# Mechanism of bile salt-induced mucin secretion by cultured dog gallbladder epithelial cells

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1. Hypersecretion of gallbladder mucin has been proposed to be a pathogenic factor in cholesterol gallstone formation. Using cultured gallbladder epithelial cells, we demonstrated that bile salts regulate mucin secretion by the gallbladder epithelium. In the present study we have investigated whether established second messenger pathways are involved in bile salt-induced mucin secretion. 2. The effect of activators and inhibitors on mucin secretion was studied by measuring the secretion of [$^3$H]*N*-acetyl-D-glucosamine-labelled glycoproteins. Intracellular cAMP content of the cells was measured using a radioimmunoassay. 3. Incubation of the cells with 10 mM taurocholate did not increase the intracellular cAMP content (25.7 versus control 22.8 pmol of cAMP/mg of protein). No stimulation of mucin secretion was observed after incubation with 1–100 $\mu$M concentrations of the calcium ionophores ionomycin and A23187. The stimulatory effect of 10 mM tauroursodeoxycholate (TUDC) on mucin secretion could not be inhibited by the addition of EDTA. Activation of protein kinase C (PKC) by 1 $\mu$g/ml phorbol 12-myristate 13-acetate (PMA) caused an increase in mucin secretion (342 % versus control 100 %), comparable with the effect of 40 mM TUDC. The effect of 10 ng/ml PMA could partially be inhibited by a concentration of 2 $\mu$M of the PKC inhibitor staurosporin. Staurosporin had no inhibitory effect on mucin secretion induced by TUDC. 4. In gallbladder epithelial cells bile salts do not stimulate mucin secretion via one of the classical signal transduction pathways. We hypothesize that bile salts act on mucin secretion via a direct interaction with the apical membrane.

## INTRODUCTION

Mucus glycoprotein, the primary secretory product of the gallbladder epithelium, has long since been implicated in the formation of cholesterol gallstones. The involvement of mucin in gallstone formation was first recognized in detailed studies of gallstone structure. Mucin has been shown to be present in the nucleus of several types of gallstones. A macromolecular complex of mucin and bilirubin has been identified as a major structural component of the gallstone matrix [1,2]. That mucin may contribute to the formation of gallstones in humans is suggested by the finding that purified human gallbladder mucin is capable of nucleating cholesterol crystals from both human [3] and model bile [4–7]. Moreover, gallbladder bile of cholesterol gallstone patients has been observed to contain increased amounts of mucin as compared with bile from control patients [1,8]. Gallbladder mucus hypersecretion and increased mucin concentration in bile are common findings in nearly all animal models of cholesterol gallstone disease. Mucin hypersecretion occurs before crystals and stones are formed, suggesting that mucin hypersecretion plays a role in the initial stages of gallstone formation [9,10].

We recently reported the successful long-term culturing and passaging of normal, well-differentiated gallbladder epithelial cells from the dog [11]; these cells form electrically leak-proof monolayers and synthesize protein and mucus glycoprotein. It was also shown that mucin secretion in these gallbladder epithelial cells could be stimulated by several secretagogues that caused an increase in intracellular cAMP, such as prostaglandin E2, prostaglandin E1, vasoactive intestinal peptide, adrenaline and isoprenaline [12]. In a more recent study [13] we have used these gallbladder cells to study mucin hypersecretion by the gallbladder epithelium. We demonstrated that mucin secretion by these cultured gallbladder cells could be stimulated by model bile solutions. Bile salts were demonstrated to be responsible for the stimulatory effect of model bile on mucin secretion and caused a dose-dependent stimulation of mucin secretion. Hydrophobic bile salts such as taurodeoxycholic acid and taurochenodeoxycholic acid were shown to be more potent stimulators of mucin secretion than the more hydrophilic bile salts tauroursodeoxycholate (TUDC) and taurocholate (TC).

Previously we have shown that the hypersecretion of mucin as caused by bile salts is a highly specific process, involving unknown interactions between the bile salt and the apical membrane of the epithelial cells. However, the exact mechanism by which bile salts are able to stimulate mucin secretion is not yet clear. In this study we have investigated the mechanism of bile salt-induced mucin secretion.

## MATERIALS AND METHODS

### Chemicals and reagents

Vitrogen was purchased from Celtrix Laboratories (Palo Alto, CA, U.S.A.). Tissue-culture plates were from Falcon (Lincoln Park, NJ, U.S.A.), and Transwell inserts (24.5 mm diameter, 3.0 $\mu$m pore size) were obtained from Costar (Cambridge, MA, U.S.A.). Cell culture media and reagents were obtained from BioWhittaker (Walkersville, MD, U.S.A.).

