MHR

S. KHURANA, ET AL. [2006] MED HYPOTHESES RES 3: 651-663.

STEROID RING HYDROXYLATION AND CONJUGATION MODULATE BILE ACID-MEDIATED CELLULAR SIGNALING: MOLECULAR BASIS OF HORMONE-LIKE BEHAVIOR

SANDEEP KHURANA, KUNRONG CHENG AND JEAN-PIERRE RAUFMAN*

DIVISION OF GASTROENTEROLOGY AND HEPATOLOGY, VA MARYLAND HEALTH CARE SYSTEM, UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE, BALTIMORE, MD 21201, USA.

REVIEW ◇ HYPOTHESIS

ABSTRACT. BEYOND THEIR KNOWN FUNCTIONS of facilitating cholesterol excretion and lipid absorption, recent studies indicate that bile acids play an important role as cell membrane and nuclear receptor ligands that alter post-receptor signaling and cell function. These findings support the novel hypothesis that bile acids are important cellular signaling molecules. Bile acid structure modifications, particularly steroid nucleus hydroxylation and conjugation, result in conformational changes that alter molecular charge and solubility. These structural characteristics determine the interaction of bile acids with cellular receptors, their access to different cellular compartments, and their interaction with subcellular components. Unconjugated hydrophobic bile acids interact primarily with death receptor pathways to induce apoptosis in hepatobiliary and colonic epithelial cells. In contrast, more soluble conjugated bile acids act as cell membrane receptor ligands that initiate post-receptor intracellular cell signaling and alter cell function. Experimental evidence indicates that conjugated secondary bile acids activate several plasma membrane receptors, including G-protein-coupled receptors and receptor tyrosine kinases. These functional interactions with cell membrane receptors on gastric chief cells, colon cancer cells and vascular endothelial cells stimulate pepsinogen secretion, colon cancer cell proliferation and vasorelaxation, respectively. As reviewed here, cellular signaling actions of bile acids depend on at least three major variables: (i) hydroxylation and conjugation of the steroid nucleus; (ii) cellular expression of relevant plasma membrane and nuclear receptors; and (iii) target cell exposure to effective bile acid concentrations. Further elucidation of molecular mechanisms underlying the cellular signaling actions of bile acids will identify new therapeutic targets for common disorders.

*ADDRESS ALL CORRESPONDENCE TO: JEAN-PIERRE RAUFMAN, M.D., DIVISION OF GASTROENTEROLOGY AND HEPATOLOGY, UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE, 22 SOUTH GREENE STREET, N3W62, BALTIMORE, MD 21201. PHONE: 410-328-1895. FAX: 410-328-8315. E-MAIL: jraufman@medicine.umaryland.edu

• MEDICAL HYPOTHESES AND RESEARCH • THE JOURNAL FOR INNOVATIVE IDEAS IN BIOMEDICAL RESEARCH •

1. INTRODUCTION

Bile acids[1] are involved in a myriad of important physiological processes that include but are not limited to bile excretion, cholesterol metabolism and elimination, and lipid and nutrient absorption. Insight into the role of bile acids in health and disease continues to expand. Bile acids, synthesized by the liver as by-products of cholesterol metabolism, are secreted into the bile canaliculus where they stimulate bile flow. This process contributes to the elimination of toxic molecules including bilirubin and xenobiotics. By regulating expression of membrane transporters located on hepatocyte canalicular and basolateral membranes, bile acids regulate cholesterol metabolism. In the biliary tree, bile acids promote cholesterol solubility and stimulate gallbladder epithelium to secrete mucin, thus reducing the likelihood of gallstone formation [2]. In the small intestine, bile acids facilitate digestion, particularly with regard to absorption of lipids and fat-soluble vitamins and nutrients. Most bile acids released in the small intestine are reabsorbed in the terminal ileum and transported back to the liver. This enterohepatic circulation allows the liver to regulate production of bile acids via a feedback mechanism and to maintain the bile acid pool [3]. A small proportion of bile acids (1-2%) that is not reabsorbed in the ileum passes into the colon.

In last decade, several investigations expanded the biological role of bile acids to include cell signaling. It is now apparent that bile acids are natural ligands for cell membrane and nuclear receptors [4,5]. Moreover, the signaling actions of bile acids vary depending on their structural modification and on the cell type examined. A pattern of bioactivity has emerged indicating that lipophilic dihydroxy and trihydroxy bile acids have pro-apoptotic actions and more water-soluble glycine and taurine conjugates of these molecules tend to have anti-apoptotic or pro-proliferative actions. Here, we review the structural modifications that modulate bile acid interaction with both cell membrane and nuclear receptors. These observations support the hypothesis that bile acid structural modifications and their consequent interaction with cellular receptors alter post-receptor signaling and cell function, thereby playing an important regulatory role in health and disease.

[1] The term *bile acid* refers to the protonated form and *bile salt* the ionized form. In this report as is common in the literature, these terms are used interchangeably. Bile acid nomenclature conforms to recommendations by Hofmann et al. [1].

2. BILE ACID STRUCTURE

Bile acids consist of four fused rings, referred to chemically as a cylcopentanophenanthrene or steroid nucleus [1]. Three of these four rings are 6-sided (labeled A-C in FIG. 1) and one is 5-sided (labeled D in FIG. 1). Carbon atom numbering is shown in FIG. 1. In humans, most bile acids have 24 carbon atoms (cholanoic acids). In C-24 bile acids, a 5-carbon aliphatic side chain, which ends in carboxylic acid, is attached at the 17β-position of the D ring. Based on the orientation of the proton at the C-5 position at the junction of rings A and B, bile acids are divided into two groups. The C-5 proton in *trans* with respect to the methyl group at C-19 renders the bile acid in a 5α-configuration with all four rings in the same plane. However, the C-5 proton in *cis* to the methyl group at C-19 renders the bile acid into a 5β-configuration with ring A at an approximate right angle to the plane of the other rings. In humans, bile acids are in the 5β-configuration.

