Bile Acids Regulate Cardiovascular Function

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Abstract

Research over the last decade has uncovered roles for bile acids (BAs) that extend beyond their traditional functions in regulating lipid digestion and cholesterol metabolism. BAs are now recognized as signaling molecules that interact with both plasma membrane and nuclear receptors. Emerging evidence indicates that by interacting with these receptors, BAs regulate their own synthesis, glucose and energy homeostasis, and other important physiological events. Herein, we provide a comprehensive review of the actions of BAs on cardiovascular function. In the heart and the systemic circulation, BAs interact with plasma membrane G-protein-coupled receptors, for example, TGR5 and muscarinic receptors, and nuclear receptors, for example, the farnesoid (FXR) and pregnane (PXR) xenobiotic receptors. BA receptors are expressed in cardiovascular tissue, however, the mechanisms underlying BA-mediated regulation of cardiovascular function remain poorly understood. BAs reduce heart rate by regulating channel conductance and calcium dynamics in sino-atrial and ventricular cardiomyocytes and regulate vascular tone via both endothelium-dependent and -independent mechanisms. End-stage liver disease, obstructive jaundice, and intrahepatic cholestasis of pregnancy are prominent conditions in which elevated serum BAs alter vascular dynamics. This review focuses on BAs as newly recognized signaling molecules that modulate cardiovascular function.
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Introduction

Bile acids (BAs) are synthesized in the liver as by-products of cholesterol metabolism and secreted into the duodenum. They journey through the small intestine, undergo metabolism by intestinal flora, are reabsorbed efficiently by specific transporters in the ileum and circulate back to the liver via the mesenteric and portal veins.[1] In the liver, BAs are transported into hepatocytes and conjugated with amino acids before excretion into bile ducts. BAs are stored in the gallbladder and released into the duodenum with meals. This cycle of BA metabolism in the liver and recovery from the intestines, designated as enterohepatic circulation, is a highly efficient system whereby more than 95% of the BA pool is recycled. In normal circumstances, the remaining small fraction of the BA pool either enters the colon to be excreted in feces or spills into the systemic circulation. This small percentage of fecal BAs is important for maintaining normal defecatory function.

In recent years, the recognized role of BAs has expanded beyond fat absorption and cholesterol metabolism. Interaction of BAs with plasma membrane G-protein-coupled (GPCRs) and nuclear receptors in various tissues triggered investigation into the role of BAs in regulating glucose homeostasis, obesity, thyroid function, and other important physiological processes; this is reviewed elsewhere.[2,3] Herein, we focus on the role of BAs and their receptors in regulating cardiovascular function in health and disease.

The role of BAs in regulating cardiovascular function was investigated sporadically in the past and more actively in recent years. In large part, this renewed interest stems from identification of BA receptors in cardiovascular tissue and recognition of BAs as vasoactive ligands that regulate vascular tone and myocardial contractility in disease. Recent advances highlight the importance of this line of investigation. As liver disease progresses toward cirrhosis, the BA pool shifts from the enterohepatic to the systemic circulation.[4] Cirrhosis is associated with increased cardiac output, reduced arterial pressure, and reduced systemic vascular resistance.[5] In cirrhosis, several mechanisms and ligands including BAs are proposed to induce the systemic and splanchnic vasodilation that yields a hyperdynamic circulation. Intrahepatic cholestasis of pregnancy (ICP) is another disorder that is accompanied by elevated serum BA levels.[6] In ICP, BA-induced cardiac arrhythmias are proposed to cause fetal morbidity and mortality.[6,7] In addition, activation of vascular farnesoid (FXR) may play a role in atherosclerosis. Hence, elucidating the interaction of BAs with cardiovascular tissues is likely to provide novel mechanistic insights into their regulatory role.

BA Metabolism

BAs have complex structural and biochemical properties that vary amongst vertebrates.[8] In humans, they are synthesized exclusively in hepatocytes as by-products of cholesterol metabolism. While preserving the cholesterol backbone (C-27 and four fused rings: A-D) (*Figure 1*), modifications consisting primarily of key hydroxylations and shortening of the C-8 to a C-5 side chain yield a predominantly C-24 BA pool.[9] BAs are divided into two groups based on the orientation of C-5 proton with respect to the C-19 methyl group. The C-5 proton in *trans* and *cis* configurations renders 5α- or 5β-BAs, respectively. The 5α-BAs have all four rings in same plane, while 5β-BAs have ring A at an approximate right angle to the other rings. The majority of human BAs are in the 5β-configuration. Conversion of cholesterol into BAs via neutral and acidic pathways involves multiple steps that are catalyzed by enzymes expressed in different hepatocyte compartments.[10] The neutral pathway is regulated by the rate-limiting 7α-hydroxylase-CYP7A1, a microsomal cytochrome P450 enzyme. In the "acidic pathway," the initial step is catalyzed by CYP27A1, and chenodeoxycholic acid (CDCA) is the main product. This pathway may contribute up to 50% of the BA pool. Cholesterol 25-hydroxylase is a minor BA synthesis pathway.

