Bile salts potentiate adenylyl cyclase activity and cAMP-regulated secretion in human gallbladder epithelium

NICOLAS CHIGNARD,1 MARTINE MERGEY,1 DANIELLE VEISIÈRE,1 ROAUL POUPON,1,2 JACQUELINE CAPEAU,1,3 ROLLAND PARC,4 ANNICK PAUL,1 AND CHANTAL HOUSSET 1,2,3

1Institut National de la Santé et de la Recherche Médicale, 2Service d’Hépatologie, Hôpital Saint-Antoine, and 3Service de Chirurgie Générale et Digestive, Hôpital Saint-Antoine, 75012 Paris; and 4Service de Biochimie, Hôpital Tenon, 75020 Paris, France

Submitted 19 July 2002; accepted in final form 31 October 2002

Chignard, Nicolas, Martine Mergey, Danielle Veisière, Raoul Poupon, Jacqueline Capeau, Rolland Parc, Annick Paul, Chantal Housset. Bile salts potentiate adenylyl cyclase activity and cAMP-regulated secretion in human gallbladder epithelium. Am J Physiol Gastrointest Liver Physiol 284: G205–G212, 2003. First published November 6, 2002; 10.1152/ajpgi.00292.2002.—Fluid and ion secretion in the gallbladder is mainly triggered by the intracellular second messenger cAMP (31) in response of absorption to secretion is mainly triggered by the carbonate-rich fluid after feeding (24, 34). The reversal through fluid absorption during fasting, secretes a bi-secretin (24). The cellular effects of the vasoactive intestinal polypeptide (VIP) (34) or by cholate induced PKC-
whereas both taurochenodeoxycholate and tauroursodeoxy-
potentiating effect was abrogated after PKC inhibition, similar extent, without affecting cAMP basal levels. This cholate increased forskolin-induced cAMP accumulation to a bile salts. Taurochenodeoxycholate and tauroursodeoxycholate increased forskolin-induced cAMP accumulation to a similar extent, without affecting cAMP basal levels. This potentiating effect was abrogated after PKC inhibition, whereas both taurochenodeoxycholate and tauroursodeoxycholate induced PKC-α and -δ translocation to cell membranes. Consistent with a PKC-mediated stimulation of cAMP production, the expression of six adenylyl cyclase isoforms, including PKC-regulated isoforms 5 and 7, was identified in human gallbladder epithelial cells. cAMP-dependent chloride secretion induced by isoproterenol, a β-adrenergic agonist, was significantly increased by taurochenodeoxycholate (TCDC), a more hydrophobic endogenous and therapeutic bile salts via PKC regulation of adenylyl cyclase activity potentiate cAMP production in the human gallbladder epithelium. Through this action, bile salts may increase fluid secretion in the gallbladder after feeding:

β-adrenergic agonist; chenodeoxycholic acid; chloride channels; ursodeoxycholic acid

THE GALLBLADDER, WHICH STORES and concentrates bile through fluid absorption during fasting, secretes a bicarbonate-rich fluid after feeding (24, 34). The reversal of absorption to secretion is mainly triggered by the intracellular second messenger cAMP (31) in response to β-adrenergic stimulation (17) and by stimulation by the vasoactive intestinal polypeptide (VIP) (34) or by secretin (24). The cellular effects of β-adrenergic agonists, of VIP and of secretin, are mediated by G protein-coupled receptors (13, 36). After activation of these receptors by their ligands, the G protein α-subunit is released and stimulates an adenylyl cyclase (AC) enzyme, which converts ATP into cAMP. Adenylyl cyclase enzymes form a superfamily of nine isoforms termed AC1–9. Whereas stimulation through the Gαs subunit is the major mechanism by which all adenylyl cyclases are activated, individual isoforms have different regulatory properties, which allow complex signal integration. AC1, -3, and -8 may be stimulated or inhibited by intracellular free calcium and by calmodulin, whereas AC2, -5, and -7 are stimulated by PKC, AC4 is stimulated by the βγ-subunit, AC6 is inhibited by low concentrations of calcium, and AC9 is insensitive to either calcium, PKC, or βγ-subunit (12).

