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Effects of Bile Acids on Hepatocellular Signaling and Secretion

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Bile acids modulate hepatocellular signaling pathways in vitro at physiological concentrations. The present paper provides a brief overview of the effects of bile acids on three key messengers in liver cells: cytosolic free calcium, protein kinase A and protein kinase C.

INTRODUCTION

Liver cell functions are modulated by a complex network of intracellular and transmembraneous signaling mechanisms. Not only hormones, but also bile acids have increasingly been recognized to trigger hepatocellular signaling mechanisms in vitro [1-12]. This article is aimed to give a brief overview on the present knowledge of bile acid-mediated modulation of hepatocellular signaling. It will focus on three key messengers of the hepatocellular signaling network: cytosolic free Ca^{++} $[Ca^{++}]_i$, protein kinase A (PKA), and protein kinase C (PKC).

EFFECTS OF BILE ACIDS ON $[Ca^{++}]_i$

Cytosolic free calcium $[Ca^{++}]_i$ is of key importance for the control of many cell functions, including secretion [13, 14]. $[Ca^{++}]_i$ levels in hepatocytes are 100-200 nM under resting conditions. Increases in $[Ca^{++}]_i$ are induced by depletion of inositol 1,4,5-trisphosphate (IP_3)-sensitive or ryanodine- and Ca^{++}-sensitive microsomal stores as well as by Ca^{++} influx across the plasma membrane [13, 14]. $[Ca^{++}]_i$ levels are renormalized by active, ATP-dependent Ca^{++} extrusion mechanisms across the plasma membrane or the membranes of the endoplasmic reticulum. $[Ca^{++}]_i$ levels are ten-thousand-fold lower than extracellular levels (10^{-3} M) and a thousand-fold lower than those within the endoplasmic Ca^{++} stores (10^{-4} M).

It has been assumed previously that bile acid-induced hepatotoxicity and cholestasis may also be mediated by changes in $[Ca^{++}]_i$ [1, 3, 4]. Because hydrophilic taurine- and glycine-conjugated ursodeoxycholic acid (TUDCA, GUDCA) protect hepatocytes from more hydrophobic bile acids in vitro and in vivo [15-17], we studied the effect of TUDCA in comparison to other bile acids on $[Ca^{++}]_i$ in groups of isolated hepatocytes in short-term culture using microspectrofluorometry [7, 8]. We observed that the hydrophilic bile acid TUDCA, at a physiological concentration of only 5 μM, induced a sustained increase of $[Ca^{++}]_i$ of about 400 nM [7], reflecting the maximum sustained response of hepatocytes

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[b] *Abbreviations:* $[Ca^{++}]_i$, cytosolic free calcium; DAG, sn-1,2-diacylglycerol; HRP, horseradish peroxidase; IP_3, inositol 1,4,5-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TLCA, taurolithocholic acid; TUDCA, tauroursodeoxycholic acid.

under physiological conditions [14]. The effect of TUDCA at low micromolar concentrations on $[Ca^{++}]_i$ was stronger than that of any other bile acid tested at equimolar concentrations [7] and was due to IP_3-independent depletion of IP_3-sensitive endoplasmic Ca^{++} stores and to stimulation of influx of extracellular Ca^{++} across the plasma membrane via Ni^{++}-sensitive Ca^{++} channels [7, 8, 10; Table 1]. In contrast to TUDCA, the primary bile acid TCA did not affect $[Ca^{++}]_i$ at low and high micromolar levels [1, 3, 7; Table 1]. The hydrophobic bile acid TLCA also induced an increase of $[Ca^{++}]_i$, but this $[Ca^{++}]_i$ increase was only transient [1, 5, 7; Table 1]. This may be due to lack of Ca^{++} influx [5] and/or due to activation of Ca^{++} extrusion mechanisms [2]. IP_3 formation was not affected by any of these bile acids [4, 7, 10] indicating that phosphoinositolbisphospate-dependent phospholipase C is not a target of bile acids (Table 1).

Table 1: Effects of bile acids on hepatocellular Ca^{++} homeostasis.

	TUDCA	TCA	TLCA
$[Ca^{++}]_i$	+	–	+
Ca^{++} influx	+	–	–*
Ca^{++} extrusion	–	–	+
Depletion of IP_3-sensitive Ca^{++} stores	+	–	+
IP_3 formation	–	–	–

From Refs.1-5, 7, 8, 10; * controversial data; see text for details.

