Biliary pancreatitis is the most common etiology of acute pancreatitis, accounting for 30–60% of cases. A dominant theory for the development of biliary pancreatitis is the reflux of bile into the pancreatic duct and subsequent exposure to pancreatic acinar cells. Bile acids are known to induce aberrant Ca\(^{2+}\) signals in acinar cells as well as nuclear translocation of NF-\(\kappa\)B. In this study, we examined the role of the downstream Ca\(^{2+}\) target calcineurin on NF-\(\kappa\)B translocation. Freshly isolated mouse acinar cells were infected for 24 h with an adenovirus expressing an NF-\(\kappa\)B luciferase reporter. The bile acid tauroliothocholic acid-3-sulfate caused NF-\(\kappa\)B activation at concentrations (500 \(\mu\)M) that were associated with cell injury. We show that the NF-\(\kappa\)B inhibitor Bay 11-7082 (1 \(\mu\)M) blocked translocation and injury. Pretreatment with the Ca\(^{2+}\) chelator 1,2-bis(o-aminophenoxy)ethane-N,N,N\(^{\prime}\),N\(^{\prime}\)-tetraacetic acid, the calcineurin inhibitors FK506 and cyclosorine A, or use of acinar cells from calcineurin \(\alpha\beta\)-deficient mice each led to reduced NF-\(\kappa\)B activation with tauroliothocholic acid-3-sulfate. Importantly, these manipulations did not affect LPS-induced NF-\(\kappa\)B activation. A critical upstream regulator of NF-\(\kappa\)B activation is protein kinase C, which translocates to the membranes of various organelles in the active state. We demonstrate that pharmacologic and genetic inhibition of calcineurin blocks translocation of the PKC-\(\delta\) isoform. In summary, bile-induced NF-\(\kappa\)B activation and acinar cell injury are mediated by calcineurin, and a mechanism for this important early inflammatory response appears to be upstream at the level of PKC translocation.

Acute pancreatitis is a painful necroinflammatory disease and a rising clinical problem. It is a major inpatient gastrointestinal diagnosis in the United States, accounting for more than 200,000 hospitalizations annually (1, 2). Acute pancreatitis has many causes, of which biliary pancreatitis is the most common, accounting for 30–60% of all cases (3, 4). Biliary pancreatitis results when gallstones or biliary sludge obstruct the distal common bile duct (5). A proposed mechanism for this etiology is the reflux of bile acids into the pancreatic duct (6, 7), which ultimately reaches the terminal ductules and acinar lumen. The most immediate effect of bile acids is their ability to elicit cytosolic Ca\(^{2+}\) signals within acinar cells (8–11). These changes in Ca\(^{2+}\) are observed at bile acid concentrations below the critical micellar concentration (8, 10).

The exposure of bile acids also initiates early inflammatory signals, notably the transcription factor or nuclear factor \(\kappa\) light chain enhancer of activated B cells (NF-\(\kappa\)B) (11–15). NF-\(\kappa\)B is a heterodimeric complex composed of members of the Rel protein family (16–18). The most common dimeric form is the p50-p65 heterodimer. Prior to activation, NF-\(\kappa\)B proteins are predominantly restricted to the cytosol by associating with members of the IkB family. Phosphorylation of IkB by IkB kinases triggers the degradation of IkB and allows translocation of NF-\(\kappa\)B to the nucleus. Upon translocation, NF-\(\kappa\)B mediates the expression of numerous inflammatory response genes, including proinflammatory cytokines, chemokines, adhesion molecules, and inducible enzymes such as cyclooxygenase 2 and inducible nitric oxide synthase (19–21). Of importance, NF-\(\kappa\)B plays a role in early inflammation during pancreatitis (22). In addition, NF-\(\kappa\)B is implicated in several other non-inflammatory pathways, including cell proliferation, differentiation, apoptosis, and survival (23, 24).

Ca\(^{2+}\) signals are linked to NF-\(\kappa\)B activation primarily through PKC (25–27). However, it is not known in the acinar cell whether additional Ca\(^{2+}\) targets are required. A sustained rise in acinar cell cytosolic Ca\(^{2+}\) is associated with NF-\(\kappa\)B activation and cell injury (11). In this study, we examined whether the Ca\(^{2+}\)/calmodulin-dependent serine/threonine phosphatase calcineurin mediates NF-\(\kappa\)B activation in acinar cells because of bile acids. Calcineurin is a heterodimer complex

**Background:** Bile acids cause activation of NF-\(\kappa\)B and lead to injury in pancreatic acinar cells, but the mechanism is unknown.

