Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR and FXR

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Regulatory network of lipid-sensing nuclear receptors: roles for CAR, PXR, LXR and FXR

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Abstract

Cloning and characterization of the orphan nuclear receptors constitutive androstane receptor (CAR, NR1I3) and pregnane X receptor (PXR, NR1I2) led to major breakthroughs in studying drug-mediated transcriptional induction of drug-metabolizing cytochromes P450 (CYP). More recently, additional roles for CAR and PXR have been discovered. As examples, these xenosensors are involved in the homeostasis of cholesterol, bile acids, bilirubin and other endogenous hydrophobic molecules in the liver: CAR and PXR thus form an intricate regulatory network with other members of the nuclear receptor superfamily, foremost the cholesterol-sensing liver X receptor (LXR, NR1H2/3) and the bile-acid-activated farnesoid X receptor (FXR, NR1H4). In this review, functional interactions between these nuclear receptors as well as the consequences on physiology and pathophysiology of the liver are discussed.

Key Words: nuclear receptors; drug-induction; lipids; bile acids; cholesterol; CAR; PXR; LXR; FXR; cytochrome P450

Metabolism of drugs and other xenobiotics in the liver is our body's primary defense against accumulation of potentially toxic, lipophilic compounds. The superfamily of cytochromes P450 (CYPs) are the best-studied class of enzymes in this task [1]. Transcriptional and post-transcriptional regulation of CYPs after exposure to certain drugs or other xenobiotics has been described several decades ago. Classically, the barbiturate phenobarbital induces its own metabolism and excretion by elevating CYP levels [2]. However, the molecular mechanisms underlying this observation remained a conundrum until the discovery and subsequent characterization of the constitutive androstane receptor (CAR, official nomenclature NR1I3) and the pregnane X receptor (PXR, NR1I2, alternatively called PAR or SXR), two members of the superfamily of nuclear receptors [3-7]. Mice with genetic ablations of CAR and PXR have significantly reduced inducibility of CYPs by a variety of drugs [8, 9]. Whereas these two receptors share some common ligands and also have an overlapping target gene pattern [10-13], the mode of activation for CAR and PXR is quite different [14]. PXR is located in the nucleus, it has a low basal activity and is highly activated upon ligand binding [14, 15]. In contrast, in the non-induced 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 is regulated by various protein phosphorylation events [16-18]. For a more detailed discussion of CAR and PXR functions in drug-mediated induction of CYPs, see some recent reviews (e.g. refs. [19-23] and references therein).

The nuclear receptor NR1I group includes xenosensors and lipid-sensing members

Compounds that induce transcription of CYPs and that activate CAR and PXR are structurally very diverse [21]. However, most of them are small in size and are highly lipophilic [24]. Whereas the CAR ligand-binding domain structure has not been solved yet, PXR crystal structures provided evidence for the high promiscuity of its ligand binding pocket [25, 26]. The binding cavity is 1150 $Å^3$ in size, substantially larger than those of many other members of the nuclear receptor superfamily, and has only a small number of polar groups in the smooth, hydrophobic ligand binding pocket [25, 26]. CAR and PXR are members of the nuclear receptor groups NR1I2 and NR1I3, respectively [27] (Fig. 1). These groups also contain the frog benzoate X receptors α and β (BXRα/β, NR1I2) which are functionally and pharmacologically distinct from the xenosensors [28, 29]. The vitamin D receptor (VDR, NR1I1) is the closest relative of the xenobiotic-activated nuclear receptors in terms of amino acid sequence similarity and belongs to the same subfamily. Fig. 1 depicts the phylogeny of these receptors from different species. The liver X receptors α and β (LXRα/β, NR1H3/2) and the farnesoid X receptor (FXR, NR1H4) have several features in common with CAR, PXR, and VDR, other members of the NR1I subfamily: they are lipid-activated nuclear receptors, they bind their ligands with relative low affinity, often in the micromolar range and they heterodimerize with the retinoid X receptor (RXR,

NR2B1/2/3) [30]. These receptors belong to the so-called type 2 nuclear receptor group, which is characterized by low ligand affinity, binding of endogenous and dietary lipids and heterodimerization with RXR [30-32]. In contrast, the “classical” steroid hormone receptors belong to the type 1 nuclear receptors that normally have high affinity ligands, which are synthesized from endogenous endocrine sources. Moreover, the steroid hormone receptors usually bind DNA as a homodimer [30-32]. In their initial characterization, ligands of the NR1I receptors were drugs and other xenobiotics for CAR and PXR, 1α,25-dihydroxyvitamin D_3 for VDR [33], oxysterols for LXR [34] and bile acids for FXR [35-37]. However, later findings showed that a number of endogenous compounds are also able to influence PXR and CAR activity and that these xenosensors share an overlapping ligand pattern with other members of the NR1I and NR1H subfamilies (Fig. 2).

