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themes

Nuclear Receptors
I. Nuclear receptors and bile acid homeostasis

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Goodwin, Bryan, and Steven A. Kliewer. Nuclear Receptors. I. Nuclear receptors and bile acid homeostasis. *Am J Physiol Gastrointest Liver Physiol* 282: G926–G931, 2002; 10.1152/ajpgi.00044.2002.—Bile acids are required for the absorption of lipids and fat-soluble vitamins. The hepatic biosynthesis of bile acids is a major pathway for the catabolism and removal of cholesterol from the body. Because of their intrinsic toxicity, bile acid synthesis, transport, and metabolism must be tightly regulated. It is now apparent that members of the nuclear receptor family of lipid-activated transcription factors are key regulators of these physiological processes. A greater understanding of these receptors should afford novel opportunities for therapeutic intervention in chronic diseases such as cholestasis and dyslipidemia.

farnesoid X receptor; pregnane X receptor; bile acids

BILE ACIDS ARE IMPORTANT PHYSIOLOGICAL agents that subserve a number of functions, including absorption, solubilization, transport, and secretion of lipids. In the liver, they participate in the generation of bile flow and the secretion of cholesterol and phospholipids, such as phosphatidylcholine. When released into the intestine, they facilitate the uptake of cholesterol, fat-soluble vitamins, and other lipids (reviewed in Ref. 35). Moreover, the biosynthesis of bile acids from cholesterol is the most significant pathway for the elimination of cholesterol from the body (36). However, because of their detergent properties, bile acids are inherently cytotoxic and perturbations in their normal transport or secretion can result in a variety of pathophysiological conditions (14). Thus it is necessary that intracellular levels of bile acids be tightly regulated. This is largely accomplished by transcriptional regulation of genes encoding proteins involved in bile acid biosynthesis, transport, and metabolism.

It is now known that synthesis of the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) from cholesterol involves at least 14 different enzymes (30). The biosynthesis of bile acids is primarily, but not exclusively, performed by hepatocytes. The first and rate-limiting step in the classic or neutral pathway of bile acid biosynthesis involves hydroxylation of cholesterol at the 7α-position and is catalyzed by cytochrome *P*-450 (CYP)7A1. This enzyme has been extensively studied both at the biochemical and molecular levels (5). The gene encoding CYP7A1 is regulated by a variety of small, lipophilic molecules including steroid and thyroid hormones, cholesterol, and bile acids. Notably, *CYP7A1* expression is stimulated by cholesterol feeding and strongly repressed by bile acids (5). Feedforward regulation by cholesterol and its oxysterol metabolites promotes catabolism of cholesterol to bile acids, thereby promoting the clearance of dietary cholesterol. Conversely, bile acids effectively downregulate the expression of *CYP7A1* and other genes involved in bile acid biosynthesis, such as sterol 12α-hydroxylase (CYP8B1), resulting in suppression of bile acid production (5).

NUCLEAR RECEPTORS REGULATE CYP7A1 EXPRESSION

It is now apparent that various members of the nuclear receptor superfamily of ligand-activated transcription factors are key regulators of *CYP7A1* expression (2). Nuclear receptors have critical roles in nearly every aspect of development and adult physiology. Family members share a common domain structure that includes a highly conserved DNA binding domain (DBD). The DBD targets the receptor to short stretches of DNA, termed response elements, in the regulatory regions of target genes. Typically, these response elements are repeats of the AGGTCA hexad (23). The COOH-terminal region of the nuclear receptors includes the conserved ligand-binding domain (LBD). The binding of a ligand to the LBD results in a conformational change that allows the nuclear receptor to interact with accessory proteins, such as steroid receptor coactivator-1, and activate the expression of target genes (11, 24).