[$^3$H]*N*-Acetyl-D-glucosamine was from Amersham Life Science (Arlington Heights, IL, U.S.A.). The cAMP radioimmunoassay kit was purchased from Immunotech International (Marseille, France). Filters with a pore size of 0.45 $\mu$m were obtained from Millipore (Molsheim, France). TUDC was from Calbiochem (La Jolla, CA, U.S.A.). Staurosporin was purchased from Boehringer Mannheim (Mannheim, Germany). Phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-dibutyrate (PDB)

Abbreviations used: LDH, lactate dehydrogenase; PDB, phorbol 12,13-dibutyrate; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PTA, phosphotungstic acid; TCA, trichloroacetic acid; TC, taurocholate; TUDC, tauroursodeoxycholate.

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and the calcium ionophores A23187 (Calcimycin) and ionomycin, as well as prostaglandin E2 were from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical grade and purchased from Sigma except where noted.

### Isolation and culture of dog gallbladder epithelial cells

Gallbladder epithelial cells were isolated from dog gallbladder as described previously [11] by trypsinization. Stock cultures were grown on 60-mm-diam. Petri dishes coated with 1 ml of vitrogen gel (1 : 1 mixture of vitrogen and medium) in Dulbecco's modified Eagle's medium, supplemented with 10 % (v/v) fetal calf serum, 2 mM L-glutamine, 20 mM Hepes, 100 IU/ml penicillin and 100 $\mu$g/ml streptomycin. Medium was changed twice a week and the cells were maintained in a 37 °C incubator with 5 % $CO_2$. The cells were passaged when confluent (every 10–14 days), using trypsin (2.5 g/l) and EDTA (1 g/l) treatment.

### Mucin secretion assay

Mucin assays were performed as described by Kuver et al. [12] with slight modifications. Dog gallbladder epithelial cells were grown to confluence on Transwell inserts without vitrogen in 6-well tissue-culture plates. This allowed separate and independent access to the apical (luminal) and basolateral (serosal) compartments, and enabled simulation of luminal events mediated by biliary bile salts. The cells were labelled overnight (16–24 h) with 2 $\mu$Ci/well of [$^3$H]*N*-acetyl-D-glucosamine, in medium containing 10 % (v/v) fetal calf serum. The precursor sugar was added to the lower compartment of the wells, exposing only the basolateral side of the cells to the label. To remove unincorporated label the cells were then washed with sterile PBS, pH 7.4, for 30 min, followed by washing for another 30 min with serum-free medium. In some experiments the cells were pre-incubated with inhibitors during the washing with serum-free medium. Next, 2 ml aliquots of serum-free medium containing the activators and/or inhibitors were added to the upper compartments and 2 ml aliquots of serum-free medium to the lower compartments of the wells and the plates were returned to the incubator. After the incubation 1 ml of medium was harvested from each Transwell and spun at 500 ***g*** for 10 min to pellet released cells. A sample (0.5 ml) of the supernatant was then mixed with 8 ml of 10 % trichloroacetic acid (TCA)/1 % phosphotungstic acid (PTA), vortexed and incubated overnight at 4 °C. The cells in the Transwells were washed once with PBS and harvested with trypsin/EDTA. After collection they were again washed with PBS and spun down at 500 ***g*** for 10 min. Aliquots of the cell pellets were sampled for protein determination as described by Lowry et al. [14]. The remaining cells were treated with 10 % TCA/1 % PTA similar to the processing of the medium samples. After precipitation overnight the samples were spun at 1500 ***g*** for 15 min, the resulting protein pellet was washed twice, first with 5 ml of 10 % TCA/1 % PTA, then with 2 ml of 90 % ethanol. Finally the pellets were dissolved in 0.5 ml of water and counted in 10 ml of scintillation fluid. Results were expressed as percentage of control in d.p.m. per mg of cell protein.

### Measurement of cell viability

Cell viability was assessed by measuring the leakage of an endogeneous cytoplasmic enzyme, lactate dehydrogenase (LDH) into the culture medium, using the method of Amador et al. [15]. Results are expressed in units of LDH activity per mg of protein. For the control incubations the LDH activity is also expressed as a percentage of the total LDH activity present in the cells.