The major driving force for both fluid and bicarbonate secretion across the gallbladder epithelium, is the extrusion of chloride ions through the apical cAMP-dependent chloride channel, CFTR (9, 31). Current evidence indicates that bile salts, the main constituents in bile, contribute to the regulation of biliary epithelial cell secretory functions (2, 8, 33). The apical sodium-dependent bile salt transporter is expressed in human gallbladder epithelial cells, and bile salt uptake affects chloride and mucin secretion in these cells (8). We have also previously shown that the tauroconjugate of ursodeoxycholic acid, a hydrophilic bile salt used in the treatment of biliary disorders (4), may generate distinct regulatory events compared with taurochenodeoxycholate (TCDC), a more hydrophobic endogenous bile salt (8). The purpose of the present study was to examine the action of TCDC and of tauroursodeoxycholate (TUDC) on the cAMP-dependent secretory pathway in the human gallbladder epithelium, including potential regulating effects on AC isoforms expressed in these cells.

MATERIALS AND METHODS

Reagents. DMEM/Ham’s F-12 (1:1) mixture was purchased from Life Technologies (Cergy Pontoise, France), Uльтрос G, from Biosepra (Villeneuve-la-Garenne, France), and human
type IV collagen, from Tebu (Les Ulis, France). Protease type XIV from \textit{Streptomyces griseus}, forskolin, and diphenylamine carboxylic acid (DPC) were purchased by Sigma (Saint-Quentin Fallavier, France). TCDC, TUDC, and glycogenodeoxycholate (GDCA) (99% pure), PMA, DIDS, BAPTA/AM, \textit{Bordetella pertussis} toxin, GF 109203X, CPT-cAMP, and IBMX were obtained from Calbiochem (Meudon, France). \textit{ATCC} was obtained from American Type Culture Collection (Rockville, MD). Ribonuclease inhibitor RNazine was purchased from Promega (Charbonnieres, France), Moloney murine leukemia virus RT, from Life Technologies (Cergy Pontoise, France), and Taq DNA polymerase, from PerkinElmer (Les Ulis, France).

\textit{Cell culture.} Gallbladder samples were obtained from patients who underwent liver or pancreas surgery. The procedure was in accordance with current French legislation. The patients who underwent liver or pancreas surgery. The procedure was in accordance with current French legislation. The patients who underwent liver or pancreas surgery. The procedure was in accordance with current French legislation.

\textit{cAMP assay.} Gallbladder epithelial cells at days 5–6 of primary culture were incubated for 10 min at 37°C, with forskolin (10 μmol/l) or isotoprenol (10 μmol/l) and/or with TCDC (0.5 mmol/l), TUDC (0.5 mmol/l), GCDC (0.5 mmol/l), or PMA (0.16 μmol/l) in DMEM/Ham’s F-12, whereas the controls were incubated with plain culture medium. Part of the cells underwent preincubation with either PMA (1.6 μmol/l) for 24 h, \textit{B. pertussis} toxin (100 μg/l) for 18 h, or GF 109203X (1 μmol/l) for 30 min. All incubations were performed at 37°C. At the end of the experiments, the cells were transferred on ice. DMEM/Ham’s F-12 containing diginton (40 μmol/l) and IBMX (1 mmol/l) was added. cAMP was assayed in the supernatant by a commercial radioimmunoassay kit (RIA; New England Nuclear-Life Science Products, Paris, France). The protein content of cell samples was determined by BCA-protein assay (Pierce, Bezon, France). Incubations with inhibitors were performed at concentrations that did not induce cytotoxicity, as ascertained by lactate dehydrogenase release (14).

\textit{RT-PCR.} Total RNA was extracted from freshly isolated and cultured cells, using RNA plus lysis solution (Quantum, Montreuil-sous-Bois, France), according to Chomczynski and Sacchi’s method (10). Total RNA from human brain tissue was provided by Clontech (Palo Alto, CA). One microgram of total RNA was denatured by heating at 72°C for 10 min, and then incubated in 25 μl of a reaction buffer containing 10 mmol/l DTT, 0.5 mmol/l dNTP, 20 units of RNazine, 5 μmol/l random hexamers, and 200 units of Moloney murine leukemia virus RT. Reverse transcription was allowed to proceed for 1 h at 37°C.