Cholic acid (CA) and CDCA are the primary human BAs (*Figure 1*). In the small intestine, bacterial 7α-dehydroxylase converts a fraction of these molecules into secondary BAs—deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. Further hepatic and bacterial modifications lead to formation

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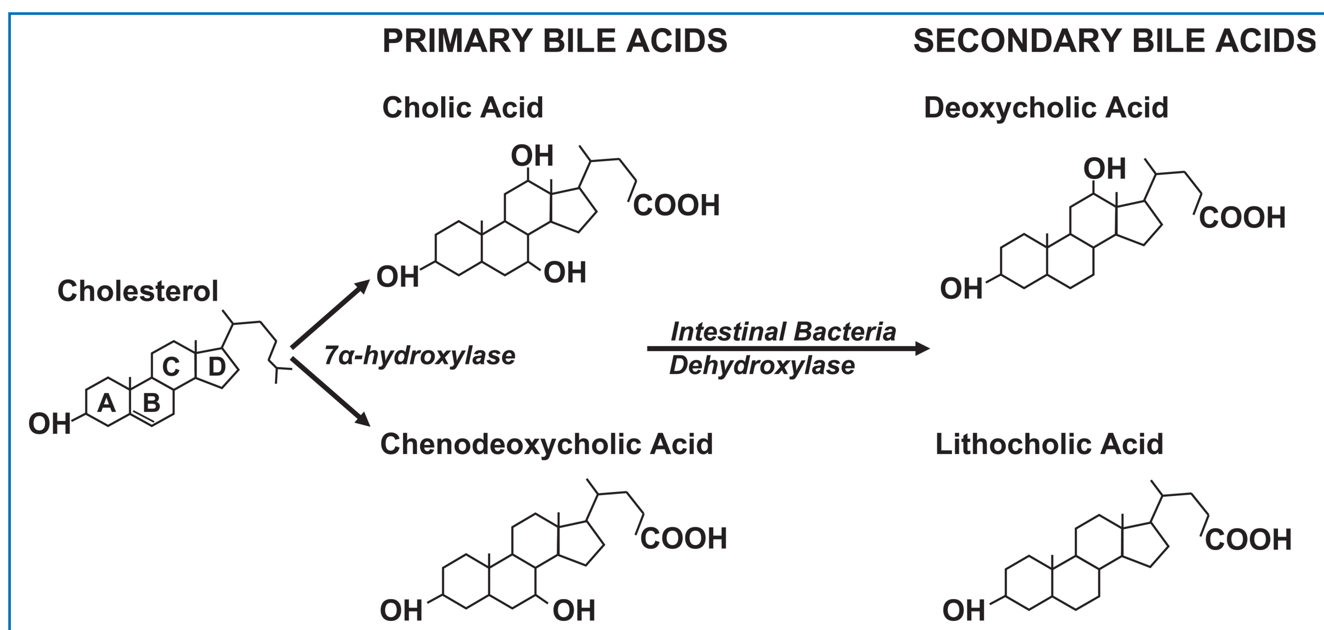

Figure 1. This scheme shows the structure of major human BAs derived from cholesterol metabolism. The steroid nucleus and ring lettering for both primary and secondary human BAs are shown. Dehydroxylases derived from intestinal bacteria regulate formation of secondary BAs. The degree of hydroxylation of the steroid nucleus determines the biochemical and functional characteristics of both primary and secondary BAs. Whereas cholic acid has three hydroxyl groups, chenodeoxycholic acid and deoxycholic acid have two, and lithocholic acid has one.

Bile acid (BA)	Abbreviation for unconjugated BA	Abbreviation for taurine-conjugated BA	Abbreviation for glycine-conjugated BA
Cholic acid	CA	CT	CG
Deoxycholic acid	DCA	DCT	DCG
Chenodeoxycholic acid	CDCA	CDCT	CDCG
Lithocholic acid	LCA	LCT	LCG
Ursodeoxycholic acid	UDCA	UDCT	UDCG
*From Hofmann et al.[9]			

Table 1. Recommended designation and abbreviations for major human bile acids.*

of tertiary BAs, for example, ursodeoxycholic acid (UDCA). In human adults, 95% of the 3- to 5-g pool consists of primary and secondary BAs. Normally, less than 5% of the BA pool escapes reabsorption to be lost in feces, whereupon it is replaced by hepatic synthesis. Before secretion into bile, BA solubility is increased through amidation of the carboxyl group with either glycine or taurine. In adults, the ratio of glycine to taurine conjugates is normally 3:1. Dietary intake of taurine, primarily in meat, dictates the size of taurine-conjugated BA pool as hepatocytes do not synthesize that amino acid. Adhering to a proposed nomenclature, taurine conjugates of CA, CDCA, DCA, LCA, and UDCA are designated cholyltaurine (CT), chenodeoxycholyltaurine (CDCT), deoxycholyltaurine (DCT), lithocholyltaurine (LCT), and ursodeoxycholyltaurine (UDCT).[11] Glycine conjugates are named likewise (e.g., cholylglycine). A summary of BA nomenclature and abbreviations is provided in *Table 1*.

In the enterohepatic circulation, BAs are transported across the cell membranes via various transport proteins.[12,13] In the liver, BAs are transported into hepatocytes by Na^+-taurocholate cotransporting polypeptide and organic anion transport polypeptide (OATP). BAs are secreted from hepatocytes by the bile salt export pump (BSEP) and multidrug resistance proteins. In the small intestine, BAs are absorbed by enterocytes via ASBT in apical membranes, transported across the cells by ileal BA binding protein, and released into portal circulation via organ solute transporters (OSTα-OSTβ). The small fraction of BAs spilled into the systemic circulation is reabsorbed by renal proximal tubule cells via ASBT and OSTα-OSTβ for hepatic reuptake. Bile duct epithelium also expresses ASBT and OSTα-OSTβ on the apical and basolateral membranes, respectively, which mediate transcellular transport of BAs.

