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COMMENTARY

BILE ACIDS AS ENDOGENOUS VASODILATORS?

ARIEH BOMZON*†‡ and PREDRAG LJUBUNCIC*

*Department of Pharmacology, Bruce Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Haifa, Israel; and †Division of Gastroenterology, Department of Medicine, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

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The idea that bile acids are endogenous vasodilators originated almost 100 years ago from experiments in which the effects of bile on the cardiovascular system were evaluated [1, 2]. Such studies showed that bile, when injected intravenously into anesthetized dogs, caused hypotension. In 1932, Meakins [3] described the effects of "catarrhal jaundice" on a patient with essential hypertension. He noted that with the onset of jaundice, the blood pressure of this patient fell rapidly to normal and only returned to its elevated value long after the jaundice had disappeared. Referring to the earlier studies of Meltzer and Salant [1] and King and Stewart [2], he postulated that the cause of the fall in blood pressure in his patient was due to a hepatic metabolite that normally was excreted in bile but had accumulated in the plasma of the jaundiced patient.

Of the many substances that are normally excreted in bile but can accumulate in the plasma of patients with liver disease, bile acids were regarded as potential hypotensive compounds because of their cellular toxicity; and the early studies, by and large, focussed on the effects of bile acid on cardiac function [4, 5]. These early experiments established that bile acids caused negative inotropism and chronotropism (subsequently confirmed by others [6–9]). It was assumed that the tendency to hypotension in jaundiced patients was due to this cardiodepressant action of bile acids.

In the early eighties, several groups showed that bile acids have vasodilator properties [10–12], and this, too, has since been confirmed by others [13–15]. Recently, Tominaga *et al.* [16] showed that bile acids are able to reduce blood pressure, as well as attenuate *in vitro* vascular reactivity to norepinephrine in spontaneously hypertensive rats. Since this mechanism is not the only process whereby vasodilatation can be induced, we and others have examined the effects of bile acids upon other known mechanisms that contribute to the maintenance of vascular tone, and these observations are the subject of this commentary.

Before discussing the effects of bile acids upon some of the known determinants of vascular tone, we feel it necessary to preface our comments with some basic information pertaining to their biosynthesis and physico-chemical properties. Additional and more specific information can be found in the comprehensive reviews written by Heaton [17], Hofmann [18] and Radominska *et al.* [19].

Bile acid biosynthesis

Bile acids are steroids synthesized from free cholesterol in the hepatocyte, and the conversion of cholesterol into bile acids represents the major pathway for cholesterol elimination from the body. They are essential for the solubilization of lipids in bile, the induction and possible maintenance of bile flow, and the absorption of fat from the gastrointestinal tract. In humans, two primary types, cholic acid and chenodeoxycholic acid, are synthesized, with the synthesis rate of cholic acid being about twice that of chenodeoxycholic acid. These primary bile acids can be conjugated by amidation with the amino acids glycine and taurine,

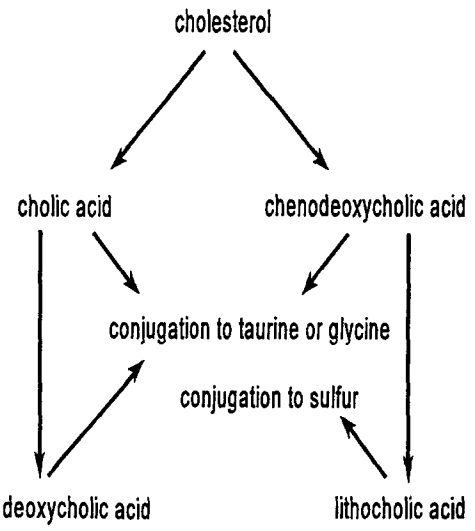

Fig. 1. Hepatic synthesis of the major bile acids from cholesterol in humans.

‡Corresponding author: Dr. Arieh Bomzon, Department of Pharmacology, Bruce Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, P.O. Box 9649, Haifa, Israel 31096. Tel. 972-4-295259; FAX 972-4-513145.

