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Endocrine functions of bile acids

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Bile acids (BAs), a group of structurally diverse molecules that are primarily synthesized in the liver from cholesterol, are the chief components of bile. Besides their well-established roles in dietary lipid absorption and cholesterol homeostasis, it has recently emerged that BAs are also signaling molecules, with systemic endocrine functions. BAs activate mitogen-activated protein kinase pathways, are ligands for the G-protein-coupled receptor TGR5, and activate nuclear hormone receptors such as farnesoid X receptor α. Through activation of these diverse signaling pathways, BAs can regulate their own enterohepatic circulation, but also triglyceride, cholesterol, energy, and glucose homeostasis. Thus, BA-controlled signaling pathways are promising novel drug targets to treat common metabolic diseases, such as obesity, type II diabetes, hyperlipidemia, and atherosclerosis.

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Introduction

Bile consists of bile acids (BAs), cholesterol, phosphatidylcholine, and bilirubin, and is secreted from the hepatocytes into the bile canaliculi. BA synthesis from cholesterol is the prime pathway for cholesterol catabolism. BAs are amphipathic molecules, which contain a sterol nucleus with hydroxyl groups and a side chain that terminates in a carboxyl group. Their amphipathic nature is essential to solubilize dietary lipids, which subsequently promotes their absorption in the digestive tract. The principal BAs include in humans the primary BAs cholic acid and chenodeoxycholic acid (CDCA), their glycine and taurine conjugates, and the secondary BAs deoxycholic acid and lithocholic acid (Figure 1A). In mice, CDCA is efficiently converted into muricholic acid and BAs are almost exclusively conjugated to taurine. Most of the BAs are present within the enterohepatic organs. Usually BAs are stored in the gallbladder; however, when a meal is ingested, they flow into the duodenum and intestine. The BAs are efficiently (95%) absorbed again by passive diffusion and active transport in the terminal ileum, and transported back to the liver via the portal vein. In the liver, the BAs are taken up at the basolateral (sinusoidal) membrane and exported again at the apical (canalicular) membrane of the hepatocytes into the bile canaliculus (transhepatic BA flux). This completes their enterohepatic circulation. Each BA molecule may complete 4–12 cycles between the liver and intestine per day (Cohen, 2003). Owing to this efficient recirculation, only a small amount of the BA pool size is derived from *de novo* biosynthesis. Several reviews describe BA biosynthesis with its enzymes, genetics, and regulation in detail (Chiang, 2002; Russell, 2003).

Besides its roles in dietary lipid absorption and cholesterol homeostasis, it has become clear that BAs are also signaling molecules. Three major signaling mechanisms have been identified. BAs activate mitogen-activated protein kinase (MAPK) pathways (Gupta *et al*, 2001; Qiao *et al*, 2003), are ligands for the G-protein-coupled receptor (GPCR) TGR5 (Maruyama *et al*, 2002; Kawamata *et al*, 2003), and activate nuclear hormone receptors such as farnesoid X receptor α (FXRα; NR1H4) (Makishima *et al*, 1999; Parks *et al*, 1999; Wang *et al*, 1999). The discovery of BAs as the endogenous FXRα ligands suggested a function for them in the enterohepatic recycling of BAs and the feedback regulation of BA biosynthesis, which is in line with the reported expression pattern of FXRα in liver and intestine (Forman *et al*, 1995). In these tissues, FXRα activation protects against accumulation of BAs, which is toxic, via mechanisms that have been reviewed recently (Chiang, 2002; Russell, 2003; Houten and Auwerx, 2004). To summarize (Figure 1B), FXRα activation in the liver leads to increased conjugation of BAs, followed by the excretion of BAs from the hepatocyte into the bile canaliculus, leading to an increase in the formation of bile. In the intestine, FXRα activation leads to increased expression of a protective BA-binding protein and the basolateral BA transporters (organic solute transporter (OST) α and β). Of particular interest is the FXRα-mediated induction of short heterodimer partner expression (SHP, NR0B2). SHP is an atypical nuclear hormone receptor that only has a ligand-binding domain and no DNA-binding domain and inhibits the activity of several nuclear receptors. The SHP induction underlies the negative feedback regulation of BA biosynthesis and uptake via a mechanism that will be described below. In this review, we will focus on the recent interest in BAs as general regulators of metabolic homeostasis and the mechanisms by which BAs can exert these functions.

