Taurodeoxycholate Activates Potassium and Chloride Conductances via an IP_3-mediated Release of Calcium from Intracellular Stores in a Colonic Cell Line (T84)

Daniel C. Devor,* M. Chandra Sekar,‡ Raymond A. Frizzell,* and Michael E. Duffey

*Department of Physiology, State University of New York at Buffalo, Buffalo, New York 14214; and Departments of *Physiology and Biophysics, and ‡Pathology and Pharmacology, University of Alabama at Birmingham, Birmingham, Alabama 35294*

Abstract

Whole-cell patch-clamp techniques and fluorescence measurements of intracellular Ca^{2+} concentration, $(Ca^{2+})_i$, were used to investigate the mechanism of taurodeoxycholate (TDC) stimulation of Cl^- secretion in the T84 colonic cell line. During perforated whole-cell recordings, the cell membrane voltage was alternately clamped to E_K and E_{Cl}. Initially, TDC (0.75 mM) stimulated inward nonselective cation currents that were composed of discrete large conductance single-channel events. This initial response was followed by activation of K^+ and Cl^- currents with peak values of 385±41 pA and 98±28 pA, respectively (n = 12). The K^+ and Cl^- currents oscillated while TDC was present and returned to baseline levels upon its removal. The threshold for activation of the oscillatory currents was 0.1 mM TDC. Taurocholate, a bile acid that does not stimulate colonic Cl^- secretion, induced no current response. The TDC-induced currents could be activated in Ca^{2+}-free bathing solutions. Preincubation of cells with the Ca^{2+} chelator, bis-(*o*-aminophenoxy)-ethane-*N,N,N',N'*-tetraacetic acid, tetra(acetoxymethy)-ester (20 μM), (BAPTA-AM), eliminated the K^+ and Cl^- current responses, although the nonselective cation channel events were still present. Replacement of bath Na^+ with $NMDG^+$ inhibited the TDC-induced nonselective cation current but did not affect the K^+ or Cl^- currents. TDC induced a transient $(Ca^{2+})_i$ rise of 575±70 nM from a baseline of 71±5 nM (n = 15); thereafter, $(Ca^{2+})_i$ either plateaued or oscillated. TDC-induced $(Ca^{2+})_i$ oscillations were observed in the absence of bath Ca^{2+}; however, removal of bath Ca^{2+} during the TDC response caused $(Ca^{2+})_i$ to return to near baseline values. Simultaneous K^+ current and $(Ca^{2+})_i$ measurements confirmed that the initial nonselective cation current was independent of $(Ca^{2+})_i$, while K^+ current oscillations were in phase with the $(Ca^{2+})_i$ oscillations. TDC induced inositol monophosphate (IP) accumulation, reflecting production of inositol 1,4,5-trisphosphate (IP_3) during TDC stimulation. The response to TDC during standard whole-cell patch-clamp was similar to that observed with perforated whole-cell recordings, except the nonselective cation current was prolonged. When heparin (1 mg/ml) was added to the pipette under these conditions, the Ca^{2+}-activated currents were inhibited, but the nonselective cation currents were unaffected. These data suggest that TDC induces a Ca^{2+}-independent nonselective cation conductance, perhaps by directly permeabilizing the plasma membrane. TDC stimulates Cl^- secretion by activating K^+ and Cl^- conductances via an IP_3-mediated release of Ca^{2+} from intracellular stores. (*J. Clin. Invest.* 1993. 92:2173–2181.) Key words: bile salts • chloride secretion • intestine • calcium

Introduction

Under normal conditions, more than 95% of the bile acids secreted by the liver into the intestine are actively reabsorbed in the distal ileum via a Na^+-dependent co-transporter. However, in certain pathological conditions including intractable diarrhea in infancy, irritable bowel syndrome, Crohn's disease, cystic fibrosis, and surgical ileal resection, active bile acid absorption is compromised (1). Bile acids malabsorbed by the small intestine are transferred to the colon where they stimulate secretion of electrolytes and water that results in diarrhea (2–5). Similarly, while bile acids are administered orally to treat various hepatic diseases (6–8) and to dissolve gallstones (9), one of the side effects of this treatment is diarrhea (7–9).

Dihydroxy bile acids are known to stimulate intestinal Cl^- secretion (2–5, 10–12), but the mechanism is not fully resolved. It has been argued that bile acids induce secretion because of epithelial cell damage that increases Cl^- permeability (13). Bile acids have also been proposed to stimulate secretion via a cAMP-mediated pathway (2, 4, 14); however, they have not been found to stimulate adenylate cyclase activity in human colon (15). Since bile acids can act as Ca^{2+} ionophores in phospholipid membranes (16, 17) and in intestinal membrane vesicles (18, 19), it has also been proposed that they induce Cl^- secretion by a Ca^{2+}-mediated pathway (20, 21). Furthermore, bile acid-induced intestinal secretion can occur in the absence of bath Ca^{2+} (3, 4, 10), but is blocked by the calmodulin antagonist, trifluoperazine (3), suggesting the possible involvement of Ca^{2+} from intracellular stores.

We used perforated and standard whole-cell patch-clamp techniques, and the intracellular Ca^{2+} indicator fura-2 to investigate the mechanism of TDC[1] stimulation of ionic conductances in the human colonic cell line, T84. We found that TDC stimulates K^+, Cl^-, and nonselective cation currents. The K^+ and Cl^- currents oscillate in phase with oscillations of $(Ca^{2+})_i$ and are activated by Ca^{2+} release from cellular stores, while the nonselective cation conductance appears to result from a direct

Address correspondence to D. C. Devor, Department of Physiology and Biophysics, BHSB Rm. 770, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294.

Received for publication 12 April 1993 and in revised form 18 June 1993.

J. Clin. Invest.

0021-9738/93/11/2173/09 $2.00
Volume 92, November 1993, 2173–2181

1. *Abbreviations used in this paper:* BAPTA, bis-(*o*-aminophenoxy)-ethane-*N,N,N',N'*-tetraacetic acid; $(Ca^{2+})_i$, intracellular calcium concentration; E_{Cl}, chloride equilibrium potential; E_K, potassium equilibrium potential; IP_3, inositol 1,4,5-trisphosphate; NMDG, *N*-methyl-D-glucamine; PIP_2, phosphatidylinositol 4,5-bisphosphate; TDC, taurodeoxycholate.

effect of TDC on the plasma membrane. In addition, the TDC-induced oscillations of $(Ca^{2+})_i$ result from breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2). Our findings suggest that TDC stimulates Cl^- secretion by activating K^+ and Cl^- conductances via an IP_3-mediated release of Ca^{2+} from intracellular stores.

Methods

Cell culture. T84 cells were grown in Dulbecco's modified Eagle's medium (DME) and Ham's F-12 (1:1) supplemented with 20 mM Hepes, 10 mM glucose, and 5% newborn calf serum in a humidified atmosphere of 95% O_2/5% CO_2 at 37°C. Experiments were performed on single, isolated cells plated onto No. 1 glass coverslips the previous day.

Solutions. The standard bath solution contained (mM): 145 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 Hepes. Sodium-free solutions contained either *N*-methyl-D-glucamine ($NMDG^+$) or choline, as noted. During K^+-free experiments, K^+ was replaced by Na^+ and 140 meq Cl^- by gluconate. The Ca^{2+}-free bath contained no added Ca^{2+} and 1 mM EGTA. For perforated whole-cell recordings, the pipette solution contained (mM): 60 K_2SO_4, 40 KCl, 5 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 Hepes. During standard whole-cell recordings, the pipette solution contained (mM): 130 KCl, 5 NaCl, 4 $MgCl_2$, 0.12 $CaCl_2$, 10 Hepes, 2 ATP, 0.5 GTP, and 0.2 EGTA. The calculated free Ca^{2+} concentration was 100 nM. In K^+-free pipette solutions, K^+ was replaced by Na^+. All solutions were adjusted to a pH of 7.4 with NaOH. All measurements were made at room temperature (22–25°C).