### Adenosine 3′,5′-cyclic monophosphate assay

Cells were grown to confluence on Transwell inserts without vitrogen in 6-well tissue-culture plates. The cells were washed with PBS, pH 7.4, for 30 min, followed by washing for another 30 min with serum-free medium. Next, 2 ml of serum-free medium (control), or solutions of 10 mM TC or 10 $\mu$M prostaglandin E2 in serum-free medium, were added to the upper compartments and 2 ml of serum-free medium to the lower compartments of the wells and the plates were returned to the incubator.

After 10, 20 or 30 min the medium was aspirated and the cells quickly washed with PBS. The cells were then quickly scraped from the insert with a flat-edged spatula and resuspended in 1 ml of deionized water, and pipetted into a test tube containing 1 ml of cold 5 % perchloric acid. After 15 min at 4 °C, the tubes were spun at 1500 ***g*** for 10 min. The protein pellet was washed with 10 % TCA/1 % PTA and then with 90 % ethanol. The protein concentration was determined as described by Lowry et al. [14]. Potassium bicarbonate (400 $\mu$l of 30 %) was added to the sample supernatant to precipitate potassium perchlorate. After 45 min at 4 °C the samples were spun at 1500 ***g*** for 10 min, and the supernatant was lyophilized overnight. The dried sample was dissolved in 1.2 ml of Tris/EDTA buffer. The adenosine 3′,5′-cyclic monophosphate (cAMP) concentration was measured using a cAMP radioimmunoassay kit from Immunotech International (Marseille, France). Results are expressed as pmol of cAMP per mg of protein [16].

## RESULTS AND DISCUSSION

### Effect of TC on intracellular cAMP levels

We have previously demonstrated that bile salts stimulate mucous glycoprotein secretion by cultured dog gallbladder epithelial cells [13]. To test the possibility that bile salts act on mucin secretion via the cAMP pathway, we examined the effect of 10 mM TC on intracellular cAMP levels in the gallbladder epithelial cells after 10, 20 and 30 min of incubation. Control cells were incubated in serum-free medium for 20 min, and the effect of 10 $\mu$M of prostaglandin E2 on intracellular cAMP levels after 10 and 20 min of incubation was also tested. After the incubations the cells were harvested and the intracellular cAMP levels measured as described in the Materials and methods section. In contrast to prostaglandin E2, no increase in cAMP could be demonstrated after each time interval following the incubation with 10 mM TC (Table 1). Thus our results do not support the hypothesis that

**Table 1 Effect of TC and prostaglandin E2 on intracellular cAMP concentrations of dog gallbladder epithelial cells**

Dog gallbladder epithelial cells were grown to confluence on Transwell inserts and incubated with either serum-free medium or solutions of 10 mM TC or 10 $\mu$M prostaglandin E2 in medium for 10, 20 or 30 min. Cells were then harvested and intracellular cAMP levels were measured as described in the Materials and methods section. Values are means of the number of wells mentioned in the table (the range is shown in parentheses).

| | Incubation time (min) | Intracellular cAMP (pmol/mg of protein) | Number of wells |
|---|---|---|---|
| Control | 20 | 22.8 (18.6–28.5) | $n = 5$ |
| TC (10 mM) | 10 | 11.8 (11.0–12.5) | $n = 2$ |
| | 20 | 25.7 (2.6–44.0) | $n = 5$ |
| | 30 | 13.3 (13.0–13.7) | $n = 2$ |
| Prostaglandin E2 (10 $\mu$M) | 10 | 233.3 (193.5–273.0) | $n = 2$ |
| | 20 | 298.1 (199.3–411.2) | $n = 5$ |

**Table 2 Intracellular and secreted mucin of dog gallbladder epithelial cells: effects of the calcium ionophores ionomycin and A23187**

Dog gallbladder epithelial cells were grown to confluence on Transwell inserts and incubated either with serum-free medium or solutions of the calcium ionophores ionomycin or A23187 in medium for 4 h. Mucin secretion, intracellular mucin content and LDH release were then measured as described in the Materials and methods section. LDH activity is expressed in units per mg of protein. The control values are also expressed as a percentage of the total LDH activity present in the cells. Values are means of duplicate wells (individual values are shown in parentheses). The experiment was performed twice with similar results.

