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Induction of Drug Metabolism: The Role of Nuclear Receptors

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***Abstract*——Induction of drug metabolism was described more than 40 years ago. Progress in understanding the molecular mechanism of induction of drug-metabolizing enzymes was made recently when the important roles of the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR), two members of the nuclear receptor superfamily of transcription factors, were discovered to act as sensors for lipophilic xenobiotics, including drugs. CAR and PXR bind as heterodimeric complexes with the retinoid X receptor to response elements in the regulatory regions of the induced genes. PXR is directly activated by xenobiotic ligands, whereas CAR is involved in a more complex and less well understood mechanism of signal transduction triggered by drugs. Most recently, analysis of these xenobiotic-sensing nuclear receptors and their nonmammalian precursors such as the chicken xenobiotic receptor suggests an important role of PXR and CAR also in endogenous pathways, such as cholesterol and bile acid biosynthesis and me-**

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tabolism. In this review, recent findings regarding xenosensors and their target genes are summarized and are put into an evolutionary perspective in regard to how a living organism has derived a system that is able to deal with potentially toxic compounds it has not encountered before.

I. Introduction

The cell membrane constitutes an efficient barrier that protects the cell from toxic, water-soluble xenobiotics. However, lipophilic substances can cross this boundary much more easily and subsequently may accumulate within the membrane and the cell until toxicity levels are reached. Thus, to protect themselves from this threat, biological organisms had to develop systems that can prevent accumulation of these compounds. Two general mechanisms have evolved for this purpose, biotransformation and transport. In protozoa, elimination of compounds may be achieved by simply increasing their efflux from the cell using transporter proteins. In multicellular organisms, additional mechanisms are required since lipophilic compounds should leave not only the cells but also the organism. Thus, lipophilic substances are biotransformed into more water-soluble metabolites that subsequently can be excreted from the body.

A gene superfamily of heme proteins, the cytochromes P450 (P450[2]), encodes for the main enzymatic system for metabolism of lipophilic substrates of diverse structures (Nelson et al., 1996; Nebert and Russell, 2002). P450s are important in the oxidative, peroxidative, and reductive metabolism of numerous endogenous compounds including steroids, bile acids, fatty acids, prostaglandins, leukotrienes, biogenic amines, and retinoids (Waxman and Azaroff, 1992). Together with dehydrogenases, reductases, and oxidases, P450s belong to the group of enzymes in the hepatic detoxification system that are responsible for primary modifications of lipophilic compounds (phase I reactions) (Ziegler, 1994). With the help of reducing equivalents from NADPH cytochrome P450 oxidoreductase, P450s catalyze mono-oxygenase reactions of lipophilic compounds allowing subsequent use of the attached hydroxyl group as a reactive group that can be used by other so-called phase II enzymes for further modifications. Phase II reactions consist mainly of glucuronidation, sulfation, attachment of glutathione, methylation, *N*-acetylation, or conjugation with amino acids. In addition, esterases, amidases, imidases, epoxide hydratases, or other hydrolytic processes increase the hydrophilicity of xenobiotic compounds (Jakoby, 1994). Finally, the intracellular levels of both parent drugs and their metabolites are regulated by transporter proteins, sometimes called phase III enzymes, localized on the sinusoidal and the apical membrane of hepatocytes, the intestine, and the kidney (Stieger and Meier, 1998; Muller, 2000; Suzuki and Sugiyama, 2000; Bohan and Boyer, 2002).

In higher animals, the number of expressed P450s is in the range of 50 to 80 with 57 P450s and 19 P450 pseudogenes known in the human genome (Nelson et al., 1996; Nebert and Russell, 2002). In contrast to bacterial P450s, these enzymes are membrane-bound and located in the endoplasmic reticulum or the inner mitochondrial membrane. Since the biosynthesis of sterols, an important component of eukaryotic membranes, requires P450-catalyzed oxidation reactions, P450s are essential for eukaryotic life (Werck-Reichhart and Feyereisen, 2000). In most species, some of these P450s are important in the metabolism of a large number of xenobiotic substrates such as drugs, carcinogens, food additives, pollutants, pesticides, or environmental chemicals in addition to the metabolism of endogenous compounds (Waxman and Azaroff, 1992). Of the 57 human P450s, approximately 15 are involved in xenobiotic metabolism. The biotransformation of xenobiotics in most cases leads to pharmacologically inactive metabolites that are subsequently excreted. However, biotransformation may also activate so-called prodrugs to pharmacologically active products or even to toxic metabolites. Similarly, nontoxic procarcinogens can be activated by P450-catalyzed reactions and thus be turned into potent carcinogens (Nebert and Gonzalez, 1987). Since P450s play key roles in biosynthetic and catabolic pathways of a variety of compounds, their expression must be highly regulated. Some P450s are expressed only in some tissues and specific cells within this tissue. Similarly, the expression pattern of a number of P450s is different in developmental stages and in females and males.

II. Drug-Mediated Induction of Cytochromes P450

A characteristic of a subset of enzymes of the P450 superfamily able to metabolize xenobiotic compounds is their relatively low basal expression in the absence of substrate and their highly elevated expression in the presence of their own substrates or other inducer compounds. In particular, members of the CYP1A, CYP2B,

[2] Abbreviations: P450, cytochrome(s) P450; PB, phenobarbital; AhR, aromatic hydrocarbon receptor; PPAR, peroxisome proliferator-activated receptor; PCN, 5-pregnen-3β-ol-20-one-16α-carbonitrile; kb, kilobase; bp, base pair(s); PXR, pregnane X receptor; PBRU, PB-responsive enhancer unit; DR, direct repeat; PBREM, PB-responsive enhancer module; NF-1, nuclear factor-1; ER, everted repeat; XREM, xenobiotic-responsive enhancer module; RXR, retinoid X receptor; CAR, constitutive androstane receptor; GRIP1, glucocorticoid receptor-interacting protein 1; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; GR, glucocorticoid receptor; CXR, chicken xenobiotic receptor; AF, activation function; SHP, small heterodimerization partner; FXR, farnesoid X receptor; VDR, vitamin D receptor; LXR, liver X receptor; HNF4α, hepatic nuclear factor 4α; PGC-1α, PPARγ-coactivator 1α.

CYP2C/H, CYP3A, and CYP4A gene subfamilies are highly inducible by some xenobiotics. This xenobiotic induction usually is tissue-specific, rapid, dose-dependent, and reversible upon removal of the inducer. The observation that rats adapt to increasing doses of the barbiturate phenobarbital (PB) with an increase in total P450 concentration and in drug metabolism (Fig. 1A) was made more than 40 years ago (Remmer, 1958, 1972; Conney et al., 1960; Remmer and Merker, 1963). This increase in drug metabolism was subsequently attributed to PB-induced transcriptional activity of P450 genes (Adesnik et al., 1981; Gonzalez and Kasper, 1982). Later, it was found that PB, other barbiturates, and numerous other compounds that exhibit a similar induction pattern and therefore are called PB-type inducers, activate transcription of CYP2A, CYP2B, CYP2C, and CYP3A genes, the same P450s activated by the dexamethasone/rifampicin-type compounds (Waxman and Azaroff, 1992). As indicated in Fig. 1B, the effect of inducer drugs is not restricted to the regulation of P450s and other drug-metabolizing enzymes or drug transporters but involves a major pleiotropic response including the up- or down-regulation of numerous genes and physiological systems including proliferation of the smooth endoplasmic reticulum (Okey, 1990; Waxman and Azaroff, 1992).

In addition to PB-type and dexamethasone/rifampicin-type inducers, other prototypical classes of compounds are represented by aromatic hydrocarbons that mainly induce CYP1As and CYP1Bs, peroxisome proliferators elevating CYP4A levels, and ethanol that increases CYP2E1 (Table 1). The dexamethasone/rifampicin class of inducers affects the same P450s as the PB-type compounds but with different relative potencies. CYP3As are more efficiently induced than CYP2Cs and CYP2Bs by the dexamethasone/rifampicin-type compounds (Waxman and Azaroff, 1992; Denison and Whitlock, 1995; Meyer, 1996; Dogra et al., 1998).

In this review, we focus on the PB- and dexamethasone/rifampicin-type induction of CYP2Bs, CYP2Cs, and CYP3As, the major drug-metabolizing P450s (Meyer, 1996). The mechanisms underlying induction by the other inducer classes are briefly discussed, but interested readers are referred to the respective reviews. The elucidation of the mechanism of induction of CYP1As by polycyclic aromatic hydrocarbons has progressed more rapidly than the PB- and dexamethasone/rifampicin-type induction mechanism. With the help of genetic polymorphisms, high-affinity ligands, and inducible cell culture systems, the aromatic hydrocarbon receptor (AhR) and its binding partner, AhR nuclear translocator, could be identified. These findings, in addition to the discovery of AhR-response elements in the flanking regions of CYP1As were the basis for further characterization of this mechanism (Hankinson, 1995; Whitlock, 1999; Ma, 2001). Soon after the peroxisome proliferator-activated receptor (PPAR) was discovered in 1990, it also became clear that this orphan nuclear receptor plays a crucial role in induction of CYP4As by peroxisome proliferators and related compounds (Johnson et al., 1996; Simpson, 1997). In contrast, ethanol affects CYP2E1 at the post-translational level by stabilization of the enzyme not involving a receptor-dependent mechanism (Gonzalez et al., 1991; Lieber, 1997).

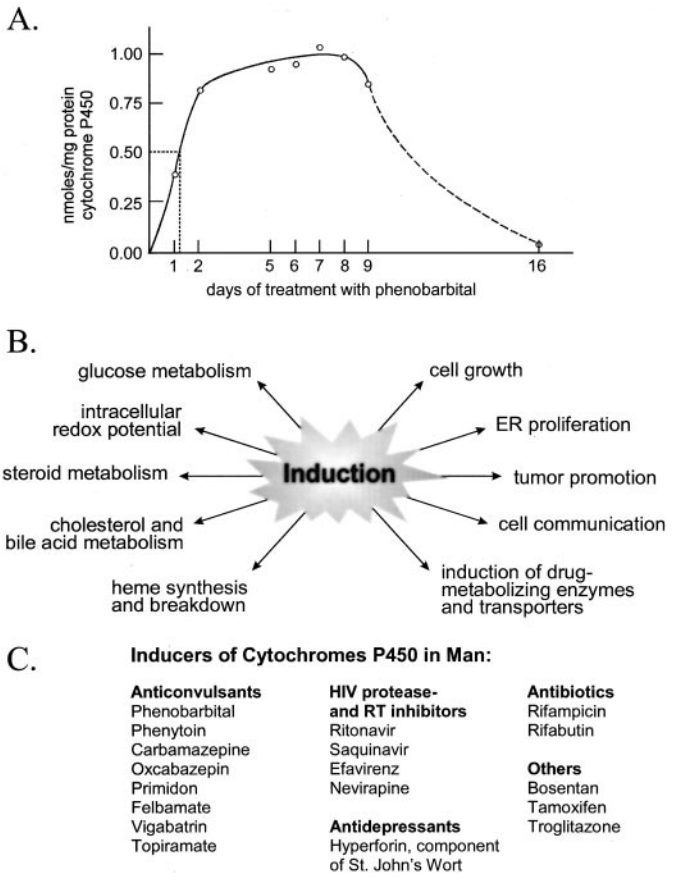


FIG. 1. History and pleiotropic dimension of induction of drug metabolism. A, discovery of phenobarbital-type induction of total cytochrome P450 and of drug metabolism in rat liver, modified from Remmer (1972). Rats were treated for 9 consecutive days with PB and total hepatic P450 protein levels were monitored showing rapid induction of P450 proteins after drug treatment and reversal of this effect upon removal of the inducer compound. B, pleiotropic effects of phenobarbital-type inducers. ER, endoplasmic reticulum. C, list of clinically relevant inducers of cytochromes P450 in man. HIV, human immunodeficiency virus; RT, reverse transcriptase.