Primary bile acids, namely, cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid), are synthesized *de novo* exclusively by the liver. When primary bile acids are released into the small intestine, bacterial 7α-dehydroxylase converts a fraction of these molecules into secondary bile acids [deoxycholic acid (3α,12α-dihydroxy-5β-cholanoic acid) and lithocholic acid (3α-monohydroxy-5β-cholanoic acid)] [6] (FIG. 2). Bile acids that undergo further hepatic and bacterial modification, such as ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholanoic acid), are known as tertiary bile acids. In adults, primary and secondary bile acids collectively constitute more

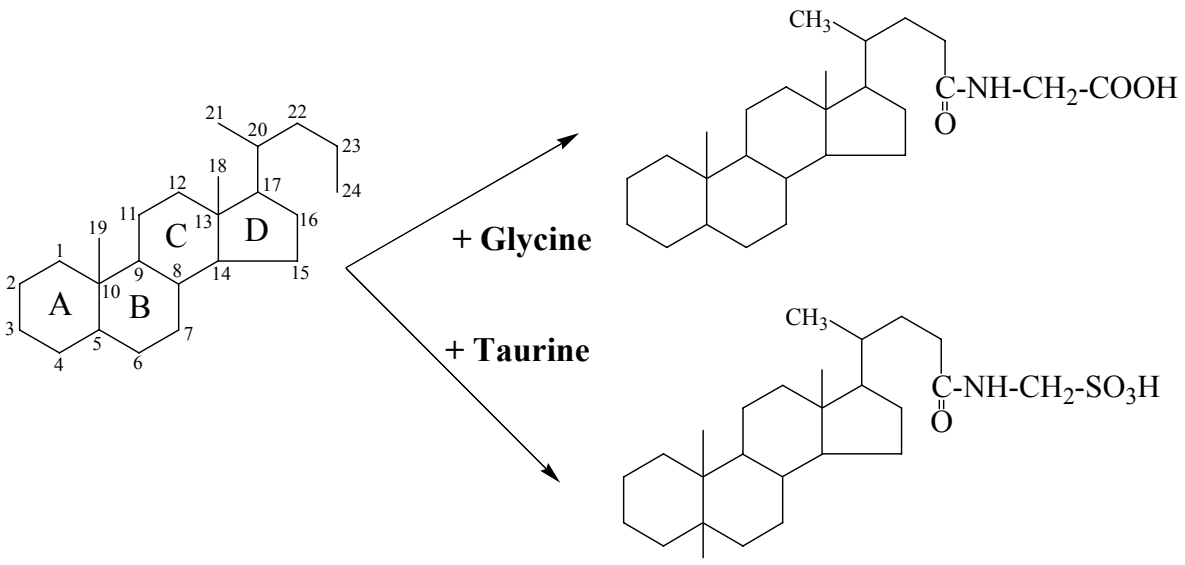

FIGURE 1. GENERAL STRUCTURE FOR NATIVE BILE ACIDS AND FOR GLYCINE AND TAURINE CONJUGATES. Steroid nucleus and conjugate structure, and ring and carbon atom lettering and numbering, respectively, are shown.

than 95% of the bile acid pool.

Before their secretion into bile, *N*-aminoacyl conjugation occurs between the carboxyl group of the bile acid and the amino group of glycine or taurine (FIG. 1). In adults, the ratio of glycine to taurine conjugates is normally 3:1. Conjugation increases bile acid solubility; more for taurine than for glycine conjugates [5]. Hepatocytes are not capable of synthesizing taurine from other sulfur-containing amino acids. Hence, dietary intake of this amino acid dictates the amount of taurine-conjugated bile acids. In the small intestine and colon, a portion of bile acids is deconjugated by bacterial enzymes, absorbed actively and returned to the liver for reconjugation.

3. BILE ACID SYNTHESIS

3.1. CHOLESTEROL 7α-HYDROXYLASE PATHWAY

Cholesterol is the primary substrate for bile acid synthesis by either the 'neutral' or 'acidic pathways' (reviewed in [6]). The neutral pathway, also known as the 'cholesterol 7α-hydroxylase pathway', is mediated by the first rate-limiting, extensively regulated 7α-hydroxylase, a microsomal cytochrome P450 (CYP) enzyme, CYP7A1, expressed exclusively in hepatocytes. In the second step, catalyzed by 3β-hydroxy-Δ5C_{27}-steroid oxidoreductase, the 3β-hydroxyl group is oxidized leading to formation of 7α-hydroxy-4-cholesten-3-one, the precursor of cholic and chenodeoxycholic acids. Microsomal sterol 12α-hydroxylase, CYP8B1, converts 7α-hydroxy-4-cholesten-3-one to 7α,12α-dihydroxy-4-cholesten-3-one. Cytosolic 3-oxosteroid-5β-reductase and 3α-hydroxysteroid dehydrogenase convert 7α,12α-dihydroxy-4-cholesten-3-one into 5β-cholestane 3α,7α,12α-triol which is a precursor of cholic acid, or alternatively into 5β-cholestane 3α,7α,-diol, a precursor of deoxycholic acid. These compounds are subsequently converted by a mitochondrial sterol 27-hydroxylase, CYP27A1, into 5β-cholestane 3α,7α,12α,27-tetrol and 5β-cholestane 3α,7α,27-triol. The final steps occur in peroxisomes yielding C-24 hydroxylated bile acid intermediates. Side chain oxidation and thiolytic cleavage forms cholic or chenodeoxycholic acids, which are then conju-

