Effects of bile acids on dog pancreatic duct epithelial cell secretion and monolayer resistance

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Effects of bile acids on dog pancreatic duct epithelial cell secretion and monolayer resistance. Am J Physiol Gastrointest Liver Physiol 283: G1042–G1050, 2002; 10.1152/ajpgi.00436.2001.—Pancreatic duct epithelial cells (PDEC) mediate the secretion of fluid and electrolytes and are exposed to refluxed bile. In nontransformed cultured dog PDEC, which express many ion transport pathways of PDEC, 1 mM taurodeoxycholic acid (TDCA) stimulated an 125I efflux inhibited by DIDS and 5-nitro-2-(3-phenylpropyl)amino benzoic acid (NPPB) and a 86Rb efflux inhibited by charybdotoxin. Inhibition by 1,2-bis(2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid (BAPTA)-AM suggests mediation via increased intracellular Ca2+ concentration, whereas the absence of lactate dehydrogenase release excludes cellular toxicity. At 1 mM, TDCA stimulated a larger 125I efflux than glycodeoxycholate; two dihydroxy bile acids, taurochenodeoxycholate and TDCA, were similarly effective, whereas a trihydroxy bile acid, taurocholate, was ineffective. In Ussing chambers, 1 mM serosal or 2 mM luminal TDCA stimulated an Imao increase from confluent PDEC monolayers. TDCA also stimulated 1) a short-circuit current (Imsc) increase from basolaterally permeabilized PDEC subject to a serosal-to-luminal Cl- gradient that was inhibited by BAPTA-AM, DIDS, and NPPB; 2) an Imsc increase from apically permeabilized PDEC subject to a luminal-to-serosal K+ gradient inhibited by BAPTA-AM and charybdotoxin. Along with the efflux studies, these findings suggest that TDCA interacts directly with PDEC to stimulate Ca2+-activated apical Cl- channels and basolateral K+ channels. Monolayer transepithelial resistance was only minimally affected by 1 mM serosal and 2 mM luminal TDCA but decreased after exposure to higher TDCA concentrations (2 mM serosal and 4 mM luminal). A secretory role for bile acids should be considered in pancreatic diseases associated with bile reflux.

Pancreatic duct epithelial cells (PDEC) secrete fluid and electrolytes. Together with acinar cells, which secrete digestive enzymes, they are the main components of pancreatic exocrine secretion. Because the pancreatic and the bile ducts share a common outflow in the duodenum, obstruction of the ampulla of Vater may cause bile to reflux backward into the pancreatic duct, exposing PDEC to bile acids. Indeed, in 1901, Opie suggested that refluxed bile is a factor in gallstone pancreatitis. Bile acids stimulate electrolyte secretion by epithelial cells from different portions of the gastrointestinal tract, such as the large bowel and the biliary tree (8, 9, 11, 12). However, to our knowledge, the secretory effects of bile acids on PDEC have not been evaluated thoroughly. Using cultured nontransformed dog PDEC that express many secretory functions characteristic of PDEC (1, 13, 20), such as mucin secretion (21), cAMP- and Ca2+-activated Cl- channels (14), and Ca2+-activated K+ channels (16), we 1) examined whether bile acids activate Cl- and K+ conductances, 2) identified the signaling pathway mediating this effect, 3) compared the secretory effects of different bile acids, 4) studied the effects of bile acids on monolayer transepithelial resistance (TER), and 5) compared the effects of bile acids on the apical or basolateral membrane of the PDEC.

MATERIAL AND METHODS

Chemicals and reagents. Taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid, glycodeoxycholic acid, taurocholic acid, charybdotoxin, 4,4’-disothiocy anostilbene-2,2’-disulfonic acid (DIDS), sodium pyrophosphate, lactate, tissue culture medium and supplements were from Sigma (St. Louis, MO). 1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM) was from Calbiochem (San Diego, CA), and 5-nitro-2-(3-phenylpropyl)amino benzoic acid (NPPB) was from Respo Biochemicals International (Natick, MA). Na125I (16 mCi/mg iodide) was from Amersham (Arlington Heights, IL), and 86RbCl (4.66 mCi/mg 86Rb+) was from NEN (Boston, MA).