Table 1. Hydrophobicity indices of the ionized forms of unconjugated and the taurine and glycine conjugates of various bile acids

Bile acid	Unconjugated	Taurine conjugate	Glycine conjugate
Lithocholic acid		+1.00	+1.05
Deoxycholic acid	+0.72	+0.59	+0.65
Chenodeoxycholic acid	+0.59	+0.46	+0.51
Cholic acid	+0.13	0.00	+0.07
Ursodeoxycholic acid	−0.31	−0.47	−0.43

The indices of taurocholic acid and taurolithocholic acid were set arbitrarily to 0 and 1, respectively. (Modified from Heuman [20], and cited with permission.)

sulfated, or glucuronidated within the liver to give rise to the conjugated bile acids, or undergo bacterial transformation or be dehydroxylated in the intestine to give rise to secondary bile acids, such as deoxycholic acid (Fig. 1).

Conjugation and the presence of hydroxyl groups on the steroid nucleus increase their hydrophilicity, and it is common to refer to "the hydrophilic–hydrophobic balance" of an individual bile acid [20]. The hydrophilic–hydrophobic balance or the relative balance between the hydrophobic and hydrophilic properties of the naturally occurring bile acids is determined by their state of ionization, the orientation, position and number of hydroxyl groups, and by the presence of the side chain ester, be it taurine, glycine, sulfate or glucuronate. Heuman [20] has derived a "hydrophobic index" for bile acids based upon the standard free energy change for partition of bile acids between a polar mobile phase and a non-polar stationary phase for quantifying bile acid structure–activity relationships. Table 1 presents the "hydrophobic index" of some of the bile acids mentioned in this commentary.

The highest plasma concentrations of bile acids are found in the portal vasculature because of their enterohepatic circulation. The total systemic bile acid concentration is less than 1 μM/L. Bile acids (predominantly as amine conjugates) circulate in the blood bound to albumin [17, 18], and under conditions when the plasma bile acid concentration exceeds the capacity of the high-affinity class of albumin binding sites, they become bound to lipoproteins [21]. The total free plasma bile acid concentration is low, in the order of nM/L. When the total systemic bile acid concentration rises to as high as 100 μM/L, as it often does in liver disease, the ratio between free and bound bile acids increases as the plasma concentrations rise. Since the bile acid pool contains a mixture of bile acids, plasma bile acid profiles vary with the different types of liver disease depending on the nature of the hepatocellular damage, the degree of biliary obstruction, and the presence of portosystemic shunts.

As a group, the bile acids are amphipathic compounds with physico-chemical properties enabling them to interact with biological membranes by insertion into the lipid bilayer with subsequent solubilization. These effects depend to a great extent on the hydrophilic–hydrophobic balance and the concentration of the bile acid, with hydrophobic bile acids being more active than high concentrations of hydrophilic bile acids. The concentration at which bile acids interact with membranes to form polymolecular aggregates or micelles is called the critical micellar concentration (CMC), and this usually occurs at concentrations greater than 1 mM. The maximum total plasma bile acid concentration in severe liver disease is lower than the CMC of actual bile (1.45 mM) or an equimolar mixture of taurine-conjugated di- and trihydroxy bile acids (1.6 mM). Hence, the vasorelaxant action of bile acids cannot be attributed solely to a detergent action.

Vasodilatation

It is well known that the free intracellular calcium concentration $[Ca^{2+}]_i$ determines vascular tone [22], and the regulation of $[Ca^{2+}]_i$ is dependent upon several calcium-transport and calcium-binding processes at the level of the plasma membrane [23]. It is not the purpose of this commentary to discuss the details of each of these calcium-regulating processes pertaining to vasorelaxation. Instead, a simplistic view of vasodilatation will be presented, together with a review of our current knowledge of the effects of bile acids on each of these processes. Fortunately, this reductionist approach has been made even more simple since bile acids do not cross the cell membrane of vascular smooth muscle cells to influence intracellular processes that participate in the regulation of $[Ca^{2+}]_i$ [15]. Hence, the effects of bile acids on only those cell membrane processes need be discussed. Thus, one can discuss vasodilatation simply in terms of processes that antagonize contraction and those that induce relaxation.

Thus, relaxation can be induced by:

1. Antagonism of surface membrane receptors that utilize phosphoinositide hydrolysis as the second messenger and, when activated, cause contraction.
2. Inhibition of phosphoinositide hydrolysis.
3. Activation of surface membrane receptors linked to the cAMP second messenger pathway, which, when activated, cause relaxation.
4. Activation of the cAMP second messenger pathway.
5. Activation of the surface membrane receptors linked to the cGMP second messenger pathway, which, when activated, cause relaxation.
6. Activation of the cGMP second messenger pathway.