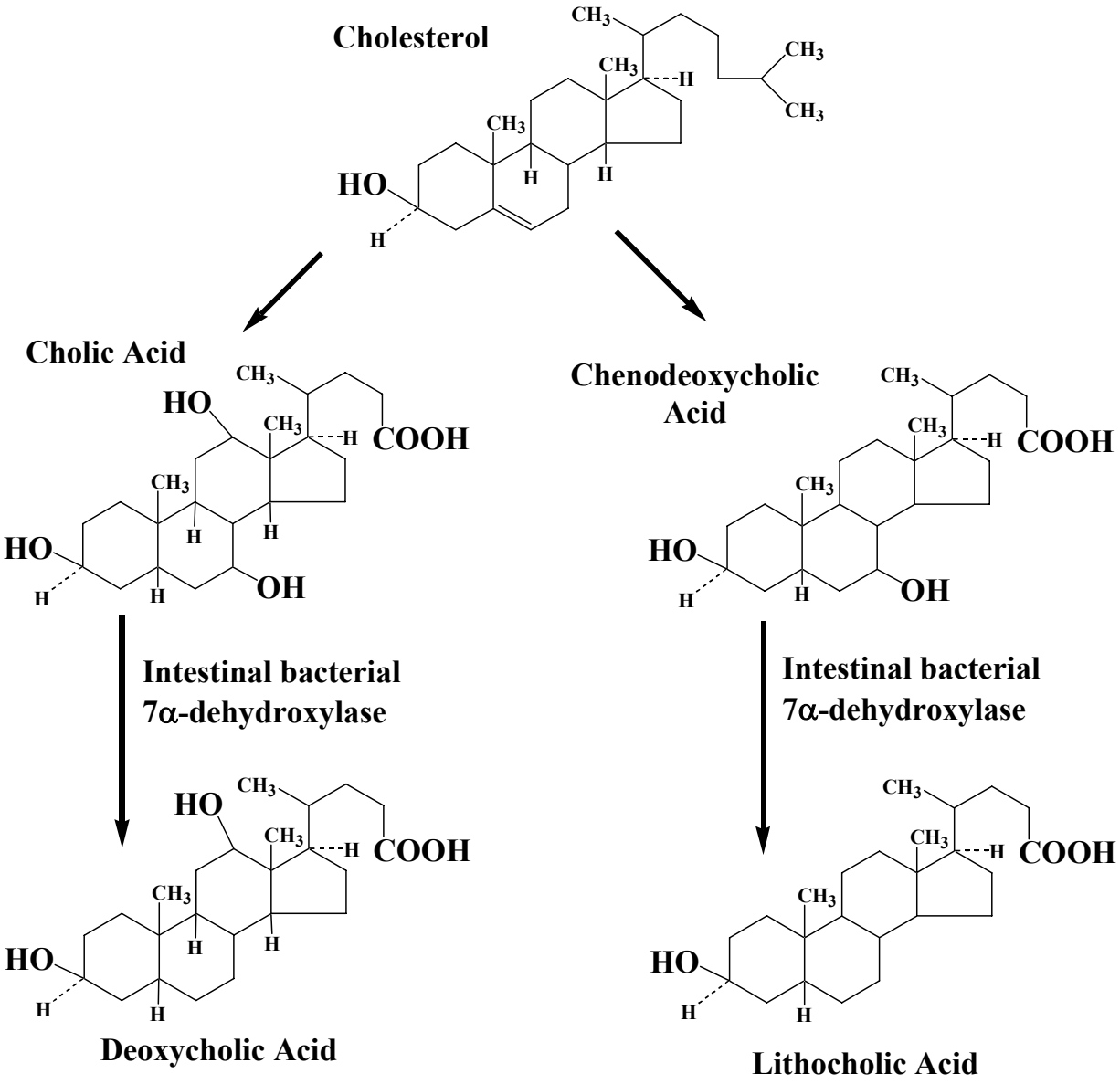

FIGURE 2. DERIVATION OF MAJOR HUMAN BILE ACIDS. This general scheme shows the major bile acid synthetic pathways from cholesterol. The bile acid -COOH and -OH groups are shown in boldface.

gated with glycine or taurine.

3.2. STEROL 27- HYDROXYLASE PATHWAY

In the sterol 27-hydroxylase pathway, commonly referred to as the 'acidic pathway', oxidative cleavage of the side chain precedes modification of the steroid nucleus. Chenodeoxycholic acid is the main product of this pathway. The initial step is catalyzed by CYP27A1. Synthesis of bile acids from extrahepatic cholesterol by this pathway may contribute up to 50% of the bile acid pool.

3.3. CHOLESTEROL 25- HYDROXYLASE PATHWAY

Cholesterol 25-hydroxylase is a minor biosynthetic pathway. The key enzyme in this pathway, CYP7B1, produces 7α-hydroxylated oxysterols that are channeled into bile acid synthesis. Only 5–16% of total bile acids are synthesized via this pathway.

4. NUCLEAR RECEPTORS: REGULATORS OF BILE ACID SYNTHESIS

Nuclear receptors are ligand-activated transcription factors that regulate gene function. Nuclear receptors with no identifiable ligands are referred to as orphan receptors. Because typical nuclear receptor ligands are small hydrophobic molecules, bile acids are excellent candidate nuclear receptor ligands. In 1999, the farnesoid X receptor (FXR), a previously cloned orphan nuclear receptor, was identified as the first bile acid nuclear receptor [7,8]. Three additional bile acid nuclear receptors were identified; rodent xenobiotic receptor (PXR) [9], its analog human xenobiotic receptor (SXR), and the vitamin D receptor (VDR).

FXR alters bile acid synthesis by influencing various steps in cholesterol metabolism and bile acid excretion. Deoxycholic and chenodeoxycholic acids are FXR ligands. $FXR^{-/-}$ mice have increased expression of CYP7A1, indicating that FXR regulates the neutral pathway of bile acid synthesis [10]. FXR also regulates bile acid excretion by altering expression of hepatocyte canalicular and basolateral membrane bile acid transporters. Total serum bile acid concentration in $FXR^{-/-}$ mice is 8-fold greater than in wildtype mice, and increases to 23-fold when the animals are fed 1% cholic acid. In contrast to wildtype animals, feeding 1% cholic acid to $FXR^{-/-}$ mice, does not reduce hepatocyte expression of CYP7A1 or Ntcp (Na-dependent taurocholate cotransporter), a hepatocyte basolateral transporter. Bsep, a major canalicular bile acid transport pump is expressed at much lower levels in $FXR^{-/-}$ mice. With a 1% cholic acid diet, Bsep expression remained unchanged in the livers of $FXR^{-/-}$ mice whereas it increases in wild-type mice. In wildtype mice, feeding a synthetic FXR ligand, GW4064, leads to increased hepatic expression of Bsep and Mdr2 (bile acid and phospholipid transporters, respectively) and decreased expression of CYP7A1 and CYP8B1 [11]. These findings indicate that FXR negatively regulates CYP7A1 function, inhibits Ntcp, and induces Bsep and phospholipid transport proteins.

The pregnane X (PXR) receptor is activated by variety of compounds such as steroids, xenobiotics and drugs [12]. In rat liver, PXR induces expression of CYP3A4 but inhibits CYP7A1. Lithocholic acid and 3-keto-lithocholic acid bind to and activate PXR. Thus, it is hypothesized that PXR inhibits bile acid synthesis and stimulates CYP3A4 to detoxify LCA.

The VDR is activated by lithocholic acid, thereby inducing CYP3A4, which detoxifies lithocholic acid in the liver and intestines. Lithocolic and 3-keto-lithocholic acids are the only bile acids that bind and activate VDR.