Xenosensors in steroid biosynthesis and metabolism

Since some of the CYPs that are regulated by PXR and CAR are involved in steroid metabolism, it is not surprising that the activities of both xenosenors are also modulated by steroids [38]: PXR is activated by pregnanes, progesterone and glucocorticoids [4, 5] whereas androstane metabolites, estrogens and progesterone affect CAR activity both positively and negatively [10, 39-41]. Transgenic expression of a human constitutively active VP16-PXR fusion protein in mouse liver massively increases steroid clearance [42]. In patients, long-term

treatment with rifampicin, a strong human PXR activator, phenobarbital or other anti-convulsants interferes with the bioavailability and therapeutic efficacy of co-administrated corticosteroids and steroid-based oral contraceptives [38, 43]. The effect of drugs and xenobiotics on endogenous steroid levels is less clear because of highly efficient compensatory mechanisms that control steroidogenesis and metabolism. However, in some cases, long-term treatment of a tuberculosis patient with rifampicin resulted in misdiagnosis of Cushing's syndrome [44]. Phenobarbital was shown to lead to developmental abnormalities in animal models due to its effect on steroid clearance which results in a demasculinized phenotype [45-47]. Similarly, human epidemiological studies suggested that prenatal exposure to phenobarbital increases the risk for reproductive development abnormalities [48, 49].

CAR and PXR confer hepatoprotection upon bile acid exposure

Under standard conditions, PXR knockout mice are viable and show no overt phenotype [8]. However, upon challenge with a bile acid-rich diet, PXR null animals suffer from a higher degree of bile acid-induced hepatotoxicity compared to wild-type littermates [50, 51]. Certain bile acids (e.g. lithocholic acid) have been shown to directly activate PXR at concentrations between 10-100 μM [50, 51]. Moreover, three bile acid precursors (7α-hydroxy-4-cholesten-3-one, 5β-cholestan-3α,7α,12α-triol, and 4-cholesten-3-one) activate mouse PXR in the low

micromolar range but are less potent activators of its human ortholog [52]. This species difference in ligand specificity extends to other xenobiotic ligands [53].
CAR is also able to confer hepatoprotection from bile acids by increasing their sulfation and excretion [54-56]. No direct binding of bile acids to CAR has been described. However, several bile acids modulate the activity of a fusion protein of GAL4-DNA binding domain combined with the CAR ligand binding domain in reporter gene assays [28]. Finally, activation of both PXR and CAR increases clearance of bilirubin from hepatocytes [42, 57]. Bilirubin does not directly bind to either CAR or PXR [57]. Instead, bilirubin activates CAR indirectly by promoting cytoplasmic-nuclear translocation, similar to the effects described for phenobarbital on CAR [57]. The overlap of endogenous lipids to activate CAR, PXR, FXR and LXR suggests a functional connection between these receptors in liver physiology. The best studied example is the regulation of cholesterol biosynthesis and metabolism to bile acids (Fig. 3).

Nuclear receptor regulation of cholesterol biosynthesis and metabolism

Cholesterol is metabolized by two different pathways. The “classic” bile acid biosynthesis pathway is exclusively found in the liver and results in the formation of the primary bile acids cholic acid and chenodeoxycholic acid. The “alternative” pathway is ubiquitous and produces oxidized cholesterols which have to be transported to the liver in order to be converted into bile acids. Under normal conditions, the classic pathway is the main bile acid biosynthetic pathway in the

liver [58-60]. This pathway is highly regulated, predominantly at its first enzymatic step, the cholesterol 7α-hydroxylase (CYP7A1). CYP7A1 expression is controlled by a variety of factors and stimuli including hormones, oxysterols, bile acids, drugs and diurnal rhythm [58, 61]. In the mouse and rat CYP7A1 promoters, LXR binds to a nuclear receptor motif arranged as a direct repeat of hexamer halfsites with a spacing of 4 nucleotides (DR-4). When activated by oxysterols or other ligands, LXR binds to this DR-4 element and strongly induces CYP7A1 transcription [62, 63]. Interestingly, LXR has much less of an effect on hamster and no effect on human CYP7A1 expression [64, 65]. This difference might be attributed to a mutation in the DR-4 site in the human CYP7A1 promoter which prevents LXR from binding [64]. The ability of LXR to induce Cyp7a1 in mice and rats makes these animals extremely resistant to a high cholesterol diet whereas other species, including man, rapidly develop hypercholesterolemia under comparable conditions. Accordingly, high cholesterol-fed mice that transgenically express human CYP7A1 in a mouse Cyp7a1 knockout background lack induction of CYP7A1 and become hypercholesterolemic [66, 67].

The rates of cholesterol biosynthesis and triglyceride biogenesis are predominantly controlled by the sterol regulatory-element binding proteins (SREBP) [68]. Of the three SREBP isoforms, SREBP-2 coordinately activates the genes for cholesterol biosynthesis when hepatic cholesterol is low. On the other hand, SREBP-1c induces triglyceride biosynthesis. SREBP-1a, a splice variant of the SREBP-1 gene, regulates all SREBP target genes. Cholesterol biosynthesis

from acetyl-CoA is a complex process involving 15 enzymatic steps and NADPH as co-factor [68]. Major metabolic intermediates in the pathway are acetoacetyl-CoA, hydroxy-methylglutaryl-CoA, mevalonate and squalene. Several of these cholesterol precursors also serve as substrates for other biosynthetic pathways, e.g. 7-dehydrocholesterol for the generation of vitamin D_3 [30]. In contrast, CAR and PXR are activated by precursors in the cholesterol biosynthesis pathway, namely isoprenoids and squalene metabolites, respectively [69, 70]. When cholesterol biosynthesis is blocked, CAR and PXR might be activated by these cholesterol precursors and subsequently inhibit cholesterol metabolism to bile acids by repressing CYP7A1 as seen after activation of PXR by other ligands [50]. This regulation could prevent cholesterol levels from dropping too low when cholesterol biosynthesis is impaired. However, the physiological relevance of these activations and the validity of this hypothesis remain to be tested. Interestingly, geranylgeranyl-pyrophosphate, another intermediate in mevalonate metabolism to cholesterol, inhibits LXR activity and thus also results in lower CYP7A1 levels [71].