Whole-cell current recordings. Currents were recorded during voltage-clamp using either the standard whole-cell configuration (22) or the perforated whole-cell patch-clamp technique (23). For the perforated patch technique, pipettes were fabricated as described previously (24, 25), and were filled completely with nystatin-containing solution. Currents were recorded as previously described (24, 25). With nystatin in the pipette, current recordings were not begun until the access resistance (R_a) of the patch had decreased to below 20 MΩ. The average R_a for all patches in this study was 11.8±0.3 MΩ, while the simultaneously determined membrane capacitance averaged 17.4±1.3 pF (n = 113). These values are similar to our previously reported values for T84 cells (24). The plasma membrane was alternately voltage-clamped between the K^+ equilibrium potential (E_K = −88 mV for perforated recordings and −83 mV for standard recordings) and the Cl^- equilibrium potential (E_{Cl} = −29 mV for perforated recordings and −2 mV for standard recordings). For experiments performed in the absence of pipette K^+, the membrane was alternately clamped between the Na^+ equilibrium potential (E_{Na} = + 3 mV) and E_{Cl} = + 60 mV.

Intracellular calcium measurements. Cells were loaded at room temperature with fura-2/AM (4 μM) using one of two protocols (*a*) 20 min in a Ca^{2+}-free bathing solution, followed by 1 h postincubation in normal growth media (26); or (*b*) 1 h in a 1:1 mixture of normal growth media: standard bathing solution (27). Similar results were obtained using either protocol.

Coverslips containing the fura-2 loaded cells were affixed to the bottom of a Plexiglas chamber that was mounted on the stage of an inverted microscope (Nikon, Diaphot, or Zeiss, IM-35) equipped for epifluorescence using a 40X oil-immersion lens with a 1.3 numerical aperture (Nikon, CF Fluor). Fura-2 fluorescence was detected using either a photomultiplier tube based system (26) or a video based system (27), as previously described. In either case, a whole-cell average value was determined.

Simultaneous fura-2 and perforated whole-cell patch-clamp recording. The ratioed average fura-2 fluorescence and whole-cell current from a cell chosen for simultaneous recordings were digitized by a Pulse code modulator (model 200-T; A. R. Vetter Co., Rebersburg, PA) and recorded onto two channels of a VCR tape as previously described (27). Simultaneous acquisition of both signals by the PCM/VCR allowed temporal comparison of the $(Ca^{2+})_i$ and membrane current signals.

Inositol phosphate determinations. T84 cells were plated onto 60 mm plastic dishes and allowed to attach for ~ 3 h in normal growth media such that they were 30–40% confluent. The cells were then labeled overnight with 2 μCi/ml [^{3}H]inositol in serum-free media. The following day, this medium was aspirated and the cells were washed three times using the standard bath solution. Cells were then bathed for 5 min before stimulation in the standard bath, to which 10 mM LiCl was added to reduce IP degradation. Cells were then stimulated at room temperature with either TDC (0.75 mM) or carbachol (100 μM) for 15 or 30 min in the continued presence of 10 mM LiCl. Stimulation was terminated by aspirating the stimulatory solution and adding 1 ml of boiling water. The cells were then scraped from the plastic dishes, transferred to tubes, and immersed in a boiling water bath for 5 min. After centrifugation, the supernatant was filtered and inositol phosphates were separated by HPLC (series 410; The Perkin-Elmer Corp., Norwalk, CT) using a 250 × 10 mm SAX column (partisil; Whatman Inc., Clifton, NJ) with the following elution profile: 5 min elution with water, 1 min linear gradient to 10 mM ammonium formate, 20 min isocratic elution at 10 mM ammonium formate, and 20 min elution with 1 M ammonium formate.

Taurodeoxycholate and taurocholate were obtained from Sigma Chemical Co. (St. Louis, MO) [bis-(*o*-aminophenoxy)-ethane-*N,N,N'N'*-tetraacetic acid, tetra(acetoxymethyl)-ester (BAPTA-AM) was obtained from Calbiochem Corp. (La Jolla, CA). Tissue culture media were obtained from GIBCO BRL (Gaithersburg, MD).

Data analysis. Statistical analysis was performed using *t*-tests and analysis of variance. In all cases, a value of $P < 0.05$ was considered statistically significant. The data are presented as mean±SE.

Results

TDC-stimulated currents. The current responses of two T84 cells to TDC (0.75 mM) during perforated whole-cell patch-clamp recording are shown in Fig. 1, *A* and *B*. The cells were bathed in standard NaCl solution and were alternately voltage-clamped between E_K and E_{Cl} at 2-s intervals (see Methods and References 19, 20). TDC stimulated outward and inward oscillatory currents that returned to baseline when TDC was removed. The currents recorded from different cells varied in the periodicity and duration of oscillations (compare Fig. 1, *A* and *B*). The outward current, recorded at E_{Cl}, is due to the flow of K^+ from cell-to-bath. The inward current, recorded at E_K, could be due to either Cl^- exit from the cell or Na^+ entry from the bath (see below). These responses were typical of 10 additional cells. The peak outward and inward currents for these 12 cells averaged 385±41 and 98±28 pA, respectively. In four additional cells, oscillatory current responses were not seen, but smaller inward currents were present (see below). The threshold concentration of TDC required to induce the oscillatory current responses was 0.1 mM, with average peak outward and inward currents of 164±48 and 35±18 pA, respectively (n = 10). In 10 patches, 0.03 mM TDC had no effect on transmembrane currents.

Before stimulating these large inward- and outward-current transients, TDC elicited a smaller inward current. Fig. 1 *C* shows expanded views of the control and TDC-induced currents at E_K and E_{Cl} at times labeled i, ii, and iii in Fig. 1 *B*. As can be seen in Fig. 1 *C*ii, TDC activated early, distinct inward-current bursts. In some experiments this current could be resolved into discrete channel events, as shown in Fig. 1 *D*. These initial inward-current events were not observed at low concentrations of TDC (0.03 mM), but were seen in 100% of patches stimulated with TDC concentrations greater than 0.1 mM (n = 37).