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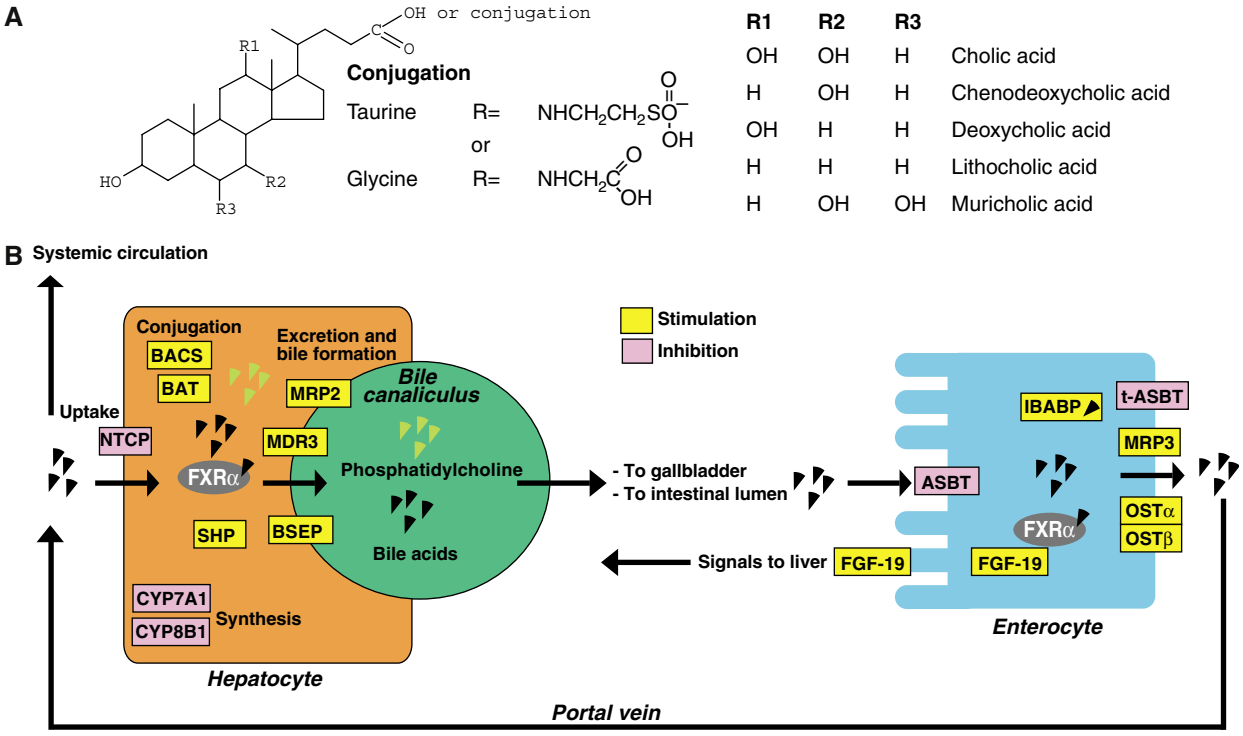

Figure 1 (**A**) Structure of selected BAs. (**B**) FXRα target genes involved in the enterohepatic recycling and detoxification of BAs. Genes whose expression is directly induced by BAs and FXRα are in yellow rectangles; those in pink rectangles are inhibited by BAs. BACS, BA-CoA synthetase; BAT, BA-CoA: amino acid *N*-acetyltransferase; BSEP, bile salt export pump; MDR3, multidrug resistance 3 *p*-glycoprotein; MRP2, multidrug resistance-associated protein 2; IBABP, ileal BA-binding protein; CYP8B1, sterol 12α-hydroxylase; NTCP, sodium taurocholate cotransporting polypeptide; ASBT, apical sodium-dependent bile salt transporter.