A potent product-mediated negative feedback inhibition underlies the regulation of bile acid synthesis from cholesterol. By activating FXR, bile acids induce the expression of the small heterodimer partner (SHP, NR0B2), a nuclear receptor lacking a DNA binding domain. Subsequently, SHP binds to the liver receptor homolog-1 (LRH-1, NR5A2) which is a potent activator of CYP7A1. This interaction decreases the transcriptional activity of LRH-1 and subsequently

lowers CYP7A1 transcription [72, 73]. Surprisingly, bile acids are able to repress Cyp7a1 expression in SHP -/- animals suggesting the presence of redundant mechanisms [74, 75]. FXR directly activates transcription of the fibroblast growth factor 19 which, via a c-jun N-terminal kinase-dependent pathway, leads to reduced CYP7A1 expression [76]. Another possible pathway of SHP-independent CYP7A1 repression might be mediated by PXR. Drugs, other xenobiotics and bile acids that activate PXR have been observed to downregulate CYP7A1 mRNA expression in hepatocytes and *in vivo* [50, 77]. Apart from its independence from SHP, the exact molecular mechanism of this repression has not been elucidated. Preliminary findings imply the hepatic nuclear factor 4α (HNF4α, NR2A1) to be involved in this process ([58] and C. H. and U. A. M., unpublished observations). HNF4α is an important regulator of CYP7A1 expression in different species and is at least in part responsible for mediating CYP7A1 repression by bile acids [76, 78, 79].

Similar to FXR, CAR and PXR promote metabolism and excretion of bile acids. They partly do so by inducing the same target genes including the canalicular bile acid transporter multidrug-resistance-associated protein 2 and 3 (MRP2/ABCC2 and MRP3/ABCC3) [80-82]. However, the xenosensors also increase alternate, compensatory pathways for lowering hepatic bile acid levels by inducing their hydroxylation, conjugation and subsequent excretion via blood and urine [50, 51, 54, 55, 83]. In contrast, FXR is predominantly responsible for triggering bile acid export from the liver into the bile duct followed by excretion of

bile acids via feces [84, 85]. PXR induces several bile acid-metabolizing CYPs, bile acid transporters and sulfotransferases that serve to detoxify bile acids such as lithocholic acid [50, 51, 77, 82, 86]. Activation of CAR by bile acids triggers yet another alternate response in the hepatocyte. In addition to CYP2Bs and CYP3As, CAR increases sulfation of bile acids [54]. By this mechanism, CAR is able to protect the liver from bile acid-induced hepatotoxicity [55]. As a fourth layer of defense, VDR is also activated by bile acids [87]. This receptor can then increase CYP2B, CYP2C and CYP3A levels [88] and subsequent bile acid metabolism [87]. VDR thus activates a similar “emergency” response to high bile acid levels as the xenosensors. The more than tenfold difference in affinity for bile acid binding to FXR and PXR implies that under physiological conditions, bile acids predominantly activate the FXR-mediated pathway and thus their normal excretion. However, in disease states where the regular ways for bile acid excretion are blocked and bile acid levels rise inside the hepatocytes (e.g. cholestasis), the xenosensors are activated by these elevated bile acid levels and subsequently promote alternate mechanisms in order to lower intrahepatic bile acid levels before they become hepatotoxic. In summary, the four nuclear receptors FXR, PXR, CAR, and VDR are functionally related inasmuch as they coordinately reduce hepatic bile acid levels by increasing bile acid metabolism and export and in part by inhibiting *de novo* biosynthesis of bile acids from cholesterol [89].

Among the drug-metabolizing CYPs, CYP3As are the major class of enzymes that hydroxylate bile acids [51, 90, 91]. Moreover, transcriptional induction of CYP3A genes by bile acids often exceeds that of CYP2B and CYP2C genes (refs. [92, 93] and Carmela Gnerre and U. A. M., unpublished observation). A number of findings suggest the presence of additional mechanisms for CYP3A regulation by bile acids. FXR-mediated induction of SHP by bile acids decreases the transcriptional activity of PXR [94]. Moreover, in mice with transgenically incorporated human CYP3A4 5'-flanking region linked to a reporter gene, increase in CYP3A4-driven reporter gene expression is not primarily dependent on the levels of circulating lithocholic acid, the primary bile acid-ligand of mouse PXR [95]. FXR activates CYP3A4 drug-responsive enhancer elements and might thus directly increase CYP3A levels [51, 83]. Alternatively, CYP3A is also induced by CAR which can bind to the same drug-responsive elements as PXR [11]. Finally, VDR is also activated by bile acids and can induce transcription of CYP3As in liver and intestine [87]. However, the role of the different bile acids in the CYP3A regulation and the receptors mediating this induction remain to be elucidated.