BA Receptors in the Circulation

BA interaction with nuclear receptors

In 1999, FXR, previously an orphan nuclear receptor, was identified as a BA receptor.[14] Since then, three additional nuclear BA receptors were identified; rodent xenobiotic receptor, that is, pregnane (PXR), its human analog, steroid and xenobiotic receptor, and the vitamin D receptor (VDR).[15–17] VDR is classified as an endocrine nuclear receptor; FXR and PXR are classified as "adopted nuclear receptors."[18] While these receptors are expressed primarily in tissues exposed to high-BA concentrations such as liver, kidneys, and gastrointestinal tract, FXR expression was also detected in cardiovascular organs such as the coronary arteries, aorta, heart, and atherosclerotic arteries.[19,20] Amongst all nuclear receptors, FXR is the most studied. Whereas CDCA is the most potent endogenous FXR agonist, FXR is also activated by DCA and LCA. Interaction of conjugated BAs with FXR requires the presence of plasma membrane BA transporters.[14] CDCA binding to FXR triggers a

conformational change that facilitates formation of a heterodimer with the retinoic acid receptor (RXR) in the cytoplasm, which then translocates into the nucleus and recognizes DNA-sequence motif in the promoter region of FXR target genes.[21]

VDR is also expressed in endothelial and vascular smooth cells and may play a role in atherosclerosis.[22] Structure-function analysis revealed that $1,25(OH)_2D_3$ (vitamin D) and LCA interact with different sets of amino acids in the ligand-binding pocket of VDR resulting in distinct conformational responses to $1,25(OH)_2D_3$ and LCA binding.[23,24] Docking models suggest that compared to $1,25(OH)_2D_3$, LCA and 3-keto-LCA are weakly accommodated in the VDR ligand-binding pocket;[23,24] such ligand and receptor-site specific interactions may explain different biological actions of $1,25(OH)_2D_3$ and LCA. Nonetheless, a direct impact of LCA-mediated regulation of cardiovascular function has yet to be demonstrated.

PXR plays a major role in xenobiotic metabolism and was shown to act as an LCA sensor.[25] Similar to FXR, PXR is expressed predominantly in the gastrointestinal tract and liver and forms a heterodimer with RXR to regulate gene transcription.[26] A recent report suggested that PXR is expressed in mesenteric arteries and may contribute to vascular adaptation during pregnancy.[27] In view of this single report, the role of PXR in vascular tone remains preliminary. Studies elucidating the interaction of PXR with BAs in regulating cardiac function are lacking.

BA Interaction with Plasma Membrane GPCRs

BAs interact with two GPCRs—muscarinic receptors and TGR5. Muscarinic receptors are widely expressed in the body, including all regions of the gastrointestinal tract, intestinal smooth muscle, and the central nervous system. In 1998, Raufman and colleagues reported that conjugated secondary BAs interact functionally with muscarinic receptors. Five muscarinic receptors (M_1 to M_5) couple to different G proteins ($M_{1,3,5}$ are coupled to $G_{\alpha q}/G_{11}$ and $M_{2,4}$ to $G_{\alpha i}/G_0$).[28] In CHO cells expressing rat M_3 muscarinic receptors (M_3R), DCA, LCA, and their taurine and glycine conjugates inhibited binding of a muscarinic radioligand,[3] [H] N-methylscopolamine. Similarly, DCT and DCG inhibited acetylcholine (ACh)-induced postreceptor signaling (increase in inositol phosphate formation and MAPK phosphorylation).[29,30] Computer modeling revealed that BA and ACh molecular surface structures show striking similarities;[29] the charge distribution on the LCT amide side chain closely resembles the charge distribution on ACh. Hence, LCT structure favors interaction with M_3R. Khurana and colleagues reported that conjugated LCA and DCA interact with rat and human (M_3R).[29–32] A recent study indicates that CT regulates cardiac contractility by interacting with M_2 muscarinic receptors;[33] M_2 and M_3 muscarinic receptors are the major subtypes that regulate cardiac function. The role of M_3R in BA-mediated modulation of cardiac function was not investigated.

In 2002, TGR5, a $G_{\alpha s}$-protein-coupled receptor that is activated by conjugated and free BAs, was discovered.[34] $G_{\alpha s}$-coupled receptors stimulate cAMP synthesis and, via PKA, activate a cAMP response element binding protein that induces expression of many genes. In cell culture, LCA and DCA were the most efficacious activators of TGR5.[34] TGR5 is widely expressed, with the greatest expression in gallbladder, spleen, intestine, adipose tissue, and immune cells. In thermogenic tissues (brown adipose tissue and muscle), BA–TGR5 interaction increases energy expenditure.[35] Recently, TGR5 expression was identified in hepatic sinusoidal endothelial cells and cardiomyocytes.[36,37]

Nonreceptor-Mediated BA Actions

In addition to GPCRs and nuclear receptors, BAs interact with large conductance Ca^{2+}-activated K^+ (BKCa) channels that regulate arterial tone. Vascular smooth muscle stretch activates BKCa to favor hyperpolarization, vessel relaxation, and preservation of tissue perfusion. That mechanism has been proposed to function as a brake on myogenic constriction and local blood flow.[38] It appears that BAs activate these channels directly to a degree that is inversely related to the number of hydroxyl groups in the BA molecule.[39] Various aspects of this interaction such as the effect of BA conjugation and conformational changes in channel structure remain to be elucidated. A summary of BA receptors, their expression in cardiovascular tissue, and their role in BA-mediated regulation of cardiovascular function are provided in *Table 2*.

BA receptors	Tissue type	Direct BA action demonstrated	References
Nuclear receptors			
FXR	Endothelial cells	Yes	20,75
	Vascular smooth muscle cells	Yes	77,78,79
	Cardiomyocytes	No	20
PXR	Mesenteric arteries	No	27
VDR	Cardiomyocytes	No	52
G-Protein-coupled receptors			
TGR5	Hepatic sinusoidal endothelial cells	Yes	36
	Cardiomyocytes	No	37
M_2R	Cardiomyocytes	Yes	33
M_3R	Endothelial cells	Yes	69
Potassium channels			
BKCa	Vascular smooth muscle cells	Yes	39,66

Table 2. Expression of BA receptors that regulate cardiovascular function.

