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Activation of CAR and PXR by Dietary, Environmental and Occupational Chemicals Alters Drug Metabolism, Intermediary Metabolism, and Cell Proliferation

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Abstract

The constitutive androstane receptor (CAR) and the pregnane × receptor (PXR) are activated by a variety of endogenous and exogenous ligands, such as steroid hormones, bile acids, pharmaceuticals, and environmental, dietary, and occupational chemicals. In turn, they induce phase I–III detoxification enzymes and transporters that help eliminate these chemicals. Because many of the chemicals that activate CAR and PXR are environmentally-relevant (dietary and anthropogenic), studies need to address whether these chemicals or mixtures of these chemicals may increase the susceptibility to adverse drug interactions. In addition, CAR and PXR are involved in hepatic proliferation, intermediary metabolism, and protection from cholestasis. Therefore, activation of CAR and PXR may have a wide variety of implications for personalized medicine through physiological effects on metabolism and cell proliferation; some beneficial and others adverse. Identifying the chemicals that activate these promiscuous nuclear receptors and understanding how these chemicals may act in concert will help us predict adverse drug reactions (ADRs), predict cholestasis and steatosis, and regulate intermediary metabolism. This review summarizes the available data on CAR and PXR, including the environmental chemicals that activate these receptors, the genes they control, and the physiological processes that are perturbed or depend on CAR and PXR action. This knowledge contributes to a foundation that will be necessary to discern interindividual differences in the downstream biological pathways regulated by these key nuclear receptors.

Keywords

Constitutive androstane receptor; pregnane × receptor; orphan nuclear receptors; drug targets; gene-environment interaction

1. Introduction

The constitutive androstane receptor (CAR, NR1I3), and the pregnane × receptor (PXR, NR1I2; also known as SXR) are members of the orphan nuclear receptor subfamily. Orphan nuclear receptors share many of the structural features found in the nuclear receptor family, but lack known physiological ligands. Based on their structural features, CAR and PXR have

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been placed in nuclear receptor group 1, subgroup I; which also contains the Vitamin D Receptor (VDR). CAR and PXR are both relatively promiscuous nuclear receptors that are activated by numerous xenobiotics, drugs, bile acids, and hormones [1,2]. Therefore, they may be considered adopted orphans that recognize a number of endobiotic and xenobiotic chemicals [3]. In turn, they act as master regulators of the phase I, phase II, and phase III enzymes and transporters critical for detoxification and elimination of steroids, bile acids, and xenobiotics [1,4–6] following heterodimerization with RXRα (NR2B1) [7,8].

Detoxification genes induced by CAR and PXR include several phase I cytochrome P450 enzymes (CYPs) [4,9–11], phase II enzymes such as Uridine diphospho-glucuronosyltransferases (UDPGT), glutathione S-transferases (GSTs) and sulfotransferases (SULTs) [12–15], and the phase III transporters such as multidrug resistance-associated protein 2 (MRP2) and multidrug resistance protein (MDR1) [16–19]. However, recent research demonstrates that CAR and PXR have broad functions that include control of intermediary metabolism [20], and liver regeneration and proliferation [21,22].

The purpose of this review is to investigate the potential for environmental, occupational, or dietary chemicals to alter CAR and PXR activity, induce or repress the transcription of target genes, and perturb metabolic processes such as drug metabolism, intermediary metabolism, and cell proliferation. Recent studies using microarrays or transgenic mice suggest that CAR and PXR are more than just xenosensors, but may also act as mitogens, stress regulators, or even important hormonal regulators that may affect ion balance [23], or fatty acid oxidation [24]. We provide summary tables that show CAR and PXR are activated by a large number of diverse environmentally relevant chemicals, discuss the changes in gene expression induced by CAR and PXR activation, and examine their downstream physiological effects on drug metabolism and other metabolic processes. We suggest that a deeper understanding of these orphan nuclear receptors may help future personalized medicine efforts through an increased recognition of a host of environmental factors that can modify individual differences in response to health interventions such as drug therapy and nutrition.

2. Activation and Promiscuity of PXR and CAR

CAR received its name from the androstanes, which act as inverse agonists because they inhibit the high amount of constitutive activity CAR demonstrates in vitro [25]. Inverse agonists are often used in transactivation assays to reduce its high constitutive activity and increase sensitivity. Thus, both activation and inactivation of CAR activity can occur. Many different androgens can inhibit CAR activity, including those used to enhance athletic performance [26]. However, the efficacy of androgenic inverse agonists is quite low or elusive for human CAR (hCAR), as they appear much more potent on mouse CAR (mCAR) [25,27]. Therefore, the translation of this research to humans has yet to be resolved.

In contrast to other nuclear receptors that contain five domains, CAR contains only three: a highly conserved DNA-binding domain; a hinge region; and a divergent ligand binding/dimerization/transcriptional activation domain [28]. This in part may explain some of the unique features of CAR including its constitutive activity [29]. CAR's high constitutive activity is a function of the AF2 domain, as the AF2 helix is rigid and one of the shortest among known nuclear receptors; allowing for the formation of additional hydrogen bonds and permitting AF2 to remain in its active conformation [29]. This shorter linker region leads to a unique arrangement between the linker helix and helix H10 of CAR. The importance of the interaction between these two regions is critical for the constitutive activation of CAR as illustrated by studies in which insertion of three amino acids to this shorter linker region perturbed CAR's constitutive activity [30].

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CAR's ligand-binding pocket is smaller and less flexible than PXR's [29,31], and for this reason CAR is probably less promiscuous than PXR [2,29]. The ligand binding pocket of mCAR and hCAR complexed to RXRα and either androstenol (mCAR) or CITCO (hCAR) is 570 and 675 $Å^3$, respectively. However, ligand binding is not a prerequisite for activation of CAR as demonstrated by phenobarbital (PB) [32]. Phosphorylation-induced translocation of CAR to the nucleus is a key step for indirect activation, as okadaic acid inhibits nuclear translocation and CAR's transcriptional activity [32,33]. More recent research indicates PB as well as other chemicals, activate CAR through the formation of reactive oxygen species via a mechanism involving the mitochondria. In turn, the upstream kinase LKB1 activates AMP Kinase (AMPK) and its subsequent phosphorylation cascade [34]. Given the size of the ligand binding domain of CAR, it has been hypothesized that the majority of CAR activators work through an indirect pathway similar to PB instead of direct binding such as performed by 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) [35].

Inside the cell, CAR is typically retained in the cytoplasm forming a complex with heat shock proteins (Hsp90), immunophilins, P-23, and cytoplasmic CAR retention protein (CCRP), [36,37]. CAR may also be found within a protein complex in the cell membrane [38]. In response to stress (activation), protein phophatase 2A (PP2A) is recruited leading to dephosphorylation of Ser-202 near the C terminus of the ligand binding domain [37,39], allowing for translocation to the nucleus, binding to RXR and its response element, and subsequent transcription (Fig. 1). In contrast, epidermal growth factor or hepatocyte growth factor activation of extracellular signal related kinase (ERK1/2) causes phosphorylation of CAR and retains it within the cytosol [40]. How these stimuli work with AMPK, which does not directly phosphorylate CAR, is not known [34].

Recent data indicates that diverse but potentially interacting stressors involving phosphorylation cascades can activate CAR. Ding et al.'s [41] results indicate that fasting/caloric restriction increases cAMP and activates protein kinase A (PKA), which in turn induces peroxisome proliferator-activated receptor coactivator-1α (PGC-1α) through the cAMP-response element binding protein (CREB). This causes PGC-1α to interact with Hepatocyte Nuclear Factor-4α (HNF-4α) and induce CAR. CAR then interacts with PGC-1α and its heterodimeric partner RXR to induce CAR target genes. There is still much to learn about how indirect activators mediate CAR activity, but taken together these data suggest roles for the mitochondria, oxidative stress, fasting, diabetes, seasonally adjusted fat deposition, and physiological/pharmacological stressors in CAR activity. Thus, multiple stressors, diet, and disease conditions may effect CAR and in turn alter drug efficacy, metabolism, and elimination in addition to chemical activation,

PXR also received its name from steroidal ligands, as the most efficacious activators of PXR are the pregnanes that are the immediate precursors of progesterone and formed by the side chain cleavage of cholesterol [9]. Because the concentrations of the individual steroids required to activate CAR and PXR are greater than those detected in biological samples, it remains uncertain whether these nuclear receptors have a high-affinity ligand or instead function as a more generalized steroid, xenobiotic or stress sensors [8,9,42,43].

In contrast to CAR that is primarily found in the cytosol [44], PXR is primarily found in the nucleus and inactive PXR may form repressor complexes with SMRT or SHP and histone deacetylases, and inhibit transcription [45,46]. Ligand activation releases the co-repressors and initiates the recruitment of co-activators such as steroid receptor co-activator 1 (SRC-1) and histone acetylases that promote transcription [45–47] (Fig. 2).

PXR is activated by a wide variety of xenobiotics because it has evolved several features, including a small number of polar residues spaced through a smooth, hydrophobic ligand-

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binding domain [31]. Computer generated models show PXR's LBD has a hydrogen acceptor region, as well as four hydrophobic regions [31,48]. Determination of the crystal structure of the PXR ligand binding domain (LBD) found its size to be about 1200 $Å^3$ [31], which is large for a LBD. The LBD of PXR can form homodimers at the β1′ strand of each monomer generating a ten-stranded intermolecular β-sheet [47]. Mutation of key residues at the homodimer interface disrupts PXR dimer formation in addition to reducing transcriptional activity and coactivator recruitment demonstrating that homodimerization plays a role in coactivator binding through the indirect stabilization of αAF and the AF-2 surface [49]. In addition, the presence of a flexible loop in helix 6 and unwinding of helix 7 is believed to be responsible for PXR's ability to bind ligands of different sizes since this loop may contract or expand in order to accommodate different chemicals. This, along with other attributes of the PXR LBD, including its ability to use only a portion of its pocket to bind and in turn be activated by ligands, allow for the PXR to serve as a more promiscuous receptor relative to other nuclear receptors [50].

Even though it is promiscuous, the LBD of PXR has evolved significantly different across vertebrate species, with similarities between human, mouse and rat, compared to the zebrafish, chicken, and frogs [51]. Therefore, PXR and CAR exhibit significant species differences in the activation profiles and therefore extrapolating even from mouse to human can be problematic. For example, mouse PXR is activated following exposure to pregnenolone 16α-carbonitrile (PCN, a prototypical CYP3A inducer), but not the human PXR activators rifampicin or hyperforin [52,53]. Recent work also indicates that PXR, similar to CAR, is a phosphoprotein; however there are species differences in the activity of phosphorylated PXR. cAMP and PKA activate mouse PXR, but repress human and rat PXR transcriptional induction of their corresponding CYP3A genes. The reduced transcriptional activity of phospho-PXR may be due to its increased affinity for the co-repressor, NCoR [54].