Human CYP3A4, but not CYP1A2, CYP2C9 or CYP2B6, catalyzes both 6α- and 4β-hydroxylation of cholesterol [96]. Interestingly, patients treated with the anti-epileptic drugs phenobarbital, carbamazepine or phenytoin have up to 20 fold elevated plasma levels of 4β-hydroxycholesterol whereas fecal levels of patients and control subjects are comparable [96]. The 52 hours half-life of this oxysterol

in plasma is extremely long compared to other oxysterols, e.g. 24-hydroxycholesterol with a half-life of 12 hours or 27-hydroxycholesterol and 7α-hydroxycholesterol, which have half-lifes shorter than 0.75 hours and 0.5 hours in human circulation, respectively [97]. Moreover, 4β-hydroxycholesterol is a poor substrate for 7α-hydroxylations by CYP7A1 whereas the two oxysterol 7α-hydroxylases CYP7B1 and CYP39A1 have no catalytic activity toward 4β-hydroxycholesterol [97]. It seems that upon activation of PXR, elevated CYP3A levels catalyze 4β-hydroxylation of cholesterol resulting in a steep increase in the plasma levels of 4β-hydroxycholesterol [96, 97]. Subsequently, this oxysterol is a potent activator of LXR [34]. Another major hepatic cholesterol metabolite, the oxysterol 24(S),25-epoxycholesterol is an activator of both mouse PXR and LXRα [98]. Interestingly, intrahepatic levels of the 24(S),25-epoxycholesterol enantiomer increase after mevalonate administration in rats suggesting that this oxysterol is a key mediator of the effect of mevalonate on downregulation of HMG-CoA reductase and on induction of CYP7A1 activity [99]. This overlap in ligands and the PXR-induced production of LXR activators thus further promotes cholesterol metabolism and excretion in a coordinate action between PXR and LXR.

The predominant bile acid hydroxylations catalyzed by CYP3As are 6α-hydroxylation reactions which are stimulated in hepatocytes by rifampicin, a strong activator of human PXR [90, 100]. Both 6α-hydroxylated chenodeoxycholic acid (hyocholic acid) as well as 6α-hydroxylated lithocholic

acid (hyodeoxycholic acid) are selective activators of LXR [93, 101]. Treatment of hepatoma cells with hyocholic acid or hyodeoxycholic acid reduces the levels of drug-induced CYPs [93]. LXR binds to drug-responsive enhancer elements in the chicken CYP2H1 and the human CYP2B6 and CYP3A4 5'-flanking regions [93]. One possible mechanistic explanation for these observations is binding of LXR to these elements followed by competition with the binding of the xenosensors and a resulting decreased transcriptional activity of the drug-responsive enhancers [93, 102]. Thus, LXR forms a negative feedback loop on the drug-inducible CYPs catalyzing the hydroxylation reactions of bile acids which result in LXR agonists. This mechanism probably ensures protection from accumulation of hydroxylated bile acids in the liver.

NR1I subfamily members regulate lipid levels in the liver

In addition to cholesterol and bile acid homeostasis, LXR and FXR play diametrically opposed roles in the regulation of lipid biosynthesis. LXR is a strong activator of SREBP-1c and thus triggers an increase in triglyceride biosynthesis in the liver [103, 104]. Moreover, independent of SREBP-1c, LXR directly activates other lipogenic genes including fatty acid synthase (FAS) [105]. In contrast, FXR transcription is increased in the fasting liver by the peroxisome proliferator activated receptor γ coactivator 1α (PGC-1α) [106]. The interaction between FXR and PGC-1α results in an induction of genes that promote triglyceride clearance and fatty acid β-oxidation concomitantly with a reduction of

lipogenic gene transcription [106]. Among the FXR-target genes, SHP is the major inhibitor of SREBP-1c induction by LXRs [107]. Another strong activator of fatty acid metabolism, the peroxisome proliferator activated receptor α (PPARα, NR1C1), also antagonizes LXR function and vice versa [108]. In the fasted liver with high levels of fatty acid β-oxidation, PPARα interferes with LXR-mediated induction of SREBP-1c [109]. On the other hand, activated LXR reduces PPARα binding to fatty acid-metabolizing gene promoters [110]. The role of the xenobiotic-sensing nuclear receptors in triglyceride homeostasis has not been extensively studied. However, a number of findings suggest a role for CAR and PXR in this process. First, CAR can bind to DNA-elements overlapping with those for PPARα in the promoter of enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, the second enzyme of peroxisomal fatty acid β-oxidation [111]. PPARα signaling on the other hand influences CAR-mediated hepatocyte proliferation after drug-treatment [112]. Phenobarbital induces Cyp4a10 and Cyp4a14, two CYPs involved in peroxisomal fatty acid oxidation, but only in the CAR null background suggesting an inhibitory role of CAR on these genes [41]. Moreover, CAR localization in the nucleus is affected by its binding to PGC-1α [113], a strong transcriptional coactivator that is regulated in the liver by fasting and feeding [114]. Finally, during caloric restriction, CAR is a regulator of thyroid hormone levels [115]. Thus, by increasing thyroid hormone metabolism, CAR contributes to the body's resistance to weight loss [115]. Recent studies furthermore suggested that CAR in the brain is involved in the regulation of dexamethasone levels in the brain which in turn influence the levels of the

glucocorticoid-receptor target genes neuropeptide Y and the neuropeptide Y receptor subtype 1 in mice [116]. This has potential implications in the regulation of food intake in these animals. However, very high concentrations of dexamethasone were used in this study and the physiological relevance of this observation *in vivo* is thus not clear.

