Activation of protein kinase C α and δ by bile acids: correlation with bile acid structure and diacylglycerol formation

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Abstract The feedback repression of cholesterol 7α-hydroxylase transcriptional activity and mRNA levels by taurocholate (TCA) occurs via a protein kinase C (PKC)-dependent signal. To determine whether bile acids could activate PKC indirectly via generation of diacylglycerol (DG), their effects on DG levels in primary cultures of rat hepatocytes were determined using a DG kinase assay. To determine whether bile acids might activate PKC isozymes more directly, their effects on PKCα and δ purified from baculovirus expression systems were examined in phosphatidylserine/phosphatidylcholine/Triton X-100 (PS/PC/TX) mixed micelles. Addition of tauroursodeoxycholate (TUDCA), taurocholate (TCA), or taurodeoxycholate (TDCA) (50 μM) to the cells rapidly (15 min) increased DG content in cultured rat hepatocytes to 105%, 155%, and 130%, respectively, as compared to untreated control cultures. Addition of TCA increased PKCα specific activity with EC_{50} of ~400 nM; maximal activity was observed with 5 μM. Other taurine-conjugated bile acids (5 μM) increased PKCα specific activity (pmol/min/μg protein) in proportion to their relative hydrophobicity: PS/PC/TX 17 ± 2; + TUDCA 29 ± 18; + TCA 68 ± 13; + TDCA 166 ± 21; and, taurochenodeoxycholate 178 ± 20 (P vs. PS/PC/TX = 0.54, 0.019, 0.002, and 0.001, respectively); unconjugated bile acids gave similar results (r^2 for activity vs. hydrophobicity index = 0.59). Taurine-conjugated bile acid interaction enthalpies, as determined by dimyristoyl-phosphatidylcholine chromatography, were more highly correlated (r^2 = 0.96) with PKCα activation than with the hydrophobicity index. TCA also stimulated the activity of purified PKCδ with EC_{50} of ~150 nM and maximally (2.7-fold) at 1 μM. Free and taurine-conjugated bile acids (1 μM) increased PKCδ activity according to their hydrophobicity index (r^2 = 0.89) and interaction enthalpies (r^2 = 0.96).—**Rao, Y–P., R. T. Stravitz, Z. R. Vlahcevic, E. C. Gurley, J. J. Sando, and P. B. Hylemon.** Activation of protein kinase C α and δ by bile acids: correlation with bile acid structure and diacylglycerol formation. *J. Lipid Res.* 1997. **38:** 2446–2454.

Supplementary key words protein kinase C activation • bile acid hydrophobicity • diacylglycerol • hepatocytes

Increasing experimental evidence suggests that bile acids regulate the transcriptional activity of several hepatic genes in direct proportion to their relative hydrophobicity, including cholesterol 7α-hydroxylase (1, 2), sterol 27-hydroxylase (3, 4), and the major histocompatibility complex class I gene (5, 6). A fundamental unanswered question remains: how do hepatocytes "sense" both the intracellular concentration and relative hydrophobicity of bile acids? In previous studies, we (7, 8) and others (5, 6) have provided evidence that bile acids may regulate hepatic genes by generating protein kinase C (PKC)-dependent signals. The mechanisms by which bile acids activate PKC, and the effects of bile acid hydrophobicity on PKC activation, however, remain to be elucidated.

The PKC superfamily consists of at least 11 isoenzymes that differ in intracellular location, tissue expression, and activating cofactors, suggesting that they play different roles in intracellular signaling (9). Members of the classical group (including PKCα, βI, βII and γ) require membrane acidic phospholipids (most importantly phosphatidylserine [PS]), membrane diacylglycerol (DG), and high concentrations of cytosolic free calcium for activation. In contrast, novel group members (including PKCδ, η, θ, and ϵ) require only mem-

Abbreviations: PKC, protein kinase C; TCA, taurocholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; GCDCA, glycochenodeoxycholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; PS, phosphatidylserine; PC, phosphatidylcholine; TX, Triton-X 100; DG, diacylglycerol; SDS, sodium dodecyl sulfate; CHAPS, (3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfate); MOI, multiplicity of infection.

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brane PS and DG for activation, and are therefore calcium-independent. These lipid requirements underlie the fact that PKC isoforms must physically associate from an inactive cytosolic pool to intracellular membranes before they become activated, a process termed PKC translocation. The third group of PKC isoforms, called atypical, include PKCζ and λ; they appear to require only PS for activation.

The process of PKC translocation from cytosol to membranes and subsequent activation is complex and incompletely understood. Recent evidence suggests that the structure of the membrane may plan an important role in the activation of PKC (10–12). Membrane components that influence PKC activation include phospholipid composition, fatty acid chain length, degree of saturation, and DG concentration (10, 11). In addition to membrane structure, an absolute requirement for activation of all PKC isoforms is the release of an autoinhibitory pseudosubstrate, an integral portion of the regulatory domain of the protein, from its catalytic site. Pseudosubstrate release requires a conformational change in the PKC protein, which occurs in the setting of optimal membrane structure, in the presence of increased membrane DG and for the classical subfamily, increased cytosolic calcium (13).

