Role of Bile Acids and Bile Acid Receptors in Metabolic Regulation

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Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of Bile Acids and Bile Acid Receptors in Metabolic Regulation. Physiol Rev 89: 147–191, 2009; doi:10.1152/physrev.00010.2008.—The incidence of the metabolic syndrome has taken epidemic proportions in the past decades, contributing to an increased risk of cardiovascular disease and diabetes. The metabolic syndrome can be defined as a cluster of cardiovascular disease risk factors including visceral obesity, insulin resistance, dyslipidemia, increased blood pressure, and hypercoagulability. The farnesoid X receptor (FXR) belongs to the superfamily of ligand-activated nuclear receptor transcription factors. FXR is activated by bile acids, and FXR-deficient (FXR−/−) mice display elevated serum levels of triglycerides and high-density lipoprotein cholesterol, demonstrating a critical role of FXR in lipid metabolism. In an opposite manner, activation of FXR by bile acids (BAs) or nonsteroidal synthetic FXR agonists lowers plasma triglycerides by a mechanism that may involve the repression of hepatic SREBP-1c expression and/or the modulation of glucose-
induced lipogenic genes. A cross-talk between BA and glucose metabolism was recently identified, implicating both FXR-dependent and FXR-independent pathways. The first indication for a potential role of FXR in diabetes came from the observation that hepatic FXR expression is reduced in animal models of diabetes. While FXR−/− mice display both impaired glucose tolerance and decreased insulin sensitivity, activation of FXR improves hyperglycemia and dyslipidemia in vivo in diabetic mice. Finally, a recent report also indicates that BA may regulate energy expenditure in a FXR-independent manner in mice, via activation of the G protein-coupled receptor TGR5. Taken together, these findings suggest that modulation of FXR activity and BA metabolism may open new attractive pharmacological approaches for the treatment of the metabolic syndrome and type 2 diabetes.

I. INTRODUCTION

Bile acids (BAs) are amphipathic molecules with a steroid backbone that are synthesized from cholesterol exclusively in parenchymal cells (hepatocytes) of the liver. BAs and related bile alcohols with a surprising complexity in structure and, therefore, with different physicochemical properties have been identified in bile samples collected from various vertebrate species (207), implying the evolution of several biochemical pathways to convert membrane-bound, water-insoluble cholesterol molecules into water-soluble, amphipathic molecules with detergent properties. In fish and in several amphibians and reptiles, there is a preponderance of bile alcohols and BAs in which the C_{27} steroid backbone of cholesterol is preserved. In contrast, in most mammals, shortening of the C_{8} side chain of cholesterol to a C_{5} side chain occurs as part of the synthetic cascade leading to BA pools in which the so-called C_{24} BAs predominate. This review will focus on metabolism and on the metabolic actions of the latter class of BAs, which are present in humans and in rodents. The conversion of cholesterol into C_{24} BAs involves multiple enzymatic steps, of which the details have been largely elucidated (see Ref. 261), which are catalyzed by enzymes predominantly or exclusively expressed in the liver. Intriguingly, the enzymes that catalyze the individual biosynthetic steps are localized in different cellular compartments, i.e., endoplasmic reticulum, cytosol, mitochondria, and peroxisomes of the hepatocytes. How exactly BA synthesis intermediates, showing a gradual increase in hydrophilicity, shuttle from one cellular compartment to the other and how they pass the various intracellular membranes is still largely unknown and represents a challenging issue in current BA research.

BAs are actively secreted by the liver into bile and discharged into the intestinal lumen upon ingestion of a meal. The multistep enzymatic conversion of cholesterol into BAs confers detergent-like properties to the latter that are crucial for their physiological functions in hepatic bile formation and absorption of dietary lipids and fat-soluble vitamins from the small intestine. Efficient reabsorption of BAs in the terminal ileum results in the accumulation of a certain mass of BAs within the body, referred to as the BA pool, which cycles between intestine and liver in the enterohepatic circulation. The existence of this circulating pool, which cycles between intestine and liver in the enterohepatic circulation, is still largely unknown. The (patho)physiological relevance of this metabolic zonation, if any, remains to be established.

During the last decades of the 20th century, BA research focused mainly on topics related to the specific physicochemical characteristics of these natural detergents, i.e., bile formation and cholestasis, cytotoxicity, gallstone formation, fat absorption, and on the role of BA synthesis in the maintenance of cholesterol homeostasis. The detergent properties of BAs, determined by the number and the orientation of hydroxyl groups present on the steroid moiety, and influenced by the conjugation profile, are crucial for most of their biological functions, i.e., promoting biliary excretion of hydrophobic compounds and facilitating intestinal fat absorption. The physical characteristics of BAs, which allow...
them to form micelles, also impose a certain risk to cells that are exposed to high concentrations of these natural detergents. When present at high concentrations, BAs may become cytotoxic. In particular, hepatocytes and bile duct cells are at risk, for instance, in conditions of disturbed bile formation or stasis of bile in the ductular system (cholestasis), and protective mechanisms appear to become active when intracellular BA concentrations are elevated. Obviously, both the maintenance of physiological control of the enterohepatic circulation and the initiation of cell protective reactions require a mode of “BA sensing” in exposed cells. A new era of BA research was initiated in 1999, when BAs were identified as the natural ligands of the nuclear receptor farnesoid X receptor/bile acid receptor (FXR/BAR or NR1H4) (190, 232, 336), which uncovered a hitherto unknown function of BAs in the control of gene expression. It became then clear that BAs themselves are directly involved in regulation of gene expression in liver and intestine via interaction with FXR, which provides such a sensor function.

It turned out that many of the genes encoding proteins involved in BA synthesis, metabolism and transport in the enterohepatic circulation are strictly controlled by BAs themselves via activation of FXR. In addition, and more surprising, it became clear that BAs also serve specific functions as “metabolic integrators” in the control of fat, glucose, and energy metabolism through modulation of gene expression, a signaling role which may be partly dependent and partly independent of the FXR signaling pathway, and involve the G protein-coupled receptor TGR5/Gpbar1. This knowledge has opened up new avenues for exploration of strategies for prevention and treatment of metabolic diseases. This review focuses on the current status of knowledge of FXR biology, i.e., the cloning and characterization of FXR (sect. iv), the role of this nuclear receptor in the control of BA synthesis and transport (sect. v), in lipoprotein (sect. vi) and glucose metabolism (sect. vii), the potential role of FXR in the development of atherosclerosis (sect. viii) and, finally, a discussion on potential roles of FXR in other pathophysiological phenomena (sect. ix). Relevant information on BA biology, which is essential for full appreciation of FXR biology, will first be discussed (sect. iii). From the discussion below, it will become evident that BAs fulfill a variety of functions in the body that go far beyond the classical role as the endogenous detergent of the body.

II. BILE ACID METABOLISM

A. Bile Acid Synthesis in the Liver

The immediate products of the BA synthetic pathways are referred to as primary BAs. Cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid) are the primary BAs formed in humans (see Fig. 1 for structure). In rodents, alternative hydroxylation reactions give rise to differently structured primary BAs, particularly the α-, β- and γ-muricholic acids (3α,6β,7α-trihydroxy-5β-cholanoic acid, 3α,6β,7β-trihydroxy-5β-cholanoic acid, and 3α,6α,7β-trihydroxy-5β-cholanoic acid, respectively). The chemical diversity of the BA pool in the body is further increased by the actions of the intestinal bacterial flora, giving rise to so-called secondary BA species. In humans, deoxycholic (3α,12α-dihydroxy-5β-cholanoic acid) and lithocholic (3α-hydroxy-5β-cholanoic acid) acids (Fig. 1) represent the major secondary BA species that are derived from cholic and chenodeoxycholic acids, respectively.

A complete description of the BA synthesis cascade is beyond the scope of this overview and can be found in excellent recent review articles (47, 261). In short, the steps leading to formation of primary BAs include the hydroxylation of cholesterol at the C7 position of the steroid ring structure or at the C24, C25, or C27 positions at the side chain, subsequent further modification of the steroid ring structure, followed by side-chain shortening. In particular, the hydroxylation step catalyzed by the microsomal cytochrome P-450 enzyme cholesterol 7α-hydroxylase (CYP7A1), the first step of the so-called “classical pathway” of BA biosynthesis that yields 7α-hydroxycholesterol (see Fig. 1), is considered to be of great regulatory importance and the activity of this enzyme is subject to complex modes of control (see sect. v). The physiological relevance of this step, and of BA synthesis in general, is evident from the phenotype of CYP7A1-deficient mice (132, 271): these mice display a very high incidence of postnatal lethality as a consequence of liver failure and fat/vitamin malabsorption. Mice that die within the first 3 postnatal weeks produce only small amounts of specific monohydroxy BA species that are hepatotoxic. Animals that survive this period, by supplementation of cholic acid to the diet of the nursing dams, start to produce “normal” BAs via pathways in which oxysterols, rather than cholesterol, serve as substrates for 7α-hydroxylation. However, their BA pool remains markedly compromised, i.e., at ~25% of normal values. A frameshift mutation in the human CYP7A1 gene that abolishes enzyme activity has been identified in three subjects with statin-resistant hypercholesterolemia and premature gallstone disease (249). A 94% reduction in fecal BA output, which by definition reflects the hepatic synthesis rate, was recorded in one of the homozygous subjects with a clear shift towards predominant formation of chenodeoxycholic acid. The latter finding indicates a preferential formation of the remaining BAs via alternative pathways. Unfortunately, no formal assessment of BA synthesis and pool size has been reported so far in CYP7A1-deficient subjects.
It was discovered in the 1960s that side-chain hydroxylated cholesterol molecules like 24-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol can serve as substrates for BA synthesis. As extensively discussed by Russell (261), available data from mouse and human studies indicate that formation of 24-hydroxycholesterol, which is particularly important for cholesterol turnover in the brain, and 25-hydroxycholesterol contributes only very modestly to BA synthesis in quantitative terms. In contrast, BA synthesis from 27-hydroxycholesterol occurs in relevant quantities in humans, as well as in mice, via a route usually referred to as the “alternative” or
“acidic” pathway. This oxysterol, which is the most abundant oxysterol species in human plasma, is synthesized by the mitochondrial sterol 27-hydroxylase (CYP27A1), an ubiquitously expressed enzyme. On the basis of studies in CYP7A1-null mice, it has been estimated that the alternative pathway contributes to ~25% of total BA synthesis in the mouse (272). The relative contribution of the oxysterol-initiated pathways to total BA synthesis in humans is difficult to assess directly. Limited data from sterol-initiated pathways to total BA synthesis in humans (272). The relative contribution of the oxy-

The products of HSD3B7 activity can subsequently take two routes: in case the 3-oxo,Δ4 intermediate interacts with microsomal sterol 12α-hydroxylase (CYP8B1), the end product will be cholic acid. Cheno-

dexoycholic acid (or muricholic acids in rodents) will be formed when 12α-hydroxylation does not occur (see Fig. 1). The activity of CYP8B1 thus determines the ratio in which the primary BAs are being formed and, thereby, the physicochemical and biological properties of the BA pool. The 12α-hydroxylated intermediates as well as those that escaped the actions of CYP8B1 are both subject to reduction of the C4 double bond by the Δ4-3-oxosteroid 5β-reductase (AKR1D1), localized in the cytosol, implying that at this point in the synthetic cascade intermediates transfer from a lipophilic membraneous (endoplasmic reticulum, ER) environment to an aqueous environment. Whether or not specific transport proteins are involved herein is not known (261). Defective Δ4-3-oxosteroid 5β-reductase has been identified as the cause of severe intrahepatic cholestasis in identical infant twins on the basis of an elevated urinary excretion and predominance of unsaturated hydroxy-oxo BAs, i.e., 7α-hydroxy-3-oxo-4-cholenoic and 7α,12α-dihydroxy-3-oxo-4-cholenoic acids (75% of total). Cheno-

dexoycholic acid and allo-BAs, i.e., BAs in which the A and B rings of the steroid backbone are in the trans- rather than in the cis-configuration, could be identified in serum and bile, albeit at low concentrations, indicative of the existence of a pathway in which side chain oxidation (see below) can occur despite incomplete modification of the steroid nucleus (278). The final step of ring modification in normal BA biosynthesis
involves reduction of the 3-oxo group to a 3α-hydroxyl group by 3α-steroid dehydrogenase (AKR1C4), a cytosolic enzyme that belongs to the aldo-keto reductase family. As stated previously, the presence of this hydroxyl group in the 3α orientation is a main determinant of the physicochemical characteristics and, therefore, of the biological actions of the end product.

The next steps in the biosynthetic cascade leading to primary BA production involve the progressive oxidation and shortening of the side chain. The first three steps of this process are performed by mitochondrial CYP27A1, previously shown to be involved in formation of 27-hydroxycholesterol in the alternative route of BA synthesis. The actions of CYP27A1 introduce a hydroxyl group at C27, then oxidize this group to an aldehyde then to a carboxylic acid (243). The ensuing oxidized intermediates are then transferred from mitochondria to peroxisomes for shortening of the side chain by yet unresolved mechanisms. This process, in which the three terminal C atoms are removed in a series of reactions that are analogous to those in fatty acid β-oxidation, involves the sequential actions of BA coenzyme A ligase to activate the BA intermediate, of 2-methylacyl-coenzyme A racemase to convert the coenzyme A conjugate to the proper 25(S)-isomer, and of branched chain acyl-coenzyme A oxidases (ACOX1 or ACOX2) to yield 24,25-trans-unsaturated derivatives. The next step involves hydration and oxidation at the Δ24 bond by the D-bifunctional enzyme. Consequently, mice as well as humans deficient in D-bifunctional protein not only accumulate very-long-chain fatty acids (e.g., pristanic acid) but also unsaturated C27 BA intermediates. The last step of side chain shortening is catalyzed by peroxisomal thiolase 2, which cleaves the C27-C25 bond to yield a C24 BA-coenzyme A and propionyl-coenzyme A. As a consequence of the prominent role of peroxisomes in BA biosynthesis, various inborn errors of metabolism caused by peroxisomal dysfunction are characterized by the presence of abnormal BA species with diagnostic value (25, 80, 302).

Prior to their secretion into the bile canicular lumen, primary BAs are conjugated at their side chains with either taurine or glycine, further enhancing their hydrophilicity. This reaction, i.e., the formation of an amide linkage between the amino acid and the C24 BA-coenzyme A thioester, is catalyzed by bile acid coenzyme A:amino acid N-acyltransferase (BAAT) (78). Most mammals are able to form both taurine and glycine conjugates: the ratio in which they are formed is determined by the availability of taurine since the enzyme has a greater affinity for this amino acid. BAAT is solely responsible for the conjugation reaction, as delineated by the fact that both taurine and glycine conjugates are absent in serum of patients with familial hypercholanaemia homozygous for a mutation in the BAAT gene (39). The normal 3:1 ratio of glycine to taurine conjugates in human bile is altered in various disease states. The percentage of taurine conjugates is, in general, higher than normal in cholestatic liver disease and lower in situations characterized by increased conjugation requirements, such as during treatment with BA sequestrants or external biliary drainage. Conjugation appears to be a prerequisite for efficient secretion of the common C24 BAs into bile. Furthermore, conjugation with glycine or taurine lowers the pKα value by ~1.3 and 5 units, respectively, compared with the unconjugated parent compound, and therefore renders conjugated BAs fully ionized at physiological pH. This will prevent back-diffusion of conjugated BAs during their passage down the biliary tree, as well as their passive reabsorption from the small intestine (see below). The conjugation reaction is highly efficient, because essentially all biliary BAs in all mammalian species studied are conjugated (207). BA infusion studies in rodents showed that conjugation is virtually complete after a single pass through the liver (100). BAAT resides in peroxisomes with variable amounts present in the cytosol of both human and rat liver (109, 294). This has led to the speculation that peroxisomal BAAT is responsible for conjugation of newly synthesized BAs present in these organelles, whereas cytosolic BAAT is involved in reconjugation of free BAs returning to the liver after deconjugation in the intestine (see sect. III.1). However, a recent study by Pellicoro et al. (240) firmly established the presence of both human and rat BAAT exclusively in peroxisomes: this finding implies that unconjugated BAs must be taken up by peroxisomes and that conjugated BAs need to be released from these organelles before they can be secreted into the bile. Putative transporter proteins involved in the various obligatory steps in intracellular BA (intermediate) transport have remained elusive so far.

Very recently, fatty acid transport protein 5 (FATP5) has been identified as another player in BA conjugation based on the phenotype of FATP5-deficient mice (68, 122). FATP5 is a liver-specific member of the FATP/Slc27 family that exhibits both fatty acid transport and BA-CoA ligase activity in vitro. It was reported that, although total BA concentrations were unchanged in bile, urine, and feces, the majority of gallbladder BAs was actually in unconjugated form in FATP5-null mice. Only primary BAs were found to be conjugated with taurine, indicative of a specific requirement for FATP5 in reconjugation during enterohepatic cycling. Potential interactions and redundancies between the various pathways involved in BA conjugation now need to be defined.