5. BILE ACIDS ACTIVATE INTRACELLULAR SIGNALING AND ALTER CELL FUNCTION

5.1. BILE ACID INTERACTION WITH PLASMA MEMBRANE G-PROTEIN COUPLED RECEPTORS

Experiments conducted in our laboratory indicated that conjugated secondary bile acids are muscarinic receptor ligands. In guinea pig gastric chief cells, lithocholyltaurine (LCT) caused dose-dependent increases in pepsinogen secretion and inositol phosphate (IP) production that was inhibited by atropine [13]. Increasing concentrations of LCT diminished pepsinogen secretion stimulated by carbachol, a more efficacious and potent cholinergic agonist, and caused dose-dependent inhibition of muscarinic radioligand binding. In CHO cells expressing rat M_3 muscarinic receptors (CHO-rM_3R), increasing concentrations of both LCT and deoxycholyltaurine (DCT), caused dose-dependent inhibition of muscarinic radioligand binding and muscarinic antagonists (atropine and N-methylscopolamine) inhibited LCT-induced IP formation [14]. DCT reduced acetylcholine-

induced IP formation and mitogen activated protein (MAP) kinase phosphorylation [15].

5.2. ROLE OF BILE ACIDS IN APOPTOSIS

Apoptosis is initiated by ligand-dependent or independent activation and oligomerization of cell surface death receptors, recruitment of a Fas-associated DD (FADD) protein, activation of procaspase-8 and -10, thereby resulting in formation of a death-inducing signaling complex (DISC) [16-18]. Cytotoxic signaling downstream of DISC differs based on cell type; Type I when activated initiator caspases cleave and activate effector caspases-3, -6, and -7 to execute cell death, or Type II when activation of effector caspases requires mitochondrial amplification. Recent research suggests that glycochenodeoxycholic acid (GCDA) induces death receptor-dependent hepatocyte apoptosis, promoting plasma membrane Fas aggregation in a Fas ligand-independent manner by altering cellular trafficking of this death receptor and increasing its cell surface density [19]. In hepatocytes, GCDA also enhances initiator caspase activation and augments mitochondrial cytochrome c release, thereby amplifying the caspase activation cascade [19].

To determine whether bile acid hydrophobicity predicts the ability to induce apoptosis in HCT116 colon cancer cells, Martinez and colleagues investigated the actions of a relatively high concentration (500 μM) of 26 conjugated and unconjugated bile acids [20]. A 12-hr treatment with ursodeoxycholic and deoxycholic acid analogues suppressed cell proliferation and extended exposure resulted in apoptosis. Deoxycholic and chenodeoxycholic acids induced apoptosis without first causing growth arrest. Glycine-conjugated deoxycholic and ursodeoxycholic acids caused growth arrest but not apoptosis. These data indicate that the ability of bile acids to induce apoptosis increases with their hydrophobicity. Likewise, Martinez-Diez, et al. demonstrated that conjugated bile acids are less likely to reduce cell viability when compared with the unconjugated parent molecules [21]. Sclottmann and colleagues reported that deoxycholic and chenodeoxycholic acids cause concentration- and time-dependent apoptosis in SW480 and HT-29 colon cancer cells [22]. In SW480 cells, deoxycholic acid-induced apoptosis was associated with activation of caspases-2, -3, -7 and -8. However, conjugated bile acids and a trihydroxy bile acid (cholic acid) did not induce apoptosis. These data support the concept that bile acid solubility is inversely related to the ability to induce apoptosis.

5.3. ROLE OF BILE ACIDS IN MUSCARINIC RECEPTOR-MEDIATED COLON CANCER CELL PROLIFERATION

For more than three decades, epidemiological and animal studies have supported an association between changes in fecal bile acids and the development of cancer of the proximal colon. Nonetheless, the molecular mechanism for this association has been elusive.

Colon epithelial cells express and most colon cancers over-express M_3 muscarinic receptors (M_3R) [23,24]. In H508 colon cancer cells, acetylcholine activates the p44/42 MAP kinase (Erk 1/2) signaling pathway and stimulates cell proliferation. Muscarinic agonists do not alter proliferation of SNU-C4 colon cancer cells that do not express M_3R. These data indicate that M_3R are involved in cell proliferation and possibly promote colon cancer growth. Additional work indicated that, as observed with other G-protein coupled receptors, muscarinic agonist-induced colon cancer cell proliferation is mediated by transactivation of epidermal growth factor receptors (EGFR) [25].

Bile acids mimic the signaling and proliferative actions of cholinergic agonists on colon cancer cells that express M_3R. Whereas deoxycholic acid caused a 15- to 43-fold increase in caspase-3 activity, a marker of apoptosis, up to 5-day incubation of H508 cells with conjugated bile acids did not increase caspase-3 activity. In fact, incubation of H508 cells with conjugated secondary bile acids activated p44/42 MAP kinase signaling and stimulated an increase in the proliferation of H508 colon cancer cells [25,26]. As observed with acetylcholine, the proliferative actions of conjugated bile acids are dependent on expression and activation of both M_3R and EGFR [25].

5.4. BILE ACIDS STIMULATE SYSTEMIC VASODILATION

Bile acids are implicated in the modulation of vascular tone, particularly in reducing systemic vascular resistance in end-stage liver disease. In bile duct ligated rats, Bomzon and colleagues demonstrated reduced contractile responses of vascular smooth muscle to noradrenaline [27]. In isolated rat mesentery preconstricted with an α_1-adrenergic receptor agonist, taurine-conjugated deoxycholic, chenodeoxycholic and ursodeoxycholic acids caused dose-dependent decreases in vascular smooth muscle tension [28]. We observed in phenylephrine-constricted rodent (rat and mouse) thoracic aortic rings that increasing doses of DCT caused a progressive decrease in vascular smooth muscle tension [29,30]. DCT-induced vasodilation was more pronounced in intact aortic segments compared to those denuded of endothelium. These findings indicate that DCT-induced decreases in vascular smooth muscle tension are endothelium-dependent.

Nitric oxide (NO), a potent endothelium-derived vasodilator, formed by endothelial NO synthase (eNOS), diffuses into the vascular smooth muscle cells where it stimulates the formation of cyclic GMP, calcium influx and smooth muscle relaxation. DCT-induced vasodilation was attenuated in rodent aortic segments with intact endothelium incubated with an eNOS inhibitor [30]. These findings indicate that in rodent thoracic aorta, DCT-induced endothelium-dependent vasodilation is

TABLE 1. CELLULAR EXPRESSION OF NUCLEAR AND PLASMA MEMBRANE RECEPTORS REPORTED TO INTERACT FUNCTIONALLY WITH BILE ACID LIGANDS.