The following primers were designed on the basis of adenyl cyclase human cDNA sequences, except for AC4 and –5 sequences, which were from \textit{Rattus norvegicus}: 5’-CTG CGA GTG TAC ACA CCA TG-3’ (sense) and 5’-GGT CTG TCA TCC ATC CGA CT-3’ (antisense) that amplify a 870-bp cDNA fragment (nucleotides 1331–2200) of AC1 (GenBank accession no. LO5500); 5’-CTG AAC GAG ATC ACG GCT GA-3’ (sense) and 5’-CGG TGG GCG AGC TAC CAT AT-3’ (antisense) that amplify a 900-bp cDNA fragment (nucleotides 901–1800) of AC2 (GenBank accession no. L21993); 5’-GGG TAT TTG GAT GTG GAG CC-3’ (sense) and 5’-GTC CCG TGT AGT ACT GGA GA-3’ (antisense) that amplify a 805-bp cDNA fragment (nucleotides 1543–2347) of AC3 (GenBank accession no. AF038681); 5’-ATC GCC AAG ATG AAC CGC CA-3’ (sense) and 5’-GCA GCT GAT CTG CAT GAA CA-3’ (antisense) that amplify a 248-bp cDNA fragment (nucleotides 1801–2048) of AC4 (GenBank accession no. M80633); 5’-ATC GCC AAG ATG AAC CGC CA-3’ (sense) and 5’-GCA GCT GAT CTG CAT GAA CA-3’ (antisense) that amplify a 248-bp cDNA fragment (nucleotides 1801–2048) of AC4 (GenBank accession no. M80633); 5’-ATC GCC AAG ATG AAC CGC CA-3’ (sense) and 5’-GCA GCT GAT CTG CAT GAA CA-3’ (antisense) that amplify a 248-bp cDNA fragment (nucleotides 1801–2048) of AC4 (GenBank accession no. M80633). Primers of human \textbeta -actin cDNA used as an internal standard were previously selected to generate a 631-bp product (28). Amplifications were achieved using 0.5 μmol/l of primers, 1.25 units of Taq DNA polymerase, and annealing temperatures of 60°C. PCR products obtained after the completion of 38 cycles were separated by electrophoresis through a 2% agarose gel stained with ethidium bromide. The authenticity of all AC isoforms PCR products obtained from gallbladder epithelial cells was verified by sequencing.

\textit{PKC immunoassays.} Gallbladder epithelial cells at days 5–6 of primary culture were incubated for 10 min, with or without PMA (0.16 μmol/l), TCDC (0.5 mmol/l), or TUDC (0.5 mmol/l) in UltraVio G-free DMEM/Ham’s F-12. Cells were then harvested and sonicated in ice-cold buffer containing 10 mmol/l Tris-HCl (pH 7.5), 0.25 mol/l sucrose, 0.2 mmol/l CaCl2, and protease inhibitor cocktail (from Roche, Meylan, France). After the addition of EDTA at a final concentration of 1 mmol/l, the samples were centrifuged at 100,000 g for 60 min. The crude membrane pellet was suspended in a buffer containing 20 mmol/l Tris-HCl (pH 7.5), 0.25 mol/l sucrose, 1 mmol/l EDTA, 1 mmol/l EGTA, 10 mmol/l 2-mercaptoethanol, protease inhibitor cocktail, and 1% Triton X-100. After sonication on ice, and centrifugation at 100,000 g for 60 min, insoluble material was discarded and the supernatant was collected as a solubilized membrane fraction. Membrane proteins (10 μg) were then subjected to electrophoresis through a 7.5% SDS polyacrylamide gel, and transferred to polyvinylidene difluoride membranes. Immunoblotting was performed using monoclonal antibodies raised against α-, β-, γ-, δ-, and ε-isoforms (Transduction Laboratories, Le Pont-de-Clai, France), at concentrations of 0.25–1 mg/l. Immunoreactivity was revealed by enhanced chemiluminescence (Amersham, Les Ulis, France).