**Results:** Pharmacologic and genetic inhibition of calcineurin reduces bile acid-induced NF-\(\kappa\)B activation and PKC-\(\delta\) translocation.

**Conclusion:** Calcineurin facilitates bile-induced NF-\(\kappa\)B activation. The mechanism is at the level of PKC activation.

**Significance:** We have identified a novel mechanism by which bile acids facilitate NF-\(\kappa\)B activation in pancreatic acinar cells.
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consisting of a catalytic domain, calcineurin A (CnA) and a regulatory Ca\(^{2+}\) binding domain, calcineurin B (CnB) (28–30). Calcineurin has several known phosphoprotein targets, notably nuclear factor of activated T-cells. Recent work, however, has implicated a critical role for calcineurin in mediating Ca\(^{2+}\)-dependent NF-κB translocation (31, 32). We and others have demonstrated that calcineurin is a primary target of aberrant Ca\(^{2+}\) signals in pancreatic acinar cells and plays a key role in the pathogenesis of both caerulein-, muscarinic-, and bile acid-induced pancreatitis (11, 12, 15, 33–35).

Here we demonstrate in pancreatic acinar cells 1) that bile acids cause NF-κB activation, 2) that NF-κB activation facilitates bile acid-induced cell injury, 3) that NF-κB activation is dependent on calcineurin, and 4) that the mechanism for this calcineurin effect is at the level of PKC translocation.

**EXPERIMENTAL PROCEDURES**

**Reagents and Animals**—All reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. Male Swiss Webster mice weighing 20–25 g (Charles River Laboratories, Inc., Wilmington, MA) were fed standard laboratory chow, given free access to water, and randomly assigned to control or experimental groups. Calcineurin Aβ-deficient (CnAβ\(^{-/-}\)) mice were of the strain B6129PF1/J and were a gift from Dr. J. Molkentin (36). Age-, sex-, and strain-matched control mice were used as WT controls (The Jackson Laboratory, Bar Harbor ME).

The PKC-δ translocation inhibitor 8V1-1 (SFNSYELGSL) and a scrambled peptide (LSETKPAV) were a gift from Dr. Daria Mochen-Rosen (37). Each of these peptides was conjugated to a TAT peptide (YGRKKRRQRRR) to make them cell-permeable.

**Preparation of Pancreatic Acini**—Groups of pancreatic acinar cells were isolated as described previously (38), with minor modifications. Briefly, the pancreas was removed and minced for 5 min in DMEM/F12 1× buffer without phenol red (Invitrogen) plus 0.1% BSA and 2 mg/ml type 4 collagenase (Worthington, Freehold, Nj). The suspension was incubated briefly for 5 min at 37 °C while shaking at 90 rpm. The buffer was removed and replaced with new collagenase buffer and then incubated for 35 min. The suspension was filtered through a 300-μm mesh (Sefar American, Depew, NY) and then washed three times with collagenase-free buffer. Acinar cells were allowed to equilibrate for 5 min at 37 °C prior to use.

**NF-κB-Luciferase Activity Assay**—Acinar cells were infected with Ad-NF-κB-luciferase 16 h prior to stimulation using a method described previously (39). Following a wash with DMEM/F12 medium, acinar cells were evenly distributed in a 48-well plate and incubated for 30 min at 37 °C. They were stimulated for 6 h with the bile acids tauroliothococholic acid-3-sulfate (TLCS) or taurocholate or the cholecystokinin analog stimulated for 6 h with the bile acids taurolithocholic acid-3-sulfate (TLCS) or taurocholate or the cholecystokinin analog.

**Cell Injury Assays**—Prior to stimulation with bile acids or caerulein, cells were washed twice with fresh buffer to clear any residual lactate dehydrogenase from the media. Acinar cells were stimulated for 4 h, unless otherwise specified, and cell injury was measured using a cytotoxicity assay for lactate dehydrogenase leakage (Promega, Madison, WI). Absorbance was measured at 490 nm 15 min after stopping the enzyme reaction. Results were expressed as percent lactate dehydrogenase released into the medium. For propidium iodide (PI) uptake, acinar cells were incubated in a 48-well plate with 50 μg/ml of PI (Sigma) for 30 min prior to addition of the bile acids. Fluorescence was measured at 536 nm excitation and 617 nm emission wavelengths over time (0–6 h). Total DNA content was measured by PI fluorescence after cell lysis with 0.5% Triton X-100.