In an attempt to better understand how bile acids regulate hepatic genes via activation of PKC, we have recently shown that one calcium-dependent PKC isoform (PKCα) and two calcium-independent isoforms (PKCδ, and ϵ) translocated from cytosol to membranes in response to the addition of TCA to primary cultures of rat hepatocytes (8). Membrane translocation of PKC isoforms by bile acids, however, does not prove bile acid-induced PKC isoform activation (i.e., pseudosubstrate release and exposure of catalytic site), and does not identify possible mechanisms for this activation.

In the present study, we provide evidence that bile acids may activate PKC α and δ by two mechanisms. In vitro, in the absence of DG, bile acids increase PKC activity probably by facilitating enzyme association with phospholipids. In cultured rat hepatocytes, bile acids also increased membrane DG content.

MATERIALS AND METHODS

Materials

Bile acids were obtained from Calbiochem or Sigma (>95% purity). Myelin basic protein, histone III-SS, L-α-phosphatidylcholine (β-oleoyl-γ-palmitoyl) and L-α-phosphatidyl-L-serine were purchased from Sigma. Sf9 cells and Sf900 II serum-free insect cell medium were from Life Technologies (Grand Island, NY). The MonoQ and phenyl-Superose columns were purchased from Pharmacia. γ-[^{32}P]-ATP was from DuPont/NEN. Recombinant baculoviruses containing full-length cDNAs encoding PKCα and PKCδ were a generous gift from Drs. Silva Stabel, (Max-Delbruck Laboratorium, Koln, Germany), P. Parker (Protein Phosphorylation Laboratory, Lincoln's Inn Fields, London, England), and D. Fabbro (Ciba-Geigy, Basal, Switzerland).

Recombinant baculovirus expression and purification of PKCα and δ

Sf9 insect cells were grown in shaker culture and infected with recombinant baculovirus encoding full-length cDNAs for PKCα or δ at a multiplicity of infection (MOI) of ~0.5. Cells were harvested when approximately 15% non-viable by Trypan blue exclusion. All purification procedures were performed at 4°C. Sf9 cells were pelleted and sonicated in Tris HCl (20 mM, pH 7.5), EDTA (0.5 mM), EGTA (0.5 mM), sucrose (0.25 M), leupeptin (250 μg/ml), benzamidine (0.5 mM), 2-ME (10 mM). The cell lysate was centrifuged at 1000 *g* for 10 min to pellet unbroken cells, and the supernatant subsequently at 100,000 *g* for 1 h. The post-100,000 *g* supernatant fluid was adjusted to 20 mM NaCl and loaded onto a Mono Q 5/5 column preequilibrated in column buffer (Tris HCl [20 mM, pH 7.5], EDTA [0.5 mM], EGTA [0.5 mM], 2-ME [10 mM]) containing 20 mM NaCl. The Mono Q column was washed and eluted at 1 ml/min with a linear NaCl gradient (20–400 mM) collecting 1-ml fractions. Active fractions were pooled, and the pooled fractions were adjusted to 1.2 M NaCl and loaded onto a 5/5 phenyl-Superose column pre-equilibrated in column buffer + 1.2 M NaCl. The phenyl-Superose column was washed and eluted at 0.5 ml/min with a decreasing NaCl gradient (1.2 M to 480 mM for 5 ml, followed by 480 mM to 0 NaCl for 10 ml). Active fractions were pooled, adjusted to 10% glycerol and 0.05% Triton X-100, and frozen at −70°C. PKC isoforms were 90–95% pure as assessed by silver stain of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels; their identity was confirmed by Western immunoblotting using isoform specific anti-PKC isoform antibodies (affinity-purified) from Gibco-BRL, as described (8).

PKC assays

Before examining the effects of bile acids on PKC isoform activity, the effects of phosphatidylserine (PS), phosphatidylcholine (PC), and Triton X-100 (TX) on PKC activities were determined. Mixed micelles were prepared by vigorously vortexing PS, PC in Tris HCl (50 mM, pH 7.5) plus TX (0.3%). Standard reaction mixtures contained TX (0.03%), PS (66 mol%) + PC (33 mol%) for PKCα, and PS (20 mol%) + PC

(80 mol%) for PKC δ; these concentrations were thereafter used in assays with bile acids unless otherwise indicated. Bile acid-containing mixed micelles were prepared by adding bile acid (final concentrations 1 nM–100 μM) to the phospholipid mixture before drying under a nitrogen gas atmosphere.