TABLE 1
The five classes of inducers of CYPs

Inducer Classes/Prototypes	Examples of Induced CYPs
Polycyclic aromatic hydrocarbons, TCDD	CYP1A1, CYP1A2, CYP1B1
Phenobarbital-type	CYP2Bs, CYP2Cs, CYP3As
Rifampicin-, dexamethasone-type	CYP3As, CYP2Cs, CYP2Bs
Ethanol, isoniazide	CYP2E1
Clofibrate-type	CYP4As

A. Induction of CYP2Bs, CYP2Cs, and CYP3As by Drugs and Xenobiotics

The major mystery in the induction of P450s by drugs and other chemicals for many years was how the cell recognizes these inducers and how the information is conveyed to the transcriptional machinery. Although CYP2B, CYP2C, and CYP3A induction by PB has been described decades ago, progress in this field has been hampered by four major peculiarities. First, the classes of PB- and dexamethasone/rifampicin-type inducer compounds constitute a variety of different substrates such as drugs, steroids, pesticides, pollutants, food additives, and many other chemicals that show no obvious quantitative structure/activity relationship, except that they are lipid-soluble molecules with a relatively low molecular weight. Moreover, most of these xenobiotics activate their target enzymes only at relatively high concentrations in the micro- to millimolar range. Any putative receptor would have to be able to accommodate all these different structures and would require considerable plasticity in its recognition site similar to the substrate binding sites of P450s (Okey, 1990; Waxman and Azaroff, 1992). Indeed, because the P450 substrate binding site exhibits a similar promiscuity toward different substrates, direct interaction of inducers with P450s and thereby release of an endogenous inducer or formation of chemically reactive, reduced oxygen species by uncoupling of the hydroxylation reactions were postulated as alternatives to the PB receptor theory (Fonné-Pfister and Meyer, 1987). Second, as an additional experimental drawback, PB-type induction of P450s is not commonly observed in primary hepatic cell culture systems where it is either qualitatively disturbed or completely absent. This might be due to the dedifferentiation process that occurs when generating continuously dividing cell systems, since drug induction and metabolism is a hallmark of highly differentiated, nondividing hepatocytes (Meyer and Hoffmann, 1999). When culture conditions were modified and included matrix components and other factors, primary rat hepatocytes that retained PB inducibility could be cultured (Waxman et al., 1990). This methodological breakthrough led to the discovery of DNA-enhancer elements that mediate induction (Trottier et al., 1995). However, despite these promising advances, the identified enhancer elements in various species apparently exhibited no obvious common features (Dogra et al., 1998). Third, in contrast to CYP1A induction, no animal models with genetic defects of induction were available that allowed mapping of important components of the PB induction machinery. Finally, the induction potency of several compounds is drastically different in different species, suggesting that multiple mechanisms or receptors may operate to produce this response (Denison and Whitlock, 1995). For example, the antibiotic rifampicin is one of the strongest inducers of human CYP3A4 but has very little effect on rodent CYP3As, whereas the antiglucocorticoid 5-pregnene-3β-ol-20-one-16α-carbonitrile (PCN) is a potent activator of rodent, but not human, CYP3As (Savas et al., 1999; Xie and Evans, 2001). Together, these features have delayed the elucidation of the mechanisms by which PB- and dexamethasone/rifampicin-type inducers change gene expression.

III. Drug-Response Elements in Inducible Cytochrome P450 Genes

Identification of PB-responsive enhancer elements went hand in hand with the establishment of suitable culture systems for primary hepatocytes from chicken, mouse, and rat. Surprisingly, chick embryo hepatocytes in primary cultures preserve PB-type drug induction of P450s (Althaus et al., 1979), whereas hepatocytes from mammals rapidly lose this ability if not cultured under special conditions (Waxman et al., 1990). Thus, the first breakthrough in isolating PB-responsive DNA elements was made by identifying a drug-responsive 4.8-kb enhancer in the flanking region of chicken CYP2H1 (Hahn et al., 1991). The first mammalian PB-responsive enhancer element was isolated from the rat CYP2B2 5′ flanking region in 1995 (Trottier et al., 1995). Later, similar drug-responsive enhancer elements in other mammalian CYP2Bs, CYP3As, and CYP2Cs could be identified (Honkakoski and Negishi, 1998b; Sueyoshi and Negishi, 2001). Interestingly, the basic mechanism of PB induction in higher animals seems to be conserved, whereas bacteria apparently use different mechanisms to react to barbiturate exposure.

A. CYP102/106 in Bacillus megaterium

Bacterial and eukaryotic P450s differ in several ways: whereas bacterial P450s are soluble, eukaryotic P450s are membrane bound. In bacteria, NADH is the predominant cofactor in contrast to NADPH in eukaryotes, and although some bacterial P450s are one-component systems, eukaryotic P450s depend on a reductase (Fulco, 1991). Despite these differences, induction of P450s by PB and other barbiturates is also observed in certain bacteria. In *B. megaterium*, PB induction of CYP102 and CYP106 was postulated to be mediated by PB removal of a repressor protein from a "barbie-box", a 17-bp DNA element with a conserved AAAG motif. The expression of the protein Bm1P1 is stimulated by the inducer and then perturbs the binding of the repressor protein Bm3R1 to the barbie-box (He and Fulco, 1991; Shaw and Fulco, 1993; Liang et al., 1995). However, this concept of PB-mediated de-repression has recently been challenged by results that show that neither mutations of the gene encoding for Bm1P1 nor mutations of the barbie-box affect PB induction of CYP106 (Shaw et al., 1998). On the contrary, Bm1P1 might even help to repress the CYP101 gene (Shaw et al., 2000). Thus, the molecular basis of PB induction in *B. megaterium* and the role of

the barbie-box in this process remain controversial. Intriguingly, conserved barbie-boxes are also found in the proximal flanking regions of chicken and mammalian P450s. As discussed below, the discovery of nuclear receptors as mediators of drug induction in higher animals implies that bacteria use a different strategy to mediate PB gene expression, since nuclear receptor genes have exclusively been observed in metazoan genomes (Mangelsdorf et al., 1995).

B. CYP6 in Insects

In the fruit fly *Drosophila melanogaster* and the house fly *Musca domesticus*, P450s of the subfamilies CYP6A and CYP6D have been isolated and shown to be responsive to PB (Feyereisen, 1999). Analysis of the flanking region of the *D. melanogaster* CYP6A2 gene revealed PB induction to be mediated by sequences within the first 428 bp upstream of the transcriptional start site (Dunkov et al., 1997). No detailed analysis of drug response elements has been reported. In contrast, comparison of 13 members of the subfamily CYP6B from the closely related tiger swallowtail *Papilio glaucus* and *Papilio canadensis*, which are inducible by a number of compounds, revealed differences in the 5′ flanking regions distal of −640 bp from the transcriptional start site. A response element to the xenobiotic xanthotoxin and to ecdysone as well as putative drug-responsive elements known to regulate vertebrate-inducible P450s are present in this region, including a binding site for AhR and an imperfect pregnane X receptor (PXR)-responsive element, which might suggest a conservation of drug-responsive elements in insects compared with those found in vertebrates (Li et al., 2002). Similar elements were recently described in the 5′ flanking region of CYP6B1 of *Papilio polyxenes* (Petersen et al., 2003). However, transcription factors that may bind to these elements and are responsible for insect xenobiotic induction have not been reported. The sequence of the *D. melanogaster* genome has revealed a much lower number of predicted nuclear receptors compared with the human and the *Caenorhabditis elegans* genomes (Enmark and Gustafsson, 2001). Recently, it has been reported that aberrant transcription of the CYP6G1 gene in *D. melanogaster* confers resistance to DDT, and the respective mutation in the CYP6G1 locus is found worldwide (Daborn et al., 2002). Furthermore, genomic comparison of the three major enzyme families responsible for insecticide resistance, the carboxylesterases, glutathione transferases, and the P450s between *D. melanogaster* and *Anopheles gambiae* revealed an expansion of these enzyme families in the mosquito genome compared with the fruit fly (Ranson et al., 2002). Understanding the signaling mechanism responsible for insecticide-mediated induction of P450s and other genes could help to develop countermeasures for insecticide resistance.

C. CYP2H1/2, CYP3A37, and CYP2C45 in Chicken

In 1991, Hahn, Hansen, and May described the first drug-responsive enhancer sequence, a 4.8-kb fragment of DNA (−5.9 to −1.1 kb) in the flanking region of chicken CYP2H1 (Hahn et al., 1991). Following this report, it took several years to identify the functional elements within this large fragment (Fig. 2). The first 1.1 kb of DNA proximal to the CYP2H1 transcriptional start site were not contributing to PB induction unlike the elements found in bacteria (Dogra and May, 1997). In fact, the presence of this 1.1-kb fragment together with the 4.8-kb enhancer largely decreased the drug response in reporter gene assays. Other experiments in chicken primary hepatocytes using the protein synthesis inhibitor cycloheximide and puromycin suggested that the mechanism of PB induction in chicken and mammals may differ. Inhibition of protein synthesis caused a superinduction of CYP2H1 in chicken primary hepatocytes exposed to phenobarbital, but this superinduction did not occur in mammalian hepatocytes (Dogra et al., 1993; Denison and Whitlock, 1995; Sidhu and Omiecinski, 1998). Moreover, this argument was initially supported when 240-bp PB-responsive enhancer sequence in the 4.8-kb enhancer (−1640 to −1400 bp) did not reveal the typical hexamer repeats of mammalian PB-responsive elements (Dogra et al., 1999). This element was predominantly active in combination with additional DNA fragments resulting in a size of the responsive domain of 556 bp. In contrast, our own studies identified a 264-bp PB-responsive enhancer unit (PBRU) at −1657 to −1393 bp that overlaps with the 240-bp element of Dogra and coworkers, as well as an additional 240-bp PBRU (−5120 to −4881 bp) further upstream in the flanking region of CYP2H1, both harboring direct repeats of hexamer half-sites with a spacing of four nucleotides (DR-4). Both of these elements mediated PB induction in reporter gene assays in the chicken hepatoma cell line LMH, similar to those elements found in mammalian CYP2B PBRUs (Handschin and Meyer, 2000; Handschin et al., 2001a). A third PB-inducible fragment is present within the first 6 kb of flanking region between −5896 and −4528 bp (Dogra et al., 1999; Handschin and Meyer, 2000). First analysis of this region, however, failed to reveal conserved PB-responsive DNA elements, and this third drug-responsive enhancer awaits further examination (Handschin et al., 2001a). The mRNA levels of PB-induced CYP2H1 are about 10 times higher than those of the closely related CYP2H2. This is due to differences in the sequence of a hepatic nuclear factor 3 site in the CYP2H2 promoter that leads to lower expression of CYP2H2 compared with CYP2H1, whereas the enhancer regions are identical between these two genes (Davidson et al., 2001). Thus, DR-4 hexamer repeats are the common theme in PB-inducible enhancers in CYP2H1/2 as well as in a chicken PB-inducible member of the CYP3A family, CYP3A37,

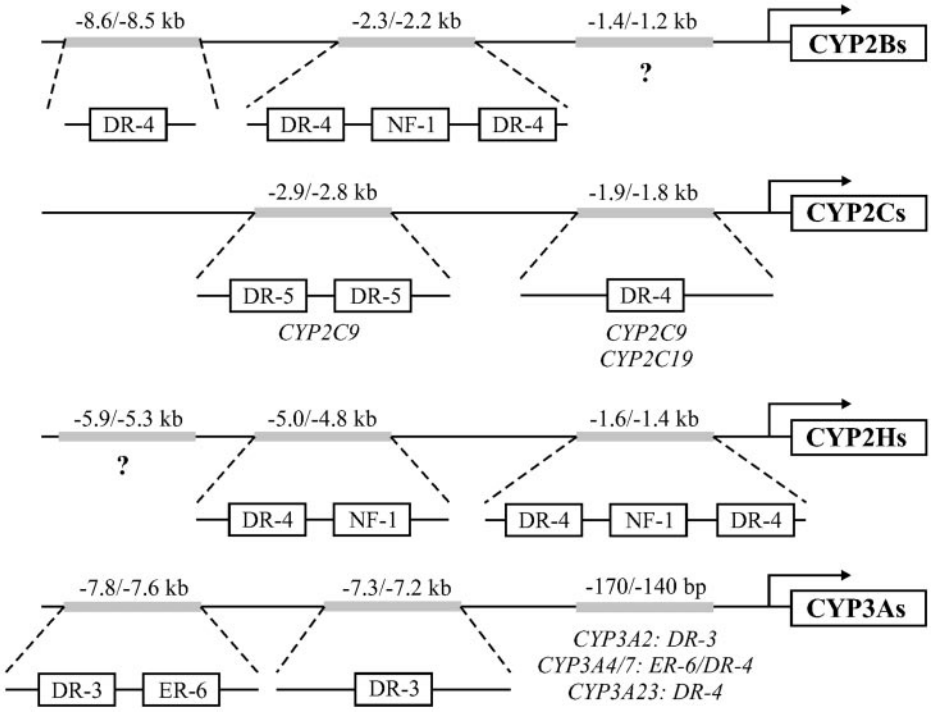


FIG. 2. Drug-responsive enhancer sequences of P450 genes. Summary of *cis*-acting drug-responsive DNA sequences in mammalian and chicken P450s. The drug-response elements are referred to in the text as PBRUs (phenobarbital-responsive enhancer units), PBREMs (phenobarbital-responsive enhancer modules), and XREMs (xenobiotic-responsive enhancer modules). See legend of Fig. 3 for nomenclature of nuclear receptor binding site.

where a 159-bp PBRU has been located at −1159 to −1037 bp (Podvinec et al., 2002). Similar to the CYP3A37 enhancer, we have observed that mutagenesis of a DR-4 site in a 239-bp PBRU (−2435 to −2197 bp) of the chicken CYP2C45 abolishes induction by PB (Baader et al., 2002). In summary, all the currently known chicken drug-inducible P450s share a conserved arrangement of DNA elements that mediate induction by PB and other xenobiotics. Moreover, these elements show a striking conservation when compared with drug-responsive enhancers in mammals as discussed below.