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The liver X receptor α (LXRα; NR1H3) has been shown to mediate the feedforward regulation of *CYP7A1* (2). LXRα is abundantly expressed in the liver and binds its cognate hormone response elements as a heterodimer with the 9-*cis* retinoic acid receptor (RXR; NR2B1) (23). LXRα is activated by cholesterol derivatives, including 24,25(*S*)-epoxycholesterol, and binds to a response element in the rat *CYP7A1* gene ~60 bp upstream of the transcription initiation site (2). Mice lacking LXRα do not induce *CYP7A1* expression in response to cholesterol feeding (29). Moreover, these animals accumulate massive amounts of cholesterol in their livers when fed a high-cholesterol diet (29). These data clearly establish LXRα as one of the body's key cholesterol sensors.

Activation of the rat *CYP7A1* promoter by LXRα is contingent on the presence of another nuclear receptor, liver receptor homolog-1 (LRH-1; NR5A2) (20). LRH-1 is the mammalian homolog of the *Drosophila fushi tarazu* F1 receptor (FTZ-F1; NR5A3) and, like FTZ-F1, binds its cognate target sequence as a monomer. LRH-1 was shown to activate the human *CYP7A1* promoter by binding to an extended nuclear receptor half-site sequence that is conserved in the mouse, rat, and hamster *CYP7A1* promoters (27). In addition to directly transactivating expression, it is proposed that LRH-1 acts as a competence factor for LXRα. Thus, in the absence of LRH-1, LXRα is incapable of forming a transcriptionally competent complex on the *CYP7A1* promoter (20). Importantly, the LRH-1 response element coincides with a so-called bile acid response element. Chiang and co-workers (5) demonstrated that this motif, in conjunction with a second site, directed the bile acid-mediated suppression of the *CYP7A1* promoter. Additionally, LRH-1 has been implicated in the transrepression of the *CYP8B1* gene (6). These studies demonstrate the central role of LRH-1 in the expression and regulation of bile acid biosynthetic genes.

FARNESOID X RECEPTOR REGULATES BILE ACID BIOSYNTHESIS AND TRANSPORT

FXR is a bile acid receptor. A major advance in our understanding of bile acid signaling came in 1999 with the discovery that endogenous bile acids are ligands for the farnesoid X receptor (FXR; NR1H4) (21, 28, 37). FXR, which is highly expressed in the liver, kidney, gut, and adrenal cortex, was named based on activation of the rat ortholog by micromolar concentrations of farnesol derivatives (10). Subsequent studies demonstrated that physiological concentrations of the primary bile acid CDCA were capable of transactivating FXR in cell-based transient transfection assays. The secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA) also activated FXR, although these compounds were less effective than CDCA (21, 28, 37). In mammals, the majority of bile acids exist as conjugates with glycine or the cysteine derivative taurine. In the absence of an active bile acid transporter, these amidated compounds do not enter the cell. Thus, to examine whether conjugated bile acids activated FXR, Parks et al. (28) coexpressed the apical sodium-dependent bile acid transporter (ASBT; SLC10A1) with FXR in transient transfection assays performed in simian kidney cells. In this setting, FXR was efficaciously activated by the tauro- and glycoconjugates of CDCA, LCA, DCA, and, to a lesser extent, CA (28). Similar results were obtained when the Na^+-taurocholate cotransporting polypeptide (NTCP; SLC10A1) was used as the bile acid transporter (37). Interestingly, CA failed to activate FXR in the absence of transporter but, in the presence of exogenously expressed NTCP, strongly activated the receptor (37). CDCA and its glyco- and tauroconjugates were shown to bind directly to FXR with EC_{50} of ~5–10 μM, which is within their physiological range (21, 28).