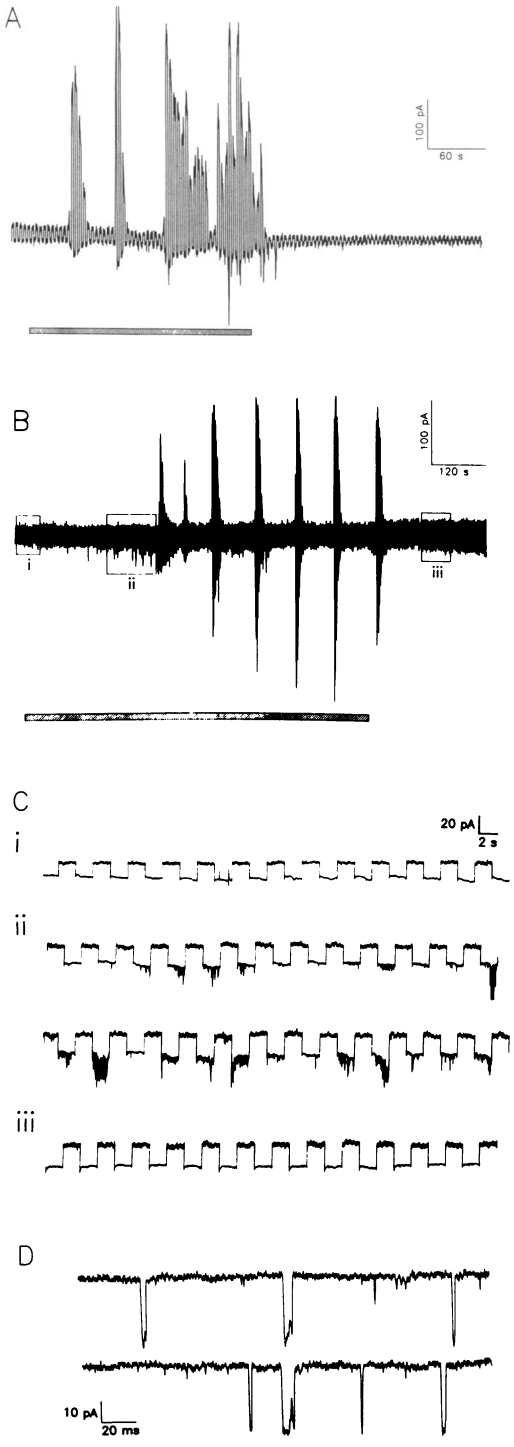

Figure 1. TDC stimulation (0.75 mM; exposure indicated by hatched bar) of membrane currents during perforated whole-cell patch-clamp recording. Currents in *A* and *B* were recorded from two different cells during voltage pulses to E_K (downward deflections) and E_{Cl} (upward deflections) at 2-s intervals using standard bath (145 mM NaCl) and pipette (60 mM K_2SO_4, 40 mM KCl) solutions. Expanded views of these currents are given in *C* for the boxes labeled *i, ii,* and *iii* in *B*. Further expansion of inward-current records during voltage-clamp to E_K is shown for a different cell in *D*.

Dihydroxy bile acids like TDC, when present in the colonic lumen, cause water and electrolyte secretion; however, similar concentrations of trihydroxy bile acids do not have this effect (5). TDC is produced via the dehydroxylation of the trihydroxy bile acid, taurocholate. Therefore, we examined the effect of taurocholate on membrane currents in T84 cells, since a response to this compound would suggest a nonspecific detergent effect. Fig. 2 shows currents recorded from a cell that was stimulated with taurocholate (0.75 mM) and then with TDC (0.75 mM). Taurocholate did not induce currents, but exposure of the same cell to TDC induced current oscillations. This result was typical of seven additional cells.

Ion replacement experiments. Bile acids are absorbed in the distal ileum via a Na^+-dependent cotransporter. To determine whether Na^+-dependent TDC entry into T84 cells was responsible for current activation, Na^+ in the bath solution was replaced with $NMDG^+$ before TDC exposure. Fig. 3 shows a typical result. TDC stimulated the large inward and outward current oscillations (Fig. 3 *A*), but in the absence of bath Na^+ the initial inward current response to TDC was not observed (Fig. 3 *B*). This suggests that activation of a nonselective cation conductance is responsible for the early inward current (see Discussion). The peak outward (428±98 pA) and inward (83±30 pA) currents were not different from those recorded with Na^+ in the bath (n = 5). These findings also reveal the ionic basis of the TDC-induced oscillating inward current. In the absence of bath Na^+, the inward current recorded at E_K must be due to the flow of Cl^- from the cell to the bath. Therefore, TDC elicits increases in both K^+ and Cl^- conductances in these cells.

In separate experiments, bath Na^+ was replaced with choline rather than $NMDG^+$. Unexpectedly, this eliminated the TDC-induced currents (Fig. 4). Subsequent reintroduction of bath Na^+ stimulated outward K^+ (334±74 pA) and inward Cl^- (114±48 pA) currents that were not different from control (n = 6). This does not reflect a requirement for bath Na^+, since replacing Na^+ with $NMDG^+$ did not inhibit the current responses (see above). Choline is an amphiphilic molecule and is the primary constituent of lecithin. Lecithin forms mixed micelles with TDC and other bile acids, and has been shown to

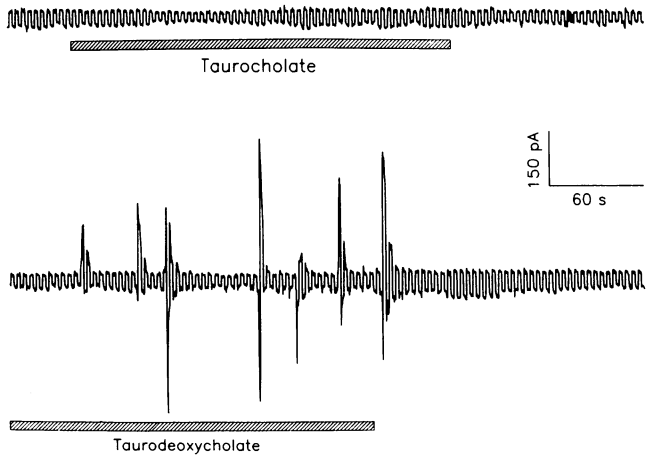

Figure 2. (*Top trace.*) Effect of the nonsecretory bile acid taurocholate (0.75 mM; exposure indicated by hatched bar) on membrane currents recorded during perforated patch-clamp recording. (*Bottom trace.*) Response of the same cell shown in the top trace to TDC exposure (0.75 mM). Top and bottom traces are from a continuous recording during alternate voltage-clamping between E_K and E_{Cl} at 2-s intervals using standard bath (145 mM NaCl) and pipette (60 mM K_2SO_4, 40 mM KCl) solutions.

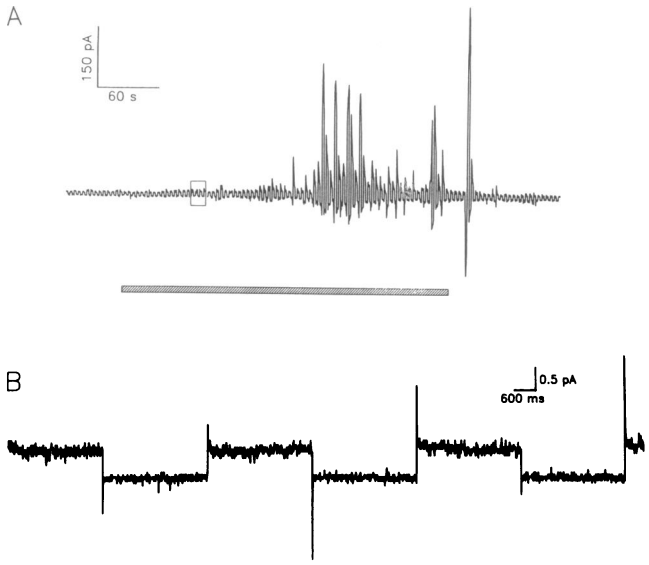

Figure 3. Effect of $NMDG^+$ replacement of bath Na^+ on TDC-induced (0.75 mM; exposure indicated by hatched bar) membrane currents recorded during perforated patch-clamp recording. Current *A* was recorded from a single cell during alternate voltage-clamping between E_K and E_{Cl} at 2-s intervals. Sodium was replaced ~ 1 min before the beginning of recording. The standard pipette solution (60 mM K_2SO_4, 40 mM KCl) was used. Expanded views of the currents recorded during voltage-clamp between E_K and E_{Cl} are shown in *B* for the corresponding box in *A*.

inhibit bile acid-induced intestinal secretion (12), presumably by reducing the concentration of free bile acid below the threshold level required to produce a secretory response. The formation of TDC:choline mixed micelles is likely to have the same effect, i.e., to inhibit the response of T84 cells to TDC. Thus, it is likely that inhibition of the response to TDC during choline substitution is due to the formation of TDC:choline mixed micelles.