| Ionophore ($\mu$M) | Mucin in medium (% of control) | Mucin in cells (% of control) | LDH in medium (units/mg of protein) |
|---|---|---|---|
| Ionomycin | | | |
| 0 | 100 (90, 110) | 100 (100, 100) | 0.16 (0.14, 0.17) (0.42% of total) |
| 100 | 460 (450, 469) | 67 (64, 69) | 21.8 (20.3, 23.2) |
| 10 | 221 (190, 251) | 92 (86, 97) | 13.8 (10.5, 17.0) |
| 1 | 104 (96, 111) | 97 (93, 100) | 0.33 (0.23, 0.44) |
| A23187 | | | |
| 0 | 100 (98, 102) | 100 (96, 104) | 0.22 (0.18, 0.25) (0.49% of total) |
| 100 | 115 (113, 116) | 90 (90, 90) | 0.25 (0.23, 0.26) |
| 10 | 122 (120, 124) | 93 (90, 95) | 0.46 (0.41, 0.50) |
| 1 | 105 (97, 112) | 90 (84, 95) | 0.44 (0.32, 0.55) |

bile salts elicit their response by increasing the intracellular cAMP content of the cells.

### Effect of calcium ionophores on mucin secretion

The role of calcium ions as a second messenger involved in the exocytosis of proteins has been well established. We investigated the effect of the calcium ionophores ionomycin and A23187 on mucin secretion by the gallbladder cells. After labelling the epithelial cells with [$^3$H]*N*-acetylglucosamine, solutions of both ionophores in serum-free medium were added to the upper compartments of Transwells containing the cells. The ionophores were added in concentrations varying from 1 to 100 $\mu$M and incubated for 4 h. In the control incubation the cells were incubated with serum-free medium. The concentration of $Ca^{2+}$ in the culture medium was 1.8 mM. Incubation of the cells with 100 $\mu$M ionomycin resulted in an increase in mucin secretion by the cells (460% versus 100% control). However, at this concentration the increase in mucin secretion was accompanied by a drastic increase in LDH leakage from the cells (Table 2). Concentrations of ionomycin that did not cause cell damage, did not affect mucin secretion. The stimulatory effect of the ionophore on mucin secretion was completely attributable to cell lysis.

After incubation with A23187, a less potent calcium ionophore, no increase in mucin secretion was observed (Table 2). No cytotoxic effect of this ionophore could be demonstrated, since no increase in LDH activity in the culture medium was observed.

The effect of the calcium-chelator EDTA on mucin secretion was also studied, both in the absence and presence of the bile salt TUDC. Cells were incubated for 4 h, either in serum-free medium, or in medium containing 0.5, 1 or 2 mM EDTA. Cells were also incubated with 10 mM TUDC, and with a combination of TUDC and 0.5, 1 or 2 mM EDTA. No effect of EDTA on basal or TUDC-stimulated mucin secretion could be demonstrated, indicating that mucin secretion by the gallbladder cells can be stimulated, independent of the extracellular $Ca^{2+}$ concentration (results not shown).

Taurine-conjugated bile salts have been demonstrated to act as $Ca^{2+}$ ionophores [17]. Also it has been shown that hydrophobic bile salts can form membrane channels specific for calcium [18]. Using cultured guinea-pig gallbladder explants Malet et al. [19] demonstrated that A23187 can stimulate glycoprotein secretion. We did not find a specific effect of calcium ionophores on mucin secretion. The results of Malet et al. might be explained by a cytotoxic effect of the calcium ionophores on the gallbladder explants. Perhaps gallbladder explants are more sensitive to calcium ionophores than are cultured gallbladder epithelial cells.

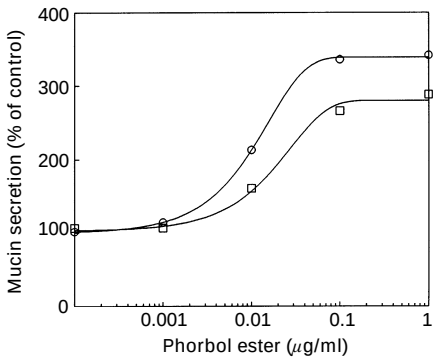


**Figure 1 Effect of different concentrations of phorbol esters on mucin secretion by dog gallbladder epithelial cells**

Dog gallbladder epithelial cells were grown to confluence on Transwell inserts and incubated either with serum-free medium or solutions of the PKC activators PMA (○) or PDB (□) in medium for 4 h. Mucin secretion was then measured as described in the Materials and methods section. Values are means of duplicate wells.