Similarly, mouse CAR is activated by the classical Cyp2b inducer TCPOBOP, but not the human CAR activator CITCO and vice versa [55]. The activation profile differences are less understood in CAR because hCAR is difficult to work with and transactivation assays are unreliable and difficult to perform; however, the use of humanized mice, primary human cells, and new techniques will help determine activation of CAR in humans [56–60]. Ultimately, both CAR and PXR have undergone evolutionary changes that make them suitable as promiscuous xenosensing nuclear receptors [61,62], but we must be aware that the evolutionary differences also indicate that data with model species may not project to human pharmacogenomics.

3. PXR/CAR-Mediated Changes in Gene Expression

PXR binds the IR-6, ER-6 and DR-3 xenobiotic response elements (XRE) localized in the promoter regions of CYP3A and other genes [8,9,63,64]. CAR binds the IR-6 and DR-4 phenobarbital response element module (PBREM) of the CYP2B promoter [63,65]. In turn, CAR and PXR recruit coactivators and histone acetylases, activate transcription, and induce a wide variety of genes, including those involved in detoxification, intermediary metabolism, and cancer (Fig. 3).

Early research suggested that PXR primarily induced the transcription of CYP3A family members, and CAR primarily induced the transcription of CYP2B family members. More recent research indicates that these receptors are involved in "crosstalk". They regulate the expression of analogous genes by stimulating similar response elements and show overlapping affinities for some ligands [27,53,66–69]. For example, human PXR has been known to induce CYP2B genes due to its affinity for binding the PBREM of CYP2B that contains two imperfect DR-4 type nuclear receptor binding sites [53], including an upstream enhancer [70]. PXR

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shows broader effects on CYP2B and CYP3A than CAR, which is a good CYP3A inducer in rodents but a much weaker CYP3A inducer in humans [71]. Thus, PXR and CAR regulate overlapping, but distinct sets of genes involved in multiple physiological processes including but not limited to protecting the cell from toxicants (Fig. 3a) [10,12].

4. Environmental, Occupational, and Dietary Activators of CAR and PXR

PXR and CAR may be activated or repressed by a large number of endogenous hormones, their synthetic derivatives, endocrine disrupters (EDCs), bile acids, bilirubin, herbal remedies, and pharmaceutical products. Table 1 contains a list of environmental, occupational, and dietary chemicals known to alter the activity of CAR and PXR. Most of these chemicals are activators. We have not included pharmaceuticals as a couple of recent reviews listed many of the pharmaceuticals known to activate these nuclear receptors [72,73]. Further, the significant number of EDCs that activate PXR suggests that it has a role in protecting cells from xenobiotic chemicals that may perturb the endocrine system. Activation of PXR by pregnanes and other metabolic products of steroids, further implicates it in the protection of the endocrine system [2].

The list of chemicals known to activate CAR is shorter than the list of chemicals that activate PXR, probably due to PXR's larger and flexible binding pocket [31]. However, there has been less research performed on the activation of CAR, primarily because of the necessity to use inverse agonists to perform transactivation assays and the lack of a good inverse agonist for studying human CAR. Many of CAR's activators are partial agonists [26] similar to PB and CAR's receptivity to indirect activation may allow it to show high promiscuity in a fashion similar to but not to the level of PXR. Thus the number of CAR activators will continue to increase (as will PXRs) as further studies are performed and new assays are developed [60]. This is further demonstrated by a recent manuscript that describes twenty-seven novel chemicals that bind and activate human CAR and provides us with a new 3D-quantitative structure activity relationship (QSAR) model that demonstrates the importance of Phe161 in S1 of the ligand binding pocket in stabilizing helix 12 [74]. However, while CAR may have a smaller role as a xenosensor it may have a greater role in recognition of endogenous chemicals and stress as indicated by CAR's involvement in hyperbilirubinemia [75], caloric restriction [76], obesity [24], fatty acid metabolism [77], and bile acid homeostasis [78], and cell proliferation [22,79]

Table 1 includes EDCs, pesticides, plasticizers, but also several traditional Chinese herbal remedies, cafestrol from coffee, linoleic acid, and Docosahexaenoic acid, a polyunsaturated fatty acid that suppresses CAR activity [80,81]. This indicates that diet as well as xenobiotic chemicals in our diet may alter our normal toxicant responses. For example, Docosahexaenoic acid in our diet suppresses our ability to respond to toxicants.

Most of the chemicals listed in Table 1 were studied in vitro, so their ability to activate CAR and PXR in vivo is not known. Short half-life due to rapid metabolism, clearance, or exposure route may reduce the likelihood that these chemicals reach the concentrations necessary to activate CAR and PXR in vivo. For example, chlorpyrifos is the most efficacious activator of CAR that we have tested [26]; however, it does not induce Cyp2b in B6/129 CAR+/+ mice suggesting that chlorpyrifos is rapidly metabolized resulting in low or no CAR activation in vivo (unpublished data). In contrast, uncharacterized or unidentified metabolites of other chemicals may activate CAR or PXR.

In addition, many chemicals alone may not reach the requisite concentrations to activate CAR or PXR, but in combination these compounds may easily reach the necessary concentrations. For example, a mixture of nonylphenol, cypermethrin, bisphenol A, phthalic acid, and chlorpyrifos at 170nM each significantly activated CAR, while none of these chemicals alone

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at these concentrations would have activated CAR [26]. Both partial and full activators were used in the chemical mixtures. Interestingly, if a full activator was present the reduced activity of partial activators need not be considered [26]. This is because partial activators acted as full activators in the presence of a full activator. Therefore, the activity of partial activators may be underestimated in vivo because humans are most likely exposed to a mixture of both full and partial activators.

To our knowledge, nobody has performed chemical mixture studies with PXR. Furthermore, because some chemicals activate both CAR and PXR and we are exposed to xenobiotic mixtures that have both CAR and PXR activators; there is a need to study the additive or interactive effects of CAR and PXR activators. Because CAR and PXR work on similar response elements, they may act in an additive fashion; however, they may also suppress each other's actions by competing for coactivators. Since we are not exposed to only one chemical at a time, and instead a milieu of chemicals, the activity of CAR and PXR in vivo is probably controlled by many factors including the mixtures we have been exposed to, the pharmaceuticals we are taking (both natural and developed), and our diet. Hence, CAR and PXR activity is probably variable at any given time within each person in a population, and this makes many if not all individuals' susceptible to idiosyncratic drug reactions.

5. Adverse Drug Reactions

While the activation of CAR and PXR are generally considered protective, several adverse effects occur because of downstream induction of detoxification enzymes by xenobiotics. Adverse drug reactions (ADRs) are one of these potential consequences. It is estimated that between 635,000 and 770,000 patients have a serious ADR each year and approximately 106,000 people die from ADRs in the United States [82]. This would make ADRs between the 4^{th} and 6^{th} leading cause of death in the United States [82]. Currently, ADRs (similar to auto fatalities and other subheadings) are not listed as a cause of death by the Centers for Disease Control and Prevention because they are considered subtotals under other diseases or injuries [83]. However, the estimates from epidemiological studies are alarming and indicate that further study is necessary [84].

Recent research suggests that pharmacogenetic data on P450 polymorphisms will ultimately explain approximately 10–20% of ADRs. However, nearly 50% of ADRs can be explained by other physiological or environmental factors [85]. This includes the induction of P450s because approximately 50–60% of drugs, nutraceuticals, and herbal remedies are metabolized by CYP3A4 and 25–30% are metabolized by CYP2B6 [53,86,87]. The activation of CAR and PXR are the primary mechanisms for the induction of many of the drug metabolizing P450s [88]. Thus, identifying the chemicals (dietary supplements, environmental chemicals, occupational chemicals, pharmaceutical agents) that activate these promiscuous nuclear receptors may help us predict ADRs due to P450 induction and save lives.

For example, PXR activation by St. John's wort and subsequent induction of CYP3A subfamily members [89,90] has been shown to cause numerous ADRs because of altered metabolism and clearance [91–94], including warfarin, immunosuppresants, and birth control pills. PXR is associated with drug resistance during chemotherapy [95,96]. PB treatment in pregnant mothers is associated with undescended testes and genital malformations in male rats and male humans, potentially due to increased testosterone metabolism [97–100]. CAR activation and the subsequent induction of CYP3A4 have been associated with increased vitamin D metabolism. This provides a potential explanation for the association between anti-epileptic drugs such as PB and phenytoin and a decline in bone mineral density [101]. Other research indicates that PB causes reduced calcium and osteomalacia by repressing CYP2D25 and CYP27A1, the enzymes primarily responsible for the 25-hydroxylation of vitamin D to vitamin

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D3 [102]. PXR activation by chemicals such as carbamazepine and rifampicin are also associated with a decline in bone mineral density due to induction of CYP3A4 and metabolism of vitamin D3 to inactive metabolites [101]. Furthermore, PXR activation can induce CYP24 and lead to vitamin D deficiency in the absence of vitamin D3 [103,104]; however, in the presence of vitamin D3 PXR represses CYP24 levels by perturbing VDR corepressor interactions [103]. Taken together, long-term activation of CAR and PXR can have harmful effects of vitamin D homeostasis and bone mineral density.

The production of the toxic metabolite of acetaminophen, 4-hydroxyacetanilide, N-acetyl-paminophenol (APAP), is significantly enhanced by CAR activation, and mice lacking CAR are not susceptible to acetaminophen toxicity [55]. Thyroid hormone concentrations are reduced following treatment with CAR activators in wild type, but not CAR-null mice [105]. Furthermore, hydroxylation of methoxychlor, polychlorinated biphenyls, and possibly polybrominated diphenyl ethers by P450s is necessary to produce their endocrine disrupting metabolites [106–109], and accordingly induction of P450s by CAR or PXR activation may enhance toxicity of these environmental pollutants.

Therefore, exposure to environmental contaminants and dietary constituents (Table 1) may induce phase I–III detoxification enzymes and lead to altered toxicity and clearance of endogenous wastes, pharmaceuticals, pesticides, and other xenobiotics due to PXR and CAR activation. This may lead to idiosyncratic drug reactions or reduced efficacy.