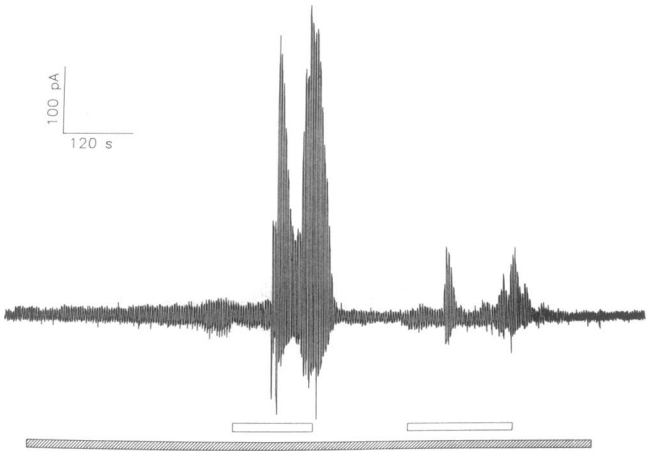

Figure 4. Inhibition of TDC-induced (0.75 mM; exposure indicated by hatched bar) membrane currents in one cell when bath Na^+ was replaced with choline during a perforated patch-clamp recording. Sodium was replaced ~ 1 min before beginning the recording. Standard Na^+-containing electrolyte solution was reintroduced for two brief intervals (*open bars*). Recordings were made during alternate voltage-clamping between E_K and E_{Cl} at 2-s intervals using the standard pipette solution (60 mM K_2SO_4, 40 mM KCl).

Signal transduction mechanisms: role of Ca^{2+}. Bile acids are known to act as Ca^{2+} ionophores (16–19). Thus, TDC could induce Cl^- secretion by enhancing Ca^{2+} entry to elevate $(Ca^{2+})_i$, as has been shown for the Ca^{2+} ionophores A23187 and ionomycin (20, 21). To examine this possibility, we determined the effect of bath Ca^{2+} removal on the TDC-induced currents. A typical result is shown in Fig. 5 *A*. Bath Ca^{2+} was removed ~ 45 s before stimulation with TDC (0.75 mM). Taurodeoxycholate induced inward- and outward-current oscillations similar to those seen in the presence of bath Ca^{2+}. The initial peak outward K^+ current averaged 401±77 pA, and the initial peak inward Cl^- current averaged 79±14 pA ($n = 9$). These currents were not significantly different from those measured in the presence of bath Ca^{2+}, suggesting that TDC is not acting as a Ca^{2+} ionophore.

We assessed the possibility that TDC induced a release of Ca^{2+} from intracellular stores by loading cells with the Ca^{2+} chelator BAPTA-AM (20 μM) for 30 min. This was followed by 1 h postincubation to permit the cells to deesterify the chelator and recover steady state $(Ca^{2+})_i$. Stimulation of the BAPTA-loaded cells with TDC (0.75 mM) produced inward current fluctuations, as shown in Fig. 5 *B*. This response was typical of the early inward currents associated with Na^+ entry through nonselective cation channels (Fig. 1, *C* and *D*); however, K^+ and Cl^- current oscillations did not occur. Similar results were obtained from 10 additional cells. In addition, re-

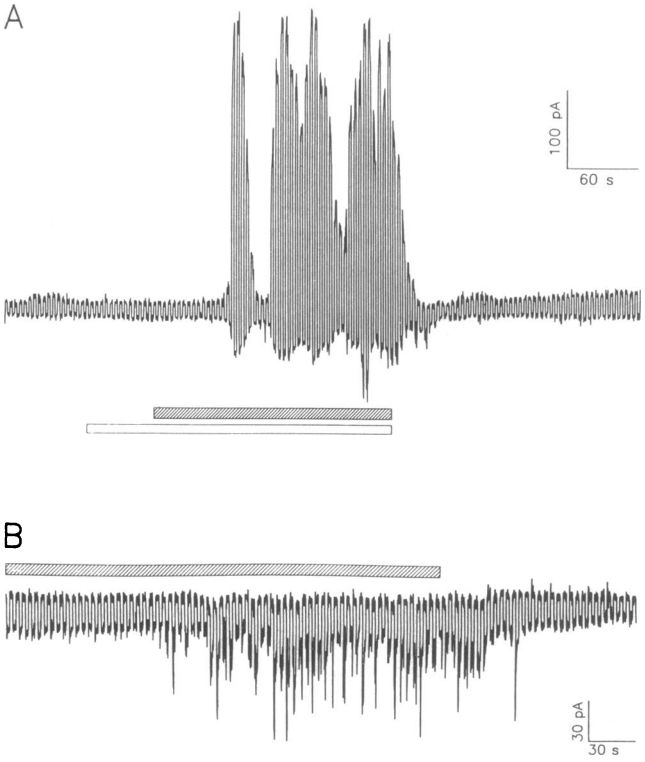

Figure 5. Effect of manipulation of extracellular and intracellular Ca^{2+} on membrane currents in response to TDC (0.75 mM; exposure indicated by hatched bar) during perforated patch-clamp recording. Recordings were made during alternate voltage-clamping between E_K and E_{Cl} at 2-s intervals using standard bath (145 mM NaCl) and pipette (60 mM K_2SO_4, 40 mM KCl) solutions, except as noted. (*A*) Effect of zero bath Ca^{2+} (1 mM EGTA; open bar) on TDC-induced membrane currents for one cell. (*B*) Response of one cell following incubation in 20 μM BAPTA-AM for 30 min.

moval of bath Ca^{2+} did not affect the inward current fluctuations in five cells (data not shown). Taken together, the results of Fig. 5, *A* and *B* suggest that the oscillating K^+ and Cl^- conductances are Ca^{2+} dependent and that their stimulation by TDC is due to the release of Ca^{2+} from intracellular stores. In contrast, TDC stimulation of the nonselective cation conductance does not require an increase in cell Ca^{2+}.

Determination of $(Ca^{2+})_i$. To determine whether TDC raises $(Ca^{2+})_i$, we loaded T84 cells with the fluorescent Ca^{2+} indicator, fura-2. Basal $(Ca^{2+})_i$ averaged 65±3 nM for all cells (n = 33). Two patterns of $(Ca^{2+})_i$ responses were observed during exposure to 0.75 mM TDC, as shown in Fig. 6. In both cases, TDC caused an initial rise in $(Ca^{2+})_i$, followed either by oscillations (Fig. 6 *A*) or a sustained, elevated plateau (Fig. 6 *B*). In either case, removal of TDC caused $(Ca^{2+})_i$ to return rapidly to baseline levels. In 15 cells, 0.75 mM TDC induced an average peak $(Ca^{2+})_i$ rise of 575±70 nM; the peak $(Ca^{2+})_i$ observed with 0.5 mM TDC was not different (548±70 nM, n = 18).

To assess the source of Ca^{2+} for the TDC-induced $(Ca^{2+})_i$ rise, cells were exposed to a zero Ca^{2+} bath ~ 30–45 s before the addition of 0.75 mM TDC. In these experiments, fluorescence ratios were determined, but $(Ca^{2+})_i$ was not calculated. Under these conditions, TDC induced an initial $(Ca^{2+})_i$ rise, followed by a series of oscillations that returned to baseline upon TDC removal (data not shown; however, see Fig. 8). The increase in fluorescence ratio averaged 2.4±0.1-fold (n = 20), a value not significantly different from the 2.5±0.2-fold increase seen in the presence of bath Ca^{2+} (n = 18).