**Western Blot Analysis for PKC Isoforms and PKC-δ Localization**—The dispersed acini were homogenized using a Dounce homogenizer (50 strokes/sample) in ice-cold homogenization buffer containing 130 mM NaCl, 50 mM Tris HCl (pH 7.5), 5 mM EGTA, 5 mM EDTA, 1.5 mM MgCl\(_2\), 10 mM NaF, 1 mM Na\(_3\)VO\(_4\), 10 mM Na\(_2\)P\(_2\)O\(_7\), 1 mM PMSF, and 10% (v/v) glycerol plus 5 μg/ml each of pepstatin, leupeptin, and aprotinin. Homogenates were centrifuged at 500 × g for 10 min at 4 °C to remove unbroken cells, nuclei, and other debris. Supernatants were recovered and ultracentrifuged at 150,000 × g for 45 min at 4 °C to separate the cytosolic and membrane fractions. The pellet was washed five times, resuspended in homogenization buffer containing 0.5% Triton X-100, sonicated five times for 10 s on ice, and incubated for 30 min at 4 °C. Lastly, the samples were centrifuged at 15,000 × g for 15 min, and the resulting supernatant was designated as the membrane fraction. Western blot analysis was performed on both fractions using a PKC-δ-specific antibody (catalog no. sc-213, Santa Cruz Biotechnology, Dallas, TX). Blots with PKC-α and PKC-ε were performed using Santa Cruz Biotechnology antibodies (catalog nos. sc-8393 and sc-1681, respectively). Densitometry was performed using Image J software (National Institutes of Health).

**Preparation of Human Acinar Cells**—Pancreatic tissue was harvested from cadaveric donors as described by Bottino et al. (40). Briefly, specimens were transported in cold preservation fluid (histidine-tryptophan-ketoglutarate) with a cold ischemia time of 11 h. Fat, connective tissue, and blood vessels were removed. The pancreas was washed in a mixture of antibiotics and then cut at the level of the neck to reveal the pancreatic duct. Catheters were placed in both sides of the transected duct, and a blend of exogenous enzymes, including collagenases and neutral proteases (Serva, GMP grade, Heidelberg, Germany) freshly dissolved in Hanks’ balanced salt solution, was prewarmed to 28–30 °C and introduced intraductally. The pancreatic organ was then transferred to a Ricordi digestion chamber, and the pancreatic tissue was disrupted mechanically as described by Ricordi et al. (41). Pancreatic cells were washed several times in cold RPMI medium supplemented with...
human serum albumin (2.5% total volume). Endocrine cell contamination was < 1%. Acinar cells were kept in calcium- and magnesium-free Hanks’ buffer, and cell injury assays were performed as described above.

**Statistical Analysis**—Data were expressed as mean ± S.E. unless stated otherwise. Statistical analysis was performed using Student’s t test. Statistical significance was defined as \( p < 0.05 \). NF-κB luciferase and propidium iodide uptake were measured as relative luminescent or fluorescent units. Time course for TLCS-induced NF-κB-luciferase activity. Cell injury was measured by lactate dehydrogenase leakage or PI uptake (Fig. 1 C and D). Values for NF-κB and propidium iodide were normalized to total DNA and expressed as fold increase relative to control.

**RESULTS**

**Bile Acids Cause NF-κB Activation, and NF-κB Mediates TLCS-induced Acinar Cell Injury**—We and others have demonstrated that bile acids cause injury to isolated pancreatic acinar cells (8, 10, 11, 15). The injury is dependent on an early, aberrant rise in cytosolic Ca\(^{2+}\), which triggers several downstream pathways. Among these is the translocation of NF-κB to the nucleus (22, 25, 42, 43). We used a reporter system to evaluate NF-κB activation by infecting acinar cells with an adenovirus containing an NF-κB-driven luciferase gene (15).