BAs regulate lipid metabolism

Besides its roles in BA homeostasis, increasing evidence is emerging for an important role of FXRα in lipid metabolism. BAs decrease their own biosynthesis from cholesterol, through an elaborate feedback inhibitory circuit, which starts by BA-mediated activation of FXRα, which will in turn induce the expression of SHP. In rodents, SHP then binds and interferes with the activity of two nuclear receptors, liver X receptor α (LXRα, NR1H3) and liver receptor homolog-1 (LRH-1, NR5A2) (Goodwin *et al*, 2000; Lu *et al*, 2000; Brendel *et al*, 2002), both necessary for transcriptional activation of the cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in BA biosynthesis from cholesterol. Significant differences exist in BA homeostasis of rodents, humans, and other model organisms. Most notably, differences have been documented in the regulation of CYP7A1 promoter activity by LXRα. The regulation of other key pathways such as the FXRα-mediated induction of SHP, however, is well conserved between species. Therefore, a high transhepatic BA flux, which translates in FXRα activation, positively correlates with hepatic and LDL-cholesterol levels, since cholesterol is not eliminated via its conversion to BAs. HDL levels are negatively correlated with transhepatic BA flux (Figure 2; Schoenfield and Lachin, 1981; Leiss and von Bergmann, 1982). Several genes with a role in HDL metabolism are FXRα targets and have been reviewed recently (Francis *et al*, 2003).

Interestingly, BAs also affect triglyceride homeostasis. In fact, for a long time it has been known that in man there is an inverse relationship between the transhepatic BA flux and hepatic VLDL production. Treatment with BA-binding resins, ileal exclusion, or bile withdrawal interrupts the enterohepatic circulation, decreasing transhepatic BA flux (Figure 2). All these interventions induce the production of VLDL (Grundy *et al*, 1971; Nestel and Grundy, 1976; Angelin *et al*, 1978). Conversely, treatment of cholesterol gallstones with the BA CDCA increases the BA pool and consequently the transhepatic BA flux, and reduces hypertriglyceridemia (Figure 2) (Miller and Nestel, 1974; Angelin *et al*, 1978).

The direct ability of BAs to modify gene expression by activating FXRα can explain these effects. Indeed, FXRα was shown to induce human and rodent apolipoprotein C-II (apoC-II) expression (Kast *et al*, 2001). ApoC-II is a coactivator of lipoprotein lipase and its induction lowers serum triglycerides. During fasting in rodents, FXRα mRNA levels are increased by two nuclear hormone receptors, peroxisome proliferator-activated receptor γ (PPARγ, NR1C3) and hepatic nuclear factor 4α (HNF4α, NR2A1) (Zhang *et al*, 2004). FXRα is furthermore coactivated by PPARγ coactivator-1α (PGC-1α) in a ligand-dependent or -independent way (Kanaya *et al*, 2004; Zhang *et al*, 2004; Savkur *et al*, 2005). PGC-1α is an important activator of hepatic gluconeogenesis, whereas in other tissues such as muscle and brown adipose tissue (BAT) it induces mitochondrial biogenesis and thermogenesis. These authors speculated that, during fasting, FXRα and PGC-1α cooperate to maintain energy homeostasis by decreasing serum TG levels via effects on VLDL clearance (Zhang *et al*, 2004).

We have shown that the induction of SHP via FXRα activation, which causes feedback inhibition of BA synthesis, also underlies the feedback regulation of hepatic fatty acid

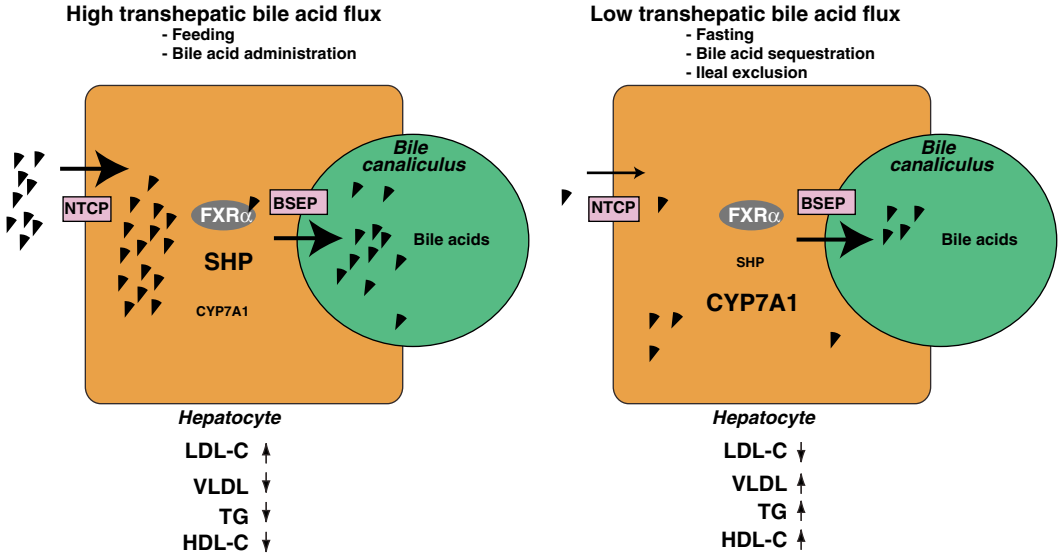

Figure 2 Respective situations that cause high (left panel) and low (right) transhepatic flux of BAs and their principle functional consequences.