PDEC culture. As previously described (21), PDEC isolated from the accessory pancreatic duct of a dog were cultured on Transwell or Snapwell inserts (Costar, Cambridge, MA) coated with 0.7 ml (for Transwell) or 0.15 ml (for Snapwell) of 20 mM HEPES, 100 IU/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml bovine insulin, 5 μg/ml human transferrin, and 5 ng/ml sodium selenite. These PDEC express a cAMP-activated Cl- channel, corresponding to the cystic fibrosis transmembrane conductance regulator, a Ca2+-activated Cl- channel (14), and Ca2+-activated K+ channels (16). Cells

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used in this report were between passages 9 and 30. Efflux studies, Ussing chamber recordings, TER measurements, and lactate dehydrogenase (LDH) assays used confluent PDEC on Transwell or Snapwell filters that were isolated from the myofibroblast feeder layer.

**Efflux studies.** Studies of the activation of Cl− and K+ conductances through cellular 125I− and 86Rb+ effluxes have been performed in 184 cells (23) and have been used extensively to characterize the Cl− and K+ conductances on PDEC (14–19).

PDEC were grown to confluence on Transwell inserts as described. The membranes and attached cells were excised from the insert and washed twice with 1 ml of experimental buffer consisting of 140 mM NaCl, 4.7 mM KCl, 1.2 mM CaCl₂, 10 mM glucose, and 10 mM HEPES, pH 7.4. The cells were then loaded with radioactive markers through a 45-min incubation at 37°C with 1.5 ml of experimental buffer containing either ~2 μCi/ml Na125I or ~1 μCi/ml 86RbCl and washed four times with 2 ml of isotope-free buffer. The isotope efflux was measured by sequential addition and removal of 1 ml of isotope-free buffer at 15-s intervals for a 5-min period. After establishing baseline efflux, 86Rb was added for the first minute; in the remaining 4 min, the secretagogue tested was included in the buffer. When inhibitors were tested, they were added at the beginning of the experiment (including the baseline monitoring period). Treatment with BAPTA-AM also included the 125I− loading period. The radioactivity of these sequential samples and the radioactivity associated with the cells at the end of the experiment were measured using a gamma counter for 125I− and a liquid scintillation counter for 86Rb+.

The radioactivity contained in the cells at a particular time point was calculated as the sum of the radioactivities released in subsequent efflux samples and remaining in the cells at the end of the experiment. The efflux rate coefficient for a certain time interval was calculated using the formula

\[ r = \frac{[\ln (R_1) - \ln (R_2)]}{(t_1 - t_2)} \]

where \( R_1 \) and \( R_2 \) are the percentages of radioactivity initially loaded that remained in the cells at times \( t_1 \) and \( t_2 \).

**Ussing chamber studies.** Confluent monolayers of PDEC cultured on Snapwell inserts were mounted in modified Ussing chambers with an aperture area of 1.0 cm². Three different configurations were studied. For studies of net electronegative transepithelial ion transport, both sides of the monolayer were bathed in Ringer solution (in mM: 115 NaCl, 1.2 CaCl₂, 1 MgCl₂, 0.4 KH₂PO₄, 2.4 K₂HPO₄, 25 NaHCO₃, and 10 glucose), warmed to 37°C with a circulating water jacket, and gently mixed and aerated with a constant flow of 5% O₂-5% CO₂. For studies of apical Cl− conductance, the basolateral surface of the PDEC was permeabilized by adding 0.20 mg/ml nystatin to the serosal compartment 15 min before the addition of TDCA. A serosal-to-luminal Cl− gradient (intracellular-to-extracellular gradient across the apical membrane) was generated by adding, to the serosal compartment, a buffer consisting of 135 mM NaCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 2.4 mM K₂HPO₄, 0.6 mM KH₂PO₄, 10 mM HEPES, and 10 mM glucose, titrated to pH 7.4 with NaOH, and, to the luminal compartment, a buffer of 135 mM sodium gluconate, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 2.4 mM K₂HPO₄, 0.6 mM KH₂PO₄, 10 mM HEPES, and 10 mM glucose, titrated to pH 7.4 with NaOH. For studies of basolateral K+ conductance, the apical membrane was permeabilized with nystatin (0.20 mg/ml, 15 min) and a luminal-to-serosal K+ gradient (intra-to-extracellular gradient across the basolateral membrane) generated using a luminal buffer of 10 mM NaCl, 1.25 mM CaCl₂, 1 mM MgCl₂, 118 mM potassium gluconate, 10 mM HEPES, and 10 mM glucose, titrated to pH 7.4 with NaOH, and a serosal buffer of 10 mM NaCl, 1.25 mM CaCl₂, 1 mM MgCl₂, 4 mM potassium glutamate, 114 mM N-methyl-d-glucamine, 10 mM HEPES, and 10 mM glucose, titrated to pH 7.4 with acetic acid.