BA/ligand	Tissue/model	Effect	Signaling/mediator	References
DCT	Rat cardiomyocyte	↓ Contraction	Not known	43
CA	Rat heart	Bradycardia	↓ Cholinergic stimulation	44,45
CT	Rat cardiomyocyte; rabbit sinoatrial node	↓ action potential duration	↓ inward Na^+ and Ca^{2+} currents, and ↑ outward K^+ currents	46,47
CT, CG	Neonatal rat cardiomyocytes	↓ contraction rate, amplitude and synchronization	Altered intracellular calcium dynamics; M_2R-mediated ↓ cAMP	33,49,50
UDCA	Mouse heart	↓ allograft rejection	Immune-mediated	58,59
UDCA	Rat cardiac ischemia-reperfusion model	↓ myocyte apoptosis	Activation of PI3K-AKT	60
DCT, CDCT	Rat aorta, portal vein, mesenteric and carotid arteries	Vasodilation	M_3R-mediated NO release;? VDCC activation	32,63,64
DCA, DCT, CDCA	Endothelial cells	NO and K^+ current generation	↑ cytoplasmic [Ca^{2+}]	68
LCT, CT, CDCT	Hepatic sinusoidal endothelial cells	NOS up-regulation and NO generation	TGR5-mediated cAMP generation	36
LCA	Cerebral arteries	Vasodilation	BKCa activation	39,66
CDCA	Endothelial cells	NOS up-regulation	FXR activation	75
GW4064	Rabbit mesenteric arteries	↓ NO sensitivity	↓ generation of cGMP	76
CDCA	Rat pulmonary artery endothelial cells	↓ ET-1 expression	FXR activation	77
CDCA, GW4064	Rat aortic smooth muscle cells	↑ AT2R expression	FXR activation	78
6ECDCA, GW4064	Rat and human aortic smooth muscle cells	↓ inflammation and migration	↓ IL-1β -mediated iNOS and COX2 expression	79
INT-747	*ApoE*$^{-/-}$ mice	↓ aortic plaque area and calcification	↓ expression of IL-1β, IL-6 and CD11b	80,81
CDCA	Endothelial cells; human esophageal cancer xenograft	↑ angiogenesis	FXR activation; COX-2-dependent VEGF production	88,89
UDCA	Chick embryo CAM; laser-induced CNV	↓ angiogenesis	Not known	90,91

↓: decreased; ↑: increased; INT-747 is a synthetic CDCA derivative. GW4064 and 6α-ethyl-chenodeoxycholic acid (6ECDCA) are synthetic FXR agonists; CAM: chorioallantoic membrane assay; CNV: choroidalneovascularization ; VDCC: Voltage-dependent calcium channels; VEGF, vascular endothelial growth factor.

Table 3. Summary of the effects of bile acids on cardiovascular tissue

BAs and Cardiac Function

Effects of BAs on cardiac function may be categorized as indirect and direct. Indirect effects involve BA and cholesterol metabolic pathways that regulate blood cholesterol levels, atherosclerotic plaque formation, and myocardial function. Direct effects require interaction of BAs with myocytes, thereby influencing myocardial conduction and contraction; these actions may be receptor dependent or independent. A summary of the effects of specific BAs on cardiovascular tissue is outlined in *Table 3*.

Human diseases associated with elevated serum BAs (>200–400 μM) and altered cardiac function include ICP, obstructive jaundice, chronic viral hepatitis, and cirrhosis.[4,40] ICP is complicated by fetal distress, preterm labor, and intrauterine death. In the fetus, both bradycardia and tachycardia ≤100 or ≥180 beats/minute) are observed.[40] Experimental and clinical studies indicate that cirrhosis is associated with impaired myocardial contractility and electrophysiological abnormalities. The syndrome of "cirrhotic cardiomyopathy" is discussed elsewhere.[41]

Clinical observations regarding the effect of bile components on the heart are not new. In 1863, Röhrig proposed that BAs might be responsible for jaundice-associated bradycardia.[42] In 1909, John King reported that in dogs, bile pigments such as biliverdin, rather than BAs, induce an atropine sensitive bradycardia and hypotension.[42] Such earlier reports suggested that bile constituents have cardiovascular effects and implicated cholinergic mechanisms. In the early 1980s, the effect of individual BAs on cardiac function was demonstrated, both *in vitro* and *in vivo*. DCT and serum from bile duct ligated rats reduced spontaneous contraction of cultured rat cardiac myocytes in a concentration- and time-dependent manner.[43] Compared to controls, 20 μM DCT reduced the rate of myocyte contraction within 2 hours (86 ± 6 and 67 ± 7 beats/minute, respectively) and by 24 hours, the rate was reduced by 60%. Higher DCT concentrations, that is, 40 and 60 μM, abolished cardiac myocyte contraction. Further, in rats, bradycardia was observed 7 days after bile duct ligation, and infusion of CA induced dose-dependent bradycardia that was inhibited by vagotomy and atropine.[44,45] These early studies provided initial evidence that BAs can exert a direct effect on cardiac function.