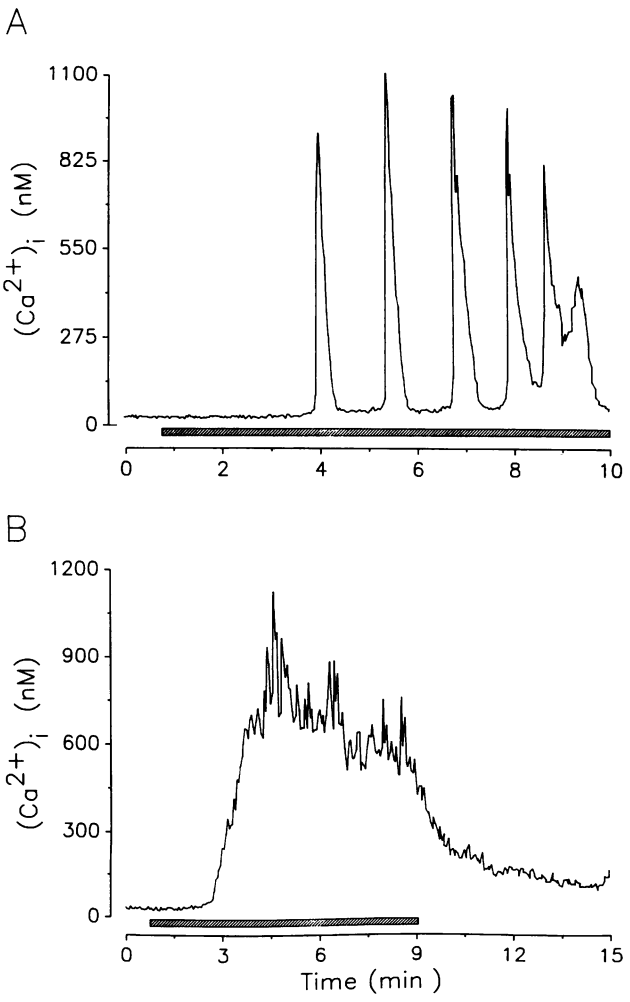

Figure 6. Intracellular Ca^{2+} concentration ($(Ca^{2+})_i$) as a function of time for two cells stimulated with TDC (0.75 mM; exposure indicated by hatched bar). $(Ca^{2+})_i$ was determined at 2-s intervals for the cell in *A* and 3-s intervals for the cell in *B*.

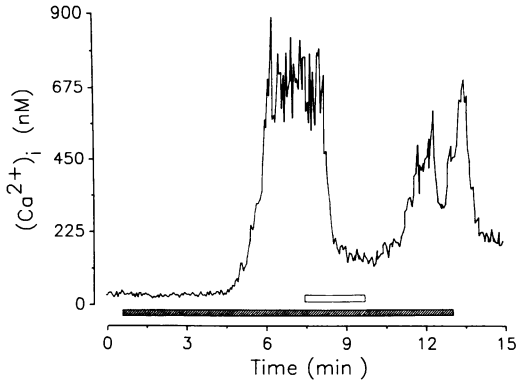

Figure 7. $(Ca^{2+})_i$ as a function of time for a cell in which bath Ca^{2+} was removed (1 mM EGTA; *open bar*) during a prolonged TDC-induced (0.75 mM; exposure indicated by hatched bar) rise in $(Ca^{2+})_i$. Data was collected at 3-s intervals. Removal of bath Ca^{2+} during oscillatory $(Ca^{2+})_i$ responses (e.g., Fig. 6 *A*) produced similar results.

To determine the requirement for bath Ca^{2+} during the TDC response, cells were stimulated with TDC (0.75 mM) and bath Ca^{2+} was removed after the response had peaked (Fig. 7). When bath Ca^{2+} was removed, $(Ca^{2+})_i$ returned rapidly to near baseline values. Reintroduction of bath Ca^{2+} caused $(Ca^{2+})_i$ to increase again. Removal of TDC also caused the $(Ca^{2+})_i$ response to rapidly return toward baseline. These results are typical of 15 additional cells and demonstrate that bath Ca^{2+} is necessary to maintain the $(Ca^{2+})_i$ response to TDC (see Discussion).

Simultaneous $(Ca^{2+})_i$ *and current measurements.* The separate $(Ca^{2+})_i$ and membrane current measurements provide indirect evidence that TDC activates K^+ and Cl^- conductances in T84 cells via the release of Ca^{2+} from intracellular stores. To more directly determine the relationship between $(Ca^{2+})_i$ oscillations and the increase in ionic conductances, we simultaneously measured the fura-2 fluorescence ratio and K^+ currents in individual cells (see Methods) in the absence of bath Ca^{2+}. A typical result is shown in Fig. 8. The upper trace shows the outward K^+ current recorded at E_{Cl}, while the lower trace shows the simultaneously recorded fura-2 fluorescence ratio. A large initial inward current was seen upon TDC exposure that was not associated with a change in $(Ca^{2+})_i$. This inward current must be due to Na^+ entry into the cell from the bath since the cell was voltage-clamped at E_{Cl}. The activation of a Ca^{2+}-independent inward Na^+ current is consistent with the inward currents we observed in BAPTA-loaded cells (see Fig. 5). After this inward current transient, TDC induced outward K^+ current oscillations whose timing coincided with the $(Ca^{2+})_i$ transients. These K^+ current and $(Ca^{2+})_i$ oscillations were observed in only 3 of 10 experiments where simultaneous patch-clamp and fluorescence measurements were performed. In the other seven cells, TDC stimulated the small inward current but did not raise $(Ca^{2+})_i$. When similar experiments were performed without fura-2 loading, outward K^+ currents

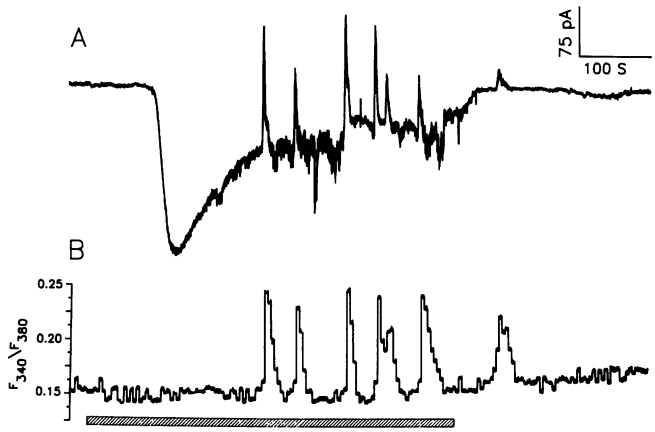

Figure 8. Simultaneous perforated patch-clamp and fura-2 fluorescence ratio (F_{340}/F_{380}) measurements for a single cell during TDC (0.75 mM; exposure indicated by hatched bar) stimulation. (*Top trace.*) Membrane K^+ current recorded during voltage-clamp to E_{Cl} using standard bath (145 mM NaCl) and pipette (60 mM K_2SO_4, 40 mM KCl) solutions. (*Bottom trace.*) Simultaneously recorded fura-2 fluorescence ratio.

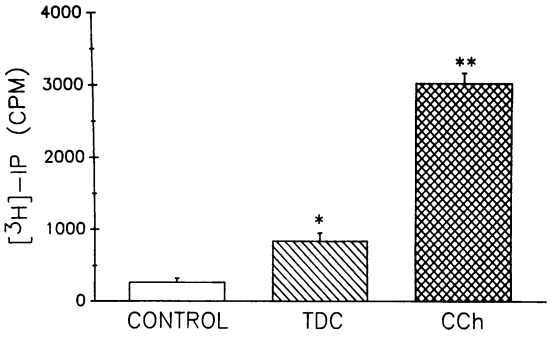

Figure 9. Agonist-stimulated IP formation in T84 cells. Taurodeoxycholate (TDC; 0.75 mM) stimulated a significant increase in IP formation (* $P < 0.05$, $n = 4$) compared to control ($n = 4$). Stimulation with carbachol (CCh; 100 M) induced a rise in IP which was significantly greater than both control and TDC-stimulated levels (** $P < 0.01$, $n = 3$).

were observed in nine of nine cells in the absence of bath Ca^{2+}. We previously found that muscarinic agonist-induced K^+ current and $(Ca^{2+})_i$ oscillations diminished during simultaneous patch-clamp and fura-2 measurements, which could reflect a loss of cellular Ca^{2+} homeostasis in fura-2 loaded cells buffered in Ca^{2+}-free media (25). Nevertheless, the findings indicate that the TDC-induced current oscillations are paralleled by $(Ca^{2+})_i$ transients that result from Ca^{2+} release from intracellular stores. The initial inward cation current is independent of changes in $(Ca^{2+})_i$.