Spontaneous tissue potential differences were measured using a bipolar voltage clamp described (Physiologic Instruments). In this system, the Lw reflects 1) for nonpermeabilized PDEC, a net electronegative ion transport across the intact epithelial monolayer; 2) for basolaterally permeabilized PDEC, Cl− flow down the serosal-to-luminal Cl− gradient across activated apical Cl− channels; and 3) for apically permeabilized PDEC, K+ flow down the luminal-to-serosal K+ gradient across activated basolateral K+ channels. Instruments were calibrated before each experiment using Vitrogen-coated membranes devoid of cells.

**Measurement of TER.** Confluent PDEC monolayers cultured on Snapwell inserts were replenished with fresh culture medium and exposed, either apically or serosally, to different concentrations of TDCA for either 2 min, 5 min, 10 min, or 24 h. TER of the monolayers was measured at the beginning of the experiment, after TDCA exposure (or after 10 min for the control and 24 h treatments), at 15 min, and after 24 h, using an epithelial voltomhmmeter (EVOM apparatus) from WPI (Sarasota, FL). TER measurements were calibrated using a Transwell membrane coated with Vitrogen but devoid of PDEC. For each monolayer, TER measurements were normalized to the value obtained at the beginning of the experiment.

**LDH activity.** To assess for cellular injury, LDH release from PDEC exposed to bile acids was monitored. The cultured PDEC and supporting filter were excised from the Transwell inserts and exposed for 5 min to 1 ml of efflux buffer containing different concentrations of bile acids. LDH released in the medium was assayed according to method of Amador et al. (3): 1.4 ml reaction buffer (77.5 mM lactic acid, 5.25 mM β-nicotinamide adenine dinucleotide, and 0.05 M sodium pyrophosphate, pH 8.8) was mixed with 100 μl supernatant, and absorption at 340 nm was measured using a 160U ultraviolet spectrophotometer (Shimadzu). The absorption change after a lag time of 1 min was determined.

**Statistics.** All observations were based on at least three experiments. Unless specified otherwise, results were expressed as means and SE, and statistical significance was determined with the unpaired Student’s t-tests or ANOVA (concentration-dependence studies) using the Statview 512+ (Abacus Concepts, Calabasas, CA) or InStat 3 (GraphPad Software) software.

**RESULTS**

**Iodide efflux studies.** The possible activation of Cl− conductances on PDEC by TDCA was evaluated by monitoring cellular 125I− efflux. As shown in Fig. 1A, TDCA, at a concentration of 1 mM, stimulated a robust increase in 125I− efflux to a peak rate coefficient of 0.726 ± 0.072 min⁻¹ 30 s after addition of the conjugated bile acid (vs. 0.155 ± 0.011 min⁻¹ for control treatment, P < 0.01, n = 3). A much smaller increase was obtained with 500 μM TDCA with a peak rate coefficient of 0.224 ± 0.026 min⁻¹ 45 s after TDCA addition (vs. 0.134 ± 0.014 min⁻¹ for control treatment, P < 0.05, n = 3). At a concentration of 100 μM,
TDCA failed to elicit an increased efflux different from control.

To verify that this effect was mediated through Cl− conductances, the effects of the inhibitors of Cl− channels, NPPB (Fig. 1B) and DIDS (Fig. 1C), were assessed. Preincubation with 500 μM of either of these two inhibitors completely abolished the subsequent response stimulated by TDCA. This inhibitory profile is similar to the one observed with the Ca2+-activated Cl− conductance previously characterized on these PDEC (14).

