molecular pharmaceutics

reviews

Xenoreceptors CAR and PXR Activation and Consequences on Lipid Metabolism, Glucose Homeostasis, and Inflammatory Response

Amélie Moreau, Marie José Vilarem, Patrick Maurel, and Jean Marc Pascussi*

Inserm, UMR-U632, 1919 route de Mende, F-34293 Montpellier, France, and University Montpellier 1, F34000 Montpellier, France

Received July 31, 2007; Revised Manuscript Received October 3, 2007; Accepted October 5, 2007

Abstract: Xenobiotic and drug metabolism and transport are managed by a large number of genes coordinately regulated by at least three nuclear receptors or xenosensors: aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR, NR1I3), and pregnane X receptor (PXR, NR1I2). Initially characterized as xenosensors, it is now evident that CAR and PXR also trigger pleiotropic effects on liver function. Recent studies have shown the existence of crosstalk between xenosensors and other nuclear receptors or transcription factors controlling endogenous signaling pathways which regulate physiological functions. This review is focused on recent observations showing that activation of CAR and PXR alters lipid metabolism, glucose homeostasis, and inflammation by interfering with HNF4α, FoxO1, FoxA2, PGC1α, or NFkB p65. Such crosstalks explain clinical observations and provide molecular mechanisms allowing understanding how xenobiotics and drugs may affect physiological functions and provoke endocrine disruptions.

Keywords: PXR; CAR; lipid metabolism; glucose metabolism; inflammation

1. Introduction

The threat represented by xenobiotics for the organism is the target of a complex strategy. Metabolism of drugs and other xenobiotics in the liver is our body's primary defense against accumulation of potentially toxic lipophilic compounds. This strategy comprises xenosensors dedicated to recognition of xenobiotics and other dangerous endogenous molecules and xenobiotic metabolizing and transporters systems (XMTS). The first step of the mechanism called "detoxification" is the xenobiotic detection by the xenosensors AhR (aryl hydrocarbon receptor), PXR (pregnane X receptor), and/or CAR (constitutive androstane receptor).

AhR is a member of the Per/ARNT/Sim (PAS) family of transcription factors, mainly involved in the regulation of CYP1s, UDP-glucuronyl transferase 1A1 (UGT1A1) and 1A6 (UGT1A6), glutathione S-transferase A2 (GSTA2), aldehyde dehydrogenase (ALDH3), or NADPH:quinone oxidoreductase (NQOR). AhR ligands are the hydrophobic environmental pollutants of polyhalogenated aromatic hydrocarbons, such as polychlorinated dibenzodioxins, dibenzofurans and coplanar biphenyls, or polycyclic aromatic hydrocarbons, e.g., benzo(*a*)pyrene, 3-methylcholanthrene, benzoflavones, and omeprazole.

In contrast, CAR and PXR belong to the nuclear receptor superfamily. They form heterodimers with the retinoic X receptor (RXR, NR1B2). They are activated by a variety of ligands and notably used in therapeutic molecules, insecticides, pesticides, and nutritional compounds. Currently, it remains uncertain whether these receptors have a high-affinity ligand or instead function as more generalized steroid/xenobiotic sensors. These xenosensors coordinate the expression of several genes encoding the most appropriate series of XMTS to inactivate and/or eliminate xenobiotics. At least four phases occur in the XMTS process: (i) xenobiotic uptake (solute carrier organic anion transporters, OATPs), (ii) xenobiotic oxidation mostly performed by the cytochrome P450 subfamilies (CYPs) 1−3, (iii) xenobiotic conjugation achieved by UGT, GST, or sulfotransferases, and (iv) xenobiotic efflux performed by transporters such as MDR1 (ATP-binding cassette, subfamily B, member 1), or MRP2 (ATP-binding cassette, subfamily C, member 2). Although the induced proteins are responsible for the

* To whom correspondence should be addressed. Mailing address: Inserm, U632, 1919 Route de Mende, F-34293 Montpellier, France. Phone: 33 4 67 61 33 69. Fax: 33 4 67 52 36 81. E-mail: pascussi@montp.inserm.fr.

10.1021/mp700103m CCC: $40.75
Published on Web 12/27/2007

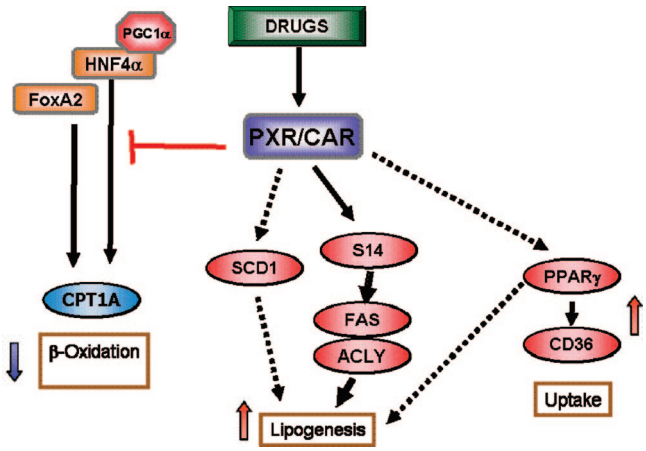

Figure 1. Schematic representation of crosstalks involved in PXR- and CAR-mediated lipid homeostasis alteration. Arrows indicate activation, while stop bars indicate repression. Pathways found only in mice (hatched arrow) or in human (double arrow) are also indicated.

metabolism, deactivation, and transport of environmental chemicals and drugs, they are also involved in bile acid, thyroid, and steroid hormone catabolism, so that xenobiotics may interfere with normal physiological functions.