Binah et al. demonstrated that BAs reduce the duration of the action potential in ventricular myocytes.[46] In rat, voltage-clamp experiments demonstrated that 1 μM CT reduced the slow inward Na^+ and Ca^{2+} currents and increased the outward K^+ current. Similarly, experiments with the rabbit sino-atrial node demonstrated that CT (30–300 μM) slowed the sinus rate by reducing diastolic depolarization accompanied by similar

depression of ion fluxes.[47] Mechanistic insights into BA-induced effects on cardiac function in ICP were provided in neonatal rat cardiomyocytes by Julia Gorelik's laboratory.[48] CT and CG (both 1 mM) reduced cardiomyocyte contraction rates by 46% and 11%, respectively. CT reduced the contraction amplitude and prevented cardiomyocyte synchronization. By comparison, CG did not affect those characteristics but, at 300 μM, altered calcium dynamics, characterized by a biphasic change in calcium wave frequency or cellular calcium overload.[49,50] These data demonstrated that BAs alter the "pacemaker" function of cardiac myocytes.

Cardiac myocytes express FXR and VDR. Since FXR and VDR are transcription factors, it is unlikely that they account for immediate changes in myocyte function that occur with BA stimulation. In contrast, BA–GPCR interaction provides a more likely mechanism. In a variety of cell systems, BAs interact with muscarinic receptors to activate intracellular signaling.[51] CT interacts with muscarinic receptor subtype 2 (M_2R) on neonatal rat cardiac myocytes to reduce intracellular cAMP and exert a negative chronotropic effect.[33] In those cells, pharmacological inhibition and siRNA-knockdown of M_2R completely abolished the effects of CT on contraction, calcium transient amplitude, and synchronization, without implicating participation of FXR and TGR5.

Like M_2R, the GPCR TGR5, is expressed on cardiac myocytes. *In vitro*, in both neonatal mouse cardiomyocytes and cardiomyocytes isolated in biliary fibrosis, LCA and CDCT stimulated phosphorylation of AKT and GSK-β suggesting TGR5-mediated activation.[37] However, these observations only provided a temporal association between TGR5 and modulation of cardiac function. More definitive experiments that establish a direct effect are lacking.

VDR may play a role in regulating cardiac function. Cardiac myocytes isolated from VDR knockout mice demonstrated accelerated contraction and relaxation rates compared to wild type controls.[52] $1,25(OH)_2D_3$ altered the contractility and rate of relaxation of cardiac myocytes.[52] Although VDR can interact with LCA and keto-LCA, it is not clear that this plays a role in BA-mediated effects.[53] Further, to postulate access to nuclear receptors, such an interaction requires transport of BAs into cardiomyocytes. Cardiac myocytes express anion transporters such as BSEP, multidrug resistance gene 3 (MDR3), and OATP; however, their role in BA-mediated regulation of cardiac function is unknown.[54]

Indirect evidence suggests that additional mechanisms may mediate BA-induced changes in cardiac function. In rabbits, using a complicated surgical model whereby biliovenous catheterization, cholecystoduodenal fistula, or exteriorization of biliary drainage was achieved after bile duct ligation; Martinez-Rodenas et al. demonstrated that bile constituents increase serum levels of atrial natriuretic peptide (ANP).[55] In patients with obstructive jaundice, investigators from the same group demonstrated that elevated ANP levels were associated with myocardial dysfunction. Internal biliary drainage not only reduced ANP levels but also improved cardiac function.[56] Despite these studies, evidence implicating specific BAs in ANP release from cardiomyocytes has yet to be demonstrated.

In nonhepatobiliary tissue, UDCA and its taurine-conjugate are the most extensively studied BAs. UDCA and UDCT inhibit apoptosis and promote cell survival, although the mechanisms remain to be determined.[57] A few studies have evaluated their role on cardiomyocyte survival. After heart transplantation, treatment with UDCA may reduce the risk of acute cardiac rejection.[58,59] In animal models, transfer of splenocytes or $CD4^+$ cells from UDCA-treated allograft recipients resulted in indefinite survival of allografts in naive secondary recipients. This effect is most likely due to UDCA-mediated regulation of immune cell function rather than to a direct effect on cardiomyocytes. In another experimental system, pretreatment of rats with UDCA (40 mg/kg over 30 minutes) protected the heart against ischemia-reperfusion injury by activating the PI3K-AKT survival pathway and reducing cardiomyocytes loss and infarct size.[60] Hence, UDCA may have cardioprotective effects but the relative roles of VDR, TGR5, or muscarinic receptors are unclear.

In summary, these observations suggest that BAs regulate cardiac function by interacting with more than one receptor and more than one cell type, though mechanistic insights are limited. The availability of knockout mice (e.g., $M_2R^{-/-}$, $M3R^{-/-}$, $FXR^{-/-}$, $VDR^{-/-}$, and $TGR5^{-/-}$ mice) may help elucidate receptor specific effects of BAs on cardiac function. However, since nuclear receptors such as FXR and PXR also influence BA metabolism, cardiomyocyte-selective gene deletion may be required to determine their cell-specific roles.

BAs and Vascular Function

The effect of BAs on vascular tone has been evaluated in a variety of vascular beds. While earlier studies demonstrated the impact of BA infusion and bile duct ligation on blood pressure, later studies demonstrated the role of BAs in isolated vascular preparations and cell culture models.