FXR regulates genes involved in bile acid homeostasis. The activation properties and expression profile of FXR suggested that it might play an important role in bile acid homeostasis. In the ileum, reabsorption of bile acids is mediated by ASBT, which is localized in the brush border membrane of enterocytes. Once in the cell, bile acids are reversibly bound to the intestinal bile acid binding protein (I-BABP). Although the precise physiological function of I-BABP is unknown, it is believed to be involved in trafficking bile acids across the enterocyte into the portal circulation. Expression of *I-BABP* is highly inducible by bile acids, implying that this gene may be directly regulated by FXR (15). Makishima et al. (21) identified an inverted repeat-1 (IR-1) element in the promoter of the mouse *I-BABP* gene and demonstrated that this motif was a functional FXR response element. In addition to I-BABP, Mangelsdorf and colleagues (21) demonstrated that suppression of *CYP7A1* by bile acids was also likely to be mediated by FXR. Thus, in a liver-derived cell line, the rank order of bile acids that suppress expression of both the endogenous *CYP7A1* gene and a reporter gene construct containing the *CYP7A1* promoter was identical to that for binding and activation of FXR (21). Importantly, the regions of the *CYP7A1* promoter that are required for bile acid-mediated suppression do not contain high-affinity FXR response elements (3).

Unfortunately, the relatively low affinity of bile acids for FXR, combined with their potential to be metabolized in vivo, meant that these natural ligands were not ideally suited for use in functional studies. Maloney et al. (22) screened combinatorial libraries of compounds using a FRET assay and identified the isoxazole GW4064 as a nonsteroidal ligand for use as a chemical tool in elucidating the genes regulated by FXR. In cell-based transient transfection assays, GW4064 activated both rodent and human FXR with EC_{50} values of 80–90 nM, ~1,000-fold more potent than CDCA (12). To identify genes whose expression is regulated by FXR in the liver, male Fisher rats were treated with GW4064, RNA was isolated, and genes that were either induced or repressed by GW4064 were determined using differential gene expression technology (12). Expression of the genes encoding *CYP7A1* and *CYP8B1* was repressed following GW4064 treatment. As discussed above, suppression of the *CYP7A1* and *CYP8B1*

genes by bile acids is an important adaptive response designed to prevent accumulation of potentially toxic bile acids. The fact that GW4064 suppressed expression of these genes provides compelling evidence that FXR mediates feedback repression of bile acid biosynthesis.

Interestingly, a gene that was strongly induced by GW4064 treatment encoded the orphan nuclear receptor small heterodimer partner (SHP; NR0B2), an unusual nuclear receptor that lacks the highly conserved DBD typically found in members of this family. SHP was originally cloned in yeast two-hybrid experiments using the orphan nuclear receptors constitutive androstane receptor (CAR) or peroxisome proliferator-activated-α as bait, but it interacts with a number of additional nuclear receptors including hepatocyte nuclear factor 4α (HNF-4α) and LRH-1 (18, 19, 25, 32). SHP represses the transcriptional activity of both these orphan receptors. In primary cultures or rat and human hepatocytes, *SHP* expression was markedly induced by both GW4064 and the naturally occurring FXR agonist CDCA, whereas *CYP7A1* expression was suppressed under the same conditions. Lu et al. (20) demonstrated that feeding mice a bile acid-supplemented diet resulted in induction of *Shp* expression that paralleled *Cyp7a1* suppression. The mouse, rat, and human *SHP* promoters were shown to be activated in an FXR-dependent manner, and a high-affinity FXR-RXR binding motif was characterized, demonstrating that SHP expression is directly regulated by FXR (12, 20). The reciprocal relationship between *SHP* and *CYP7A1* regulation, together with the established inhibitory effects of SHP on nuclear receptor activity, suggested that SHP might repress *CYP7A1* expression. Indeed, expression of SHP repressed the activity of the rat *CYP7A1* promoter in Hep G2 cells (Fig. 1) (12).

How does SHP repress transcription of the *CYP7A1* promoter? It appears that SHP exerts much of its effect through interaction with the orphan nuclear receptor LRH-1. SHP interacted strongly with LRH-1 in both a mammalian two-hybrid assay and an in vitro pull-down assay (12, 20). Moreover, SHP efficiently repressed both the LRH-1-dependent activation of the rat *CYP7A1* promoter and the ability of LRH-1 to act as a competence factor for LXRα (discussed above) (12, 20). Induction of SHP expression also results in inhibition of both the LRH-1- and HNF-4α-mediated transactivation of *CYP8B1* expression (7, 40) (Fig. 1). In addition, SHP is reported to suppress the activity of the rat NTCP promoter (8). Interactions between SHP and other nuclear receptors, most notably LRH-1, are likely to be important for the coordinate repression of a number of genes by bile acids.