Although the term conjugation is generally used to denote the N-acyl amidation of BAs with glycine or taurine, it is important to realize that BAs can be subjected to other conjugation reactions, i.e., phase II metabolic reactions similar to those involved in the inactivation of endo- and xenobiotics. Sulfation was first recognized as a pathway in BA metabolism by Palmer (229), who identified the
3α-sulfates of glyco- and taurolithocholic acid in human bile. Since then, ester glucuronidation at the C24 position, ether glucuronidation at the C5 and C6 nuclear sites, and N-acetylglucosaminidation at the C7 position of BAs with a β-hydroxy group such as ursodeoxycholic acid have been described (see Ref. 318 for review). Sulfation of BAs, i.e., the transfer of a sulfonyl group from 3-phosphoadenosine-5-phosphosulfate to the 3α-hydroxy group of hydrophobic BA species like lithocholic acid, is catalyzed by the sulfotransferase SULT2A1 (SULT2A9 in mice) (42). Glucuronide conjugation of BAs can be catalyzed by several of the ubiquitous UDP glucuronosyltransferases. In humans, UGT2B4 mediates glucuronide conjugation at the 6α-hydroxy position while UGT2B7 transfers glucuronosyl moieties to 3α- and 6α-hydroxy groups (87, 244, 320). UGT1A3 uniquely modifies the C24-carboxyl group at the side chain of BAs, giving rise to BA ester glucuronides (319). Whereas sulfated glycolithocholic and taurolithocholic acids are low abundant but normal components of the human BA pool (229), BA glucuronide formation appears to be a quantitatively minor pathway in human BA metabolism (113, 306). BA conjugation has been proposed as an adaptive response in cholestatic liver diseases to allow for effective urinary clearance of these more hydrophilic metabolites. Indeed, increased concentrations of sulfated and glucuronidated BAs have been found in urine of patients with cholestatic liver diseases. However, in the absence of cholesclerosis, sulfated and glucuronidated BAs are primarily excreted into bile, in humans (306) as well as in rodents (159, 162). In contrast to expectations, it appeared that neither sulfation nor glucuronidation reduced hepatotoxicity of monohydroxylated BAs in rodents. Sulfated glycolithocholic acid (160, 355), but not its taurine-conjugated counterpart, as well as lithocholic acid-3-O-glucuronide (226) showed a cholestatic potency equal to or higher than that of the parent compound lithocholic acid upon intravenous administration to rats. The functional consequences of sulfation or glucuronidation on newly identified BA signaling pathways (see below) have not been systematically addressed.

B. Bile Acid Biotransformations by Intestinal Bacteria

The microbial flora of the large intestine strongly impacts on BA metabolism and, vice versa, bile and BAs contribute to suppression of significant bacterial coloni- zation of the small intestine. The intensive interactions between intestinal flora and BAs and their implications have been the subject of excellent recent reviews (16, 257). Within the context of the current review, it is of importance to appreciate that colonic bacteria contribute to the salvage of BAs that escape from uptake in the distal ileum and thus spill over in the colon. Although ileal BA uptake is highly effective (~95%), frequent cycling of the BA pool, i.e., up to 12 cycles/day in humans, results in deposition of 400–800 mg BAs into the colon for bacterial biotransformation each day. Major structural modifications that occur in the large intestine include deconjugation, oxidation of hydroxy groups at C3, C7 and C12, and 7α/7β-dehydroxylation. Deconjugation, i.e., enzymatic hydrolysis of the N-aclamide bond between BA and glycine or taurine at the C24 position, is essentially complete in the human large intestine. BA hydrolases belonging to the family of choloxylic hydrolases (EC 3.5.1.24) have been isolated and/or characterized from several species of bacteria (257). Oxidation and epimerization of C20, C7, and C12 hydroxy groups is performed by hydroxysteroid dehydrogenases expressed by various strains of bacteria. Epimerization, i.e., interchange between α- and β-configuration, involves the generation of stable oxo-BAs of which several can be found in human feces (276). Since deoxycholic and lithocholic acids represent the predominant BA species in human feces (276), 7α-dehydroxylation of cholic and chenodeoxycholic acids represents the most quantitatively important biotransformation in the human colon. Surprisingly, current estimates indicate that this pathway is found in only 0.0001% of total colonic flora, by species belonging to the Clostridium genus (142). Importantly, the dehydroxylation reaction appears to be restricted to free BAs, implicating a functional interplay between deconjugation and dehydroxylation activities in the large intestine (257). This combined action increases hydrophobicity and pKₐ of the BAs, thereby permitting their absorption by passive diffusion across the colonic epithelium and entry into the BA pool. Because the human liver is incapable to rehydroxylate deoxycholic acid, unlike rodent liver, this secondary BA constitutes a significant part of the circulating BA pool in humans (~35%). In contrast, lithocholic acid does not build up in the pool; this has been ascribed to its efficient sulfation prior to secretion into bile which limits intestinal reabsorption, possibly due to interactions with calcium in the small intestinal lumen (161), hence preventing its accumulation in the enterohepatic circulation.

C. Transport of Bile Acids in the Enterohepatic Circulation

Conservation of the pool of hydrophilic, largely ion- ized BA molecules within the enterohepatic circulation requires the coordinate action of several transporter proteins expressed at the apical and basolateral membranes of liver and intestinal epithelial cells. In view of the size of the BA pool, physiological fluctuations in BA flux through the enterohepatic system during the day (because the dynamics hereof are controlled by ingestion of meals) and the relatively small escape of BAs from the circulation, it
is evident that the capacity of the transporter systems involved must be important. The molecular characterization, function, and modes of regulation of the various BA transporters have been reviewed in a recent issue of *Physiological Reviews* (317) and several other excellent overview articles (27, 305). In addition, defects in transporter systems related to human cholestatic liver diseases have been subject of several, well-referenced reviews (76, 163, 235). Hence, only a condensed description of the systems involved will be provided here.

Newly synthesized BAs and those returning from the intestine during the course of their enterohepatic cycle are actively secreted by the hepatocytes into the bile canalicular lumen by the action of the Bile Salt Export Pump (BSEP), a member of the ATP binding cassette superfamily of transporter molecules (symbol ABCB11). Active secretion of BAs provides a major driving force for bile formation (317) and is therefore of crucial importance for hepatobiliary removal of a variety of endo- and xenobiotics from the body via the hepatobiliary pathway. In addition, BA secretion drives the secretion of phospholipids and cholesterol into bile. Secretion of these lipids also depends critically on the activities of ABC transporters, i.e., MDR3 (ABCB4) and the ABCG5/ABCG8 heterodimer for phospholipids and cholesterol, respectively (76). Cosecretion of BAs and these lipids, allowing for mixed micelle formation in bile, is crucial for protection of the biliary system from the detergent actions of high biliary BA concentrations (291).

Primary bile, containing BAs and associated lipids in the millimolar concentration range, is stored in the gallbladder, where bile is further concentrated by absorption of water, and discharged into the intestinal lumen upon ingestion of a fatty meal through cholecystokinin (CCK)-induced gallbladder contraction. In the small intestinal lumen, BAs act as detergents to solubilize and facilitate the absorption of dietary fats and lipid-soluble vitamins in the small intestine. Due to their relatively low $pK_a$ value, passive absorption of conjugated BAs is minimal and their intraluminal concentrations remain high along the length of the small intestine. BAs are actively and very effectively reabsorbed by the actions of specific transporter systems only in the terminal ileum, i.e., the apical sodium-dependent BA transporter (ASBT/Slc10a2) and the basolaterally localized heterodimeric organic solute transporter Osta/Ostβ. The role in vectorial transport of BAs of the cytosolic intestinal BA-binding protein (IBABP) that is attached, in the cytoplasmic compartment, to ASBT (93) through the ileal enterocytes remains enigmatic (158). BAs escape reabsorption and enter the colon where they may be converted into secondary BAs that is absorbed returns to the liver via the portal blood via the Na\textsuperscript{+}-taurocholic acid cotransporting polypeptide (NTCP or Slc10a1), which has a substrate specificity essentially limited to conjugated BAs (102), or by Na\textsuperscript{+}-independent systems represented by members of the superfamily of organic anion transporting polypeptides (OATP/SLCO) (103). Of these, OATP1A2, OATP1B1, and OATP1B3 exhibit overlapping substrate specificities for conjugated and unconjugated BAs as well as neutral steroids, steroid sulfates, and selected organic cations (103). After their uptake and, if necessary, reconjugation, BAs must be transported to the canalicular pole of the hepatocytes to be secreted again into the bile, which completes their enterohepatic circulation. Processes involved in the transcellular movement of BAs across the hepatocytes are yet poorly defined. Several intracellular binding proteins (e.g., glutathione-S-transferases, fatty acid binding proteins, 3-hydroxysteroid dehydrogenase) have been implicated, but their roles have not been formally proven. Additionally, free BAs may reach the canalicular membrane by rapid diffusion or, when high BA loads need to be translocated, partitioning of hydrophobic BAs into organelles such as ER and Golgi apparatus may occur. Whether or not physiological changes in transcellular BA flux, e.g., during the postprandial phase or in conjunction with (diet-induced) changes in BA pool size or composition (23), affect intracellular localization of BAs is not known.

D. Kinetics of the Enterohepatic Circulation of Bile Acids

During the day, there is a massive transport of BAs through different compartments of the body. The magnitude of this flux is not constant but highest during the immediate postprandial phase, since gallbladder contraction initiates BA cycling. Although hepatic extraction of BAs by the liver from portal blood is highly efficient, a postprandial rise of plasma BA levels occurs in healthy subjects, which may be of relevance for the signaling functions in peripheral tissues and organs (see below). On their way through the enterohepatic circulation, BAs can interact with various potential “modulators” of their metabolism, such as food components with BA-binding properties in the intestinal lumen and biotransforming enzyme systems present in the intestinal flora and in the liver. Furthermore, the dynamics of enterohepatic cycling will be influenced by mechanical factors such as gallbladder contraction and intestinal motility. To be able to understand the physiology of BA metabolism as well as pathophysiological consequences of disturbances in the various processes described in the preceding paragraphs, it is necessary to have an accurate insight in the concentrations of individual BA species in the various compartments as
well as in kinetic parameters that define their behavior in the enterohepatic circulation, i.e., the size of the circulating pool, its (fractional) turnover rate as a measure of absorption efficiency, and the rate at which BAs are being synthesized.

Over the years, there has been considerable progress in analytical techniques that allow for accurate assessment of concentrations of individual BA species and their conjugates in plasma, bile, urine and feces, which were largely developed for diagnostic purposes. Application of these techniques has been of great benefit for the identification of inborn errors in BA metabolism and, at the same time, of major relevance for phenotyping of new mouse models. However, concentration measurements only do not provide full insight in the dynamics of (disturbances in) BA metabolism. Hepatic BA synthesis, an important determinant of whole body cholesterol turnover, can be derived from the rate of fecal BA output since, under steady-state conditions, fecal loss equals hepatic synthesis rate. As already discussed by Setchell et al. (276), however, accurate measurement of fecal BA loss in humans is not trivial. Other approaches have been developed for this purpose, such as the rate of release of $^{14}$CO$_2$ from orally administered $^{[26,14]}$Ccholesterol (70) or of $^3$H$_2$O from $^{[24,25-3H]}$cholesterol (58) as a measure of cholesterol side chain oxidation. Both methods were found to correlate reasonably well with fecal BA output, yet the use of radiolabeled tracers evidently limits the use of these approaches in human studies. As alternatives, quantitative measurement of intermediates in the BA synthetic cascade in blood plasma, such as 7a-hydroxycholesterol (61) and 7a-hydroxy-4-cholesten-3-one (89), have been proposed to reflect hepatic CYP7A1 activity and thus BA synthesis. These assays are definitively very useful for comparison of (patient) groups (19) and for monitoring of physiological variations in CYP7A1 activity that occur during the day-night cycle (88, 185); however, they do not provide insight in actual quantitative changes in BA synthesis.

For the actual determination of BA synthesis rate and other relevant parameters such as pool size and fractional turnover rate, tracer methodologies are required. Already in 1957, Lindstedt (179) introduced an isotope dilution approach for assessment of cholic acid kinetic parameters in human volunteers, involving oral administration of $^{[24,14]}$Ccholeic acid and repeated sampling of duodenal bile. Later, Duane et al. (71) have validated the use of $^{[22,23-3H]}$cholic acid for this purpose. On the basis of the assumption of a one-compartment model, the decay of specific activity of cholic acid in subsequent bile samples is described by an exponential curve. Conversion to the logarithm and extrapolation of the curve to time point zero, being the time of administration, allowed for assessment of pool size. The slope of the logarithmic curve reflects the fractional turnover (FTR in pools/day) of the pool, i.e., the fraction that is renewed per time interval. Multiplying pool size and FTR establishes a value for the absolute turnover, which equals the hepatic synthesis rate under steady-state conditions.

Pioneering mass spectrometric studies by Klein et al. (156) in the 1970s allowed for the replacement of radioactive tracers by stable isotopically labeled ($^{2H}$, $^{13}$C) BAs for kinetic measurements, thereby facilitating their use in patient groups also including pregnant women and children (e.g., Ref. 344). Although avoiding radiation hazard, this technique remained invasive because bile sampling was part of the procedure. Owing to major advances in gas chromatography-mass spectrometric techniques and novel derivatization procedures, Stellaard et al. (304) completed the technical development enabling accurate isotope ratio measurements for BAs in serum samples in the normal concentration range. Direct comparison of the serum sampling technique with the bile sampling technique proved isotopic equilibrium between serum and biliary BAs in the postprandial state. The technique, originally designed for simultaneous assessment of kinetic parameters of both primary BAs, i.e., cholic and chenodeoxycholic acids, was subsequently extended to also include deoxycholic acid pool size, fractional turnover, and the input rate of this secondary BA from the colon (303). This technique has been successfully applied to establish kinetic parameters of BA metabolism in various patient populations (20, 234). Furthermore, its application allowed the demonstration of strong effects of diet composition on human BA metabolism (23, 135), implying that diet may affect BA signaling functions (see below). Finally, refinement of analytical techniques has allowed for a significant down-scaling of the amount of plasma required for accurate measurements which makes the technique applicable for use in rodents (125). In this case, because biliary BA output rates can also be determined in the same experiment, an estimate of intestinal absorption efficiency and of the cycling time of the pool can be obtained (158). For instance, it was found in rats that the frequently used immunosuppressive drug cyclosporin A reduced hepatic cholic acid synthesis by 71% without inducing significant changes in cholic acid pool size, due to an accompanying 65% reduction in the fractional turnover rate of the pool. More efficient cholic acid reabsorption (1.4 ± 0.5% loss per cycle in treated rats versus 4.3 ± 0.5% loss per cycle in controls) associated with upregulation of ileal ASBT protein expression appeared to account for maintenance of BA pool size under these conditions (126). This finding illustrates the large metabolic consequences of a relatively small increase in absorption efficiency from 95.7% in controls to 98.6% in treated animals, underscoring the important role of the intestine in the maintenance of BA homeostasis. Similar reductions in BA synthesis were observed in children treated with cyclosporin A after liver transplantation (118).
E. Bile Acids and Thermogenesis

A novel concept indicating a signaling role of BAs in the control of energy metabolism independent of FXR was conceived in 2006 (342), based on the observation that addition of cholic acid to the diet increases energy expenditure in brown fat in mice and prevents the development of high-fat-induced obesity and insulin resistance. This metabolic effect of BAs was found to be critically dependent on induction of the cAMP-dependent thyroid hormone activation enzyme D2 (type 2 iodothyronine deiodinase, DIO2) since it was lost in D2-deficient mice. DIO2 converts inactive thyroxine into active 3,5,3’-triiodothyronine in cells and plays a crucial role in regulating thermogenesis in BAT by controlling the expression of the uncoupling protein (UCP)-1. These effects appeared to be independent of FXR and were suggested to be mediated by cAMP production induced by BAs binding to the G protein-coupled receptor TGR5 (also known as Gpbar1). This intriguing concept requires further evaluation, e.g., identification of the high-fat-associated factor that is essential for the effect to occur, and assessment of its relevance in species like humans that lack brown fat, etc. In addition, data obtained with recently generated Gpbar1-deficient mice are not entirely consistent with the proposed sequence of events. Vassileva et al. (331) demonstrated that Gpbar1 is predominantly expressed in gallbladders rather than in organs/tissues involved in energy metabolism and showed that Gpbar1-deficient mice are resistant to cholesterol gallstone formation upon feeding with a lithogenic diet. The authors showed that Gpbar1−/− mice display disturbed feedback inhibition of CYP7A1 gene expression upon BA feeding. Yet, in vivo studies in Gpbar1−/− mice yielded apparently conflicting results, since weight gain upon high-fat, lithogenic [0.5% (wt/wt) cholic acid] diet feeding was found to be similar (331) or significantly increased in Gpbar1−/− compared with wild-type mice. In the latter case, a gender-dependent manner effect was noted (female, but not male, Gpbar1−/− mice showed increased body weight) (195). Clearly, the exact role of the Gpbar1 in BA-mediated effects on energy metabolism still needs to be defined, although these results highlight the crucial role of BAs in regulating body weight.