and triglyceride biosynthesis and VLDL production (Watanabe *et al*, 2004). In fact, SHP interferes with the expression of sterol regulatory element-binding protein-1c (SREBP-1c). SREBPs are transcription factors that are activated by proteolytic processing and are mainly known for their control of genes involved in cholesterol homeostasis, effects mainly mediated by SREBP-2 (Horton *et al*, 2002). SREBP-1c is the master regulator of fatty acid and triglyceride biosynthesis and controls the expression of genes involved in various aspects of lipogenesis, such as acetyl-CoA carboxylase 1 (ACC1), ACC2, fatty acid synthase, glucose-6-phosphate dehydrogenase, and malic enzyme (Horton *et al*, 2002). Importantly, expression of SREBP-1c is enhanced by insulin, which explains why insulin enhances the conversion of glucose to fatty acid (Foretz *et al*, 1999; Shimomura *et al*, 1999). Activation of LXRα and LXRβ by agonists has also been shown to induce SREBP-1c, accounting for the triglyceride-raising effects of LXR agonists (Repa *et al*, 2000; Schultz *et al*, 2000). We propose a mechanism by which SREBP-1c, LXRα, LRH-1, FXRα and SHP cooperate in regulating lipid homeostasis (Figure 3). SREBP-1c and CYP7A1 expression is elevated by oxysterol-induced LXRα activation in cooperation with LRH-1, which increases triglyceride and BA biosynthesis. FXRα-mediated induction of SHP interferes with the activity of LXRα and LRH-1 to induce SREBP-1c and CYP7A1, and hence will inhibit lipogenesis and BA biosynthesis.

SHP expression levels are controlled by FXRα and as a consequence positively correlated with transhepatic BA flux. Interruption of the enterohepatic circulation will decrease transhepatic BA flux and consequently decrease SHP expression and thus increase BA biosynthesis. An increase in the BA pool will have the opposite effect (Figure 2). Therefore, SHP seems to be a pivotal factor in linking BA pool size with triglyceride levels. Consistent with this, SHP expression levels will be inversely correlated with serum triglyceride levels, a prediction confirmed by several studies. Hypertriglyceridemia and mild obesity have been associated with mutations in the SHP gene in Japanese patients (Nishigori *et al*, 2001). Later studies confirmed that genetic variation in SHP can influence birth weight and BMI, although it seems not to be a common cause of obesity (Hung *et al*, 2003; Mitchell *et al*, 2003). In contrast to humans with mutations in the SHP gene, $SHP^{-/-}$ mice have no hypertriglyceridemia and obesity; however, the study of these mice did show that the profound lowering of hepatic triglycerides observed with a CA diet was significantly attenuated in the $SHP^{-/-}$ mice (Kerr *et al*, 2002; Wang *et al*, 2002). $FXR\alpha^{-/-}$ mice show increased serum and hepatic triglyceride levels due to an increase in the production of VLDL, which coincides with a decreased SHP expression (Sinal *et al*, 2000).

Several other genes, including apolipoproteins A-V, C-III, E, PPARα, syndecan-1, and VLDL receptor, that directly affect lipid and lipoprotein metabolism, have also been reported as FXRα target genes (reviewed in Claudel *et al* (2005) and references herein). Taken together, all these genes enhance triglyceride and VLDL metabolism and consequently lower serum VLDL and triglyceride levels, consistent with the described effect of BAs. In view of the atypical nature of the FXRα response elements identified in this set of genes, additional experiments are necessary to dissect whether they are physiologically relevant FXRα targets and to carefully define their exact contributions in the triglyceride-lowering effects of BAs.