Experimental and clinical observations

A functional link between xenobiotics and lipid levels has been confirmed by a number of observations and findings in cell culture, animals and patients. As examples, blocking of *de novo* cholesterol biosynthesis using different inhibitors such as squalestatin, lovastatin or fluvastatin increases CYP2B1/2 in rat primary hepatocytes and in rat liver *in vivo* [117-119]. Phenobarbital-treatment of rats changed the expression of various genes in the cholesterol-biosynthesis pathway [120-122] whereas rats fed a high-cholesterol diet or spontaneous hyperlipidemic rats with elevated cholesterol levels have a reduced basal and phenobarbital-induced CYP2B levels [123, 124]. Furthermore, PB-induction of CYP2Bs in obese fa/fa Zucker rats is almost completely lost [125]. In contrast, nutritional obesity has very small and enzyme-specific effects on PB-induction of various CYPs [126, 127]. Long-term treatment of rats with phenobarbital leads to considerable changes in the lipoprotein levels [128, 129]. Serum biochemistry and microarray analysis of rats that were repeatedly treated with phenobarbital show induced cholesterogenesis with a corresponding elevation in serum total

cholesterol, impaired glycolysis and stimulated lipolysis in the liver [122]. Treatment of rats and with imidazoles also resulted in elevated plasma HDL levels and expression of hepatic apolipoprotein A1 [130]. Interestingly, this elevation of cholesterol and apolipoprotein A1 levels was only observed in wild-type but not in PXR knockout mice [130].

The widespread, long-term use of phenobarbital as an anti-epileptic drug allowed a number of studies regarding the effect of phenobarbital on lipid profiles in patients. Several groups reported significant changes in plasma and hepatic lipid profiles, especially after long-term treatment (e.g. see refs. [131-136]) whereas other studies failed to detect a significant correlation between phenobarbital treatment and changes in lipid levels (e.g. see ref. [137]). It is possible that induction of cholinesterase in epileptic patients treated with phenobarbital contributes to the changes in lipid levels [138]. Beneficial effects of phenobarbital on hyperbilirubinemia [139] and of phenobarbital and rifampicin on cholestasis [140] have been observed for decades. Recently, the xenosensors PXR and CAR have been identified to mediate at least some of these therapeutic effects [50, 51, 57, 141].

Species differences in hepatic detoxification

Marked differences in the way different species deal with foreign compounds have been described [53, 142]. First, CYP orthologs differ in their basal expression in different species: e.g., CYP3As are very abundant in humans and

key enzymes in steroid and xenobiotic metabolism whereas CYP3A levels, in the absence of induction, are relatively low in rodents [143]. In addition, these genes are differentially induced by drugs and other xenobiotics. As example, human, but not rodent CYP3As are strongly induced by rifampicin. In contrast, pregnenolone 16α-carbonitrile very potently increases mouse and rat CYP3As whereas it hardly changes human CYP3A4 levels [142]. Moreover, when drug-responsive elements in the 5'-flanking regions of CYPs were isolated, no apparent feature conserved between species was found (e.g. see ref. [144]). For years, it was therefore not clear whether these species use similar molecular mechanisms for hepatic detoxification [145]. These species differences make extrapolation of pharmacokinetic and pharmacodynamic data from animal models to man virtually impossible.

The discovery of the drug-sensing nuclear receptors PXR and CAR was a breakthrough in understanding the species-specific differences in hepatic drug detoxification. It turned out that many aspects of drug-induction of CYPs by nuclear receptors are highly conserved in evolution [28, 146-148]. As example, the mammalian xenosensors and the chicken xenobiotic receptor (CXR) [149] can be used interchangeably in many cell culture-based assays [146]. Also, despite their sequence differences, drug-responsive elements found in CYP 5'-flanking regions from rodents, man and chicken can be activated by the xenobiotic-sensing nuclear receptors from all of these species [146]. Like its mammalian orthologs, CXR is activated by drugs, other xenobiotics and bile

acids [93, 149]. The high specificity of certain compounds to induce CYPs in a species-selective way can largely be explained by the divergent ligand-binding domains of the xenosensor orthologs [8, 25, 53]. Thus, rifampicin is a good ligand for human PXR, but not for the rodent ortholog whereas pregnenolone 16α-carbonitrile only activates mouse PXR.