III. CLONING AND CHARACTERIZATION OF FARNESOID X RECEPTOR

A. Cloning of FXR and Gene Structure

Using a yeast-based interaction trap with the human nuclear receptor RXRα ligand binding domain (LBD) as a bait, Seol et al. (274) isolated mouse liver cDNAs encoding for the RXR-interacting protein 14, which exhibited a primary sequence with notable homologies with other members of the nuclear receptor superfamily (RIP14). RIP14 expression was detected in liver and kidney. These cDNAs corresponded to distinct isoforms of the same protein with either a 38-amino acid NH2-terminal extension (RIP14-2), or a 4-amino acid (MYTG) central insertion (RIP14-1). RIP14 bound as a heterodimer with RXRα to various previously identified hormone response elements, such as the ecdysone response element (EcRE) from the Drosophila hsp27 promoter, direct repeats with a 5-bp spacer (DR5), DR2 and DR4, and on inverted repeats of the IR0 and IR1 types. On the contrary, RXR-RIP14 heterodimers were devoid of any affinity for DR0, DR1, DR3, and IR2 to IR5 response elements. A general decrease of the DNA binding activity was noted for the RIP14-2 isoform, harboring the NH2-terminal extension. A few months later, Forman et al. (83) reported the cloning of the rat homolog of RIP14, a 469-amino acid protein able to dimerize with RXRα, by screening a regenerating rat liver cDNA library with a truncated mouse RIP14 cDNA (83). Intermediates of the mevalonate pathway such as juvenile hormone and farnesol activated RXR-rRIP14 heterodimers at 50 μM, and a synthetic RXR ligand (LG1069) could synergize with farnesol in transcriptional assays. The subsequently called “farnesoid X receptor” was shown, as RIP14, to be expressed mostly in liver and kidney, and also in the adrenal cortex and in intestinal villi from E19.5 rat embryo and adults. Of note however, recent reports described the RXR ligand 9-cis-retinoic acid as an inhibitor of FXR transcriptional activity (48, 144), suggesting that distinct RXR ligands impact differently on FXR-controlled transcription. Farnesol was also described as a PPARα ligand (104, 225), and interestingly, farnesyl pyrophosphate, an intermediate on the cholesterol synthesis pathway, is unable to activate FXR, but is a promiscuous ligand activating the thyroid hormone receptor α and other steroid receptors (57).

In 1997, a report showed that RIP14/mouse FXR could not be activated by farnesol. However, a high concentration (5 μM) of the synthetic retinoid TTNPB [(E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)pro- pent-1-yl]benzoic acid] potently activated mouse FXR in transient transfection assays in trophoblast tumor JEG3 cells (360). TTNPB was later identified as a FXR ligand based on coactivator recruitment assays (232). FXR was deorphanized 2 yr later, with the recognition that it could be activated by major BAs (190, 232, 336) and thus renamed BAR, for bile acid receptor. Chenodeoxycholic acid (CDCA), cholic acid (CA), deoxycholic acid (DCA), and lithocholic acid (LCA) were shown to activate FXR at physiological concentrations (~100 μM). Interestingly, the 7β epimer of CDCA, ursodeoxycholic acid (UDCA), was inactive in such assays, hinting at specific structure–activity relationships which were explored later and which are detailed below. Due to the lack of a labeled
high-affinity reference ligand, evidence for a direct interaction of FXR with BAs came initially from coactivator recruitment assays, which probe for ligand-induced structural transitions occurring in the LBD. Furthermore, the identification of FXR as a BA-activated transcriptional regulator of the mouse intestinal BA binding protein (I-BABP) and indirectly of CYP7A1 in human hepatoma cells (HepG2) clearly pointed to a critical role of FXR in BA metabolism.

Both the unique mouse and human gene (NR1H4) encode four isoforms, initially termed α1 (RIP14-2), α2, β1, and β2 (RIP14-1) (367). The identification of FXRβ (NR1H5), a pseudogene in primates (227) but a receptor for lanosterol, an intermediate in the cholesterol synthesis pathway, in other mammals, led to a new nomenclature designing the four NR1H4 isoforms as FXRα1 (previously mFXRα1), FXRα2 (previously mFXRα2), FXRα3 (previously mFXRβ1), and FXRα4 (previously FXR β2) (Fig. 2).

The murine FXRα gene (termed hereafter FXR) is composed of 11 exons and 10 introns. Two distinct promoters, located upstream of exon 1 and exon 3, drive the expression of either FXRα1 and α2 or FXRα3 and α4, respectively. As a consequence, FXRα1 and -2 isoform transcripts emanate from exon 1, whereas FXRα3 and -4 transcripts start from exon 3. An alternative splice donor site is located downstream of exon 5 and produces either α1 and -3 isoforms, which contain a MYTG insertion, or isoforms which lack this additional sequence (α2 and -4) (see Fig. 2). Exons 4–11 are shared between all isoforms, suggesting that all FXR isoforms will be activated non-selectively by FXR ligands (367). Huber et al. (123) showed that the structures of the FXR gene and of the encoded isoforms are highly homologous across several species (Syrian hamster, mouse, rat, and human) and that the human gene also generates four isoforms (123). As of today, little is known about the specific biological functions of each isoform, although functional differences have been highlighted (see below).

B. Tissue Distribution Patterns of FXR

High levels of FXR isoforms were detected in the adult C57BL/6J female mouse liver (367). Mouse liver and small intestine express equal amounts of each FXR isoform, whereas kidney and stomach exclusively express FXRα3/4. In contrast, heart and adrenal glands express only FXRα1/2. Other mouse organs, such as lung and fat, exhibit low amounts of FXRα1/2, whereas FXR is undetectable in brain, spleen, or skeletal muscle. In adult human tissues, an exclusive FXRα1/2 expression is found in adrenals and liver, whereas colon and kidney exclusively express FXRα3/4. Equal amounts of these isoforms are found in small intestine and duodenum, and FXR is not detectable in brain, heart, lung, and skeletal muscle (123). However, histochemical techniques suggest the presence of FXR in biopsies of human cardiac muscle, small intestine, and adrenal glands (22). A role in vascular biology can be inferred from FXR expression in human vascular smooth muscle cells from coronary arteries and aortas, as well as in atherosclerotic plaques (22) and in

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**Fig. 2.** Structure of the mouse farnesoid X receptor (FXR) gene and transcripts. **A:** structure of the FXR gene with exons numbered from 1 to 11. **B:** pre-mRNAs with the alternative splice site at exon 5, introducing a 12-bp sequence encoding for the “MYTG” insert. **C:** mRNAs encoding for each isoform. **D:** FXR protein structures. The A/B, C (DNA binding domain), D (hinge region), and E (ligand binding) domains are indicated.
cultured rat endothelial cells (110). FXR is also detected in white adipose tissue from mice (37, 258) and is one of the numerous nuclear receptors expressed in human immune cells. FXR mRNA is indeed detected in human peripheral blood mononuclear cells and subsets of lymphocytes and monocytes (270), but not in mouse peritoneal macrophages (98). The placenta transfers a large variety of substances from the mother to the fetus, and is also the main route for the elimination of toxic compounds produced by the fetal liver, including BAs. The trophoblast is a layer of chorionic villi and is a key element in the placental barrier function. FXR mRNA is expressed in cytokeratin-7-positive human trophoblast cells isolated from human placenta at term, and also in three human choriocarcinoma cell lines. Therefore, FXR may play a role in the control of the hepatobiliary-like excretory function of the placenta (275).

FXR expression is affected in several conditions. FXR is not detectable during rat kidney embryonic development, but is highly expressed in proximal tubules of adult animals (310). Similarly, the expression of FXR in the rat ileum increases from birth to adulthood (128). Metabolic and hormonal cues also modulate FXR expression. For example, FXR expression is repressed during the acute phase response and by interleukin-1 and tumor necrosis factor-α (153). High glucose concentrations (25 mM) upregulate and insulin represses FXR expression in rat primary hepatocytes (74). Glutamine, an amino acid whose facilitated uptake through the transporter ASCT2 is essential for the rapid growth of cancer cells, induces FXR expression in HepG2 hepatoma cells (31, 198). Finally, fasting, which is known to promote overexpression of the transcriptional regulator PGC1α (PPAR coactivator 1α), upregulates FXRa3/4 expression in the liver. A mechanistic study showed that this process is dependent on a DRI response element located upstream of the second, internal FXR promoter. This response element binds the nuclear receptors PPAR-γ and HNF4, whose transcriptional activity increases upon interaction with PGC1α, establishing a molecular link between fasting and FXR expression (366).

Several pathological conditions have been correlated to an altered FXR expression. FXR expression is decreased in the liver of models of genetically or chemically induced diabetes in rats (74). In contrast, diabetic db/db mice display a higher expression of FXR isoforms in liver (364). Two mutations (−1g>t and M1V) in the FXR sequence have been isolated from patients with intrahepatic cholestasis of pregnancy (ICP), resulting in a reduced FXR expression (329). FXR isoform expression is directly correlated to ATP8B4/FIC1 (a P-type adenosine triphosphatase) activity or expression (2, 44), whose deregulation through mutation(s) leads to progressive familial intrahepatic cholestasis type 1 (PFIC1).

Therefore, FXR is a nuclear receptor predominantly expressed in the gastrointestinal tract, whose regulation hints at roles in glucose and lipid metabolism. However, other roles have yet to be explored when considering the distribution of FXR expression. Notably, the regulation of the FXR promoter by HNF1α/TCF1 (182), which is mutated in the rare monogenic form of maturity-onset diabetes of the young (MODY)-3 and implicated in the growth and function of islet β cells, suggests additional role(s) which still have to be deciphered.

C. FXR as a Ligand-Regulated Transcription Factor

As a member of the nuclear receptor (NR) family, FXR acts as a ligand-modulated transcription factor whose role is to increase or decrease the transcriptional activity of regulated promoters in a coordinate fashion. Two general mechanisms contribute to the transcriptional regulation of eukaryotic promoters: step 1) the nuclear receptor releases corepressor (CoR) complexes and interacts with multiprotein coactivator complexes upon agonist binding, which modify the chromatin structure, yielding access to general transcription factors (GTFs) and RNA polymerase II (pol2) to promoter DNA; step 2) the activator stimulates pol2 and GTFs binding to DNA to promote the formation of the preinitiation complex (PIC). About 300 coactivators for NRs that favor step 1, including SWI/SNF complexes, CBP/p300, or p160-related factors (SRC-1, -2 and -3), have been described (see Refs. 169, 181 for reviews). Step 2 seems to rely mostly, if not exclusively, on the mediator complex, which is a molecular bridge between activators and pol2, although a repressive role has also been described both in yeast (9) and in mammalian cells (82). Being necessary for the activation process, mediator also plays a role in transcriptional initiation (335).

Several coactivators play a role in FXR-mediated transcription. DRIP205, a subunit of the mediator complex interacting with a number of NRs, is recruited by FXR in a ligand-dependent manner and is required for FXR-induced transcription as demonstrated by siRNA studies (246). FXR ligand-dependent loading of CARM-1, a secondary coactivator for nuclear receptors with protein methylase activity, is detected on the BSEP promoter in HepG2 cells. This interaction is correlated to methylation of histone H3 (R17) at this locus, a posttranslational modification associated with gene activation (14), thus suggesting a direct correlation between FXR activation, CARM1 recruitment, chromatin structure alteration, and transcriptional activation (4). Quite similarly, another protein methylase, PRMT-1, has also been described as a coactivator for FXR (259). CARM1 being known to interact indirectly with nuclear receptors through members of the p160 family (SRC1, SRC2/TIF2/GRIP1, SRC3/AIB1/
pCIP) (43, 297), p160 proteins are thus likely to play a role in FXR-mediated transcriptional activation. SRC-2 interacts in a ligand-dependent manner with DNA-bound RXR-FXR heterodimers, and SRC-1, widely used in cell-free systems as a probe for ligand-induced FXR transconformation, coactivates FXR (4, 69, 86, 172, 246). However, SRC-1 and SRC-2/TIF-2 do not seem to have a physiological role in FXR-mediated transcription, since SRC-1 and/or SRC-2 gene invalidation does not have any detectable influence on hepatic physiology (242). PGC-1α, a well-characterized coactivator for PPAR with broad metabolic functions, also interacts physically and functionally with FXR (266, 366), although the territory of expression of PGC-1α in normal conditions (brown fat, skeletal muscle) as well as the lack of effect of PGC-1α gene knockout on hepatic physiology (285) raise questions about the physiological relevance of this interaction. However, PGC-1α expression is known to vary in several instances, including fasting, upon stimulation of the cAMP signaling pathway and in type 2 diabetes animal models (354). In these situations, PGC-1α might become a prominent FXR coactivator, as its FXR coactivating properties are more potent than those of SRC-1 (141). How PGC-1α interacts with FXR remains a matter of debate, since FXR has been shown to either interact with the NH2 terminus of PGC1α (1–400) through its DBD (366), or to bind to the FXR LBD in an AF2- and charge clamp-dependent manner, or even to exhibit a RXR activation-dependent binding mode (140, 141, 266).

Thus the ligand-specific recruitment of cofactors to FXR is a common theme in the literature which, however, remains poorly defined, despite its major implications for understanding the biology of FXR and exploiting its full potential as a therapeutic target. Moreover, most investigations have focused on coactivators already identified as partners for other nuclear receptors, leaving open the possibility that other proteins might modulate significantly FXR transcriptional activity in vivo. Of note, coactivators such as PGC1β, Tip60, and pCAF do not exhibit significant coactivating properties on FXR-mediated transcription (141). The G protein pathway suppressor 2 (GSP2) component of the NCoR corepressor complex acts unexpectedly as a potentiator of FXR action on the human CYP8B1 FXR response element (263). The transformation/transcription domain associated protein TRRAP, a constituent of the so-called “TBP-free TAF II-containing-type HAT” coactivator complexes has also been shown to possess FXR coactivating properties (324). Finally, much like other intracellular, small lipophilic transport proteins such as CRABP-2 or FABP (64, 314) which act as coactivators for retinoic acid receptors or PPARs, IBABP binds to and coactivates FXR in intestinal cells (211). The multiplicity and diversity of FXR coactivators probably reflect the existence of very subtle regulatory pathways of FXR transcriptional activity, which remain to be fully apprehended, without forgetting corepressors, the role of which remains to be fully evaluated.

### D. Natural and Synthetic Ligands of FXR

Seminal papers published in 1999 already mentioned above identified the major mammalian BAs as activators of FXR (190, 232, 336). The use of bile extracts, as well as other natural extracts from plants, used to treat lipid metabolism disorders, prompted the search for potential FXR ligands in these extracts. The observation that the synthetic retinoid TTNPB could activate FXR gave the impetus for a search for nonsteroidal compounds, activating or repressing this nuclear receptor. These three axes of research (BA derivatives, natural extracts, synthetic FXR ligands) yielded an impressive amount of data identifying FXR modulators. Reports are sometimes contradictory or to be interpreted with caution, due to the multiplicity of experimental models and/or the poor selectivity of the identified ligands. Most BAs indeed display significant cell toxicity at concentrations above 100 μM, limiting their use as chemical tools to study FXR functions. In this respect, it is important to take into account experimental conditions that led to the classification of FXR ligands (see Table 1), and to keep in mind that BAs are promiscuous ligands; BAs also activate VDR [LCA, LCA-acetate (1, 189)] and PXR [LCA (299, 347)] or repress CAR (206). These properties, underlining the concerted action of VDR, FXR, PXR, and CAR in protecting against BA toxicity in the liver and intestinal tract (97, 262), may be confounding when analyzing structure-activity relationships. Finally, FXR-independent activities of BAs, such as activation of the TGR5 membrane receptor or of kinase-controlled pathways (see below), are potential confounders when studying FXR biological properties. Despite these difficulties, a few rules in structure-activity relationships have emerged, and they will be described after reviewing the main structural properties of FXR ligands and its LBD.