Because it has been demonstrated previously that bile acids may stimulate an increase in intracellular Ca2+ concentration ([Ca2+]i; see Refs. 7, 8, 22), we determined whether the effect of TDCA on 125I efflux was mediated by an increase in [Ca2+]i using the intracellular Ca2+ chelator BAPTA-AM. As shown in Fig. 1D, preincubation with 50 μM BAPTA also totally abolished the subsequent stimulation by 1 mM TDCA.

The 125I effluxes stimulated by deoxycholic acid conjugated to either taurine or glycine were compared next. As shown in Fig. 2A, at 1 mM, the response obtained with glycodyoxycholic acid was smaller than the response to TDCA (peak efflux rate coefficient: 0.587 ± 0.082 min−1 with glycodyoxycholic acid vs. 1.233 ± 0.094 min−1 with TDCA, P < 0.01, n = 3).

The effects of the three bile acids that account for ~90% of the bile acids in the lumen of the bile ducts (chenodeoxycholic acid, cholic acid, and deoxycholic acid) were also evaluated at the same concentration of 1 mM and when conjugated to taurine. As shown in Fig. 2B, the 125I effluxes stimulated by taurochenodeoxycholic acid and TDCA were equivalent; in contrast, no efflux was detected with 1 mM taurocholic acid (efflux rate coefficients 45 s after bile acid addition: 1.09 ± 0.006 min−1 for taurochenodeoxycholic, 1.108 ± 0.011 min−1 for TDCA, and 0.16 ± 0.006 min−1 for taurocholic acid, n = 3).

86Rb+ efflux studies. As previously reported, these PDEC also express Ca2+-activated K+ conductances that can be studied through measurements of 86Rb+ efflux (16). Because the findings above suggested that TDCA caused a [Ca2+]i increase to stimulate Ca2+-activated Cl− channels, the effect of this [Ca2+]i increase on Ca2+-activated K+ channels was also evaluated. As shown in Fig. 3A, 1 mM TDCA also stimulated...
an increased $^{86}$Rb$^+$ efflux in a concentration-dependent manner. Unlike the $^{125}$I$^-$ efflux, the $^{86}$Rb$^+$ efflux was sustained over time. Indeed, 4 min after the addition of TDCA, the $^{86}$Rb$^+$ efflux rate coefficients were at stable values of $0.045 \pm 0.007$, $0.088 \pm 0.008$, and $0.145 \pm 0.007$ min$^{-1}$ for control, $0.75$ mM, and $1$ mM TDCA ($P < 0.01$ for $1$ mM vs. control and for $1$ mM vs. $0.75$ mM; $P < 0.05$ for $0.75$ mM vs. control, $n = 3$), respectively. This effect was also inhibited by $100$ nM charybdotoxin, an inhibitor of Ca$^{2+}$-activated K$^+$ channels, from an efflux rate coefficient of $0.164 \pm 0.009$ to $0.049 \pm 0.009$ min$^{-1}$ $4$ min after the addition of $1$ mM TDCA ($P < 0.01$, $n = 3$; Fig. 3B).

\textbf{Ussing chamber studies.} As previously reported, the cultured dog PDEC exhibit tight junctions, generating the high transepithelial monolayer electrical resistance necessary for studies in Ussing chambers (16, 17, 19). They are also polarized with apical and basolateral membranes that may differ in their ability to mediate the secretory effects of bile acids. We therefore compared the effects of TDCA added to either the luminal or serosal compartment of Ussing chambers.

Confluent PDEC monolayers were mounted in Ussing chambers, and the net electrogenic transepithelial ion transport was stimulated by bile acids monitored through the $I_{sc}$. As shown in Fig. 4A, when added to the serosal compartment contiguous with the basolateral side of the PDEC, TDCA stimulated a sustained increase in $I_{sc}$. This response occurred in a dose-dependent manner, with peak $I_{sc}$ increases of $1.9 \pm 0.1$, $3.2 \pm 0.5$, $5.0 \pm 0.5$, and $6.6 \pm 0.1$ $\mu$A/cm$^2$, respectively, for $0.4$, $0.6$, $0.8$, and $1$ mM TDCA ($n = 3$, statistical significances shown in Fig. 4A, inset). As shown in Fig. 4B, when added to the luminal compartment, $1$ mM TDCA only stimulated a minimal $I_{sc}$ increase. Higher concentrations, however, elicited significant responses in a dose-dependent manner, with peak $I_{sc}$ increases of $0.7 \pm 0.2$, $1.3 \pm 0.2$, $1.1 \pm 0.2$, $2.4 \pm 0.4$, and $5 \pm 0.3$ $\mu$A/cm$^2$, respectively, for $1$, $1.25$, $1.5$, $1.75$, and $2$ mM TDCA ($n = 4-5$, statistical significances detailed in Fig. 4B, inset).