Whereas these two receptors share some common ligands and regulate overlapping sets of target genes, their mode of activation is quite different. PXR is located in the nuclei; it has a low basal activity and is highly activated upon ligand binding. In contrast, in the noninduced state, CAR resides in the cytoplasm. After treatment with activators such as phenobarbital, CAR shuttles to the nucleus to activate its target genes. Moreover, CAR localization and activity are regulated by various protein phosphorylation events.

Xenobiotics toxicity is currently described as resulting from two major contributions: the chemical reactivity of metabolites generated through biotransformation, leading to covalent binding to DNA, proteins and lipids, and drug−drug interactions. Now, a new aspect should be considered as recent developments reveal that xenosensors are able to establish crosstalk with many signaling pathways so that xenobiotics may affect other biological functions either positively or negatively. This review summarizes recent reports on the incidence of CAR and PXR activation on lipid metabolism, glucose homeostasis, and inflammation and evaluates potential adverse physiological consequences of PXR and CAR activation.

2. Effect of CAR and PXR Activation on Lipid Metabolism

Lipid homeostasis is accomplished by complex physiological mechanisms. Disruption of lipid formation and catabolism has been implicated in various metabolic diseases such as obesity and diabetes. Hepatic lipid homeostasis is tightly maintained by balanced lipid formation (lipogenesis), catabolism (β-oxidation), lipid uptake, and secretion (Figure 1). A number of clinical observations have shown that many drugs, now identified as CAR and/or PXR activators, affect lipid metabolism in patients. Notably, treatment with rifampicin (a potent human PXR activator) appears to induce liver steatosis in tuberculosis patients[1] as well as the steatogenic effect in rat liver.[2,3] In addition, carbamazepine, a recently characterized PXR activator,[4] is known to induce sporadic events of steatosis/steatohepatitis.[5] Finally, long-term use of phenobarbital (CAR activator) as an antiepileptic drug has shown significant changes in patients' hepatic and plasma lipid profiles.[6,7]

2A. CAR and PXR Inhibit Lipid Catabolism (β-Oxidation). Several observations suggest that CAR and PXR activation provokes a decrease of β-oxidation-related gene expression. For example, CAR interferes with one of the major activators of fatty acid metabolism by binding to DNA elements overlapping with the peroxisome proliferator-activated receptor α (PPARα) in the promoter of enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, the second enzyme of peroxisomal fatty acid β-oxidation.[8] In addition, phenobarbital decreases both the mitochondrial carnitine palmitoyltransferase 1 (CPT1) and the enoyl CoA isomerase (ECI) mRNA content in wild type mice but not in CAR −/− mice,[9] and the rodent PXR agonist pregnenolone 16α-carbonitrile (PCN) down-regulates the mRNA level of CPT1 in wild-type but not in PXR −/− mice.[10]

The transcriptional regulation of CPT1 is tightly regulated by several transcription factors and cofactors such as the hepatocyte nuclear factor 3 β (FoxA-2), PPARα, thyroid hormone receptor (TR), and the peroxisome proliferator activated receptor γ coactivator 1 α (PGC1α). At least two independent pathways could be involved in CAR- and PXR-

(1) Morere, P. Information obtained by liver biopsy in 100 tuberculous patients. *Sem. Hop.* **1975**, *51* (31–34), 2095–102.
(2) Piriou, A. Fatty liver induced by high doses of rifampicin in the rat: possible relation with an inhibition of RNA polymerases in eukariotic cells. *Arch. Toxicol. Suppl.* **1979**, (2), 333–7.
(3) Truhaut, R. Liver steatogenic power of high doses of rifampicin in rats. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D* **1978**, *286* (6), 493–7.
(4) Oscarson, M. Transcriptional profiling of genes induced in the livers of patients treated with carbamazepine. *Clin. Pharmacol. Ther.* **2006**, *80* (5), 440–456.
(5) Grieco, A. Fatty liver and drugs. *Eur. Rev. Med. Pharmacol. Sci.* **2005**, *9* (5), 261–3.
(6) Calandre, E. P. Serum lipids, lipoproteins and apolipoproteins A and B in epileptic patients treated with valproic acid, carbamazepine or phenobarbital. *Acta Neurol. Scand.* **1991**, *83* (4), 250–3.
(7) Luoma, P. V. Microsomal enzyme induction, lipoproteins and atherosclerosis. *Pharmacol. Toxicol.* **1988**, *62* (5), 243–9.
(8) Kassam, A. The peroxisome proliferator response element of the gene encoding the peroxisomal beta-oxidation enzyme enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase is a target for constitutive androstane receptor beta/9-cis-retinoic acid receptor-mediated transactivation. *J. Biol. Chem.* **2000**, *275* (6), 4345–50.
(9) Ueda, A. Diverse roles of the nuclear orphan receptor CAR in regulating hepatic genes in response to phenobarbital. *Mol. Pharmacol.* **2002**, *61* (1), 1–6.
(10) Nakamura, K. Nuclear pregnane X receptor cross-talk with FoxA-2 to mediate drug-induced regulation of lipid metabolism in fasting mouse liver. *J. Biol. Chem.* **2007**, *282* (13), 9768–76.

mediated CPT1 gene repression by interfering with positive regulators of CPT1 gene transcription, one involving FoxA-2 and the other involving HNF4α.