6. CAR and PXR variants

Various forms of CAR and PXR can be coded. Some of these are transcriptional splicing variants and others are single nucleotide polymorphisms (SNPs) that may or may not show perturbed activity compared to their more common counterpart. These splicing variants and polymorphisms may inhibit typical receptor activity by competing with active nuclear receptors or may show alterations in activity or ligand binding profiles that increase the sensitivity of some individuals to pharmacotherapies. This may be an area that needs further patient control studies over the next few years to ensure that sensitive individuals do not receive particular drugs [110]. Furthermore, racial differences in PXR variants have been observed.

Studies have found 15 single nucleotide polymorphisms (SNP) in PXR exons, from which 12 caused amino acid coding changes. In the DNA binding domain (DBD) four SNPs were found, with 3 SNPs in exon 2 and 1 SNP in exon 3. In the ligand binding domain (LBD) 10 SNPs were found, with the majority in exon 4 [111,112]. Caucasians and African-Americans exhibited specific race variants in exon 4. The Caucasian specific allele (V140M) showed reduced rifampin and corticosterone activation, and enhanced basal levels of transactivation in reporter plasmids that contained PXR binding elements in LS174T cells. The African-American specific (D163G) allele showed complete loss of basal transactivation for CYP3A4 and DR3 reporter constructs [112]. Thus, race could play a role in CYP3A4 expression and induction, and therefore in metabolism, elimination, and sensitivity to specific chemicals. Other studies have demonstrated a reduction of human PXR function in the variants: R98C, R122Q, Q158K, R381W and I403V with R98C showing the most dramatic effects [111,113,114]. The Q158K polymorphism affects about 2% of the Chinese population and decreases activity to some ligands [114].

Furthermore, three splicing variants are found in PXR: PXR 1, PXR 2, and PXR 3, where PXR 2 and PXR 3 lack five amino acids that are part of the ligand binding pocket of PXR [115]. PXR 2 also shows a different transcriptional start site and proximal promoters [116]. PXR 1 accounts for the majority of total PXR transcripts with PXR 2 (2–7%) and PXR 3 (<1%) representing only a small portion of liver transcripts [115,116]. Transactivation does appear to significantly differ between the three variants [116].

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CAR SNPs and splicing variants have also been discovered. Studies indicate that a F234A mutation in CAR1 causes a complete loss of CAR's constitutive activity [117]. Twenty-six CAR SNPs were described in a Japanese population, in which 15 were identified in the 5′-flanking region, 3 were in the exons and 10 were in the introns. The study found no significant difference in a wide array of diseases when comparing the prevalence of the SNPs and a control population [118]. However, Hispanics appear to have enhanced CAR activity relative to Caucasians based on their transcriptome, including the CYP2 family members [119].

There are at least 22 different splicing variants of CAR and some of these variants show compromised binding to DNA and reduced transactivation [120–122]. A specific CAR splicing variant termed CAR2, which may represent about 30% of the total transcripts in a human liver, has an additional four amino acids in its LBD that alters its ligand binding characteristics [123,124]. This variant is extremely sensitive to the plasticizer di(2-ethylhexyl)phthalate (DEHP) and it activated CAR2 at nanomolar concentrations and greatly induce transcription levels of CYP2B6 and CYP3A4. Because CAR2 is not found in rodent models and DEHP is ubiquitous in plastics (260 million pounds per year and used in intravenous tubing); these results have significant implications for DEHP liver toxicity and for the role of DEHP or IV infusate as a potential confounder in adverse drug reactions to acetaminophen and other pharmaceuticals [124].

7. CAR, PXR, and Cholestasis

Cholestasis occurs when bile flow in the liver is blocked and may occur intra or extrahepatically. Intrahepatic cholestasis occurs as a result of sepsis, viral hepatitis, alcoholism, medications, pregnancy, and genetic disorders such as Dubin-Johnson and Crigler-Najjar. Several causes of intrahepatic cholestasis involve inflammation. Extrahepatic cholestasis primarily occurs due to bile duct blockage by gallstones, cysts, tumors, surgical, or other physical trauma. Cholestasis usually results in increased serum levels of bile acids, bilirubin, cholesterol, and other fatty acids [125]. Several nuclear receptors are involved in bile acid homeostasis and metabolism, including farnesoid × receptor (FXR, also known as the bile acid receptor BAR), liver × receptor (LXR), retinoic acid receptor (RAR), retinoid × receptor (RXR), liver receptor homolog 1 (LRH-1), small heterodimer partner (SHP), and hepatocyte nuclear factors 1 and 4 (HNF-1 and 4) [126]. FXR appears to play the greatest role in bile acid homeostasis, however, the genetic disorders Dubin-Johnson and Crigler-Najjar, arise due to defects in two detoxification genes: multidrug resistance-associated protein 2 (Mrp2) and UDP-glucuronosyltransferase 1a1 (Ugt1a1) respectively [127,128]. Dubin-Johnson neonates are treated for mild jaundice; however, Crigler-Najjar requires a liver transplant to prevent death [129]. These two genes are not primarily regulated by FXR, but by two other nuclear receptors, the pregnane × receptor (PXR) and constitutive androstane receptor (CAR) [14]. Unless cholestasis is ameliorated, liver damage and toxicity occurs.

Bile acid metabolism and excretion is tightly controlled and appears to have redundant signaling to ensure bile acids do not build up in the liver. CAR and PXR have been shown to regulate several detoxification enzymes and transporters important to bile acid metabolism including cyp2b10 and cyp3a11, sulfotransferase 2a1 (Sult2a1), dehydroepiandrosterone sulfotransferase (STD), Mrp 2,3,4, and organic anion transporting protein 2 (Oatp2) (Fig. 3a, 4) [4,9,14,15]. Oatp2 is involved in bile acid import from sinusoidal blood flow into hepatocytes. Bile acids are then hydroxylated by P450s, followed by sulfation mediated by Sult2a1 or STD. Sulfated bile acids are then primarily transported into the bile canals by Mrp2 for fecal excretion, but may also be transported by Mrp3 and 4 basolaterally for renal excretion. Mice whose bile duct was ligated, mimicking cholestasis caused by bile duct blockage, showed decreased bile acid levels in serum and increased polyhydroxylated bile acids in serum and urine when treated with the PXR and CAR agonists, PCN and PB [130]. In addition, PXR

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activation caused an increase in multidrug resistance-protein 2 (Mdr2) and sodium dependent bile acid transporter (Asbt), which are important in hepatocholangiocyte shunting of bile acids [130].

Concentrations of the secondary bile acid lithocholic acid (LCA) from 125 mg/kg to 250 mg/kg have been shown to activate PXR in vivo [131–135]. In vitro, 100 μM of LCA, but not chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), or cholic acid (CA), was shown to activate PXR [131]. In addition, LCA activates CAR [43]. Diets high in the primary bile acid CA provided to PXR-null mice lead to hepatorenal failure even in mice treated with the potent CAR agonist, TCPOBOP, further demonstrating the critical importance of PXR in bile acid detoxification. Cholesterol metabolites, oxysterols and bile alcohols are also PXR ligands, and these endobiotics may cause toxicity in the absence of PXR, even if CAR is activated and ameliorating bile acid toxicity [132]. Though PXR and CAR have been shown to increase the metabolism of bile acids, CAR/PXR-double knockout animals showed a decrease in liver damage and serum bile levels. This is counter-intuitive; however, it may be attributed to CAR/PXR mediated transrepression of Cyp7a1, Cyp7b1, and Cyp8b1 that are important enzymes in bile acid catabolism [133].

Even though Oatp2 and P450s are involved in the influx and initial metabolism of bile acids, sulfation and subsequent efflux by transporters appear to constitute the primary means of bile acid homeostasis mediated by CAR and PXR. PXR induces STD when activated by LCA, and more importantly CAR induction of Sult2a1 and the subsequent sulfation of LCA, appears to be an important regulator that prevents the buildup of this highly toxic bile acid in the intestine and liver [78,136]. Interestingly Mrp4 null mice showed a decrease in Sult2a1 suggesting some interplay between these two detoxification genes, perhaps mediated by CAR, which has been shown to transcriptionally regulate both genes [16].

Dubin-Johnson syndrome results in retention of bile acids in hepatocytes due to a mutation in the Mrp2 canilicular transporter [127]. Obstructive cholestasis similarly results in bile acid buildup in the liver, due to a blockage of bile ducts, preventing bile acid excretion by the gall bladder for fecal excretion. In turn, increased bile acid concentrations activate FXR which transcriptionally regulates the corepressor SHP. SHP acts as a negative feedback mechanism on FXR, preventing further bile acid catabolism [137]. In response to obstructive cholestasis, SHP represses HNF4 transactivation of HNF1 resulting in the downregulation of several influx transporters including Oatp1 and Ntcp, thus reducing bile acid uptake into the liver [138, 139]. Additionally SHP acts as a corepressor to several other nuclear receptors including RXR, CAR, and PXR; however, Mrp3 and Mrp4 are still induced during obstructive cholestasis in order to transport excess bile acids basolaterally for renal excretion [140,141]. Excess bile acids in cholestasis also leads to inflammation and interestingly it has been shown that proinflammatory cytokines IL-1β and TNFα lead to a SHP-independent reduction of several nuclear receptors, and a subsequent downregulation of influx and efflux transporters [142]. The mechanism for continued Mrp3 and Mrp4 induction overcoming these repression mechanisms is unknown but may involve the CAR agonist bilirubin as it is common to get hyperbilirubinemia in conjunction with bile acid buildup (Fig. 4) [75].

Bilirubin has been shown to activate CAR and induce Oatp2, Gsta1 and Gsta2, Ugt1a1, and Mrp2 (Fig. 3a) [75]. Bilirubin is transported by Oatp2 and initially conjugated by Gsta1 and Gsta2 to prevent transport back to the blood. It is then diglucuronidated by Ugt1a1 and finally transported into the canilicular space by Mrp2 for clearance from the body [75]. Bilirubin's ability to activate CAR may allow for induction of detoxification genes also involved in bile acid metabolism in the presence of inhibitory mechanisms such as inflammation or SHP-mediated transrepression [143]. Lastly, it was recently reported that bilirubin conjugation is an exergonic reaction, which may provide a protective mechanism during liver toxicity by

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providing the energy requirement needed during cholestasis for bile acid detoxification and elimination [144]. This further supports the idea that CAR and PXR play a role in energy metabolism outside of cholesterol and bile acid homeostasis (see section below on energy metabolism).

In summary, both CAR and PXR regulate several key genes involved in the detoxification and subsequent elimination of bile acids, and therefore protect the liver from cholestatic damage. Furthermore, activation of CAR and PXR may provide additional protection from jaundice, bile acid toxicity and cholestasis, as suggested by the use of traditional herbal medicines for bilirubin toxicity [15] (Table 1). However, the use of these medicines in young children under 6 months is controversial due to the low levels of CAR and PXR during this age [145].