RECEPTORS ACTIVATED	BILE ACID LIGANDS	CELL TYPES	REFERENCES
NUCLEAR RECEPTORS			
FXR	Conjugated and unconjugated cholic, chenodeoxycholic, lithocholic and deoxycholic acids	Hepatocytes Intestinal epithelial cells Vascular smooth muscle cells Cardiomyocytes Renal cells Cancers	[11,42,45,46]
SXR/PXR	Conjugated deoxycholic and lithocholic acids	Hepatocytes	[47]
VDR	Lithocholic and keto-lithocholic acids	Hepatocytes Enterocytes	[48]
PLASMA MEMBRANE RECEPTORS			
TRAIL-R2/DR5	Chenodeoxycholylglycine	Hepatocytes	[49]
Fas	Chenodeoxycholylglycine	Hepatocytes	[19]
G-PROTEIN-COUPLED RECEPTORS			
M_3R	Conjugated lithocholic and deoxycholic acids	Gastric chief cells Colon cancer cells	[13,26]
TGR5	Conjugated and unconjugated cholic, chenodeoxycholic, lithocholic and deoxycholic acids	Monocytes/macrophages Intestinal cell lines Enteroendocrine cells	[35-38]
RECEPTOR TYROSINE KINASES			
EGFR	Conjugated deoxycholic acid Deoxycholic and chenodeoxycholic acids	Colon cancer cells Cholangiocytes	[25] [32]
Insulin	Deoxycholic acid	Hepatocytes	[39]

mediated by NO.

In aortic segments from $M_3R^{-/-}$ mice, acetylcholine- and DCT-induced vasodilation is reduced in comparison to effects in wildtype animals [30]. These data suggest that in the systemic circulation bile acids interact with vascular endothelial cell membrane receptors, thereby stimulating vasodilation by an NO-dependent mechanism. Since end-stage liver disease is associated with the formation of multiple collateral vessels that lead to the shunting of the blood from the portal to the systemic circulation, thereby elevating bile acids levels in the systemic circulation, we hypothesize that secondary conjugated bile acids play an important role in reducing systemic vascular resistance.

6. STRUCTURAL DETERMINANTS OF BILE ACID SIGNALING

As shown in TABLE 1, the spectrum and complexity of bile acid interaction with plasma membrane or nuclear receptors depends on at least three major factors; steroid nucleus hydroxylation, steroid nucleus conjugation, and target receptor expression. It is evident that these factors depend on the expression of a number of mammalian and bacterial genes that regulate biochemical transformations underlying bile acid synthesis and post-synthetic molecular modifications, as well as those that regulate target receptor expression. As with any mammalian signaling system, this multitude of regulatory steps allows for fine-tuning, amplification, and precise cellular targeting of the signal. The presence of bile acids in amphibians, reptiles, fish, and birds speaks to the antiquity of this signaling system [31].

6.1. STEROID NUCLEUS HYDROXYLATION

As illustrated in FIG. 2, primary and secondary bile acids vary in the number of hydroxyl groups attached to the steroid nucleus. Cholic acid has three hydroxyl groups, chenodeoxycholic and deoxycholic acids have two hydroxyl groups, and lithocholic acid has one hydroxyl group attached to the steroid nucleus.

Nuclear receptors are activated primarily by mono- and di-hydroxylated bile acids. FXR is activated by di-hydroxylated bile acids, thereby inhibiting CYP7A1, the rate-limiting enzyme involved in conversion of cholesterol to bile acids and thus suppressing bile acid synthesis. SXR and PXR are activated to a lesser extent by di-hydroxylated bile acids. Mono-hydroxylated bile acids activate PXR, SXR and VDR. Activation of PXR/SXR results in induction of CYP3A, a cytochrome enzyme that detoxifies xenobiotics. Compared to dihydroxylated bile acids, mono-hydroxylated bile acid-induced activation of FXR is minimal.

Plasma membrane receptors are activated primarily by di-hydroxylated bile acids, but the effects vary depending on the cell type examined. In vascular endothelial cells, taurine-conjugated deoxycholic acid interacts with M_3R to cause nitric oxide-mediated vasodilation [30]. In cholangiocytes and colon cancer cells, deoxycholyltaurine activates EGFR and promotes cell proliferation [25, 32]. However, in colon cancer cells, this action appears to be mediated by interaction with M_3R, the release of an EGFR ligand, and consequent transactivation of EGFR [25]. In contrast, in hepatocytes, deoxycholic acid and conjugated chenodeoxycholic acid, another di-hydroxylated bile acid, are reported to promote Fas activation and apoptosis [33]. However, more recent studies indicate that in hepatocytes, taurine-conjugated cholic and deoxycholic acids stimulate p44/42 MAP kinase activation by a G-protein coupled receptor and EGFR-dependent mechanism [34]. The changes in hepatocyte function that result from these bile acid effects and the responsible G-protein coupled receptor have yet to be elucidated.

A novel G-protein-coupled receptor, designated TGR5, was described as a 'bile acid responsive receptor'. Surprisingly, all hydroxylated bile acids are reported to interact with TGR5 to promote intracellular cAMP production [35,36]. Moreover, the function of TGR5 activation is unknown although it may play a role in glucose homeostasis [37]. Recently, novel evidence was presented that interaction of serum bile acids with TGR5 may be important for the regulation of diet-induced thermogenesis [38]. In this context, it is of interest that

a previous study indicated that deoxycholic acid also enhances the activity of the insulin receptor [39].

With respect to non-apoptotic signaling, the findings described above indicate that conjugated di-hydroxylated bile acids are the most active ligands. These bile acids interact with various cell membrane receptors to modulate cell function, including secretion, cell proliferation and vasorelaxation [13,25,30]. The structural requirements for interaction of bile acids with TGR5 do not readily conform to this paradigm. However, currently information regarding TGR5 is based on limited data [35-38].

6.2. STEROID NUCLEUS CONJUGATION

Besides steroid nucleus hydroxylation, taurine and glycine conjugation modifies the interaction of bile acids with various receptors in different cell types.

With respect to hydroxylation, the interaction of bile acids with nuclear receptors described above is limited only to unconjugated bile acids. FXR is activated by unconjugated chenodeoxycholic and deoxycholic acids, whereas the conjugated forms of these bile acids activate FXR only in cells that co-express bile acid transporters. Unconjugated lithocholic, chenodeoxycholic and deoxycholic acids activate PXR and SXR, whereas the interaction of conjugated bile acids with these nuclear receptors is not clearly defined.