#### 1. Bile acids and bile acids derivatives

The primary BA CDCA was initially described as the most potent FXR ligand in transient transfection assays, with an EC50 of ~50 μM (190). DCA and LCA also displayed a significant ability to activate FXR, albeit with a lower maximal efficiency. UDCA was inactive in these experimental conditions that led to the classification of FXR ligands (see Table 1), and to keep in mind that BAs are promiscuous ligands; BAs also activate VDR [LCA, LCA-acetate (1, 189)] and PXR [LCA (299, 347)] or repress CAR (206). These properties, underlining the concerted action of VDR, FXR, PXR, and CAR in protecting against BA toxicity in the liver and intestinal tract (97, 262), may be confounding when analyzing structure-activity relationships. Finally, FXR-independent activities of BAs, such as activation of the TGR5 membrane receptor or of kinase-controlled pathways (see below), are potential confounders when studying FXR biological properties. Despite these difficulties, a few rules in structure-activity relationships have emerged, and they will be described after reviewing the main structural properties of FXR ligands and its LBD.
<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Species</th>
<th>Response Element</th>
<th>Position</th>
<th>Models</th>
<th>Ligands</th>
<th>Regulation</th>
<th>Comments</th>
<th>Reference</th>
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<tbody>
<tr>
<td>ALAS-1</td>
<td>H</td>
<td>IR-1</td>
<td>−13906</td>
<td>Hepatocytes, liver slices</td>
<td>50 μM CDCA, 50 μM CA, 50 μM UDCA, 25 μM LCA, 1 μM GW4064</td>
<td>Up</td>
<td>The IR-1 FXRE is not conserved in rodents, and GW4064 represses ALAS-1 expression in mice</td>
<td>Peyer et al., 2007</td>
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<tr>
<td>a2-Crystallin</td>
<td>H</td>
<td>IR-1</td>
<td>+1037</td>
<td>Cholangiocytes, hepatocytes, HepG2</td>
<td>250 μM CDCA, 10 μM GW4064</td>
<td>Up</td>
<td>FXRα2 and -α4 activate this gene more efficiently</td>
<td>Lee et al., 2005</td>
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<tr>
<td>ApoAI</td>
<td>H</td>
<td>negFXRE</td>
<td></td>
<td>HepG2, human primary hepatocytes</td>
<td>75 μM CDCA, 5 μM GW4064</td>
<td>Down</td>
<td>FXR binds to this negative FXRE as a monomer. The negative FXRE has a sequence (GATCCTTGAACTCT) which is close to the negative FXRE found in the UGT2B7 promoter</td>
<td>Claudel et al., 2002</td>
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<tr>
<td>ApoAV</td>
<td>H</td>
<td>IR-8</td>
<td>−103</td>
<td>Hep3B, CV1</td>
<td>1 μM GW4064, 100 μM CDCA</td>
<td>Up</td>
<td>Not inducible in HepG2 cells nor in primary hepatocytes from cynomolgus liver</td>
<td>Prieur et al., 2003</td>
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<tr>
<td>ApoCII</td>
<td>H</td>
<td>IR-1</td>
<td>+214*</td>
<td>HepG2</td>
<td>100 μM CDCA, 100 μM CA</td>
<td>Up</td>
<td>The IR-1 overlaps a T3 response element. *Numbering refers to the HCR-1 locus in the ApoE/C gene cluster</td>
<td>Kardassis et al., 2003</td>
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<tr>
<td>ApoCIII</td>
<td>H</td>
<td>IR-1</td>
<td></td>
<td>HepG2, in vivo (1% cholate for 5 days)</td>
<td>100 μM CDCA, DCA, LCA, CA, 1 μM GW4064, 100 μM Androsterone</td>
<td>Up</td>
<td>FXR is permissive to RXR-mediated activation</td>
<td>Kast et al., 2003</td>
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<tr>
<td>ApoCIII</td>
<td>M, H</td>
<td>negFXRE (DR-1)</td>
<td>−739</td>
<td>HepG2, mouse and human primary hepatocytes, in vivo</td>
<td>GW4064</td>
<td>Down</td>
<td>The DR-1 binds also RXR-HNF4 heterodimers</td>
<td>Claudel et al., 2003</td>
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<td>ASCT2</td>
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<td>−502</td>
<td>Hepatocytes, liver slices</td>
<td>50 μM CDCA, 10 μM GW4064</td>
<td>Up</td>
<td>The resinoid LG1305 inhibits CDCA-mediated activation of the promoter</td>
<td>Bungard et al., 2005</td>
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<td>AT2R</td>
<td>R</td>
<td>IR-2</td>
<td>−531</td>
<td>CV1, in vivo (rat, GW4064100 mg/kg, 7 days)</td>
<td>50 μM CDCA, 10 μM GW4064</td>
<td>Up</td>
<td>The resinoid LG1305 inhibits CDCA-mediated activation of the promoter</td>
<td>Zhang et al., 2007</td>
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<td>BACS</td>
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<td>1 μM GW4064, 100 μM CDCA</td>
<td>Up</td>
<td>The BAT FXRE is not inducible in CV1 cells. The resinoid LG1305 potentiates CDCA-mediated activation of the promoter</td>
<td>Pircher et al., 2003</td>
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<td>BAT</td>
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<td>+2615</td>
<td>CV1, in vivo (rat, GW4064100 mg/kg, 7 days)</td>
<td>1 μM GW4064, 100 μM CDCA</td>
<td>Up</td>
<td>The resinoid LG1305 inhibits CDCA-mediated activation of the promoter</td>
<td>Pircher et al., 2003</td>
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<td>BSEP/ABCB11</td>
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<td>−64</td>
<td>HepG2</td>
<td>100 μM DCA, 100 μM CDCA, 10 μM LCA</td>
<td>Up</td>
<td>Not inducible in NIH 3T3, MDCK, and weakly responsive in Caco2 cells</td>
<td>Gerloff et al., 2002</td>
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<td>BSEP/ABCB11</td>
<td>H</td>
<td>HepG2, primary human hepatocytes</td>
<td>100 μM CDCA, 1 μM GW4064</td>
<td>Up</td>
<td>LCA decreases BSEP expression</td>
<td>Yu et al., 2002</td>
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<td>BSEP/ABCB11</td>
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<td>IR-1</td>
<td>−63</td>
<td>HepG2</td>
<td>100 μM CDCA</td>
<td>Up</td>
<td>Induction potentiaized by 1 μM 9-cis-retinoic acid</td>
<td>Ananthanarayanan et al., 2001</td>
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<td>BSEP/ABCB11</td>
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<tr>
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<td>DDAH1</td>
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<td>+90,000</td>
<td>HEK, in vivo (GW4064 for 9 days)</td>
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<td>DHEA sulfotransferase/SULT2A4</td>
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<td>eNOS</td>
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<td>FAS</td>
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<td>FGF19</td>
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<tr>
<td>Fibrinogen α, β, and γ</td>
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<td>IBABP</td>
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<td>IBABP</td>
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<td>ICAM-1</td>
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<td>−309</td>
<td>HepG2, in vivo (CA-rich diet)</td>
<td>1 μM GW4064</td>
<td>Up</td>
<td>The IR-1 is embedded into an imperfect DR5 retinoic acid response element</td>
<td>Qin et al., 2005</td>
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<td>INSIG-1</td>
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<td>IR-1</td>
<td>+6733, +11543</td>
<td>In vivo (GW4064 10 days), AML12</td>
<td>1 μM GW4064, 100 μM CDCA</td>
<td>Up</td>
<td>Regulated by two intronic FXREs; FXRα2 is a more potent activator of this promoter than FXRα1</td>
<td>Hubbert et al., 2007</td>
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<td>Kininogen</td>
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<td>IR-1</td>
<td>−66</td>
<td>Primary human hepatocytes, HepG2</td>
<td>CDCA, GW4064</td>
<td>Up</td>
<td>RRX acts synergistically with FXR, and the RRX-FXR is permissive to retinoid-mediated transcription</td>
<td>Zhao et al., 2003</td>
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<td>MDR3</td>
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<td>IR-1</td>
<td>−1970</td>
<td>Primary human hepatocytes</td>
<td>CDCA, GW4064</td>
<td>Up</td>
<td>Huang et al., 2003</td>
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<td>MRP2/ABCC2</td>
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<td>ER-8</td>
<td>−401</td>
<td>HepG2, primary mouse hepatocyte</td>
<td>100 μM CDCA</td>
<td>Up</td>
<td>Kast et al., 2002</td>
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<td>NaS-1/SLC13a1</td>
<td>M</td>
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<td>ca. −10,000</td>
<td>In vivo (GW4064, 30 mg/kg, 4 days), HepG2</td>
<td>1 μM GW4064</td>
<td>Up</td>
<td>Lee et al., 2007</td>
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<td>OAT2</td>
<td>H</td>
<td>DR-1</td>
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<td>LMH</td>
<td>100 μM CDCA, DCA, CA, UDCA; 50 μM LCA</td>
<td>Up</td>
<td>Acts by competing with HNF4α</td>
<td>Popowski et al., 2005</td>
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<td>OATP8/SLC21A8</td>
<td>C</td>
<td>IR-1</td>
<td></td>
<td>In vivo (30 mg/kg GW4064 for 4 days, ex vivo (adrenalin); H295R</td>
<td>1 μM GW4064</td>
<td>Up</td>
<td>IR-1 FXRE conserved in mouse and human. The FXRE are also regulated by RXR-LXR heterodimers (Okuwaki et al., 2007)</td>
<td>Jung et al., 2002</td>
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<td>OSTα and -β</td>
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<td>IR-1</td>
<td>−1375, −1295 (OSTα); −134 (OSTβ)</td>
<td>In vivo (0.2% CA diet or 100 mg/kg GW4064 for 5 days), CT26</td>
<td>100 μM CDCA</td>
<td>Up</td>
<td>Opposite effect of FXR through SHP-mediated inhibition of LRH-1 and through FXRE-mediated activation</td>
<td>Lee et al., 2006</td>
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<tr>
<td>OSTα and -β</td>
<td>M</td>
<td>IR-1</td>
<td>−1221 (OSTα); −5 (OSTβ)</td>
<td>In vivo (GW4064 for 5 days), CT26</td>
<td>100 μM CDCA</td>
<td>Up</td>
<td>Frankenberg et al., 2006</td>
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<td>PLTP</td>
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<td>IR-1</td>
<td>−249</td>
<td>Murine primary macrophages, HuH7, HepG2</td>
<td>GW4064, CDCA</td>
<td>Up</td>
<td>The FXRE is also responsive to LXRα and -β</td>
<td>Mak et al., 2002</td>
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<tr>
<td>PLTP</td>
<td>M</td>
<td>IR-1</td>
<td>−407, −393</td>
<td>HepG2</td>
<td>100 μM CDCA</td>
<td>Up</td>
<td>The IR-1 is not conserved in human</td>
<td>Tu and Albers, 2001</td>
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<tr>
<td>PLTP</td>
<td>H</td>
<td>IR-1</td>
<td>−339</td>
<td>CV1, in vivo</td>
<td>100 μM CDCA, 1% CA (30 days)</td>
<td>Up</td>
<td>FXR is not permissive to RXR-mediated activation</td>
<td>Urizar et al., 2000</td>
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<td>PPAR-α</td>
<td>H</td>
<td>Imperfect DR5</td>
<td>−599</td>
<td>HepG2, primary human hepatocytes</td>
<td>5 μM GW4064, 50 μM CDCA</td>
<td>Up</td>
<td>The mouse PPAR-α gene is not regulated by FXR</td>
<td>Pinneda et al., 2003</td>
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<tr>
<td>PXR</td>
<td>M</td>
<td>IR-1</td>
<td>−40,000</td>
<td>In vivo (CA-rich diet, 450 mg/kg or 50 mg/kg GW4064), HEK</td>
<td>1 μM GW4064</td>
<td>Up</td>
<td>Three functional IR-1 were identified in intron 2</td>
<td>Jung et al., 2006</td>
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### Table 1.—Continued

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<tr>
<th>Gene Name</th>
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<tr>
<td>SHP</td>
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<td>-291</td>
<td>In vivo (rat, 30 mg/kg GW4064 for 7 days), primary hepatocytes</td>
<td>1 μM GW4064, 100 μM CDCA</td>
<td>Up</td>
<td>The IR-1 is conserved in human, mouse (−320) and rat (−309)</td>
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<td>STD</td>
<td>R</td>
<td>IR-0</td>
<td>-193</td>
<td>HepG2, Caco2</td>
<td>25 μM CDCA</td>
<td>Up</td>
<td>FXR is permissive to RXR-mediated activation</td>
<td>Song et al., 2001</td>
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<td>SYNDENCA</td>
<td>H</td>
<td>DR-1</td>
<td>-921</td>
<td>HepG2, HuH7</td>
<td>10 μM GW4064, 250 μM CDCA</td>
<td>Up</td>
<td>The SCD promoter is specifically responsive to FXRα2 and -α3 isoforms</td>
<td>Anisfeld et al., 2003</td>
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<td>UGT2B4</td>
<td>H</td>
<td>B4-BARE</td>
<td>-1193</td>
<td>Primary human hepatocytes, HepG2</td>
<td>5 μM GW4064, 50 μM CDCA</td>
<td>Up</td>
<td>RXR activation decreases FXR-mediated transcriptional activation and decreases FXR binding to the B4-BARE element</td>
<td>Barbier et al., 2003</td>
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<td>UGT2B7</td>
<td>H</td>
<td>Negative FXRE</td>
<td>-148</td>
<td>Caco2, HepG2</td>
<td>30 μM LCA, 75 μM CDCA</td>
<td>Down</td>
<td>The negative FXRE has a sequence (GATCCTGTGATAT) that is close to the negative FXRE found in the hApoAI promoter</td>
<td>Lu et al., 2005</td>
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<td>VPAC1</td>
<td>h</td>
<td>IR-1</td>
<td>-919, +33, +156</td>
<td>Gallbladder epithelial cells</td>
<td>20 μM GW4064</td>
<td>Up</td>
<td>9-cis-retinoic inhibits GW4064-mediated activation of VPAC1 gene. The functionality of FXREs has not been established</td>
<td>Chignard et al., 2005</td>
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Species: H, human; M, mouse; R, rat. The "models" column indicates cellular or animal models in which the activity of the FXRE has been characterized; the "ligands" column indicates which ligand(s) and concentration have been used. Comments highlight some of the salient features of the FXR-regulated promoter.
may be conditioned by the expression of membrane transporter(s). This barrier may be circumvented by converting the carboxylic extremity of BA into an alcholic function (85). Classification of BAs as FXR ligands is based on their behavior in cell-free coactivator recruitment assays. While CDCA was consistently reported as a molecule promoting SRC-1 recruitment, and thus classified as an agonist (EC\textsubscript{50} = 8 μM) (190, 232, 237, 359), DCA, LCA, and CA were inactive in this assay but antagonized CDCA-induced SRC-1 recruitment (172, 190, 232, 359). Intriguingly, LCA could be classified as a weak agonist on the basis of its capacity to induce BSEP expression in HepG2 cells (359), much like UDCA. There is therefore no strict correlation between BA affinity for FXR, its capacity to promote SRC-1 interaction with FXR, and its biological activity, suggesting that either coactivator recruitment assays are not predictive of the transcriptional outcome, or that SRC-1 is not the appropriate model system, or that the transcriptional outcome is a function of multiple parameters including the dimerization partner and the DNA binding sequence, as suggested for FXR (172) and demonstrated for other nuclear receptors (204).

The use of BA derivatives in ligand-binding and transcriptional assays has provided interesting structure-activity relationships that are in line with the reported crystallographic structure of the FXR LBD (see below). Taken together, these studies (52, 85, 221, 222, 236, 237) have shown that 1) introduction of a 6α alkyl substituent is compatible with in vitro and in vivo activity, but polar and hydrogen-bonding substituents are deleterious for activity; 2) 6α-ethyl CDCA (6αECDCDA) is a potent FXR agonist with an activity in transcriptional assays three orders of magnitude higher than the parent compound CDCA; 3) introduction of a bulky substituent in either the 3β or the 7β position led to a decreased activity of the molecule; 4) derivatization of the C\textsubscript{24} group by carbamate moieties generates molecules exhibiting agonist or antagonistic properties; 5) an intact cholesterol side chain (C\textsubscript{27}) is not compatible with FXR agonism or antagonism, but a C\textsubscript{25} or C\textsubscript{25}'-hydroxylated bile alcohol is as effective as CDCA; 6) epimerization at C\textsubscript{12} of the hydroxyl group abolished FXR activation; and 7) the 5α epimerization yields bile alcohols that are agonists, on the basis of a coactivator recruitment assay, whereas 5β bile alcohols are antagonists. Removal of the 3-hydroxyl group has no effect on coactivator recruitment. Other steroidal molecules such as androsterone also activate FXR (340). Interestingly, BA “extranuclear” activities, dependent on the binding of BA to the G protein-coupled membrane receptor TGR5, can be triggered selectively with enantiomeric LCA and CDCA (147) or other substituted BAs (239).

2. Natural extracts

The extract from the gum resin of Commiphora mukul, guggulipid, is used in traditional medicine for its hypolipidemic effects. This has prompted investigators to isolate the active principle from this extract (327, 346). A mix of Z- and E- [4,17(20)-pregnadiene-3,16-dione] or guggulsterone (GS) was initially shown to antagonize FXR target genes activation by CDCA in vitro [IBABP, SHP, BSEP (228, 327, 346)] and to decrease hepatic cholesterol in vivo, a surprising observation when considering the phenotype of FXR\textsuperscript{−/−} mice. On the contrary, GS was reported to increase BSEP expression in another study (56), a complex effect that may be attributable to GS-mediated control of AP-1 activity (66). However, GS displayed, like BAs, agonist activity on CYP7A1 expression in HepG2 cells, an effect probably relayed through activation of the pregnane X receptor (PXR). Indeed, further investigations demonstrated that GS is a promiscuous ligand able to inhibit (327) or activate PXR in HepG2 cells (29, 228). A 10 μM GS concentration also significantly induces progesterone receptor (PR) activity in transient transfection assays (29, 32). Indeed, GS binds to PR and to other steroid receptors with an affinity in the high nanomolar range and is an antagonist for the androgen, glucocorticoid, and mineralocorticoid receptors (32). Thus, despite its relative lack of selectivity, GS is a gene-selective modulator of FXR activity, since displaying a molecular behavior typical of antagonists in cell-free coactivator binding assays (327, 346) but exhibiting gene-specific agonist or antagonist activities. Molecular modeling suggested that this peculiar behavior may be attributed to the occurrence of a specific GS docking site in the FXR LBD, although this has not been backed up by crystallographic or mutational studies (199).