8. CAR/PXR in energy metabolism

The liver is a major player in the regulation of energy metabolism by enhancing glucose production through increased gluconeogenesis and glycogenolysis. It maintains blood glucose levels, as well as induces fatty acid oxidation and ketogenesis, and reduces lipogenesis for the prevention of hepatic triglyceride storage during fasting, starvation and stress conditions. These complex pathways are regulated by pancreatic hormones that induce genes for glucose and lipid metabolism [20]. CAR and PXR are not only key regulators of the detoxification processes of endogenous and exogenous compounds from our body, but also play a major role in hepatic energy metabolism. For example, chronic PB treatment was shown in 1985 to decrease plasma glucose and increase insulin sensitivity of diabetic patients [146], probably due to its activation of CAR and repression of the forkhead transcription factor 1 (FOXO1) [147]. Microarray studies showed a decrease in the gluconeogenic and fatty acid β-oxidation genes phosphoenolpyruvate carboxykinase 1 (PEPCK) and carnitine palmitoyltransferase 1 (CPT1) following PB treatment in wild-type but not CAR-null mice [12,148]. In contrast, CPT1 was increased by VP-PXR [12] (Fig. 3b).

Further studies found that CAR and PXR directly bind to FOXO1 and inhibits FOXO1 function. In turn, CAR and PXR repress the FOXO1-regulated transcription of the gluconeogenesis genes glucose-6-phophatase (G6Pase) and PEPCK1 genes in an insulin-like manner [147]. Similarly, insulin induces the phosphorylation and inactivation of FOXO1 and represses transcription of genes involved in gluconeogenesis and detoxification.

However, there is contradicting evidence between diabetic murine models. In Zucker rats Cyp2b1/2 and CAR are downregulated, but in leptin receptor-null mice (db/db) Cyp2b10 and CAR are induced [149]. The induction of RXR and HNF4α in the db/db model [149] may explain the Cyp2b10 induction, as RXR is the heterodimer partner of CAR, and HNF4α regulates CAR expression. Cyp2b, 3a, and 4a are also induced in streptozotocin-generated diabetic mice and rats, but levels decreased upon insulin treatment [150]. Low levels of insulin provided to media also decreased Cyp2b, 3a, and 4a in primary rat hepatocytes treated with CAR and PPARα agonists suggesting interplay between insulin and the xenobiotic response [151]. It was shown several years ago that insulin represses P450 induction by xenobiotics and in diabetic livers, which has consequences on an individuals ability to metabolize drugs [152].

In contrast, fasting increases CAR responsive genes such as Cyp2b10, ugt1a1, sult2a1, and Oatp2. Genes not regulated by CAR such as PGC-1α and CAR itself were also induced during fasting. CAR and PXR are generally thought to inhibit PGC-1α's role in regulating PEPCK1 and G6Pase in a similar manner to FOXO1 as their activation causes competition for PGC-1α leading to the dissociation of PGC-1α from the hepatocyte nuclear factor 4 alpha (HNF4α) complex [20]. CAR induction as well as the induction of the CAR coactivator PGC1α by fasting may lead to increased expression of CAR regulated genes because

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PGC-1α also binds HNF4α inducing HNF4α interaction with hCAR's promoter elements and subsequent induction of CAR expression [153,154]. Interestingly, cAMP, which is increased by fasting, also lead to a similar gene expression profile as fasting [41] CAR translocation to the nucleus is activated by the AMPK cascade due to an increase in available cAMP [35, 155], further indicating that CAR activation plays a role in the fasting response.

However, while CAR and PXR activation may protect individuals from diabetes by down-regulating genes involved in gluconeogenesis, they also increase lipogenesis [156,157]. Activation of CAR by TCPOBOP decreases HDL cholesterol levels in part through a CAR-mediated reduction in apolipoprotein A–I [158]. Other apolipoproteins such as apolipoprotein CI, CII, and CIV are down-regulated by PXR activation [12]. Research also demonstrates that activated PXR increases the expression of stearoyl-CoA desaturase 1 (SCD-1) and down-regulates CPT1A and mitochondrial 3-hdroxy-3-methylglutarate-CoA synthase 2 (HMGSC2) genes in the liver of fasting mice. This leads to increases in hepatic triglycerides, represses hepatic energy metabolism, decreases β-oxidation and ketogenesis, induces lipid accumulation in the liver, and causes microvesicular steatosis [156,157]. These effects by PXR are thought to occur due to the inhibition of FOXA2 by PXR [156], and the increased transcription of the hepatic free fatty acid transporter, CD36 [157].

Further demonstrating CAR's role in increased lipogenesis, Maglich et al. [24] showed serum triglyceride levels increased in wildtype mice after a high fat diet and TCPOBOP treatment. Similarly treated CAR-null mice did not show increases in triglycerides. In addition, obese mice (ob/ob) that express high serum triglyceride concentrations did not show this phenotype when crossed to the CAR-null background [24]. Activation of CAR significantly repressed PPARα activity, a key regulator of liver fatty oxidation, and decreased the expression of Cyp4a14, CPT1α, and cytosolic acyl CoA thioesterase (CTE) in the liver [24], providing more evidence that CAR competes for PGC-1α with PPARα. These data provide a putative mechanism for the development of CAR inverse agonists as pharmacological treatments for metabolic disorders such as metabolic syndrome, type 2 diabetes, non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD). Further, recent work indicates that CAR activation worsens hepatic injury in a dietary model of NASH [159]. However, the benefit of CAR activation on gluconeogenesis would be lost and may provide further complications.

Interestingly, ob/ob mice that express high serum triglycerides also express higher than normal levels of hepatic Cyp2b10 [24]. The experiments and data below provides a mechanism for this correlation. Increased unsaturated fatty acids, increased hepatic triglyceride accumulation, decreased plasma lipid levels, increased CYP levels, and hepatic enlargement were measured as a consequence of loss of CYP function in the P450 oxidoreductase (POR) – null mouse model [77]. This suggests that CYPs are important in the metabolism and clearance of unsaturated fatty acids, and they are regulated in order to increase clearance. The use of double (CAR-null/POR-null) and (PXR-null/POR-null) showed a loss of CYP induction in addition to a loss of CYP activity, indicating that CAR and to a lesser extent PXR, is activated by unsaturated fatty acids and in turn induces CYP proteins such as Cyp2b, Cyp2c, and Cyp 7a1 [77]. Taken together with the data indicating CAR represses PPARα activity by competing for PGC-1α CAR and to a lesser extent, PXR may take over PPARα's duties when under stress. Overall, this data suggests CAR and PXR help compensate for lipid imbalances that could lead to metabolic disorders.

However, others have measured reduced triglyceride production following treatment of mice with the CAR activators, TCPOBOP and PB, and the PXR activator, PCN. The decreased triglyceride levels were associated with induction of insulin-induced gene 1(Insig-1) through a DR4 element. Insig-1 in turn inhibits the release of the sterol regulatory element-binding protein 1 (SREBP-1) from the endoplasmic reticulum, which reduces the transcription of genes

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involved in fatty acid and triglyceride synthesis [160]. Interestingly, SREBP-1 can inhibit CAR and PXR mediated induction of CYPs and this may also help explain the reduced clearance of drugs in obese patients [161].

Taken together, data suggest that activation of CAR and PXR by environmental toxicants and dietary constituents may be beneficial for patients with metabolic disorders such as type II diabetes by reducing serum glucose levels. However, activation of these receptors may also lead to increased triglyceride levels, a complication of type II diabetes. Therefore, dietary activation of CAR and PXR is probably not beneficial to the patient especially since type II diabetes is often associated or complicated by obesity. Some pharmacologists believe inverse agonists may actually be best suited for amelioration of metabolic disorders [24].

Lastly, it has been reported that CAR is a regulator of thyroid hormone levels during caloric restriction [76], therefore, associating CAR with the maintenance of a hormone critically involved in energy homeostasis. Studies showed that CAR-mediated induction of detoxification enzymes (Sult2a1, Ugt1a1)increased T4 clearance, which led to decreased T4 and T3 levels [105]. Interestingly, PCBs are CAR activators and also decrease T4 and T3, while increasing thyroid hypertrophy and TSH levels [162–165]. Furthermore, these effects are typically stronger in females [162], which reportedly have greater CAR levels and activity [19,166–168].

Other studies report that T4 and T3 levels were not altered by CAR activation, but thyroid stimulating hormone (TSH) and hypertrophy of the thyroid gland occurred in both murine and human models [76]. Recent studies suggest that CAR does not reduce T3, but instead reduces T3 activity by inducing Dio1 a type 1 deiodinase, which converts T4 into T3 and rT3 [169]. Dio1 1 is upregulated by treatment with PB, and down-regulated by partial hepatectomy [169] Therefore, down-regulation of Dio 1, increases rT3 and represses T3 responsive genes such as tyrosine aminotransferase (TAT) and basic transcription element binding protein (BTEB), and Cpt1 [169]. This also provides a negative mechanism (inhibition of rT3 production) by which CAR activation regulates liver regeneration and hyperplasia.

These studies provide another mechanism by which CAR may play a role in fasting induced lipogenesis, as well as liver regeneration. In summary, the activation of CAR by fasting appears to prevent undo energy loss by reducing thyroid hormone activity. In turn, CAR-null mice lost more weight from adipose tissue and not lean mass, when compared to wild-type mice treated with CAR activators [76]. Figure 5 briefly summarizes the basic mechanisms that CAR and PXR use to modulate energy metabolism.

9. Hepatocarcinogenesis

Researchers have recently found a link between chronic exposure to xenobiotics and hepatomegaly that could lead to liver tumor formation in a PXR or CAR-requisite manner in rodents [22,79,170–172]. CAR has been implicated in liver tumor formation due to xenobiotic stress and is required for PB and TCPOBOP-induced promotion of liver tumors upon exposure to genotoxic compounds [22,79]. Barbituates have historically been correlated with liver tumor formation in murine experiments [173–175], and an increase in CAR expression in G1 phase is associated with cell proliferation [176]. Further, a global proteomics study following PB-treatment of wild-type rats found that a possible mechanism for carcinogenesis was through increased xenobiotic metabolism enzymes, especially CYP2B and other oxidoreductases, increased cell cycle activity, and a general induction of several pro-carcinogenic proteins [44]. PB was also prescribed to patients with hepatic injury due to its ability to decrease the time for hepatic regeneration, and CAR activation by PB or TCPOBOP has been correlated with hepatomegaly of the liver in response to toxicants. The increase in hepatocyte number

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and size is not uncommon to toxicants and aids in the livers ability to detoxify and eliminate toxicants quickly.