The characterization of two additional FXR target genes, namely multidrug resistance-associated protein 2 (MRP2; ABCC2) and the bile salt export pump (BSEP; ABCB11), has reinforced the role of FXR in bile acid homeostasis (1, 16). BSEP is critical for the ATP-dependent transport of bile acids across the hepatocyte canalicular membrane into bile. Ananthanarayanan et al. (1) demonstrated that BSEP was directly regulated by FXR and identified an IR-1 element in the promoter proximal region of this gene. MRP2 (also known as the canalicular multispecific organic anion transporter) is involved in the transport of organic anions, including sulfated and glucuronidated bile salts and conjugated xenobiotics. MRP2 mRNA levels were induced in human or rat hepatocytes by either CDCA or GW4064, and an FXR-response element was identified in the promoter of the rat MRP2 gene (16). Thus FXR can increase expression of two canalicular bile acid transport proteins and simultaneously suppress bile acid

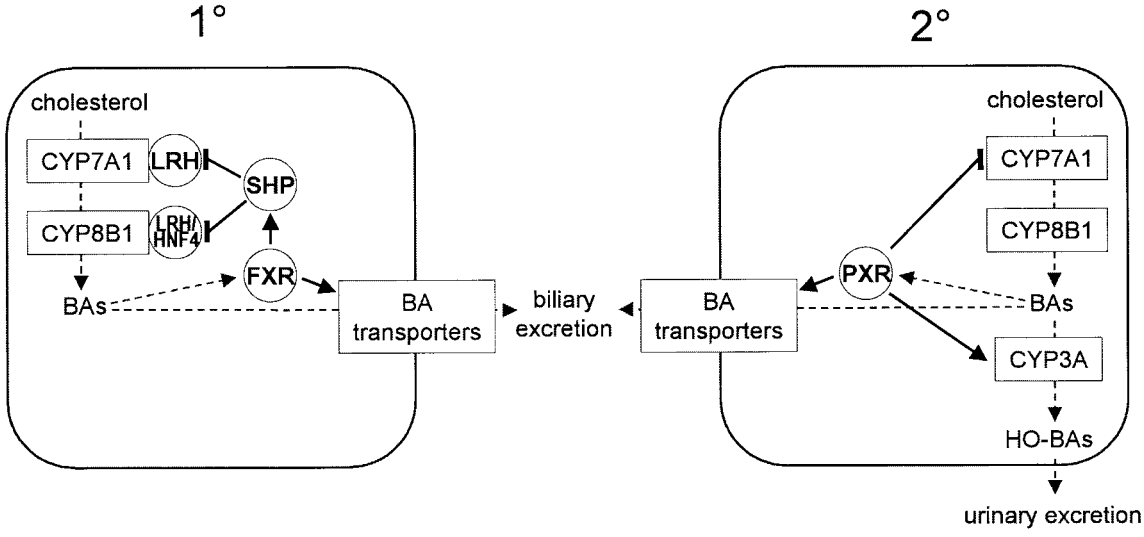

Fig. 1. Regulation of bile acid (BA) homeostasis by farnesoid X receptor (FXR) and pregnane X receptor (PXR). FXR is the primary (1°) BA sensor. FXR represses *CYP7A1* [cytochrome *P*-450 (CYP)] and BA synthesis by inducing small heterodimer partner (SHP) and stimulates expression of BA transporters. PXR represents a second line of defense (2°) against lithocholic acid and perhaps other toxic BAs. PXR represses *CYP7A1* and BA synthesis through an unknown mechanism and stimulates expression of BA transporters and *CYP3A*. LRH, liver receptor homolog; HNF4, hepatocyte nuclear factor 4; HO-BAs, hydroxylated bile acids.

biosynthesis, thereby preventing the accumulation of potentially harmful bile acids in the hepatocyte.