D. CYP2Bs, CYP3As, and CYP2Cs in Mammals

A seminal breakthrough in identifying mammalian CYP2B PBRUs was reported in 1995 by Trottier and coworkers who isolated a 163-bp PB-responsive enhancer fragment in the rat CYP2B2 5′ flanking region situated at −2318 to −2155 bp upstream of the transcription start site (Trottier et al., 1995). Drug induction of this PBRU in vivo was confirmed by in situ DNA injections in rat liver (Park et al., 1996). Soon thereafter, a DNA fragment located at −1404 to −971 sharing high similarity to the rat CYP2B2 PBRU was reported to regulate drug induction of mouse Cyp2b10 (Honkakoski et al., 1996). In both sequences, candidate transcription factor binding sites were predicted, most strikingly repeats of hexamer half-sites that resembled known nuclear receptor binding sites (Honkakoski and Negishi, 1997; Stoltz et al., 1998). The mouse Cyp2b10 enhancer could subsequently be reduced to a 51-bp PB-responsive enhancer module (PBREM) located at −2339 to −2289 bp, which responded to a variety of xenobiotics in reporter gene assays in mouse primary hepatocyte cultures (Honkakoski et al., 1998a). A characteristic of the mouse Cyp2b10 PBREM, the subsequently identified human CYP2B6 PBREM (Sueyoshi et al., 1999), and the rat CYP2B2 PBRU is a conserved arrangement of two DR-4 elements separated by a putative nuclear factor-1 (NF-1) binding site (Fig. 2). Site-specific mutations of the hexamers within the DR-4 sites dramatically decrease PB induction of these elements (Honkakoski et al., 1998b; Liu et al., 1998; Ramsden et al., 1999; Stoltz and Anderson, 1999; Paquet et al., 2000; Liu et al., 2001). Wang and coworkers (2003a) further analyzed the CYP2B6 5′ flanking region and were able to isolate an additional PB-responsive element located 8.5-kb upstream of the transcriptional start site that contains a DR-4 element. The functional role of the NF-1 site in the PBREM is much less clear compared with the DR-4 elements. In transgenic mice that contain 2.5 kb of CYP2B2 flanking region, specific mutations of the NF-1 site abolished binding of NF-1 but retained full inducibility by PB, thus suggesting no functional role of NF-1 in drug induction of CYP2B2 (Ramsden et al., 1999). However, experiments using in vivo footprinting techniques revealed that the NF-1 binding site is protected under normal conditions and that this protected region is enlarged after PB treatment (Kim and Kemper, 1997; Kim et al., 2000). Moreover, NF-1 binding increased drug induction in reporter gene assay using *Drosophila*

embryo extract to assemble chromatin (Kim et al., 2001). These and another report (Stoltz and Anderson, 1999) suggest that NF-1 contributes to drug induction mediated by these PBRUs. Recently, this configuration of two functional DR-4 elements separated by a NF-1 site has also been found in the chicken CYP2H1 264-bp PBRU (Podvinec et al., 2002). Finally, in vivo injection experiments have shown that additional sequences flanking the two DR-4 elements and the NF-1 sites are also contributing to drug responsiveness, namely an uncharacterized site at the 3′ flank and an additional nuclear receptor binding element at the 5′ flank (Rivera-Rivera et al., 2003).

Because of the presence of a barbie-box similar to that found in bacteria, regions proximal to mammalian CYP2B promoters were also analyzed for their ability to confer PB induction (Kemper, 1998). After PB treatment, increased binding of phosphorylated proteins to a positive element (−98 to −69 bp) in the CYP2B1/2 flanking region was observed in rat livers in vivo (Prabhu et al., 1995; Nirodi et al., 1996; Sultana et al., 1997). These proteins have eluded identification so far (Samudre et al., 2002). In contrast, in transgenic mice expressing either 800 bp or 19 kb of CYP2B2 flanking region, only the strain with the 19 kb showed responsiveness to PB (Ramsden et al., 1993, 1999). In other experiments, no specific protein binding to the barbie-box in the proximal promoter region was observed, and targeted disruption of the barbie-box did not affect PB inducibility of CYP2B genes (Kemper, 1998; Sueyoshi and Negishi, 2001). These findings from various laboratories provide compelling evidence that the distal enhancer elements harboring the DR-4 sites are the predominant regulatory DNA elements in drug induction of these P450s.

Mammalian CYP3A genes were initially analyzed to map regions responsive to both classical glucocorticoids and antiglucocorticoids (Quattrochi and Guzelian, 2001). The identified regions proved to be more heterogeneous compared with the highly conserved CYP2B PBRUs. In the proximal promoter between −170 and −140 bp, DR-3 elements in the rat CYP3A2, everted repeats with a spacing of six nucleotides (ER-6) in the human CYP3A4 and CYP3A7 and a DR-4 element in the rat CYP3A23 were identified, as shown in Fig. 2 (Miyata et al., 1995; Quattrochi et al., 1995; Barwick et al., 1996; Huss et al., 1996; Huss and Kasper, 1998; Pascussi et al., 1999; Bertilsson et al., 2001). Furthermore, when testing 13 kb of the CYP3A4 5′ flanking region, an important 230-bp xenobiotic-responsive enhancer module (XREM) was discovered at −7836 to −7606 bp that apparently accounts for a major proportion of the drug induction response and harbors DR-3 and ER-6 sites that respond to both dexamethasone/rifampicin- and PB-type inducers (Goodwin et al., 1999, 2002a). This XREM and the upstream enhancer module found in the CYP2B6 flanking region are both essential for maximal induction of CYP3A4 and CYP2B6, respectively (Goodwin et al., 1999; Wang et al., 2003a). However, the exact contribution of the hexamer repeats near the promoter, the XREM, and an additional DR-3 at −7287 to −7273 bp is not known. Since mutations of each of these sites decrease reporter gene activity in the range of 20 to 50%, none of these sites seems to be responsible for mediating induction of CYP3As, and all of these elements apparently contribute to drug induction (Quattrochi and Guzelian, 2001; Sueyoshi and Negishi, 2001).

Of the mammalian drug-inducible CYP2Cs, PBRUs have been reported in human CYP2C9, CYP2C8, and CYP2C19 (Fig. 2). In the CYP2C9 flanking region, a PBRU located at −1856 to −1783 bp that contains a DR-4 site confers induction by PB and rifampicin (Gerbal-Chaloin et al., 2002) similar to the DR-4 in the chicken CYP2C45 (Baader et al., 2002). Recently, a more distal enhancer between −2900 and −2841 bp in the flanking region of CYP2C9 has been characterized, and two DR-5 sites were identified (Ferguson et al., 2002c). In the human CYP2C8 5′ flanking region, two DR-4 sites have been identified in a 400-bp fragment that are responsive to preferentially dexamethasone/rifampicin-type inducer compounds (Ferguson et al., 2002b). The human CYP2C19 flanking region is very similar to that of CYP2C9. Thus, analysis of the two homologous drug-enhancer regions revealed that the more proximal element at −1874 bp is mainly responsible for drug induction and differs from the CYP2C9 element only by one nucleotide (Ferguson et al., 2002a).

E. Other Mammalian Drug-Inducible Cytochromes P450

Induction by PB- and dexamethasone/rifampicin-type compounds has been observed for a range of P450s other than those discussed above. Most strikingly, PB activates members of the CYP1A and CYP2A subfamily in mammals (Dogra et al., 1998; Kemper, 1998). Although no DNA response elements have been identified so far, PB induction of at least CYP1A2 seems to be independent of the presence or absence of the AhR (Zaher et al., 1998; Sakuma et al., 1999).

IV. Nuclear Receptors Involved in Drug Induction of Cytochromes P450

The gene superfamily of nuclear receptors includes a number of ligand-dependent and ligand-independent transcription factors that are usually characterized by a zinc finger DNA binding domain and C-terminal ligand binding domain as depicted in Fig. 3A (Mangelsdorf et al., 1995; Enmark and Gustafsson, 1996; Nuclear Receptors Nomenclature Committee, 1999). Nuclear receptors were prime candidates for mediating hepatic drug induction for several reasons (Waxman and Azaroff, 1992). First, their ligands are normally small and lipophilic, properties strikingly similar to those of xenobiotic and endobiotic inducer compounds such as steroids, bile ac-

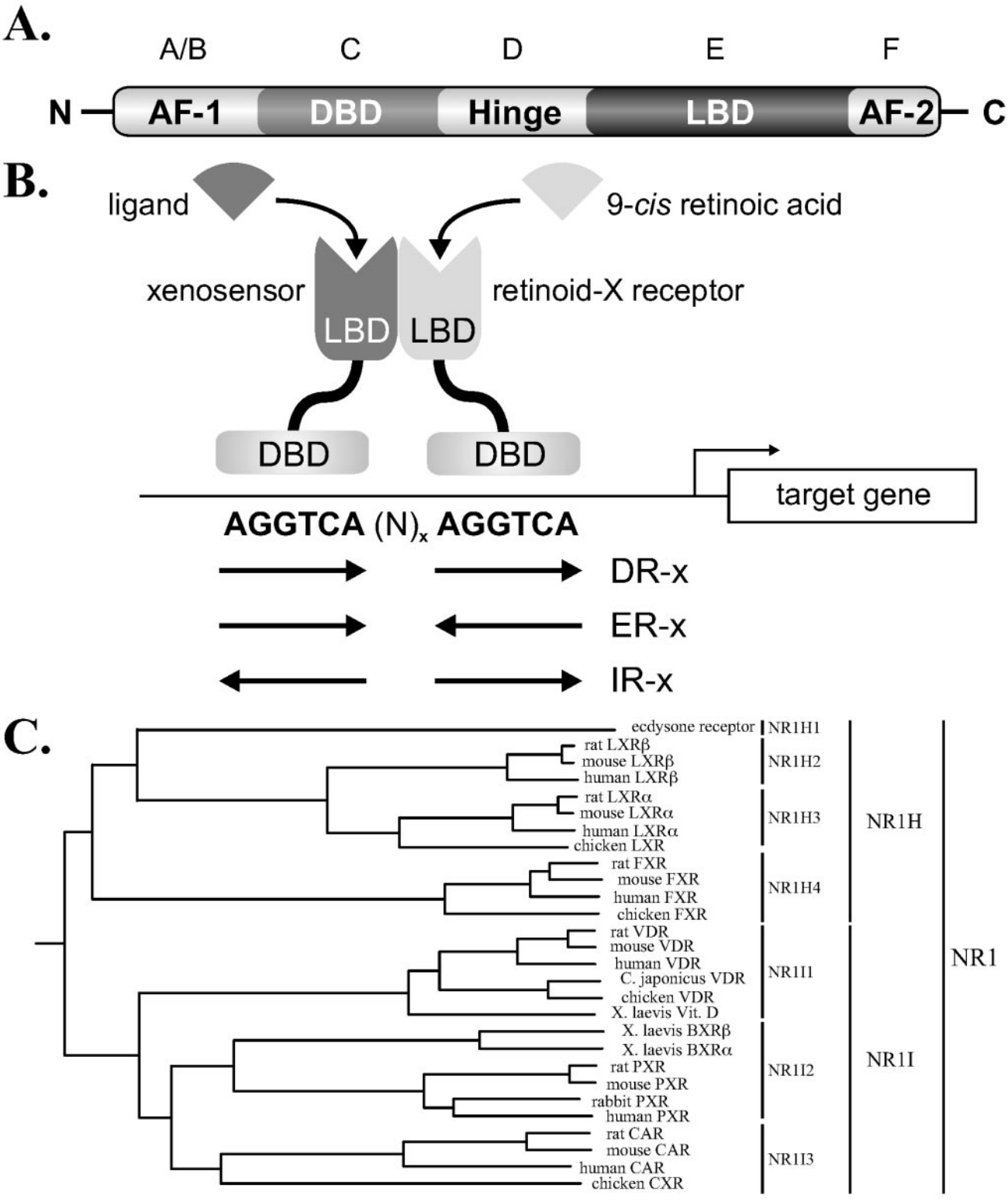


FIG. 3. Structure, DNA binding, and phylogeny of nuclear receptors. A, structure of nuclear receptors. Members of the nuclear receptor superfamily consist of four modular domains: a highly variable N-terminal region that in some receptors harbors an activation function (AF-1), a DNA binding domain (DBD) consisting of two zinc-finger motifs, a flexible hinge domain, and the ligand binding domain (LBD) that also contains an activation function (AF-2). B, DNA binding of nuclear receptors. The xenobiotic-sensing nuclear receptors bind as heterodimers with the RXR to repeats of the nucleotide hexamer AGG/TTCA with variable spacing. The hexamers can be arranged either as direct repeats (DR), everted repeats (ER), or inverted repeats (IR). C, phylogeny of the xenobiotic-sensing and closely related nuclear receptors. Comparison of the amino acid sequences of the xenobiotic-sensing and other nuclear receptors reveals high similarity between the PXRs (official nomenclature, NR1I2), CARs and CXR (NR1I3), and the vitamin D, bile acid, and cholesterol sensors VDR (NR1I1), FXR (NR1H4), and LXR (NR1H2/3), respectively.

ids, or fatty acids. Second, nuclear receptors bind to DNA elements consisting of repeats of hexamers in different kinds of arrangements such as those found in drug-responsive enhancers of P450s (Fig. 3B). Third, the tissue-specific expression of a subset of nuclear receptors is identical to the tissue specificity of drug induction. Finally, closely related members of the nuclear receptor subfamilies NR1I and NR1H (Fig. 3C) play key roles in many physiological processes where P450s are involved. These include steroid, vitamin D, cholesterol, lipid, or bile acid biosynthesis and metabolism (Waxman and Azaroff, 1992; Beato et al., 1995; Mangelsdorf and Evans, 1995; Mangelsdorf et al., 1995; Enmark and Gustafsson, 1996; Waxman, 1999; Honkakoski and Negishi, 2000).