Other natural extracts have also been shown to contain FXR activators. Stigmasterol, a soy lipid-derived phytosterol, suppresses ligand-induced expression of FXR target genes involved in adaptation to cholestasis in HepG2 cells (40). The diterpene cafestol, isolated from unfiltered coffee brew, is a hypercholesterolemic agent and an agonist for FXR and PXR displaying an efficiency in cultured hepatoma cells (HepG2) similar to that of reference compounds (CDCA and PCN, respectively) (255). More surprisingly, cafestol did not induce FXR target genes in the liver in vivo, but activated the FGF15 gene in the small intestine, hence triggering an inhibitory signaling enterohepatic loop (129). This probably reflects the low bioavailability of cafestol, or its rapid metabolism in the liver (255) and further illustrates the role of the ileum as a critical FXR target organ (115). The prenylated chalcone xanthohumol from beer hop activates the BSEP promoter in transcriptional assays in hepatoma cells (EC\textsubscript{50} = 5 μM), and lowers hepatic and plasma triglycerides in diabetic KK-A\textsuperscript{2} mice, suggesting that it may be a FXR activator.
3. Synthetic compounds

As mentioned previously, naturally produced FXR ligands have a moderate selectivity, thus prompting the development of synthetic molecules displaying a strong selectivity and affinity for FXR. The initial observation that TTNPB activates FXR, albeit with a low affinity (360), provided a lead compound for the synthesis of stilbene derivatives (192). GW9047 emerged as a compound fulfilling the “selectivity” criterion, but is a weak activator in transcriptional assays. Further pharmacomodulation around this structure led to the identification of GW4064 [3-(2,6-dichlorophenyl)-4-(3’-carboxy-2-chloro-stilben-4-yl)-oxy-methyl-5-isopropyl-isoxazole], a potent and selective FXR agonist (EC\textsubscript{50} = 90 nM), active both in vivo and in vitro (94, 192, 288). Although displaying a limited bioavailability, GW4064 has gained a widespread use as a powerful and selective FXR ligand and has reached the status of “reference compound” in this field. Using again TTNPB as a lead compound, Dussault et al. (75) adopted a strategy to reinforce its selectivity towards FXR. They modified the TTNPB backbone to prevent RAR binding, and such a pharmacophore modification yielded AGN29 and AGN31. These molecules turned out to be efficient agonists of FXR with an EC\textsubscript{50} value around 1 \mu M, and as well as of RXR, as expected from the property of the parent compound (24). Further pharmacomodulation around retinoid structures led to the identification of AGN34, a dual RXR-FXR antagonist displaying gene-specific properties. Of note, the synthetic FXR agonist LG268 was shown to activate FXR/RXR heterodimers through the RXR moiety (75), but was later shown to inhibit GW4064, 6αEDCA, and CDCA-induced FXR transcriptional activity (144, 259). Using a benzopyran structure to identify novel FXR ligands, Downes et al. (69) optimized the structure of lead compounds to identify biaryl cinnamates, amongst which fexaramine and fexarine emerged as potent and highly selective FXR agonists (EC\textsubscript{50} = 38 and 36 nM, respectively). A comparative gene profiling study underlined distinctive properties of fexaramine, GW4064, and CDCA to activate target genes in primary human hepatocytes, demonstrating that each of these ligands, in addition to the AGN compounds, may be considered as selective BA receptor modulators or SBARMs. Possible explanations for these differential activities could be a differential positioning and filling of the ligand-binding pocket by these ligands, or a RXR-induced allosteric regulation of FXR ligand-binding property. Indeed, GW4064 requires RXR to exhibit maximal efficacy in transcriptional assays. However, a difficulty in comparing ligand effects is that target cells, such as hepatocytes, produce BAs at nonnegligible rates [58 \mu g/96 h, equivalent to 16 \mu M for HepG2 cells (171)], and that, as mentioned above, BAs may elicit FXR-independent effects. Finally, 1,1-biphosphonate esters are potent FXR agonists displaying hypocholesterolemic and antiproliferative effects (218), and the sulfonyamide T9001317 activates FXR in the micromolar range, concentrations well above its reported affinity for LXR and PXR (20–40 nM) (203).

E. Ligand-Binding Properties of FXR

An important step in exploiting FXR as a molecular target in metabolic diseases is to establish structure-activity relationships, with the aim of identifying ligands with optimized biological properties. Early mutational studies identified several amino acids of the FXR LBD that are critical for ligand binding or ligand discrimination. Considering sequence homologies with LXR, Urban et al. (325) suggested that F268 in the human FXR LBD is critical for ligand recognition. Based on the differential response of hFXR vs mFXR to CDCA, N354 and I372 were identified as responsible for the high sensitivity of hFXR to CDCA, and mutation of the corresponding amino acids in mFXR could “humanize” this receptor (55). However, these amino acids are not involved in a direct interaction with CDCA (see Fig. 3C).

Crystallographic data of fexaramine and of 3-deoxy-CDCA or 6ECDCA complexed to the human or rat FXR LBD, respectively (69, 200), brought significant insights in the mechanism of activation of FXR by its cognate ligands (Fig. 3). Of note, amino acids involved in ligand-receptor interaction are conserved between rat and human FXRs. These studies have revealed not only features of the FXR LBD similar to those of other nuclear receptor LBDs, such as its general organization in a 12 σ-helix bundle, but also unanticipated characteristics. As for other NRs, the COOH-terminal LBD acts as a molecular switch, undergoing major structural transitions enabling the selective recruitment of coactivators by forming a charge clamp and a hydrophobic groove with which LXXLL motifs from coactivator molecules will interact (69, 200). More unanticipated was the opposite orientation of these ligands in the LBD compared with other steroid receptors, as well as the occurrence of a second docking site for the LXXLL motif. However, a recent report described the binding mode of synthetic MFA-1 FXR agonist as similar to that of steroids to steroid receptors, underlining a structural versatility which expands drug design strategies in this field (293).

When considering BAs, the ligand-binding pocket (LBP) has to accommodate a steroid nucleus with specific conformational and physicochemical properties, with a concave hydrophilic face (α face) and a convex hydrophobic face (β face), which is the privileged site for
interaction with other hydrophobic molecules (Fig. 3). The $\alpha$ face may harbor several hydroxyl groups in the 3, 7, and/or 12 positions, and these hydroxyl groups directly affect BA affinity for FXR. Indeed, the 7-hydroxy group interacts with $rY366$, conferring a high affinity to CDCA, whereas the lack of this hydroxyl group in LCA decreases the affinity for FXR. Introducing bulky substituent on the $\beta$ face has a general deleterious impact on the affinity for FXR (85). Importantly, optimal activation of FXR is dependent on the proper positioning of helix H3 versus helix H12, generating the LXXLL docking groove. CDCA, like fexaramine, engages interactions with helix 3 and helix 7 through the steroid 7\(\alpha\) hydroxy group, resulting in a highly stable H3-H12 structure and full agonist activity. In contrast, partial agonists such as DCA and LCA lack the 7\(\alpha\)-hydroxyl group, loosening this structure and compromising stable coactivator recruitment. Furthermore, the FXR LBP cannot accommodate a 12\(\alpha\)-hydroxyl group. Therefore, CA and DCA display a weaker affinity for FXR. Generating additional contacts within a hydrophobic cavity strongly increases the affinity for FXR, as seen for 6-\(E\)CDCA. Quite strikingly, the carboxylic extremity of BAs is oriented towards the entry of the LBP, thus explaining why derivatization of this function, as seen in
conjugated BAs, does not preclude high-affinity binding to FXR and allows FXR activation by these compounds. However, structural variations introduced on the carboxyl end of BAs may yield substantial variations in FXR activity (238).

Isolated LXXLL motifs bind to the FXR LBD with a low affinity (~160–500 μM), whereas receptor interaction domains (RIDs) from coactivators encompassing two or three LXXLL motifs display an increased affinity (~1 μM) for the FXR LBD. Structural and physical studies of the FXR-coactivator complex showed that the FXR LBD binds 2 LXXLL motifs in an anti-parallel fashion, with one being located in the “classical” groove and secured by a charge clamp, whereas the second binding site lacks this charge clamp and is located on helix 3. Data were compatible with the association of a single coactivator polypeptide to the FXR LBD, suggesting that the second LXXLL interaction surface increases FXR affinity for a single coactivator molecule (200). However, this hypothesis has not yet been tested in mutational studies.

F. DNA-Binding Properties of FXR and FXR Target Genes

FXR can bind to DNA to so-called FXREs either as a monomer or a heterodimer with RXR. The latter configuration is the most common and generally associated with gene activation, whereas the monomeric form may also be associated with gene repression. Early studies (165, 274) established that the preferred consensus FXRE is an inverted repeat (IR) of the core hexanucleotidic AGGTCA sequence separated by one base pair (IR-1). Sequences flanking this IR-1 were not found to influence RXR-FXR affinity for DNA. In addition, FXR could bind in vitro to IR-0, but not to IR-2, IR-3, IR-4, and IR-5 sites. A direct repeat (DR) geometry with a similar core sequence AGGTCA is compatible with RXR-FXR binding, and DR-2, DR-4, and DR-5, but not DR-0, DR-1, or DR-3 sequences, can form complexes with RXR-FXR heterodimers. The identification of FXR target genes, which regulate diverse cellular processes (Fig. 4), has shown that FXR can bind FXREs with a remarkably diverse geometry, ranging from IR-1 to inverted repeat (ER)-8 sites (summarized in Table 1). While the structure of hormone response elements has been shown to dictate the activity of several other nuclear receptors such as the thyroid hormone receptor (117, 332), the glucocorticoid receptor (292, 298) or the retinoic acid receptor (208), this phenomenon has not yet been appreciated for FXR.

G. FXR Polymorphisms

The etiology of some BA metabolism disorders is poorly characterized and thus, in view of the central role of FXR in the maintenance of BA homeostasis, genetic variations in its sequence have been sought in several pathologies. As mentioned above, heterozygous variants of FXRα1&2 have been isolated from ICP patients (329). The −1g>t, +1a>g (M1V), and 518t>c (M173T) affect the stability or the activity of FXR, whereas the 238c>t (W80R) did not display any functional defect in vitro. The −1g>t mutation affects protein translation efficiency, although this destabilization was not observed when assessing the translation efficiency of this cDNA in in vitro heterologous systems (196). Other single nucleotide polymorphisms were detected in several ethnic groups, albeit with a much lower frequency compared with the −1g>t mutation (2.5–12.1% vs. 0–0.5%). Two SNPs altered the coding sequence [+643c>t (H215Y), +646g>t (A216S)], but the functional consequences of these mutations were not studied (196). A study involving three cases of idiopathic BA malabsorption did not reveal any mutation in the FXR gene (205).

IV. FARNESOID X RECEPTOR-MEDIATED REGULATION OF BILE ACID SYNTHESIS AND TRANSPORT

As described in section ii, hepatic BA biosynthesis and intestinal reabsorption must be rigorously coordinated to maintain a constant BA pool size. The biosynthetic and transport processes involved in enterohepatic cycling are essentially regulated through complex transcriptional networks involving not only FXR and other nuclear receptors, but also other signaling pathways.

A. Transcriptional Regulation of Bile Acid Biosynthesis

The importance of CYP7A1 and of CYP8B1 in generating an appropriate primary BA pool has been described in section ii. Studies with FXR-null (FXR<sup>−/−</sup>) mice have unequivocally demonstrated the physiological role of this BA-activated nuclear receptor in the control of BA metabolism (288). Increased hepatic CYP7A1 mRNA levels but, very surprisingly, reductions of total bile salt pool size and fecal bile salt loss are observed in FXR<sup>−/−</sup> mice. Counterintuitively, these data would imply that in the absence of functional FXR, BA synthesis would actually be suppressed. Other mouse models with increased CYP7A1 expression showed, as expected, increased BA synthesis rates and expansion of BA pool sizes. This may imply that FXR deficiency has an impact on the maintenance of BA pool size at other levels, for instance, in the intestine. To address this issue, Kok et al. (158) evaluated parameters of the enterohepatic circulation of CA, quantitatively the major BA species in the mouse, in relation to bile formation and the expression of transport proteins in...
another mouse model of FXR deficiency. For quantitation of cholic acid kinetic parameters, a stable isotope dilution technique was used. This study demonstrated that, in accordance with derepressed transcription of CYP7A1, FXR−/− mice showed an increased cholic acid synthesis rate without any effect on its fractional turnover rate. Interestingly, the calculated intestinal cholic acid reabsorption was markedly increased, leading to a significantly enlarged BA pool size. The different procedures used for generation of FXR null mice might be responsible for the apparently discrepant findings of Sinal et al. (288) and Kok et al. (158): a direct comparison of both strains using the same methodologies to analyze bile acid metabolism has not been reported so far.

Both CYP7A1 and CYP8B1 are transcriptionally regulated by several nuclear receptors. A complex regulatory sequence containing overlapping binding sites for the orphan NRs HNF4α and LRH-1, and the retinoic acid receptors, conveys a positive regulation of the Cyp7A1 gene upon HNF4α or LRH-1 binding. Interestingly, HNF4α is coregulated by the transcriptional coactivator PGC1α, whose expression and activity are modulated by insulin and fasting, two signals known to modulate BA synthesis (59, 321). The FXR-mediated repression of CYP7A1 occurs through a direct regulation of the small heterodimer partner (SHP) gene by FXR. SHP, a peculiar NR devoid of a DNA-binding domain, exerts a repressive activity upon dimerization with several transcription factors, including LRH-1 and HNF4α (94, 184). This occurs through diverse mechanisms, which may involve the recruitment of a Sin3A/Swi-Snf complex to the CYP7A1 promoter (15, 149). A similar SHP-mediated feedback regulation of the
Bile Acids and Bile Acid Receptors in Metabolic Regulation

human CYP8B1 gene has been reported (62, 353, 362). However, SHP-independent regulatory pathways have also been described, which may account for the fact that the loss of SHP in mice increased the levels of hepatic CYP7A1 and CYP8B1 by only twofold, which is much less than observed in mice with a depleted BA pool size (150, 338). Further investigations pointed to the role of FXR-mediated upregulation of FGFR19 expression in human hepatocytes (114), and of FGFR15 in murine intestine (129), initiating an autocrine/paracrine loop activating the FGFR7 receptor (45). Note, that FGFR’s action requires the presence of Klotho and βKlotho, which are single-pass transmembrane proteins, to activate the FGFR4 signaling pathway. The synthesis and excretion of BAs are strongly increased in βKlotho-deficient mice, as well as the expression of CYP7A1 and CYP8B1. BA-dependent induction of SHP is significantly attenuated in the liver, but not in the intestine of βKlotho-null mice (133). Mice deficient for FGFR4 exhibit an increased BA pool size and increased CYP7A1 expression (357). FGFR4-overexpressing mice display an opposite phenotype (358). The role of FGFR19 signaling in the maintenance of diurnal variation in hepatic BA synthesis in humans was elegantly demonstrated by Lundasen et al. (185). Interestingly, these molecular investigations shed light on the nature of the putative intestine-derived molecule regulating BA synthesis that explains the paradox that intravenous TCA administration fails to regulate CYP7A1 in rats while duodenal TCA infusion does (230).

B. Transcriptional Regulation of Bile Acid Export and Reabsorption

Hepatic BA transporter expression is, to a considerable extent, regulated by FXR, as detailed by Trauner and Boyer (317). On the one hand, secretion of BAs by hepatocytes into the canalicular lumen is essentially dependent on the expression of the ATP-dependent bile salt export pump BSEP/ABCB11, whose expression is directly regulated by FXR (3). The functional importance of BSEP is underlined by pathological consequences of BSEP mutations, leading to PFIC type 2 (308). The ABC transporters MDR3/ABCB4 and MRP2/ABCC2, responsible for hepatobiliary transport of phospholipids and of hydrophilic organic anions like divalent BA conjugates, respectively, are also upregulated by FXR (120, 145). On the other hand, FXR downregulates the expression of the sinusoidal/basolateral sodium-taurocholate cotransporting polypeptide NTCP/SLC10A1, through which 80% of BA uptake occurs. This regulation is species specific and may involve SHP (67). More surprisingly, the expression of another basolateral BA import pump, OATP1B3/SLC01B3, is upregulated by FXR. This unexpected response has been interpreted as a means for the hepatocyte to maintain its xenobiotics disposal potential, even during cholestasis (139).