Each PKC assay also contained final concentrations of 20 μM γ[^{32}P]-ATP, 20 mM Tris HCl (pH 7.5) 0.2 μg substrate (histone III-SS for PKCα and myelin basic protein for PKCδ), 200 μM Ca^{2+} for PKCα, 20 mM Mg^{2+}, purified PKC (5 to 10 ng). Incubations were performed at 30°C for 5 min, and were terminated by spotting onto Whatman P81 anion exchange paper. Papers were washed three times in 1% phosphoric acid to removed unreacted ATP. The degree of substrate phosphorylation was determined by liquid scintillation spectrometry.

Determination of DG content in primary cultures of rat hepatocytes

Bile acids (50 μM) were individually added to hepatocytes 24 h after plating and incubated from 5 to 20 min (8). Cells (0.85×10^5) were then harvested in 0.8 ml cold 1 M NaCl. Samples were extracted by a modification of the method of Bligh and Dyer (13, 14). Cells were extracted with 3 ml chloroform–methanol 1:2 (vol/vol). The monophase was mixed and 1 ml 1.0 M NaCl plus 1 ml chloroform were added. After centrifugation at 5,000 *g* for 2 min, the lower chloroform phase was assayed for DG within 72 h. The DG concentration was determined using a commercially available DG assay system specific for the *sn*-1,2-diacylglycerol (Amersham). The assay is based on the DG kinase-mediated formation of [^{32}P]phosphatidic acid from DG and [γ-^{32}] ATP (15, 16). DG content of each sample was calculated using a standard curve and normalized to μmoles cellular phospholipid. Total cellular phospholipid concentration was determined by the method of Fiske and SubbaRow (17).

RESULTS

Effects of taurine-conjugated bile acids on DG production in primary cultures of rat hepatocytes

In order to identify possible mechanisms of PKC activation by bile acids in intact cells, we examined the ability of bile acids of different structure to increase the production of DG in cultured rat hepatocyte membranes. As depicted in **Fig. 1**, three taurine-conjugated bile acids stimulated DG production in hepatocellular membranes in approximate proportion to their relative hydrophobicity (TCA and TDCA>TUDCA).

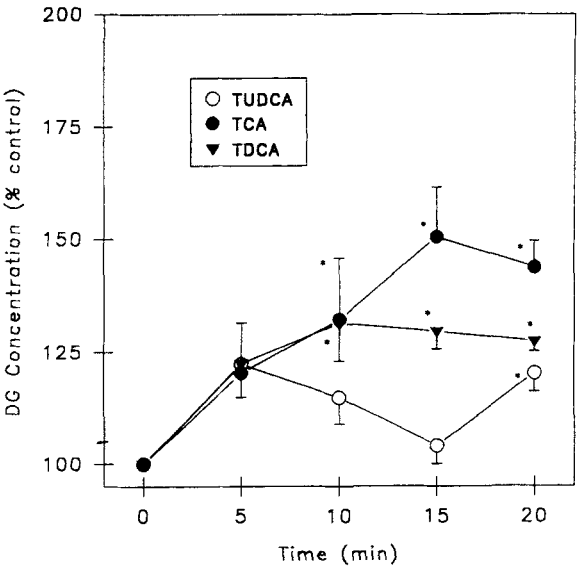

Fig. 1. Effects of taurine-conjugated bile acids on diacylglycerol (DG) in membranes of primary cultures of rat hepatocytes. Cellular DG concentrations were determined after the individual addition of 50 μM tauroursodeoxycholate (TUDCA), taurocholate (TCA), or taurodeoxycholate (TDCA) to primary hepatocytes in culture using Amprep Minicolumns and the DG Kinase kit. DG concentrations were normalized to total cellular phospholipid as described in Materials and Methods. The concentration of DG at 0 time was 20.5 nmol/μmol phospholipid or 3.2 nmol/mg whole cell protein. *P at least <0.05 (n = 3 to 6 experiments from separate hepatocyte preparations, determined in duplicate).

Optimization of PKC isoform activity

Before examining the effects of bile acids on PKC α and δ activity in vitro, assay conditions were optimized for phospholipid concentration in the absence of DG (data not shown) for each isoform. Individual mixed micelle components were mixed with TCA in order to determine whether the bile acid were capable of substituting for a particular phospholipid. As shown in **Fig. 2A and 2B**, neither PKCα nor PKCδ activity was enhanced by the addition of TCA to pure PS micelles. In contrast, the addition of TCA to PC/PS mixed micelles increased the activities of both isoforms additively over levels in PC/PS mixed micelles alone.