The studies above suggest that TDCA stimulated net electrogenic ion transport from nonpermeabilized PDEC. Because the efflux studies suggest that TDCA activated Cl$^-$ and K$^+$ channels, we characterized the effects of this bile acid on Cl$^-$ and K$^+$ conductances of PDEC mounted in Ussing chambers. To study apical Cl$^-$ conductances, the basolateral membrane of PDEC was permeabilized with nystatin added to the serosal compartment, and the monolayer was subjected to a $135$ mM serosal-to-luminal Cl$^-$ gradient. As shown in Fig. 5, $1$ mM serosal TDCA stimulated an increase in $I_{sc}$ that was inhibited by $50$ $\mu$M BAPTA-AM (98% inhibition, Fig. 4A, inset), $500$ $\mu$M DIDS (98% inhibition, Fig. 4B and inset), and $500$ $\mu$M NPPB (75% inhibition, data not shown). These findings are consistent with the $^{125}$I$^-$ efflux studies, suggesting that Cl$^-$ flow is mediated through apical Ca$^{2+}$-activated Cl$^-$ channels (14, 17, 18).

Activation of basolateral K$^+$ channels was also examined by permeabilizing the apical membrane of PDEC with luminal nystatin and subjecting the monolayer to a luminal-to-serosal K$^+$ gradient of $114$ mM. As shown in Fig. 6A, $1$ mM serosal TDCA also stimulated an increased $I_{sc}$ in this configuration. This effect was inhibited by $50$ $\mu$M BAPTA-AM (98% inhibition, Fig. 6A and inset) and $100$ nM charybdotoxin (92% inhibition, Fig. 6B and inset), suggesting mediation through the Ca$^{2+}$-activated K$^+$ channels previously identified in these cells (16).

\textbf{Measurements of TER.} TER is another electrophysiological property of PDEC monolayers that may be affected by bile acids. Figure 7 shows that the effects of TDCA on monolayer TER also differ according to the side of exposure. From the serosal compartment (Fig. 7A), transient treatments with $1$ mM TDCA (2, 5, and

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Fig. 2. Effect of different bile acids on $^{125}$I$^-$ efflux. $^{125}$I$^-$ efflux from PDEC was determined as detailed in MATERIALS AND METHODS, and the efflux rate coefficient was calculated and shown. In A, means and SE from 3 experiments are shown. A: after 1 min of baseline determination, $1$ mM of deoxycholic acid conjugated to either taurine (○) or glycine (●) was added. B: after 1 min of baseline determination, $1$ mM of either deoxycholic acid (●), chenodocholic acid (●), or cholic acid (●), all conjugated to taurine, was added.

Fig. 5. $1$ mM serosal TDCA stimulated an increase in $I_{sc}$ that was inhibited by $50$ $\mu$M BAPTA-AM (98% inhibition, Fig. 4A, inset), $500$ $\mu$M DIDS (98% inhibition, Fig. 4B and inset), and $500$ $\mu$M NPPB (75% inhibition, data not shown). These findings are consistent with the $^{125}$I$^-$ efflux studies, suggesting that Cl$^-$ flow is mediated through apical Ca$^{2+}$-activated Cl$^-$ channels (14, 17, 18).

Fig. 6. A: transient treatments with $1$ mM TDCA (2, 5, and 10 min) increased $I_{sc}$ that was inhibited by $50$ $\mu$M BAPTA-AM (98% inhibition, Fig. 6A and inset) and $100$ nM charybdotoxin (92% inhibition, Fig. 6B and inset), suggesting mediation through the Ca$^{2+}$-activated K$^+$ channels previously identified in these cells (16).
10 min) only produced temporary TER decreases of up to 12 ± 2% (n = 6), resolving when TDCA was removed (15-min time point). However, 24 h after treatment, TER was only 40–45% of the initial value. Continuous exposure to 1 mM TDCA resulted in a marked TER decrease of 95% after 24 h. When PDEC were exposed serosally to 2 mM TDCA, the resulting decrease in TER increased with time. Indeed, after only 10 min of TDCA exposure, the TER was 22 ± 3% of its original value; after 24 h, this value decreased to 5%.