Recent studies examining the effects of bile acids on acinar cell pathophysiology have used TLCS (8, 10, 44, 45), which is the least hydrophilic of the naturally occurring bile acids. TLCS induces Ca\(^{2+}\) signals at submillimolar concentrations that are below its critical micellar concentration (46). We found that TLCS (500 \( \mu \text{M} \)) markedly increased NF-κB-luciferase activity and that the NF-κB inhibitor BAY 11-7082 reversed this effect by 50% (Fig. 1A, \( p < 0.05 \)). Further, we established a time course for TLCS-induced NF-κB-luciferase activity that demonstrates a maximal increase above control 6 h after TLCS treatment (Fig. 1B, \( p < 0.05 \)). For this reason, we chose the 6-h time point for all of the NF-κB luciferase experiments. We then examined two markers of injury, lactate dehydrogenase leakage or PI uptake (Fig. 1, C and D). The NF-κB inhibitor BAY 11-7082 reduced each of the two indices by 51 and 59% compared with TLCS, respectively (\( p < 0.05 \)). Similar results were observed with the less potent bile acid taurocholic acid (Fig. 1, E and F, TC). The findings indicate that NF-κB mediates bile-induced acinar cell injury.
Calcineurin Mediates Acinar Cell NF-κB Activation Because of TLCS—To determine whether Ca\(^{2+}\) elevations are sufficient to induce acinar cell NF-κB activation, we used the Ca\(^{2+}\) ionophores ionomycin and A23187. Neither ionophore caused a significant increase above control levels (Fig. 2A). To examine whether TLCS-induced NF-κB activation is Ca\(^{2+}\)-dependent, acinar cells were treated with the intracellular Ca\(^{2+}\)-chelator 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) 30 min prior to TLCS exposure. BAPTA reduced NF-κB activation in our model system by 95% (p < 0.05, Fig. 2B). Similar findings with BAPTA were observed following treatment with caerulein (Fig. 2C). The results suggest that cytosolic Ca\(^{2+}\) elevations are necessary but not sufficient to induce NF-κB activation.

To examine whether calcineurin is involved in NF-κB activation, we used the specific calcineurin inhibitors FK506 and cyclosporine (CsA). Both inhibitors work by forming a complex with calcineurin that prevents access of substrates to its catalytic site (47). Importantly, both inhibitors reduced the activation of NF-κB (Fig. 2B), suggesting that TLCS-induced NF-κB activation is mediated by calcineurin. To complement this pharmacologic approach, we isolated pancreatic acinar cells from mice deficient in the acinar predominant calcineurin isoform Aβ (CnAβ\(^{-/-}\)) (36, 48). We have shown previously that CnAβ deficiency protects against neurogenic-induced zymogen activation and cell injury in vitro (48) as well as bile acid-induced pancreatitis in vivo (15). Here, we demonstrate that acini from these mice do not elicit NF-κB activation in response to TLCS stimulation (Fig. 2D).

LPS-induced NF-κB Activation Does Not Involve Calcineurin—To know whether calcineurin-mediated NF-κB activation is specific for bile acid exposure, we examined another known activator of NF-κB, LPS, which is a bacterial endotoxin that binds to the innate immune receptor, toll-like receptor 4 (TLR4). Two downstream pathways are activated by TLR4, each of which is thought to directly activate NF-κB (49–51). The MyD88-dependent pathway recruits the kinases IRAK1 and IRAK4, which phosphorylate TRAF6, leading to the activation of the IkB kinase complex, allowing for NF-κB translocation (52, 53). The MyD88-independent pathway is not fully understood but is thought to involve Trif adaptor molecules and RIP1 and RIP3 to activate NF-κB (54–56). TLR4 is found in the acinar cell and is known to mediate bile injury (57).

We found that LPS induced a 3.9-fold increase in NF-κB-luciferase (p < 0.05, Fig. 3A). However, neither BAPTA nor the two calcineurin inhibitors had any effect on NF-κB activation because of LPS. As a positive control, preincubation with the global PKC inhibitor GF 109203X (1 μM) brought luciferase down to control levels. The CnAβ-deficient acinar cells had similar levels of NF-κB activation with LPS as the wild-type cells (Fig. 3B). Taken together, the results indicate that calcineurin selectively mediates bile acid-induced NF-κB activation.

Calcineurin Mediates Bile Acid-induced PKC Translocation—PKC constitutes a family of serine/threonine kinases, several of which are critical upstream mediators of NF-κB. There are 10 PKC isoforms, and they are divided into three classes: conventional (α, βI, βII, and γ), novel (δ, ε, θ, and η),
**FIGURE 3.** LPS-induced NF-κB activation in pancreatic acinar cells. **A**, NF-κB-luciferase activity was measured from acinar cells stimulated with LPS (10 μg/ml). Cells were pretreated with the intracellular Ca²⁺ chelator BAPTA (20 μM), the calcineurin inhibitors FK506 (10 μM) or CsA (1 μM), or the PKC inhibitor GF109203X (GFX, 1 μM). **B**, NF-κB luciferase activity was measured from wild-type or CnAB⁻/⁻ acinar cells stimulated with LPS (n = 3). Ctl, control. # and *, p < 0.05 relative to control or LPS, respectively.

