PXR and CAR at the crossroad of drug metabolism and energy metabolism

Jie Gao, Wen Xie

Center for Pharmacogenetics and Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA 15261 (J.G., W.X.); Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA 15261 (W.X.)
Running Title: Nuclear receptors integrate drug and energy metabolism

Corresponding Author: Dr. Wen Xie, Center for Pharmacogenetics, 633 Salk Hall, University of Pittsburgh, Pittsburgh, PA 15261. Telephone, 412-648-9941; Fax, 412-648-1664; E-mail, wex6@pitt.edu

Number of text pages: 25
Number of figures: 1
Number of references: 65
Numbers of words in abstract: 125

Abbreviations: AMPK, AMP-activated protein kinase; CAR, constitutive androstane receptor; CYP, cytochrome P450; LXR, liver X receptor; PB, phenobarbital; PCN, pregnenolone-16α-carbonitrile; PXR, pregnane X receptor; SREBP, sterol regulatory element binding protein; TCPOBOP, 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene; TH, thyroid hormone
Abstract

The pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) are two closely related and liver enriched nuclear hormone receptors originally defined as xenobiotic receptors. PXR and CAR regulate the transcription of drug metabolizing enzymes and transporters, which are essential in protecting our bodies from the accumulation of harmful chemicals. An increasing body of evidence suggests that PXR and CAR also have an endobiotic function to impact energy homeostasis through the regulation of glucose and lipids metabolism. Interestingly and conversely, disruptions of energy homeostasis, such as those observed in obesity and diabetes, also have a major impact on drug metabolism. This review will focus on recent progress in our understanding of the integral role of PXR and CAR in drug metabolism and energy homeostasis.
1. PXR and CAR as master regulators of drug metabolism and drug transporter

Over the long period of evolution, every organism has developed a complex defense system to prevent the accumulation of toxic xenobiotics and endogenous metabolites. While many of the water-soluble chemicals are readily eliminated by transporter proteins, lipophilic compounds often require biotransformation to become more water-soluble before being excreted. The enzymes responsible for biotransformation include Phase I and Phase II enzymes. The cytochrome P450 (CYP) enzymes belong to a superfamily of heme-containing Phase I enzymes that catalyze mono-oxygenase reactions of lipophilic compounds facilitated by the reducing power of the NADPH cytochrome P450 oxidoreductase. Phase II enzymes catalyze the conjugation of water-soluble groups to xeno- and endobiotics. Conjugation reactions include glucuronidation, sulfation, methylation, and N-acetylation. In many cases, biotransformation leads to metabolic inactivation of chemicals. However, biotransformation may also activate the so-called pro-drugs to pharmacologically active products or even to toxic metabolites (Pascussi et al., 2008; Handschin et al., 2003).

Most drug metabolizing enzymes are inducible in response to xenobiotics, which represents an adaptive response of our bodies to chemical insults. The molecular basis for this inducible defense system remained largely unknown until 1998, when pregnane X receptor (PXR, alternatively termed steroid and xenobiotic receptor, or SXR, in humans) was discovered (Blumberg et al., 1998; Kliewer et al., 1998). PXR is expressed predominantly in the liver and intestine, and activated by a wide variety of
natural and synthetic compounds. Upon activation, PXR forms a heterodimer with RXR, and activates the transcription of drug metabolizing enzyme and transporter genes. Examples of PXR target genes include Phase I CYP3As and CYP2Cs, Phase II UDP-glucuronosyltransferase 1A1 (UGT1A1) and sulfotransferases (SULTs), and drug transporters MDR1 and MRP2. PXR has since been established as a xenosensor and master regulator of xenobiotic responses. The essential role of PXR in xenobiotic regulation and in dictating the species specificity of xenobiotic responses was confirmed through the creation and characterization of PXR null mice (Xie et al., 2000; Staudinger et al., 2001), as well as humanized PXR transgenic mice (Xie et al., 2000; Ma et al., 2007).