### Effect of protein kinase C (PKC) activators on mucin secretion

There have been numerous reports that bile salts can mediate a response through an activation of PKC [20–25]. We investigated the effect of two different activators of PKC on mucin secretion by the gallbladder epithelial cells. The phorbol esters PMA and PDB have both been reported to specifically activate calcium-dependent PKC isoenzymes. The phorbol esters were added to the cells in concentrations varying from 0.1 ng/ml to 1 $\mu$g/ml. After an incubation for 4 h with either control medium or the PKC activators, both phorbol esters were found to be potent stimulators of mucin secretion (Figure 1). At a concentration of 1 $\mu$g/ml a maximum increase in mucin secretion was observed for both PMA as well as PDB (342% and 289% of control, respectively), although PMA was found to be more effective. Only the highest concentration of PMA used caused an increase in LDH leakage from the cells, so that the observed increase in mucin secretion could not be ascribed solely to a cytotoxic effect of the activators (Table 3). Phorbol esters appear to mimic the effect of bile salts on mucin secretion. With both TUDC and the phorbol ester PMA the same maximum stimulation of mucin secretion could be achieved; 40 mM TUDC causes an increase in mucin secretion of approx. 350% of control [12] versus 1 $\mu$g/ml of PMA, an increase of 342% of control. To further study the possibility that bile salts stimulate mucin secretion via activation of PKC, it was investigated whether the effect of PMA and TUDC on mucin secretion could be inhibited by the PKC inhibitor staurosporin [26]. The cells were labelled overnight and then first washed with PBS for 30 min. Control cells were incubated in serum-free medium for 30 min and then incubated for 4 h with either serum-free medium or medium containing 10 ng/ml PMA. Other cells were pre-incubated for 30 min in medium containing 0.5, 1, 2 or 4 $\mu$M staurosporin and then

**Table 3 Effect of different concentrations of phorbol esters on mucin secretion by dog gallbladder epithelial cells**

Dog gallbladder epithelial cells were grown to confluence on Transwell inserts and incubated either with serum-free medium or solutions of the PKC activators PMA or PDB in medium for 4 h. Mucin secretion and LDH release were then measured as described in the Materials and methods section. LDH activity is expressed in units per mg of protein. The control values are also expressed as a percentage of the total LDH activity present in the cells. Values are means of duplicate wells (individual values are shown in parentheses). The experiment was performed twice with similar results.

| Phorbol ester (ng/ml) | Mucin in medium (% of control) | LDH in medium (units/mg of protein) |
|---|---|---|
| PMA | | |
| 0 | 100 (93, 107) | 0.38 (0.37, 0.39) (1.04% of total) |
| 1000 | 342 (319, 365) | 0.73 (0.71, 0.76) |
| 100 | 336 (310, 362) | 0.60 (0.57, 0.64) |
| 10 | 213 (199, 226) | 0.57 (0.47, 0.66) |
| 1 | 115 (110, 120) | 0.42 (0.42, 0.43) |
| 0.1 | 102 (95, 108) | 0.42 (0.41, 0.42) |
| PDB | | |
| 0 | 100 (96, 104) | 0.49 (0.30, 0.69) (1.79% of total) |
| 1000 | 289 (282, 295) | 0.57 (0.54, 0.61) |
| 100 | 267 (255, 278) | 0.54 (0.47, 0.60) |
| 10 | 161 (156, 165) | 0.42 (0.34, 0.49) |
| 1 | 107 (105, 109) | 0.23 (0.20, 0.26) |
| 0.1 | 107 (100, 113) | 0.30 (0.29, 0.31) |

**Table 4 Effect of staurosporin on PMA- and TUDC-stimulated mucin secretion by dog gallbladder epithelial cells**

Dog gallbladder epithelial cells were grown to confluence in Transwell inserts and then pre-incubated either in serum-free medium (control) or in medium containing 0.5–4 $\mu$M of staurosporin for 30 min. The cells were then incubated for 4 h in serum-free medium (control) or in medium containing a combination of 10 ng/ml PMA with 0.5–4 $\mu$M of staurosporin, or in medium containing a combination of 10 mM TUDC with 0.5–4 $\mu$M of staurosporin. Mucin secretion was then measured as described in the Materials and methods section. Values are means of duplicate wells (individual values are shown in parentheses). The experiment was performed three times with similar results.