Fox (Fork head box) genes encode a subgroup of the helix-turn-helix class of remarkably conserved transcription factors (>50 genes in man). FoxA-2 is a transcriptional activator of important metabolic genes in the liver that are suppressed by the action of insulin. At low insulin levels, FoxA-2 has been shown to regulate positively β-oxidation by controlling the transcription of target genes including CPT1.[11] Activation of phosphatidylinositol 3-kinase-Akt by insulin induces FoxA2 phosphorylation, nuclear exclusion, and inhibition of FoxA-2-dependent transcriptional activity.[12] Using *Pxr−/−* and wild-type starving mice, Nakamura et al.[10] recently reported that activated PXR physically interacts with FoxA-2 through their ligand and DNA binding domains, respectively. This interaction prevents the binding of FoxA-2 to its DNA response elements in CPT1 promoter. Whether such interaction between CAR and FoxA-2 occurs is currently unknown.

In addition, it has been shown that HNF4 can activate CPT1A gene transcription through binding to a nuclear receptor motif arranged as a direct repeat of hexamer half-sites with a spacing of 1 nucleotide (DR-1) located 3 kb upstream of the transcription start site,[13] while TR increases rat CPT1A gene expression through thyroid hormone receptor binding to a thyroid hormone receptor responsive element located 2.9 kb upstream of the transcription start site.[14] As other nuclear receptors, HNF4α and TR require cofactors recruitment to efficiently increase the transcription rate of their target genes. PGC1α is one of these cofactors. Indeed, PCG1α has been shown to be necessary for full transactivation of CPT1A by HNF4α and TR.[8,13,15] However, PGC1α is also required for numerous nuclear receptors transcriptional activities, such as PPARα, PPARγ, TR, RXR, GR (glucocorticoid receptor), CAR, and PXR. Thus, PGC1α appears to represent a convergence point of crosstalk between xenobiotic and other signaling pathways. Recently, it has been proposed that PGC-1α intracellular level is present in limiting amounts in hepatocytes. Notably, it has been demonstrated that ligand-activated CAR and PXR interfere with HNF4α signaling by targeting the common coactivator PGC1α, via the so-called "squelching effect". Both CAR and PXR interact with PGC1α.[16,17] The increase of CAR/PGC1α or PXR/PGC1α complex formation, and the concomitant decrease of HNF4α/PGC-1α complex formation, leads to the down-regulation of CYP7A1 gene expression.[16,18,19] In addition, it has been observed that ligand-activated PXR directly and strongly interacts with HNF4α and blocks HNF4α interaction with coactivator PGC1α.[16] Since HNF4α and PGC1α are jointly involved in the control of CPT1A, it is likely that this crosstalk applies to this gene.

2B. PXR Increases Fatty Acid Uptake in Mice Liver. Recently, it has been reported that PXR activation causes hepatic steatosis characterized by a marked accumulation of triglycerides in mice liver.[10,20] Further analysis revealed that CD36 was induced in mice hepatocyte in a PXR-dependent manner.[20] CD36 is a fatty acid translocase involved in long-chain fatty acid (LCFA) transport. This class B scavenger receptor is involved in high-affinity peripheral fatty acid uptake, and mice lacking CD36 exhibit increased plasma free fatty acid and triglyceride (TG) levels.[21] CD36 transcription is activated by fatty acids and by the nuclear transcription factor PPARγ.[22] Recently, Zhou and colleagues have shown that mice CD36 gene promoter contains a functional PXR DR3-type response element,[20] thus confirming that CD36 is a direct transcriptional target of PXR. In addition, hepatic lipid accumulation and CD36 induction were also observed in the hPXR "humanized" mice treated with the hPXR agonist rifampicin.

(11) Wolfrum, C. Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes. *Nature* **2004**, *432* (7020), 1027–32.

(12) Wolfrum, C. Insulin regulates the activity of forkhead transcription factor Hnf-3beta/Foxa-2 by Akt-mediated phosphorylation and nuclear/cytosolic localization. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (20), 11624–9.

(13) Louet, J. F. The coactivator PGC-1 is involved in the regulation of the liver carnitine palmitoyltransferase I gene expression by cAMP in combination with HNF4 alpha and cAMP-response element-binding protein (CREB). *J. Biol. Chem.* **2002**, *277* (41), 37991–8000.

(14) Zhang, Y. Peroxisomal proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1 alpha) enhances the thyroid hormone induction of carnitine palmitoyltransferase I (CPT-I alpha). *J. Biol. Chem.* **2004**, *279* (52), 53963–71.

(15) Napal, L.; Marrero, P. F.; Haro, D. An intronic peroxisome proliferator-activated receptor-binding sequence mediates fatty acid induction of the human carnitine palmitoyltransferase 1A. *J. Mol. Biol.* **2005**, *354* (4), 751–9.

(16) Li, T.; Chiang, J. Y. Mechanism of rifampicin and pregnane X receptor inhibition of human cholesterol 7 alpha-hydroxylase gene transcription. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2005**, *288* (1), G74–84.