More recently, tumor formation was shown to be CAR-dependent in mice chronically treated with TCPOBOP or PB [22,79]. CAR-null mice treated with the genotoxin diethylnitrosamine (DEN), a known liver carcinogen, followed by the CAR activator PB showed no tumor formation. In contrast, wild-type mice showed eosinophilic formation and hepatocarcinomas by 39 weeks [79]. Similarly, in separate studies, wild-type but not CAR-null mice co-treated with DEN and TCPOBOP developed tumors. The mechanism by which CAR promotes cancer involves an increase in the oncogene Mdm2 (double minute oncogene), which can directly inhibit the apoptotic and tumor suppressive actions of p53 [22].

c-Myc expression is induced in TCPOBOP-treated wild-type mice, but CAR-null mice showed no induction of c-myc, indicating that it is a downstream target gene of CAR [177]. Studies suggest that in response to TCPOBOP treatment, c-Myc binds to the forkhead box M1 (FoxM1) promoter and therefore shows overlapping transcription profiles [177]. c-Myc has been known to regulate genes involved in cell proliferation, cellular growth, apoptosis, angiogenesis, cell adhesion and differentiation [178,179], and therefore induction of c-myc is another mechanism by which CAR may increase hepatocellular carcinoma and hepatomegaly.

Several microarray experiments have shown a host of genes activated in response to CAR-stressors that are responsible for proliferation and growth of cells [180,181] (Fig. 5). These CAR-dependent genes generally fall in three large categories. The first category is anti-apoptotic genes. CAR activation leads to a downregulation of pro-apoptotic genes such as Bax (Bcl-2-associated × protein), Bak (Bcl-2 antagonistic killer), and death-inducer obliterator 1 (Dio 1). CAR activation also leads to an induction of the survival proteins Gadd45b/MyD118, and myeloid cell leukemia factor-1 (Mcl-1). Gadd45b inhibits apoptosis by activating p38 and/or inactivating Jun N-terminal Kinase (JNK) [180]. Mcl-1 is a special interest because it further interacts with and inhibits Bax and Bak activity and in turn promotes survival [182].

The second class of genes altered by CAR activation is involved in increased DNA replication in S phase. The primary players associated CAR-mediated increases in DNA replication are proliferating cell nuclear antigen (PCNA), Rho guanine nucleotide exchange factor 7 (Arhgef7), ribonucleoside-diphosphate reductase M2 subunit (Rrm2), and the proliferation marker antigen KI-67 (Ki-67) [22,182–184]. These genes probably work in close conjunction with the third class of pro-proliferative genes altered by CAR activation; genes involved in cell cycle progression.

CAR activation alters the expression of both G1 to S phase and S to G2 checkpoints. For example, increases in phosphorylated retinoblastoma (pRb) have been measured, and induction of cyclins A2, D1, cell division protein kinase 2 (cdk2), and the transcription factor E2F occur in a CAR-dependent manner (Fig. 3c). These changes allow for progression from G1 to S-phase. Furthermore, cyclin B1, which induces progression from S to G2-phase, is also induced by CAR activation [168,185]. A summary of the pro-carcinogenic genes induced by CAR is provided in Figure 3c, and Dai et al [44] provides a list of proteins effected by CAR activation. Therefore, the evidence suggests CAR activation induces a three-pronged attack that leads to increased cell cycle progression, DNA replication, and cell survival, ultimately leading to hepatomegaly of the liver and potentially hepatocarcinoma (HCC) (Fig. 6). However, it is our opinion that the repression of p53's tumor suppressor activity and activation of pro-apoptotic functions may be the key step in CAR-mediated tumor formation. Many of the genes described in this review have been characterized primarily through microarray analysis, however, the oncogene ubiquitin-protein ligase E3 (Mdm2-also known as p53-binding protein) has functionally been shown to be involved in CAR mediated tumor formation by binding p53's

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transcriptional activation domain, promoting export of p53 and targeting it for proteolysis [22].

CAR activation was also shown to have an epigenetic effect on methylation patterns in PB induced tumors. One-hundred four different methylation sites in precancerous lesions and tumors caused by PB treatment were observed, as opposed to CAR-null mice resistant to PB-induced tumors [186]. Altered methylation patterns in promoter regions appear to be linked to the tumorigenesis of other cancers [187].

Even though CAR activation has been positively correlated with increased HCC tumors in mice, human tumorigenesis has not been positively correlated to PB, phenytoin, and other CAR activators [188]. Therefore, the risk to humans may be difficult to address or low. PB has not been shown to be genotoxic in any assay, and genotoxicity may be required for tumorigenesis in humans [189]. Debate on the possibility of extrapolating from rodent models persists, specifically with liver cancer [188]. However, it may prove appropriate for scientists to continue to consider potential effects of CAR activators on human health and liver cancer, because of the activation of CAR by several hepatic stressors and the difficulty in estimating chronic concurrent activation of CAR in a human model.

Staudinger et al. [172] demonstrated hepatomegaly and increased PCNA staining in conjunction with PXR-mediated PCN-induced CYP3A levels and activity. PXR is also involved in hepatomegaly, tumor promotion, and liver cancer, and PXR-null mice are resistant to the effects of pregnane-16α-carbonitrile (PCN) in this regard [170]. Recently, PXR was also shown to be required for liver regeneration. The lack of PXR led to the inactivation of STAT3 (Signal Transducers and Activators of Transcription 3), and suppression of the second stage of liver proliferation [171]. Overall, fewer studies have been performed with PXR and prototypical PXR inducers have not been previously correlated with liver cancer formation in the way that the CAR activator, PB has. Studies have also found that the activation of PXR has anti-apoptotic effects against human colon cancer cells [21].

10. Sexual dimorphism

Several reports indicate that CAR shows greater expression or activity in females than males. For example, in mice TCPOBOP increased liver proliferation in females more than males [168]. Females showed greater CYP induction following treatment with the plasticizer and moderate CAR activator nonylphenol [190] than did males; however, males and females showed little difference in sensitivity to TCPOBOP [167]. CAR activation increases NASH [24,159], and females show greater fibrotic severity [191]. PCBs which activate CAR and PXR (some also inactivate PXR in humans), have greater effects on thyroid hormones and thyroid hyperplasia in females than males [162], presumably due to the greater expression and activation of CAR in human females [166]. This in turn increases CAR-mediated thyroid hormone clearance [105]. It is interesting to speculate that sex differences in CAR activity may in part explain the female predominance of human CYP2B6 [166]. In contrast, male rat thyroid adenomas occur at lower doses than in female rats following pyrethroid treatment [192]. Overall, there are several occurrences of greater sensitivity in females to CAR activators.

There are several potential reasons for the greater response in females to CAR activation, including higher mRNA expression of CAR in female liver than male liver in rodents [19], and humans [166]. However, significantly greater protein expression has not been measured [167]. Furthermore, estradiol is a CAR agonist in mice [193], and xenobiotic activators may work in an additive fashion to increase CAR activity. Androgens and their metabolites also repress CAR activity, which may decrease the efficaciousness of CAR in males [25]. The metabolism of testosterone and in particular the 6α/15α-hydroxlase ratio is decreased greater than 2.5-fold in female CAR-null mice compared to their wild-type counterparts. The 6α/

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15α-OH ratio, which is typically much greater in females, is in part controlled by androgens and considered a biomarker of androgen disruption or androgen status [194]. It is interesting that this ratio is effectively masculinized in female CAR-null mice [167]. This suggests that CAR is a key player in the recognition of androgen status in the liver, consistent with its repression by androstanes and other androgens [25].

The nuclear receptors RXRα and HNF4α are involved in the basal regulation of sexually dimorphic CYPs [195–197]. Both of these receptors interact with CAR either directly or indirectly to help regulate CAR expression and CYP expression. HNF4α and CAR expression levels are associated with the expression of several CYPs including the repression of Cyp2b9 and Cyp2b13 in males [198]. Loss of CAR or HNF4α effects the expression of some CYPs including Cyp2b13 in males indicating a role for CAR or HNF4α/CAR interactions in regulating sexually dimorphic expression of CYPs in mice [167,197]. Current data in humans and mice suggests that HNF4a expression regulates CAR expression which regulates CYP expression, except in cases in which HNF4a directly regulates CYPs [167,196–198].

Several CYP3A subfamily members are sexually dimorphic. For example, Cyp3a41 and Cyp3a44 are female specific in mice [199,200]; while Cyp3a9 is female predominant in adult female rats [201]. CYP3A4 shows higher expression in human females [202], and this appears to increase the clearance rate of several drugs in females. Overall, gender differences in pharmacokinetics are rare, but when present may be clinically significant. Drugs cleared faster in women than men include verapamil, cyclosporine, erythromycin, nifedipine, diazepam, prednisolone, methylprednisolone, ifosfamide, and alfentanil [202,203]. Tirilazad, a drug extensively metabolized by CYP3A4 [204], is a clinically important example of a drug cleared faster in females. This drug used for subarachnoid hemorrhage was denied approval because it is not as efficacious in women since it is cleared approximately 40–60% faster in women than men [204,205]. Humanized PXR and CYP3A4 models may help us elucidate the mechanisms of gender differences in CYP3A4 expression that cause human gender differences in drug metabolism and clearance [206].

Whether PXR plays a direct role in the gender differences in CYP3A expression is unknown, but there is circumstantial evidence. PXR-null male mice demonstrate greater CYP3A activity, while PXR-null female mice demonstrate reduced CYP3A activity compared to wild-type mice [199], indicating that PXR may play a different role in each gender. In addition, liver levels of PXR decrease with age in male B6 mice, but increase with age in female B6 mice [207]. This may help explain some of the CYP3A expression differences by gender as data indicates that CYP3A expression is associated with PXR expression in humans [145,198]. Lastly, PXR and CYP3A levels increase during pregnancy, probably to protect the mother from high concentrations of steroids [208].

Greater sensitivity to PXR ligands has also been observed in females. For example, St. John's wort mediated induction of CYP3A4 is greater in human females than males [91]. TCPOBOP and PCN induced CYP3A4 more in females than there age and gender match male counterparts in a humanized CYP2D6/CYP3A4 transgenic mouse model [209]. DDT, a CAR and PXR agonist, demonstrated significantly greater induction of CYP3A1 and CYP3A2 in female rats compared to male Wistar rats. In contrast, female rats demonstrated reduced sensitivity to DDT when investigating CYP2B induction, but showed similar maximal induction at the highest doses [210]. Dexamethasone down-regulates the female-predominant Cyp3a41 in mice in a PXR-dependent manner while males did not demonstrate down-regulation of any Cyp3a subfamily member [199]. The female specific Cyp3a44 is only induced in males but not females following treatment with both PXR and CAR activators [200].