Monolayer TER was more resistant to luminal TDCA exposure. Only transient decreases by 15–20% from the original TER were detected when PDEC were exposed to 2 mM luminal TDCA for up to 10 min. Different from transient serosal exposure to 1 or 2 mM TDCA, PDEC monolayers recovered their original TER value after 24 h when exposed transiently to 2 mM luminal TDCA. However, after continuous exposure, the corresponding TER decreased to 74 ± 6.3% of its original value after 24 h. With 3 mM luminal TDCA, the transient TER decreases were more pronounced; 5 min after a 10-min exposure to TDCA, the TER was 16.5 ± 4.1% of its original value. However, after tran-

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**Fig. 3.** Effect of TDCA on $^{86}$Rb efflux. $^{86}$Rb efflux from PDEC was determined as detailed in MATERIALS AND METHODS, and the efflux rate coefficient was calculated and shown. In A and B, means and SE from 3 experiments are shown. A: After 1 min of baseline determination, TDCA was added at the different final concentrations shown. B: After 1 min of baseline determination, 1 mM TDCA was added in the absence (●) or presence (○) of 100 nM charybdotoxin.

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**Fig. 4.** Effect of TDCA on net electrogenic ion transport by PDEC monolayers. Monolayers of confluent PDEC were mounted in Ussing chambers, as described in MATERIALS AND METHODS, and the short-circuit current ($I_{sc}$) was measured at 10-s intervals. After ~5 min of baseline determination, TDCA was added at the different final concentrations shown. The $I_{sc}$ increases shown (calculated by subtracting the $I_{sc}$ recorded just before the addition of TDCA) were obtained from monolayers cultured and studied at the same time. A: TDCA was added to the serosal compartment in contact with the basolateral membrane of the PDEC. These tracings are representative of 3 experiments, and the average peak $I_{sc}$ increases (highest $I_{sc}$ within 10 min of TDCA addition − $I_{sc}$ just before addition of TDCA) are shown in the inset [significant differences from 0 (†), 0.2 (♯), 0.4 (©), and 0.6 ( &) mM, $P < 0.05$ by ANOVA]. B: TDCA was added to the luminal compartment in contact with the apical membrane of the PDEC. These tracings are representative of 5 experiments, and the average peak $I_{sc}$ increases (highest $I_{sc}$ within 10 min of TDCA addition − $I_{sc}$ just before addition of TDCA) are shown in the inset [significant differences from 0 (†), 0.75 (©), 1.25 ( &) and 1.75 ( *) mM, $P < 0.05$ by ANOVA].
sient exposure, the PDEC monolayers still regained their original TER values after 24 h. Only at a higher concentration of 4 mM luminal TDCA were there marked sustained TER decreases to 10 and 5% of the initial values 15 min and 24 h, respectively, after exposure.

Cellular LDH release. Because bile acids are detergents, the toxicity of these bile acids against PDEC was also evaluated by monitoring LDH release from cells exposed to bile acids. As shown in Fig. 8, after treatment with 0.5 and 1 mM TDCA for 5 min, minimal LDH was released in the supernatant, similar to the control treatment (LDH activity in arbitrary units; control: 120 ± 20.8, 1 mM TDCA: 96.7 ± 28.5, n = 3). A significant increase in LDH was released in the supernatant with 2 mM TDCA; with 5 mM TDCA, the amount of LDH released was equivalent to the amount released with 1 mM Triton X-100, used as positive control (LDH activity: 2 mM TDCA, 893 ± 91; 5 mM TDCA, 8,803 ± 2,112; and Triton X-100, 7,316 ± 1,839).