\textit{Chloride efflux assay.} Chloride efflux was measured as described (15). Gallbladder epithelial cells at days 5–6 of primary culture were loaded with 35Cl (5 μCi/ml) in efflux buffer containing (in mmol/l) 140 NaCl, 4 KCl, 1 KH2PO4, 2 MgCl2, 1 CaCl2, 10 glucose, and 10 HEPES, pH 7.4, for 1 h at...
37°C. Cells were washed rapidly three times with 1 ml of isotope-free buffer, which was then replaced at 2-min intervals, before stimulating agents were added in 1 ml of efflux buffer, whereas efflux buffer alone was added to the controls. The following agents were tested: isoproterenol (10 μmol/l) and CPT-cAMP (0.2 mmol/l) alone or combined with TCDC (0.5 mmol/l) and TUDC (0.5 mmol/l). Where indicated, the efflux buffer at all time points contained DPC (1 mmol/l) or DIDS (1 mmol/l) chloride channel blockers, or BAPTA/AM (50 μmol/l), a chelator of intracellular calcium. At the end of experiments, the cells were solubilized in 1 mol/l NaOH, and samples were counted for radioactivity. The efflux was calculated as the ratio of radioactivity in the efflux sample at a given time to the total radioactivity present in the cells during the previous 2-min interval. Increases in chloride efflux expressed in percent were calculated as the ratio between the peak of chloride efflux occurring 2–4 min after stimulation and the chloride efflux measured immediately before stimulation (basal level).

**Statistical analysis.** Comparisons were made using the Student's t-test and the paired Wilcoxon signed-rank test. n = Number of human samples; P < 0.05 was considered significant.

**RESULTS**

**Bile salt regulation of cAMP production in gallbladder epithelial cells.** In gallbladder epithelial cells exposed to TCDC or TUDC alone, the cAMP content showed no significant change, whereas in cells exposed to forskolin, which causes AC activation, cAMP content rose from 1.2 ± 0.1 to 166 ± 38 pmol/mg protein (n = 11; P < 0.002) (Fig. 1), and in those exposed to IBMX, which causes phosphodiesterase inhibition, cAMP content rose from 1.4 ± 0.1 to 2.7 ± 0.1 pmol/mg protein (n = 3; P < 0.05). These results indicated that bile salts alone affected neither AC nor phosphodiesterase activities in gallbladder epithelial cells. By contrast, as shown in Fig. 1, when TCDC or TUDC were added in combination with forskolin, the cAMP content rose to levels (242 ± 55 and 241 ± 63 pmol/mg protein, respectively), which were significantly higher than those elicited by forskolin alone (Fig. 1). The production of cAMP was increased to a similar extent by both tauroconjugates and also by the glycoconjugate GCDC. GCDC augmented the rise in cAMP elicited by forskolin from 152 ± 17 to 216 ± 28 pmol/mg protein (n = 3; P < 0.05). The physiological relevance of these results was supported by the findings that bile salts elicited the same potentiation of forskolin-induced cAMP accumulation in freshly isolated cells as in cultured cells (not shown). Because these effects of bile salts on cAMP production required concomitant AC stimulation, we inferred that they potentiated AC activity. To provide support to this possibility, we analyzed the pattern of AC expression in human gallbladder epithelial cells.

**Pattern of AC expression in gallbladder epithelial cells.** Transcripts of six AC isoforms, 3–7 and 9, were detected by RT-PCR in all freshly isolated gallbladder epithelial cells issued from twelve different donors (Fig. 2A), whereas in all these preparations, the transcripts of AC1, -2, and -8 were undetectable (Fig. 2C). All AC amplification products obtained from gallbladder epithelial cells were authenticated by sequencing. Those of AC4 and -5 showed >80% homology with *Rattus norvegicus* sequences. In cultured cells, sustained expression of all the isoforms identified in freshly isolated cells, was recorded up to at least 6 days of primary culture (Fig. 2B).