The constitutive androstane receptor (CAR) is a sister xenobiotic receptor of PXR. Purified first from hepatocytes as a protein bound to the phenobarbital responsive element in the CYP2B gene promoter, CAR was subsequently shown to bind to the CYP2B gene promoter as a heterodimer with RXR. It is generally believed that endogenous CAR resides in the cytoplasm of hepatocytes. Upon exposure to its agonist phenobarbital (PB) or 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), CAR translocates from the cytoplasm to the nucleus and triggers the transcription of its target genes. Transfected CAR exhibited a high basal activity, and thus was once termed a “constitutively active receptor”. The name of constitutive androstane receptor was conceived due to the binding and inhibition of CAR activity by androstanes (Forman et al., 1998). CAR null mice showed a lack of induction of Cyp2b10 and many other Phase I and Phase II enzymes and drug transporters by TCPOBOP and PB in the liver.
and small intestine (Wei et al., 2000).

2. Drug metabolism can be affected by energy metabolism

Drug metabolism can be affected by various pathophysiological factors, including diabetes and liver diseases that may affect the expression or activity of drug metabolizing enzymes and transporters. Nuclear receptors, including the xenobiotic receptors PXR and CAR and sterol sensor liver X receptor (LXR), may function as the links between drug metabolism and energy metabolism.

CYP enzyme down-regulation has been well documented in animal models of obesity, steatosis, and nonalcoholic steatohepatitis (NASH) (Leclercq et al., 1998; Su et al., 1999). Down-regulation of constitutive CYP expression in rat liver was proportional to the extent of hepatic lipid accumulation. Moreover, the capacity of drug metabolism was reported to be impaired in human patients with obesity, hepatic steatosis and NASH (Blouin et al., 1999; Fiatarone et al., 1991; Cheymol et al., 2000). Liver lipid accumulation, especially in the early stage of steatosis, can significantly down-regulate several important CYPs (Zhang et al., 2007), which is consistent with the decreased CYP expression in liver microsomes derived from patients with steatosis and NASH (Fisher et al., 2009; Donato et al., 2006; Donato et al., 2007). A recent report showed that the polyunsaturated fatty acids (PUFAs) can down-regulate PB-induced CYP2B expression in a CAR dependent manner in rat primary hepatocytes (Finn et al., 2009), which was consistent with an earlier report that PUFAs can attenuate PB-induced nuclear accumulation of CAR (Li et al., 2007). Since hepatic steatosis
often leads to increased free fatty acid (FFA) levels, the inhibitory effect of FFAs on CAR provides a plausible explanation for the negative effect of steatosis on drug metabolism. Hepatic steatosis is often associated with increased lipogenesis, in which the sterol regulatory element binding protein 1 (SREBP-1) is a key lipogenic transcription factor. It has been reported that SREBP-1 inhibited the transcriptional activities of PXR and CAR by functioning as a non-DNA binding inhibitor and blocking the interaction of PXR and CAR with nuclear receptor co-factors (Roth et al., 2008a).

The effect of hepatic steatosis on drug metabolism can also be mediated by the lipogenic nuclear receptor LXR through the crosstalk between LXR and CAR. LXRs, both the α and β isoforms, were defined as sterol sensors. In rodents, LXR activation increases hepatic cholesterol catabolism and formation of bile acids by inducing cholesterol 7α-hydroxylase (Cyp7a1) (Peet et al., 1998). LXRs were later found to be an important regulator of SREBP-1c and lipogenesis (Repa et al., 2000). It has been reported that LXR-deficient mice fed with a high-cholesterol diet showed significantly higher induction of Cyp2b10 mRNA level upon PB treatment compared with wild type mice, suggesting that LXR may repress PB-mediated CAR activation and Cyp2b10 induction in vivo (Gnerre et al., 2005). Our lab recently showed that LXRα and CAR are mutually suppressive and functionally related in vivo. Specifically, loss of CAR increased the expression of lipogenic LXR target genes, leading to increased hepatic triglyceride accumulation; whereas activation of CAR inhibited the expression of LXR target genes and LXR ligand-induced lipogenesis. Conversely, a combined loss of
LXR α and β increased the basal expression of xenobiotic CAR target genes; whereas activation of LXR inhibited the expression of CAR target genes and sensitized mice to xenobiotic toxicants. The mutual suppression between LXRα and CAR was also observed in cell culture and reporter gene assays (Zhai et al., 2010).

