

CONCLUSIONS

The discovery of CAR and PXR has greatly increased our understanding of the signaling pathways and transcriptional events that regulate a large set of liver-expressed genes with diverse biological functions, including drug metabolism and disposition. Many drugs and other foreign compounds have the ability to interact with CAR and/or PXR as agonists, antagonists, and inverse agonists or by other mechanisms, and may thereby contribute to drug interactions. Screening of lead compounds early during the drug development process for interactions with CAR and PXR may provide the opportunity to eliminate drug candidates that have a high potential for drug interactions and other adverse effects. Identification of CAR or PXR-activating compounds in a herbal mixture may lead to the development of novel products with an improved safety profile.

The following points should be considered when designing studies to investigate the effects of drugs and other chemicals on CAR and PXR.

1. Experiments must be performed not only with rodent nuclear receptors, but with human receptors, given the many examples of species differences in CAR and PXR activation by drugs and other chemicals (Tables 1, 3, and 4). A relevant example in the context of drug development is troglitazone, which activates human PXR, but not rat or mouse PXR (Jones et al., 2000). Other mammalian species, such as rhesus monkey, pig, dog, and rabbit, may be more suitable animal models. Among these, the rhesus monkey may be the best because its PXR ligand-binding domain displays the highest amino acid sequence identity (96%) to human PXR (Moore et al., 2002). The importance of including human receptors is also illustrated by the fact that a compound may be an agonist in one species, but an inverse agonist in another species (Table 1).
2. Conclusions about one xenobiotic receptor (e.g., PXR) may not be drawn from findings obtained from another receptor (e.g., CAR) due to the low (~45%) amino acid identity between their ligand-binding domains. As an example, rifampicin activates human

PXR, but it does not activate human CAR (Moore et al., 2000b). Other examples are shown in Tables 1 and 3.

3. Caution should be used when comparing quantitative nuclear receptor activity values obtained for a given compound in different cell-based reporter gene assays. For example, the extent of PXR activation by paclitaxel was 3 to 6-fold in one study (Luo et al., 2002), but 50-fold in another study (Synold et al., 2001). This may relate to the differences in the conditions (e.g., cell type, reporter construct) used in each study.
4. Cell-based reporter gene and other assays should include clinically relevant concentrations in experiments designed to determine whether a compound is a physiologically or pharmacologically relevant nuclear receptor modulator. For example, meclizine can be shown to act as an inverse agonist of human CAR when analyzed at an *in vitro* concentration of 20 μM (Huang et al., 2004b); however, this concentration is 100-fold greater than the peak plasma concentration achieved following a therapeutic dose in humans (Fouda et al., 1978).
5. The battery of *in vitro* assays used to identify nuclear receptor modulators may give false positive results, even when the *in vitro* concentration of the drug reflects its pharmacological level *in vivo*. For example, based on *in vitro* receptor activation assays, ritonavir is an agonist of human PXR (Synold et al., 2001). However, ritonavir does not increase the clearance of drugs known to be metabolized by the PXR target CYP3A4 in humans *in vivo* (Fellay et al., 2005).
6. The use of *in vitro* assays to predict the *in vivo* effects of a CAR or PXR modulator is complicated by the finding that the relative abundance of PXR and CAR in an individual human patient may determine whether the overall response to a given compound may reflect its inverse agonist activity with CAR or its agonist activity toward PXR (Ding and Staudinger, 2005b). Thus, pathophysiological conditions (Langmann et al., 2004; Pascussi et al., 2003) and other factors that alter CAR and PXR expression may have implications for interindividual responses to drugs or other substances.

ABBREVIATIONS

ACTR	activator of the thyroid and retinoic acid receptor
ASC-2	signal cointegrator-2
CAR	constitutive androstane receptor
CCRP	CAR cytoplasmic retention protein
CITCO	6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-[3,4-dichlorobenzyl)oxime
CYP	cytochrome P450
DRIP205	vitamin D receptor interacting protein 205
GRIP	glucocorticoid receptor interacting protein
HSP90	heat shock protein 90
MDR1	multi-drug resistance 1 gene
NCoR1	nuclear hormone receptor corepressor 1
PBP	peroxisome proliferator-activated receptor-binding protein
PBREM	phenobarbital-responsive enhancer module
PCN	pregnenolone 16α-carbonitrile
PXR	pregnane X receptor
RXR	retinoid X receptor
SHP	small heterodimer partner
SMRT	silencing mediators of retinoid and thyroid hormone

SRC-1	steroid co-activator 1
TCPOBOP	1,4-bis[2-(3,5-dichloropyridyloxy)]benzene
TRAP	thyroid hormone receptor associated protein

DEDICATION

This article is dedicated to the memory of Dr. David Kupfer, a true friend and colleague who made many important contributions to our understanding of CYP enzymes and their role in the metabolism of steroids, drugs, and environmental chemicals.

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