Conjugation alters the interaction of bile acids with plasma membrane receptors. As indicated in the steroid hydroxylation segments, taurine-conjugated lithocholic acid interacts with muscarinic receptors on gastric chief cells to promote inositol phosphate formation and stimulate pepsinogen secretion whereas the unconjugated bile acid does not induce these effects [13]. In colon cancer cells, unconjugated deoxycholic acid activates caspases and induces apoptosis, but taurine and glycine conjugates of the same bile acid interact with M_3R, transactivate EGFR and stimulate p44/42 MAP kinase signaling, thereby inducing cell proliferation [25]. In a similar manner, conjugation alters the signaling actions of bile acids on hepatocytes and liver cancer cells [19,34,40]. In human HuH-7 cells, the glycine conjugate of chenodeoxycholic acid enhances the expression of TRAIL-R2/DR5 and Fas to initiate death-signaling [19] whereas in rat McNtcp.24 cells, the taurine conjugate promotes cell survival via PI3K-dependent signaling [40]. The pattern of bile acid conjugation also alters the ability of these molecules to stimulate DNA synthesis in regenerating mouse liver [41]. The observation that conjugation of bile acids alters interaction with different cell types and that specific conjugation with either taurine or glycine further modulates bile acid actions on cell signaling has been noted by several groups [14,19,34,40].

6.3. TARGET RECEPTOR EXPRESSION

From the previous segments, it is apparent that in addition to structural modification of the steroid nucleus, the actions of bile acids also depend on the cell type examined and, in particular, the receptors expressed by these cells. Moreover, it is important to recognize that either physiological or pathological actions of bile acids on a particular cell type can only occur in a setting (organ) where that cell is exposed to biologically active concentrations of the relevant bile acid. For example, it is likely that bile acid effects on hepatoma or cholangiocarcinoma cells are important, since these cells are exposed to effective concentrations of bile acids. For cell types in other locations, the physiological relevance of bile acid interaction with cell receptors must be proved by showing that effective concentrations of the relevant bile acid are achieved in the organ of interest.

Nuclear bile acid receptors, including FXR, PXR, SXR and VDR, are expressed predominantly in hepatocytes and enterocytes, cells that are most involved in lipid and nutrient absorption and enterohepatic circulation. Recent investigation has revealed FXR expression in the vasculature, heart, kidney, lymph nodes and cancers, including metastatic bronchial adenocarcinoma and sarcomas [42]. The distribution of FXR across different cell lines is a new finding and the physiological and pathological implications are areas of future investiga-

tion.

As indicated above, bile acids interact with various cell surface receptors. M_3R are widely distributed in the gastrointestinal tract and vascular endothelium. The expression of muscarinic receptors in gastrointestinal epithelial cells and colon cancer cells that are continuously exposed to bile acids is the basis of our working hypothesis that interaction of bile acids with these receptors plays a regulatory role in colon cancer growth. In cirrhosis, systemic vascular endothelial cells are exposed to high levels of bile acids. Hence, bile acid interaction with vascular M_3R may play a role in mediating the hemodynamic changes associated with cirrhosis.

Our laboratory has shown that bile acids activate M_3R and EGFR, thereby inducing colon cancer cell proliferation. EGFR is expressed abundantly in the epithelial lining of all the hollow organs, including the entire gastrointestinal, respiratory, biliary and urinary tract, and by various cancers. Several investigators have shown that bile acid-induced activation of post-receptor signaling is associated with cell survival. Using a small intestinal epithelial cell line, Wang and colleagues showed that deoxycholyltaurine stimulates anti-apoptotic signaling, including activation of NF-κB [43,44]. These signaling mechanisms play an important role in mediating bile acid-induced cellular restitution following mucosal injury [45]. Collectively, these observations support the hypothesis that bile acids interact functionally with epithelial cells expressing EGFR and that this interaction stimulates cell proliferation. These effects are likely to be beneficial when cell proliferation is necessary to heal mucosal injury, but they are detrimental when they stimulate the growth of hepatobiliary or colon cancers.

7. CONCLUSIONS

The data reviewed here demonstrate that conjugated bile acids are receptor ligands that interact not only with nuclear receptors but also with cell surface receptors on gastric chief cells, colon cancer cells and vascular endothelial cells. Bile acid interactions with plasma membranes are associated with activation of post-receptor signaling and measurable changes in target organ function; pepsinogen secretion, cellular proliferation, and vasodilation, respectively. Hence, experimental data support our central hypothesis that bile acids play an important role as cell membrane receptor ligands that alter post-receptor signaling and cell function.

The pattern that emerges from these observations leads us to refine our hypothesis and suggest that, much like conventional peptide and steroid hormones, bile acids are signaling molecules whose actions are modulated by structural modifications. Whereas *unconjugated* bile acids are primarily pro-apoptotic, the finding that *conjugated bile* acids are primarily pro-proliferative and anti-apoptotic allows us to generate additional testable hypotheses regarding the interaction of bile acids with muscarinic and other plasma membrane receptors. These include, but are not limited, to the following:

- Bile acid-induced stimulation of gastric pepsinogen secretion *in vivo* may play a role in mediating the pathological findings associated with 'bile gastritis'.

- Bile acid-induced colon cancer cell proliferation may play an important promoting role in the growth of colonic neoplasms. This is particularly true in the proximal colon, where lesions are more likely exposed to concentrations of conjugated secondary bile acids that activate muscarinic receptors (Hamilton J, Xie G, Raufman J-P, et al, unpublished observations).

- Bile acid-induced NO release from aortic endothelial cells may play a role in stimulating angiogenesis and neovascularization. Moreover, end-stage liver disease is associated with decreased hepatic NO production and increased splanchnic and systemic circulation NO that correlates with a shift of the bile acid pool to the systemic circulation. Hence, bile acids may play an important role in regulating intrahepatic vascular and sinusoidal tone and reducing systemic vascular resistance.

As reviewed here, present evidence indicates that cellular actions of bile acids depend on three

major variables: hydroxylation and conjugation of the steroid nucleus, bile acid concentration, and cellular expression of relevant plasma membrane and nuclear receptors. Clearly, the potential role of bile acids in activating intracellular cell signaling, although compelling on the basis of the experiments outlined above, requires additional study. For example, the molecular mechanisms underlying bile acid-induced transactivation of receptor tyrosine kinases via M_3R activation requires exploration, as does the impact on cell cycle regulation. These issues can best be addressed by a combination of *in vitro* and *in vivo* approaches. Elucidation of the molecular mechanisms underlying the regulatory and signaling actions of bile acids will identify new therapeutic targets for common disorders.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Office of Research Development, Medical Research Service, Department of Veterans Affairs (J.P.R.) and by NIH grant CA107345 (J.P.R.).