The AMP-activated protein kinase (AMPK) can also mediate the effect of energy metabolism on drug metabolism. As an important energy sensor, AMPK is activated in response to stresses, such as starvation, exercise and hypoxia, which deplete cellular ATP supplies. Upon activation, AMPK increases energy production such as lipid oxidation, while it decreases energy-consuming processes such as gluconeogenesis and lipogenesis, in order to restore energy balance (Long et al., 2006). Recent reports suggest that activation of AMPK is essential for PB induction of drug metabolizing enzymes in mouse and human livers (Rencurel et al., 2005; Rencurel et al., 2006; Shindo et al., 2007). The induction of Cyp2b10 by PB in primary mouse hepatocytes was associated with an increased AMPK activity, and the treatment of AMPK agonist AICAR triggered the nuclear accumulation of CAR in mouse livers (Shindo et al., 2007). In primary human hepatocytes, treatment with AICAR or metformin, another AMPK agonist, alone can induce CYP2B6 and/or CYP3A4 expression as efficient as PB, whereas overexpression of the dominant-negative mutant AMPKα1 or use of AMPK inhibitor completely blocked the PB induction of CYP2B6 and CYP3A4 (Rencurel et al., 2006). The essential role of AMPK in the induction of Cyp2b10 was further demonstrated in AMPKα1/α2 liver-specific knockout mice, in which the PB- and AICAR induction of Cyp2b10 was blunted (Rencurel et al., 2006).
The link between PB treatment and AMPK activation remains to be firmly established. It was proposed that PB may alter mitochondrial function and trigger the generation of reactive oxygen species, which subsequently activate LKB1 kinase and AMPK phosphorylation (Blattler et al., 2007). The expression of CAR and its target genes has been reported to be regulated by the circadian clock controlled PAR-domain basic leucine zipper (PAR bZip) family of transcription factors. Mice deficient of three PAR bZip proteins were hypersensitive to xenobiotic compounds, and the deficiency in detoxification may contribute to their early aging (Gachon et al., 2009). Interestingly, AMPK can also regulate the circadian clock by phosphorylating and destabilizing the clock component cryptochrome 1 (Lamia et al., 2009). Together, these reports suggest the connections between molecular clocks, energy metabolism, and nuclear receptor signaling pathways.

Diabetes, a major manifestation of the metabolic syndrome, also has an impact on drug metabolism. Drug clearance was significantly increased in untreated patients of type 1 diabetes, which can be reversed by insulin treatment (Zysset et al., 1988; Goldstein et al., 1990). This result was consistent with the observation in a streptozotocin-induced type 1 diabetic mouse model, in which the mice exhibited an increased Cyp2b10 basal expression that can be corrected by insulin treatment (Sakuma et al., 2001). The increased Cyp2b expression in type 1 diabetic mice seemed to be CAR dependent, and was associated with increased activities of PGC-1α and AMPK (Dong et al., 2009a). The increased expression of CYP2b in type 1 diabetes is reminiscent of starvation conditions during which the insulin level is decreased, and the
expression of both cyp2b10 and CAR is induced (Maglich et al., 2004). Consistent with the observations in type 1 diabetes, insulin deprivation enhanced both dexamethasone- and β-naphthoflavone-induced expression of Cyp3a and Cyp1a in rat primary hepatocytes (Sidhu et al., 1999). In contrast, the expression of Cyp2b and 2e was suppressed by insulin in rat hepatoma cells (De et al., 1995). The molecular mechanism for the interaction between the insulin pathway and PXR/CAR activity was suggested by a recent study showing that Forkhead box O1 protein (FOXO1), a member of the insulin sensitive transcription factor family, can interact with and function as a coactivator to CAR- and PXR-mediated transcription (Kodama et al., 2004). It was believed that in conditions of insulin deficiency, such as starvation and type 1 diabetes, FOXO1 translocates into the nucleus, becomes activated, and increases the transcriptional activity of CAR and PXR.