**FIGURE 4.** Calcineurin mediates TLCS-induced PKC-δ translocation. **A**, expression of PKC-α, PKC-δ, and PKC-ε was determined by Western blot analysis from either a rat acinar cell line or primary mouse acinar cells. **B**, PKC-δ expression was examined from both cytosolic and membrane fractions of mouse acinar cells in the presence of TLCS with or without the calcineurin inhibitors FK506 or CsA or from CnAβ-deficient acinar cells. **C**, densitometry measurements were taken from both fractions and are expressed as membrane/cytosol and normalized to the maximal response. **D** and **E**, NF-κB-luciferase activity and cell injury were measured from acinar cells stimulated with TLCS with or without the specific PKC-δ translocation inhibitor δV1-1. RLU, relative luminescence units. # and *, p < 0.05 relative to control or TLCS, respectively.
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![Graph showing NF-κB Activation](image)

**FIGURE 5. Calcineurin and NF-κB mediate TLCS-induced acinar cell injury in human acinar cells.** Human acinar cells were obtained from cadaveric specimens and stimulated with TLCS (500 μM) in the presence or absence of the calcineurin inhibitor FK506 (10 μM) or the NF-κB inhibitor BAY 11-7082 (1 μM). Cell injury was measured as percent lactate dehydrogenase release. # and *, p < 0.05 relative to control or TLCS, respectively.

and atypical (ζ, and δ/λ) (58–60). The PKC isoforms expressed in the rat acinar cell are α, δ, ε, and ζ (61–65), but PKC-ζ does not translocate to the membrane in response to secretagogue stimulation (62, 66). Of the former three isoforms, we found that only PKC-α and PKC-δ are expressed in the primary mouse acinar cells (Fig. 4A). PKC-δ has been implicated in a host of acinar cell functions, including secretion (62), FK506 translocation (65, 66), and PKD1 activation (67). Bile acids failed to cause translocation of PKC-α (data not shown). There was, however, a 10-fold increase in cytosol-to-plasma membrane translocation of PKC-δ (Fig. 4C). Pharmacologic inhibition with FK506 and CsA or genetic deletion of CnAα each reduced PKC-δ translocation (Fig. 4, B and C). In addition to loading equal amounts of protein, the sodium potassium ATPase and GAPDH were examined in a sample experiment to control for membrane and cytosol loading, respectively (data not shown).

Lastly, to examine the role of PKC-δ on TLCS-induced NF-κB activation we used a peptide (δV1-1) that inhibits PKC-δ translocation. The peptide corresponds to a specific sequence in the V1 region that is responsible for anchoring PKC-δ to its translocation site (37). Thus, the peptide competitively inhibits the binding of PKC-δ (66). We demonstrate that δV1-1 blocks TLCS-induced NF-κB luciferase activity and cell injury by 75 and 100% relative to control, respectively (Fig. 4, D and E). Incubation with the scrambled peptide did not reduce any of these parameters (data not shown). The results demonstrate that bile acid-induced translocation of PKC-δ is dependent on calcineurin and that it mediates NF-κB activation.

**Calcineurin and NF-κB Mediate TLCS-induced Acinar Cell Injury in Human Acinar Cells**— It is important to know the relevance of the current findings to humans. For this reason, we obtained live human pancreatic acinar cells from a 24-year-old male cadaveric donor who died from a motor vehicle accident. The cold ischemia time was ~11 h. The cells were stimulated with TLCS in the presence or absence of FK506 or BAY 11-7082. Similar to what was seen in the rodent model system, TLCS (500 μM) caused an increase in lactate dehydrogenase leakage (p < 0.05, Fig. 5). In addition, FK506 and the NF-κB inhibitor each reduced cell injury by 82 and 70%

**DISCUSSION**

The key finding of this study is that bile acid-induced NF-κB activation is dependent on calcineurin. The role of NF-κB in the exocrine pancreas is controversial (68). Some studies argue that NF-κB plays a protective role in pancreatitis (69, 70), whereas others have suggested that it worsens disease (25, 71–73). Our data support the latter claim that NF-κB activation facilitates the early stage of injury within the acinar cell.