A sustained $[Ca^{++}]_i$ increase is the key signal for the stimulation of regulated exocytosis in a number of secretory cells, including pancreatic acinar cells, platelets, and chromaffine cells [18]. In the liver cell, stimulation of exocytosis may lead to targeting and insertion of tranport proteins in the canalicular membrane, thereby regulating bile excretory function [19, 20]. In experimental cholestasis, the process of hepatocellular exocytosis is impaired [21, 22].

The effect of bile acids on hepatocellular exocytosis was studied in the isolated perfused rat liver. In livers preloaded with horseradish peroxidase (HRP), an established marker of the vesicular pathway, TUDCA, but neither TCA nor TCDCA induced a sustained stimulation of biliary HRP secretion [8]. This effect was Ca^{++}-dependent and was not observed when extracellular Ca^{++} was lowered to levels below 0.2 mM, low enough to prevent an agonist-induced sustained increase of $[Ca^{++}]_i$, yet adequate to prevent Ca^{++}-deprivation-induced cholestasis in the perfused rat liver [8]. Thus, TUDCA stimulates hepatocellular exocytosis by Ca^{++}-dependent mechanisms.

EFFECTS OF BILE ACIDS ON PROTEIN KINASE A

Cyclic AMP is the physiological activator of protein kinase A (PKA) and is formed from ATP by adenylate cyclase. Cyclic AMP is implicated in numerous hepatocellular processes including bile secretion [13]. The effect of dihydroxy bile acids on cAMP formation has been studied in isolated hamster hepatocytes [11]. UDCA and CDCA did not affect basal levels of cAMP although UDCA impaired glucagon-induced increases of cAMP in a protein kinase C-dependent way [11]. Thus, dihydroxy bile acids do not directly affect PKA activity in hepatocytes.

EFFECTS OF BILE ACIDS ON PROTEIN KINASE C

The protein kinase C (PKC) family of isoenzymes regulates numerous functions of the liver cell including bile formation [13, 23-25]. At least 10 different PKC isoforms have been identified in man [26, 27], and five of them (α, β II, δ, ε, ζ) have been described in liver cells [9, 12]. Activation of PKC isoforms involves 2 major steps [26, 27]: (i) translocation of the protein from cytosol to the membranes with binding to membrane phosphatidylserine residues, translocation of the conventional isoforms α, β, and γ being Ca^{++}-dependent; (ii) binding of the physiological activator sn-1,2-diacylglycerol (DAG) to the regulatory site of the PKC molecule leading to (iii) activation of the membrane-bound enzyme.

(i) Translocation of PKC isoforms by bile acids

TUDCA at low 10 μM concentrations induced translocation of the Ca^{++}-sensitive α-isoform from cytosol to the membranes as observed by Western blotting studies and confirmed by immunohistochemistry [9]. However, the δ-, ε-, and ζ-isoform were not affected by TUDCA in isolated hepatocytes in short-term culture [9]. Translocation of α-PKC by TUDCA was also observed in primary hepatocyte cultures by Stravitz et al. [12] as well as in isolated hamster hepatocytes by Bouscarel et al. [11]. Thus, TUDCA apparently induces translocation of only the Ca^{++}-sensitive α-isoform in hepatocytes at low micromolar concentrations (Table 2).

Table 2: Effect of bile acids on hepatocellular protein kinase C

	TUDCA	TCA	TLCA
Translocation to membranes of:			
α-PKC	+	–/+	– **
ß II-PKC	– *	– *	
δ-PKC	–	–/+	– **
ε-PKC	–	–/+	+ **
ζ-PKC	–	–	– **
DAG formation	+	+	+
Activation of membrane-bound total PKC	+	+	+

Compiled from Refs. 9,11,12; * controversial data; ** Beuers et al., Hepatology 26:262A, 1997; see text for details.

In contrast, immunoblotting and immunohistochemical studies did not reveal an effect of TCA at low 10 μM concentrations on the distribution of PKC isoforms in isolated hepatocytes in short-term culture [9] whereas TCA at higher concentrations caused translocation of the α, δ, and ε isoforms in primary hepatocytes cultured for at least 18 hr [12; Table 2]. These differences may depend on the differences in the experimental conditions (e.g., metabolically active vs. resting state depending on the period of culture; hormones added to the culture medium; rate of bile acid uptake; bile acid concentrations chosen). Preliminary data indicate that the hydrophobic bile acid taurolithocholic acid (TLCA) induces translocation of only the ε-isoform at low micromolar concentrations in freshly isolated rat hepatocytes (Beuers et al., Hepatology 26:262A, 1997; Table 2).