3. Energy metabolism can be affected by drug metabolism and xenobiotic receptors PXR and CAR

3.1 Effects of CYPs and PXR/CAR on hepatic lipid metabolism

CYP enzymes are known to involve in the biotransformation of both xenobiotics such as drugs as well as endobiotics such as cholesterol, steroid hormone, bile acids, and prostanoids. While the role of CYPs in drug metabolism has been well recognized and extensively studied, the endobiotic functions of CYPs and to what extent they will affect systemic or tissue-specific homeostasis are less understood. Because all CYPs
receive electrons from a single donor, cytochrome P450 reductase (CPR, NADPH:ferrihemoprotein reductase) (Smith et al., 1994), deletion of CPR will in principle inactivate all CYPs. Mouse models with liver-specific deletion of CPR were generated by two independent groups (Gu et al., 2003; Henderson et al., 2003). Liver CRP null mice, in which the Cre expression was under the control of the albumin promoter, showed dramatically decreased liver microsomal CYP and heme oxygenase activities as expected (Pass et al., 2005). Surprisingly, the liver of CPR null mice exhibited hepatomegaly and massive hepatic steatosis by two months of age, accompanied by severely reduced circulating cholesterol and triglyceride levels. These results have clearly implicated CYP activity in hepatic lipid homeostasis. Given the fact that the liver CPR null mice have their CPR gene deletion occur neonatally, as controlled by the albumin promoter, a subsequent study was designed to investigate the effect of CYP inactivation in adult mice by using a conditional knockout strategy. The conditional knockout of CRP was achieved by crossing the CRP floxed mice with the CYP1A1-Cre transgenic mice in which the expression of Cre recombinase is controlled by the rat CYP1A1 gene promoter. The CYP1A1-Cre transgene has a low basal expression, but its expression can be markedly induced by a pharmacological administration of 3-methlycholanthrene, an aryl hydrocarbon receptor agonist, leading to a time-dependent and hepatic specific deletion of CRP (Finn et al., 2007). Associated with the time-dependent reduction of CPR expression in the liver after the treatment of 3-methlycholanthrene in adult mice, these animals developed fatty liver and had reduced non-fasting plasma cholesterol and triglyceride levels. The hepatic steatosis is
mainly accounted by the accumulation of triglycerides. The source of accumulated triglycerides was believed to be dietary fatty acids, because a fat-deficient diet can reverse the steatotic phenotype (Finn et al., 2009).

The xenobiotic receptor CAR also plays an important role in hepatic lipid homeostasis. Activation of CAR by its agonist TCPOBOP has been reported to inhibit the expression of hepatic lipogenic genes and alleviate hepatic steatosis in high-fat diet fed mice and ob/ob mice (Gao et al., 2009; Dong et al., 2009b). Several possible but not mutually exclusive molecular mechanisms have been proposed to explain the inhibitory effect of CAR on lipogenesis. In one study, it was suggested that activation of CAR or PXR reduced the level of SREBP-1 by inducing Insig-1, a protein blocking the proteolytic activation of SREBPs (Roth et al., 2008b). Our own study suggested that CAR may inhibit lipogenesis by inhibiting the LXR agonist responsive recruitment of LXRα to the Srebp-1c gene promoter (Zhai et al., 2010).