Bile acids trigger inflammatory pathways, leading to acute pancreatitis (8, 10, 11, 15, 33). It is still unclear, however, how bile acids acts on the acinar cell. One study suggests the involvement of the G protein-coupled bile acid receptor (Gpbar1), also known as Tgr5 (10). Mice lacking Gpbar1 were protected against early indices of biliary pancreatitis in vivo and in vitro. Other reports have examined bile acid transporters on the plasma membrane (11). Kim et al. demonstrated acinar cell expression of both the apically localized sodium taurocholate cotransporting polypeptide and the basolateral bicarbonate-dependent bile acid exchanger OATP1 (11).

Whether as a ligand for a surface receptor or directly transported into the cell, bile acids trigger a cascade of events, which results in cytosolic Ca^{2+} elevations (8, 10, 11). In the intact acinar cell, bile acids potentiate Ca^{2+} release from the endoplasmic reticulum and vesicular Ca^{2+} stores. This occurs through opening of two major endoplasmic reticulum Ca^{2+} channels, the ryanodine receptor (8, 45, 74) and the inositol 1,4,5-trisphosphate receptor (6). TLCS-induced Ca^{2+} transient reductions were reduced by the inositol 1,4,5-trisphosphate receptor inhibitor caffeine (45), the ryanodine receptor inhibitor dantrolene (8), or in two photon-permeabilized acinar cells by the ryanodine receptor inhibitor ruthenium red (74). Recently, inositol 1,4,5-trisphosphate receptors and store-operated Ca^{2+} entry have been shown to contribute to bile acid-induced acinar cell pathology (8, 11, 74–76). Intracellular Ca^{2+} release is followed by depletion of Ca^{2+} pools and subsequent opening of store-operated Ca^{2+} entry channels (77). The net result is higher or sustained cytosolic Ca^{2+} levels.

Sustained Ca^{2+} results in the activation of Ca^{2+} binding targets such as the serine/threonine phosphatase calcineurin. Our laboratory initially demonstrated that calcineurin is an important mediator of acinar cell zymogen activation following caerulein stimulation in vitro (35). Subsequently, using an in vivo model of acute pancreatitis, we found that administration of calcineurin inhibitors attenuated several disease outcomes, including hyperamylasemia, serum cytokine levels, and histological injury (34). Using a mouse model of biliary pancreatitis, we demonstrated that both pharmacologic and genetic inhibition of calcineurin markedly reduce histological severity of acute pancreatitis following infusion of bile acids into the pancreatic duct (15).

Bile acid exposure with either deoxycholic acid (12), taurocholate (13, 14), TLCS (15), or a mixture of bile acids (11, 15)
has been known to increase NF-κB DNA binding activity in acinar cells. In this study, we demonstrate that bile acid-induced NF-κB activation is dependent upon calcineurin. The mechanism for this dependence appears to be upstream at the level of PKC translocation.

Several studies have examined the interplay of calcineurin in the NF-κB activation pathway. For example, in a study by Frantz et al. (78), the authors describe a synergistic role for calcineurin with PKC to inactivate the endogenous NF-κB inhibitor IκB.

Several studies describe a role for calcineurin with PKC (43, 79–82). An early report by Werlen et al. (80) demonstrated that calcineurin preferentially synergizes with PKC to activate IL-2 and the MAP kinase JNK in T lymphocytes. Another study by Trushin et al. (43) demonstrated that simultaneous activation of PKC and calcineurin resulted in the synergistic activation of the IκB kinase complex, a critical regulator of NF-κB.

In addition, there are at least two studies that directly examine the effect of calcineurin on PKC translocation. Murat et al. (83) described cross-talk between the calcineurin pathway and PKC in cardiomyocytes to regulate hypertrophy. They demonstrated that cyclosporine completely inhibited translocation of the Ca$^{2+}$-independent PKC isoforms ε and ζ. A second study in cardiomyocytes by Vincent et al. (79) demonstrated a similar role for calcineurin on PKC-ε translocation. Taken together, these data indicate that calcineurin cooperates with PKC in select cell types.

Of the major PKC isoforms expressed in the acinar cell, we found the presence of PKC-α and PKC-δ in primary mouse acinar cells. Of the two, we found that bile acids caused translocation of PKC-δ and that this translocation was mediated by calcineurin. Previous work demonstrated that cholecystokinin causes PKC-δ activation (62, 66, 67). However, this study is the first demonstration in acinar cells of a dependence on calcineurin. In summary, bile-induced NF-κB activation and acinar cell injury is mediated by calcineurin, and the mechanism for this important early inflammatory response appears to be upstream at the level of PKC-δ translocation.

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