In 1983, Lautt and colleagues demonstrated in cats that intravenous administration of CT-induced vasodilation of mesenteric and hepatic arteries.[61] Studies using rat portal veins, isolated 3–5 days after bile duct ligation, demonstrated an attenuated contractile response to noradrenalin.[62] Similarly, isolated rat hind limb preparations incubated with CT had blunted noradrenalin-mediated contraction. Pak et al. evaluated the effects of dihydroxylated BAs (i.e., UDCT, CDCT, and DCT) on vascular tone.[63] In rats, intravenous infusion of increasing doses of CDCT and DCT increased mesenteric arterial blood flow and reduced arterial pressure; UDCT infusion had no effect. In isolated preparations of mesenteric and carotid arteries and portal vein, CDCT and DCT induced dose-dependent (1 μM to 10 mM) vasodilation, mimicking *in vivo* experiments. Again, UDCT had no effect. Further investigation suggested that receptor-operated and voltage-gated calcium channel modulation may play a role in BA-induced vasodilation.[64]

Role of Endothelium

The role of endothelium in BA-mediated vasodilation has been investigated with varying results. Pak et al. reported that endothelium denudation did not alter DCT-mediated vasodilation of rat mesenteric arteries.[64] Ljubuncic et al. made similar observations; endothelial denudation did not alter DCA-mediated vasodilation of rat aorta.[65] Both studies observed that incubation of endothelium-intact vessels with L-NAME, a nitric oxide synthase (NOS) inhibitor, did not alter DCT- and DCA-mediated vasodilation of rat mesenteric arteries and abdominal aorta, respectively.[64,65] Bukiya et al. found that LCA mediates nonendothelial-dependent vasodilation of cerebral arteries by directly activating BKCa channels on vascular smooth muscle.[66] In contrast, noradrenalin- and 5-hydroxytryptamine-mediated contraction was accentuated in endothelium-denuded renal

arteries from mongrel dogs with bile duct ligation.[67] In the same preparation, ACh-mediated vasodilation was abolished by endothelial removal. Nakajima et al. employed fluorescence microscopy to determine the effect of BAs on bovine aortic and human umbilical vein endothelial cells.[68] DCA, CDCA, and DCT induced concentration-dependent increase in cytoplasmic Ca^{2+} ($[Ca^{2+}]i$) and NO production.

Investigations in our laboratory favor a role for the endothelium in BA-mediated vasodilation. In rat and mouse aorta, we demonstrated that DCT mediates concentration-dependent vasodilation that is attenuated by endothelium denudation or incubation with L-NAME.[32] Further, DCT-induced vasodilation (0.1 μM–1 mM) was reduced by a synthetic ACh: BA hybrid that acts as an M_3R antagonist.[69] Similarly, ACh- and DCT-mediated relaxation of mouse aortic rings was reduced by M_3R gene ablation.[32] These data support the interpretation that systemic vasodilatory actions of DCT are mediated by an M_3R-dependent mechanism. The role of TGR5 in BA-mediated vasodilation is less clear. One report suggests that TGR5 is expressed in hepatic sinusoidal endothelial cells. In these cells, LCT, CT, and CDCT increased cAMP-induced endothelial NO synthase (eNOS) mRNA expression, eNOS Ser1177 phosphorylation, and NO production.[36] However, the study did not directly link TGR5 stimulation to the BA-mediated eNOS activation. Absence of specific TGR5 inhibitors is a major limitation and studies using TGR5 knockdown are lacking. The expression and role of TGR5 in other vascular beds is not known.

Role of Potassium Channels

BA-mediated activation of Ca^{2+}-dependent K^+ currents in cultured endothelial cells has been demonstrated.[68] BAs also activate Ca^{2+}-dependent K^+ channels in vascular smooth muscle cells. In rabbit, mesenteric artery smooth muscle cells, using patch-clamp techniques, Dopico et al. demonstrated that BAs reversibly activate BKCa channels.[39] In pressurized cerebral resistance arteries, endothelium-independent vasodilation by LCA was blocked by the BKCa channel blocker, iberiotoxin. LCA failed to stimulate vasodilation in arteries from BK β-1 subunit knockout mice implicating an important role for the BK β-1 subunit in LCA activation.[66] It appears that LCA effects are mediated by interaction with the second transmembrane domain of the BK β-1 subunit.[70,71] Collectively, these data demonstrated that BAs stimulate receptor-independent vasodilation by activating smooth muscle K^+ channels.

Role of Nuclear Receptors

Studies of the role of FXR in vascular function were sparked by identification of its expression in the vasculature.[20] Generation of FXR and PXR null mice broadened the scope of these investigations, but study of *Fxr*$^{-/-}$ mice, in particular, must be interpreted with caution. FXR (and PXR) directly impact BA metabolism, and *Fxr*$^{-/-}$ mice have eight-fold elevations of BA concentrations.[72–74]

Since nuclear receptors are transcription factors, it is expected that they regulate vascular function by altering the expression of vasoactive molecules and other receptors. In cultured endothelial cells, CDCA and GW4064 (a chemical FXR agonist) increased eNOS expression.[75] In contrast, chronic stimulation of FXR impaired NO-dependent vasodilation due to blunted increase of cGMP in smooth muscle cells.[76] These observations suggest that short- and long-term FXR stimulation have differential effects on NO generation and sensitivity. Further, in endothelial cells, FXR ligands increased FXR expression and reduced ET-1 expression;[77] in vascular smooth muscle cells, FXR ligands increased angiotensin type 2 receptor expression.[78] These data provide evidence that in vascular tissue, FXR, in addition to regulating its own expression, alters the generation of vasodilatory and vasoconstrictor molecules.

FXR also appears to regulate vascular inflammation and, thereby, may affect generation of atherosclerosis. In vascular smooth muscle cells, FXR ligands inhibited IL-1β-mediated induction of iNOS and COX-2.[79] Apolipoprotein E-deficient (*ApoE*$^{-/-}$) mice fed INT-747, a CDCA derivative, for 12 weeks had 95% reduction in aortic plaque formation; reduced aortic expression of IL-1β, IL-6, and CD11b mRNA; and increased expression of FXR.[80] FXR was also induced during osteogenic transformation of bovine calcifying vascular cells (CVCs) and in the aorta of partially nephrectomized *ApoE*$^{-/-}$ mice.[81] In CVCs, INT-747 inhibited phosphate-induced mineralization and triglyceride accumulation, a feature of atherosclerotic calcification. FXR inhibition augmented mineralization of CVCs and blocked the anticalcific effect of INT-747. It is not clear if TGR5 plays a role in some effects of INT-747 in these models; the role of FXR in atherosclerosis appears to be complex and needs to be further elucidated.