(17) Shiraki, T. Activation of orphan nuclear constitutive androstane receptor requires subnuclear targeting by peroxisome proliferator-activated receptor gamma coactivator-1 alpha. A possible link between xenobiotic response and nutritional state. *J. Biol. Chem.* **2003**, *278* (13), 11344–50.

(18) Bhalla, S. Ligand-activated pregnane X receptor interferes with HNF-4 signaling by targeting a common coactivator PGC-1alpha. Functional implications in hepatic cholesterol and glucose metabolism. *J. Biol. Chem.* **2004**, *279* (43), 45139–47.

(19) Miao, J. Functional inhibitory cross-talk between constitutive androstane receptor and hepatic nuclear factor-4 in hepatic lipid/glucose metabolism is mediated by competition for binding to the DR1 motif and to the common coactivators, GRIP-1 and PGC-1alpha. *J. Biol. Chem.* **2006**, *281* (21), 14537–46.

(20) Zhou, J. A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. *J. Biol. Chem.* **2006**, *281* (21), 15013–20.

(21) Coburn, C. T. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J. Biol. Chem.* **2000**, *275* (42), 32523–9.

(22) Feng, J. Induction of CD36 expression by oxidized LDL and IL-4 by a common signaling pathway dependent on protein kinase C and PPAR-gamma. *J. Lipid Res.* **2000**, *41* (5), 688–96.

2C. PXR Increases Hepatic Expression of Transcription Factors and Enzymes Involved in Lipogenesis. *In vivo* in mice, pharmacological activation of PXR, or the use of a constitutively activated mutant of PXR have shown that the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) and two lipogenic enzymes, i.e., stearyl CoA desaturase (SCD1) and fatty acid elongase, were induced at mRNA levels in a PXR-dependent manner.[10,20] Moreover, both PXR-mediated triglyceride accumulation and lipogenesis-related gene induction seems to be independent of the activation of the lipogenic transcriptional factor SREBP1c (sterol regulatory element binding transcription factor 1),[20] suggesting that these effects were independent of the classical SREPB1c lipogenic pathway. Indeed SREPB1c mRNA and protein expression levels (both the precursor and the mature forms) are unaffected after PXR activation in mice[20] and in human hepatocyte (Moreau et al. Manuscript in preparation).

The role of SCD1 and fatty acid elongase as accessory lipogenic enzymes is well documented; however, the role and the importance of PPARγ in lipogenesis is less understood. PPARγ is a member of the nuclear receptor superfamily and a key regulator of adipogenesis and lipid storage. While PPARγ2 expression is limited exclusively to adipose tissue, PPARγ1 is expressed at relatively low levels in many tissues including the liver. Although the mechanism of induction of PPARγ by PXR is not yet known, the finding that expression of this receptor is dependent on PXR suggests a potential crosstalk between both pathways, the consequence of which is hepatic steatosis. Interestingly, two recent reports have shown that overexpression of PPARγ in mice induced hepatic steatosis.[23,24] Northern blotting and gene expression profiling results showed that lipogenesis-related genes including adipsin, adiponectin, fasting-induced adipose factor, fat-specific gene 27 (FSP27), CD36, SCD1, and malic enzyme (ME), among others, were markedly induced in PPARγ transgenic mice. These results suggest that a high level of PPARγ in mouse liver is sufficient for the induction of adipogenic transformation of hepatocytes with adipose tissue-specific gene expression and lipid accumulation. However, we failed to observe any up-regulation of PPARγ1, PPARγ2, or CD36 in primary culture of human hepatocytes treated with typical hPXR agonist such as rifampicin or SR12813 (Moreau et al. Manuscript in preparation), suggesting that there may be an important species-specificity in the ability of PXR to activate these lipogenesis-related target genes or that additional factors lacking in cultured hepatocytes, are required for these up-regulation, in contrast to the *in vivo* situation.

Interestingly, we recently observed that S14 (or THRSP or SPOT14), a protein suspected to be implicated in lipogenesis, is a PXR target gene in human hepatocytes in primary culture and mouse liver (Moreau et al. Manuscript in preparation). S14 is a small (about 17 kDa) acidic (pI 4.65) protein with no sequence similarity to any other functional motif.[25] It is predominantly expressed in tissues producing lipids such as the liver, white and brown adipose tissues, and the lactating mammary glands. Thyroid hormone regulates S14 gene expression both in vivo and in vitro,[26,27] and functional Thyroid Hormone Response Elements (TREs) in the rat and human S14 gene were mapped.[26] Although the biochemical mechanism of its action is unknown, it is clear that S14 protein acts to transduce hormone- and nutrient-related signals to genes involved in lipid metabolism.[25] Accumulated evidence suggests that S14 could play an important role in the induction of lipogenic enzymes.[25,29–31] Notably, antisense experiments performed in mice hepatocytes have demonstrated that S14 regulates the activation of lipogenic enzymes transcription, notably ATP citrate lyase (ACLY) and the fatty acid synthase (FASN), as well as the triacylglycerol formation by stimuli such as carbohydrate feeding and thyroid hormone administration.[29] In addition, S14 overexpression in MCF-7 human breast cancer cells promoted neutral lipid vacuoles accumulation.[30] We observed a delayed up-regulation of ACLY and FASN in human hepatocytes after long-term activation of PXR. These results are in contrast with those previously reported by Zhou and colleagues in mice, where both FASN and ACLY mRNA levels were not affected by PXR activation.[20] Thus, here again, it appears that the effect of PXR on lipogenic genes expression remain complex and require further investigation as PXR induces CD36 and PPARγ gene expression *in vivo* in mice but not in human hepatocytes, while PXR induces the S14/FASN-ACLY cascade in primary human hepatocyte but not *in vivo* in mice.