Targeted disruption of the FXR gene. Definitive evidence that FXR functions as a bile acid receptor was provided by mice lacking functional FXR. Sinal et al. (33) reported serum bile acid concentrations in the $FXR^{-/-}$ mice were elevated compared with wild-type animals. Furthermore, gross hepatotoxicity was evident in $FXR^{-/-}$ but not wild-type animals when fed a bile acid-enriched diet. Expression of SHP in the $FXR^{-/-}$ mice was dramatically lower than that in $FXR^{+/+}$ mice, and bile acid feeding resulted in upregulation of SHP in the wild-type but not $FXR^{-/-}$ animals. Importantly, the bile acid-dependent suppression of *Cyp7a1* and *Cyp8b1* was lost in the $FXR^{-/-}$ mice, clearly implicating FXR in this process. In addition, expression of the genes encoding BSEP and the enteric I-BABP was highly inducible by bile acid feeding in wild-type mice but was virtually undetectable in the $FXR^{-/-}$ animals irrespective of diet (33). This study further established a central role for FXR in bile acid homeostasis.

FXR and triglyceride levels. In addition to its role in the regulation of bile acid biosynthesis and transport, FXR also appears to play a role in specific aspects of lipid metabolism. When administered to Fisher or Zucker diabetic fatty rats, GW4064 causes a decrease in circulating triglycerides and a concomitant increase in high-density lipoprotein (HDL) cholesterol levels (J. Way and B. Goodwin, unpublished observations) (22). The molecular mechanism underlying this observation remains to be elucidated. However, the fact that FXR directly regulates the expression of phospholipid transfer protein (PLTP), a secreted protein involved in the transfer of phospholipids and cholesterol from triglyceride-rich lipoproteins to plasma HDL, and apolipoprotein C-II, a cofactor for lipoprotein lipase, suggests an extended role for FXR in controlling lipoprotein levels (9). In support of this, $FXR^{-/-}$ mice exhibit elevated levels of low-density lipoprotein and very-low-density lipoprotein, a proatherogenic serum lipid profile (33).

PXR—A SECOND LINE OF DEFENSE AGAINST BILE ACIDS

PXR regulates CYP3A. The body must defend itself against a vast array of lipophilic chemicals that are either derived from the environment (xenobiotics) or synthesized by the body itself (endobiotics). If these chemicals were not metabolized and excreted in a timely fashion, they might accumulate to toxic concentrations. The CYP450 family of heme-containing monooxygenases often catalyzes the first step in the conversion of lipophilic chemicals to polar derivatives that can be more readily excreted from the body. Members of the CYP3A subfamily play a particularly important role in this chemoprotection. These enzymes have very broad substrate specificities and are involved in the oxidative metabolism of a plethora of potentially harmful endogenous and xenobiotic substances, including bile acids (26, 39). Notably, CYP3A concentrations can be dramatically induced by a variety of structurally diverse compounds (26). Often, these chemical "inducers" are also substrates for the CYP3A enzymes. Thus induction of *CYP3A* provides a physiological mechanism for amplifying the body's detoxification response during periods of prolonged chemical challenge.

Although the effects of xenobiotics and endobiotics on *CYP3A* gene expression were well established, the molecular mechanism underlying this induction was poorly understood. In 1998, a novel nuclear receptor was reported and named the pregnane X receptor (PXR) (17). There are now a wealth of data demonstrating that PXR serves as a key regulator of *CYP3A* expression. PXR is highly expressed in the liver and intestine, the same tissues in which *CYP3A* is expressed and regulated. PXR binds as a heterodimer with RXR to xenobiotic response elements that have been characterized in the promoters and enhancers of *CYP3A* genes. Importantly, PXR is activated by the diverse chemicals that are known to induce *CYP3A*, including the macrocyclic antibiotic rifampicin, the glucocorticoid dexamethasone, and the synthetic pregnane pregnenolone 16α-carbonitrile (PCN) (13). Finally, *Cyp3a11* is not stimulated by xenobiotics in mice lacking functional PXR (34, 38). Moreover, these PXR-null mice are hypersensitive to treatment with chemicals such as the sedatives tribromoethanol and zoxazolamine that are metabolized by CYP3A isozymes (38). These genetic data demonstrated unequivocally that PXR regulates *Cyp3a11* in response to xenobiotics. Recent studies have revealed that PXR regulates a number of additional genes involved in the solubilization and excretion of xenobiotics (reviewed in Ref. 13). Thus PXR regulates a whole program of genes involved in all aspects of xenobiotic metabolism.

