Bile acid-induced secretion in polarized monolayers of T84 colonic epithelial cells: structure-activity relationships

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Bile acid-induced secretion in polarized monolayers of T84 colonic epithelial cells: structure-activity relationships. Am J Physiol Gastrointest Liver Physiol 292: G290–G297, 2007. First published August 10, 2006; doi:10.1152/ajpgi.00076.2006.—Bile acid epimers and side-chain homologues are present in the human colon. To test whether such bile acids possess secretory activity, cultured T84 colonic epithelial cells were used to quantify the secretory properties of synthetic epimers and homologues of deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA). In our study, chloride secretion was measured as changes in short-circuit current (ΔIsc, in μA/cm²) with the use of voltage-clamped monolayers of T84 cells mounted in Ussing chambers. Bile acids were added at 0.5 mM, a concentration that did not alter transepithelial resistance. Data were expressed as peak ΔIsc (means ± SD). When added bilaterally, DCA stimulated a ΔIsc response of 15.7 ± 12.5 μA/cm². The 12β-OH epimer of DCA was less potent (ΔIsc = 8.0 ± 1.7 μA/cm²), whereas its 3β-OH epimer had no effect. CDCA stimulated secretion (ΔIsc = 8.2 ± 5.5 μA/cm²), whereas both its 7β-OH and 3β-OH epimers were inactive, as was lithocholic acid. HomodCA (1 additional side-chain carbon) was active (ΔIsc = 7.8 ± 4.8 μA/cm²), whereas norDCA (1 fewer carbon) and dinorDCA (2 fewer carbons) were not. Taurine conjugates of DCA and CDCA stimulated secretion (ΔIsc = 12.3 ± 7.5 and 8.8 ± 4.8 μA/cm², respectively) from the basolateral side but not the apical side. Uptake of taurine conjugates from the basolateral but not the apical side was shown by mass spectrometry. These studies indicate marked structural specificity for bile acid-induced chloride secretion and show that modification of bile acid structure by colonic bacteria modulates the secretory properties of these endogenous secretagogues.

bile acid homologues; conjugated bile acids; bile acid physiology

The conjugated and unconjugated forms of the two common natural dihydroxy bile acids, chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA), induce secretion in the perfused mammalian colon, whereas cholic acid (a trihydroxy bile acid) and its conjugates do not (4, 10, 20, 23, 32). Secretion induced by perfused dihydroxy bile acids in humans is concentration dependent and is evoked when the luminal concentration of bile acids approaches the critical micellization concentration, i.e., a concentration greater than 1 mM. Secretory responses induced by bile acids at such concentrations are generally associated with histological evidence of epithelial injury and a consequent increase in paracellular permeability (4, 8, 20, 23).

Multiple mechanisms appear to be involved in mediating secretory responses to bile acids in the intact colon. First, on the basis of studies that used cultured epithelial cells, bile acids can have a direct secretory effect on the colonic enterocyte (6, 7, 12). Second, bile acids can activate mast cells, and consequent histamine release appears to contribute to bile acid-induced secretion (9). Third, bile acids stimulate intrinsic neural arcs that likely contribute to secretory responses through the release of neurohumoral mediators such as vasoactive intestinal peptide (32).

In humans, the major biliary bile acids are conjugates of cholic acid and CDCA (primary bile acids) and DCA (a secondary bile acid). Most of the bile acids released into the intestine are reabsorbed in the ileum; however, with each cycle of the enterohepatic circulation, a small proportion (3–5%) of the bile acid pool enters the large intestine. Here, bile acids undergo extensive modification by luminal bacteria. First, the bile acids are deconjugated to form the corresponding unconjugated bile acids. Once deconjugated, bile acids may then undergo 7-dehydroxylation, with cholic acid being converted to DCA and CDCA to lithocholic acid (11). Accordingly, in humans, the dominant colonic bile acids are DCA and lithocholic acid, although lesser concentrations of primary unconjugated bile acids are also present. In addition to 7-dehydroxylation, colonic bacterial enzymes can also oxidize (dehydrogenate) the hydroxy groups at C-3, C-7, and C-12 (14). The resulting oxo groups may, in turn, be reduced to their corresponding α-hydroxy or β-hydroxy epimers. Bile acids with modified side chains also occur, as nor (C-24-nor) bile acids have been reported to be present in fecal content (31). Thus, colonic bile acids are an extremely complex mixture of mostly unconjugated mono- and disubstituted bile acids.