**Signaling pathways mediating bile salt regulation of cAMP production in gallbladder epithelial cells.** The pattern of AC expression in gallbladder epithelial cells pointed to PKC and to G protein βγ-subunit as potential intracellular signals regulating cAMP production in these cells, via the stimulation of AC5 and -7, and of AC4 and -7 isoforms, respectively. Confirmation that PKC regulation of cAMP production occurs in gallbladder epithelial cells was provided by testing the effect of PKC activation, in cells exposed to the phorbol ester PMA (0.16 μmol/l). In cells exposed to PMA, cAMP levels elicited by forskolin increased from 112 ± 25 to 172 ± 28 pmol/mg protein (n = 5; P < 0.05) (Fig. 3). PKC downregulation was then achieved in cells incubated with PMA at high concentration (1.6 μmol/l) for 24 h, and resulted in the suppression of TCDC potentiating effect on cAMP production (Fig. 4A). By contrast, in cells treated with *B. pertussis* toxin, despite effective inhibition of Gβγ release from G protein as ascertained by an increase in forskolin-stimulated cAMP formation (Fig. 4B), the potentiating effect of TCDC remained significant (Fig. 4B). Therefore, whereas TCDC regulation of cAMP production required PKC activation, no evidence was found to indi-
Bile salt regulation of cAMP-dependent chloride secretion in gallbladder epithelial cells. To test the influence of bile salts on cAMP-dependent secretion, chloride efflux was measured in cells exposed to isoproterenol, a β-adrenergic agonist, and to bile salts, either separately or simultaneously. In cells exposed to isoproterenol, the cAMP content rose from 1.3 ± 0.3 to 518 ± 153 pmol/mg protein and further to 635 ± 143 pmol/mg protein when isoproterenol was combined with TCDC (n = 5; P < 0.05 vs. isoproterenol alone). Both isoproterenol and bile salts (TCDC or TUDC), when added separately, stimulated chloride secretion in the cells (Fig. 7, A and B). When isoproterenol and TCDC or TUDC were added simultaneously, chloride secretion was significantly higher than in cells exposed to either isoproterenol or bile salts alone (Fig. 7, A–C). The chloride channel blocker DIDS significantly reduced chloride secretion stimulated by TCDC or TUDC (Fig. 7B). Moreover, BAPTA/AM, a chelator of intracellular calcium, caused 80 and 75% inhibitions in TCDC- or TUDC-induced secretions, respectively, providing further evidence that calcium-dependent chloride channels were involved. By contrast, in cells exposed to isoproterenol alone (although DPC, a nonselective chloride channel blocker, significantly decreased chloride secretion) DIDS had no effect (Fig. 7A), consistent with cAMP-dependent chloride secretion in response to β-adrenergic stimulation. Similar inhibition profiles were observed when chloride secretion was triggered by isoproterenol in conjunction with TCDC, TUDC (Fig. 7C), or PMA (not shown). Furthermore, the potentiating effect of TCDC on chloride secretion induced by isoproterenol was abrogated by the PKC inhibitor.

Fig. 2. Pattern of adenylyl cyclase (AC) expression in gallbladder epithelial cells. RT-PCR analysis of AC3–7, and -9 in freshly isolated cells (A), in cells at day 6 of primary culture (B), and of AC1, -2, and -8 in human brain tissue used as a positive control (C, lanes 1), in freshly isolated cells (lanes 2) and in cultured cells (lanes 3), was performed. AC amplification products are visualized as unique bands distinct from β-actin. β-actin PCR products were diluted (1:4) before electrophoresis. Gels are representative of results obtained from the analysis of 12 different human samples.

Fig. 3. Effect of PKC activation on cAMP production in gallbladder epithelial cells. PMA (0.16 μmol/l) and forskolin (10 μmol/l) were added to gallbladder epithelial cells either separately or simultaneously, whereas the controls remained in plain culture medium. cAMP content was measured by RIA and reported to cell protein content. Data represent means ± SE of experiments performed in duplicate using 5 different human samples. *P < 0.05.

Bcискать что G protein βγ-subunit contributes to this regulation.