Bile acid effects on PKC activity in vitro

In the presence of optimized PC and PS concentrations, PKC α and δ activities were next determined after the addition of TCA over a wide concentration range. As shown in **Fig. 3**, the addition of TCA (10 nM–5 μM) increased the activities of both isoforms with EC_{50} of ~500 nM; peak activity was observed in the presence of 25 mol% TCA for PKCα and 3 mol% TCA for PKCδ. Interestingly, TCA in concentrations of 10 μM–100 μM

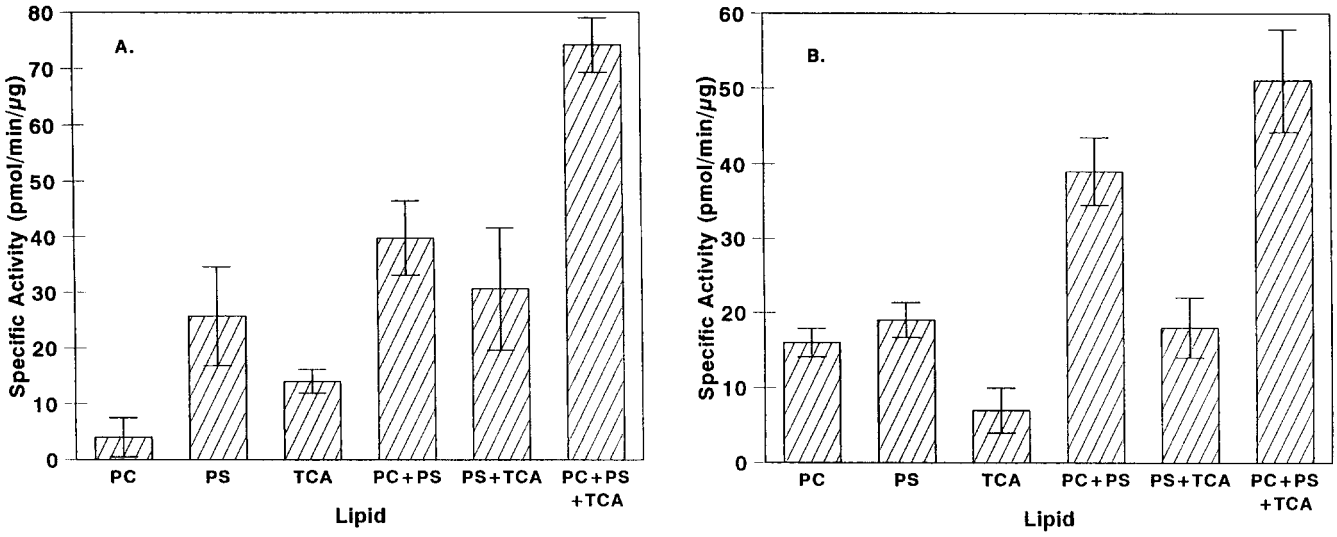

Fig. 2. Effects of phospholipid and taurocholate on activation of PKCα and PKCδ in vitro. (A). Specific activities of PKCα using reaction mixtures containing either 33 mol% phosphatidylcholine (PC), 66 mol% phosphatidylserine (PS), 5 μM taurocholate (TCA), PC + PS, PS + TCA, or PC + PS + TCA. (B) Specific activities of PKCδ using standard reaction mixtures containing either 80 mol% PC, 20 mol% PS, 5 μM TCA, PC + PS, PS + TCA, or PC + PS + TCA (mean ± SE of 3 separate experiments, performed in duplicate).

inhibited the activities of both isoforms, such that the TCA-induced enhancement in activity was completely reversed at 100 μM.

Effects of bile acid structure on bile acid-induced PKC α and δ activation

We next examined the ability of free and conjugated bile acids of widely varying structure to activate PKC α and δ in vitro. As shown in **Fig. 4**, PKCα specific activity was increased by taurine-conjugated bile acids (5 μM) in the following order: PC/PS alone 17 ± 2, +TUDCA 29 ± 18, +TCA 68 ± 13, +TDCA 166 ± 21, and +TCDCA 178 ± 20 pmol/min per μg protein. In a different set of experiments, free and taurine-conjugated bile acids stimulated both PKCα and δ activity roughly in proportion to their hydrophobicity index (18) as determined by elution from C18 reverse phase (methanolic mobile phase) HPLC (r^2 = 0.60 and r^2 0.89, respectively; **Figs. 5A and 5B**). Note that the relationship between PKC activation and bile acid hydrophobicity indices becomes less linear with the more hydrophobic bile acids (especially with TCDCA and TDCA, the latter being more hydrophobic than the former by this method; 18). However, a much stronger relationship between bile acid structure and PKC activity is evident when PKC activity is expressed according to the interaction enthalpies (ΔH) of taurine conjugated bile acid to an artificial membrane HPLC column (dimyristoyl-PC covalently linked to silica microspheres in an aqueous mobile phase; r^2 of both activities 0.96 versus ΔH for taurine-conjugated bile acids; Fig. 5C) (19).