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Taken together, both CAR and PXR show greater sensitivity in females than males. This may be related to steroid hormones as the androgens suppress CAR activity and progesterone increases PXR activity. Therefore, future research may demonstrate that increased drug clearance, drug-nutraceutical interactions, drug-toxicant, and other adverse drug reactions occur because of PXR and/or CAR activation and specific precautions may be appropriate. This enhanced activity in human females may also contribute to other diseases, but also provide benefits as high levels of specific toxicant receptors are associated with longevity [211].

11. Relevance to Personalized Medicine

Personalized medicine aims to find people at risk to specific conditions, or managing an individual's care by considering age, gender, weight, diet, environment, and genetic status. Our response to drugs varies greatly and not just from individual to individual, but within the same individual at different times. Certainly CAR and PXR are part of this equation as they respond to a milieu of different chemicals in our environment and in turn induce drug metabolizing enzymes and transporters. Patients exposed to plasticizers or surfactants through IV bags or industrial detergents may have induced enzymes and in turn respond differently than other patients or differently than they would have two weeks earlier. Similarly, farm workers through pesticide exposure may show induced enzymes and differential sensitivity to drugs. However, we cannot forget to consider other factors that can modulate CAR and PXR activity. For example, a patient's diet may have significant effects on their ability to detoxify and eliminate drugs. Several dietary compounds activate CAR, PXR, or both. There are also other factors that need to be considered as fat in the diet (depending on the source) and obesity may alter and potentially significantly repress an individual's ability to metabolize drugs relative to their body weight. Diabetes is another condition that can complicate a typical pharmacotherapy regimen for diseases that may otherwise be easily treated because of poor metabolism. These factors should be considered.

Young children have always shown significant sensitivity to a variety of chemicals, including pesticides and drugs. The reduced expression of CAR and PXR provides a potential mechanism. Furthermore, there are a variety of splicing variants and SNPs that may cause sensitivity to drugs in some individuals. This is an area that warrants further research and testing using pharmacogenetics approaches so that clinicians can make the correct decisions regarding which drug to prescribe and its attendant dosing regimen.

Lastly, CAR and PXR may also serve as drug targets for diseases such as HCC, diabetes, and cholestasis. While we search for better treatment for these diseases, both inhibitors and activators of these receptors may further complicate pharmacotherapy and provide a greater need for personalized medicine. These individuals will show perturbed drug metabolism since direct perturbations in CAR and PXR activity by these drugs will alter the metabolism of other drugs as well as dietary constituents and environmental toxicants.

12. Concluding Remarks and Future Outlook

Exposure to a wide range of dietary compounds, herbal remedies, occupational chemicals, and environmental pollutants that individually or collectively activate CAR or PXR may have a wide range of effects on the genes we express and the phenotype in which we express these changes. This includes increased detoxification, altered hormonal or lipid metabolism, increased cell proliferation, decreased intermediary carbohydrate metabolism, and potentially lipogenesis. Thus, CAR and PXR activation by a plethora of activators from dietary constituents to xenobiotics and nutraceuticals, gives us excuses for our reduced activity and increased obesity. However, it also indicates that we must be aware of what we are exposed because the effects of CAR and PXR activators may be physiologically relevant to multiple systems and far reaching. Medical professionals will need to become more aware of the issues

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regarding activation of CAR and PXR in each patient as they attempt to individualize disease diagnosis and treatment, and the downstream biological effects of these key nuclear receptors for their ability to influence drug metabolism as we are exposed to greater amounts of pharmaceuticals, nutraceuticals, and environmental chemicals in society.

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Abbreviations

Asbt	sodium dependent bile acid transporter
ADR	adverse drug reactions
Arhgef7	Rho guanine nucleotide exchange factor 7
AMPK	AMP activated protein kinase
APAP	4-hydroxyacetanilide, N-acetyl-paminophenol
Bak	Bcl-2 antagonistic killer
BAR	Bile acid receptor
Bax	Bcl-2-associated × protein
BTEB	basic transcription element binding protein
CA	cholic acid
CAR	Constitutive androstane receptor
CCRP	cytoplasmic CAR retention protein
CDCA	chenodeoxycholic acid
cdk2	cell division protein kinase 2
CITCO	6-(4-Chlorophenyl)imidazo[2,1-O-3,4-b][1,3]thiazole-5-carbaldehyde dichlorobenzyl) oxime
CPT1	carnitine palmitoyltransferase 1
CREB	cAMP-response element binding protein
CTE	cytosolic Acyl-CoA thioesterase
CYP	cytochrome P450
DBD	DNA binding domain
DCA	deoxycholic acid
DEHP	di(2-ethylhexyl)phthalate
DEN	diethylnitrosamine
Dio1	type 1 5′-deiodinase
Dio 1	death-inducer obliterator 1
Gadd45b	growth arrest and DNA-damage-inducible, beta
EDCs	endocrine disruptors
ERK1/2	extracellular signal related kinase

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FOXA2	forkhead box A2
FOXM1	forkhead box M1
FOXO1	forkhead transcription factor 1
FXR	farnesoid × receptor
GSTs	glutathione S-transferases
HMGSC2	3-hdroxy-3-methylglutarate-CoA synthase 2
HNF-1	hepatocyte nuclear factor 1
HNF-4α	Hepatocyte Nuclear Factor-4α
Hsp	heat shock protein
Insig-1	insulin-induced gene 1
JNK	Jun N-terminal kinase
Ki-67	proliferation marker antigen Ki-67
LBD	ligand binding domain
LCA	lithocholic acid
LRH-1	liver receptor homolog 1
LXR	liver × receptor
Mcl-1	myeloid cell leukemia factor-1
Mdm2	double minute oncogene
MDR1	multidrug resistance protein 1
MRP2	multidrug resistance-associated protein 2
NAFLD	non-alcoholic fatty liver disease
NASH	on-alcoholic steatohepatitis
NCoR	nuclear receptor co-repressor
Oatp2	organic anion transporting protein 2
PB	phenobarbital
PBREM	phenobarbital response element module
PCBs	polychlorinated biphenyls
PCN	pregnenolone 16α-carbonitrile
PCNA	proliferating cell nuclear antigen
PEPCK	phosphoenolpyruvate carboxykinase 1
PGC-1α	peroxisome proliferator-activated receptor coactivator-1α
PKA	protein kinase A
POR	P450 oxidoreductase
PP2A	protein phophatase 2A
PXR	pregnane × receptor
pRB	phosphorylated retinoblastoma

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RAR	retinoic acid receptor
Rrm2	ribonucleoside-diphosphate reductase M2 subunit
RXR	retinoid × receptor
SCD-1	stearoyl-CoA desaturase 1
SHP	small heterodimer partner
SMRT	silencing mediator of retinoid and thyroid hormones
SNPs	single nucleotide polymorphisms
SRC-1	steroid receptor co-activator 1
SREBP-1	sterol regulatory element-binding protein 1
STAT3	Signal Transducers and Activators of Transcription 3
STD	dehydroepiandrosterone sulfotransferase
SULTs	sulfotransferases
SXR	steroid and xenobiotic receptor
TAT	tyrosine aminotransferase
TCPOBOP	1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP)
UDPGT	Uridine diphospho-glucuronosyltransferases
VDR	vitamin D receptor
XRE	xenobiotic response element

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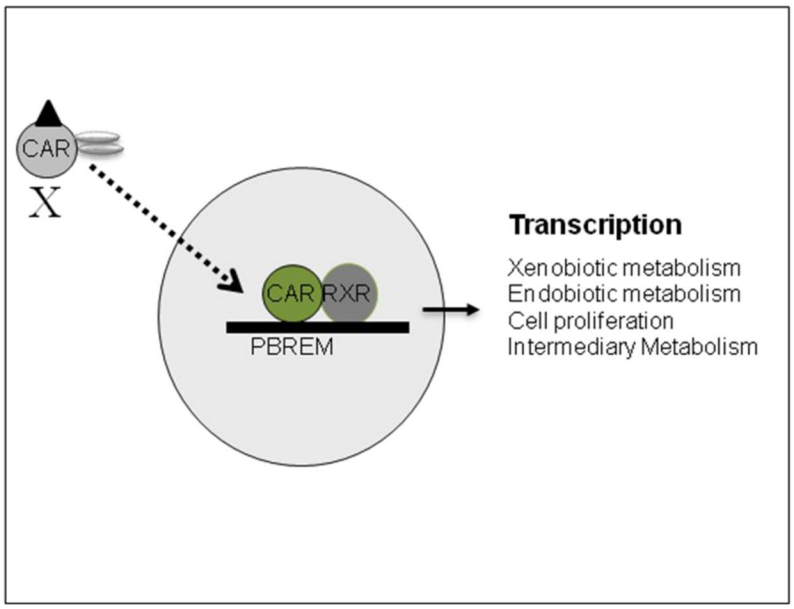

Fig. 1. Activation of CAR

Chemical (X) activation of CAR triggers its release from cytoplasmic retention proteins such as heat shock protein 90 (hsp90) (*ovals*) and cytoplasmic CAR retention protein (CCRP) (*triangle*) allowing for its translocation to the nucleus, and subsequent interaction with RXR, requisite co-activators, and histone acetylases needed to assemble and activate the basal transcriptional machinery.

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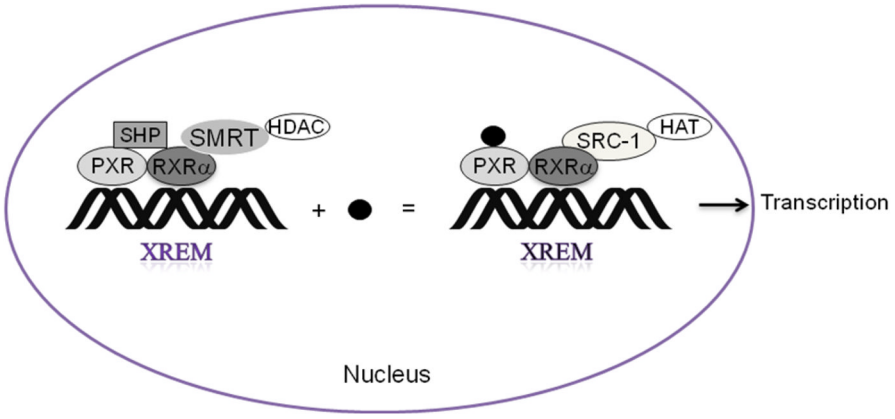

Fig. 2. Activation of PXR

Chemical (●) activation of PXR triggers the release of co-repressors and histone deacetylases, and the subsequent interaction of PXR with co-activators and histone acetylases, leading to transcription of target genes.