Phosphoinositide turnover. The mechanism of TDC-induced cellular Ca^{2+} release could involve production of IP_3 via breakdown of PIP_2. We measured the accumulation of IP, a breakdown product of IP_3, during TDC stimulation to address this possibility (Fig. 9). Stimulation of T84 cells with TDC (0.75 mM) for 30 min elicited a threefold increase in IP accumulation above control values ($P < 0.05$). Carbachol (100 μM) produced an 11-fold increase in IP accumulation ($P < 0.05$), similar to a previous report (28). At 15 min TDC and carbachol produced increases in IP that fell between the 30 min stimulated and control values (data not shown). A TDC-induced increase in phospholipid turnover has been previously reported for isolated rat colonic crypts (29).

Effect of heparin on TDC activation. While our findings indicate that TDC increases phospholipid turnover in T84 cells, they do not show that IP_3 production is causally related to Ca^{2+} release and the activation of K^+ and Cl^- conductances. It has recently been demonstrated that bile acids induce the release of Ca^{2+} from intracellular stores in hepatocytes in an IP_3-independent fashion (30). Therefore, we assessed the role of IP_3 in $(Ca^{2+})_i$ release using heparin, a blocker of IP_3 receptors (31).

Heparin was introduced into T84 cells using the open-cell patch-clamp technique, which permits substances contained within the patch pipette to enter the cell interior. Before assessing the effects of the IP_3 receptor antagonist, control experiments were performed to determine the effects of TDC in this recording mode, inasmuch as all other records were obtained under perforated whole-cell conditions. With the standard whole-cell technique, TDC reversibly stimulated an outward K^+ current during voltage clamp to E_{Cl} and an inward current during voltage clamp to E_K. These effects were similar to those obtained with perforated whole-cell recordings, except that the inward currents were larger than found previously. The results of ion-replacement studies (not shown) indicate that this was due primarily to the activation of a larger nonselective cation current. Therefore, we focused our attention on the TDC-stimulated K^+ current recorded at E_{Cl} (0 mV) where nonselective cation currents would be small. The source of this larger nonselective cation current in the open-cell mode is unknown, but differences in agonist-induced currents have been observed previously when open-cell and perforated recording techniques were compared (24, 32).

When present in the pipette solution, heparin (1 mg/ml) abolished or reduced the TDC-stimulated K^+ current. 11 of 18 cells studied with pipette heparin showed no detectable response to TDC. In seven other cells, the responses were significantly smaller than those observed under control conditions (Table I). Heparin also reduced the TDC-stimulated inward current. Inasmuch as both the K^+ and Cl^- currents are Ca^{2+} dependent, we anticipate that heparin reduces these currents by blocking IP_3 receptors.

Discussion

The dihydroxy bile acid, taurodeoxycholate (TDC), activates K^+ and Cl^- conductances in T84 intestinal secretory cells. In

Table I. Effect of Heparin on TDC-stimulated Outward K^+ Current (I_K) in T84 Cells

			Pooled data	
	n	I_K (pA)	*n*	I_K (pA)
Control	9	216±63	—	—
	11	0		
Heparin	7	68±23*	18	27±12*

* Significantly different from control ($P < 0.05$).

addition, a nonselective cation conductance was activated early in the response to TDC.

Ca^{2+}-insensitive nonselective cation conductance. A Ca^{2+}-insensitive nonselective cation conductance was seen in the initial inward-current responses to TDC and could be resolved into discrete single-channel events (Fig. 1 *D*). It is not likely that these channels are activated by increased cell Ca^{2+} since their activity could be elicited by TDC after BAPTA loading (Fig. 5 *B*). The same conditions abolished the K^+ and Cl^- currents stimulated by TDC (see below). In addition, the nonselective cation conductance was activated by TDC in the absence of any change in $(Ca^{2+})_i$ during simultaneous patch-clamp and $(Ca^{2+})_i$ measurements (Fig. 8). Two experiments showed that the inward current through this conductance pathway was carried by Na^+. First, removal of Na^+ from the bath abolished the early inward current response to TDC during perforated patch recording (Fig. 3). Second, outward single-channel currents were observed in open-cell recordings with Na^+ in the pipette (data not shown). The single-channel conductance, calculated from these records is ~ 300 pS (20 pA current at + 60 mV). Similarly, the single-channel conductance, calculated from the data of Fig. 1 *D* (assuming a reversal potential of 0 mV for a nonselective cation channel) is ~ 300 pS.

We do not know the nature of this nonselective cation conductance pathway. TDC may open or alter an existing channel, and T84 cells are known to express a nonselective cation channel, although its single-channel conductance is much smaller (~ 25 pS; References 24, 33, 34) than that found here. Alternately, TDC itself may conduct cation currents in a nonselective manner. Bile acids have been reported to act as channels that conduct mono- and divalent cations (16, 17).

Ca^{2+}-sensitive K^+ and Cl^- conductances. Our initial observation that membrane currents oscillate during TDC exposure (Fig. 1) suggested that the effects of TDC may be Ca^{2+}-mediated, since agonist-induced $(Ca^{2+})_i$ oscillations occur in these cells in response to cholinergic stimulation (26). The TDC-activated K^+ and Cl^- conductances are Ca^{2+}-sensitive since loading of the cells with BAPTA blocks the TDC-induced oscillating currents (Fig. 5 *B*). This finding is similar to the block of muscarinic agonist-activated K^+ current oscillations we observed in T84 cells when the whole-cell pipettes contained EGTA (25). In support of this idea, we observed $(Ca^{2+})_i$ oscillations during TDC exposure (Fig. 6), and simultaneous current and $(Ca^{2+})_i$ measurements showed that the K^+ currents oscillate in phase with $(Ca^{2+})_i$ (Fig. 8).

A Ca^{2+}-sensitive K^+ conductance pathway has been identified previously in isolated T84 cells (25). Recent measurements of membrane potential and current in isolated rat (35) and guinea pig crypts (36) have shown that the rise in $(Ca^{2+})_i$ stimulated by muscarinic agonists can activate a basolateral membrane K^+ conductance. Evidence of a role for Ca^{2+} in the response to TDC is also provided by findings from intact rabbit colon, where bile acid-stimulated K^+ secretion, which apparently involves an apical membrane K^+ conductance, was blocked by the calmodulin antagonist, trifluoperazine (3). A Ca^{2+}-sensitive Cl^- conductance has also been recorded from T84 cells (33, 37).

Role of bath Ca^{2+} in bile acid-induced Cl^- secretion. TDC induces a release of Ca^{2+} from intracellular stores, and the resulting rise in $(Ca^{2+})_i$ activates both K^+ and Cl^- conductances. Furthermore, both the TDC-induced increase in $(Ca^{2+})_i$ and activation of ionic conductances can occur independently of bath Ca^{2+} (Fig. 5 *A* and 8). Our findings are consistent with previous data showing that bile acid-induced secretion in the colon (3, 4) and jejunum (10) does not require bath Ca^{2+}. Bile acids have also been shown to stimulate a rise in $(Ca^{2+})_i$, which paralleled Cl^- secretion in T84 monolayers (11). These authors reported an average change in $(Ca^{2+})_i$ of only 25 nM during TDC (0.5 mM) stimulation. In the absence of bath Ca^{2+} the TDC-induced short circuit current (I_{sc}), was reduced, but under these conditions, Dharmsathaphorn et al. (11) were unable to detect any $(Ca^{2+})_i$ change. However, the $(Ca^{2+})_i$ chelator 1,2-bis-5-methyl-amino-phenoxylethane-*N,N,N'*-tetraacetoxymethyl acetate (MAPTAM) inhibited both the increase in $(Ca^{2+})_i$ and I_{sc} (11), suggesting that the change in $(Ca^{2+})_i$ may have been beyond the limits of detection in the absence of bath Ca^{2+}.