Interestingly, *Fxr*$^{-/-}$ mice have a proatherogenic lipid profile but do not display enhanced atherosclerosis, even when fed a high-fat/high-cholesterol diet.[72, 82, 83] *Fxr*$^{-/-}$male mice generated on an *ApoE*$^{-/-}$ background and fed an atherogenic diet had more severe atherosclerosis and reduced survival compared to controls.[82] In contrast, Guo et al. reported fewer atherosclerotic lesions in female *Fxr*$^{-/-}$/*ApoE*$^{-/-}$ mice.[84] These conflicting observations suggest that studies are needed to adequately assess the functional impact of long-term FXR stimulation and inhibition, and further *in vivo* studies, using organ-specific gene ablation, are required to determine the impact of BA–FXR interactions on atherosclerosis. In addition, the role of vascular FXR in regulating vascular tone in cirrhosis or ICP has not been evaluated. Specifically, mice with selective knockout of *Fxr* in endothelial and vascular smooth muscle may be needed to delineate the role of vascular FXR in BA-mediated changes in vascular tone.

Compared to FXR, the roles of PXR and VDR in vascular function have received little attention. PXR mRNA is expressed in mouse mesenteric arteries, but it is not clear if PXR is expressed in endothelial cells, vascular smooth muscle cells, or both.[27] Compared to nonpregnant mice, arteries from pregnant mice have reduced phenylephrine-induced constriction and enhanced bradykinin-induced vasodilation, an effect that is eliminated by PXR knockout.[27] Further experiments suggested that PXR-dependent increases in vasorelaxation may be caused by activation of cytochrome P450 epoxygenases. In contrast, no studies evaluated the role of VDR in vascular tone. Although VDR activators reduce vascular calcification, a role for BAs has not been shown.[22]

Finally, evidence regarding BA-mediated neural regulation of vascular tone is limited. Patients with obstructive jaundice have decreased sympathetic and vagal components of the baroreflex that correlates inversely with plasma ANP levels.[85] Preserved baroreflex is associated with improved survival in sepsis.[86] However, these observations are preliminary and studies in isolated arterial preparations are lacking.