(23) Schadinger, S. E. PPARgamma2 regulates lipogenesis and lipid accumulation in steatotic hepatocytes. *Am. J. Physiol. Endocrinol. Metab.* **2005**, *288* (6), E1195–205.

(24) Zhang, Y. L. Aberrant hepatic expression of PPARgamma2 stimulates hepatic lipogenesis in a mouse model of obesity, insulin resistance, dyslipidemia, and hepatic steatosis. *J. Biol. Chem.* **2006**, *281* (49), 37603–15.

(25) LaFave, L. T.; Augustin, L. B.; Mariash, C. N. S14: insights from knockout mice. *Endocrinology* **2006**, *147* (9), 4044–7.

(26) Campbell, M. C.; Anderson, G. W.; Mariash, C. N. Human spot 14 glucose and thyroid hormone response: characterization and thyroid hormone response element identification. *Endocrinology* **2003**, *144* (12), 5242–8.

(27) Feng, X. Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray. *Mol. Endocrinol.* **2000**, *14* (7), 947–55.

(28) Oppenheimer, J. H. Regulation of gene S-14 by triiodothyronine in liver. *Horm. Metab. Res. Suppl.* **1987**, *17*, 1–5.

(29) Kinlaw, W. B. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. *J. Biol. Chem.* **1995**, *270* (28), 16615–8.

(30) Sanchez-Rodriguez, J. The spot 14 protein inhibits growth and induces differentiation and cell death of human MCF-7 breast cancer cells. *Biochem. J.* **2005**, *390* (Pt 1), 57–65.

(31) Zhu, Q. The Spot 14 protein is required for de novo lipid synthesis in the lactating mammary gland. *Endocrinology* **2005**, *146* (8), 3343–50.

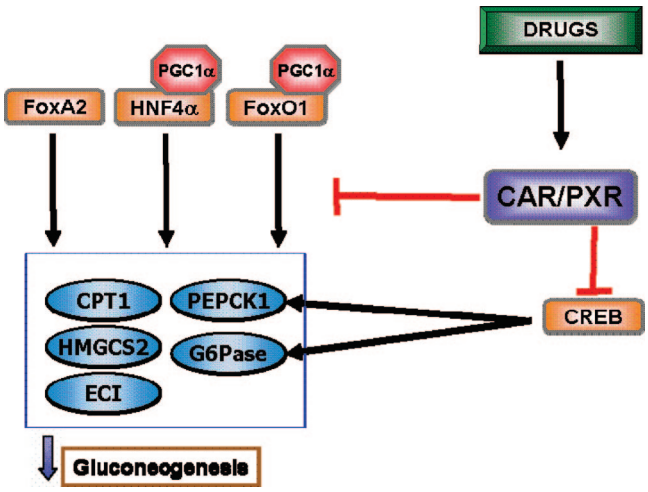

Figure 2. Schematic representation of crosstalks involved in PXR- and CAR-mediated glucose homeostasis alteration. Arrows indicate activation, while stop bars indicate repression.

3. Effect of CAR and PXR Activation on Glucose Metabolism

Hepatic gluconeogenesis is tightly controlled by insulin and glucagon and plays a major role for survival during fasting or starvation (Figure 2). Genes involved in gluconeogenesis include notably phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). G6Pase is the critical enzyme that controls the serum level of glucose by catalyzing the dephosphorylation of glucose-6-phosphate generated from both gluconeogenesis and glycogenolysis. PEPCK catalyzes the formation of phosphoenolpyruvate from oxaloacetate, with the release of carbon dioxide and GDP. In the liver, gluconeogenesis is controlled positively by glucocorticoids, cAMP, and glucagon and negatively by insulin and glucose. Previous observations revealed functional links between insulin- and xenobiotic-mediated pathways.[32] It has long been known that treatment with drugs, which are now known as activators of nuclear receptors CAR and PXR, represses hepatic gluconeogenic enzymes and genes.[9,18,33,34] Phenobarbital has been shown to decrease plasma glucose in diabetic patients.[35] Treatment with the mouse PXR activator PCN decreased blood glucose levels in fasting wild-type but not *Pxr−/−* mice.[10] In addition, *pepck1* and *G6Pase* genes are up-regulated in the transgenic mice expressing constitutively activated PXR.[20] Although the exact mechanism remains unclear, most of the data obtained so far strongly suggest that CAR and PXR activation repress the glucogenic pathway by interfering with transcription factors or cofactors involved in the transcriptional regulation of these gluconeogenic enzymes.