BAs and energy homeostasis

BAs have been reported to inhibit diet-induced obesity and prevent the development of insulin resistance (Ikemoto *et al*, 1997), suggesting effects on energy homeostasis. We have recently shown that administration of BAs to mice increases energy expenditure in BAT, preventing obesity and insulin resistance (Figure 3; Watanabe *et al*, 2006). This metabolic effect of BAs is critically dependent on induction of the cAMP-dependent thyroid hormone activating enzyme type 2 iodothyronine deiodinase (D2), since it is lost in mice with a targeted disruption of D2 ($D2^{-/-}$). BA treatment of brown adipocytes, but also human skeletal myocytes, increases D2 activity and oxygen consumption. These effects

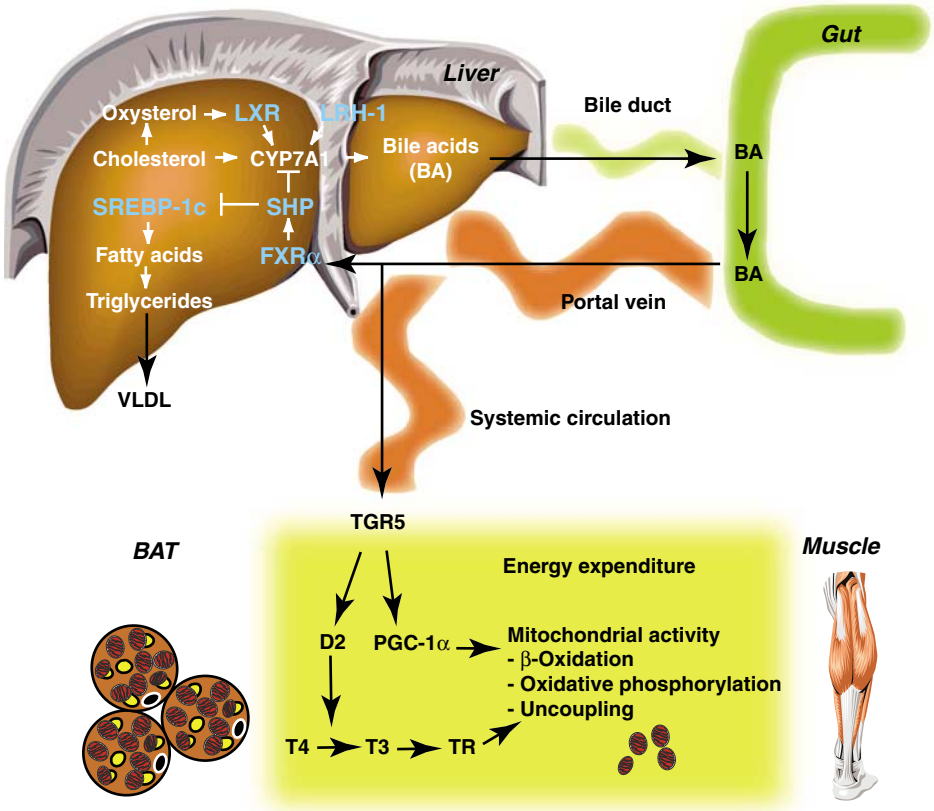

Figure 3 Schematic representation of the control of BAs on BA, triglyceride and energy homeostasis. TR, thyroid hormone receptor.

are independent of FXRα, and instead are mediated by increased cAMP production, that stems from the binding of BAs with the GPCR TGR5. TGR5 is a novel GPCR which responds to BAs by inducing receptor internalization, activation of MAPK pathways, and cAMP production (Maruyama *et al*, 2002; Kawamata *et al*, 2003). The most thermogenically important tissues of rodents (BAT) and humans (skeletal muscle) are specifically targeted by this mechanism because they coexpress D2 and TGR5 (Watanabe *et al*, 2006).

FXRα has been shown to regulate directly the expression of fibroblast growth factor-19 (FGF-19, mouse ortholog FGF-15). This secreted growth factor signals from the intestine to the liver through the cell-surface FGF receptor 4 (FGFR4) tyrosine kinase, resulting in an alternative pathway that potently suppresses CYP7A1 expression in a SHP-independent fashion (Figure 1B; Holt *et al*, 2003; Inagaki *et al*, 2005). Interestingly, transgenic mice which overexpress human FGF-19 display improved metabolic rate and decreased adiposity. This was attributed to increased BAT mass and enhanced liver β-oxidation due to decreased ACC2 expression (Tomlinson *et al*, 2002). A decreased ACC2 expression reduces the levels of malonyl-CoA that inhibit carnitine palmitoyl transferase 1 enzyme activity, the rate-limiting enzyme involved in the import of fatty acids into the mitochondrial matrix prior to their β-oxidation. Thus, the FXRα-mediated FGF-19 induction could also signal in an endocrine manner, mediating in part the effects of BAs on energy homeostasis.