Other aspects in the biology of the NR1I and NR1H nuclear receptors show divergent evolution. Foremost, the two xenosensors, PXR and CAR have only been found in mammals whereas other vertebrate genomes including fish or chicken encode only one xenosensor [28, 149, 150]. Similarly, only one xenobiotic-sensing nuclear receptor has been found in the *C. elegans* genome [151]. Future studies may show why mammals have two xenosensors and how those affect drug-induction and lipid homeostasis. However, in addition to the species-specificity in the drug-detoxification machinery, there are considerable variations in the hepatic lipid homeostasis. Distinct serum lipoprotein levels have been found in different species (e.g., see ref. [152]). Another example, the different regulation of CYP7A1 by LXR in mice, rats and man has been discussed above. Extrapolation of data obtained in rodents regarding drug regulation of lipid homeostasis might therefore only be of limited use.

Conclusions

Although it appears paradoxical because the potential for drug-drug interactions and adverse drug reactions may increase [153], therapeutic targeting of CAR and PXR might be beneficial under certain conditions. Inhibition of CAR either by genetic ablation or by using CAR inverse agonists decreases acetaminophen-induced hepatotoxicity [154]. On the other hand, increasing CAR activity most likely ameliorates neonatal jaundice by increasing bilirubin conjugation and clearance [155]. Moreover, drug-mediated activation of both PXR and CAR is potentially beneficial in cholestasis [156]. Increasing our knowledge of the functions of CAR and PXR in hepatic detoxification as well as their roles in regulating lipid homeostasis in concert with other nuclear receptors such as FXR, LXR and PPAR could lead to novel approaches in the therapy of diseases related to these processes. In summary, work on CAR and PXR in recent years clearly shows that these nuclear receptors are more than mere xenosensors. Both receptors seem to be involved in the regulation of a variety of endogenous pathways and thus not only respond to xenobiotic challenges, but also to metabolic and nutritional stress [157].

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Abbreviations

CAR, constitutive androstane receptor; PXR, pregnane X receptor; CYP, cytochrome(s) P450; LXR, liver X receptor; FXR, farnesoid X receptor; VDR, vitamin D receptor; DR, direct repeat; SHP, small heterodimer partner; LRH-1, liver receptor homolog-1; SREBP, sterol regulatory element binding protein; FAS, fatty acid synthase; PGC-1, peroxisome proliferator-activated receptor γ coactivator 1; PPAR, peroxisome proliferator-activated receptor; CXR, chicken xenobiotic receptor

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Figure Legends

Figure 1. Phylogenetic tree of the nuclear receptors from the subfamilies NR1I and NR1H. The tree was generated comparing the full-length amino acid sequences of the respective receptors and shows the relationship between the drug-sensing nuclear receptors CAR (NR1I3), PXR (NR1I2), the vitamin D_3-receptor VDR (NR1I1), the bile acid-activated FXR (NR1H4) and the oxysterol-sensing LXRα/β (NR1H3/2) from different species. The scale bar represents 0.1 amino acid substitution per site.

Figure 2. Endogenous and xenobiotic lipophilic compounds activate a number of nuclear receptors which control their intra- and extra-hepatic levels. The members of the nuclear receptor subfamilies NR1I and NR1H are activated by various xenobiotics and endogenous lipids. In general, the receptors subsequently regulate the metabolism and excretion of these compounds. A high redundancy exists for several substance classes to bind to multiple receptors. See text for details.

Figure 3. FXR, LXR, PPAR, CAR, PXR, and VDR control hepatic lipid homeostasis. Oxysterol-activated LXR increases metabolism of cholesterol to bile acids. Moreover, LXR also stimulates lipogenesis by inducing SREBP-1c and other lipogenic genes. Simultaneously, LXR inhibits PPARα-mediated fatty acid oxidation by interfering with PPARα-binding to its target sites. In contrast,

PPARα, FXR, CAR, PXR and VDR have counter-regulatory effects on LXR in the regulation of triglyceride as well as cholesterol and bile acid levels. Moreover, xenosensor and LXR functions are diametrically opposed in the regulation of drug- and bile acid-metabolizing CYPs. See text for details.

Figure 1 (EPS)