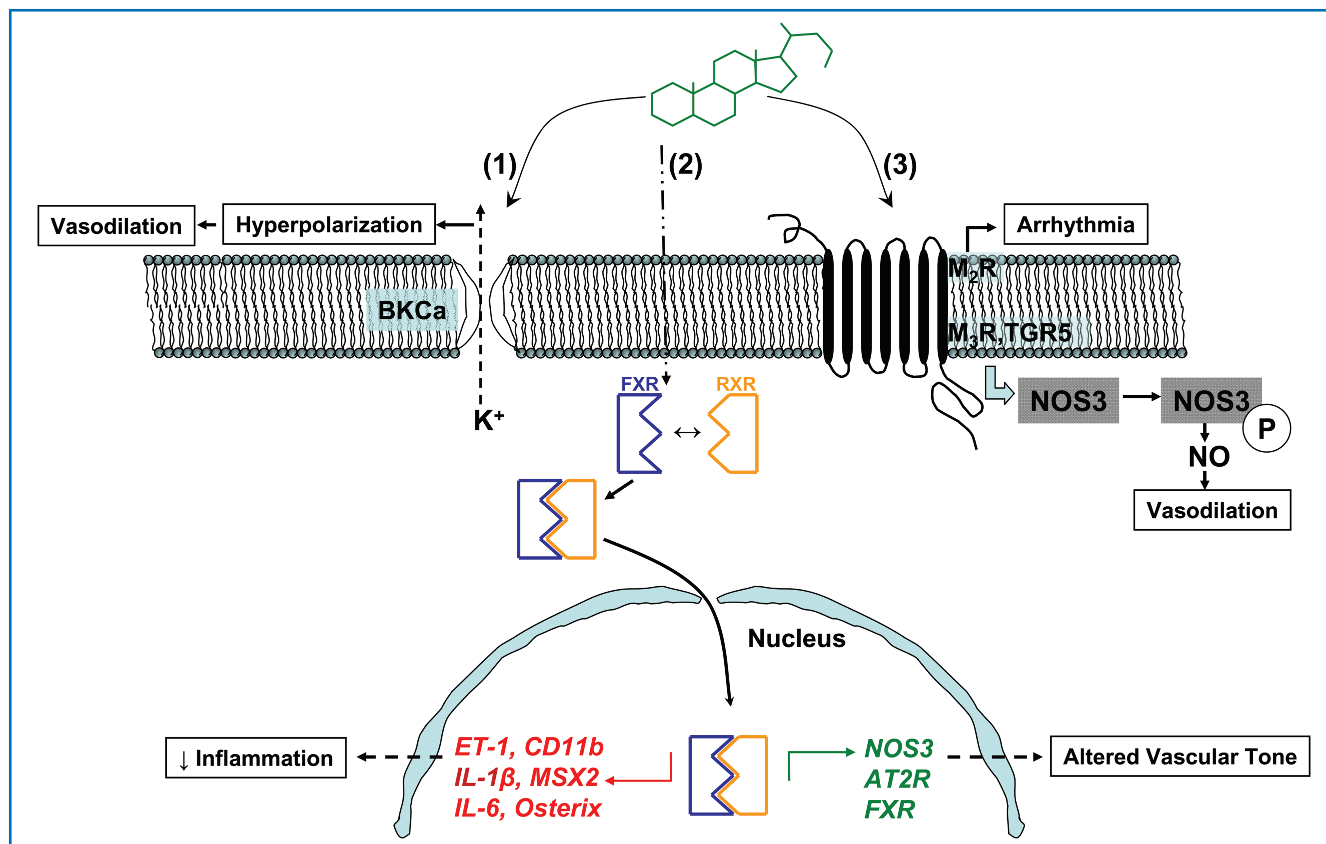

Figure 2. This cartoon outlines the interaction of BAs with various cellular molecules. (A) Nonreceptor-mediated interaction of BAs with BKCa that leads to K^+ efflux and hyperpolarization and relaxation of vascular smooth muscle. (B) BA interaction with nuclear receptors. In the cytoplasm, BA binding to FXR triggers dimerization with RXR that leads to translocation of FXR into the nucleus, where FXR binds to target gene regulatory elements. Downward (red) and upward (green) arrows indicate down-regulation and up-regulation of molecules, respectively. MSX2 and osterix are osteogenic transcription factors. (C) BA interaction with GPCRs that can lead to negative chronotropic response (for M_2R) in cardiac myocytes and NO generation (for M_3R and TGR5) in endothelial cells. AT2R: angiotensin type 2 receptor ; BKCa: big potassium, calcium-activated channels; CD 11b: cluster of differentiation 11b; ET-1: endothelin-1; IL-1,6: interleukin-1 and -6); M_2R: muscarinic receptor subtype 2; M_3R: muscarinic receptor subtype 3); MSX2: muscle segment homebox 2; NOS3: nitric oxide synthase 3.

BAs and Neovascularization

Neovascularization in response to injury results in organ repair or dysfunction. Cirrhosis is characterized by intrahepatic vascular remodeling with capillarization of sinusoids, formation of intrahepatic shunts, and extrahepatic portal-systemic collaterals.[87] Due to the shift of the BA pool to the systemic circulation, the role of BAs in neovascularization associated with cirrhosis is plausible. *In vitro*, CDCA increased endothelial cell motility, matrigel tube formation, and focal adhesion plaques; all were inhibited by FXR siRNA.[88] In a xenograft mouse model of human esophageal cancer, intraperitoneal administration of CDCA (50 mg/kg twice weekly for 5 weeks) increased tumor volume, neovascularization, and COX-2-dependent production of vascular endothelial growth factor.[89] In contrast, in chick embryo chorioallantoic membrane assay, UDCA-inhibited angiogenesis.[90] Further, in a rat model of laser-induced choroidal neovascularization (CNV), intraperitoneal administration of UDCA (500 mg/kg) and UDCT (100 mg/kg) for 14 days reduced fluorescein leakage and the size of CNV lesions.[91] Contrasting effects of CDCA and UDCA on promotion and inhibition of angiogenesis, respectively, suggest that different BAs may have opposing effect on neovascularization. However, studies evaluating the role of BAs in neovascularization in animal models of cirrhosis are lacking.

Conclusions and Perspective

BAs regulate cardiovascular function by receptor-dependent and -independent mechanisms and can modify vascular tone by interacting with muscarinic receptors and transcription factors such as FXR and PXR (*Figure 2*). Although some studies failed to demonstrate direct interaction of BAs with FXR, the bulk of evidence indicates that short-term effects of BAs induce vasodilation and long-term effects modulate transcription of vasoactive molecules. These studies defined hormone-like actions of BAs and their pharmacological potential to regulate cardiovascular function. Many questions remain regarding the interaction of BAs with cardiovascular tissue. For example, while FXR activation modifies expression of vasoactive and proatherogenic molecules, the mechanistic role of BAs other than CDCA remains to be defined. FXR is activated by unconjugated CDCA and DCA, whereas conjugated forms of these BAs activate FXR only in cells that coexpress BA transporters. While expression of BA transporters such as MDR3 and OATP in cardiac myocytes is established and a few reports suggest that similar anion transporters are expressed in choroid plexus endothelium,[92,93] their expression and role in the systemic vasculature remains to be elucidated. Similarly, the role of PXR, VDR, and TGR5 in regulating vascular and cardiac function has received minimal

attention. Despite expression of VDR and TGR5 in cardiovascular tissue, evidence regarding BAs interaction with these receptors in regulation of cardiovascular function is at best circumstantial.

The ability of BAs to interact with transcription factors, a variety of GPCRs (TGR5, M_3R, and M_2R) and potassium channels indicates that BAs are promiscuous signaling molecules. While such interactions may mediate acute events such as vasodilation, long-term events have not been adequately investigated. Studies are needed to provide mechanistic insight into long-term effects of GPCR–BA interactions and their role in resetting cardiovascular function. These insights may be particularly relevant for disorders such as cirrhosis and ICP, where serum BAs concentrations can be very elevated. Amidation and hydroxylation play major roles in BA solubility and interaction. The effect of BA hydroxylation on BK channel activity was elegantly demonstrated by Dopico et al.[39] Similarly, the effect of hydroxylation on BA interaction with muscarinic receptors has been explored.[29] However, the impact of amidation on BA interaction with vascular transcription factors is unknown.

Vascular beds differ in the mechanisms that regulate vascular tone. While TGR5 appears to play a role in regulating the hepatic microcirculation, the impact of BAs in regulating vascular tone in the renal or cerebral circulation is not clear. Renal failure is a major cause of mortality in end-stage liver disease and FXR is expressed in renal tissue; however, few studies have identified a role for BAs in regulation of the renal circulation.[94] It is clear that BAs regulate cardiovascular function by multiple mechanisms. In view of the findings, such as, cardiovascular expression of FXR, PXR and TGR5, and BA-mediated activation of K^+ channels, the use of tissue-selective knockout mice is likely to provide additional mechanistic insight into the actions of BAs in cardiovascular tissue.

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