We found that removal of bath Ca^{2+} during the TDC response causes $(Ca^{2+})_i$ to return to near baseline levels (Fig. 7). Thus, while bath Ca^{2+} is not required to initiate the response to TDC, it clearly plays a role in maintaining an elevated $(Ca^{2+})_i$. The mechanism that underlies this requirement for bath Ca^{2+} after initiation of the response to TDC is unknown. Since bile acids can act as Ca^{2+} ionophores in cell membranes (18, 19) and phospholipid membrane vesicles (16, 17), TDC may contribute to loss of cell Ca^{2+} during prolonged stimulation by TDC. Indeed, if the nonselective cation pathway activated by TDC is capable of conducting Ca^{2+} (see above), it could also contribute to cell Ca^{2+} loss. In addition, during the response to agonist Ca^{2+}, efflux is stimulated following the rise in cell Ca^{2+} in an attempt to maintain cellular Ca^{2+} homeostasis. When bath Ca^{2+} is removed during stimulation, cell Ca^{2+} falls below basal values (27).

Mechanism of the bile acid-induced rise in $(Ca^{2+})_i$. Measurement of IP, a breakdown product of IP_3, demonstrated that the TDC-stimulated Ca^{2+} rise is accompanied by mobilization of IP_3 (Fig. 9). These data are consistent with reports of increased IP_3 production in isolated colonic crypts during bile acid stimulation (29). Bile acids have also been shown to increase IP_3 production in guinea pig hepatocytes (30). We found that heparin, a known blocker of IP_3 receptors (31), blocked the TDC-induced membrane currents (Table I). Thus, in T84 cells TDC induces the release of Ca^{2+} from intracellular stores via an IP_3-dependent mechanism. In keeping with this conclusion, recent experiments in hepatocytes show that bile acids mobilize Ca^{2+} from the same intracellular pool that releases Ca^{2+} during an increase in intracellular IP_3 production (38–40).

The mechanism by which bile acids stimulate polyphosphoinositide turnover is unclear. Bile acids may directly activate phospholipase C (PLC) since they have been shown to stimulate a twofold increase in IP_3 accumulation, and a reciprocal fall in PIP_2 in isolated colonic crypts of rats (29). Alternately, it has been suggested that PLC may not have access to all polyphosphoinositides in the cell membrane, and that membrane disruption or reorganization promoted by bile acids may make polyphosphoinositides available for degradation by PLC (41). Finally, it is possible that TDC activates a G-protein which acts on PLC so that IP_3 is generated in a manner analogous to receptor-mediated mechanisms. Additional experiments are needed to differentiate among these possibilities.

We do not know the mechanisms responsible for Ca^{2+} oscillations during exposure of these cells to TDC. As in other

systems, these oscillations may represent a balance between agonist-induced intracellular Ca^{2+} release and the uptake and efflux mechanisms which attempt to maintain a low $(Ca^{2+})_i$. Muscarinic agonists also evoke Ca^{2+} oscillations in these cells (26).

Bile acids as detergents. Bile acids are well known anionic detergents and are used in purification and reconstitution of membrane proteins. It has been suggested that this detergent action is responsible for the increased intestinal mucosal permeability associated with the secretory effect of bile acid stimulation (13, 42). However, there are several reasons why these detergent effects are not likely to be involved in the activation of specific ionic conductances observed in the current study. First, taurocholate (a nonsecretory bile acid) is also an anionic detergent, but it does not activate ionic conductances in T84 cells. Second, the cells continued to respond to TDC in the absence of bath Ca^{2+}, which would not be likely if a detergent-induced leak pathway for Ca^{2+} was responsible for the secretory effects of TDC. Third, bile acid exposure does not cause an increase in $(Ca^{2+})_i$ in some cell types, e.g., platelets or neuroblastoma cells (43), whereas increased $(Ca^{2+})_i$ would be expected in all cells if the effects were nonspecific. Fourth, there was no detectable leak of fura-2 from the cells during the Ca^{2+} measurements.

A model for secretion. Several pathological conditions result in a compromised ileal bile acid reabsorption (1). This results in an increased movement of bile acids into the colon where they stimulate fluid and electrolyte secretion, resulting in diarrhea (2–5). Bile acids require two hydroxyl groups in the α configuration at the 3,7 position, 3,12 position, or 7,12 position of the steroid ring to induce secretion in the intestine (13). We have used the colonic cell line, T84, to assess the cellular mechanism whereby bile acids induce Cl^- secretion. Our observations demonstrate that the dihydroxy bile acid TDC (3α-OH, 12α-OH) stimulates the release of Ca^{2+} from intracellular stores, with the subsequent activation of plasma membrane ionic conductances, while the trihydroxy bile acid TC (3α-OH, 7α-OH, 12α-OH) does not (Fig. 2). These results are consistent with the more general observation that dihydroxy bile acids stimulate fluid and electrolyte secretion in humans while trihydroxy bile acids do not (5), although a complete characterization of the structure-function relationship was not attempted in this cell line.

The Cl^- secretory process involves the coordinated activity of several ion-transport mechanisms, including the Na^+:K^+ ATPase, Na^+:K^+:$2Cl^-$ cotransporter and K^+ channels located at the basolateral membrane, as well as apical membrane Cl^- channels. Dharmsathaphorn and his colleagues (11) have proposed that Ca^{2+}-mediated agonists induce secretion, at least in part, by activating the basolateral membrane K^+ conductance. Activation of this K^+ conductance would hyperpolarize the cell membranes, thereby increasing the electrochemical driving force for Cl^- exit across the apical membrane. We found that free bile acids stimulate IP_3 production, Ca^{2+} release from intracellular stores, and activation of plasma membrane conductances for both K^+ and Cl^-. These findings indicate that bile acid-induced colonic Cl^- secretion utilizes both signal transduction mechanisms and ion channels that are utilized by other Ca^{2+}-mediated agonists.

Acknowledgments

We would like to gratefully acknowledge the excellent technical assistance of Karlin Henze.

This work was supported by an Alabama Heart Association grant to M. C. Sekar and National Institutes of Health Research grants DK-31091 to R. A. Frizzell and DK-38026 to M. E. Duffey.

References

1. Balistreri, W. F., J. E. Heubi, and F. J. Suchy. 1983. Bile acid metabolism: Relationship of bile acid malabsorption and diarrhea. *J. Pediatr. Gastroenterol. Nutr.* 2:105–121.

2. Binder, H. J., C. Filburn, and B. T. Volpe. 1975. Bile salt alteration of colonic electrolyte transport: role of cyclic adenosine monophosphate. *Gastroenterology.* 68:503–508.

3. Freel, R. W. 1987. Dihydroxy bile salt-induced secretion of rubidium ion across the rabbit distal colon. *Am. J. Physiol.* 252:G554–G561.

4. Freel, R. W., M. Hatch, D. L. Earnest, and A. M. Goldner. 1983. Dihydroxy bile salt-induced alterations in NaCl transport across the rabbit colon. *Am. J. Physiol.* 245:G808–G815.

5. Mekhjian, H. S., S. F. Phillips, and A. F. Hofmann. 1971. Colonic secretion of water and electrolytes induced by bile acids. Perfusion studies in man. *J. Clin. Invest.* 50:1569–1577.

6. Cotting, J., M. J. Lentze, and J. Reichen. 1990. Effects of ursodeoxycholic acid treatment on nutrition and liver function in patients with cystic fibrosis and longstanding cholestasis. *Gut.* 31:918–921.

7. Kaplan, M. M. 1991. Medical approaches to primary sclerosing cholangitis. *Semin. Liver Dis.* 11:56–63.

8. Kaplan, M. M. 1989. Medical treatment of primary biliary cirrhosis. *Sem. Liver Dis.* 9:138–143.

9. Talamini, M. A., and T. R. Gadacz. 1990. Gallstone dissolution. *Surg. Clin. North Am.* 70:1217–1230.

10. Ammon, H. V., D. S. Cho, R. L. Loeffler, and K. L. Reetz. 1986. Effects of taurodeoxycholate on in vivo water and solute transport in rat jejunum in absence and presence of calcium. *Am. J. Physiol.* 250:G248–G251.