In contrast to the inhibitory effect of CAR on lipogenesis, activation of PXR seemed to promote lipogenesis. Transgenic mice expressing a constitutively activated PXR showed hepatomegaly and marked hepatic steatosis, and treatment of mice with a PXR agonist elicited a similar effect. PXR induced lipogenesis was independent of the activation of SREBP-1c, and was associated with the induction of fatty acid translocase (FAT/CD36), peroxisome proliferator-activated receptor γ2 (PPARγ2), and stearoyl-CoA desaturase-1 (SCD-1) (Zhou et al., 2006). Further promoter analyses showed that both CD36 and PPARγ2 are direct transcriptional targets of PXR (Zhou et al., 2006; 2008). A recent report showed that the thyroid hormone-responsive spot 14...
protein (THRSP), whose expression correlates with lipogenesis, was a PXR target gene (Breuker et al., 2010). Therefore, the induction of spot 14 may have also contributed to the lipogenic effect of PXR. Treatment of mice with the PXR agonist PCN is known to alleviate the lithocholic acid-induced hepatotoxicity (Xie et al., 2001; Staudinger et al., 2001). It was recently reported that the PCN-mediated stimulation of lipogenesis contributes to the protection from lithocholic acid-induced hepatotoxicity (Miyata et al., 2010).

The steatotic effect of PXR was also associated with suppression of several genes involved in fatty acid β-oxidation. Activation of PXR by its agonist PCN can inhibit lipid oxidation by down-regulating the mRNA expression of carnitine palmitoyltransferase 1α (CPT1α) and mitochondrial 3-hydroxy-3-methylgluturate-CoA synthase 2 (HMGCS2), two key enzymes involved in β-oxidation and ketogenesis, in a PXR dependent manner. Mechanistically, it has been shown that PXR can directly interact with Forkhead box A2 (FoxA2), and prevent FoxA2 binding to the Cpt1a and Hmgcs2 gene promoters (Nakamura et al., 2007).

Finally, unlike CAR, activation of PXR had little effect on the lipogenic effect of LXR (Zhai et al., 2010).

3.2 Effects of PXR and CAR on hepatic glucose metabolism

Chronic treatment with PB has been reported to decrease plasma glucose levels and improve insulin sensitivity in diabetic patients (Lahtela et al., 1985). Treatment with PB suppressed the expression of gluconeogenic enzymes PEPCK1 and G6Pase in mouse
liver (Ueda et al., 2002) and in primary rat hepatocytes (Argaud et al., 1991). The regulation of the gluconeogenic pathway by PXR was initially suggested by the suppression of PEPCK and G6Pase in VP-hPXR transgenic mice (Zhou et al., 2006). Treatment of wild type mice with the PXR agonist PCN also suppressed cAMP-dependent induction of G6Pase in a PXR-dependent manner (Kodama et al., 2004). Several possible but not mutually exclusive molecular mechanisms have been proposed to explain the inhibitory effect of PXR and CAR on gluconeogenesis. It was reported that PXR can form a complex with phosphorylated CREB in a ligand-dependent manner to prevent CREB binding to the CRE and inhibit CREB-mediated transcription of G6Pase (Kodama et al., 2007). PXR and CAR can also physically bind to FoxO1 and suppress its transcriptional activity by preventing its binding to the insulin response sequence (IRS) in the gluconeogenic enzyme gene promoters (Kodama et al., 2004). PXR and CAR may also inhibit hepatocyte nuclear factor-4α (HNF4α) activity by competing for the DR1 binding motif in the gluconeogenic enzyme gene promoters (Miao et al., 2006). The in vivo significance of CAR-mediated suppression of gluconeogenesis was supported by two recent reports that activation of CAR ameliorated hyperglycemia and improved insulin sensitivity in ob/ob mice and high-fat diet fed wild type mice (Gao et al., 2009; Dong et al., 2009b).