BA uptake by enterocytes is a highly critical step in BA metabolism (see sect. ii). The expression of the murine apical sodium-dependent BA transporter (ABST)/intestinal BA transporter (IBAT) is positively controlled by LRH-1, which, as described for CYP7A1 in hepatocytes, is repressed by a FXR-induced SHP-mediated repression (45). Repression of hABST expression is likely the result of a distinct mechanism, involving a negative interference of SHP with the positive regulation by the glucocorticoid and/or retinoic acid receptors (137, 215). The expression of the fatty acid binding protein 6 (FABP6/BABP), whose role is likely to sequester excess intracellular BA and to serve as a coactivator for FXR, results from a direct transcriptional regulation by FXR (95, 128).

C. Cholestatic Liver Diseases

Cholestasis, functionally defined as an impairment or absence of bile flow, may result from functional defects in the primary bile formation process at the level of the hepatocytes or the cholangiocytes (intrahepatic cholestasis) or from a (usually mechanical) obstruction of bile flow at the level of bile ducts (extrahepatic cholestasis). Impaired function of hepatobiliary transport systems (sect. ii) due to genetic defects, e.g., BSEP/ABCB11 in PFIC2 or MDR3/ABCB4 in PFIC3 (235) or secondary to exposure to certain drugs, hepatotoxic endobiotics, or the presence of sepsis, are key in the pathogenesis of most forms of intrahepatic cholestasis (317). Treatment options are very limited and, apart from liver transplantation for some of the inherited forms of cholestasis, mainly aimed at reduction of symptoms (jaundice, pruritus). Independent of the underlying origin, cholestasis results in intrahepatic and systemic accumulation of substances normally excreted into bile (cholephils). Particularly, accumulation of detergent BAs that are normally secreted into bile in millimolar amounts may lead to liver cell damage, inflammation, and eventually organ failure. A series of adaptive reactions occurs during cholestasis that contributes to a diminished BA toxicity: inhibition of endogenous synthesis, induction of phase I and phase II detoxification reactions to render BAs more hydrophilic, and induction of alternative transport routes to eliminate BAs. FXR, but also other nuclear receptors like PXR, VDR, and CAR, are involved in the orchestration of these compensatory reactions (370). Modulation of FXR activity has been proposed as an attractive target for treatment of cholestatic liver diseases. Given the complexity of the bile formation process and the various etiologies that may underlie cholestatic liver disease, it is evident that FXR agonism will not provide a panacea. Therapeutic strategies should be focused not (only) on restoration of biliary
excretion function but predominantly on the reduction of hepatocellular uptake via NTCP/OATPs, stimulation of detoxification systems (hydroxylation, conjugation), and alternative pathways for elimination of BAs via the kidneys. Studies in bile duct-ligated (BDL) mice, a model for obstructive extrahepatic cholestasis, would indicate that, particularly for this form of cholestasis, a FXR antagonist might be beneficial. FXR-null mice were found to be less sensitive to BDL-induced liver damage (301, 334) likely due to the fact that these mice, in contrast to wild-type mice, did not maintain BSEP/ABCB11 expression. Maintenance of BA transporting capacity into the occluded biliary system resulted in increased biliary pressure and induction of bile infarcts, as well as to bile ductular proliferation in wild-type mice (334). In addition, FXR-null mice were reported to adapt to biliary obstruction by enhanced BA hydroxylation, likely catalyzed by induction of CYP3A11, leading to enhanced urinary excretion (194). Studies in rat models of chemically induced intrahepatic cholestasis and also, counterintuitively, of BDL-induced extrahepatic cholestasis showed that activation of FXR with the synthetic agonist GW4064 resulted in significant reductions in serum alanine aminotransferase, aspartate aminotransferase, as well as other markers of liver damage (180). GW4064 also decreased the incidence and extent of necrosis as well as decreased inflammatory cell infiltration and bile duct proliferation. On the basis of analysis of gene expression profiles, the beneficial effects of FXR activation have been ascribed to reduction of BA synthesis genes, such as CYP7A1, and induction of genes involved in biliary transport, such as the phospholipid transporter Mdr2/Abcb4 (180). Similarly, 6-ECDCA has been shown to exert protective effects in estrogen-induced cholestasis in rats by suppression of BA synthesis genes, reduction of NTCP expression and induction of BSEP/ABCB11, Mdr2/ABCB4 as well as Mrp2/ABCC2, the latter being involved in hepatobiliary transport of divalent BA conjugates (81). Feeding of the hepatotoxic monohydroxy BA lithocholic acid resulted in a more severe cholestatic phenotype in wild-type mice than in FXR-null mice, ascribed to higher LCA sulfotransferase activity in the latter (155). Lithocholic acid has been proposed to act as a FXR antagonist and, thereby, to decrease BSEP/ABCB11 expression (359). FXR activation has also been shown to induce overall gene expression of alternative basolateral BA transporters like Mrp3/ABCC3 and Mrp4/ABCC4, specific for divalent BA conjugates, and of the bidirectional BA transporter Osta/Ostβ (371). FXR induces UGT2B4 expression and activity in human hepatocytes, indicating a feed-forward reduction of BA toxicity in humans by glucuronidation (12). In addition, upregulation of Mrp2/ABCC2, Mrp4/ABCC4 and Osta/Ostβ on the basolateral surface of renal tubular cells in the kidney will increase the overall elimination capacity for such hydrophilic BA metabolites from the body (10). Part of the beneficial effects of FXR stimulation, either as a consequence of cholestasis or induced by a ligand, may be secondary to activation of PXR gene transcription by FXR (138). PXR is known to induce a variety of BA-detoxifying pathways, including cytochrome P-450s and several of the transporter genes, and to repress CYP7A1 expression (299). Recent data have also implicated the xenobiotic receptor CAR in protection from BA-induced hepatotoxicity (97, 361). Thus understanding the complex network of adaptative responses that collectively protect liver cells, as well as other cells in the body, from the toxic actions of BAs, and potentially also other harmful cholephilic, provides opportunities for development of new therapeutics. This will however require a tailored design for specific disease entities and will strongly depend on whether or not bile flow is completely absent. Particularly important in this respect is the role of the intestine in conservation of BAs. Lanzini et al. (168) reported a strongly reduced fractional turnover of the BA pool in patients with primary biliary cirrhosis (PBC). Thus, in this case, there seems to be an undesired, more effective conservation of BAs while enhanced removal would be preferable. As argued by Hofmann (112), this appears to be an adaptive response to low intraluminal BA concentrations with the undesired consequence of exposing the liver to even more BAs. In addition, low intraluminal BAs will be associated with low intestinal FGF19 expression and, hence, a less effective suppression of hepatic BA synthesis. Whether induction of intestinal FGF15 contributes to the suppression of CYP7A1 expression in cholestatic rodents treated with an FXR agonist remains to be established.

V. FARNESOID X RECEPTOR AND LIPOPROTEIN METABOLISM

The ability of BAs to affect lipid metabolism is known since the 1970s. Since its discovery in 1995, a role for FXR in the control of both triglyceride and cholesterol metabolism has been established, identifying FXR as a molecular link between BAs and plasma lipids.

A. FXR and LDL-Cholesterol Metabolism

FXR impacts on cholesterol metabolism through the repression of CYP7A1, the rate-controlling enzyme for cholesterol catabolism into BAs. CYP7A1 induction stimulates the conversion of cholesterol to BAs, resulting in a relative deprivation of hepatic microsomal cholesterol content, followed by upregulation of LDL-receptor (LDL-R) expression and activity which consequently reduces plasma LDL-cholesterol (LDL-C) levels. This mechanism underlies the hypcholesterolemic effect of BA sequestrants, like cholestyramine and colesevelam (131).
Moreover, human CYP7A1 deficiency results in a statin-resistant hypercholesterolemia (250). The fact that BA sequestrants lower LDL-C suggests that a synthetic FXR agonist might reduce LDL-C levels. In traditional Indian medicine, oleogum resin (known as guggul) from the guggul tree, Commiphora mukul, has been used to treat various diseases, including hypercholesterolemia. A number of uncontrolled clinical studies carried out in India demonstrated the hypolipidemic activity of guggulsterone with, on average, 10-30 and 10-20% decreases in total cholesterol and triglyceride, respectively. However, individual variations in response to guggulsterone treatment have been noted with ~70-80% responders and 20-30% nonresponders (65, 323). In contrast to these data, a United States randomized controlled trial demonstrated that guggulsterone treatment did not improve the plasma lipid profile of hypercholesterolemic patients, and even leads to a modest increase in LDL-C (313). This demonstrates that guggulsterone does not reduce LDL-C in humans at the doses commonly used. However, it cannot be definitively excluded that a more potent or specific FXR antagonist might have the potential to decrease LDL-C levels.

On the other hand, in vitro studies performed in human hepatocyte cell lines demonstrate that CDCA increases LDL-R gene expression and activity, a potential beneficial effect in the prevention of atherosclerosis (38, 210, 315). This CDCA-mediated induction of LDL-R may involve a mitogen-activated protein (MAP) kinase-mediated stabilization of LDL-R mRNA (210). More recently, CDCA, as well as GW4064, have been shown to potentiate LDL-R activity in response to statin treatment in human hepatocytes. It has been proposed that FXR activation leads to the repression of the Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9), a natural inhibitor of LDL-R, thereby enhancing LDL-R activity (167). These in vitro studies suggest that FXR activation by CDCA or synthetic agonists may be useful for lowering LDL-C levels in humans. It should be noted, however, that a 3-wk treatment with CDCA reduced LDL-R mRNA levels in the liver of subjects who underwent cholecystectomy for gallstone disease, while PCSK9 mRNA levels were not altered (219). In humans, short-term (20 days) treatment with CDCA did not alter LDL-C levels (341), whereas long-term administration (2 yr) of CDCA in patients with gallstone disease resulted in a 10% increase of LDL-C (269). The reason for this discrepancy between in vitro and in vivo studies remains unclear, but in vivo treatment with CDCA may impact additional signaling pathways in the liver and in the intestine. FXR also controls intestinal cholesterol absorption since FXR<sup>−/−</sup> mice have been reported to exhibit increased dietary cholesterol absorption (166). However, this may simply represent a secondary consequence of the enlarged BA pool size in these animals.

Clearly, additional in vivo studies with specific FXR agonists are needed to resolve the question of the role of FXR in controlling LDL-C levels.

B. FXR and HDL-Cholesterol Metabolism

HDL carries cholesterol from the peripheral organs to the liver, where it can be excreted into the bile either as free cholesterol or after conversion into BAs, in a process called reverse cholesterol transport. FXR has a profound effect on HDL metabolism and remodeling, which stems seemingly from liver-specific processes. On the one hand, FXR<sup>−/−</sup> mice display elevated plasma HDL-C and apolipoprotein (apo) A-I concentrations (158, 288). FXR represses human and murine apoA-I gene expression both in vitro and in vivo, via a monomeric FXR binding site in the promoter (50). This negative FXRE is also a functional LRH-1 binding site, suggesting that FXR-mediated repression may also involve a FXR-induced SHP-mediated inhibition of LRH-1 transcriptional activity (63). Consistent with this, feeding apoA-I transgenic mice with CA lowers plasma HDL cholesterol and apo-AI levels. On the other hand, hepatic and ileal apo-AI expression is unaffected in FXR<sup>−/−</sup> mice (166). In vivo kinetic studies indicated that elevated plasma HDL-C levels observed in FXR<sup>−/−</sup> mice are due to a reduced, selective uptake of HDL-cholesteryl esters by the liver, potentially linked to a decreased expression of the scavenger receptor B1 (SR-B1), rather than to enhanced production. Accordingly, a CA-enriched diet decreases hepatic SR-B1 expression, both at the mRNA and protein level (166). Similar to FXR-mediated repression of CYP7A1, the reported BA-induced down-regulation of SR-BI may implicate the activation of the FXR > SHP > LRH-1 cascade (191). FXR enhances the expression of the phospholipid transfer protein (PLTP; Ref. 326) and represses the expression of hepatic lipase (290), demonstrating a role for FXR in HDL remodeling.

In vivo treatment with the FXR agonist GW4064 significantly improves hypercholesterolemia in mouse models of insulin resistance (37, 364), an effect unfortunately associated with a decrease in HDL-C (343). These latter results are in accordance with human studies in which BA sequestrants increase HDL-C concentrations (5, 282), whereas CDCA administration results in decreased HDL-C levels (13, 170). Additional studies are needed to establish whether FXR activation may modify HDL composition, hence its function. Intriguingly, treatment with CDCA of patients with cerebrotendinous xanthoma increased cholesteryl ester transfer protein (CETP) activity (154). Kinetic studies in humans are clearly required to investigate further the relationships between FXR and HDL formation, catabolism, and functions. In addition, the contribution of intestinal factors to the regulation of HDL metabolism should be evaluated.
C. FXR and Triglyceride Metabolism

Already in the 1970s, several clinical studies highlighted a reciprocal relationship between BA and triglyceride metabolism. The first evidence came from the observation that treatment of dyslipidemic patients with BA-sequestering resins, such as cholestyramine, resulted in an increase in plasma triglyceride and very-low-density lipoprotein (VLDL) levels, which are potentially undesirable effects (5, 17, 54). Furthermore, elevated plasma triglyceride concentrations were found in patients exhibiting decreased BA synthesis linked to CYP7A1 deficiency (250), and some patients with monogenic familial hypertriglyceridemia were found to have a defect in ileal BA absorption (5). Conversely, CDCA used for the treatment of patients with cholesterol gallstone disease decreased plasma triglyceride levels and was therefore suggested as a potential drug for the treatment of hypertriglyceridemia (5, 13, 18). The ability of endogenous and synthetic FXR agonists to act as hypotriglyceremic agents has also been evaluated in rodent models. CA lowers hepatic triglyceride accumulation, VLDL secretion, and elevated serum triglycerides in KK-A(y) mice, a mouse model of hypertriglyceridemia (343). GW4064 treatment lowered plasma triglyceride levels in rats (192), as well as in chow-fed mice in an FXR-dependent manner (49, 186, 364). Furthermore, GW4064 treatment also improves hypertriglyceridemia in mouse models of insulin resistance such as ob/ob and db/db mice (36, 364). Interestingly, CDCA significantly reduces plasma triglycerides and VLDL-cholesterol in fructose-fed hamsters, due to a reduction in the rate of VLDL production (21). Altogether, these results suggest that FXR activation may improve combined hyperlipidemia, a common feature of the metabolic syndrome in humans. Interestingly, FXR agonists also reduced the elevated free fatty acid (FFA) levels in insulin-resistant rodents, although the underlying mechanisms remain elusive (21, 364).

Both the production of triglyceride-rich VLDL particles and their clearance from the circulation have been proposed as key processes regulated by several BAs. Indeed, BAs inhibit production of VLDL by cultured human (178), rat (177), and mouse (77) hepatocytes in a dose-dependent and BA species-dependent fashion. Some of these in vitro effects of BAs may be FXR independent, since taurocholic acid is equally effective in suppressing VLDL production in hepatocytes isolated from wild-type and FXR−/− mice (77). Since treatment with UDCA, which is unable to activate FXR, does not alter plasma triglyceride levels in humans, FXR may act as a molecular link between BA and triglyceride metabolism (41, 170). In accordance with this hypothesis, FXR−/− mice exhibit a lipid profile characterized by increased serum triglyceride levels, linked to an increased hepatic synthesis of apoB-containing lipoproteins (mainly VLDL) (166, 288).

FXR activation impacts on triglyceride metabolism at different levels (Fig. 5). FXR is involved in the control of hepatic de novo lipogenesis, one source of fatty acids used for the assembly of VLDL. FXR activation by BAs or synthetic agonists represses the expression of the transcription factor SREBP-1c and its lipogenic target genes in mouse primary hepatocytes and in liver (343, 366). Based on studies in SHP-deficient (SHP−/−) mice, it has been proposed that the induction of SHP mediates the downregulation of SREBP-1c expression in response to BA treatment (338, 343). Counterintuitively, decreased, and not increased, SREBP-1c mRNA was observed in ob/ob/SHP−/− double mutants (119). More disturbing was the observation that transgenic mice constitutively expressing SHP in the liver exhibited an enhanced expression of SREBP-1c mRNA levels and a concomitant accumulation of hepatic triglycerides (28). Thus acute induction of SHP expression following FXR activation and transgenic SHP overexpression have opposite effects on triglyceride accumulation in the liver. Additional studies tend to call into question the central role of SREBP-1c as a mediator of the hypotriglyceremic effect of FXR. First, in combined hyperlipidemic hamsters, CDCA treatment did not significantly decrease SREBP1-c mRNA levels, whereas triglyceride production was efficiently reduced (21). Second, hepatic SREBP-1c mRNA levels are not increased, but reduced, in FXR−/− mice compared with controls (73, 366). However, it has not yet been determined whether FXR alters SREBP-1c cleavage, hence modulating the level of biologically active nuclear SREBP-1c protein. Third, reduced SREBP-1c levels are observed in livers of diabetic mice fed with the BA-binding resin colestimide (157). To complicate the issue further, a recent study demonstrated that FXR enhances the transcription of fatty acid synthase (FAS), a key lipogenic enzyme, through direct binding to an IR-1 site in the FAS promoter (197). Such a FXR-mediated induction of FAS could be a mechanism by which BAs may bypass SHP inhibition on lipogenesis, thus maintaining an adequate fatty acid pool for cholesterol esterification in specific situations, for instance when cholesterol and BAs are chronically elevated. Taken together, these data suggest that FXR activation may control de novo lipogenesis through both SREBP-1c-dependent and -independent mechanisms. As detailed in section \( \alpha \beta \), FXR modulates the kinetics of the response to dietary carbohydrate intake, since the maximal induction of glycolytic and lipogenic genes occurs earlier during the refeeding phase in FXR−/− than in wild-type mice. Lack of FXR therefore leads to an enhanced glycolytic flux which provides substrates for lipogenesis (Fig. 6) (74). Triglycerides derived from de novo lipogenesis efficiently mobilize apoB and induce VLDL assembly (72, 92). Consistent with this observation, hepatic VLDL production is significantly increased upon refeeding FXR−/− mice with a carbohy-
drate-rich diet (73). It is of importance to note, however, that this FXR-lipogenesis-VLDL hypothesis is mainly based on gene expression data and that no actual measurements of lipogenesis rate have been reported so far. Moreover, hepatic lipogenesis should not be considered as the sole driving force for VLDL production by the liver. Indeed, various animal models have been described in which increased hepatic lipogenesis is associated with liver steatosis without any effect on VLDL production (11, 345).