3A. CAR and PXR Interfere with the Gluconeogenic Function of HNF4α. Hepatic gluconeogenic enzymes such as PEPCK1 are repressed in phenobarbital treated rodents[36] but not in CAR knockout mice.[9,34] In addition, PXR activation has been reported to decrease both *G6Pase* and *Pepck1* genes expression in mice,[10] while *G6Pase* and *Pepck1* genes were found to be down-regulated in transgenic mice bearing a constitutively activated mutant of hPXR.[20] The effect of CAR and PXR on CPT1 gene expression has already been discussed (see section 2A). On the other hand, the CAR-mediated repression of PEPCK1 seems to involve at least two molecular mechanisms: (i) the competition with HNF4α for the binding of a DR1 element within the PEPCK gene promoter and (ii) the inhibition of the HNF4α transcriptional activity by the squelching of PGC1α.[19] These two mechanisms seem to permit a knock down of the gluconeogenic function of HNF4α. Note that PXR is also able to recruit PGC1α, suggesting that PXR activation could also provoke PEPCK1 down-regulation by interfering with HNF4α.

3B. CAR and PXR Inhibits FoxO1 Activity. FoxO1 belongs to the fork head family of transcription factors which are characterized by a distinct fork head domain. FoxO1 positively controls the expression of genes involved in gluconeogenesis and it represents the target of insulin repressive action on the gluconeogenesis pathway. Insulin activates the PI3K-Akt pathway leading to the phosphorylation FoxO1 which is no longer retained in the nucleus and is unable to bind PGC-1α, and thus makes it transcriptionally inactive. FoxO1 has been shown to interact with several nuclear receptors in a ligand-dependent (ER) or -independent (TR or RAR) manner and behaves either as corepressor (HNF4, GR, PR, ER)[37] or coactivator (RAR, TR).[38] Kodama et al. reported on the crosstalk between FoxO1 and CAR/PXR.[34] By performing a yeast two-hybrid screening, these authors identified FoxO1 as a CAR-binding protein, acting as a coactivator of CAR transcriptional activity. In addition, these authors demonstrated that FoxO1 similarly binds to and activates PXR. On the other hand, both CAR and PXR inactivate FoxO1 transcriptional activity by preventing its binding to its responsive element IRS in target genes

(32) Tien, E. S.; Negishi, M. Nuclear receptors CAR and PXR in the regulation of hepatic metabolism. *Xenobiotica* **2006**, *36* (10–11), 1152–63.
(33) Argaud, D. Inhibition of gluconeogenesis in isolated rat hepatocytes after chronic treatment with phenobarbital. *Biochem. J.* **1991**, *280* (Pt 3), 663–9.
(34) Kodama, S. Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Mol. Cell. Biol.* **2004**, *24* (18), 7931–40.
(35) Lahtela, J. T.; Arranto, A. J.; Sotaniemi, E. A. Enzyme inducers improve insulin sensitivity in non-insulin-dependent diabetic subjects. *Diabetes* **1985**, *34* (9), 911–6.
(36) Fox, G. R.; Virgo, B. B. The effects of phenobarbital, atropine, L-alpha-methyldopa, and DL-propranolol on dieldrin-induced hyperglycemia in the adult rat. *Toxicol. Appl. Pharmacol.* **1985**, *78* (3), 342–50.
(37) Fan, W. Insulin-like growth factor 1/insulin signaling activates androgen signaling through direct interactions of FoxO1 with androgen receptor. *J. Biol. Chem.* **2007**, *282* (10), 7329–38.
(38) Zhao, H. H. Forkhead homologue in rhabdomyosarcoma functions as a bifunctional nuclear receptor-interacting protein with both coactivator and corepressor functions. *J. Biol. Chem.* **2001**, *276* (30), 27907–12.

promoters such as PEPCK and G6Pase. In addition, as PGC1α is also a cofactor of FoxO-1 and CREB-mediated regulation of hepatic gluconeogenesis such as PEPCK,[39,40] another mechanism implicating a squelching as the one described between CAR, PXR, and HNF4α for PGC1α could be envisaged.

3C. PXR Inhibits FoxA-2 Activity. FoxA-2 is a key regulatory factor that activates both Cpt1a and mitochondrial 3-hydroxy-3-methylglutarate-CoA synthase 2 (Hmgcs2) gene expression in fasting mouse liver.[11] Hmgcs2 is involved in ketone bodies (notably 3-hydroxybutylate) production by the liver through the ketogenesis pathway. These ketone bodies are the major energy source to the extra-hepatic tissues and organs when the blood glucose level is low (after fasting or prolonged exercise for example). As previously described for CPT1 (section 1-A), PXR binding to FoxA2 leads to the decrease of *Hmgcs2* gene expression.[10] This is consistent with the observation that PCN treatment decreases blood 3-hydroxybutylate levels in rodents.