Further investigations of the PKC-dependent pathway showed that, on forskolin stimulation, the elevations in cAMP levels triggered by PMA, TCDC, or TUDC were significantly reduced by the PKC inhibitor GF 109203X at 1 μmol/l (Fig. 5). These inhibitions were effective, whereas the cAMP response to forskolin alone was not significantly different in GF 109203X-treated cells (148 ± 71 pmol cAMP/mg protein) compared with controls (136 ± 61 pmol cAMP/mg protein, n = 4, P = not significant). GF 109203X at 1 μmol/l inhibits PKC-α, -β, -γ, -δ, and -ε isoforms. Among these isoforms, only PKC-α, -δ, and -ε were detected by immunoblot analyses in cultured gallbladder epithelial cells. The addition of PMA, TCDC, or TUDC to these cells caused an increase in the amounts of membrane-bound PKC-α and -δ (Fig. 6), whereas no membrane-bound PKC-ε was detectable in these cells, irrespective of whether or not they were exposed to PMA, TCDC, or TUDC (data not shown). These data suggest that both TCDC and TUDC potentiate the production of cAMP in gallbladder epithelial cells, through AC regulation by PKC-α and/or -δ isoforms.
The effect of isoproterenol combined with TCDC, compared with that of isoproterenol alone, decreased from 147/100 ± 13 (n = 4; P < 0.05) to 107/100 ± 45% (n = 4; P = 0.9), on inhibition by GF 109203X, whereas the response to isoproterenol alone, was not significantly modified by GF 109203X (not shown). These results suggest that on β-adrenergic stimulation, rather than additional activation of calcium-dependent channels, further activation of the cAMP production by means of AC potentiation through PKC is the major mechanism by which bile salts increase chloride secretion. In support of this conclusion, TCDC was unable to further enhance chloride secretion elicited by the cAMP analog CPT-cAMP, which elicits cAMP-dependent chloride secretion without activating AC (30/100 ± 5 vs. 27 ± 4% over basal n = 3; P = not significant).

Fig. 5. Effect of PKC signaling pathway in bile salt regulation of cAMP production. Gallbladder epithelial cells were pretreated or not with GF 109203X (1 μmol/l) for 30 min, exposed to forskolin (10 μmol/l) alone or in combination with PMA (0.16 μmol/l) and then used as a positive control of PKC activation with TCDC (0.5 mmol/l) or with TUDC (0.5 mmol/l). cAMP content was measured by RIA and reported to cell protein content. Data normalized during pairing are expressed as a percentage over cAMP content in cells exposed to forskolin alone. Data represent means ± SE of experiments performed in duplicate using 4 different human samples. *P < 0.05 vs. similar stimulus in the absence of GF 109203X.

GF 109203X. The effect of isoproterenol combined with TCDC, compared with that of isoproterenol alone, decreased from 147 ± 13 (n = 4; P < 0.05) to 107 ± 45% (n = 4; P = 0.9), on inhibition by GF 109203X, whereas the response to isoproterenol alone, was not significantly modified by GF 109203X (not shown). These results suggest that on β-adrenergic stimulation, rather than additional activation of calcium-dependent channels, further activation of the cAMP production by means of AC potentiation through PKC is the major mechanism by which bile salts increase chloride secretion. In support of this conclusion, TCDC was unable to further enhance chloride secretion elicited by the cAMP analog CPT-cAMP, which elicits cAMP-dependent chloride secretion without activating AC (30 ± 5 vs. 27 ± 4% over basal n = 3; P = not significant).

Fig. 6. Membrane translocation of PKC isoforms. Gallbladder epithelial cells were exposed to either PMA (0.16 μmol/l), TCDC (0.5 mmol/l), or TUDC (0.5 mmol/l) for 10 min. Controls were maintained in plain medium. Membrane proteins (10 μg) prepared from 3 different cell preparations were pooled and subjected to PKC-α and -δ immunoblot-enhanced chemiluminescence analysis.
DISCUSSION