SHP was recently also reported to inhibit PGC-1α expression and energy production in BAT, as concluded from the resistance of $SHP^{-/-}$ mice to diet-induced obesity (Wang *et al*, 2005). Further studies are, however, required to define whether this effect is directly due to the absence of SHP in BAT in the $SHP^{-/-}$ mice (normally SHP is expressed to very low levels in BAT) or perhaps to an indirect mechanism, subsequent to altered whole body BA homeostasis. It is expected that the characterization of tissue-specific SHP deficient animals will shed light on this issue in the near future.

BAs and glucose metabolism

An assessment of the efficacy and tolerability of cholestyramine in patients with dyslipidemia and type 2 diabetes, unexpectedly revealed that glycemic control was also improved with cholestyramine therapy (Garg and Grundy, 1994). Recently several studies addressed an eventual role of BAs in glucose metabolism, however, the contribution of FXRα in mediating the effects of BAs on glucose homeostasis is still highly debated. Whereas hepatic FXRα expression was reported to be increased by fasting, suggesting an inhibitory role of insulin levels on FXRα gene expression (Zhang *et al*, 2004), another study indicated that FXRα levels are decreased in animal models of diabetes and could be normalized by insulin addition (Duran-Sandoval *et al*, 2004). The role of FXRα in the regulation of gluconeogenesis is also unclear at present. BAs have been reported to inhibit gluconeogenesis via downregulation of phosphoenolpyruvate carboxykinase (PEPCK) mRNA levels in a FXRα-dependent and -independent manner (De Fabiani *et al*, 2003; Yamagata *et al*, 2004). In another paper, however, FXRα activation was shown to activate PEPCK via a mechanism that involved induction of PPARα and TRB3 (Stayrook *et al*, 2005). TRB3 is a fasting

inducible inhibitor which prevents activation of the serine/threonine kinase Akt/PKB and mediates activation of gluconeogenesis during fasting and insulin resistance (Koo *et al*, 2004). Two recent studies indicated that FXRα activation might increase hepatic glycogen levels (Duran-Sandoval *et al*, 2005; Zhang *et al*, 2006). The control of FXRα on whole body glucose homeostasis, however, seems rather small and is limited to specific timepoints in the fasting/(re)feeding cycle.

The concept that the impact of BAs on whole body glucose homeostasis is indirect and the consequence of their major effects on body weight has gained support recently. In fact, via activation of the TGR5-D2 signaling pathway, BAs robustly increase mitochondrial activity and oxidative phosphorylation in BAT (in rodents) and skeletal muscle cells (in humans), which was associated with a remarkable insulin sensitization both in genetic and diet-induced models of diabesity (Watanabe *et al*, 2006). The effects of BAs on FGF-19 production could also contribute to enhanced mitochondrial activity (Tomlinson *et al*, 2002). In line with this, a number of independent and converging observations in animal models and humans have linked reduced mitochondrial function and oxidative phosphorylation with the development of type 2 diabetes (Lowell and Shulman, 2005). Finally, activation of TGR5 was also shown to induce the production of glucagon-like peptide-1, *in vitro* which improves insulin secretion, and also via this way could ameliorate whole body glucose homeostasis (Katsuma *et al*, 2005). This effect warrants further investigation *in vivo*.

BA signaling pathways as therapeutic targets to treat metabolic disease

As described in the previous sections, BAs have evolved over the last years from regulators of BA homeostasis to general metabolic integrators. It is therefore not too surprising that a number of BA-activated signaling pathways have become attractive targets in our fight against common metabolic diseases, such as obesity, type 2 diabetes, hyperlipidemia, and atherosclerosis.

FXRα agonists

GW4064, the first reported high-affinity synthetic nonsteroidal FXRα agonist, was identified using high-throughput screening and combinatorial chemistry. GW4064 protects against cholestatic liver damage (Liu *et al*, 2003) and cholesterol gallstone disease (Moschetta *et al*, 2004), and lowers also serum triglyceride levels in KK-A^y and *ob/ob* mice (Watanabe *et al*, 2004) and in Fisher rats (Maloney *et al*, 2000). 6α-ethyl-CDCA (6-ECDCA), a high affinity steroidal FXRα agonist, has anticholestatic properties and protects the liver against LCA-induced necrosis and fibrosis (Pellicciari *et al*, 2005).