3D. PXR Interferes with the cAMP-Response Element Binding Protein (CREB) Pathway. Finally, it has been proposed recently that PXR could inhibit the *G6Pase* gene expression by interfering with the cAMP-response element binding protein (CREB) pathway.[41] Glucagon is the gluconeogenic hormone that increases the transcription of hepatic genes coding for rate-limiting enzymes responsible for gluconeogenesis such as G6Pase and PEPCK1. Glucagon activates cAMP-dependent protein kinase (PKA), which phosphorylates CREB.[42] Once phosphorylated, CREB binds to cAMP-responsive elements (CRE) and activates the transcription of CRE-bearing genes such as G6Pase[43] and PEPCK1.[44] Recently, a crosstalk between PXR and CREB has been described in mice hepatocytes and in human hepatoma HuH7 cell line.[41] The authors showed that drug activation of PXR results in the repression of the cAMP-mediated induction of the *G6Pase* gene as well as in that of the PKA/CREB-mediated activation of the *G6Pase* promoter in human hepatocarcinoma cells. Indeed, repression by hPXR of PKA-mediated G6P promoter activation was delineated to the CRE sites. This is reminiscent to the inhibition of CREB by the thyroid receptor.[45]

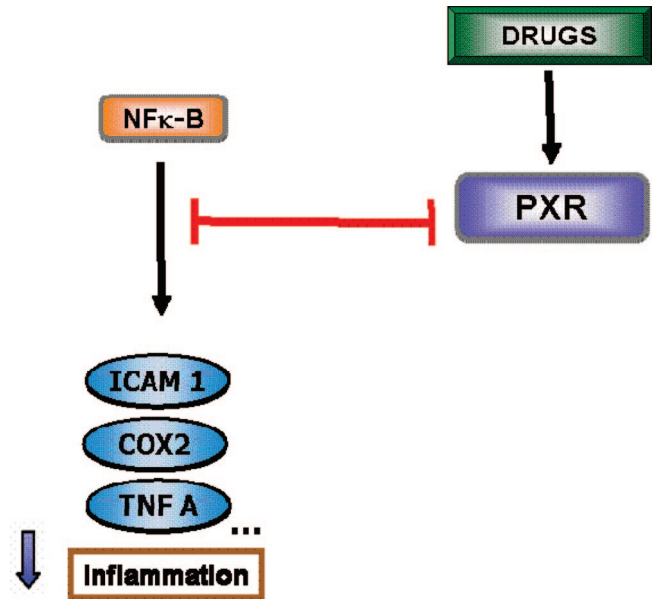

Figure 3. Schematic representation of the mutual repression between PXR and NF-κB signaling pathways. Arrows indicate activation, while stop bars indicate repression.

4. PXR and Inflammation

Inflammation and proinflammatory cytokines suppress the expression of several hepatic transporters and metabolic enzymes, often resulting in cholestatic liver disease (Figure 3). While comparing the effect of inflammation on hepatic gene regulation in wild-type versus PXR-null mice, Teng and colleagues observed that PXR plays a role in the down-regulation of several hepatic proteins during inflammation.[46] In addition, it has long been known that exposure to xenobiotic chemicals can impair immune function. For example, rifampicin tends to suppress humoral and cellular immunological responses in liver cells,[47] and its immunosuppressive role has been well described in humans.[48,49] However, the molecular mechanisms underlying both of these phenomena have remained largely unknown.

(39) Herzig, S. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* **2001**, *413* (6852), 179–83.
(40) Yoon, J. C. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* **2001**, *413* (6852), 131–8.
(41) Kodama, S., et al., Human nuclear pregnane X receptor cross-talk with CREB to repress cAMP activation of the glucose-6-phosphatase gene. *Biochem. J.*, **2007**.
(42) Rosenberg, D. Role of the PKA-regulated transcription factor CREB in development and tumorigenesis of endocrine tissues. *Ann. N.Y. Acad. Sci.* **2002**, *968*, 65–74.
(43) Lin, B.; Morris, D. W.; Chou, J. Y. The role of HNF1alpha, HNF3gamma, and cyclic AMP in glucose-6-phosphatase gene activation. *Biochemistry* **1997**, *36* (46), 14096–106.
(44) Park, E. A. The role of the CCAAT/enhancer-binding protein in the transcriptional regulation of the gene for phosphoenolpyruvate carboxykinase (GTP). *Mol. Cell. Biol.* **1990**, *10* (12), 6264–72.
(45) Mendez-Pertuz, M.; Sanchez-Pacheco, A.; Aranda, A. The thyroid hormone receptor antagonizes CREB-mediated transcription. *EMBO J.* **2003**, *22* (12), 3102–12.
(46) Teng, S.; Piquette-Miller, M. The involvement of the pregnane X receptor in hepatic gene regulation during inflammation in mice. *J. Pharmacol. Exp. Ther.* **2005**, *312* (2), 841–8.
(47) Paunescu, E. In vivo and in vitro suppression of humoral and cellular immunological response by rifampicin. *Nature* **1970**, *228* (5277), 1188–90.
(48) Doria, G.; Agarossi, G. Inhibition of the immune response in vitro by rifampicin and derivatives. *Scand. J. Respir. Dis. Suppl.* **1973**, *84*, 23–6.
(49) Ibrahim, M. S. Antibiotics and immunity: effects of antibiotics on mitogen responsiveness of lymphocytes and interleukin-2 production. *Chemioterapia* **1988**, *7* (6), 369–72.