7. Preventing transmembrane influx of extracellular calcium by blocking voltage-dependent calcium channels.
8. Enhancing potassium efflux by opening potassium channels, thus reducing transmembrane calcium influx leading to a reduction in the $[Ca^{2+}]_i$ and relaxation.
9. Increasing the cellular extrusion of $[Ca^{2+}]_i$ through activation of the calcium pump or sodium–calcium (Na^+–Ca^{2+}) exchange, leading to a reduction in the $[Ca^{2+}]_i$ and relaxation.

Bile acids and antagonism of surface membrane receptors

Of the many receptors that are present on the cell membrane of the vascular smooth muscle cell and utilize phosphoinositide hydrolysis as the second messenger, the effect of bile acids on the α_1-adrenoceptor is the most well studied. Bomzon and Tominaga and their respective colleagues have shown that the unconjugated and the glycine and taurine conjugates of cholic acid blunt the contractile response to norepinephrine in a concentration-dependent manner [12, 16]. Moreover, the degree of attenuation is greater with a hydrophobic bile acid, such as deoxycholic acid, than with a less hydrophobic (or more hydrophilic) bile acid, such as taurocholic acid. We recently confirmed this hydrophobic bile acid-dependent relationship on attenuation by showing a linear correlation between the degree of attenuation of the *in vitro* contractile response to norepinephrine and bile acids whose hydrophobic indices are greater than +0.25 [24].

In addition, we have demonstrated that hydrophobic bile acids over the concentration range of 10^{-6}–10^{-4} M/L attenuated the *in vitro* contractile responses to the α_1-adrenoceptor agonist, phenylephrine, and 5-hydroxytryptamine [24]. The inhibitory effect of taurodeoxycholic acid on the α_1-adrenoceptor in the rat portal vein, superior mesenteric arterial ring, and perfused mesentery has also been described recently by Pak *et al.* [15]. Using a competitive antagonist radioligand binding assay with [^{3}H]prazosin in a rat vascular smooth muscle membrane preparation with and without 10^{-4} M/L deoxycholic acid, we demonstrated that the bile acid reduced the affinity of the α_1-adrenoceptors without changing the number of receptors [24]. When an equimolar concentration of taurocholic acid was used, the binding constants for the α_1-adrenoceptors were not affected when compared with controls [24]. In another recent study to understand the vasodilator action of bile acids, Pak *et al.* [15] found that taurodeoxycholic acid attenuates the pressure response to arginine-vasopressin (V_1 receptors) in the isolated perfused rat mesentery.

Since receptor activation is also linked to the opening of receptor- or ligand-operated calcium channels, it is also possible that the bile acids interfere with transmembrane calcium flux using this pathway. In 1988, Better and Bomzon [25] presented evidence that receptor-operated calcium channels were unaffected by bile acids. In contrast, Pak *et al.* [15] suggested that these channels are adversely affected by bile acids restricting calcium entry.

Bile acids and phosphoinositide hydrolysis

The initiation of phosphoinositide hydrolysis involves stimulation of the G-protein, which then activates phospholipase C leading to the hydrolysis of phosphatidylinositol-4,5-biphosphate (PIP_2) and a subsequent increase in $[Ca^{2+}]_i$ mediated by diacylglycerol and inositol triphosphate (IP_3) and the activation of protein kinase C. There are no published reports in smooth muscle, be it vascular or non-vascular, on the effects of bile acids on this pathway or on ligands, such as adenosine, that inhibit this pathway. However, there are several reports describing both stimulatory and inhibitory effects of various bile acids upon protein kinase C activity in epithelial cells where bile acids do gain entry into the intracellular environment [26, 27]. These studies have demonstrated that amphipathic bile acids interact with the phospholipid-binding domain to stimulate protein kinase C by providing the hydrophobic environment required for its activation. Although these studies may not be applicable to bile acid-induced vasorelaxation, it is possible that some of the membrane events may actually take place. These studies need to be repeated in the vascular smooth muscle to determine if these events are relevant to bile acid-induced vasorelaxation.