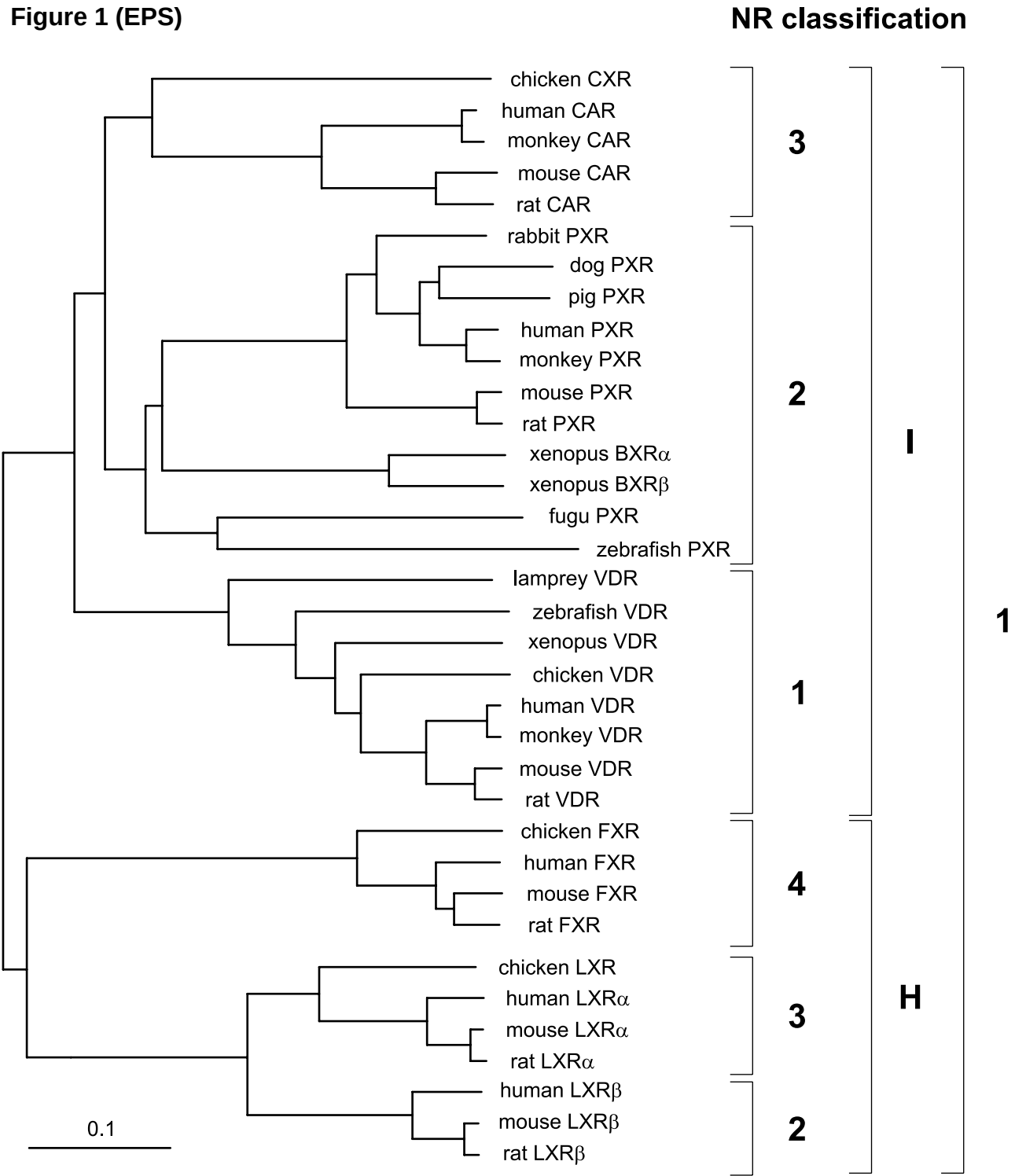

NR classification
chicken CXR
human CAR
monkey CAR
mouse CAR
rat CAR
rabbit PXR
dog PXR
pig PXR
human PXR
monkey PXR
mouse PXR
rat PXR
xenopus BXRα
xenopus BXRβ
fugu PXR
zebrafish PXR
lamprey VDR
zebrafish VDR
xenopus VDR
chicken VDR
human VDR
monkey VDR
mouse VDR
rat VDR
chicken FXR
human FXR
mouse FXR
rat FXR
chicken LXR
human LXRα
mouse LXRα
rat LXRα
human LXRβ
mouse LXRβ
rat LXRβ
0.1
3
2
1
4
3
2
I
H
1

Figure 2 (EPS)