DISCUSSION

PDEC mediate the secretion of fluid and electrolytes (mainly bicarbonate), one of the two main functions of the exocrine pancreas. Because these cells are exposed to refluxed bile acids after ampullary obstruction and because bile acids stimulate secretion from colonic and biliary epithelia, we examined the secretory effects of bile acids on PDEC. Nontransformed, well-differentiated, and polarized cultured dog PDEC served as the...
In this model, TDCA activated both Cl⁻ and K⁺ conductances. Indeed, it stimulated an increased ¹²⁵I⁻ efflux, inhibited by the Cl⁻ channel blockers NPPB or DIDS, and a ⁸⁶Rb⁺ efflux inhibited by the K⁺ channel blocker charybdotoxin. When PDEC were examined in Ussing chambers, TDCA also stimulated an $I_{sc}$ increase from basolaterally permeabilized cells subject to a serosal-to-luminal Cl⁻ gradient and from apically permeabilized cells subject to a luminal-to-serosal K⁺ gradient. These $I_{sc}$ increases were also inhibited, respectively, by the Cl⁻ channel inhibitors NPPB and DIDS and the K⁺ channel inhibitor charybdotoxin. As previously reported, these efflux and $I_{sc}$ responses are characteristic of those mediated by the apical Ca²⁺-activated Cl⁻ channels and basolateral Ca²⁺-activated K⁺ channels (14, 16). Inhibition by BAPTA-AM, a cell-permeant Ca²⁺ chelator, of TDCA-stimulated ¹²⁵I⁻ efflux and $I_{sc}$ increases from basolaterally permeabilized PDEC subject to a serosal-to-luminal Cl⁻ gradient and apically permeabilized PDEC subject to a luminal-to-serosal K⁺ gradient further verifies that activation of the Cl⁻ and K⁺ channels is mediated through an increase in [Ca²⁺]. A toxic effect of TDCA at the concentrations tested was excluded by the absence of increased LDH leakage after exposure to ≤1 mM TDCA. These findings are consistent with the activation, through increased [Ca²⁺], by similar concentrations of bile acids of K⁺ and/or Cl⁻ conductances on colonic T84 cells and biliary cells and the stimulation by taurocholate-cholate of exocytosis in hepatocytes (6, 8, 9, 22).

PDEC polarization and the presence of monolayer resistance allowed us to examine the polarity of TDCA effects. At 1 mM, only serosal, but not luminal, TDCA stimulated an $I_{sc}$ increase from intact monolayers; a higher concentration (2 mM) was required to produce a model since they were used successfully to characterize the secretory effects of histamine (acting via H₁ receptors), UTP and ATP (via P₂Y₂ and P₂Y₁₁ receptors), and trypsin (via proteinase-activated receptor-2; see Refs. 15, 17, 18, and 19). In addition, these PDEC are derived from the main pancreatic duct of the dog, the first pancreatic structure exposed to refluxed bile.

![Graph A](image1.png)
![Graph B](image2.png)

**Fig. 7.** Effects of TDCA on transepithelial resistance (TER) of PDEC monolayers. PDEC monolayers were either untreated (control) or treated with 1, 2, 3, or 4 mM TDCA added to either the serosal (A) or luminal (B) compartment for 2 min, 5 min, 10 min, or 24 h. Monolayer TERs were measured, as described in MATERIALS AND METHODS, before treatment, at the end of treatment (or at 10 min for the control and 24-h exposures), at 15 min, and at 24 h. The mean ± SE of the TERs, normalized to the initial value before treatment, are shown (n = 6). Statistical difference from 100% ($P < 0.05$, t-test, 5 degrees of freedom). For the serosal exposure, the initial TERs were, respectively, 706 ± 13, 604 ± 9, 592 ± 19, 653 ± 24, 796 ± 29, 621 ± 55, 711 ± 67, 693 ± 94, and 757 ± 109 Ω-cm² for the control, 1 mM/2 min, 1 mM/5 min, 1 mM/10 min, 1 mM/24 h, 2 mM/2 min, 2 mM/5 min, 2 mM/10 min, and 2 mM/24 h treatments. For the luminal exposure, the initial TERs were, respectively, 839 ± 35, 861 ± 56, 849 ± 67, 807 ± 43, 860 ± 44, 929 ± 90, 904 ± 112, 929 ± 142, 917 ± 130, 901 ± 119, 875 ± 145, 943 ± 149, and 907 ± 160 Ω-cm² for the control, 2 mM/2 min, 2 mM/5 min, 2 mM/10 min, 2 mM/24 h, 3 mM/2 min, 3 mM/5 min, 3 mM/10 min, 3 mM/24 h, 4 mM/2 min, 4 mM/5 min, 4 mM/10 min, and 4 mM/24 h treatments.