REFERENCES

[1] HOFMANN AF, SJOVALL J, KURZ G, RADOMINSKA A, SCHTEINGART CD, TINT GS, VALACEVIC ZR AND SETCHELL DR [1992] A proposed nomenclature for bile acids. J Lipid Res 33: 599-604.

[2] KLINKSPOOR JH, TYTGAT GN, LEE SP AND GROEN AK [1996] Mechanism of bile salt-induced mucin secretion by cultured dog gallbladder epithelial cells. Biochem J 316 (Pt 3): 873-877.

[3] HOFMANN AF [1989] Current concepts of biliary secretion. Dig Dis Sci 34: 16S-20S.

[4] CHIANG JY [2003] Bile acid regulation of hepatic physiology: III. Bile acids and nuclear receptors. Am J Physiol Gastrointest Liver Physiol 284: G349-G356.

[5] RAUFMAN JP, CHENG K AND ZIMNIAK P [2003] Activation of muscarinic receptor signaling by bile acids: physiological and medical implications. Dig Dis Sci 48: 1431-1444.

[6] FUCHS M [2003] Bile acid regulation of hepatic physiology: III. Regulation of bile acid synthesis: past progress and future challenges. Am J Physiol Gastrointest Liver Physiol 284: G551-G557.

[7] PARKS DJ, BLANCHARD SG, BLEDSOE RK, CHANDRA G, CONSLER TG, KLIEWER SA, STIMMEL JB, WILLSON TM, ZAVACKI AM, MOORE DD AND LEHMANN JM [1999] Bile acids: Natural ligands for an orphan nuclear receptor. Science 284: 1365-1368.

[8] MAKISHIMA M, OKAMOTO AY, REPA JJ, TU H, LEARNED RM, LUK A, HULL MV, LUSTIG KD, MANGELSDORF DJ AND SHAN B [1999] Identification of a nuclear receptor for bile acids. Science 284: 1362-1365.

[9] ZHANG J, KUEHL P, GREEN ED, TOUCHMAN JW, WATKINS PB, DALY A, HALL SD, MAUREL P, RELLING M, BRIMER C, YASUDA K, WRIGHTON SA, HANCOCK M, KIM RB, STROM S, THUMMEL K, RUSSELL CG, HUDSON JR, SCHUETZ EG AND BOGUSKI MS [2001] The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. Pharmacogenetics 11: 555-572.

[10] Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G and Gonzalez FJ [2000] Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell 102: 731-744.

[11] MOSCHETTA A, BOOKOUT AL AND MANGELSDORF DJ [2004] Prevention of cholesterol gallstone disease by FXR agonists in a mouse model. Nat Med 10: 1352-1358.

[12] WATKINS RE, WISELY GB, MOORE LB, COLLINS JL, LAMBERT MH, WILLIAMS SP, WILLSON TM, KLIEWER SA AND REDINBO MR [2001] The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. Science 292: 2329-2333.

[13] RAUFMAN JP, ZIMNIAK P AND BARTOSZKO-MALIK A [1998] Lithocholyltaurine interacts with cholinergic receptors on dispersed chief cells from guinea pig stomach. Am J Physiol 274: G997-G1004.

[14] RAUFMAN JP, CHEN Y, CHENG K, COMPADRE C, COMPADRE L AND ZIMNIAK P [2002] Selective interaction of bile acids with muscarinic receptors: a case of molecular mimicry. Eur J Pharmacol 457: 77-84.

[15] RAUFMAN JP, CHEN Y, ZIMNIAK P AND CHENG K [2002] Deoxycholic acid conjugates are muscarinic cholinergic receptor antagonists. Pharmacology 65: 215-221.

[16] ARAGANE Y, KULMS D, METZE D, WILKES G, POPPELMANN B, LUGER TA AND SCHWARZ T [1998] Ultraviolet light induces apoptosis via direct activation of CD95 (Fas/APO-1) independently of its ligand CD95L. J Cell Biol 140: 171-182.

[17] BELTINGER C, FULDA S, KAMMERTOENS T, MEYER E, UCKERT W AND DEBATIN KM [1999] Herpes simplex virus thymidine kinase/ganciclovir-induced apoptosis involves ligand-independent death receptor aggregation and activation of caspases. Proc Natl Acad Sci USA 96: 8699-8704.

[18] HIGUCHI H AND GORES GJ [2003] Bile acid regulation of hepatic physiology: IV. Bile acids and death receptors. Am J Physiol Gastrointest Liver Physiol 284: G734-

G738.

[19] FAUBION WA, GUICCIARDI ME, MIYOSHI H, BRONK SF, ROBERTS PJ, SVINGEN PA, KAUFMANN SH AND GORES GJ [1999] Toxic bile salts induce rodent hepatocyte apoptosis via direct activation of Fas. J Clin Invest 103: 137-145.

[20] POWELL AA, LARUE JM, BATTA AK AND MARTINEZ JD [2001] Bile acid hydrophobicity is correlated with induction of apoptosis and/or growth arrest in HCT116 cells. Biochem J 356: 481-486.

[21] MARTINEZ-DIEZ MC, SERRANO MA, MONTE MJ AND MARIN JJ [2000] Comparison of the effects of bile acids on cell viability and DNA synthesis by rat hepatocytes in primary culture. Biochim Biophys Acta 1500: 153-160.

[22] SCHLOTTMAN K, WACHS FP, KRIEG RC, KULLMANN F, SCHOLMERICH J AND ROGLER G [2000] Characterization of bile salt-induced apoptosis in colon cancer cell lines. Cancer Res 60: 4270-4276.

[23] FRUCHT H, JENSEN RT, DEXTER D, YANG WL AND XIAO Y [1999] Human colon cancer cell proliferation mediated by the M3 muscarinic cholinergic receptor. Clin Cancer Res 5: 2532-2539.

[24] FRUCHT H, GAZDAR AF, PARK J-A, OIE H AND JENSEN RT [1992] Characterization of functional receptors for gastrointestinal hormones on human colon cancer cells. Cancer Res 52: 1114-1122.

[25] CHENG K AND RAUFMAN JP [2005] Bile acid-induced proliferation of a human colon cancer cell line is mediated by transactivation of epidermal growth factor receptors. Biochem Pharmacol 70: 1035-1047.