Bile acids and activation of surface membrane receptors linked to the cAMP second messenger system

To date there are only studies that have examined the effect of bile acids on the response of surface membrane receptors linked to the cAMP second messenger system, and the results are conflicting. Four hours after the obstruction of the bile duct in an anesthetized dog and the subsequent plasma accumulation of bile constituents, including bile acids, Levy *et al.* [28] showed that the renal vasodilator response to dopamine was blunted. In contrast, Pak *et al.* [15] reported that the vasodilating effect of taurodeoxycholic acid in various vascular preparations was unaffected by propranolol, suggesting that bile acid-induced vasorelaxation is not mediated by β_2-adrenoceptors.

There are also several reports indicating that bile acids may exert their relaxant action on vascular smooth muscle through activation of the cAMP second messenger pathway. Sunagane *et al.* [29] showed that the bile acids ursodeoxycholic acid and deoxycholic acid produced their relaxant action by accelerating calcium efflux in the isolated guinea pig gall bladder. This effect was coupled with elevated levels of tissue cyclic AMP content and inhibition of calcium uptake. Dibutyryl cyclic AMP mimicked the effects of the bile acids on calcium efflux and muscle relaxation but showed no effect on cellular calcium uptake. The stimulatory effect of bile acids upon cAMP has also been shown in a non-muscular preparation by Potter *et al.* [30]. Using the adult rabbit distal colon *in vitro*, they showed that 50 μM taurodeoxycholic acid significantly increases cyclic AMP, leading to enhanced chloride secretion.

Bile acids and activation of surface membrane receptors linked to the cGMP second messenger system

In 1984, Levy *et al.* [28] demonstrated that bile constituents could alter the sensitivity of the renal vasculature to various vasodilators that utilized the cGMP second messenger system, such as bradykinin and acetylcholine. At that time, it had only recently been established that acetylcholine exerted its vasodilator action through the release of endothelium-derived relaxant factor [31]. Today, it is well established that the mechanism of vasodilatation of acetylcholine and bradykinin is mediated by the release of nitric oxide from the endothelial cell, which diffuses into the vascular smooth muscle to activate guanylate cyclase to cause relaxation [32, 33]. In addition to activation of this pathway by nitric oxide, other endogenous compounds, such as atrial natriuretic peptide, stimulate surface membrane receptors that utilize this same pathway to effect vasorelaxation [34]. Hence, the effects of bile acids upon two different pathways need addressing. First, do bile acids modify the release of endothelium-derived relaxant factors such as nitric oxide and prostacyclin? Second, do bile acids modify the response of surface membrane receptors on vascular smooth muscle cells that utilize cGMP as the second messenger?

In response to the first question, Parl *et al.* [35] showed that bile duct ligation in the rat, where plasma bile acid concentrations are elevated, causes severe configurational changes of the endothelium cell. If these conformational changes act in the same manner as shear stress [36–38], it may be argued that bile acids promote the release of nitric oxide and prostacyclin release. Said *et al.* [39] showed that hydrophobic bile acids could relax precontracted endothelial-intact arterial rings with the EC_{50} of vasodilator response curves to deoxycholic acid and chenodeoxycholic acid being 2 μM in both instances. It has since been demonstrated that this relaxation still occurs when the arterial ring is denuded of its endothelium [15, 24] or following inhibition of nitric oxide synthase [15]. Finally, Calcamuggi *et al.* [40] showed that bile acids depress endothelial release of prostacyclin, providing further evidence that bile acid-induced vasorelaxation is not endothelium dependent.

In response to the second question, there are no published studies of the effects of bile acids on surface membrane receptors on vascular smooth muscle cells that utilize cGMP as the second messenger system.

Bile acids and voltage-dependent calcium channels

The effect of bile acids on voltage-dependent channels has not been investigated extensively, but the reports are unequivocal. Said *et al.* [39] and Pak *et al.* [15] have both shown that contractions induced by KCl in isolated rat arterial rings and the isolated perfused mesentery, respectively, are reduced significantly by preincubation with bile acids. Moreover, Said *et al.* [24] found that this effect is species dependent, with hydrophobic acids attenuating the response at lower molar concentrations than hydrophilic acids do. From these studies, it appears that the vasodilator effect of bile acids may be mediated by interfering with voltage-dependent calcium channels. Other investigations using non-vascular cells have also shown that bile acids interfere with voltage-dependent calcium channels [9, 41, 42].