Several other mechanisms may contribute to the triglyceride-lowering effect of FXR activation (Fig. 5). In human primary hepatocytes, FXR ligands induce the expression of PPARα and of its target gene pyruvate dehydrogenase kinase-4 (PDK-4), which may promote fatty acid oxidation (245, 265). CDCA treatment represses the expression of microsomal triglyceride transfer protein (MTP), which plays a critical role in the assembly and secretion of VLDL (111). Notably, FXR also controls genes governing triglyceride clearance. Indeed, FXR activation increases apoC-II expression (146), an activator of lipoprotein lipase (LPL) activity and decreases both apoC-III (49) and ANGPTL3 (342), which are LPL inhibitors. In addition, FXR induces the expression of the VLDL receptor in human and mouse liver, possibly enhancing the clearance of triglyceride-rich lipoproteins (289). Finally, the expression of syndecan-1, a transmembrane heparin sulfate proteoglycan involved in the clearance of remnant particles, is induced by FXR ligands in cultured human liver cells (7). Altogether, these observations suggest that FXR activation also promotes the clearance of circulating triglycerides. Additional studies, including lipoprotein kinetic studies in humans, are necessary to delineate the precise role of FXR activation on triglyceride metabolism.

VI. FARNESOID X RECEPTOR AND GLUCOSE HOMEOSTASIS

A. FXR Expression in Diabetes

Several groups have demonstrated that the BA profile is perturbed in diabetes (296). The BA pool is significantly increased in several rat models of type 1 diabetes such as...
spontaneously diabetic Wistar rats (108) and streptozotocin- or alloxan-treated rats (216). Changes in the BA pool size and hepatobiliary BA secretion were directly dependent on the insulin-deficient state and were reversed with insulin therapy (217, 330). Moreover, liver-specific insulin receptor knockout mice (LIRKO) exhibited enlarged gallbladders with increased bile volume, suggestive for, but not proving, the existence of an enlarged BA pool (201). Altogether, these results suggest that the negative-feedback mechanism regulating BA synthesis is defective in type 1 diabetes. More recent studies indicated that FXR might be one of the molecular links between altered BA metabolism and diabetic states. Indeed, hepatic FXR mRNA levels are increased in diabetic db/db mice (364). The reason for this discrepancy between diabetic rat and mouse models remains unclear. However, these latter results in db/db mice are consistent with in vitro data, since FXR gene expression is stimulated by glucose and repressed by insulin in vitro in primary rat hepatocytes (74). FXR appears to be regulated by glucose via the pentose phosphate pathway because the addition of xylitol, a precursor of xylulose-5-phosphate, increases FXR expression to a comparable level to that induced by d-glucose (74). In addition, glucose increases nuclear FXR DNA-binding activity and subsequently FXR transcriptional activity on target genes such as SHP and apo-CIII (74).

In a more physiological setting, FXR mRNA levels vary with the nutritional status. While fasting specifically increases hepatic FXRα 3/4 expression, a high-carbohydrate refeeding subsequently reduces FXR mRNA levels (73, 366). This fasting-induced FXR expression appears to be sustained by cAMP and PGC-1α, both of which enhance FXR gene transcription (366). Furthermore, hepatic expression of FXRβ, but not of FXRα, displays a clear diurnal rhythm, like the majority of other nuclear receptors involved in metabolic homeostasis. White adipose FXRα and FXRβ expression is also subjected to circadian variation (352). Therefore, FXR expression appears to be tightly regulated by the metabolic status, indirectly supporting a role for this nuclear receptor in metabolic diseases.

B. FXR and Hepatic Glucose Metabolism

In the fasted state, the flux of newly synthesized glucose is sustained by the activation of gluconeogenesis. To date, the precise role of FXR in the regulation of hepatic glucose metabolism remains a controversial issue. A first observation in this field was the observation that FXR activation induces phosphoenolpyruvate carboxykinase (PEPCK) expression (35, 300). For a long time, PEPCK has been considered to be the rate-controlling enzyme of gluconeogenesis. However, in vivo studies clearly demonstrate that mice with a 95% reduction in PEPCK mRNA have a nearly normal blood glucose concentration after a 24-h fast (279, 280). Other rate-controlling steps in gluconeogenesis are catalyzed by glucose-6-phosphatase (G6Pase) and fructose 1,6-bisphosphatase.
Glucoseogenic enzymes are regulated at the transcriptional level by hormones controlling glucose homeostasis. Glucagon and glucocorticoids, which have strong glucoseogenic actions, induce PEPCK expression, whereas insulin, which suppresses hepatic gluconeogenesis, represses PEPCK expression (106). Since type 2 diabetes is characterized by an increased hepatic glucose output, which contributes to fasting hyperglycemia, pharmacological modulation of glucoseogenic gene expression appears an attractive possibility for type 2 diabetes treatment (188).

1. In vitro studies

Numerous in vitro studies have focused on the transcriptional regulation of glucoseogenic enzymes by BAs and led to conflicting results. On the one hand, CDCA treatment of human hepatoma cell lines has been shown to decrease PEPCK, as well as G6Pase and FBP1 expression (60, 349). It is still unclear whether these pathways are regulated in a FXR-dependent manner. As on the CYP7A1 promoter, CDCA decreases the activity of hepatocyte nuclear factor 4-α (HNF-4α), a positive regulator of PEPCK gene expression (60). Chromatin immunoprecipitation experiments demonstrated that CDCA impairs the recruitment of PGC-1 and CBP (CAMP response element-binding protein (CREB) binding protein) by HNF-4 to the PEPCK promoter (349). Importantly, treatment with the nonsteroidal FXR agonist GW4064 did not modify HNF-4 activity, suggesting an FXR-independent mechanism (60). Other data suggest that FXR may decrease glucoseogenic enzyme expression via induction of SHP. Notably, SHP has been shown to prevent CBP recruitment by HNF-4α on the PEPCK and FBP1 promoters, and by Foxo1 on the G6Pase promoter (349). However, the FXR dependency of this pathway remains to be established. In keeping with a potential role for SHP in gluconeogenesis, transiently transfected SHP inhibits PEPCK promoter transactivation by the glucocorticoid receptor (26), HNF-4α (26), HNF-3 (152), and C/EBP (231). More recently, it has also been suggested that CDCA represses PGC-1 promoter activity in a SHP-dependent manner (348). Unexpectedly, basal glucose production was found to be significantly lower in SHP−/− primary hepatocytes (337), raising doubts about the proposed role and mechanisms of SHP in gluconeogenesis.

On the other hand, a role for FXR in the induction of glucoseogenic gene expression can be inferred from several observations. Fasting markedly induces hepatic expression of PGC-1, which subsequently stimulates the entire program of genes involved in hepatic gluconeogenesis by acting as a coactivator for GR and HNF-4α (248). PGC-1 coactivates FXR-target gene promoter activity in vitro, either in a ligand-independent (141) or in a ligand-dependent manner (266, 366). Thus it is plausible that FXR activation participates in the induction of gluconeogenesis. In agreement with this hypothesis, FXR activation by CDCA, GW4064, or fexaramine leads to a dose-dependent increase of PEPCK mRNA levels in rat hepatoma cell lines, as well as in primary rat hepatocytes (300). Of potential functional relevance, FXR activation also increases glucose output by primary rat hepatocytes in vitro (300). Results are more discordant in primary mouse hepatocytes: GW4064 either induces (364) or has no effect on PEPCK gene expression (36). However, basal (i.e., nonpharmacologically stimulated) PEPCK mRNA levels are decreased by 60% in hepatocytes isolated from FXR−/− mice compared with controls, suggesting that FXR expression is required for proper PEPCK regulation (36).

2. In vivo studies

In accordance with a putative repressive effect of FXR on glucoseogenic genes expression, feeding C57BL/6J mice with 1% CA-supplemented diet during 7–8 days decreases hepatic PEPCK, G6Pase, and FBP1 mRNA levels (60, 349). Furthermore, feeding a CA-enriched diet has been shown to decrease PEPCK and G6Pase mRNA levels in wild-type but not FXR−/− or SHP−/− mice. This decrease was associated with decreased fasting blood glucose only in wild-type mice, reinforcing the hypothesis that the FXR-SHP negative regulatory cascade represses gluconeogenesis (186). However, these results remain difficult to reconcile with those obtained in SHP−/− mice. Both PEPCK and G6Pase basal expression are indeed reduced in the liver of SHP−/− mice. This expression profile is well correlated with a reduced in vivo glucose output from livers of SHP−/− mice (337). The reason for this apparent discrepancy remains unclear. In contrast to the results with CA feeding, in vivo treatment with the FXR agonist GW4064 has been shown to induce PEPCK mRNA levels in a FXR-dependent manner. However, this regulation was also associated with a decrease in plasma glucose levels (300, 364). Taken together, these results suggest that BAs may impact additional FXR-independent signaling pathways involved in glucose metabolism that make the integrated response in vivo more complex. To complicate the issue further, the regulation of glucoseogenic enzymes expression by FXR seems to be modulated by the diabetic state. In contrast to what was observed in C57BL/6J mice, Zhang et al. (364) found that oral GW4064 treatment (30 mg · kg−1·day−1) reduced PEPCK and G6Pase expression, and improved hyperglycemia in db/db diabetic mice. Similar results were obtained following adenoviral-mediated hepatic overexpression of constitutively active FXR (364). In contrast, another study performed in female ZDF rats showed that oral GW4064 administration increased PEPCK mRNA levels in a dose-dependent manner, whereas high doses of GW4064 (30
mg\(^{-1}\cdot kg\(^{-1}\cdot \text{day}^{-1}\)) decreased G6Pase mRNA levels. Unfortunately, blood glucose levels were not reported in this work (118).

A complementary approach to assess the role of FXR in hepatic glucose metabolism is based on the phenotypic analysis of the FXR\(^{-/-}\) mice. Metabolic adaptation to prolonged fasting was investigated in this mouse model. While the long-term fasting adaptation is not altered by FXR deficiency, the kinetics of metabolic changes during short-term fasting are altered, leading to an accelerated and transient drop in glycemia (36). In line with this observation, the short-term fasting-associated induction of PEPCK (36, 186) and of PGC-1 (186) mRNA levels is blunted in FXR\(^{-/-}\) mice. This fasting-hypoglycemia may also be linked to an impaired glycogenolysis since hepatic glycogen content is significantly reduced in FXR\(^{-/-}\) mice (36). Conversely, the FXR agonist GW4064 increases hepatic glycogen synthesis and storage in vitro in primary hepatocytes, as well as in vivo in db/db mice (364). In accordance with a role for FXR in the control of the kinetics of glucose metabolism, FXR\(^{-/-}\) mice subjected to an overnight fasting followed by refeeding with a high-carbohydrate diet exhibit an accelerated induction of glycolytic and lipogenic genes compared with wild-type mice (73). The accelerated response of FXR\(^{+/-}\) mice to high-carbohydrate refeeding seems to be due to a potentiation of carbohydrate-induced signaling. FXR activation by GW4064 attenuated glucose-induced mRNA expression as well as promoter activity of several glucose-regulated genes, such as L-pyruvate kinase and acetylCoA carboxylase 1, in rodent primary hepatocytes (73). Additional studies are needed to determine whether FXR can interfere with the transcriptional activity of the carbohydrate response element-binding protein (ChREBP), a major mediator of glucose action (247).

Taken together, these data provide evidence for a critical role of BAs in the dynamic regulation of glucose metabolism in the liver. As underlined above, FXR acts through a complex network to drive the expression of gluconeogenic genes. The exact underlying molecular mechanisms, as well as the relative contribution of FXR-dependent and FXR-independent pathways, remain to be determined. One might suggest, however, that FXR acts mainly as a metabolic sensor to direct metabolic fluxes during the fasting-refeeding transition. The enhanced expression of Cyp7A1 during fasting would increase the BA pool, allowing improved digestion and absorption of fats after a meal. Concomitantly, FXR expression is induced, but its transcriptional activity is likely decreased due to the absence of endogenous ligands during fasting caused by impaired enterohepatic cycling. Upon meal ingestion, the increased enterohepatic circulation of BAs activates FXR, leading to inhibition of de novo lipogenesis and glycolysis, to promote glycogen storage. Then, the rise of plasma insulin levels upon refeeding reduces FXR expression, which may contribute to a redirection of the glucose flux to glycolysis in the interprandial state (Fig. 6).

An additional argument for a link between BA and glucose metabolism arises from the observation that BA sequestrants reduce blood glucose levels in animal and human studies (reviewed in Ref. 296). Recently, coleste- midide treatment has been shown to prevent weight gain, insulin resistance, and diabetes in a rodent model of diet-induced obesity (157). In humans, a slight, but significant, improvement of glycemic control was initially reported upon cholestyramine treatment (90). Similar results were subsequently reported for colesevelam (369) and colestimide (350). While the precise mechanisms sustaining this hypoglycemic effect remain unclear, it has been suggested that BA sequestrants may interfere with the enteroinsular axis. In a small open-labeled study of patients with type 2 diabetes, a 1-wk treatment with colestimide increased the 2-h postprandial plasma glucagon-like peptide(GLP)-1 level. In parallel with this stimulation of GLP-1 secretion, both 1-h and 2-h postprandial plasma glucose levels were significantly decreased in the same study (311). In addition, in vitro studies demonstrated that BAs promote GLP-1 secretion through the activation of TGR5 in the murine enteroendocrine STC-1 cell line (143).

C. FXR and Insulin Sensitivity

Three independent studies identified a role for FXR in regulating insulin sensitivity (37, 186, 364). FXR deficiency leads to impaired glucose tolerance and insulin resistance. Hyperinsulinemic-euglycemic clamp studies confirmed that FXR\(^{-/-}\) mice display a peripheral insulin resistance reflected by a reduced peripheral glucose disposal (37, 186). Consistent with these observations, insulin signaling is impaired in peripheral insulin-sensitive tissues such as skeletal muscle and white adipose tissue (37, 186). Discordant data concerning the level of hepatic insulin sensitivity in FXR\(^{-/-}\) mice are reported in the literature. Some studies found a reduced inhibition of hepatic glucose output during low-dose insulin clamp (186) and an impaired hepatic insulin signaling in FXR\(^{-/-}\) mice (186, 364). In contrast, FXR deficiency was also shown to be associated with normal hepatic insulin sensitivity and signaling (37, 73). The reason for this discrepancy is unclear, but may be linked to different genetic backgrounds [C57Bl6/J (186, 364) vs. C75Bl6/N (37, 73)] of the mice and/or in the insulin dose used during the clamp. Based on these results, a prediction would be that FXR activation promotes insulin sensitivity. In accordance with this hypothesis, treatment with GW4064 improved insulin sensitivity in both db/db, KK-A(y) (364), and ob/ob (37) mice. Similar results were obtained following adeno- viral-mediated overexpression of constitutively active FXR in the liver of db/db mice (364).
The molecular mechanisms behind the insulin-sensitizing effect of FXR remain ill-defined. Since FXR is not expressed in skeletal muscle, it is conceivable that FXR deficiency alters indirectly insulin signaling in this tissue. A hypothesis is that FXR deficiency promotes ectopic lipid deposition in insulin target tissues, a phenomenon usually referred to as “lipotoxicity” (264). Indeed, FXR−/− mice have elevated circulating FFA levels (37, 186) and increased intramuscular triglyceride and FFA contents (186). Furthermore, the level of insulin receptor substrate-1 (IRS-1) serine-307 phosphorylation, which is linked to fatty acid-induced insulin resistance (356), is increased in skeletal muscle of FXR−/− mice (186). A similar mechanism could also operate in liver, since hepatic triglyceride content is increased in FXR−/− mice (73, 186). Conversely, GW4064 treatment reduces neutral lipid accumulation in the liver of db/db mice (364). It should be underlined that the contribution of the FXR-SHP cascade in the insulin-sensitizing effect of FXR ligands seems unlikely, since SHP−/− mice show increased whole body insulin sensitivity in wild-type and ob/ob mice (119, 337).