11. Dharmsathaphorn, K., P. A. Huott, P. Vongkovit, G. Beuerlein, S. J. Pandol, and H. V. Ammon. 1989. Cl^- secretion induced by bile salts. A study of the mechanism of action based on a cultured colonic epithelial cell line. *J. Clin. Invest.* 84:945–953.

12. Wingate, D. L., S. F. Phillips, and A. F. Hofmann. 1973. Effect of glycine-conjugated bile acids with and without lecithin on water and glucose absorption in perfused human jejunum. *J. Clin. Invest.* 52:1230–1236.

13. Chadwick, V. S., T. S. Gaginella, G. L. Carlson, J-C. Debongnie, S. F. Phillips, and A. F. Hofmann. 1979. Effect of molecular structure on bile acid-induced alterations in absorptive function, permeability, and morphology in the perfused rabbit colon. *J. Lab. Clin. Med.* 94:661.

14. Potter, G. D., J. H. Sellin, and S. M. Burlingame. 1991. Bile acid stimulation of cyclic AMP and ion transport in developing rabbit colon. *J. Pediatr. Gastroenterol. Nutr.* 13:335–341.

15. Simon, B., P. Cyzgan, A. Stiehl, and H. Kather. 1978. Human colonic adenylate cyclase: effects of bile acids. *Eur. J. Clin. Invest.* 8:321–323.

16. Abramson, J. J., and A. E. Shamoo. 1979. Anionic detergents as divalent cation ionophores across black lipid membranes. *J. Membr. Biol.* 50:241–255.

17. Oelberg, D. G., L. B. Wang, J. W. Sackman, E. W. Adcock, R. Lester, and W. P. Dubinsky. 1988. Bile salt-induced calcium fluxes in artificial phospholipid vesicles. *Biochim. Biophys. Acta.* 937:289–299.

18. Maenz, D. D., and G. W. Forsyth. 1984. Calcium ionophore activity of intestinal secretory compounds. *Digestion.* 30:138–150.

19. Maenz, D. D., and G. W. Forsyth. 1982. Ricinoleate and deoxycholate are calcium ionophores in jejunal brush border vesicles. *J. Membr. Biol.* 70:125–133.

20. Bolton, J. E., and M. Field. 1977. Ca ionophore-stimulated ion secretion in rabbit ileal mucosa: relation to actions of cyclic 3′,5′-AMP and carbamylcholine. *J. Membr. Biol.* 35:159–173.

21. Frizzell, R. A. 1977. Active chloride secretion by rabbit colon: calcium-dependent stimulation by ionophore A23187. *J. Membr. Biol.* 35:175–187.

22. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.

23. Horn, R., and A. Marty. 1988. Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J. Gen. Physiol.* 92:145–159.

24. Devor, D. C., and M. E. Duffey. 1992. Carbachol induces K^+, Cl^-, and nonselective cation conductances in T84 cells: a perforated patch-clamp study. *Am. J. Physiol.* 263:C780–C787.

25. Devor, D. C., S. M. Simasko, and M. E. Duffey. 1990. Carbachol induces oscillations of membrane potassium conductance in a colonic cell line, T84. *Am. J. Physiol.* 258:C318–C326.

26. Devor, D. C., Z. Ahmed, and M. E. Duffey. 1991. Cholinergic stimulation produces oscillations of cytosolic Ca^{2+} in a secretory epithelial cell line, T84. *Am. J. Physiol.* 260:C598–C608.

27. Morris, A. P., K. L. Kirk, and R. A. Frizzell. 1990. Simultaneous analysis of cell Ca^{2+} and Ca^{2+}-stimulated chloride conductance in colonic epithelial cells (HT-29). *Cell Regul.* 1:951–963.

28. Dickinson, K. E. J., R. A. Frizzell, and M. C. Sekar. 1991. Activation of T84 cell chloride channels by carbachol involves a phosphoinositide-coupled muscarinic M_3 receptor. *Eur. J. Pharmacol.* 225:291–298.

29. Craven, P. A., J. Pfanstiel, and F. R. DeRubertis. 1987. Role of activation of protein kinase C in the stimulation of colonic epithelial proliferation and reactive oxygen formation by bile acids. *J. Clin. Invest.* 79:532–541.

30. Capiod, T., L. Combettes, J. Noel, and M. Claret. 1991. Evidence for bile acid-evoked oscillations of Ca^{2+}-dependent K^+ permeability unrelated to a D-myo-inositol 1,4,5-trisphosphate effect in isolated guinea pig liver cells. *J. Biol. Chem.* 266:268–273.

31. Worley, P. F., J. M. Baraban, S. Supattapone, V. S. Wilson, and S. H. Snyder. 1987. Characterization of inositol trisphosphate receptor binding in brain. *J. Biol. Chem.* 262:12132–12136.

32. Amedee, T., W. A. Large, and Q. Wang. 1990. Characterization of chloride currents activated by noradrenaline in rabbit ear artery cells. *J. Physiol.* 428:501–516.

33. Cliff, W. H., and R. A. Frizzell. 1990. Separate Cl^- conductances activated by cAMP and Ca^{2+} in Cl^--secreting epithelial cells. *Proc. Natl. Acad. Sci. USA.* 87:4956–4960.

34. Halm, D. R., G. R. Rechkemmer, R. A. Schoumacher, and R. A. Frizzell. 1988. Apical membrane chloride channels in a colonic cell line activated by secretory agonists. *Am. J. Physiol.* 254:C505–C511.

35. Bohme, M., M. Diener, and W. Rummel. 1991. Calcium- and cyclic-AMP-mediated secretory responses in isolated colonic crypts. *Pfluegers Arch. Eur. J. Physiol.* 419:144–151.

36. Walters, R. J., and F. V. Sepulveda. 1991. A basolateral K^+ conductance modulated by carbachol dominates the membrane potential of small intestinal crypts. *Pfluegers Arch. Eur. J. Physiol.* 419:537–539.

37. Anderson, M. P., and M. J. Welsh. 1991. Calcium and cAMP activate different chloride channels in the apical membrane of normal and cystic fibrosis epithelia. *Proc. Natl. Acad. Sci. USA.* 88:6003–6007.

38. Combettes, L., B. Berthon, E. Doucet, S. Erlinger, and M. Claret. 1989. Characteristics of bile acid-mediated Ca^{2+} release from permeabilized liver cells and liver microsomes. *J. Biol. Chem.* 264:157–167.

39. Combettes, L., M. Dumont, B. Berthon, S. Erlinger, and M. Claret. 1988. Effect of the bile acid taurolithocholate on cell calcium in saponin-treated rat hepatocytes. *FEBS (Fed. Eur. Biochem. Soc. Lett.* 227:161–166.

40. Combettes, L., M. Dumont, B. Berthon, S. Erlinger, and M. Claret. 1988. Release of calcium from the endoplasmic reticulum by bile acids in rat liver cells. *J. Biol. Chem.* 263:2299–2303.

41. Plantavid, M., L. Rossignol, H. Chap, and L. Douste-Blazy. 1986. Studies of endogenous polyphosphoinositide hydrolysis in human platelet membranes. Evidence that polyphosphoinositides remain inaccessible to phosphodiesterase in the native membrane. *Biochim. Biophys. Acta.* 875:147–156.

42. Fasano, A., G. Budillon, S. Guandalini, R. Cuomo, G. Parrilli, A. M. Cangiotti, M. Morroni, and A. Rubino. 1990. Bile acids reversible effects on small intestinal permeability: an *in vitro* study in the rabbit. *Dig. Dis. Sci.* 35:801–808.

43. Coquil, J-F., B. Berthon, N. Chomiki, L. Combettes, P. Jourdon, C. Schteingart, S. Erlinger, and M. Claret. 1991. Effects of taurolithocholate, a Ca^{2+}-mobilizing agent, on cell Ca^{2+} in rat hepatocytes, human platelets and neuroblastoma NG108-15 cell line. *Biochem. J.* 273:153–160.