[26] CHENG K, CHEN Y, ZIMNIAK P, RAUFMAN JP, XIAO Y AND FRUCHT H [2002] Functional interaction of lithocholic acid conjugates with M3 muscarinic receptors on a human colon cancer cell line. Biochim Biophys Acta 1588: 48-55.

[27] BOMZON A, FINBERG JP, TOVBIN D, NAIDU SG AND BETTER OS [1984] Bile salts, hypotension and obstructive jaundice. Clin Sci (Lond) 67: 177-183.

[28] LEE SS, PAK JM, MEDLICOTT SM AND BOMZON A [1995] Vasodilatory responses of isolated arteries of cirrhotic rats. Clin Sci (Lond) 89: 227-232.

[29] KHURANA S, CHACON I, XIE G, YAMADA M, WESS J, RAUFMAN JP AND KENNEDY RH [2004] Vasodilatory effects of cholinergic agonists are greatly diminished in aorta from $M3R^{-/-}$ mice. Eur J Pharmacol 493: 127-132.

[30] KHURANA S, YAMADA M, WESS J, KENNEDY RH AND RAUFMAN JP [2005] Deoxycholyltaurine-induced vasodilation of rodent aorta is nitric oxide- and muscarinic M_3 receptor-dependent. Eur J Pharmacol 517: 103-110.

[31] HASLEWOOD GA [1967] Bile salt evolution. J Lipid Res 8: 535-550.

[32] WERNEBURG NWQ, YOON JH, HIGUCHI H AND GORES GJ [2003] Bile acids activate EGF receptor via a TGF-alpha-dependent mechanism in human cholangiocyte cell lines. Am J Physiol Gastrointest Liver Physiol 285: G31-G36.

[33] QIAO L, STUDER E, LEACH K, MCKINSTRY R, GUPTA S, DECKER R, KUKREJA R, VALERIE K, NAGARKATTI P, EL DEIRY W, MOLKENTIN J, SCHMIDT-ULLRICH R, FISHER PB, GRANT S, HYLEMON PB AND DENT P [2001] Deoxycholic acid (DCA) causes ligand-independent activation of epidermal growth factor receptor (EGFR) and FAS receptor in primary hepatocytes: inhibition of EGFR/mitogen-activated protein kinase-signaling module enhances DCA-induced apoptosis. Mol Biol Cell 12: 2629-2645.

[34] DENT P, FANG Y, GUPTA S, STUDER E, MITCHELL C, SPIEGEL S AND HYLEMON PB [2005] Conjugated bile acids promote ERK1/2 and AKT activation via a pertussis toxin-sensitive mechanism in murine and human hepatocytes. Hepatology 42: 1291-1299.

[35] KAWAMATA Y, FUJII R, HOSOYA M, HARADA M, YOSHIDA H, MIWA M, FUKUSUMI S, HABATA Y, ITOH T, SHINTANI Y, HINUMA S, FUJISAWA Y AND FUJINO M [2003] A G protein-coupled receptor responsive to bile acids. J Biol Chem 278: 9435-9440.

[36] MARUYAMA T, MIYAMOTO Y, NAKAMURA T, TAMAI Y, OKADA H, SUGIYAMA E, NAKAMURA T, ITADANI H AND TANAKA K [2002] Identification of membrane-type receptor for bile acids (M-BAR). Biochem Biophys Res Commun 298: 714-719.

[37] KATSUMA S, HIRASAWA A AND TSUJIMOTO G [2005] Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem Biophys Res Commun 329: 386-390.

[38] WATANABE M, HOUTEN SM, MATAKI C, CHRISTOFFOLETE MA, KIM BW, SATO H, MESSADDEQ N, HARNEY JW, EZAKI O, KODAMA T, SCHOONJANS K, BIANCO AC AND AUWERX J [2006] Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature 439: 484-489.

[39] HAN SI, STUDER E, GUPTA S, FANG Y, QIAO L, LI W, GRANT S, HYLEMON PB AND DENT P [2004] Bile acids enhance the activity of the insulin receptor and glycogen synthase in primary rodent hepatocytes. Hepatology 39: 456-463.

[40] RUST C, KARNITZ LM, PAYA CV, MOSCAT J, SIMARI RD AND GORES GJ [2000] The bile acid taurochenodeoxycholate activates a phosphatidylinositol 3-kinase-dependent survival signaling cascade. J Biol Chem 275: 20210-20216.

[41] BARBERO ER, HERRERA MC, MONTE MJ, SERRANO MA AND MARIN JJ [1995] Role of amidation in bile acid effect on DNA synthesis by regenerating mouse liver. Am J Physiol 268: G1051-G1059.

[42] BISHOP-BAILEY D, WALSH DT AND WARNER TD [2004]

Expression and activation of the farnesoid X receptor in the vasculature. Proc Natl Acad Sci USA 101: 3668-3673.

[43] STRAUCH ED, YAMAGUCHI J, BASS BL AND WANG JY [2003] Bile salts regulate intestinal epithelial cell migration by nuclear factor-kappa B-induced expression of transforming growth factor-beta. J Am Coll Surg 197: 974-984.

[44] TOLEDO A, YAMAGUCHI J, WANG JY, BASS BL, TURNER DJ AND STRAUCH ED [2004] Taurodeoxycholate stimulates intestinal cell proliferation and protects against apoptotic cell death through activation of NF-kappaB. Dig Dis Sci 49: 1664-1671.

[45] STRAUCH ED, WANG JY AND BASS BL [2001] Bile salt stimulates intestinal epithelial cell migration through TGFβ after wounding. J Surg Res 97: 49-53.

[46] WANG H, CHEN J, HOLLISTER K, SOWERS LC AND FORMAN BM [1999] Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 3: 543-553.

[47] XIE W, RADOMINSKA-PANDYA A, SHI Y, SIMON CM, NELSON MC, ONG ES, WAXMAN DJ AND EVANS RM [2001] An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. Proc Natl Acad Sci USA 98: 3375-3380.

[48] MAKISHIMA M, LU TT, XIE W, WHITFIELD GK, DOMOTO H, EVANS RM, HAUSSLER MR AND MANGELSDORF DJ [2002] Vitamin D receptor as an intestinal bile acid sensor. Science 296: 1313-1316.

[49] HIGUCHI H, BRONK SF, TAKIKAWA Y, WERNEBURG N, TAKIMOTO R, EL-DEIRY W AND GORES GJ [2001] The bile acid glycochenodeoxycholate induces trail-receptor 2/DR5 expression and apoptosis. J Biol Chem 276: 38610-38118.

RECEIVED ON 12-20-2005.
ACCEPTED ON 1-13-2006.