Recent data suggest that FXR plays a role in adipocyte differentiation and function. FXR expression increases progressively during adipocyte differentiation in vitro, both in 3T3-L1 cells and mouse embryonic fibroblasts (MEFs) cells (37, 258). With the use of MEFs as a model system, it has been shown that FXR deficiency leads to an impaired adipogenic program with a defective triglyceride accumulation (37). Conversely, the synthetic FXR ligand 6α-ECDDA/INT-747 promotes adipocyte differentiation and lipid storage in 3T3-L1 adipocytes (258). Consistent with these in vitro data, FXR−/− mice exhibit a moderate lipoatrophic phenotype that may contribute to their impaired insulin sensitivity. Moreover, GW4064 treatment improves insulin signaling and insulin-induced glucose uptake in 3T3-L1 differentiated adipocytes (37, 258). However, several questions remain to be answered to better understand the role of FXR in white adipose tissue. What is the molecular nature of the endogenous FXR ligands in the adipocyte? What are the FXR target genes in this tissue?

Recently, fibroblast growth factor 19 (FGF19, or FG19, the mouse ortholog FG15), a member of the FGF family of proteins, has emerged as a new regulator of metabolic homeostasis (307). FGF19 expression is induced in a FXR-dependent manner in intestinal epithelial cells, in response to BA released into the intestinal lumen upon feeding. Then, FGF19 circulates through the portal vein to act on hepatocytes to reduce BA synthesis (114, 129). Interestingly, resistance to both diet-induced obesity and insulin desensitization were observed in transgenic mice overexpressing human FGF19 (316). A direct inhibition of hepatic acetyl CoA carboxylase 2 and stearoyl-coenzyme A desaturase-1, and a subsequent increase in fatty acid oxidation, may contribute to the decreased hepatic triglyceride content and improved insulin sensitivity in response to increased FGF19 levels (84, 316). More recently, FGF19 has been shown to increase glucose uptake in 3T3-L1 adipocytes in vitro (164). Activation of FGF19 signaling in response to injected FG19 is less prominent in white adipose tissue than in liver, suggesting that this potential extrahepatic activity of FGF19 is dependent on supraphysiological circulating levels of FGF19, as observed in transgenic mice (164). On the basis of these results, it can be suggested that part of the FXR-mediated insulin-sensitizing effect may involve FGF19 signaling pathways. Thus study of tissue-specific FXR knockout mice is essential to unravel the respective contribution of hepatic, intestinal, and adipose FXR in the control of insulin sensitivity.

D. FXR and Energy Expenditure

Adaptive thermogenesis is defined as heat production in response to variations in environmental temperature or diet. Hence, it protects the organism from cold exposure and regulates energy balance after dietary changes (183). In rodents, brown adipose tissue (BAT) is the major site of thermogenesis.

FXR mRNA expression is not detected in mouse BAT (34, 342), excluding a direct action of FXR on adaptive thermogenesis. In contrast, SHP, a direct FXR target gene, appears to be a negative regulator of thermogenesis in BAT by inhibiting PGC-1 expression. Accordingly, SHP−/− mice show increased energy expenditure, and resistance to diet-induced obesity (339). However, two additional independent studies failed to detect SHP mRNA in mouse BAT (34, 342), raising doubts about the physiological role of SHP in this tissue. Interestingly, SHP mutations have been associated with low birth weight, insulin resistance, and obesity in a Japanese cohort of young children (220). However, these findings were not confirmed in two subsequent studies on Caucasian subjects performed in the United Kingdom (127, 202). FXR deficiency does not alter the metabolic rate measured under basal un-stressed conditions, despite elevated plasma BA concentrations (34). In contrast, FXR appears to be involved in the regulation of adaptive thermogenesis since FXR−/− mice display an accelerated entry into torpor upon fasting and cold intolerance. Both these altered responses may be linked to the lipoatrophic phenotype of FXR−/− mice, with reduced glycogen and triglyceride stores in liver and adipose tissue, respectively (34). Under these dynamic conditions, FXR contributes to the control of metabolic fuel stores that are essential to sustain heat production by BAT. These results identify a role for FXR as a modulator of energy homeostasis.
VII. FARNESOID X RECEPTOR AND ATHEROSCLEROSIS

A. A Role for FXR in the Vessel Wall?

Recently, several studies have focused on a potential new role for FXR in the vasculature. In vitro studies demonstrated that FXR is expressed in both vascular smooth muscle cells (VSMCs; Refs. 22, 363) and endothelial cells (110, 253). In contrast, FXR is not expressed in macrophages, such as differentiated THP-1 macrophage-like cells (241) and peritoneal macrophages (98).

1. VSMCs

As detailed in section mB, FXR is expressed in the vessel wall, in VSMCs from the coronary artery and aorta, as well as within atherosclerotic lesions and human primary saphenous vein smooth muscle cells (22, 174, 363), although expression is lower than in rat liver or HepG2 human hepatoma cells. Activation of FXR with the synthetic FXR ligands 6ECDCA (237) and GW4064 (192) induces expression of the FXR target genes SHP and PLTP in rat smooth muscle cells (RASMCs) (174, 363). This effect was also observed in response to CDCA treatment in one study (363) but not in another (22). This discrepancy casts doubt on the identity of endogenous FXR ligands outside the enterohepatic circulation. From a functional point of view, FXR activation has been shown to induce RASMC apoptosis. Interestingly, FXR activators synergize with the RXR ligand 9-cis-retinoic acid to induce cell death (22). In addition, 6ECDCA and GW4064 inhibit VSMCs inflammatory responses and migration.

Synthetic FXR ligands inhibit interleukin-1β-induced expression of inducible nitric oxide synthase (iNOS) and of cyclooxygenase-2 in RASMCs, by reducing NF-kB expression of inducible nitric oxide synthase (iNOS) and of syntheses in response to lipopolysaccharide-stimulated ET-1 expression and secretion. FXR was found to modulate ET-1 expression via inhibition of the activator protein AP-1 (110). Therefore, it can be hypothesized that FXR may have anti-inflammatory actions through a transrepression mechanism, similar to that observed with peroxisome proliferator-activated receptors (PPARs) (256). FXR also directly enhances transcriptional activation of the endothelial nitric oxide synthase (eNOS) gene promoter, leading to increased NO production in vascular endothelial cells (173). Thus FXR may positively influence the vascular tone.

2. Endothelial cells

A low expression level (~1,000-fold less than in the liver) of FXR was detected in several models of endothelial cells such as rat pulmonary artery endothelial cells (RPAEC) (110), human (HAEC) (253), and bovine aortic endothelial cells (BAEC) (173), as well as primary human umbilical vein endothelial cells (HUVECs) (253). Like in VSMCs, activation of FXR by CDCA leads to SHP induction in RPAEC (110). Interestingly, FXR is positively autoregulated in RPAEC, since CDCA treatment strongly increases FXR expression (110). The endothelium plays a crucial role in regulating the vascular tone. Imbalance between the vasodilating agent NO and the vasoconstrictor endothelin-1 (ET-1) contributes to endothelial dysfunction, especially in insulin resistance and diabetes (254).

The FXR ligands CDCA and GW4064 repress the basal and lipopolysaccharide-stimulated ET-1 expression and secretion. FXR is expressed in both vascular smooth muscle cells induced by platelet-derived growth factor (PDGF)-β (174). Moreover, FXR directly enhances the transcription of the angiotensin type 2 receptor (AT2R) in RASMCs by binding to an IR2 FXRE in the AT2R promoter. The upregulation of AT2R results in an inhibition of angiotensin II-mediated ERK signaling in RASMCs (363). Of clinical relevance, overexpression of AT2R and inactivation of the ERK signaling pathway has been suggested to prevent neointimal proliferation (212), to partly mediate the hypertensive effects of AT1R blockers (267), and to mediate the cardioprotective effect of several PPARγ ligands (204). While FXR activation does not alter expression of the AT1R in RASMCs, it should be kept in mind that CDCA represses, in hepatocytes, angiotensinogen expression in a SHP-dependent manner (286). Altogether, these in vitro results suggest that FXR activation in VSMCs may have a beneficial effect on vascular inflammation, neointimal proliferation, and blood pressure levels. However, additional studies, especially in FXR−/− mice, are needed to further characterize the effect of FXR activation in an intact organism and to assess whether these signaling pathways are FXR dependent.
not CA, induce the expression of intracellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), and E-selectin in a dose-dependent manner in HUVEC. In contrast, GW4064 only increases adhesion molecule expression at high concentrations (5 μM) and to a lesser extent than CDCA, suggesting that BAs operate in a FXR-independent manner. This effect seems to be mediated through the elevation of reactive oxygen species and the subsequent stimulation of the NFκB and p38 MAP kinase signaling pathways. Of functional relevance, this induction of adhesion molecules promotes adhesion of monocytes to endothelial cells, a crucial step in the initiation of the atherosclerosis process (253).

3. Liver-mediated vascular effects

FXR can also impact on vascular functions via its actions in the liver. Consequences of FXR activation on lipoprotein metabolism are reviewed above (see sect. VI).

Atherosclerosis is a chronic inflammatory disease. Excessive inflammation within the arterial wall is a risk factor for cardiovascular disease and can promote atherogenesis (107). High concentrations of hydrophobic BAs induce the expression of a number of inflammatory genes in the liver, which may contribute to atherogenesis in mice fed an atherogenic diet supplemented with 0.5% cholate (176, 252, 333). In addition to a direct cytotoxic effect, this inflammatory response is also mediated through activation of several protein kinases including PKC (252, 309), ERK (251), and c-Jun NH2-terminal kinase (JNK) (99). Interestingly, hepatic FXR appears to be downregulated during the acute phase response in rodents, similar to other nuclear receptors such as PPARα and LXR (79, 153). This suggests that FXR may modulate the expression of genes participating in the inflammatory response. Notably, treatment of mice with CA induces the expression of ICAM-1, VCAM-1, serum amyloid A2, and TNF-α. Moreover, in vitro experiments in human hepatocytes demonstrate that FXR increases the transcriptional activity of the human ICAM-1 promoter (252). Based on these results, FXR activation in liver could be associated with a deleterious proinflammatory profile.

Recent data indicate that FXR activation may alter anticoagulation processes. The first indication came with the demonstration of a FXR-mediated upregulation of kininogen expression in human hepatocytes (368). Human kininogen is the precursor for the two-chain kinin-free kininogen, which exerts antiadhesion, anti-inflammation, and antiplatelet aggregation activities, and for bradykinin which is a potent vasodilator and a mediator of inflammation (193). Thus the increased production of human kininogen may lead to antiadhesion and antithrombosis. In contrast, another study found that FXR induces human fibrinogen expression in an isoform- and species-specific manner (6). Fibrinogen is a key component of the coagulation pathway, and it has been suggested to be a cardiovascular risk factor in a prospective cohort study (268). Therefore, additional in vivo studies are needed to assess the physiological consequences of FXR activation on coagulation processes.

Finally, concordant data have demonstrated that FXR is involved in the BA-mediated repression of paraoxonase-1 (PON1) (101, 285). PON1 is an enzyme that circulates in plasma associated with HDL. By preventing LDL oxidation, PON1 exerts anti-inflammatory effects and appears to be atheroprotective in both humans and mice (284). A CA-containing atherogenic diet reduces the expression of PON1 in C57Bl/6J mice (283). Studies with FXR−/− mice and FXR agonists demonstrate that BA-mediated repression of PON1 expression is mediated by FXR. As previously reported for CYP7A1 (129), FXR-mediated repression of PON1 involves the induction of FGF-19, its subsequent binding to FGFR4, and activation of the JNK pathway (101, 285). Thus FXR activation in the intestine may promote atherosclerosis risk by repressing both hepatic CYP7A1 and PON1 through the ileal induction of FGF19.

B. In Vivo Role of FXR in Mouse Models of Atherosclerosis

Since in vitro studies have led to conflicting conclusions concerning the potential role of FXR in the pathogenesis of atherosclerosis (Fig. 7), evaluation of the overall consequence of FXR deficiency in a whole body organism is mandatory to fully appreciate its role in cardiovascular diseases. Unfortunately, the reported data are controversial, and discrepancies might stem from the distinct atherosclerosis mouse models, gender, or the diet used in each study (98, 105, 365). On the basis of the observation that FXR−/− mice exhibit a proatherogenic lipid profile (288), accelerated atherosclerosis would be anticipated in these mice. However, FXR−/− mice did not display enhanced atherosclerosis susceptibility even when fed a high-fat/high-cholesterol diet (105, 365). Therefore, backcrosses with genetic mouse models of accelerated atherosclerosis were used to assess the consequence of FXR deficiency on atherosclerosis progression in vivo. Hanniman et al. (105) found that male FXR−/− mice bred on an ApoE-deficient genetic background (FXR−/−/ApoE−/−) fed an atherogenic diet exhibited more severe atherosclerosis and a significantly reduced survival rate. In striking contrast, Guo et al. (98) reported fewer atherosclerotic lesions in female FXR−/−/ApoE−/− mice, and male FXR−/− mice crossed with LDL-R-deficient mice (FXR−/−/LDL-R−/−) also displayed less pronounced atherosclerotic lesions than LDL-R−/− mice (365). The atheroprotective effect of FXR deficiency in both male FXR−/−/
LDL-R−/− and female FXR−/−/ApoE−/− mice may be linked to a change in lipoprotein composition with a decrease of LDL-C, paralleled by an increase of VLDL-C concentrations (98, 365). Such a LDL-C decrease was not observed in female FXR−/−/LDL-R−/− mice, which, accordingly, did not display reduced atherosclerotic lesions (365). More disturbing is the observation that LDL-C concentrations are increased in male FXR−/−/ApoE−/− (105). The reason(s) for this discrepancy is currently unclear. Interestingly, FXR deficiency was found to be associated with reduced foam-cell formation in macrophages isolated from both female FXR−/−/LDL-R−/− mice, which, accordingly, did not display reduced atherosclerotic lesions (365). More disturbing is the observation that LDL-C concentrations are increased in male FXR−/−/ApoE−/− (105). The reason(s) for this discrepancy is currently unclear.

In conclusion, although it appears that FXR modulates both systemic and vascular parameters of atherosclerosis, its exact effect on the atherogenic process is far from clear. Hence, it is currently impossible to predict vascular consequences of in vivo FXR modulation with specific synthetic agonists or antagonists. It would be of great interest to assess this question in an atherosclerosis-prone animal model, such as the rabbit or the guinea pig. Moreover, the use of tissue-specific FXR knockout mice would be of considerable interest to determine the relative contribution of systemic lipidic and/or inflammatory indirect effects versus a direct impact of FXR in the vasculature.
VIII. OTHER PATHOPHYSIOLOGICAL ASPECTS OF FARNESOID X RECEPTOR BIOLOGY

As it could be predicted from its expression pattern, FXR regulates not only metabolism, but also processes critical for the functioning of several organs and protection of the organism. Elevated BAs stimulate liver growth after partial heptectomy, a phenomenon which is FXR dependent (121). FXR activation contributes to cell cycle entry of hepatocytes by inducing the expression of transcription factors that regulate cell cycling, and appears therefore as a critical sensor of liver functionality (91, 115). The protective role of FXR is also underlined by the increased prevalence of hepatocellular adenoma and carcinoma in old (15 mo) male and female FXR−/− mice, a tumorigenic response which is preceded by general liver injury and inflammation (151, 351). This probably relates to the cytotoxic effects of BA, and accordingly, a CA-enriched diet favors chemically induced liver tumor progression. In line with this, BA sequestrants decrease tumor appearance in treated mice (151, 351). In addition, a role for FXR in breast cancer has been suggested by several reports, although they appear somewhat contradictory. The proliferation of breast cancer MCF7 cells is induced by farnesol, and this process was described as an estrogen receptor (ER)-dependent process (136) that correlates to a direct physical ER-FXR interaction. However, both ER-positive (MCF7) and ER-negative (MDA-MB-468) cell lines exhibited an increased apoptosis in response to GW4064 or DCA treatment in a FXR-dependent manner (287, 312). Guggulsterone also induced apoptosis of ER-negative MDA-MB-231 cells (282). Consistent with a protective role for FXR, FXR activation decreased aromatase expression, probably through SHP-mediated inhibition of LRH-1 (312).

Obstruction of bile flow is known to promote intestinal bacterial overgrowth and systemic infection. FXR−/− mice exhibit an increased bacterial flora and a defective epithelial barrier function (130), underlining the complexity of the BA enterohepatic cycle which also relies on the conversion of primary BA to secondary BA by the gut flora (281).

Finally, FXR plays also a role in kidney pathophysiology, by preventing the deleterious effects of a high-fat diet on renal disease. FXR activation counteracted lipid-induced SREBP1c and proinflammatory cytokine expression in renal mesangial cells and in high-fat diet-fed mice. Moreover, FXR activation prevented induced synthesis of oxidative stress enzymes, glomerulosclerosis, and proteinuria in vivo, suggesting that FXR modulation could be a new therapeutic avenue for treating diabetic kidney disease (134).

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Bile Acids and Bile Acid Receptors in Metabolic Regulation


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