A. Constitutive Androstane Receptor

Within the CYP2B PBRU structure, the two DR-4 sites, called NR1 and NR2, are not equivalent in terms of activation potency by drugs. The more distal DR-4 site (NR1) is more conserved among man, rodents, and chicken (Paquet et al., 2000; Zelko and Negishi, 2000). Thus, the NR1 site was used in affinity purifications for isolation of proteins binding to this sequence and mediating drug induction. This approach led to the identification of the murine nuclear receptor CAR to bind as a heterodimer with the retinoid X receptor (RXR) to the mouse Cyp2b10 NR1 but not to the minimally different, noninducing corresponding fragment from Cyp2b9 (Honkakoski et al., 1998b). Apart from mouse, CAR orthologs have also been described in man, monkey, and rat (Baes et al., 1994; Choi et al., 1997; Yoshinari et al., 2001). Moreover, binding of CAR to the NR1 site predominantly occurred in liver extracts of PB-treated mice and to a much lesser degree in untreated control animals. Subsequently, CYP2Bs in rat have also been shown to be regulated by the rat CAR ortholog but, in addition, require binding of the transcription factor Sp1 to the CYP2B1 proximal promoter (Muangmoonchai et al., 2001; Xiong et al., 2002). In transient transfection assays and in stably transfected HepG2 cells, CAR triggered high basal activity of reporter genes regulated by the mouse Cyp2b10 and the human CYP2B6 PBREMs (Sueyoshi et al., 1999), as expected by the initial reports describing CAR as a constitutively active receptor (Baes et al., 1994; Choi et al., 1997; Yoshinari et al., 2001). Thus, CAR activity after drug induction has to be regulated by additional mechanisms than just ligand binding. Different mechanisms of how CAR can be activated by drugs have been proposed so far, none of them explaining the whole process of signal transduction (Fig. 4). First, although CAR normally resides in the cytoplasm of untreated mouse liver and hepatocytes, it undergoes a cytosolic-nuclear translocation upon PB stimulation, at least in mouse liver and primary rat hepatocytes (Kawamoto et al., 1999; Maglich et al., 2003). This process is controlled by protein phosphorylation events and can be inhibited by using the protein phosphatase inhibitor okadaic acid. Furthermore, the translocation event appears to be mediated by a leucine-rich xenochemical response signal in the C-terminal part of CAR (Zelko et al., 2001). The composition of the protein complex in which CAR is retained in the cytoplasm has not been elucidated. Recent reports described that the nuclear receptor coactivator glucocorticoid receptor-interacting protein 1 (GRIP1) enhances CAR activity and increases cytoplasmic nuclear translocation of CAR in untreated mice (Min et al., 2002a). A second level of CAR activation has been observed in stably transfected HepG2 cells where CAR was located in the nucleus but could be inhibited by administration of certain androstanols (Sueyoshi et al., 1999). These androstanols have been found to work as inverse agonists of CAR activity, the inhibition being reversed by treatment with inducer compounds (Forman et al., 1998; Tzameli and Moore, 2001). However, it is unknown whether this reversal of inhibition is due to a direct interaction of inducers with CAR. In addition to derepression, direct activation of CAR by a few chemicals has been reported. The chemical 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) is one of the strongest inducers in mouse but hardly affects CYP2B levels in man. Accordingly, TCPOBOP strongly binds to and activates mouse CAR but not human CAR (Moore et al., 2000b; Tzameli et al., 2000). Differences in activation of CAR in mouse and man are most likely due to the divergent ligand binding domain of the CAR orthologs from these species (Moore et al., 2000b). Furthermore, CAR activity in the nucleus also seems to be under the regulation of protein phosphorylation events. Experiments using calcium/calmodulin kinase inhibitors revealed changes in CAR-mediated drug induction even in the case where CAR was located in the cell nucleus (Zelko and Negishi, 2000).

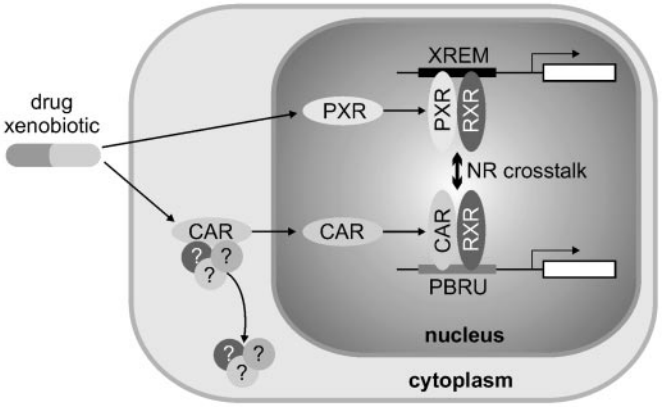


FIG. 4. Activation of the mammalian xenobiotic-sensing nuclear receptors PXR and CAR. After entering the cell, xenobiotics and other activators either 1) trigger cytoplasmic-nuclear translocation of CAR by promoting the release of so far unknown proteins, or 2) directly activate PXR in the nucleus. Subsequently, both PXR and CAR heterodimerize with RXR, bind to their respective response elements, and increase transcription of target genes. In the flanking regions of several genes, response elements have been found that are activated by both PXR and CAR and thus allow direct cross talk of these two receptors.

Finally, an additional regulation of CAR mRNA and activity was reported to occur via the glucocorticoid receptor (GR), which induces CAR transcription via a distal GR-responsive element at −4.4 kb in the human CAR 5′ flanking region (Pascussi et al., 2000b, 2003a).

CAR knockout mice reveal virtually absent induction of Cyp2b10 by TCPOBOP and PB in the liver and small intestine (Wei et al., 2000, 2002; Maglich et al., 2002; Ueda et al., 2002a). Moreover, TCPOBOP and PB induction of Cyp1a1, Cyp2a4, Cyp3a11, and a range of phase II enzymes and transporters is impaired in the livers of CAR knockout mice (Maglich et al., 2002; Ueda et al., 2002a; Wei et al., 2002). Comparison of wild-type and CAR-null mice also revealed complete absence of liver hypertrophy and hyperplasy as well as altered metabolism of different compounds resulting in altered sensitivity to toxins (Wei et al., 2000). Thus, the acetaminophen-metabolizing enzymes Cyp1a2, Cyp3a11, and glutathione *S*-transferase are activated in a CAR-dependent manner after treatment with acetaminophen in wild-type, but not in CAR knockout, mice (Zhang et al., 2002). This finding could be recapitulated in "humanized" mice where the endogenous CAR was ablated, and human CAR under the control of the albumin promoter was expressed in the liver (Zhang et al., 2002). The results obtained with the animal models clearly indicate a crucial role of CAR in mediation of drug induction of certain inducer compounds. However, the molecular mechanism of CAR-mediated signal transduction and the relative contribution of CAR to the total drug effect on gene expression remain enigmatic (Fig. 4). The recently described ligand and activator with high affinity for human CAR provides an opportunity to learn more about CAR signal transduction in human liver (Maglich et al., 2003).

B. Pregnane X Receptor

PXR, alternatively called steroid and xenobiotic receptor or pregnane-activated receptor, has been independently discovered in mice and humans by three groups in 1998. These investigators used either homology cloning or database mining techniques (Bertilsson et al., 1998; Blumberg et al., 1998b; Kliewer et al., 1998; Lehmann et al., 1998). Later, PXR orthologs in rat, rabbit, dog, pig, and monkey have been cloned (Zhang et al., 1999; Jones et al., 2000; Savas et al., 2000; Moore et al., 2002). PXR has subsequently been shown to bind to the DR-3 and ER-6 elements found in CYP3A drug-responsive enhancers and to be activated by a variety of steroids, drugs, and other xenobiotics. Like CAR, PXR transcription is stimulated by activators of GR, and in addition, PXR expression is inhibited by interleukin-6 during acute-phase response, which might explain the observed down-regulation of drug-induced P450s in infections (Pascussi et al., 2000a, 2001, 2000c; Beigneux et al., 2002; Jover et al., 2002). In contrast to other members of the nuclear receptor superfamily, amino acid sequence comparison of the ligand binding domains of different PXR orthologs revealed an unusual high divergence (Jones et al., 2000). This divergence explains the species differences observed in P450 induction by different drugs as demonstrated by site-directed mutagenesis of the mouse PXR ligand binding domain. Four amino acids of the mouse sequence were changed into their corresponding human counterparts, which led to a typical "human" activation pattern (Watkins et al., 2001). Similarly, PXR knockout mice that express the human PXR as transgene exhibit a human-typical response to different inducer compounds (Xie et al., 2000a). As depicted in Fig. 4, in contrast to CAR, PXR is found exclusively in the nucleus (Sueyoshi and Negishi, 2001), and a direct correlation between ligand binding and receptor activation has been demonstrated (Jones et al., 2000). Interestingly, one of the most potent inducers of human PXR discovered so far is hyperforin, a component of extracts from the herb St. John's wort (Moore et al., 2000a; Wentworth et al., 2000). St. John's wort is only one example of the many herbal remedies which are widely used with the potential to interact with drugs and lead to unwanted herb-drug interactions (Zhou et al., 2003). It is thus of considerable importance to elucidate the molecular mechanisms underlying these interactions to prevent adverse effects of herbal remedies (Raucy, 2003).

In PXR knockout animals, induction of Cyp3a11 by PCN is impaired, and basal levels of this gene are increased (Xie et al., 2000a; Staudinger et al., 2001b). However, Cyp3a11 can still be activated by PB. Similarly, PCN induction of Cyp2b10 is abolished in liver and intestine. In contrast, PCN inhibition of Cyp7a1 is abolished in $PXR^{-/-}$ animals. Cyp7a1 is the first enzyme of cholesterol metabolism to bile acids in the liver. Similarly, the expression of Cyp1a1 in the intestine is also derepressed in PXR-null mice compared with PCN-treated wild-type animals (Maglich et al., 2002). Although neither the CAR- nor the PXR knockout animals show an overt phenotype under standard laboratory conditions, constitutive activation of PXR in a transgenic mouse line expressing PXR fused to a VP16-activator domain led to a severe phenotype characterized by growth retardation, hepatomegaly, and liver toxicity (Xie et al., 2000a). Obviously, PXR plays a key role in drug induction, and because of its direct activation by ligands, PXR constitutes an attractive drug target. Activators of PXR include calcium channel blockers, statins, antidiabetic drugs, human immunodeficiency virus protease inhibitors, and anticancer drugs among many other drugs (Kliewer et al., 1998, 2002; Jones et al., 2000; Drocourt et al., 2001; Dussault et al., 2001; Synold et al., 2001; Goodwin et al., 2002b; Kliewer and Willson, 2002; Liddle and Goodwin, 2002). Many of these drugs are clinically relevant inducers at therapeutic doses in humans (Fig. 1C).