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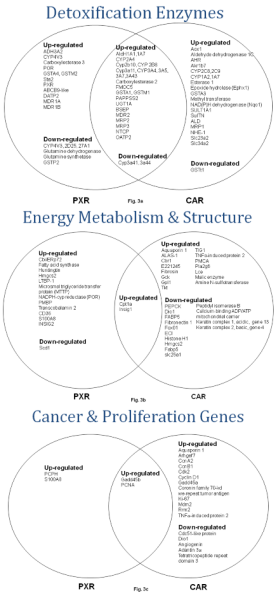

Fig. 3. Genes up- or down-regulated by CAR or PXR

Activation of CAR and PXR alters the transcription of a large number of genes associated with (**A**) detoxification of therapeutic drugs and xenobiotics, (**B**) energy metabolism, and (**C**) hepatomegaly and liver cancer (see also microarray data from references [12,148,180,264].

Abbreviations: ABCB9-like: ATP-binding cassette B9 similar; ADH3A2: Alcohol dehydrogenase 3A2; ALD: ATP-binding cassette; Aldh1A1 and A7: Aldehyde dehydrogenase; AHR: Aryl Hydrocarbon reception; Akr1b7: Aldo-keto reductase family 1 member B7; ALAS1: Aminolevulinate synthase H; Aox1: Aldehyde oxidase 1; Arhgef7: Rho guanine nucleotide exchange factor; BSEP: Domain Organization of Bile Salt Transporter (BSEP); Cbi/ERp72: Intestinal Calcium binding protein/Endoplasmic reticulum protein 72; CcnA2: Cyclin A3CcnB1; Cyclin B1Cdk2: Cyclin-dependent kinase; CD36: An integral membrane protein which is 88kDa glycoprotein found on platelets, monocytes, erythroblasts, megakaryocytes, endothelial cells, cardiac and skeletal muscle and adipocytes; Cpt1a: carnitine palmitoyltransferase 1 A; CYP: Cytochrome P450; DATP2: Na+-independent organic anion-transporting polypeptide 2; Dio 1: Type 1 deiodinase; Ephx1: Epoxide hydrolase; ECI: Enoyl-CoA isomerase; FMOC5: Flavin containing monooxygenase 5; Gadd: Growth-arrest and DNA-damage-inducible; GST: Glutathione-S-transferase A4; Hmgcs2: 3-hydroxy-3-methylglutaryl-Coenzyme A synthase (HMG CoA Synthase); INSIG2: Insulin-induced gene2; Lce: Long chain fatty acyl elongase; LTBP-1: Latent TGFβ binding protein; Ki-67: Ki-67; Mdm2: murine double minute oncogene; MDR1A: multi-drug resistance 1A; MRP: multi-drug resistant associated protein; MTTP: Microsomal triglyceride transfer protein; NHE-1: Solute carrier family 9 member 1; Nqo1: NAD(P)H dehydrogenase SULT1A1; NTCP: Na+-taurochlorate-cotransporting polypeptide; OATP2: Organic anion transport protein 2; PAPSS2: 3′-phosphoadenosine 5′-phosphosulfate synthase 2; PCNA: Proliferating cell nuclear antigen; PCPH: Ectonucleoside triphosphate diphosphohydrolase; PEPCK: Phosphoenolpyruvate carbonoxykinase; Pla2g6: Phospholipase A2; PMBP: Progesterone membrane binding protein; PMCA: ATPase, CA2+ transporting plasma membrane 2; POR: NADPH-Cyp reductase; PXR: pregnane × receptor; Rrm2: Ribonucleotide reductase M2; S100A8: cal: granulin A or migration inhibitory factor-related protein 8 (MRP8); Scd1: Stearoyl-CoA desaturase 1; Slc: Solute carrier family; Sta2: hydroxysteroid preferring sulfotransferase 2; SultN: sulfotransferase family 1D member 1; TIG1: Retinoic acid receptor responder 1; Tkt: Transketolase; UGT1A: Uridine diphosphate-glucuronosyltransferase 1A.

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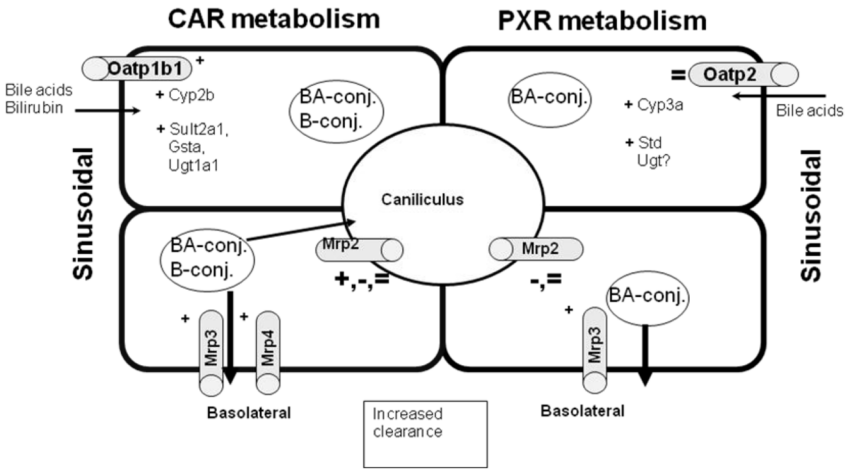

Fig. 4. Detoxification of bile acids and bilirubin during cholestasis

Right-PXR activation by bile acids (LCA, DCA, and CA) causes induction of genes involved in bile acid metabolism and transport. Initially, induction of Oatp2 transport leads to increased influx of bile acids into hepatocytes. In turn, induction of Cyp3a and STD causes increased bile acid conjugation. During cholestasis Mrp2 is increased or unchanged, leaving induction of the basolateral transporter Mrp3 for increased excretion of bile acids for renal secretion. ***Left***-CAR activation by LCA or bilirubin leads to a similar mechanism of metabolism and transport as PXR activation. LCA activation of CAR induces Cyp2b and Sult2a1, and induction of Mrp3 and Mrp4 initiates increased basolateral transport. Activation of CAR by bilirubin during cholestasis induces Oat1b1, and several conjugases such as GSTA1, GSTA2, and Ugt1a1 that conjugate bilirubin. Induction of Mrp2 by bilirubin allows for canilicular excretion of bilirubin and perhaps LCA, which normally downregulates Mrp2 during cholestasis. **Abbreviations:** Oatp: Organic anion transporting protein; Cyp: cytochrome P450; Sult: sulfotranferase; STD: dehydroepiandrosterone sulfotranferase; GST: gluthathione S-transferase; Ugt: UDP-glucuronyl transferase; Mrp: multidrug resistance-associated protein; BA-bile acid; B-bilirubin; conj-conjugated; + induction; − downregulation; = no change.

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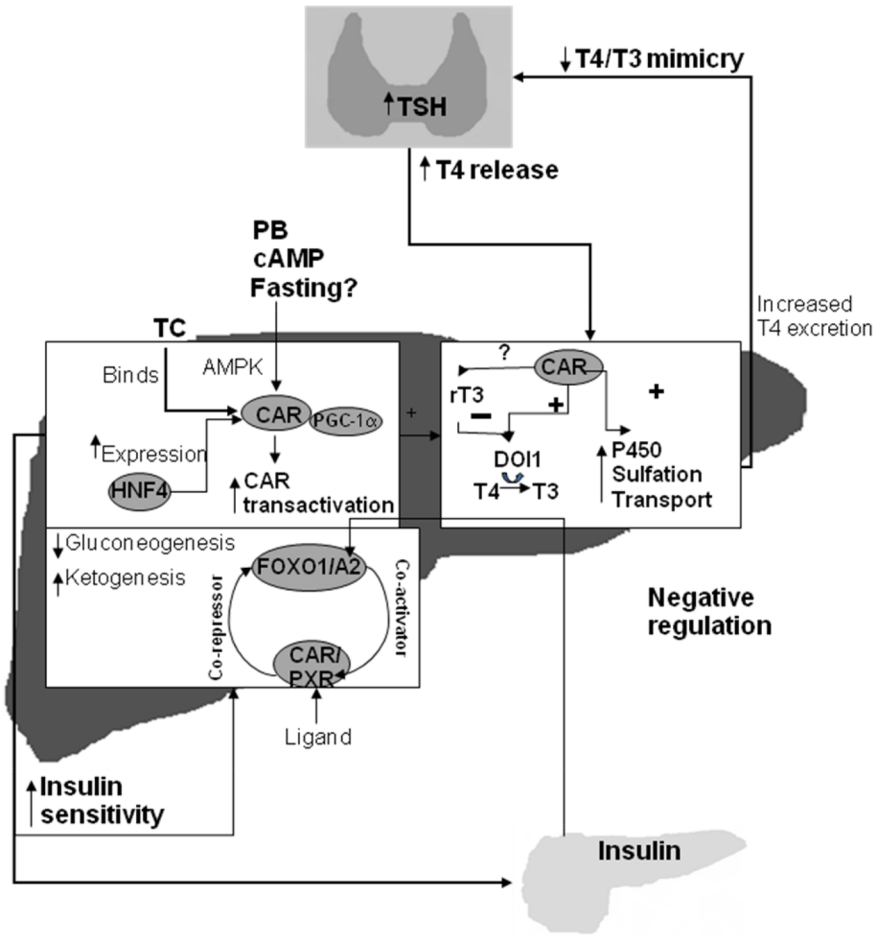

Fig. 5. CAR activation leads to altered energy metabolism

CAR agonists (PB, TC) and low energy (fasting, and high cAMP) activate CAR, which is transcriptionally regulated by HNF4a. Increased CAR activation may lead to increased sulfation and excretion of thyroid hormones (T3/T4). Low serum thyroid levels cause increased thyroid stimulating hormone leading to thyroid hypertrophy. CAR inhibits rT3 activity by induction of deiodinase 1 (DOI1), which catalyzes the conversion of T4 to T3. CAR activation also represses FoxO1 activity leading to decreased gluconeogenesis and increased ketogenesis. PB treatment has been known to increase insulin sensitivity and decrease serum glucose levels in diabetics probably through interplay of these mechanisms.