(ii) Accumulation of sn-1,2-diacylglycerol (DAG)

The formation of sn-1,2-diacylglycerol (DAG) and the binding of DAG to the DAG binding site in the C1 region of the PKC molecule is required for activation of the enzyme [26, 27]. DAG formation is mainly mediated by phospholipase C and phospholipase D isoforms in mammalian cells [28]. Phospholipase C isoforms tend to induce a transient increase of DAG mass with a maximum after 1-2 min, whereas a sustained increase of DAG mass in mammalian cells may mainly result from cleavage of phosphatidylcholine by the sequential action of phospholipase D and phosphatidic acid hydrolase [28, 29].

A number of bile acids, including TUDCA, TCA, TCDCA, and TLCA, induce a sustained accumulation of DAG mass in isolated hepatocytes in short-term culture as determined by a combined radioenzymatic and chromatographic technique [9] (Table 2). These findings together with the observation that TUDCA and other bile acids do not affect IP_3 levels in hepatocytes [see above; 4, 7, 10], suggest that bile acid-induced DAG accumulation is mainly caused by cleavage of phosphatidylcholine as mediated by the sequential activation of phospholipase D and phosphatidic acid hydrolase [9].

(iii) Activation of membrane-bound PKC

Activation of total membrane-bound PKC (all isoforms) by bile acids was expected since a prerequisite for activation by bile acids, i.e., stimulation of hepatocellular DAG accumulation has been demonstrated. However, the pattern of membrane-bound PKC isoforms depends on the bile acid to which the cell is exposed. Indeed, TUDCA as well as TCA induced a sustained activation of total membrane-associated PKC in isolated hepatocytes in short-term culture [9] in line with their effect on DAG accumulation in isolated hepatocytes (Table 2). The stimulating effect of bile acids on hepatocellular PKC activity was confirmed by studies performed in vitro [30]. In those studies, activation of PKC was related to the degree of hydrophobicity of a bile acid [30], a phenomenon which was not observed in hepatocytes in short-term culture [9]. The reason for these differences remains unclear, although the sinusoidal uptake rates of bile acids may differ under different culture conditions.

MODEL OF TUDCA-INDUCED STIMULATION OF HEPATOCELLULAR SECRETION

UDCA improves biochemical and histological parameters [31] and prolongs survival in patients with primary biliary cirrhosis [32, 33]. Similar effects of UDCA on biochemical (and partly histological) parameters have been described in other chronic cholestatic diseases, such as primary sclerosing cholangitis [34-36], cystic fibrosis-associated liver disease [37], and progressive familial intrahepatic cholestasis [38]. However, the effect of UDCA on survival time has not been established, due to the limited number of patients who have participated in controlled trials. The mechanism of action of UDCA is still unclear although experimental evidence suggests that stimulation of hepatocellular secretion by UDCA conjugates may be of key importance for the beneficial effect of UDCA in cholestatic disorders [15, 39].

The differential effects of bile acids on signaling and secretory mechanisms of the liver cell led us to propose a model of TUDCA-induced stimulation of hepatocellular secretion [7-9]: After carrier-mediated uptake into the hepatocyte, TUDCA induces a sustained increase of $[Ca^{++}]_i$ by depleting IP_3-sensitive microsomal Ca^{++}-stores independently of IP_3 and by stimulating Ca^{++} influx across the plasma membrane via Ni^{++}-sensitive Ca^{++}-channels. The Ca^{++} increase may induce translocation of the Ca^{++}-sensitive a-isoform of PKC to the membranes as seen in other cell systems. Bile acid-induced accumulation of DAG

may then activate the membrane-associated α-PKC. TUDCA stimulates sustained, Ca^{++}-dependent vesicular exocytosis which may also be mediated by α-PKC. We speculate that TUDCA may enhance the secretory capacity of the cholestatic liver cell as it does in normal liver by stimulating apical exocytosis and thereby inducing targeting and insertion of transport proteins into the canalicular membrane [8, 9, 40].

SUMMARY

Two of the key messengers of the hepatocellular signaling network, $[Ca^{++}]_i$ and protein kinase C (but not cAMP-dependent protein kinase A), are directly modulated by bile acids at physiological concentrations in isolated liver cells. The functional implications of bile acid-induced signaling in liver cells are still incompletely understood.