C. The Evolution of Xenosensors: Lessons Learned from the Chicken Xenobiotic Receptor

The similarity between chicken and mammalian PBRUs led us to attempt to clone the avian orthologs of the mammalian xenosensors PXR and CAR. Surprisingly, only one nuclear receptor responsive to drugs, the chicken xenobiotic receptor (CXR), was identified. No additional avian receptors related to this receptor family were observed (Handschin et al., 2000). When comparing the amino acid sequences of CXR, PXRs, and CARs, we found that CXR is about equally related to the mammalian PXRs as it is to the mammalian CARs as depicted in Fig. 5 (Handschin et al., 2000). In regard to their function as xenosensors, the mammalian PXRs and CARs and the chicken CXR are interchangeable as shown by activation of mouse, rat, and human PBRUs in the drug-inducible chicken hepatoma cell line LMH and by the binding of PXR and CAR to the chicken CYP2H1 PBRU (Handschin et al., 2001b). Thus, despite the apparent difference in the number of xenosensors, the basic molecular mechanism of drug induction is conserved from birds to mammals. In a recent report, Dogra and coworkers (2003) described that the coactivator CBP/p300 increases the activity of CXR and stimulates PB-induced but not basal expression of CYP2H1. In their model, coactivator proteins such as CBP/p300 and p/CAF link factors binding to distal enhancer sites such as CXR with the proximal promoter upon drug stimulation and then promote chromatin acetylation and the subsequent increase in transcription of CXR target genes similar to proposed models in mammals.

When testing different drugs, steroids, xenobiotics, bile acids, and benzoates, CXR turned out to be one of the most promiscuous receptors, with a broad spectrum of drugs that activate or inhibit compared with the mammalian xenosensors (Moore et al., 2002). Interestingly, only one nuclear receptor related to PXR and CAR has been found in zebrafish (Moore et al., 2002), and when searching the recently published *Fugu rubripes* genome for PXR and CAR orthologs (M. Podvinec, unpublished observations). These receptors also are equally related to the mammalian PXRs and mammalian CARs (Fig. 5). Interestingly, even in the *C. elegans* genome, a single nuclear receptor related to CXR, PXR, and CAR called nhr-8 was found to be activated by different toxins and contributes to xenobiotic resistance (Lindblom et al., 2001). In mammals, cloning attempts on the basis of the mouse and human sequences were successful for the isolation of pig, dog, rabbit, and rat PXR; all have a very high similarity with the sequence of the mouse and human orthologs (Zhang et al., 1999; Jones et al., 2000; Savas et al., 2000; Moore et al., 2002).

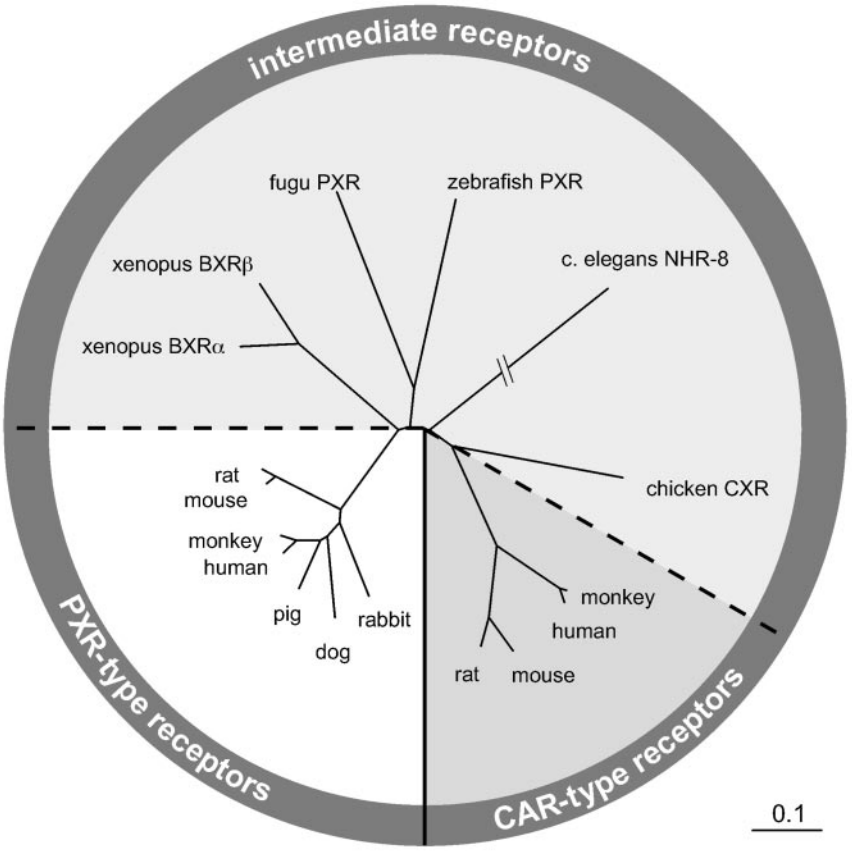


FIG. 5. Phylogeny of the nuclear receptor subfamilies NRI2 and NRI3. Full-length amino acid sequences of the NR1I2 (CAR) and NR1I3 (PXR) subfamily members were compared, and an unrooted phylogenetic tree was derived. The scale bar represents 0.1 substitutions per site. The branch for the *C. elegans* receptor nhr-8 is not drawn to scale.

Thus, the single xenosensors found in nonmammalian species likely represent the ancestral genes that in mammals diverged into two receptors, PXR and CAR. The reason for this duplication of xenobiotic-sensing nuclear receptors in mammals is not clear, but it may reflect the specific challenges in diet and environment that the different species encountered. Moreover, the xenosensors found in nonmammalian species resemble more PXR-type receptors in terms of direct ligand activation. This raises the questions of why and how the unusual nuclear receptor CAR has evolved. Further comparative genomics of additional xenosensors from other species including *D. melanogaster* should shed more light on this issue. The exact roles of the related benzoate X receptors α and β identified in xenopus are not known (Blumberg et al., 1998a; Nishikawa et al., 2000). However, benzoate X receptors α and β are clearly pharmacologically distinct from the described xenosensors, and in addition, their expression pattern exhibits no similarities to those found for PXRs, CARs, and CXRs in mammals and chicken (Heath et al., 2000; Grün et al., 2002; Moore et al., 2002). Thus, xenobiotic-sensing nuclear receptors in amphibians remain to be cloned and characterized.

D. Structure of the Xenosensors

Several puzzles concerning drug induction were clarified by solving the crystal structures of the nuclear receptors involved in this process. The extreme structural variety of inducer compounds hardly fits with the hypothesis of a common receptor. However, when PXR was crystallized and the structure analyzed, it became clear that PXR not only has a much larger ligand binding domain compared with other nuclear receptors, it also was possible for the cocrystallized ligand SR12813, a synthetic biphosphonate, to bind to PXR in three different conformations (Watkins et al., 2001). Hyperforin, one of the psychoactive components of St. John's wort and a potent activator of PXR, induces a structural change in the PXR conformation and considerably increases the size of the ligand binding pocket (Watkins et al., 2003). Whether the possibility for PXR ligands to bind in different conformations also has an impact on their activation potential remains to be investigated (Ekins and Schuetz, 2002). Analysis of the 28 amino acids shaping the ligand binding pocket can, in principle, explain the species differences in drug induction (Watkins et al., 2001). In comparison with other known nuclear receptor structures, PXR shares the same general confirmation. However, the size of the ligand binding cavity is much larger and mostly coated with hydrophobic residues that can accommodate lipophilic inducer compounds. In addition to the 12 helices found in classical nuclear receptor ligand binding domains, PXR has a large, flexible loop that apparently provides additional flexibility when binding bulky ligands and further explains the promiscuity of this receptor (Gillam, 2001). Interestingly, mutation of a single histidine at position 407 in human PXR into an alanine resulted in high constitutive activity and dramatically increased basal expression of PXR-activated reporter gene assays (Ostberg et al., 2002). The insights about the structure of the PXR ligand binding domain could now help to predict PXR activators and ligands in drug discovery and development (Ekins and Erickson, 2002; Ekins et al., 2002).

For CAR, no crystal structure has been reported yet. Molecular modeling of the CAR ligand binding domain on the basis of other nuclear receptor structures combined with site-directed mutagenesis provided some insights into the function of CAR (Dussault et al., 2002; Xiao et al., 2002; Andersin et al., 2003; Jacobs et al., 2003; Moore et al., 2003). The foremost questions regarding CAR are whether CAR has a ligand binding domain similar in size compared with PXR and whether its structure reflects the constitutive activity. A three-dimensional model based on the related PXR crystal structure predicts that CAR lacks the flexible surface loop found in PXR and thus would be less promiscuous for direct ligand binding (Xiao et al., 2002). However, the volume of the ligand binding pocket of these two receptors seems to be similar, allowing CAR to putatively accommodate compounds of different structures as observed for PXR (Dussault et al., 2002; Xiao et al., 2002). Strikingly, several features found only in the CAR model may account for its constitutive activity. Between helix 11 and helix 12, site of the classical transactivation domain in nuclear receptors, CAR has a short loop and a C-terminal helix that fix the ligand binding domain in a conformation normally found in ligand-activated nuclear receptors even in absence of CAR ligands (Dussault et al., 2002). Moreover, charge-charge interactions between the C-terminal activation domain and helix 4 apparently favor ligand-independent activation, as verified by site-directed mutagenesis of key residues in this intramolecular interaction. In contrast to the charge clamp in classical endocrine nuclear receptors, three hydrophobic amino acids in the AF-2 domain were observed to be of more importance than the lysine in helix 3 and the glutamate in helix 12 for the interactions of CAR with coactivator proteins (Andersin et al., 2003). In summary, CAR uses some of the classical conserved motifs and coregulator proteins as described for other nuclear receptors, but its structure has differences which might account for its constitutive activity. Ligand-mediated repression of CAR may be caused by replacement of coactivator proteins by corepressors. These predicted structural features of CAR are strikingly different from classical nuclear receptors and open the discussion about the evolution of such a configuration. Hopefully, more decisive answers will be provided when the CAR crystal structure is solved. All of these interpretations have to be seen in regard to the fact that most inducers seem to activate CAR by an indirect mechanism leading to cytoplasmic-nuclear translocation not involving direct

ligand activation. Predictions of the nature of compounds that trigger this translocation and activation therefore might not be achieved by knowing the structure of CAR and may require other experimental approaches.

E. Other Target Genes of Pregnane X Receptor and Constitutive Androstane Receptor

Although P450s have obviously been the primary focus in the characterization of xenosensor targets and are the primary focus of this review, numerous other genes have been reported to be regulated by these nuclear receptors. This makes sense, since inducer drugs have been known to increase the expression of not only phase I enzymes (functionalization reactions), but also phase II enzymes (conjugation reactions), drug-transporters, and related enzyme systems for endogenous substrates for these reactions (Table 2). Thus, a role of CAR and PXR has been proposed in the regulation of human bilirubin UDP-glucuronosyltransferase (Sugatani et al., 2001), dehydroepiandrosterone sulfotransferase, 3′-phosphoadenosine 5′-phosphosulfate synthetase 2 (an en-

TABLE 2
Target genes of the xenosensors CXR, PXR, and CAR[a]