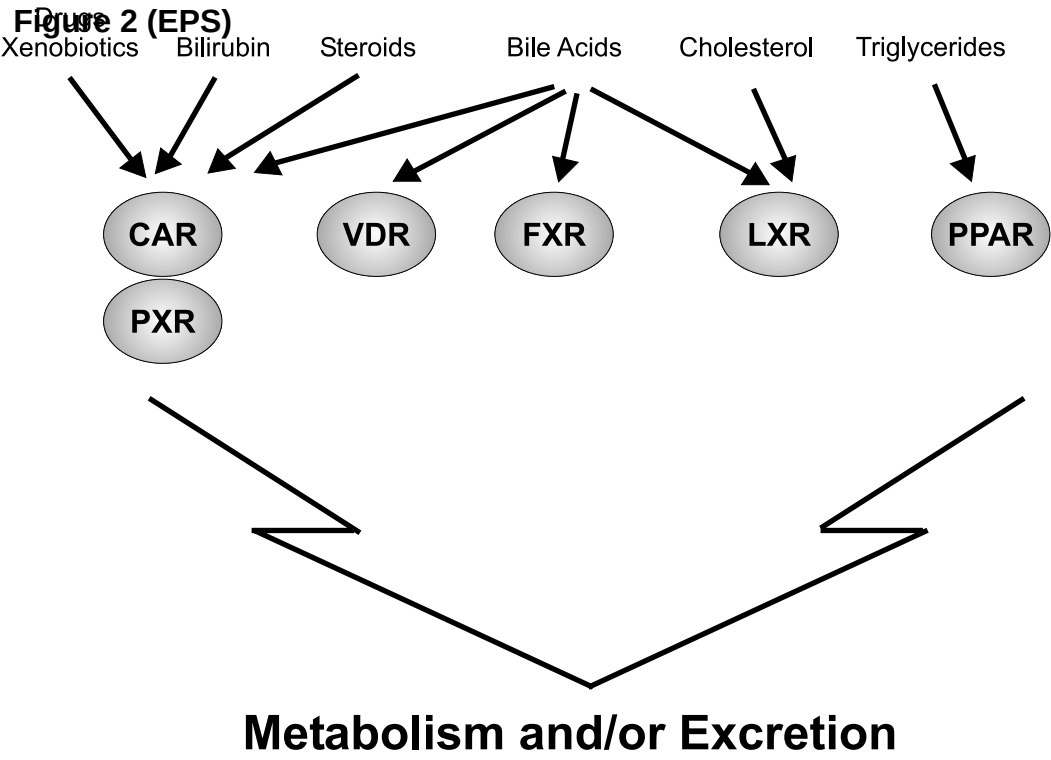
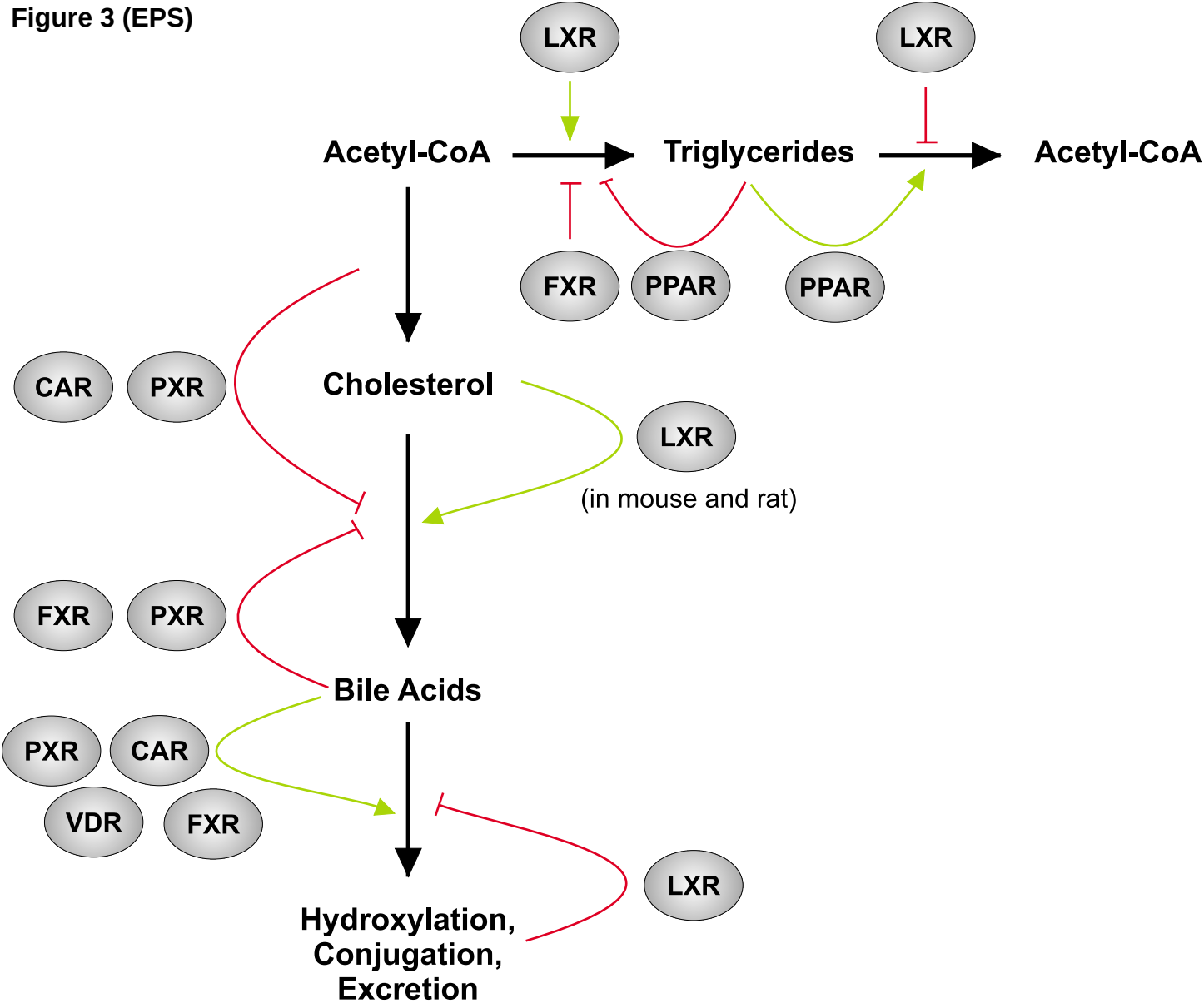

Drugs
Xenobiotics
Bilirubin
Steroids
Bile Acids
Cholesterol
Triglycerides
CAR
PXR
VDR
FXR
LXR
PPAR
Metabolism and/or Excretion

Figure 3 (EPS)


LXR
LXR
Acetyl-CoA
Triglycerides
Acetyl-CoA
FXR
PPAR
PPAR
CAR
PXR
Cholesterol
LXR
(in mouse and rat)
FXR
PXR
Bile Acids
PXR
CAR
VDR
FXR
LXR
Hydroxylation,
Conjugation,
Excretion