Bile acids are PXR ligands. For a number of years, it has been known that the rodent PXR agonist PCN suppressed transcription of the *CYP7A1* gene (4). Staudinger et al. (34) recently demonstrated that this is a PXR-dependent phenomenon. Interestingly, PCN does not stimulate expression of *Shp* (B. Goodwin, unpublished observations), which suggests that PXR represses *Cyp7a1* through a mechanism distinct from that of FXR.

In addition to repressing *Cyp7a1* and bile acid synthesis, PXR also stimulates the expression of other genes such as *CYP3A*, *MRP2*, and organic anion transporting polypeptide 2 (*OATP2*; SLC21A6), which encode proteins that contribute to the metabolism and clearance of bile acids (16, 34). The coordinate regulation of these genes suggested that PXR might regulate bile acid homeostasis. Two groups recently demonstrated that PXR is regulated directly by bile acids (34, 39). PXR was activated by the secondary bile acid LCA and its 3-keto metabolite. LCA and 3-keto LCA bound to PXR with IC_{50} values of ~10 μM (34). LCA is an extremely toxic bile acid that causes severe liver damage and biliary cholestasis when administered to rodents. Under normal physiological conditions, LCA concentrations are very low. However, they can reach micromolar concentrations in the livers of cholestatic

patients and in rodent models of cholestasis. The discovery that PXR is activated by LCA and regulates genes involved in the synthesis and metabolism of bile acids suggested that this receptor constitutes a second line of defense against the accumulation of pathophysiological levels of noxious bile acids (Fig. 1). A corollary of this hypothesis is that a potent PXR agonist should protect against bile acid-mediated toxicity. Indeed, Selye (31) showed many years ago that the PXR agonist PCN is strongly protective against the severe toxicity and mortality caused by LCA in rodents. These protective effects require PXR, thereby demonstrating a role for this receptor in protecting the liver against toxic bile acids (34, 39).

Can the hepatoprotective actions of PXR agonists be exploited therapeutically to treat liver disease? Interestingly, there is evidence that this might already be the case. The PXR ligand rifampicin has been used to treat pruritus caused by cholestasis and, in some cases, has been reported to promote complete remission of the disease. Moreover, the herb St. John's wort, which is a potent activator of PXR, has been used for centuries as a tonic for liver diseases, including cholestasis. It remains to be determined whether these effects are mediated through PXR. If this turns out to be the case, more potent PXR agonists may prove efficacious in the treatment of cholestasis, a disease for which there is no effective therapy.

SUMMARY AND PERSPECTIVES

The identification of nuclear receptors as key regulators of bile acid biosynthesis, transport, and metabolism has greatly advanced our understanding of bile acid signaling and homeostasis. The fact that nuclear receptors are activated by small lipophilic molecules makes them exquisitely amenable to pharmacological manipulation. FXR and PXR agonists, either alone or in combination, may have utility in the treatment of cholestasis. The precise role of FXR in regulating plasma lipid concentrations is unresolved, but, clearly, the potential for a novel approach to the treatment of dyslipidemia warrants further investigation. What of LRH-1 and SHP? To date, ligands have not been identified for these nuclear receptors, and they remain true "orphans." However, these proteins appear to contain the hydrophobic ligand-binding pocket that is characteristic of the lipid-regulated nuclear receptors, raising the intriguing possibility that it may be possible to develop drugs to modulate their activity. A greater understanding of the physiological pathways regulated by all of these receptors should provide exciting new opportunities for the treatment of a range of diseases associated with the dysregulation of bile acid homeostasis.

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