Class	Gene	Organism	Response Element	Receptor	Reference
Drug oxidation (phase I)	Cyp1a1	Mouse	?	CAR	Maglich et al., 2002
	CYP1a2	Mouse	?	CAR	Maglich et al., 2002
	CYP2a4	Mouse	?	CAR	Maglich et al., 2002
	CYP2B1/2	Rat	DR-4	CAR, PXR	Sueyoshi and Negishi, 2001
	CYP2B6	Human	DR-4	CAR, PXR	Sueyoshi and Negishi, 2001
	CYP2b10	Mouse	DR-4	CAR, PXR	Sueyoshi and Negishi, 2001
	CYP2C8	Human	DR-4	PXR	Ferguson et al., 2002b
	CYP2C9	Human	DR-4	CAR, PXR	Gerbal-Chaloin et al., 2002
	CYP2C19	Human	DR-4	CAR	Ferguson et al., 2002a
	CYP2C45	Chicken	DR-4	CXR	Baader et al., 2002
	CYP2H1	Chicken	DR-4	CXR	Handschin and Meyer, 2000
	CYP3A2	Rat	DR-3	PXR	Sueyoshi and Negishi, 2001
	CYP3A4	Human	ER-6, DR-3, DR-4	PXR, CAR	Sueyoshi and Negishi, 2001
	CYP3A7	Human	ER-6	PXR	Sueyoshi and Negishi, 2001
	CYP3a11	Mouse	?	PXR, CAR	Maglich et al., 2002
	CYP3A23	Rat	DR-4	PXR	Sueyoshi and Negishi, 2001
	CYP3A37	Chicken	DR-4	CXR	Podvinec et al., 2002
	Aldh1	Mouse	?	PXR, CAR	Maglich et al., 2002
	Est1	Mouse	?	CAR	Ueda et al., 2002a
	F-monoox.	Mouse	?	CAR	Ueda et al., 2002a
Drug conjugation (phase II)	GST	Mouse	?	CAR	Maglich et al., 2002
	Sultn	Mouse	?	CAR	Maglich et al., 2002
	Std	Mouse	IR-0	PXR	Sonoda et al., 2002
	UGT1A1	Human	DR-4	CAR	Sugatani et al., 2001
	Ugt1a1	Mouse	?	PXR	Maglich et al., 2002
Drug import/export	MDR1	Human	DR-4	PXR	Geick et al., 2001
	Mdr1a	Mouse	?	PXR, CAR	Maglich et al., 2002
	Mdr1b	Mouse	?	PXR	Maglich et al., 2002
	Mrp1	Mouse	?	CAR	Maglich et al., 2002
	Mrp2	Mouse	ER-8	PXR, CAR	Kast et al., 2002
	Mrp3	Mouse	?	PXR, CAR	Maglich et al., 2002
	Oatp2	Mouse	?	PXR	Maglich et al., 2002
	OATP2	Rat	DR-3	PXR	Guo et al., 2002
Essential accessory proteins	ALAS1	Chicken	DR-4	CXR	Fraser et al., 2002
	Alas1	Mouse	?	PXR, CAR	Maglich et al., 2002
	Methyltransferase	Mouse	?	CAR	Ueda et al., 2002a
	PAPS synthase	Mouse	?	CAR, PXR	Ueda et al., 2002a
	Por	Mouse	?	PXR, CAR	Maglich et al., 2002
Receptors	AhR	Mouse	?	CAR	Maglich et al., 2002
	CAR	Mouse	?	PXR	Maglich et al., 2002
	ear-2	Human	?	CAR	Sugatani et al., 2001
	PXR	Mouse	?	PXR	Maglich et al., 2002
Other enzymes and proteins	Aquaporin 1	Mouse	?	CAR	Ueda et al., 2002a
	cAMP-reg. PP	Mouse	?	CAR	Ueda et al., 2002a
	IGFBP1	Human	?	CAR	Sugatani et al., 2001
	HD	Mouse	DR-2	CAR	Kassam et al., 2000
	iNOS	Human	DR-4	PXR, CAR	Toell et al., 2002
	Semaphorin-3	Mouse	?	CAR	Ueda et al., 2002a
	SOD3	Human	?	CAR	Sugatani et al., 2001

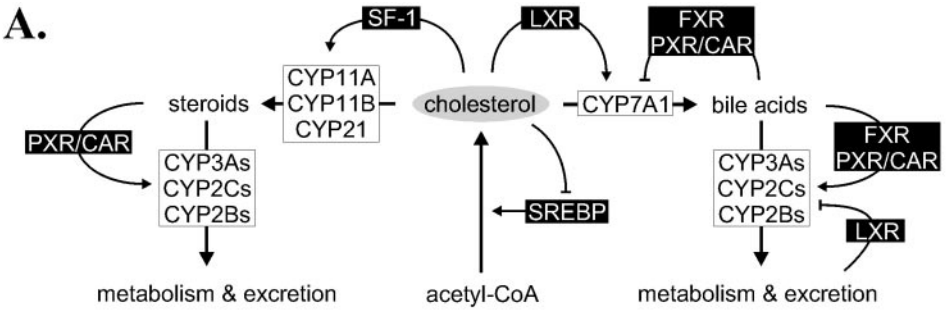
[a] List of target genes that are induced by any of the xenosensors. Genes included were identified as direct target genes with response elements or found to be reduced in PXR or CAR knockout animals. References are either reviews or primary papers describing the respective genes. See text for details or further references. Abbreviations: Aldh, aldehyde dehydrogenase; Est, esterase; GST, glutathione *S*-transferase; Sultn, sulfotransferase; Std, dehydroepiandrosterone sulfotransferase; UGT, UDP-glucuronosyl transferase; Mdr, intestinal P-glycoprotein; Mrp, multidrug-resistance protein; Oatp, organic anion transporting peptide; F-monoox, flavin containing mono-oxygenase; Por, cytochrome P450 oxidoreductase; iNOS, inducible nitric-oxide synthase; cAMP-reg. PP, cAMP-regulated phosphoprotein; SOD, superoxide dismutase; IGFBP, insulin-like growth factor-binding protein; HD, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase.

zyme that is involved in the synthesis of the donor sulfate group) (Sonoda et al., 2002), hydroxysteroid sulfotransferase (Duanmu et al., 2002), and glutathione *S*-transferase (Falkner et al., 2001; Zhang et al., 2002). Transporters regulated by the xenosensors are mostly drug- or bile acid-transport proteins and include the multidrug-resistance proteins 2, 3, and 4 (Schuetz et al., 2001; Cherrington et al., 2002; Kast et al., 2002; ; Xiong et al., 2002; Staudinger et al., 2003), the intestinal P-glycoprotein (Geick et al., 2001; Synold et al., 2001), and the organic anion transport protein 2 (Staudinger et al., 2001b; Guo et al., 2002). Other PXR, CAR, or CXR target genes were anticipated, such as the first and rate-limiting enzyme in heme biosynthesis, the 5-aminolevulinic acid synthase (Fraser et al., 2002). Other regulated genes were unexpected, for example the activation of expression of the human inducible nitric oxide synthase (Toell et al., 2002). To analyze the pleiotropic induction response (Fig. 1B), DNA-expression microarrays with cDNA derived from CAR- or PXR-deficient mice and from the humanized mice expressing human PXR recently have expanded the list of putative xenosenor target genes (Maglich et al., 2002; Ueda et al., 2002a; Rosenfeld et al., 2003), although the observed effects on mRNA expression may of course also represent secondary effects (Ueda et al., 2002a). Interestingly, expression of PXR and CAR themselves as well as of the AhR seems to be auto-regulated by these two xenosensors (Maglich et al., 2002). All of the genes analyzed so far are positively affected by the respective xenobiotic-sensing nuclear receptor, and a list of these genes can be found in Table 2. However, these drugs are also known to repress a number of genes (Frueh et al., 1997). Thus, the list of genes up- or down-regulated by CAR, PXR, and CXR is expected to grow in the future when additional genes are analyzed for their ability to be activated or repressed by drugs.

V. Endogenous Roles of the Xenosensors

Most drug-metabolizing P450s also hydroxylate various endogenous compounds such as steroids, cholesterols, lipids, vitamins, or bile acids (Fig. 6A). Similarly, in addition to being activated by drugs and xenobiotics, endogenous compounds have been shown to affect CAR, PXR, and CXR, which allows speculation about the evolutionary origin or a putative physiological role of these xenosensors beyond drug metabolism. For example, the β amino acid taurine increases induction of CYP3As by rifampicin but not by PB, but the physiological significance of this observation is not clear (Matsuda et al., 2002). Since tocopherols and tocotrienols are metabolized in part by P450s, it is not surprising that all forms of vitamin E are able to activate human PXR and increase CYP3A4 and CYP3A5 levels in HepG2 cells (Landes et al., 2003). However, the fact that α- and γ-tocotrienol are more potent inducers than rifampicin was not to be expected and implies a potential for certain forms of vitamin E to interfere with the metabolism of other drugs (Brigelius-Flohe, 2003). Different steroids activate and repress PXR and CAR. Thus, PXR activity is increased by synthetic glucocorticoids, pregnane derivatives, progesterone and some of its hydroxylated metabolites, cortisol, cortisone, estradiol, dihydrotestosterone, dehydroepiandrosterone, and other steroids to various extent (Bertilsson et al., 1998; Blumberg et al., 1998b; Kliewer et al., 1998; Lehmann et al., 1998; Moore et al., 2002; Ripp et al., 2002). This suggests an important role for PXR in maintaining serum levels of certain steroids and steroid hormones (Blumberg and Evans, 1998). Steroid hormones have divergent effects on CAR: whereas estrone and 17β-estradiol activate CAR, progesterone, 17α-ethynyl-3,17β-estradiol, androgens, and androstanols have an inhibitory effect (Forman et al., 1998; Kawamoto et al., 2000; Negishi and Honkakoski, 2000; Makinen et al., 2002). Due to its steroid sensitivity, CAR may contribute to the sexually dimorphic expression of CYP2B1 in Wistar-Kyoto rats (Yoshinari et al., 2001). Hepatic proliferation stimulated by the mouse CAR-activator TCPOBOP also differs in male and female mice; the females show a higher labeling index along with increased expression of cyclin D1, cyclin A, E2F, Cyp2b10, and elevated phosphorylation of pRb and P107 as compared with males (Ledda-Columbano et al., 2003). Repression of CAR by androstanols, testosterone, and progesterone and activation of CAR by estrogens are only observed in the mouse and not with human CAR. Structure-function analysis of the mouse and human orthologs revealed a threonine residue in the mouse CAR ligand binding domain and a corresponding methionine in human CAR to be responsible for this steroid sensitivity (Ueda et al., 2002b). Interestingly, both PXR and CAR have been reported to be influenced by some of the endocrine disruptors such as methoxychlor (Blizard et al., 2001), phthalic acid, nonylphenol (Masuyama et al., 2000), and also by organochlorine pesticides (Coumoul et al., 2002) and the antihormones cyproterone acetate and spironolactone (Schuetz et al., 1998). All these various observations suggest a role of these receptors in mediating physiological and pharmacological actions of endocrine factors.

More recently, CXR and PXR have been found to be activated by different bile acids and thus provide hepatoprotection from deleterious effects of pathologically elevated levels of bile acids by inducing their inactivation by CYP3As (Staudinger et al., 2001b; Xie et al., 2001; Handschin et al., 2002). In mice lacking Cyp27a1, the sterol 27-hydroxylase required for both the classical and the alternate bile acid-biosynthesis pathways, Cyp3a11 levels are dramatically elevated (Dussault et al., 2003; Goodwin et al., 2003). In these mice, three potentially toxic bile acid precursors were isolated that potently activate mouse PXR. The activation of human PXR by these intermediates is small, however. Apart



B.

Interacting receptor	Gene affected	Enzyme function	Regular receptor	Reference
CAR	CYP3A1	Drug metabolism	PXR	Smirlis et al., 2001
	CYP3A4	Drug metabolism	PXR	Sueyoshi et al., 1999
	Cyp3a11	Drug metabolism	PXR	Ueda et al., 2002a
	CYP3A23	Drug metabolism	PXR	Xie et al., 2000b
	Cyp4a10/14	Lipid peroxidation	PPAR	Ueda et al., 2002a
	HD	Lipid peroxidation	PPAR	Kassam et al., 2000
CXR	CYP7A1	Cholesterol metabolism	?	Handschin et al., 2002
LXRα	CYP2B6	Drug metabolism	CAR	Handschin et al., 2002
	CYP2H1	Drug metabolism	CXR	Handschin et al., 2002
	CYP3A4	Drug metabolism	PXR	Handschin et al., 2002
PXR	CYP2B1	Drug metabolism	CAR	Smirlis et al., 2001
	CYP2B6	Drug metabolism	CAR	Xie et al., 2000b
	Cyp2b10	Drug metabolism	CAR	Xie et al., 2000b
	Cyp7a1	Cholesterol metabolism	LXR	Staudinger et al., 2001b
TR	Cyp2b10	Drug metabolism	CAR	Makinen et al., 2002
VDR	CYP2B6	Drug metabolism	CAR	Drocourt et al., 2002
	Cyp2b10	Drug metabolism	CAR	Makinen et al., 2002
	CYP2C9	Drug metabolism	CAR	Drocourt et al., 2002
	CYP3A4	Drug metabolism	PXR	Thummel et al., 2001

FIG. 6. Endogenous and exogenous functions of xenobiotic-sensing nuclear receptors. A, activity of the xenobiotic-sensing nuclear receptors PXR and CAR can be modulated by a variety of xenobiotic and endogenous factors which leads to an increase or decrease of a battery of enzymes involved in the biosynthesis or metabolism of these compounds. Their metabolites in turn can again influence hepatic drug induction either positively or negatively. Since the same xenosensors are affected by these substances, they influence the metabolism of one another. Therefore, the xenobiotic-sensing nuclear receptors play key roles in maintaining hepatic cholesterol, steroid, and bile acid homeostasis by interacting with a number of other nuclear receptors and transcription factors. B, cross talk between nuclear receptors. Interactions have been listed where CXR, PXR, or CAR modulates target genes of other nuclear receptors or vice versa.