| | Mucin in medium (% of control) |
|---|---|
| PMA (10 ng/ml) + staurosporin | |
| Control | 100 (100, 100) |
| 0 | 253 (244, 263) |
| 0.5 $\mu$M | 208 (199, 217) |
| 1 $\mu$M | 152 (150, 155) |
| 2 $\mu$M | 139 (138, 140) |
| 4 $\mu$M | 160 (156, 163) |
| TUDC (10 mM) + staurosporin | |
| Control | 100 (89, 111) |
| 0 | 257 (238, 277) |
| 0.5 $\mu$M | 287 (275, 299) |
| 1 $\mu$M | 300 (295, 304) |
| 2 $\mu$M | 284 (278, 290) |
| 4 $\mu$M | 290 (264, 316) |

incubated for 4 h in medium containing 10 ng/ml PMA in combination with again 0.5, 1, 2 or 4 $\mu$M staurosporin. The results are shown in Table 4. PMA at a concentration of 10 ng/ml caused an increase in mucin secretion of 253% as compared with the control incubation without PMA (100%). Concentrations of staurosporin above 1 $\mu$M had an inhibitory effect on mucin secretion by the PMA-stimulated gallbladder cells. Maximal inhibition to 139% of control was obtained with 2 $\mu$M inhibitor. Increasing the staurosporin concentration did not result in a further inhibition of mucin secretion. This is probably due to a cytotoxic effect of staurosporin on the gallbladder cells.

**Table 5 Intracellular and secreted mucin of dog gallbladder epithelial cells: effect of pre-incubation with PMA or TUDC**

Dog gallbladder epithelial cells were grown to confluence on Transwell inserts and incubated for 30 min with either serum-free medium or solutions of PMA or TUDC in medium. The cells were then washed and incubated for 4 h with serum-free medium. Mucin secretion and intracellular mucin content were then measured as described in the Materials and methods section. Values are means of duplicate wells (individual values are shown in parentheses).

| | Mucin in medium (% of control) | Mucin in cells (% of control) |
|---|---|---|
| Control | 100 (100, 100) | 100 (97, 103) |
| PMA (10 ng/ml) | 229 (228, 230) | 88 (84, 92) |
| TUDC (10 mM) | 91 (90, 91) | 92 (90, 93) |

Similar experiments were carried out with cells incubated with 10 mM TUDC. The results are shown in Table 4. TUDC-stimulated mucin secretion could not be inhibited by staurosporin.

We then investigated whether PMA and TUDC have to be in constant contact with the cells to exert their stimulatory effects. Cells were labelled with [$^3$H]*N*-acetylglucosamine and washed with PBS and serum-free medium as described. The cells were then pre-incubated for 30 min with control medium, 10 ng/ml PMA or 10 mM TUDC. After the pre-incubation the cells were washed quickly with serum-free medium and then finally incubated for 4 h in serum-free medium and mucin secretion was measured. As can be concluded from Table 5, only the pre-incubation with PMA resulted in an increase in mucin secretion after 4 h, 229% versus 100% control. The bile salt had no stimulatory effect on mucin secretion after this time interval. The same results were obtained after a 15 min pre-incubation (results not shown). In contrast to the phorbol ester, bile salts need to be in constant contact with the cells to exert their effect on mucin secretion.

Taken together, our experiments have not provided evidence for a PKC-mediated effect of bile salts on mucin secretion. Although bile salts and phorbol esters were equally effective stimulators they showed different mechanisms of action. However, it cannot be excluded that bile salts exert their influence on the same intracellular cascade, but downstream from the point of action of phorbol esters.

Our experiments show that multiple intracellular second messenger systems are involved in the regulation of mucin secretion by gallbladder epithelial cells. In a previous study it has been shown that an increase in cAMP stimulates mucin secretion. We now demonstrate that in addition PKC activation also causes mucin hypersecretion. Activation of this pathway might be an important trigger for increased mucin secretion in the inflamed gallbladder.

The exact mechanism by which bile salts act on mucin secretion requires further study. Although the effect of bile salts on mucin secretion appears to be instantaneous [13], no prolonged effect was observed, since pre-incubation with bile salt did not result in a lasting effect on mucin secretion by the dog gallbladder epithelial cells. Our results demonstrate that continuous presence of bile salts is required. In hepatocytes bile salts have been shown

to stimulate exocytotic pathways [27–29]. In these studies signal transduction pathways have been implicated to play a role. Our study points more to a direct effect of the bile salts on the apical membrane of the cells. Numerous examples of bile salt interactions with biological membranes have been described [30–32]. These processes show a similar dependency on bile salt hydrophobicity as the effect on mucin secretion. We speculate that the interaction of bile salts with the apical membrane of the dog gallbladder epithelial cells facilitates fusion of intracellular mucin vesicles with the cell membrane, thereby accelerating exocytosis.

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