![Graph C](image3.png)

**Fig. 8.** Lactate dehydrogenase (LDH) leakage from PDEC treated with TDCA. PDEC were treated with different concentrations of TDCA or with 1% Triton X-100, and the LDH released into the supernatant was assayed as described in MATERIALS AND METHODS. The means and SE from 3 determinations are shown.

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luminal effect. In T84 cells, <1 mM TDCA also produced only a serosal effect, and ≥1 mM TDCA was required for luminal action. It was postulated that the higher luminal concentrations of bile acids disrupted T84 cell monolayer integrity, allowing bile acids to reach the basolateral membrane and stimulate secretion (9). In this report, it is possible that TDCA interacts directly with the apical membrane of PDEC to stimulate secretion. Indeed, 2 mM luminal TDCA decreased monolayer TER by only 15–20%, which may be inadequate for rapid TDCA leakage in the serosal compartment. Furthermore, the onsets of action after serosal and luminal TDCA addition were similar, without the delay expected for transepithelial leakage.

The secretory effects of the different bile acid types were examined. Because biliary bile acids are also conjugated to glycine, we demonstrated that 1 mM glycodeoxycholic acid also stimulated an increase in 

$$^{125}I^-$$ efflux, albeit of smaller amplitude. The effects of cholic acid and chenodeoxycholic acid, which, together with deoxycholic acid, account for 90% of biliary bile acids, were also evaluated. Conjugated to taurine and tested at 1 mM, chenodeoxycholic acid and deoxycholic acid were equivalent in their secretory response; in contrast, cholic acid did not produce a significant response. Of note, taurocholic acid also did not activate conductances in T84 cells (8). Because taurocholic acid, like TDCA, is a strong detergent, its lack of activity further argues against the secretory effect of TDCA resulting from a nonspecific detergent action. Structural differences may account for these different effects as follows: both chenodeoxycholic acid and deoxycholic acid are dihydroxylated (at positions 3 and 7 for chenodeoxycholic acid and at positions 3 and 12 for deoxycholic acid), whereas cholic acid is trihydroxylated (at positions 3, 7, and 12).

The effects of TDCA on PDEC TER are consistent with the previous findings that bile acids at concentrations >1 mM increased the permeability of bovine pancreatic duct explants (2) and of the main pancreatic duct in different animal models of bile-induced pancreatitis (4, 5, 10). Again, higher luminal TDCA concentrations were also necessary to affect monolayer TER. Marked immediate and delayed decreases in TER followed transient treatment with 2 mM serosal TDCA and with 4 mM luminal TDCA. Of note, transient exposure to 1 mM serosal TDCA caused delayed TER decreases after 24 h, suggesting that serosal bile acids produce long-lasting effects. On the other hand, transient exposure to 2–3 mM luminal TDCA only caused a transient TER decrease, with full recovery after 24 h. The PDEC used in this study are well differentiated and polarized, with apical and basolateral membranes differentially exhibiting receptors for ATP, UTP, histamine, and trypsin (15, 17, 18, 19). The differential effects of luminal and serosal TDCA most likely reflect different interaction with apical and basolateral membranes.

Because serum concentrations of bile acids are in the micromolar range, it is unlikely that systemic bile acids will cause any serosal effect on PDEC under physiological conditions. On the other hand, our findings may be relevant to gallstone pancreatitis in which refluxed bile may yield conjugated bile acid concentrations in pancreatic juice in excess of 2 mM. The stimulation of secretion by subtoxic concentrations of TDCA may be a defense mechanism for diluting and washing off refluxed bile acids before critically toxic concentrations are reached. The relative resistance of the apical membrane to the toxic effects of TDCA further illustrates the importance of the barrier function of the pancreatic ductal mucosa.

In summary, we have shown that dihydroxy bile acids activate Cl− and K+ conductances on PDEC and that at higher concentrations impair epithelial barrier function. These effects will be relevant to conditions associated with bile reflux and disruption of the pancreatic ductal epithelial barrier.

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