Recent studies begin to solve pieces of the puzzle. Indeed, a recent publication demonstrated mutual inhibition between PXR and NF-κB (nuclear factor of κ light polypeptide gene enhancer in B-cells 1), providing a potential molecular mechanism that links xenobiotic metabolism and inflammation.[50,51] The NF-κB family consists of five members, namely p65 or Rel A, Rel B, c-Rel, p50, and p52 and is a key regulator of inflammation and the innate and adaptive immune responses. p65 normally remains in the cytoplasm bound to the inhibitory protein inhibitor of NF-κB (IκB). Activating signals, such as proinflammatory cytokines, reactive oxygen species, and viral products lead to phosphorylation, ubiquitination, and proteosome-dependent degradation of IκB, allowing NF-κB to translocate to the nucleus and directly regulate the expression of its target genes.[52] Zhou and colleagues[51] demonstrated that drugs that typically activate human PXR, such as rifampicin, suppressed the expression of typical NF-κB target genes, such as prostaglandin-endoperoxide synthase 2 (COX-2), tumor necrosis factor alpha (TNFα), intercellular adhesion molecule 1 (ICAM-1 or CD54), and several interleukins. In contrast, hepatocytes derived from the PXR null mice showed elevated NF-κB target gene expressions compared to cells from the wild-type animals, suggesting that PXR plays a role in suppressing the NF-κB-regulated gene expression. Moreover, the PXR null mice exhibited signs of heightened inflammation in their small bowels. Interestingly, the increased inflammation in the PXR null mice appeared to be specific for the small bowel. The reason for this tissue specificity could be due to the loss of negative regulation of PXR on NF-κB, or due to the inadequate metabolism and clearance of toxic xeno- and endobiotic substances from this tissue.[53] Intriguingly, a recent report suggests that reduced expression and/or functional polymorphisms of PXR are associated with inflammatory bowel diseases.[54] These observations collectively suggest that deregulation of PXR expression or activity may predispose the gastrointestinal track to inflammatory injuries.

In addition, it was found recently that transrepression of NF-κB target genes by PPARγ is mediated by SUMOylation of PPARγ.[55] Interestingly, the PXR ligand-binding domain also contains a consensus SUMOylation site. Further investigation will reveal whether SUMOylation of PXR mediates transrepression of NF-κB signaling pathway.

5. Conclusion

Chronic therapies with CAR or PXR activators are complicated by their propensities to cause drug interactions by inducing hepatic drug-metabolizing enzymes. In addition, PXR and CAR target genes (enzymes and transporters) are not only involved in xenobiotics detoxification but are also controlling the homeostasis of many endogenous chemicals, including steroid hormones, bile acids, and bilirubin. Accordingly, deregulation of these enzymes and transporters may contribute to human diseases such as cholestatic liver diseases, hyperbilirubinemia, and jaundice (for a review, see ref 56). These new findings bring elements about potential secondary effects which can lead to or result from endocrine disruption.

The crosstalk described in this review demonstrate that signaling pathways controlling xenobiotic/drug metabolism and disposition are embedded within a tangle of regulatory networks controlling notably the metabolism of cholesterol (see review, ref 57), glucose and lipids, and inflammation. These interactions are expected to modify profoundly our vision of xenobiotic/drug toxicity. They show that xenobiotics/drugs affect physiological functions with the possibility of discovering new functions for xenosensors, thus opening the way to interesting pharmacological opportunities, notably in the treatment of inflammatory diseases, obesity, or diabetes.

However, this is just the beginning of the story. Indeed, there are now approximately 50 nuclear receptors working with >200 coactivators, most of which have not yet been fully characterized. The extreme flexibility and versatility of nuclear receptors opens the prospect of regulating their transcriptional activity by ligands, post-translational modifications, partners, coreceptors, and promoter context. While nuclear receptors activate batteries of genes, coactivators activate batteries of nuclear receptors and transcription factors. In addition, coactivators exist as multiprotein complexes, are subjected to transcriptional regulation (like PGC-1α), post-translational modification, and controlled degradation, and exhibit polymorphism, which are expected to influence the activity of their partners. We are therefore far from having a wide and clear view of the tangle of regulatory networks in which the signaling pathways controlling xenobiotic/drug metabolism and disposition are embedded.

Acknowledgment. This study was financially supported by Technologie Servier, Orléans, France (A.M.), the European Community (LSHGCT- 2005-512096, Steroltalk) (P.M, J.-M. P.), and ANR JCJC-05-47810 (J.-M.P).

MP700103M

(50) Xie, W.; Tian, Y. Xenobiotic receptor meets NF-kappaB, a collision in the small bowel. *Cell Metab.* **2006**, *4* (3), 177–8.

(51) Zhou, C. Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. *J. Clin. Invest.* **2006**, *116* (8), 2280–2289.

(52) Vermeulen, L. Regulation of the transcriptional activity of the nuclear factor-kappaB p65 subunit. *Biochem. Pharmacol.* **2002**, *64* (5–6), 963–70.

(53) Langmann, T. Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes. *Gastroenterology* **2004**, *127* (1), 26–40.

(54) Dring, M. M. The pregnane X receptor locus is associated with susceptibility to inflammatory bowel disease. *Gastroenterology* **2006**, *130* (2), 341–8, quiz 592.

(55) Zelcer, N.; Tontonoz, P. SUMOylation and PPARgamma: wrestling with inflammatory signaling. *Cell Metab.* **2005**, *2* (5), 273–5.

(56) Pascussi, J. M., et al. The Tangle of Nuclear Receptors that Controls Xenobiotic Metabolism and Transport: Crosstalk and Consequences. *Annu. Rev. Pharmacol. Toxicol.* **2007**.

(57) Handschin, C.; Meyer, U. A. Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR, and FXR. *Arch. Biochem. Biophys.* **2005**, *433* (2), 387–96.