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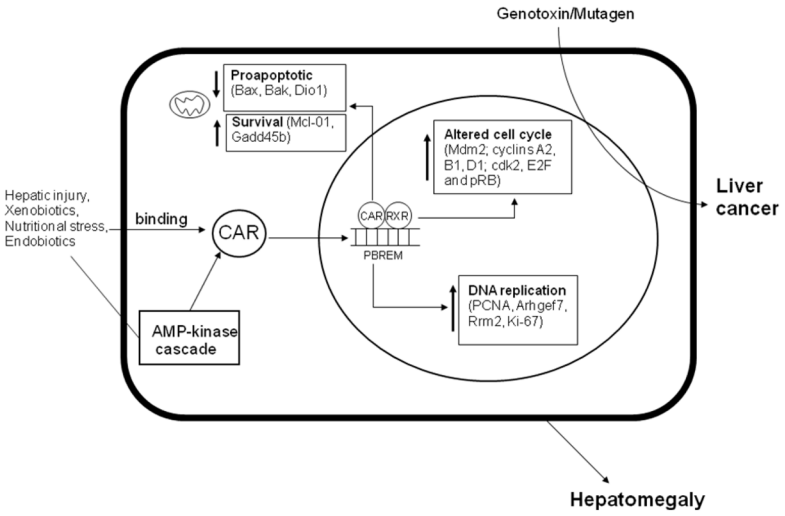

Fig. 6. Liver cancer progression induced through CAR

CAR activation activates a three-pronged attack that causes increased cell cycle progression, DNA replication, and cell survival. Continued activation ultimately triggers hepatomegaly of the liver and potentially hepatocellular carcinoma.

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Table 1

Environmental, occupational, and natural products that activate or inactivate PXR or CAR.

Chemicals/Herbs	PXR	CAR	Source
Alachlor	Rat Human(+)[212,213]	Mouse(+)[26]	Pesticide
Amitrol	Human(+) [214]		Pesticide
Androstanol	Human(+) [69]	Mouse Human(−)[25,27]	Testosterone Metabolite
Androstenol		Mouse(−)[25]	Testosterone Metabolite
Arsenite		Mouse(+)[26]	Chemical
Artemisinin	Human(+)[215]	Human(+)[215]	Herbal medicine
Artemisia capillaris		Human(+)[15]	Herbal medicine
Atractylodes lancea, A. macrocaphala	Human(+)[216]		Herbal medicine
Azo dyes (ortho-aminoazotoluene; methyl-4-dimethylaminobenzene		Mouse(+) [217]	Paint
Benzophenone	Rat(+) [212]		Packaging
Benzyl butyl phthalate	Human(+)[218]		Plasticizer
Beta-carotene	Human(+)[219]		Diet
Bisphenol-A	Human(+)[220]	Mouse(+)[26]	Consumer Products
Butylate		Mouse(+)[26]	Pesticide
Cafestrol from coffee	Mouse(+)[221]		Coffee
Chlordane	Human Mouse(+)[11,222,22]		Pesticide
Chlordecone	Human(+)[11]		Pesticide
Chlorpropham		Mouse(+)[26]	Pesticide
Chlorpyrifos	Human(+)[11]	Mouse(+)[26]	Pesticide
Commiphora mukul	Human(+) Mouse(+)[224,225]	Mouse(−)[224,225]	Herbal Medicine
Corticosterone	Human Rat Mouse(+)[8,226]	Mouse(+)[26]	Steroid Hormone
Coumestrol	Human (−)[227] Human (+)[228]		Phytoestrogen
Cypermethrin	Human(+)[11]	Mouse(+)[26]	Pesticide
Cyproconazole		Mouse(+)[229]	Pesticide
Daidzein	Human(+)[228]		Phytoestrogen
Danshen (tanshinone I, tanshinone IIA, cryptotanshinone)	Human(+)[230]	Human(+)[230]	Herbal medicine
DBP(Di-n-butylphthalate)	Rat(+)[231]	Rat(+)[231]	Plasticizer and Solvent
DDE(Dichlorodiphenyldichloroethylene)	Human Rat(+)[214,232]	Rat(+)[232]	Pesticide byproduct
o,p – DDT(1,1,1-Tichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane)	Human(+) Mouse(+)[11,213,233]	Mouse Rat(+)[33,232]	Pesticide
DEHP (Phthalic acid)	Human Mouse(+)[214,220,234]	Mouse Human(+)[26,124]	Plasticizer
Dehydroepiandrosterone (DHEA)	Human(+)[235]	Mouse(+)[235]	Hormone Precursor
DHT(5α-dihydrotestosterone)	Human Rabbit Rat Mouse(+)[226, 212,27]		Testosterone Metabolite
Dexamethasone	Mouse Human(+)[236,69]		Pharmaceutical
Dicyclohexylphthalate	Human(+)[214]		Plasticizer
Dieldrin	Human(+)[214]	Mouse(+)[67]	Pesticide

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Chemicals/Herbs	PXR	CAR	Source
Di-n-hexylphthalate	Human(+)[214]		Plasticizer
Dihydroandrosterone		Mouse (−)[26]	Testosterone Metabolite
n-Dipentylphthalate	Human(+)[214]		Plasticizer
n-Dipropyl phthalate	Human(+) [214]		Plasticizer
Docosahexaenoic acid (essential fatty acid—inhibits CAR activity)		Rat(−)[81]	Essential Fatty acid
Endosulfan	Human(+)[11,222]	Mouse(+)[26]	Pesticide
Endrin	Human(+)[11]		Pesticide
Ergovaline (ergot alkaloid)*	Rat(+)[237]		Chemical Made By Fungi
17β-Estradiol	Human(+) [8,233]	Human Rat Mouse (+) [193,238]	Female Hormone
Estrone		Mouse Rat Human(+)[193]	Female Hormone
17α-Ethinylestradiol	Human(+)[228]		Oral Contraceptive
Fenitrothion		Mouse(+)[26]	Pesticide
Fenvalerate	Human(+)[214]		Pesticide
Garlic		Rat(+)[239]	Plant
Genistein	Human(+)[228]		Isoflavones
Gingko biloba	Rat Human(+) [240,241]* [242, 243]	Rat Human (+)[240,241]* [243]	Herbal Medicine
Glycyrrhiza uralensis Fisch	Human(+) [244]		Herbal Medicine
Hypericum perforatum	Human(+) [90]		Herbal Medicine
Imazalil		Mouse(+)[26]	Pesticide
Isopimpinellin (coumarins)	Mouse(+)[245]	Mouse(+)[245]	Diet
Kava Kava* (Piper methysticum)	Rat Human(+) [246]*[247]		Herbal medicine
Kepone		Mouse(+) [26]	Pesticide
Ketoconazole	Human(−)[248]		Antifungal Drug
Lindane	Human (+) Rat (+)[11,212]		Pesticide
Linoleic acid		Mouse(+)[77]	Unsaturated fatty acid
MEHP	Human Mouse(+)[249]	Mouse(+)[26]	Plasticizer
Metolachlor		Mouse(+)[26]	Pesticide
Methoxychlor	Rat Human(+)[11,212,213]	Rat Mouse(+)[26,33,250]	Pesticide
mono-OH-Methoxychlor		Rat(+)[250]	Metabolite Pesticide
bis-OH-Methoxychlor		Rat(+)[250]	Metabolite Pesticide
Monosodium methane arsenate		Mouse(+)[26]	Pesticide
4-nitrotoluene	Human(+)[214]		Byproduct industrial process
trans-Nonachlor	Human Mouse(+)[11,226,228,236]	Mouse(−)[251]	Pesticide component
Nonylphenol	Human Rat Mouse(+)[190,218,234]	Human(+)[190]	Plasticizer
Norbolethone		Mouse(+)[26]	Anabolic steroid
Octachlorostyrene	Human(+)[214]		Refining Byproduct

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Chemicals/Herbs	PXR	CAR	Source
Octylphenol	Human(+)[218]		Plasticizer
Parathion		Mouse(+)[26]	Pesticide
PBDEs (polybrominated diphenyl ethers)	Human(+)[252]		Flame retardant
PCBs (highly chlorinated) (Polychlorinated biphenyls)	Human Mouse Rat(+)[223,228,253]	Mouse(+)[33]	Industrial Use
Pentachlorophenol		Mouse(+)[26]	Pesticide
Perfluorocarboxylic acid		Mouse(+)[254]	Surfactant
Perfluorooctanoic acid (PFOA)		Mouse(+)[255]	Surfactant
Poria cocos	Human(+)[216]		Fungus
PCN (pregnenolone 16α-carbonitrile)	Mouse(+)[233,256]		Steroid
Pregnenolone	Rabbit Rat Mouse Human(+)[226, 236]		Steroid Hormone
Progesterone	Mouse Human Rabbit Rat(+)[9,226, 228]	Mouse(+)[193]	Steroid Hormone
Propachlor		Mouse(+)[26]	Pesticide
Rhizoma curcumae	Human(+)[216]		Herbal medicine
Rifampicin	Human Mouse(+)[236]		Pharmaceutical
Schisandra chinensis Baill	Human(+)[244]		Herbal medicine
Spironolactone	Mouse Human(+)[223,226,236]		Pharmaceutical
SSS-Tributylphosphorotrithioate		Mouse(+)[26]	Pesticide
Stigmasterol	Mouse(−)[257]		Phytosterol
Tanzanian Herbal Plant Extracts	Human(+)[258]		Herbal medicines
TCPOBOP(1,4-bis[2-(3,5-dichlorpyridyloxy)] benzene)		Mouse(+)[259]	Xenobiotic
Tetrahydrogestrinone		Mouse(−)[26]	Anabolic steroid
Tian Xian	Human(+)[260]		Herbal medicine
Toxaphene	Human(+)[214]		Pesticide
Triclosan	Human(+)[228]	Mouse(+)[26]	Antifungal Antibacterial
Triclopyr		Mouse(+)[26]	Pesticide
Vinclozolin	Rat(+)[212]		Pesticide
OH-Vitamin D3	Human(+)[228]		Vitamin D Metabolite
Vitamin E (alpha tocopherol)	Human(+)[261]		Vitamin
Vitamin K2	Human(+)[262]		Vitamin
Zearalenone	Human(+)[263]		Mycoestrogen

The species used to test CAR/PXR activation/inhibition is provided next to the reference

*Based on P450 induction; gene expression profiles

(+) indicates activation; (−) induction inhibition

Sinz et al. [73] presents a list of a number of pharmaceutical activators of hPXR and Yamada et al. [72] presents a list of a number of CYP2B inducers, including several phytochemicals, pesticides and many pharmaceuticals.