Bile acids and potassium channels

Two different studies have shown that the relaxant action of bile acids is not mediated by potassium channels [15, 43]. Although these studies are the only ones involving a smooth muscle preparation, the results of studies in other non-vascular systems have shown that bile acids can affect potassium channels or increase potassium conductance. Kotake *et al.* [41] showed that bile acids increase potassium efflux in the rabbit sino-atrial node; and Devor *et al.* [42] showed that 750 μM taurodeoxycholic acid, but not 100 μM taurocholic acid, increases potassium conductance in isolated colonic epithelial cells [42].

Bile acids and the calcium pump

Since bile acids are vasodilators, one would anticipate that bile acids may activate the calcium pump to hasten the extrusion of $[Ca^{2+}]_i$. This has not been studied extensively in vascular smooth muscle cells and, based upon studies in epithelial cells, it appears that calcium efflux is enhanced by bile acids. The effect of bile acids on transmembrane calcium flux has been studied extensively in isolated hepatocytes and other *in vitro* systems where it has been shown that bile acids actually increase $[Ca^{2+}]_i$ by promoting calcium uptake in hepatocytes, behaving as calcium ionophores. Using red blood cells to investigate the mechanism of bile acid-induced hemolysis, Oelberg *et al.* [44] observed that bile acids at concentrations below the CMC stimulated $^{45}Ca^{2+}$ uptake 4- to 25-fold, the magnitude of which was related, in part, to the hydrophobicity of the bile acids. Subsequently, Zimniak *et al.* [45] showed that taurine conjugates of bile acids behave as calcium ionophores, causing an increase in $[Ca^{2+}]_i$ through mechanisms that bypass the regulatory systems that maintain cellular calcium homeostasis. Since then, other groups have shown that bile acids can increase $[Ca^{2+}]_i$ [46, 47].

Bile acids and sodium–calcium exchange

Two studies have shown that incubating smooth muscle with 1 or 10 μM ouabain does not modify the vasorelaxant action of taurocholic acid, taurodeoxycholic acid or taurochenodeoxycholic acid [15, 43], ruling out the participation of Na^+–K^+ ATPase or membrane hyperpolarization. More definitive proof of involvement of the sodium–calcium exchanger was provided recently by Romero *et al.* [43]. Using cells from guinea pig ileum smooth muscle strips, they showed that taurocholic acid at concentrations up to 1 mM (a concentration below the CMC) stimulates Na^+ uptake and Ca^{2+} efflux and relaxes guinea pig ileum smooth muscle strips and cultured cells [43]. They also showed that these effects could be blocked with 3′,4′-dichlorobenzamil, corroborating the involvement of the sodium–calcium exchanger.

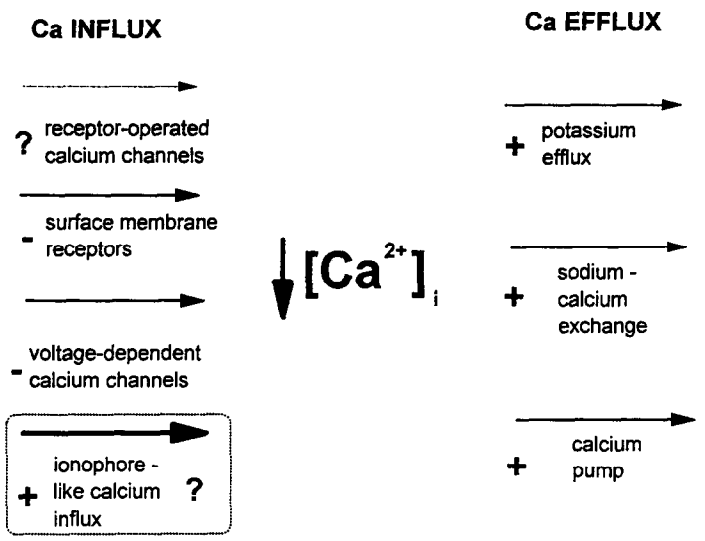

Fig. 2. A schematic diagram outlining the calcium regulatory processes present in the cell membrane of vascular smooth muscle. Key: (+) represents a stimulatory effect; (−) represents an inhibitory effect; and (?) represents an effect whose significance is unknown or questionable.