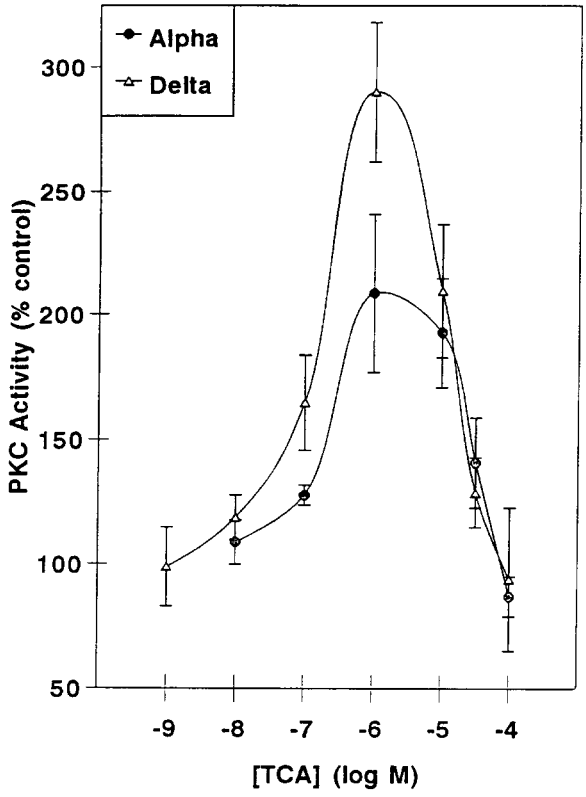

Fig. 3. Concentration-dependent activation of PKCα and δ by taurocholate. Reaction mixtures optimized for highest activity contained specified amounts of phospholipids for PKCα and PKCδ (Fig. 2 legend). Control (100%) specific activities were 47 ± 8 and 40 ± 11 pmol/min per μg protein for PKCα and PKCδ, respectively (mean ± SE of 3 separate experiments, performed in duplicate).

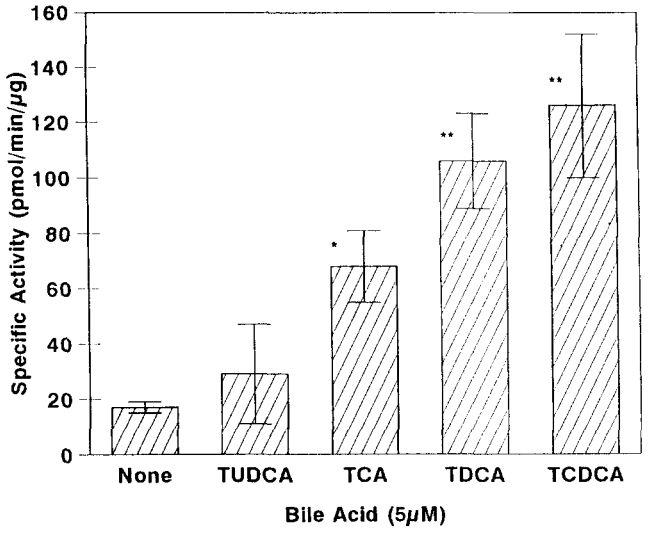

Fig. 4. Effect of taurine-conjugated bile acids on the specific activity of PKCα. The lipid components of the standard reaction mixture (Materials and Methods) contained 33 mol% PC + 66 mol% PC and either tauroursodeoxycholate (TUDCA), taurocholate (TCA), taurodeoxycholate (TDCA), or taurochenodeoxycholate (TCDCA) all at 5 μM. *$P < 0.02$ vs. PS/PC and **$P < 0.003$ vs. PS/PC (mean ± SE of 6 experiments, performed in duplicate).

In order to demonstrate that the effects of bile acids were not due to nonspecific detergent effects, SDS (5 μM) and the bile acid-derived detergent, CHAPS (5 μM), were also tested. Neither agent had any significant effect on PKCα activity in the presence of the same PC/PS concentrations (106 ± 11% and 116 ± 14% of PC/PS alone, respectively; mean of 6 determinations ± SE). Similarly, SDS and CHAPS increased PKCδ activity slightly but insignificantly (110 ± 7% and 129 ± 12% of PC/PS alone, respectively). These controls suggest that bile acids may increase PKC isoform activity by a mechanism independent of its detergent properties.

Effect of diacylglycerol and glycochenodeoxycholate on the activation of PKCα

DG activates PKC by directly binding to specific site(s) in the PKC regulatory domain. In order to explore the possibility that bile acids activate PKC by acting at the DG binding site, we next examined the effect of glycochenodeoxycholate (GCDCA) on DG-stimulated PKCα activity. The presence of GCDCA (10 μM) increased PKCα activity at all DG concentrations (**Fig. 6**). A DG concentration of 35 mol% yielded maximal stimulation under these experimental conditions. At higher DG to GCDCA molar ratios, PKCα activity was consistently above that of DG alone. The additive effect of DG and bile acid suggest that they stimulate PKC activity by different mechanisms.