3.3 CAR may impact energy metabolism and fasting response by affecting thyroid hormone metabolism

It has been reported that the expression of CAR was inducted during long-term fasting,
possibly due to the up-regulation of PGC-1α (Ding et al., 2006). Moreover, CAR-deficient mice were defective in fasting adaptation and lost more weight during calorie restriction (Maglich et al., 2004). One possible mechanism by which CAR affects fasting response is through the effect of CAR on thyroid hormone (TH) metabolism. Treatment of wild type mice with a CAR agonist decreased serum T₄ level, (Qatanani et al., 2005). The decrease in T₄ level was associated with a concomitant increase in the serum thyroid-stimulating hormone (TSH) level in wild type but not in CAR⁻/⁻ mice. The effect of CAR on TH homeostasis was believed to be achieved through the regulation of TH-metabolizing Phase II enzymes. Activation of CAR induced the expression of glucuronosyltransferases UGT1A1 and 2B1, and sulfotransferases SULT2A1, 1C1, and 1E1 in wild type mice but not in CAR⁻/⁻ mice. UGT1A1 and UGT2B1 are responsible for T₄ and T₃ glucuronidation, respectively (Visser et al., 1993a; Visser et al., 1993b). SULTs are important for the inactivation of THs by blocking outer ring deiodination and causing irreversible inactivation of THs (Visser et al., 1994). However, the net effect of CAR on TH homeostasis remains controversial. It was reported that activation of CAR decreased the serum rT₃ level, resulting in the de-suppression of thyroid hormone target genes during partial hepatectomy (Tien et al., 2007).

4. Conclusions and perspectives

Figure 1 summarizes the major interactions between drug metabolism and energy metabolism and the central roles of PXR and CAR in these crosstalks. Induction or
suppression of CYPs and other drug-metabolizing enzymes can alter drug metabolism and clearance. Our understanding of the regulation of drug metabolism and activities of PXR and CAR by liver energy status may offer possible explanations for the variation of drug responses associated with metabolic diseases and provide rationale for appropriate dose adjustment. On the other hand, activation of PXR and CAR could have a significant impact on energy homeostasis by affecting lipogenesis, gluconeogenesis, and fatty acid oxidation. It is tempting to speculate that pharmacological modulation of PXR and CAR may be beneficial in managing metabolic diseases.

Among the remaining challenges, although PXR has been shown to affect lipid and glucose metabolism, the *in vivo* role of PXR in obesity and type II diabetes remains to be demonstrated. The endogenous ligands for PXR and CAR that elicit the metabolic functions of these two receptors also remain to be identified.
References


Dong B, Qatanani M and Moore DD (2009a) Constitutive androstane receptor mediates


Goldstein S, Simpson A and Saenger P (1990) Hepatic drug metabolism is increased in
poorly controlled insulin-dependent diabetes mellitus. *Acta Endocrinol* (Copenh) **123**:550-556.


Footnotes:

The original research of ours described in this article was supported in part by National Institutes of Health (NIH) grants [ES014626 and DK076962], and a Molecular Pharmacology Fellowship funded by the University of Pittsburgh Department of Pharmacology and Chemical Biology.
Fig. 1. Summary of the major interactions between drug metabolism and energy metabolism and the central roles of PXR and CAR in these crosstalks. Note the drug metabolism and energy status can be affected by pathophysiological conditions, such as obesity, diabetes, and fatty liver. AMPK, AMP-activated protein kinase; CAR, constitutive androstane receptor; CREB, cAMP response element-binding protein; DME, drug metabolizing enzymes; FFA, free fatty acids; FOXA2, Forkhead box A2 protein; FOXO1, Forkhead box O1 protein; HNF4α, hepatocyte nuclear factor 4α; LXR, liver X receptor; PXR, pregnane X receptor; SREBP, sterol regulatory element-binding protein.