Synthesis

In interpreting these studies, it appears that the mechanism of action of bile acids, in particular the hydrophobic species, is to limit calcium entry, leading to a fall in $[Ca^{2+}]_i$ or preventing its rise in vascular smooth muscle cells (Fig. 2). This effect is achieved by preventing calcium entry through voltage-dependent calcium channels and acting as non-selective antagonists to surface membrane receptors that utilize phosphoinositide hydrolysis as the second messenger. Through this non-selective antagonism, calcium entry via receptor-operated channels may be reduced, and this, too, contributes to restricted calcium entry into the cell and the fall in $[Ca^{2+}]_i$. Although surface membrane receptor systems that lead to vasorelaxation appear to be affected adversely by the bile acids, their role in bile acid-induced vasodilatation is unclear. The calcium ionophore-like action of bile acids, described in epithelial cells, has yet to be found in vascular smooth muscle cells and, until demonstrated, this phenomenon has no relevance to the mechanism of bile acid-induced vasorelaxation. The effects of bile acids on the membranal processes of calcium efflux have not been well studied, and there is preliminary but compelling data to indicate that bile acids may activate these processes. The mechanisms of their activation need elucidation, especially if they are activated in response to increases in $[Ca^{2+}]_i$; and here the calcium ionophore-like action of the bile acids may become relevant, as it may be the trigger to activate these processes. Thus, if this ionophore-like effect can be shown to also exist in vascular and non-vascular smooth muscle cells, it will be necessary to re-evaluate the mechanisms of bile acid-induced vasorelaxation with emphasis on the processes of intracellular calcium sequestration and extracellular calcium extrusion.

Mediators of bile acid-induced vasorelaxation

What mediates the vasorelaxant action of bile acids? There are very few studies that have examined the mechanism of action and mediators of bile acid-induced vasorelaxation, and here it is necessary to draw upon our knowledge from other cell systems where this has been studied more extensively and to extrapolate the data to the vascular smooth muscle cell. Bile acid-induced changes in membrane fluidity and bile acid-induced generation of oxygen free radicals have been proposed. Bile acids can stimulate membrane phospholipid turnover, leading to a rise in intracellular free arachidonic acid, which, in turn, leads to an increase in the local concentration of vasoactive eicosanoids such as prostacyclin. As mentioned previously, bile acids depress prostacyclin release from the endothelium [40]. If bile acids depress eicosanoid synthesis in the vascular smooth muscle cell, it is possible that bile acid-induced vasorelaxation is not mediated through this pathway.

Bile acids and membrane fluidity

Cell membranes have fundamental physiological functions, in addition to their action as selective boundaries. Actually, most of the cellular biochemical and biophysical events occur or are initiated in the membrane where strict structural and dynamic features provide the control mechanisms [48]. Cell membranes consist of a dynamic, phospholipid cholesterol bilayer in which proteins are embedded and transverse the lipid core. In an imperfect analogy, intrinsic membrane proteins such as receptors, enzymes, transporters or ion channels could be considered as solute molecules in a lipid solvent. The membrane bilayer is in a fluid state, enabling membrane proteins to migrate within this "two-dimensional" fluid. An optimal fluidity and

Table 2. Effect of incubating rat vascular smooth muscle membranes with 10^{-4} M deoxycholic acid on cholesterol and phospholipid content

	Cholesterol (μg/mg protein)	Phospholipid (μg/mg protein)
Control	224.4 ± 5.8	315.5 ± 18.4
10^{-4} M Deoxycholic acid	226.3 ± 7.2	315.6 ± 15.9

Values are means ± SD, N = 20.

lipid environment hypothesis has been proposed and confirmed by receptor function studies [49].

The term "membrane fluidity" can be defined as the relative motional freedom of the lipid molecules. Molecular motion within membranes is restricted to two dimensions (anisotropy) [50]. The determinants of fluidity are numerous and include the degree of saturation and length of the fatty acyl side chains, the size and shape of the phospholipid head groups, the cholesterol:phospholipid molar ratio, and the protein content. Thus, the membrane environment contributes to the function of the membranes by providing a medium for membrane-related functions, such as correct signal transduction, orderly transport, and ion influx and efflux.