Effects of bile acids on calcium-induced PKCα activation

The ability of bile acids to increase the sensitivity of PKCα to calcium was next explored in the presence of PC/PS mixed micelles. The addition of calcium had no significant effect on the activity of the calcium-independent isoform, PKCδ, nor on the ability of TDCA to increase its activity (data not shown). In contrast, the inclusion of TDCA (10 μM) in a reconstituted PKCα assay decreased the calcium requirement for activation by approximately 50%, within a physiological range for free intracellular calcium (i.e., 10^{-8} to 10^{-6} M; **Fig. 7**) TDCA decreased the EC_{50} for calcium even more dramatically in the presence of supraphysiologic concentrations (10^{-5} to 10^{-3} M).

DISCUSSION

Previous studies from several laboratories have shown that bile acids can regulate gene expression in both hepatocytes (6, 20) and colonic adenocarcinoma cells (21). Hydrophobic bile acids appear to be powerful regulators of cholesterol 7α-hydroxylase (1, 2, 20), sterol 27-hydroxylase (3, 4), and MHC class I genes in hepatocytes (5), while hydrophilic bile acids have minor effects. We have previously reported that repression of cholesterol 7α-hydroxylase by bile acids in primary adult rat hepatocyte cultures can be blocked by specific inhibitors of PKC (7). In HepG2 cells, the induction of MHC class I genes by bile acids has also been shown to be blocked by specific PKC inhibitors (5). Bile acids at physiological concentrations (12.5–100 μM) increased PKC activity in membranes prepared from primary rat hepatocytes in proportion to their hydrophobicity index (8). The addition of bile acids to hepatocyte cultures was associated with a rapid (5–30 min) translocation of PKC α, δ, and ϵ mass (2- to 6-fold increase) to hepatocyte membranes. Although bile acids have been reported to regulate a number of genes in cells from several different tissues, probably through the activation of PKC, little is known about the detailed mechanism(s) of activation of PKC by bile acids. In the present study, we examined the effects of different bile acids and phospholipids on the activity of a selected member of the classical and novel groups of PKC, both of which are activated by bile acids in primary hepatocytes.

Bile acids may activate hepatic PKC by stimulating the formation of DG, a known activator of classical and novel isoforms of PKC. In primary adult rat hepatocytes, the addition of a physiological concentration of