In previous investigations, we have shown that bile salts, below critical micellar concentrations (32), regulate secretory functions in the human gallbladder epithelium (8, 14). The present study provides evidence that bile salts potentiate cAMP-regulated secretion in this epithelium. Both TCDC and TUDC increased forskolin-induced cAMP accumulation without affecting basal levels, consistent with a regulation of adenylyl cyclase activity. The analysis of AC expression in a large number of preparations allowed us to show for the first time that human gallbladder epithelial cells express six isoforms of AC, namely AC3–7 and -9, whereas AC1, -2, and -8 are not expressed. We deduced from this analysis that some of the isoforms expressed in gallbladder epithelial cells could be positively regulated by PKC (AC5 and -7) (22, 26) or by the $\beta\gamma$-subunit of B. pertussis toxin-sensitive Gs proteins (AC4 and -7) (21, 39). The potentiating effect of TCDC was insensitive to B. pertussis toxin (18), making unlikely the possibility that bile salts modulate cAMP synthesis through the release of $\beta\gamma$-subunit from Gs protein in gallbladder epithelial cells. By contrast, in support to PKC regulation, the potentiating effects of bile salts on cAMP production were mimicked by PMA and were abrogated by GF 109203X, which acts as a competitive inhibitor of ATP-binding sites for PKC-$\alpha$, $\beta$, $\gamma$, $\delta$, and $\epsilon$ isoforms (35). The potentiation of cAMP production by bile salts was also suppressed by downregulation of the same isoforms after long-term exposure to PMA (7, 14). Immunooanalyzes showed that, among PMA and GF 109203X inhibitable isoforms, only PKC-$\alpha$ and $\delta$ translocated to the membranes of gallbladder epithelial cells exposed to bile salts. TCDC and TUDC were both effective in inducing PKC-$\alpha$ and $\delta$ translocation, in keeping with similar effects on cAMP formation. The signals induced by TUDC were more intense, in agreement with what we previously found by measuring membrane-bound PKC activity (8), although the difference with the effect of TCDC was not significant. The pattern of AC expression in gallbladder epithelial cells raise the possibility that bile salts may have additional effects on AC and cause inhibition of isoforms, such as AC3 or -6, expressed in these cells. AC6 is inhibited by free intracellular Ca$^{2+}$ (38), at concentrations (IC$_{50}$ of 200 nmol/l) within the range of those triggered by TCDC in gallbladder epithelial cells (~260 nmol/l) (14), whereas AC3 is inhibited by calmodulin-dependent protein kinase II (37), an enzyme activated by TCDC and by TUDC in gallbladder epithelial cells (8, 14). We found that the pattern of AC isoforms expressed in human gallbladder epithelial cells is different from that in human hepatocytes (unpublished observation). Because a distinct pattern of AC expression may result in a different balance between the positive and nega-
tive regulatory signals generated by bile salts, this may be part of the reason why bile salts exert inhibitory instead of potentiating effects on cAMP formation in hepatocytes (5). The switch between fluid absorption and secretion that occurs in the gallbladder after feeding is triggered by intracellular cAMP. cAMP accumulates in gallbladder epithelial cells in response to VIP (16), secretin (20), and β-adrenergic stimulation as shown in both present and previous reports (29, 30). Higher levels of cAMP were elicited in the present study when isoproterenol was used instead of forskolin, which may be because isoproterenol via β-adrenergic receptors activates all AC isoforms, including AC9 expressed at high levels in gallbladder epithelial cells, whereas forskolin stimulates all isoforms with the exception of AC9. In keeping with a potentiation of cAMP production, TCDC and TUDC potentiated the stimulation of chloride secretion by isoproterenol. In the absence of any other stimulus, bile salts also stimulated chloride secretion. On the basis of DIDS and BAPTA/AM inhibiting effects and of previous demonstration that TCDC induces a rapid rise of intracellular Ca²⁺ concentration in gallbladder epithelial cells (14), we may postulate that the secretory response to bile salts alone was mediated by calcium-dependent chloride channels (25). By contrast, in the presence of concomitant β-adrenergic stimulation, bile salts increased chloride secretion mainly via a cAMP-dependent pathway. Chloride secretion in this setting was inhibited by DPC but not by DIDS, consistent with an effect on the cAMP-dependent chloride channel, CFTR (3, 6, 9, 11, 15, 19, 27). It was previously shown that bile salts increase secretin-induced cAMP levels and secretin receptor gene expression in rat choangiocytes (1), and that ductal bile secretion is increased in bile acid-fed rats (2). Because cAMP-dependent chloride secretion promotes bicarbonate and fluid secretion in the gallbladder (9, 31), the present findings suggest that bile salts potentiate hormonal and neurogenic stimulation of fluid secretion that will facilitate the progression of bile in bile ducts and assist gallbladder emptying after feeding. Given that TUDC induces lower mucin secretion than TCDC (8), the present findings showing similar effects of TCDC and TUDC on cAMP-dependent anion secretion reinforce the concept that ursodeoxycholic acid may alter the ratio between mucin and hydroelectrolytic secretion in a direction that will increase bile fluidity.

The authors are very grateful to Jacques Hanoune and Joëlle Masliah of Institut National de la Santé et de la Recherche Médicale for helpful discussion.

This work was supported by a grant from the Association “Vaincre la mucoviscidose.”

REFERENCES

Bile salts and cAMP-dependent secretion