from these bile acid intermediates, 3-ketolithocholic acid has been reported as a mouse PXR activator, whereas human PXR is stimulated by lithocholic acid, 3-ketolithocholic acid, ursodeoxycholic acid, and, to a lesser extent, by the cholic acid, chenodeoxicholic acid, and deoxycholic acid (Schuetz et al., 2001; Staudinger et al., 2001b). PXR therefore might be an attractive target for treatment of cholestasis to increase metabolism and subsequent excretion of bile acids. In fact, rifampicin, PB and more recently ursodeoxycholic acid have been used to relieve symptoms in cholestatic patients for years without knowing that the desired effect might be due to the activation of PXR and thereby P450s, conjugating enzymes and transporters important for the elimination of these compounds (Chawla et al., 2001; Goodwin and Kliewer, 2002; Kliewer and Willson, 2002). In addition to promoting metabolism of bile acids, PXR and CXR also inhibit expression of CYP7A1, the first and rate-limiting enzyme in the metabolism of cholesterol to bile acids, and thereby these receptors prevent formation of more bile acids when activated (Repa and Mangelsdorf, 2000; Staudinger et al., 2001b; Handschin et al., 2002). However, due to this inhibition, the xenosensors might also participate in regulating cholesterol and oxysterol levels. The mechanism of this inhibition is still unclear, although it seems to be independent of the bile acid-mediated up-regulation of the small heterodimer partner (SHP) by the farnesoid X receptor (FXR) as deduced from results obtain using SHP knockout animals (Kerr et al., 2002; Wang et al., 2002). Intriguingly, guggulsterone, a plant sterol that lowers serum cholesterol in man, strongly inhibits human CYP7A1 by activating PXR, whereas it has no effect on the FXR-mediated CYP7A1 repression (Owsley and Chiang, 2003). The xenosensors therefore play important roles in the metabolism of both xenobiotic and endobiotic lipophilic compounds and form a fine-tuned regulatory network together with other transcription factors to ensure a

tightly controlled homeostasis of these lipid compounds. Accordingly, bile acid and drug toxicity is more severe in CAR- and PXR-null mice compared with wild-type mice (Wei et al., 2000; Xie et al., 2000a, 2001; Staudinger et al., 2001b). In addition to their hepatoprotective role concerning bile acids, CAR and PXR are also important in coordinating storage, glucuronidation, and canalicular export of bilirubin, the oxidative end product of heme catabolism (Roy-Chowdhury et al., 2003). Bilirubin itself can activate CAR, and mice lacking functional PXR or CAR are defective in dealing with chronically elevated bilirubin levels (Huang et al., 2003; Xie et al., 2003).

Other factors known to influence drug induction of P450s include cytokines during inflammation and other diseases; radical oxygen species; and fasting and feeding (Cheng and Morgan, 2001). The mechanisms mediating repression or induction of P450s during fasting or feeding periods are not known, and no clear picture of how caloric intake influences drug-inducible P450s has emerged yet (Morgan et al., 1998). Increased cytokine levels during inflammation, however, lead to a decrease in the levels of the respective P450s that might be explained by the recent findings of cytokine-mediated repression of PXR, CAR, and RXR in the liver (Beigneux et al., 2000, 2002; Jover et al., 2002; Pascussi et al., 2000c). Finally, in the case of high P450 activity in the liver, radical oxygen species and nitric oxide are known to accumulate and subsequently decrease P450 expression (Hirsch-Ernst et al., 2001; Morgan et al., 2001). In the CYP2B1 5′ flanking region, this inhibition is conveyed via a PBRU, but the factors involved are still nebulous. Similarly, CAR and PXR have been found to mediate and increase the levels of superoxide dismutase and inducible nitric oxide synthase, both enzymes involved in the defense against radical oxygen species (Sugatani et al., 2001; Toell et al., 2002). Thus, a broader role of PXR and CAR emerges inasmuch as these receptors not only confer hepatoprotection against xenobiotic compounds, but also against accumulation of endobiotic compounds including bile acids, radical oxygen species, and other endogenous mediators that could accumulate to toxic levels.

A. Receptor Cross Talk in Hepatic Drug Induction

Xenosensors expectedly are part of a complex network of transcription factors in vivo (Karpen, 2002; Akiyama and Gonzalez, 2003; Pascussi et al., 2003b). Thus, it is not surprising that these nuclear receptors interact with a variety of other proteins as well as one another (Fig. 6B). Between CAR and PXR, a considerable redundancy exists with regard to overlapping ligand and activator spectrum and the binding of both receptors to the DNA-response elements of one another with overlapping affinity (Jones et al., 2000; Xie et al., 2000b; Goodwin et al., 2001, 2002a; Smirlis et al., 2001; Makinen et al., 2002). PXR and CAR might thus compensate for the loss or malfunction of one another to a certain degree, which might explain the lack of an obvious phenotype in the PXR- or CAR knockout animals.

In addition, the activator spectrum of PXR, CAR, and CXR indicates that these xenosensors share ligands with other receptors, such as thiazolidinedione troglitazone, which activates both PXR and PPARs, SR-12813 which binds to both PXR and FXR (Jones et al., 2000), or endogenous steroids that influence PXR, CAR, and the respective steroid hormone receptors. Thus, competition for ligands might constitute one level of receptor cross talk. Interestingly, mice lacking a functional GR exhibit lower levels of Cyp2b induction by steroids and lower levels of Cyp2b and Cyp3a induction by PB (Schuetz et al., 2000). A more recent study showed that GR-activation can enhance CAR- and PXR-mediated induction of CYP2B6 (Wang et al., 2003b). The mechanism of this GR-mediated modulation of steroid and drug induction of P450s is not clear. However, the GR is an essential but distinct component of this effect. Apart from PXR and CAR, binding of the vitamin D receptor (VDR), of the thyroid hormone receptor, and of the liver X receptor (LXR) to drug-responsive enhancers in CYP2Bs, CYP2Cs, CYP3As, and CYP2H1 has been observed (Thummel et al., 2001; Drocourt et al., 2002; Handschin et al., 2002; Kocarek et al., 2002; Makinen et al., 2002). None of these P450s has been shown to metabolize 1α,25-dihydroxyvitamin D_3, and thus it is unclear what the role of induction of these P450s by vitamin D represents. However, prolonged treatment with rifampicin or PB lowers circulating levels of active metabolites of vitamin D, and thus PXR and CAR may control enzymes in vitamin D biogenesis or metabolism (Schmiedlin-Ren et al., 2001; Thummel et al., 2001; Drocourt et al., 2002). Since expression levels of thyroid hormone receptor in the liver are much lower when compared with those of CAR, the physiological relevance of the observed interaction also is questionable (Makinen et al., 2002). In contrast, activation of LXR by oxysterols or by hydroxylated bile acids has an inhibitory effect on drug induction of P450s in avian hepatocytes (Handschin et al., 2002). This interaction between LXR and xenosenors may represent a carefully balanced system that ensures metabolism of bile acids via the positive effect of xenosensors on P450s and prevents accumulation of hydroxylated bile acids by the inhibitory action of LXR. The antagonizing effects of xenosenors and LXR on CYP7A1 provide further regulation of both intrahepatic cholesterol and bile acid levels (Staudinger et al., 2001a; Handschin et al., 2002). Although LXR and the xenosenors may directly compete for binding to DR-4 sites within PBRUs, it is not clear how LXR inhibits these enhancers, whereas other LXR-responsive DR-4 sites are activated upon LXR binding. In addition, other mechanisms by which so far unknown precursors in the cholesterol biosynthesis pathway activate drug-metabolizing P450s have been proposed; these mechanisms could explain the P450 induction observed after treat-

ment of rat hepatocytes and chicken hepatoma cells with statins which inhibit cholesterol biosynthesis by an effect on 3-hydroxy-3-methylglutaryl-CoA reductase (Kocarek et al., 1998; Kocarek and Mercer-Haines, 2002; Ourlin et al., 2002).

In lipid metabolism, many genes of peroxisomal β-oxidation are under control of PPAR, including enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase. Surprisingly, CAR binding to a PPAR-responsive enhancer element in this gene has been described but not to a similar element in another gene in peroxisomal β-oxidation, acyl-CoA oxidase (Kassam et al., 2000). CAR activates the PPAR binding site in the enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase and also interferes with PPAR induction of this enzyme. However, the exact role of CAR in peroxisomal β-oxidation remains unknown. Similarly, activators of PPARα have an effect on Cyp2a5 and Cyp2b10 in mice but the biological significance of this observation again is not clear (Cai et al., 2002).

The nuclear receptor hepatic nuclear factor 4α (HNF4α) interacts with PXR by enhancing its transcription during liver development (Watt et al., 2003). In HNF4α$^{-/-}$ embryos, expression of PXR in the liver is severely reduced (Li et al., 2000). More recently, an HNF4α binding site has been identified in the CYP3A4 gene and it has been observed that mice with a conditional hepatic deletion of HNF4α exhibit lower basal and inducible levels of Cyp3a11 (Tirona et al., 2003). Similarly, fetal hepatocytes containing floxed HNF4α alleles that are infected with adenoviral Cre recombinase exhibit lower expression of both Cyp3a11 and PXR (Kamiya et al., 2003). Moreover, an HNF4α binding site has been found in the PXR promoter that seems to be required for promoting PXR transcription in fetal liver development (Iwahori et al., 2003; Kamiya et al., 2003). These findings suggest that the liver specific factor HNF4α is required for a complete physiological activation of hepatic drug-inducible P450s.

At pharmacological doses, retinoic acids have been found to repress CAR-mediated activation of Cyp2b10 in mouse hepatocytes (Kakizaki et al., 2002). Under these conditions, the all-*trans* retinoic acid receptor may compete with CAR for their common heterodimerization partner RXR (Kakizaki et al., 2002). Alternatively, retinoic acids might activate permissive nuclear receptor heterodimers that have a negative effect on drug-inducible P450s, as proposed for LXR/RXR-heterodimers (Handschin et al., 2002). The essential role of RXR in hepatic drug induction has been demonstrated in RXRα-deficient mouse models that show impaired function of PXR and CAR. In these mice, TCPOBOP-induced hepatomegaly and morphological changes including endoplasmic reticulum proliferation are no longer observed (Wan et al., 2000; Cai et al., 2002).

Cross talk between xenosensors and other transcription factors can also be observed by competition for common coactivating or corepressing proteins. For example, SHP had initially been discovered in a yeast two-hybrid screen using CAR as bait (Seol et al., 1996). Since then, interactions between SHP and a variety of other members of the nuclear receptor superfamily, including HNF4α, the liver receptor homolog-1, and RXR, among many others, have been described. In all these interactions, SHP inhibits the activity of its binding partners (Goodwin et al., 2000; Lee et al., 2000; Lu et al., 2000). Cofactors that are shared by both xenosensors and other nuclear receptors include the p160 coactivators steroid receptor coactivator-1 and GRIP1 as well as the corepressor silencing mediator for retinoid and thyroid receptors, which predominantly interact with CAR or PXR in a ligand-dependent manner (Forman et al., 1998; Moore et al., 2000b; Tzameli et al., 2000; Muangmoonchai et al., 2001; Min et al., 2002a,b; Takeshita et al., 2002). Competition for common binding partners can lead to interactions between different nuclear receptors, as shown in the case of CAR that inhibits estrogen receptor action by binding to and squelching GRIP1 (Min et al., 2002b).

VI. Clinical Relevance of Induction

A. Altered Kinetics of Drugs

Induction of P450s and other drug-metabolizing enzymes can alter intestinal and hepatic clearance of drugs and consequently the serum levels of drugs or hormones that are metabolized by these enzyme systems. Induction undoubtedly contributes to interindividual and intraindividual variation in drug response and can cause drug-drug or drug-hormone interactions. Drugs given concomitantly with other drugs or even in combination with plant extracts such as St. John's wort or grapefruit juice have the potential to cause inefficacy of drug treatment or adverse drug reactions. Therefore, knowledge of the enzymes that metabolize a certain compound combined with knowledge on its inducers and inhibitors is now a common feature of package inserts or drug information sheets to anticipate and prevent these adverse effects. For example, problems associated with the antidiabetic drug troglitazone could partially be explained by the discovery that it activated PXR in addition to its effect on PPARγ. Subsequently, a troglitazone derivative, rosiglitazone, was negatively tested for PXR activation (Jones et al., 2000). Rosiglitazone is therefore a much safer compound to use and is the antidiabetic drug of choice today. All the inducers listed in Fig. 1C have been involved in drug-drug interactions. A number of websites and books deal with these interactions; for instance, see http://www.medicine.iupui.edu/flockhart/, http://www.hiv-druginteractions.org or http://www.fda.gov/cder/consumerinfo/druginteractions.htm (Rodriguez, 2002).