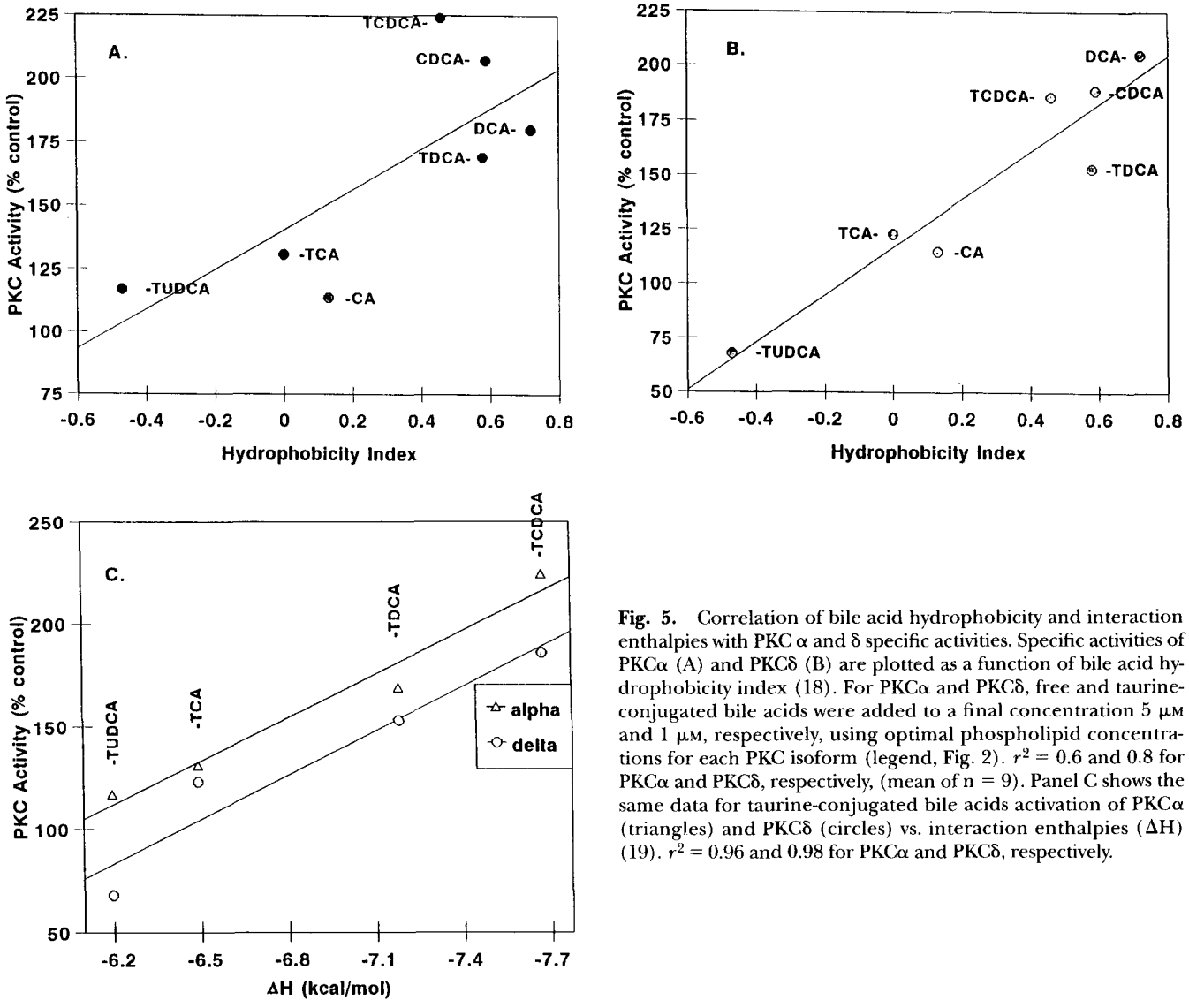

Fig. 5. Correlation of bile acid hydrophobicity and interaction enthalpies with PKC α and δ specific activities. Specific activities of PKCα (A) and PKCδ (B) are plotted as a function of bile acid hydrophobicity index (18). For PKCα and PKCδ, free and taurine-conjugated bile acids were added to a final concentration 5 μM and 1 μM, respectively, using optimal phospholipid concentrations for each PKC isoform (legend, Fig. 2). $r^2 = 0.6$ and 0.8 for PKCα and PKCδ, respectively, (mean of n = 9). Panel C shows the same data for taurine-conjugated bile acids activation of PKCα (triangles) and PKCδ (circles) vs. interaction enthalpies (ΔH) (19). $r^2 = 0.96$ and 0.98 for PKCα and PKCδ, respectively.

taurine-conjugated bile acids resulted in the rapid formation of DG (Fig. 1). Beuers et al. (22) have recently reported that bile acids stimulate the formation of DG in isolated primary rat hepatocytes. In contrast to our data, these investigators (22) showed that tauroursodeoxycholic acid, a hydrophilic bile acid, stimulated DG to the same extent as taurocholic acid. The explanation for this difference is not yet clear, but could be due to differences in bile acid concentrations and culture conditions employed in the two studies. The mechanism(s) of stimulation of increased levels of DG in hepatocytes by bile acids is currently unknown, but may involve the stimulation of phospholipase C or D. Previous studies of Takenawa and Nagai (23) showed that purified phosphatidylinositol-specific phospholipase C activity was stimulated in vitro by deoxycholic acid. Both PKC and certain phospholipases have C2-type membrane interacting domains, which may be involved in lipid binding (24). Thus, hydrophobic bile acids may be capable of the activation of both PKC and phospholipases in a similar manner. Therefore, bile acids interacting with membranes may effect two sequential steps in signal transduction in hepatocytes.

The in vitro activation of PKCα and δ by bile acids in the present study correlated loosely with the hydrophobicity index (18) but more strongly with membrane interaction enthalpies of the bile acid (Fig. 5C); hydrophobic bile acids were generally more powerful activators of PKC than hydrophilic bile acids (Fig. 6). Recent studies (25) have shown that adsorption of different taurine-conjugated bile acids to lecithin vesicles increased with their hydrophobicity (TUDCA< TCA< TCDC<TDCA). Studies by Cohen and Leonard (19) using dimyristoyl-phosphatidylcholine chromatography

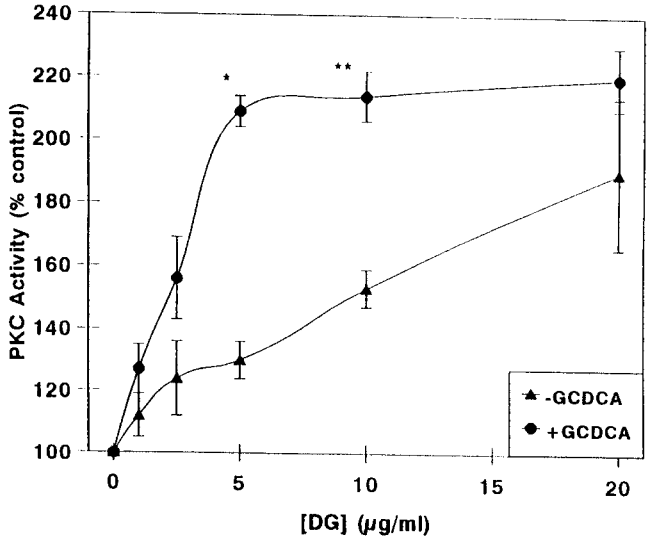

Fig. 6. Effect of diacylglycerol (DG) concentration on activation of PKCα in the presence of glycochenodeoxycholate (GCDCA). Enzyme assays were performed in the presence of 4 μg/ml phosphatidylserine (PS) (triangles) and PS + (10 μM GCDCA) (circles). Varying concentrations (10 to 68 mol%) DG were added to reaction mixtures. *$P < 0.0001$ (mean ± SE) of 7 experiments.