FXR antagonists and modulators

Guggulsterone, fexaramine and AGN34 are gene-selective FXRα modulators, which means that they can affect the expression of various FXRα target genes in different ways (antagonists or agonists) (Cui *et al*, 2003; Downes *et al*, 2003; Dussault *et al*, 2003). This phenomenon offers the possibility to develop FXRα modulators in which therapeutic effects are separated from undesired side effects. Most data are available for guggulipid, a plant extract containing the sterol guggulsterone, which is used to lower LDL cholesterol. This beneficial effect was attributed to its FXRα antagonizing activities (Urizar *et al*, 2002; Wu *et al*, 2002). In a recent clinical trial, however, guggulipid slightly but significantly increased LDL cholesterol (Szapary *et al*, 2003). In addition, guggulsterone lacks specificity for FXRα, since it interacts with numerous other nuclear receptors ((Meyer *et al*, 2005) and references herein).

TGR5 agonists

Activation of TGR5 induces energy expenditure *in vivo* and in cultured cells (Watanabe *et al*, 2006) and increases GLP-1 expression in cell lines (Katsuma *et al*, 2005). Due to these properties, synthetic TGR5 agonists could curb weight gain and improve glucose homeostasis, as claimed in the recent patent literature (Hinuma *et al*, 2005). Further *in vivo* work will be required to test whether these pharmacological compounds have the same efficacy as BAs, natural TGR5 agonists.

BAs as endocrine signaling factors, perspectives for the future

It is clear that BAs can affect metabolism, one question that remains is under which physiological conditions BAs exert this function. For this, it might be helpful to recapitulate where and when BAs can be found. BAs are together with other metabolites secreted from hepatocytes into the bile canaliculi. Usually bile is stored in the gallbladder, however, when a meal is ingested, it flows into the duodenum. The BAs are absorbed again by passive diffusion and active transport from the terminal ileum, and transported back to the liver via the portal vein, which completes their enterohepatic recirculation (Cohen, 2003). The first pass extraction of BAs by the liver is remarkably efficient (70–90%). Thus after a meal, BA levels in the hepatocyte will increase. The hepatic extraction rate of BAs usually remains constant during the fasting state and during digestion (Cohen, 2003). Consequently, a significant amount of BAs can spill over into the systemic circulation. Therefore, after a meal, BA levels will not only increase in the portal vein and the liver but also in the systemic circulation (Ho, 1976; Engelking *et al*, 1980). Indeed fasting serum BAs are usually below 5μM, whereas postprandial levels rise up to 15 μM (Everson, 1987). As a consequence of this phenomenon, serum BA levels vary during the day following a rhythm dictated by the ingestion of meals.

Based on these physiological observations it makes sense that BAs will exert their signaling functions mainly during feeding and active digestion. Under these conditions BAs are present in the intestine, return to the liver and spill over in the systemic circulation. BAs could hence be a signal to the liver and other organs that a meal has been ingested and that nutrients such as triglycerides will become available. BAs will then elicit a physiological response that is composite of FXRα-dependent (e.g. increased secretion of FGF-19) and -independent signaling (e.g. activation of the GPCR TGR5). A good example of such composite and converging signaling activities is given by the fact that BAs decrease hepatic VLDL production via the induction of the FXR–SHP–SREBP-1c pathway (Watanabe *et al*, 2004) and increase the (extra-)hepatic metabolism of VLDL and fatty acids through stimu-

lation of β-oxidation via activation of the TGR5–cAMP–D2 pathway (Figure 3) (Watanabe *et al*, 2006).

This new hormonal signaling role for BAs also forces us to think about the potential role of FXRα in non-enterohepatic tissues. Indeed, FXRα is also expressed at a high level in the kidney and the adrenal cortex (Forman *et al*, 1995). However, to activate FXRα, an intracellular receptor, BAs need specific transporters such as OSTα/β to cross the cell membrane in these tissues (Lee *et al*, 2006). Since evidence of such transport has not been demonstrated *in vivo*, one needs to consider the existence of novel natural FXRα agonists in these tissues.

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