B. Genetic Variants of Pregnane X Receptor and Constitutive Androstane Receptor

Large interindividual variation in drug effects are a well recognized problem in pharmacotherapy. Among the primary reasons for this variability are genetic polymorphisms in drug-metabolizing enzymes including P450s (Meyer and Zanger, 1997; Meyer, 2000; Lamba et al., 2002). One may suspect that genetic polymorphisms in drug-responsive enhancers and promoters and in xenosensors may play an equally important role in how an individual responds to drugs. So far, genetic polymorphisms in the PXR gene (Hustert et al., 2001; Zhang et al., 2001) and in the drug-responsive elements of CYP3A7 (Burk et al., 2002) have been observed. Interestingly, all four PXR polymorphisms described in one report (Zhang et al., 2001) were located in the 5′-part of the gene, either affecting the N terminus or the DNA binding domain of the protein. Thus, there seems to be a selective pressure on rigid conservation of the PXR ligand binding domain, maybe by the constraint of fitting an as yet unknown endogenous ligand (Forman, 2001). Of the six PXR missense mutations described by Hustert and coworkers, three actually result in altered basal and drug-induced activity of the protein (Hustert et al., 2001). However, large-scale analysis of patient samples has yet to confirm a correlation between these polymorphisms and interindividual variability in drug induction, and thus their clinical relevance remains unknown at this time (Lamba et al., 2002). Interestingly, two splice variants in breast tissue (Dotzlaw et al., 1999) and seven splice variants of human PXR in tissue from a single human liver also have been observed (Fukuen et al., 2002). The relative expression levels of these variants varied considerably in liver samples from different patients, which might contribute to interindividual differences in PXR target gene expression. Surprisingly, no polymorphisms of the CAR gene have been described so far. However, substantial interindividual differences in expression of human CAR but not human PXR have been reported that correlate with the interindividual differences observed for CYP2B6 levels (Chang et al., 2003). Moreover, four splice variants of human CAR have recently been described that differ in their ability to bind to DNA, activate transcription, and bind coactivators (Auerbach et al., 2003). The clinical relevance of all these variations remains unresolved.

VII. Open Questions

The discovery of the crucial role of xenobiotic-sensing nuclear receptors in the regulation of drug-metabolizing enzymes was a major breakthrough in our understanding of the regulation of these genes. Genetic ablation of the genes encoding for CAR and PXR in mouse models results in severely disturbed expression of several key components of the detoxification machinery after challenge by drugs and xenobiotics. However, despite this giant leap in understanding the principle underlying detoxification mechanisms, there remain many open questions (Corcos and Lagadic-Gossmann, 2001; Honkakoski et al., 2003).

A. Mechanisms of Constitutive Androstane Receptor Translocation and Activation

The mechanism by which CAR activates its target genes remains largely unknown. Cytoplasmic-nuclear transfer of CAR in mouse hepatocytes is stimulated by different compounds, but only TCPOBOP has been shown to bind directly to CAR, and the effect of most PB-type inducers seems to be indirect. It is not clear whether these compounds alter the phosphorylation status of certain proteins or trigger the release of CAR from factors that retain it in the cytoplasm. Recently, the nuclear receptor coactivator GRIP1 has been implicated in facilitating the cytoplasmic-nuclear transfer of CAR in a ligand-independent manner in rat (Min et al., 2002a). In contrast to other nuclear receptors that undergo a similar translocation, the AF-2 domain is not necessary in the case of CAR, whereas removal of this domain in the GR or the VDR abolishes transfer (Zelko and Negishi, 2000). The discovery of the xenochemical response signal in the CAR C terminus might allow isolating proteins that specifically interact with this peptide. Intriguingly, in a yeast two-hybrid screen using full-length human CAR as bait, a member of the proteasome complex called MIP224 (MB67-interacting protein 224) has been observed (Choi et al., 1996). It might be possible that degradation of CAR protein in the cytoplasm plays an important role in regulating its activity, since coexpression of MIP224 reduces constitutive activity of CAR (Choi et al., 1996). In any case, it will be interesting to study why mammals have developed two receptor systems for detoxification. It is not obvious why we should have one receptor, PXR, that is located in the nucleus and activate gene transcription after binding of ligands, whereas the other receptor, CAR, is constitutively active and relies on complex regulation involving shuttling from the cytoplasm to the nucleus, a myriad of phosphorylation events, as well as direct binding of agonists and reverse agonists.

B. Cofactors Involved in Pregnane X Receptor- and Constitutive Androstane Receptor-Mediated Signal Transduction

In the last few years, it has been increasingly realized the nuclear receptors are involved in numerous physiological functions (Mangelsdorf et al., 1995). Recent findings regarding the function of numerous coactivators and corepressors have added an additional dimension of complexity to gene regulation by nuclear receptors (Rosenfeld and Glass, 2001; Hermanson et al., 2002). For example, by binding to different nuclear receptors, the PPARγ-coactivator 1α (PGC-1α) controls different processes such as adaptive thermogenesis in brown ad-

ipose tissue, gluconeogenesis in the liver, or muscle fiber type determination (Lowell and Spiegelman, 2000; Vidal-Puig and O'Rahilly, 2001; Turner, 2002; Puigserver and Spiegelman, 2003). In the case of the xenobiotic-sensing nuclear receptors PXR and CAR, knowledge about cofactor binding is still rudimentary. Although binding of steroid receptor coactivator-1, GRIP1, or silencing mediator for retinoid and thyroid receptors to these nuclear receptors has been shown, the in vivo role of these interactions is not clear yet. Interaction of CAR and PXR with the repressor SHP has been demonstrated (Seol et al., 1996; Ourlin et al., 2003). Moreover, repression of PXR activity (Ourlin et al., 2003) as well as increased PXR-transcript levels in the SHP-null mice have been reported (Kerr et al., 2002). These interactions suggest an important role of the xenosensors and SHP in the controlling and maintaining of cholesterol and bile acid homeostasis in the liver. However, as in the case of PGC-1 or the corepressor Sharp (SMRT/HDAC1-associated repressor protein) that are induced at the transcriptional level under certain conditions (Shi et al., 2001; Yoon et al., 2001; Lin et al., 2002), additional coactivators or corepressors that explain the pleiotropic response to drugs and xenobiotics mediated by the xenosensors may well exist. It is likely that this complex response will be governed by the interactions of multiple nuclear receptors and cofactors, and many of these proteins are not known yet. Intriguingly, recent findings described an interaction between PGC-1α and the xenosensors CAR and PXR (Shiraki et al., 2003). Apparently, binding of PGC-1α increases localization of CAR to nuclear speckles. However, the physiological relevance of this localization and the link between xenobiotic-induced drug metabolism and the energy sensor PGC-1α remain to be elucidated.

C. The Xenosensors as Drug Targets

The key role of CAR and PXR in drug induction and the ability to modulate their activity by pharmacological compounds establish them as prime targets for modulation and control of drug metabolism. One could imagine that specific inhibition of one or both receptors might be used to decrease the levels of metabolism of a specific drug and thus increase the serum levels and efficacy of this compound. This concept has been established by treatment of acetaminophen-overdosed mice with androstanols, inverse agonists of mouse CAR. Inhibition of CAR prevented accumulation of acetaminophen metabolites in the liver and thus could prevent hepatotoxicity to a large extent (Zhang et al., 2002). On the other hand, it might be useful to specifically activate xenosensors to increase metabolism and excretion of unwanted compounds. In mice, catatoxic steroids that activate PXR are able to reduce bile acid-associated hepatotoxicity by stimulating hydroxylation, conjugation, and excretion of excess bile acids (Staudinger et al., 2001a; Xie et al., 2001). Thus, activators of PXR might constitute a valuable therapeutic modality in patients with increased levels of hepatic bile acids as found in cholestasis (Willson et al., 2001). In fact, this is already done with ursodeoxycholic acid treatment of cholestasis. Similarly, potent activators of CAR and PXR might be therapeutically usefull in the treatment of neonatal, acquired and genetic forms of jaundice by promoting a decrease in bilirubin levels (Huang et al., 2003; Roy-Chowdhury et al., 2003; Xie et al., 2003). Moreover, ligand-dependent recruitment of coactivator or corepressor proteins might allow designing selective PXR or CAR modulators that have either an activating or repressive effect on these receptors in specific tissues under certain conditions (Gillam, 2002). Although the antineoplastic compound ecteinascidin-743 has been shown to inhibit human PXR (Synold et al., 2001), specific, high-affinity activators and inhibitors for human PXR and CAR have yet to be discovered and tested. Recent work by Maglich and coworkers (2003) describes the characterization of a novel human CAR agonist that is highly selective, very potent, induces human CAR cytoplasmic-nuclear translocation, and increases human CAR target genes. Of course, the potential of this compound, 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime (CITCO), for therapy remains to be established.

D. The Mystery of How Cells Recognize Phenobarbital-Type Inducers

PB has been found to change the expression of more than 100 genes in chicken, mouse, and rat either by inducing or repressing them (Frueh et al., 1997; Garcia-Allan et al., 2000; Bulera et al., 2001; Gerhold et al., 2001). Among these are phase I and phase II enzymes, drug transporters, enzymes of the heme biosynthesis pathway, and many others. In fact, PB elicits pleiotropic effects in the livers (Fig. 1B) that are characterized by proliferation of smooth endoplasmic reticulum, stimulation of liver weight gain, liver tumor promotion in rodents, and a general stabilization of liver microsomal enzymes (Okey, 1990; Waxman and Azaroff, 1992). First, it is not clear how many of these effects of PB are mediated by the xenosensors PXR and CAR. It has been shown that CAR knockout animals also lack the proliferation of the smooth endoplasmic reticulum after PB and TCPOBOP treatment (Wei et al., 2000). However, several genes have been found that are inducible by PB even in the absence of CAR or PXR such as 5-aminolevulinic acid synthase or enzymes involved in cholesterol biosynthesis (Maglich et al., 2002; Ueda et al., 2002a). PXR and CAR might compensate for the loss of one another in PB induction of these specific genes but not in the induction of others, like Cyp3a11 or Cyp2b10. This hypothesis could be tested in a PXR/CAR-double knockout mouse model (Xie and Evans, 2002; Sonoda et al., 2003). However, there are hints pointing in the direction that PB might change gene expression through addi-

tional mechanisms then activating PXR and CAR (Kakizaki et al., 2003; Yamamoto et al., 2003). Although PB binding to neither CAR nor PXR could be conclusively shown so far, PB somehow influences cytoplasmic-nuclear translocation of CAR at least in mice. Moreover, PB induction of P450s and other genes is heavily influenced by protein phosphorylation and dephosphorylation events. For example, phosphorylation of a 34 kDa, so far unidentified nuclear protein has been found to be increased after PB induction in mouse liver and primary hepatocytes (Baffet and Corcos, 1995). Moreover, inhibition or activation of several protein kinases and phosphatases has profound impact on drug-inducible P450 levels in chicken, mouse, and rat (Salonpaa et al., 1994; Sidhu and Omiecinski, 1995; Dogra and May, 1996; Sidhu and Omiecinski, 1996; Sidhu and Omiecinski, 1997; Honkakoski and Negishi, 1998a; Galisteo et al., 1999; Ganem et al., 1999; Kawamura et al., 1999; Handschin and Meyer, 2000; Marc et al., 2000; Handschin et al., 2001b). Inhibition of protein synthesis has an important influence on P450 induction in chicken and rodents, suggesting that apart from nuclear receptors, other proteins might play an important role in mediating the response to drugs and, at least in the case of chicken, imply the presence of a "labile repressor" protein (Burger et al., 1990; Dogra et al., 1993; Sidhu and Omiecinski, 1998). Thus, the identity of the "true" PB target or targets in the cell remains unknown, and PB-triggered effects might go far beyond the xenobiotic-sensing nuclear receptors CAR, PXR, and CXR. In any case, finding answers to the question of how our body has adapted itself to deal with foreign compounds that it has never encountered before remains a fascinating challenge for the future.

VIII. Outlook

Our understanding of the mechanisms underlying hepatic drug induction has improved enormously in recent years. Future goals in this field might include a further unraveling of the complex network of receptors, transcription factors, and other proteins that regulate the carefully balanced system under normal conditions, during disease, in obesity, or aging and challenged by xenobiotics, diet, and endogenous compounds. Moreover, most of the research focus has centered on the liver and the intestine, whereas other tissues like kidney, lung, or brain have not been studied much. Similarly, we do not have a clear idea yet of what the endogenous role and the endogenous ligands (if any) of the xenobiotic-sensing nuclear receptors might be. Thus, the coming years will hopefully yield a wealth of interesting new findings that will help to understand both the molecular details of transcriptional regulation of genes in general and the regulation of the biotransformation of lipophilic compounds as an essential defense mechanism.

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