Bile acids can disperse water-soluble lipids into clear aqueous solutions. Hence, it is possible that they can increase the fluidity of membranes. We have studied the effect of membrane-fluidizing agents such as 2-(2-methoxyethoxy) ethyl 8-(*cis*-2-*n*-octyl-cyclopropyl)octanoate (A_2C), on *in vitro* vascular responsiveness. The contractile response to different agonists is indeed attenuated [24]. We have also determined the effect of bile acids on vascular smooth muscle membrane fluidity by measuring the cholesterol and phospholipid content and by fluorescent anisotropy with the probe 1,6-diphenyl-1,3,5-hexatriene (DPH). Table 2 shows the effect of 10^{-4} M deoxycholic acid on vascular smooth muscle membranes prepared from rat aortae. At this concentration, the hydrophobic bile acid did not modify the cholesterol or phospholipid content of membranes after 40 min of incubation, suggesting that bile acids do not modify the cholesterol:phospholipid molar ratio, which is one of the determinants of membrane fluidity.

We have confirmed this finding by fluorescent anisotropy using DPH as a probe. Figure 3 presents the results of an experiment in which the effect of increasing concentrations of deoxycholic acid on vascular smooth muscle membranes prepared from rat aortae was investigated. Over the concentration range 10^{-6} –10^{-4} M, deoxycholic acid did not alter membrane fluidity when compared to different concentrations of A_2C.

Bile acids and oxygen free radical generation

Hydrophobic bile acid-induced membrane damage appears to involve the production of oxygen free radicals. In epithelial cells, hydrophobic bile acids such as deoxycholic acid and chenodeoxycholic acid increase membrane phospholipid turnover and oxygen free radical production [51]. The generation of free radicals can lead to tissue injury by attacking membrane lipids, thiol proteins, or nucleic acid causing lipid peroxidation, which, in turn, leads to accumulation of highly toxic products: lipoxides, such as 4-hydroxynonal, and malondialdehyde [51]. Evidence for the role of oxygen free radicals mediating bile-acid induced membrane damage was provided recently by Sokol *et al.* [52], who showed that bile acids, especially the hydrophobic species, enhance lipid peroxidation in hepatocytes. We have shown that deoxycholic acid causes a concentration-dependent increase in lipid peroxidation, as measured by the generation of malondialdehyde in a rat vascular smooth muscle membrane preparation (Fig. 4).

In our minds, these preliminary studies confirm that increases in vascular smooth muscle membrane fluidity can alter vascular reactivity *in vitro*. At this stage, we have been unable to establish that bile acids do indeed increase membrane fluidity. One of the reasons may be that the DPH probe determines the static component of membrane fluidity [53], and bile acids may, in fact, increase the dynamic component, which is not measured by this probe. Hence, experiments on membrane fluidity involving probes to measure the dynamic component, such as 16-(9-anthroyloxy) palmitic acid (16-AP), and even other techniques need to be performed in order to establish whether bile acids do indeed increase membrane fluidity. On the other hand, we have demonstrated that bile acids can cause lipid peroxidation in vascular smooth membranes and, in doing so, have confirmed in this system what has been described in hepatocyte membranes. This being so, what is the relationship between vasodilatation or restricted calcium entry into vascular smooth muscle cells, membrane fluidity, oxidative attack and bile acids?

Vasodilatation, membrane fluidity and oxidative attack

In the 1980s, several groups of investigators observed that oxygen free radicals such as the superoxide anion radical, hydrogen peroxide, and the hydroxyl radical cause arteriolar relaxation with resultant vasodilatation [54]. The effect of oxidative attack on surface membrane receptors and ion channels in vascular smooth muscle membranes has not been studied. However, the effects on other receptors and enzyme transporters have been studied in other cell systems. Recently, Kaneko *et al.* [55] and Ghosh *et al.* [56] found that the affinities of cardiac α- and β-adrenoceptors, and neuronal muscarinic receptors, respectively, were depressed by oxygen free radicals. They speculated that the mechanism by which oxygen free radicals may modify these receptors or membrane proteins was initiated by membrane lipid peroxidation and the production of acyl derivatives and malondialdehyde. In another study, Jourd'Heuil *et al.* [57] demonstrated that peroxidation of the guinea pig microvillus membrane reduces sodium-dependent glucose transport but increases the rigidity of membrane, as measured by a reduction in membrane fluidity.