REFERENCES

1. Anwer, M.S., Engelking, L.R., Sullivan, D., Zimniak, P., and Lester, R. Hepatotoxic bile acids increase cytosolic Ca^{++} activity of isolated rat hepatocytes. Hepatol. 8:887-891, 1988.
2. Anwer, M.S., Little, J.M., Oelberg, D.G., Zimniak, P., and Lester, R. Effect of bile acids on Ca^{++} efflux from isolated rat hepatocytes and perfused rat livers. Proc. Soc. Exp. Biol. Med. 191:147-152, 1989.
3. Combettes, L., Dumont, M., Berthon, B., Erlinger S., and Claret, M. Release of calcium from the endoplasmic reticulum by bile acids in rat liver cells. J. Biol. Chem. 263:2299-2303, 1988.
4. Combettes, L., Berthon, B., Doucet, E., Erlinger, S., and Claret, M. Characteristics of bile acid-mediated Ca^{++} release from permeabilized liver cells and liver microsomes. J. Biol. Chem. 264:157-167, 1989.
5. Combettes, L., Berthon, B., Doucet, E., Erlinger, S., and Claret, M. Bile acids mobilise internal Ca^{++} independently of external Ca^{++} in rat hepatocytes. Eur. J. Biochem. 190:619-623, 1990.
6. Beuers, U., Thiel, M., Bardenheuer, H., and Paumgartner, G. Tauroursodeoxycholic acid inhibits the cytosolic Ca^{++} increase in human neutrophils stimulated by formyl-methionyl-leucyl-phenylalanine. Biochem. Biophys. Res. Commun. 171:1115-1121, 1990.
7. Beuers, U., Nathanson, M.H., and Boyer, J.L. Effects of tauroursodeoxycholic acid on cytosolic Ca^{++} signals in isolated rat hepatocytes. Gastroenterol. 104:604-612, 1993.
8. Beuers, U., Nathanson, M.H., Isales, C.M., and Boyer, J.L. Tauroursodeoxycholic acid stimulates hepatocellular exocytosis and mobilizes extracellular Ca^{++}, mechanisms defective in cholestasis. J. Clin. Invest. 92:2984-2993, 1993.
9. Beuers, U., Throckmorton, D.C., Anderson, M.S., Isales, C.M., Thasler, W., Kullak-Ublick, G.A., Sauter, G., Koebe, H.G., Paumgartner, G., and Boyer, J.L. Tauroursodeoxycholic acid activates protein kinase C in isolated rat hepatocytes. Gastroenterol. 110:1553-1563, 1996.
10. Bouscarel, B., Fromm, H., and Nussbaum, R. Ursodeoxycholate mobilizes intracellular Ca^{++} and activates phosphorylase α in isolated hepatocytes. Am. J. Physiol. 264:G243-G251, 1993.
11. Bouscarel, B., Gettys, T.W., Fromm, H., and Dubner, H. Ursodeoxycholic acid inhibits glucagon-induced cAMP formation in hamster hepatocytes: a role for PKC. Am. J. Physiol. 268:G300-G310, 1995.
12. Stravitz, R.T., Rao, Y.P., Vlahcevic, Z.R., Gurley, E.C., Jarvis, W.D., and Hylemon, P.B. Hepatocellular protein kinase C activation by bile acids: implications for regulation of cholesterol 7a-hydroxylase. Am. J. Physiol. 271:G293-G303, 1996.
13. Nathanson, M.H. and Schlosser, S.F. Calcium signaling mechanisms in liver in health and disease. Progr. Liver Dis. 12:1-27, 1997.
14. Exton, J.H. Role of phosphoinositides in the regulation of liver function. Hepatol. 8:152-166, 1988.
15. Kitani, K. Hepatoprotective effect of ursodeoxycholate in experimental animals. In: Paumgartner, G., Stiehl, A., Barbara, L., and Roda, E., eds. *Strategies for the treatment of hepatobiliary diseases*. Lancaster., England: Kluwer Academic Publishers., 1990. pp. 43-56.
16. Heuman, D.M., Mills, A.S., McCall, J., Hylemon, P.B., Pandak, W.M., and Vlahcevic, Z.R. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts: in vivo studies in the rat. Gastroenterol. 100:203-211, 1991.
17. Galle, P.R., Theilmann, L., Raedsch, R., Otto, G., and Stiehl, A. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human hepatocytes. Hepatol. 12:486-491, 1990.