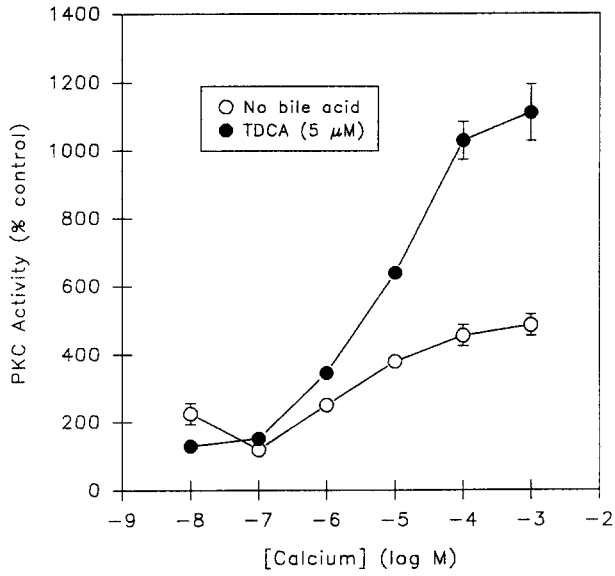

Fig. 7. Effects of calcium concentration on PKCα specific activities in the presence and absence of taurodeoxycholate. The specific activities of PKCα were determined under standard assay conditions in the presence (closed circles) of taurodeoxycholate (10 μM) or absence (open circles). Mean ± SE of 3 independent determinations, performed in duplicate.

showed that taurine-conjugated bile acids interacted with increasing affinity in the order TUDCA<TCA< TDCA<TCDCA. The affinities of taurine-conjugated bile acids in the latter system are highly correlated with the degree of activation of PKC in our studies (Fig. 5C). It is unknown why ΔH is more highly correlated with PKC than the hydrophobicity index, but may indicate that dimyristoyl-phosphatidylcholine chromatography resembles a phospholipid membrane more than C18 reverse phase chromatography.

Most models of PKC activation involve the binding and insertion of the inactive cytosolic enzyme into membranes (10–12, 26). Activation of PKC is markedly enhanced in vitro by acidic phospholipids such as phosphatidylserine (PS), DG, and Ca^{2+} (27–29). Moreover, activation of PKC has been shown to be strongly influenced by the physiochemical properties of the lipid membrane (10–12, 30, 31). Alteration of membrane structure or properties that have been demonstrated to alter PKC activation include: DG induced structural changes (30–33); species of phospholipid(s), fatty acid chain length, degree of fatty acid unsaturation (33–36), and membrane curvature (30, 37, 38). A possible common mechanism to explain these observations is that PKC might recognize an optimal head group spacing or specific membrane phospholipid domains which might be generated by bile acid binding (10, 11, 30, 39, 40). Bile acids are believed to bind to membranes via their hydrophobic backbone with hydrophilic hydroxy and conjugated amino acids oriented toward the aqueous phase.

We do not believe that the mechanism of activation of PKC by bile acids is by direct binding to the enzyme in a manner analogous to DG or phorbol esters for the following reasons. First, bile acids did not activate PKC in the absence of phospholipids (Fig. 2). Second, the activation of PKC is correlated with the membrane enthalpy of the bile acid added to the reaction mixture (Fig. 5). Third, the effect of bile acid concentration on both PKCα and δ activity showed biphasic saturation kinetics. Such kinetics cannot be explained by a single binding site for bile acids on PKC (Fig. 3). Finally, under certain assay conditions, the presence of DG and bile acids showed a significant additive increase in PKC activity (Fig. 4). Although we cannot totally eliminate the possibility of a bile acid binding site(s) on PKC, the bulk of the data is consistent with bile acid effects on membranes.

In summary, the current study provides strong evidence that hydrophobic bile acids activate specific isoforms of PKC probably by two distinct mechanisms: bile acids may activate specific isoforms of PKC by generating DG and may activate PKC by binding to membranes, possibly inducing distinct phospholipid domains or structures recognized by PKC. The data presented in this paper may have relevance as to how hepatocytes physiologically "sense" the concentration and hydrophobicity of the bile acid pool, and further imply that

PKC is involved in the regulation of hepatic cholesterol and bile acid homeostasis in the liver.

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