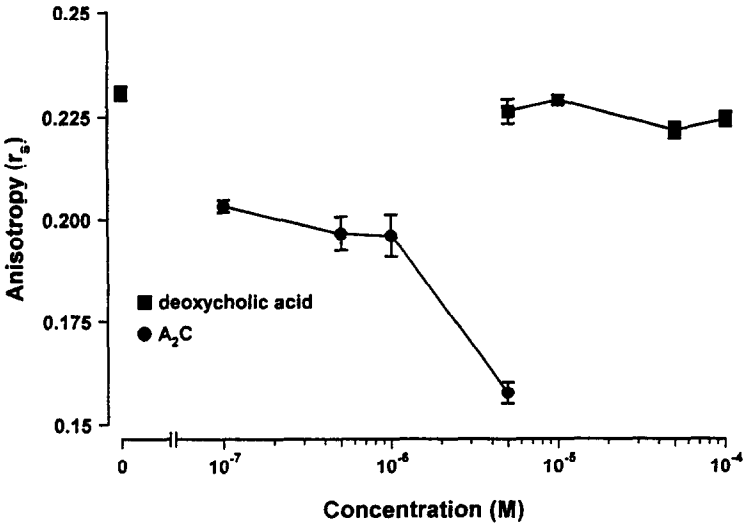

Fig. 3. Effect of increasing concentrations of deoxycholic acid and A_2C on rat vascular smooth muscle membranes. Values are means ± SD, N = 3–6 determinations at each point. (Bomzon A, Jardine G, Shaffer EA and Meddings JB, unpublished data.)

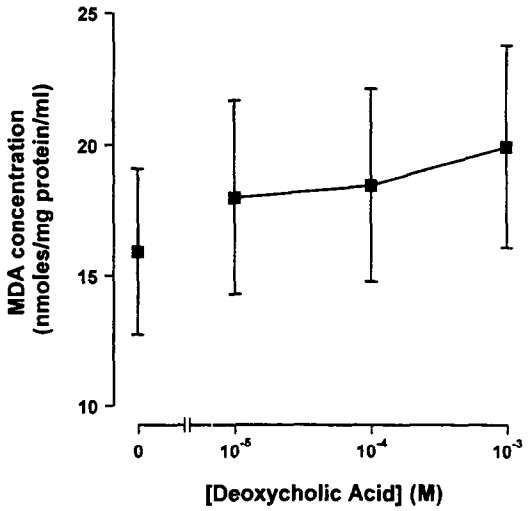

Fig. 4. Effect of increasing concentrations of deoxycholic acid on malondialdehyde (MDA) production in a crude rat aorta homogenate. Values are means ± SD, N = 7. (Ljubuncic P and Bomzon A, unpublished data.)

Conclusions

Since bile acids stimulate lipid peroxidation, presumably through the generation of oxygen free radicals, we propose that bile acid-induced oxidative attack damages those protein components of the vascular smooth membrane that regulate calcium influx, viz. surface membrane receptors and voltage-dependent calcium channels, to restrict calcium entry, leading to vasorelaxation. The role of bile acid-induced increases in membrane fluidity, be it a direct effect of the bile acids themselves, or secondary due to lipid peroxidation, needs further clarification. Although our preliminary data have not yet established that bile acids increase membrane fluidity, we still propose that a bile acid-induced increase in membrane fluidity may lead to a conformational change in the surface membrane receptors and calcium channels causing diminished function and restricted calcium entry. These factors represent the underlying mechanism whereby bile acids cause vasorelaxation. In view of the few groups studying the mechanism of bile-acid-induced vasorelaxation, it will take some time before sufficient knowledge and understanding of the action(s) of bile acids on cellular calcium homeostasis will be accumulated to validate this hypothesis on the mechanism of bile-acid induced vasorelaxation.

As a phenomenon, there is little doubt that bile acid-induced vasodilatation exists *per se*, and it has little physiological importance in the regulation of arterial vascular tone in healthy individuals. It probably has some importance in the portal vein circulation where it may participate in the portal vasodilatation associated with digestion when the portal vein concentrations of bile acids are high due to their enterohepatic circulation. In the pathophysiological environment of liver disease where the plasma bile acid concentrations are increased, bile acid-induced vasorelaxation may contribute, in part, to the tendency to hypotension in these patients. This occurs because the bile acids are no longer restricted to the enterohepatic circulation and spill over to enter the systemic circulation. Here, the free or unbound bile acids, especially the hydrophobic ones, need only increase from less than 1 μM/L to 5 μM/L to dilate resistance vessels and thus contribute to the cardiovascular complications of liver disease.

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