18. Burgoyne, R.D. and Morgan, A. Regulated exocytosis. Biochem. J. 293:305-316, 1993.
19. Benedetti, A., Strazzabosco, M., Ng, O.C., and Boyer, J.L. Regulation of activity and apical targeting of the Cl^-/HCO_3^- exchanger in rat hepatocytes. Proc. Natl. Acad. Sci. USA 91:792-796, 1994.
20. Boyer, J.L. and Soroka, C.J. Vesicle targeting to the apical domain regulates bile excretory function in isolated rat hepatocyte couplets. Gastroenterol. 109:1600-1611, 1995.
21. Larkin, J.M. and Palade, G.E. Transcytotic vesicular carriers for polymeric IgA receptors accumulate in rat hepatocytes after total biliary obstruction. J. Cell Sci. 98:205-216, 1991.
22. Barr, V.A. and Hubbard, A.L. Newly synthesized hepatocyte membrane proteins are transported in transcytotic vesicles in the bile duct-ligated rat. Gastroenterol. 105:554-571, 1993.
23. Bruck, R., Nathanson, M.H., Roelofsen, H., and Boyer, J.L. Effects of protein kinase C and cytosolic Ca^{++} on exocytosis in the isolated perfused rat liver. Hepatol. 20:1032-1040, 1994.
24. Corasanti, J.G., Smith, N.D., Gordon, E.R., and Boyer, J.L. Protein kinase C agonists inhibit bile secretion independently of effects on the microcirculation in the isolated perfused rat liver. Hepatol. 10:8-13, 1989.
25. Roelofsen, H., Ottenhoff, R., Oude Elferink, R.P.J., and Jansen, P.L.M. Hepatocellular organic-anion transport is regulated by protein kinase C. Biochem. J. 278:637-641, 1991.
26. Nishizuka, Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258:607-614, 1992.
27. Hug, H. and Sarre, T.F. Protein kinase C isoenzymes: divergence in signal transduction? Biochem. J. 291:329-343, 1993.
28. Nakamura, S. and Nishizuka, Y. Lipid mediators and protein kinase C activation for the intracellular signaling network. J. Biochem. 115:1029-1034, 1994.
29. Exton, J.H. Signaling through phosphatidylcholine breakdown. J. Biol. Chem. 265:1-4, 1990.
30. Rao, Y.P., Hylemon, P.B., Vlahcevic, Z.R., and Stravitz, R.T. Activation of protein kinase C isoforms by bile acids in vitro [Abstract]. Hepatol. 24:310A, 1996.
31. Poupon, R.E. and Poupon, R. Ursodeoxycholic acid therapy of chronic cholestatic conditions in adults and children. Pharmacol. Ther. 66:1-15, 1995.
32. Heathcote, E.J., Lindor, K.D., Poupon, R., et al. Combined analysis of French, American and Canadian randomized controlled trials of ursodeoxycholic acid therapy in primary biliary cirrhosis. Gastroenterol. 108:A1082, 1995.
33. Lindor, K.D., Therneau, T.M., Jorgensen, R.A., Malinchoc, M., and Dickson, E.R. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. Gastroenterol. 110:1515-1518, 1996.
34. Beuers, U., Spengler, U., Kruis, W., Aydemir, Ü., Wiebecke, B., Heldwein, W., Weinzierl, M., Pape, G.R., Sauerbruch, T., and Paumgartner G. Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial. Hepatol. 16:707-714, 1992.
35. Stiehl, A., Walker, S., Stiehl, S., Rudolph, G., Hofmann, W.J., and Theilmann, L. Effect of ursodeoxycholic acid on liver and bile duct disease in primary sclerosing cholangitis. A 3-year pilot study with a placebo-controlled study period. J. Hepatol. 20:57-64, 1994.
36. Lindor, K.D. and the Mayo PSC/UDCA study group. A randomized trial of ursodeoxycholic acid in the treatment of primary sclerosing cholangitis. Gastroenterology 110:A-844, 1996.
37. Colombo, C., Crosignani, A., Assaisso, M., Battezzati, P.M., Podda, M., Giunta, A., Zimmer-Nechemias, L., and Setchell, K.D.R. Ursodeoxycholic acid therapy in cystic fibrosis-associated liver disease: a dose-response study. Hepatol. 16:924-930, 1992.
38. Jacquemin, E., Hermans, D., Myara, A., Habes, D., Debray, D., Hadchouel, M., Sokal, E.M., and Bernard, O. Ursodeoxycholic acid therapy in pediatric patients with progressive familial intrahepatic cholestasis. Hepatol. 25:519-523, 1997.
39. Beuers, U., Kullak-Ublick ,G.A., and Paumgartner, G. Mechanisms of action of ursodeoxycholic acid in cholestatic syndromes. In: Strazzabosco, M., Benedetti, A., and Alvaro, D., eds. *Vanishing bile duct syndromes.* Kluwer Academic., Lancaster., UK., 1997. pp. 230-237.
40. Häussinger, D., Saha, N., Hallbrucker, C., Lang, F., and Gerok, W. Involvement of microtubules in the swelling-induced stimulation of transcellular taurocholate transport in perfused rat liver. Biochem. J. 291:355-360, 1993.