Previous studies in animal models have compared the secretory effects of natural bile acids as well as those in which hydroxy groups have been replaced by oxo (keto) groups (4, 10). However, there is no information presently available on the secretory properties of bile acids that have been modified by epimerization or by alteration of side-chain length. Thus, in the present paper, we have used polarized monolayers of T84 cells, a well-established reductionist model of human colonic epithelium, to investigate the secretory effects of a spectrum of synthetic epimers of DCA and CDCA. In addition, the ability of side-chain homologues of DCA to stimulate secretion was assessed. Finally, experiments measuring bile acid uptake were performed to elucidate why conjugated bile acids, such as taurodeoxycholate, are prosecretory only when present on the basolateral side of colonic epithelia. The data presented pro-
vide new information on the structural requirements for bile acids to have a prosecretory effect and indicate that bacterial modification of bile acids as well as the length of the side chain may play an important role in regulating the secretory activity of luminal bile acids.

MATERIALS AND METHODS

**Bile acids and other reagents.** Bile acids were obtained from a variety of sources, as indicated, and purified by adsorption chromatography on silica gel columns using methanol-chloroform mixtures. CDCA and ursodeoxycholic acid (UDCA) were gifts of Diamalt (Raubling, Germany). DCA and cholic acid were purchased from Fluka (Buchs, Switzerland; now Sigma-Aldrich, St. Louis, MO). 3β-Hydroxy epimers of CDCA and DCA were prepared by tosylation followed by a dimethylformamide inversion reaction, as described by Iida and Chang (15). The 12β-hydroxy epimer of DCA was also a gift of Diamalt; its properties have been reported previously (29). 24-norDCA was prepared from DCA as described (30). 24-homoDCA was a gift of P. Arya (Canadian Medical Research Council, Ottawa, Canada). The taurine conjugates of DCA and CDCA were prepared as described by Tserng et al. (33). The chemical structures of the bile acids used in the present study are shown (in protonated form) in Fig. 1.

![Fig. 1. Chemical structures (shown in protonated form) of the bile acids investigated in this study.](image-url)
Chemical identity of peaks was confirmed by the fragmentation droplets and to prevent particulate matter from entering the analyzer. Bile acids were added at time 0, and resulting short-circuit response ($I_{sc}$) responses were normalized to $\mu$A/cm$^2$. Data are means ± SD.

sota, FL). It has been shown previously that increases in $I_{sc}$ across T84 cell monolayers are equivalent to electronegative chloride secretion (7). $I_{sc}$ measurements were carried out in Ringer solution containing (in mM) 140 Na$^+$, 5.2 K$^+$, 1.2 Ca$^{2+}$, 0.8 Mg$^{2+}$, 119.8 Cl$^-$, 25 HCO$_3^-$, 2.4 H$_2$PO$_4^-$, and 10 glucose. Results were normalized and expressed as $\Delta I_{sc}$ (in $\mu$A/cm$^2$).

Mass spectrometry: To determine whether bile acids were taken up by the cells and whether bile acid biotransformation occurred, tandem electrospray mass spectrometry was used. T84 cells were grown as monolayers on permeable filter supports as described above. Cells were washed in Ringer solution and after equilibration for 30 min were treated with bile acids on the apical and/or basolateral side. After treatment with bile acids for 20 min, monolayers were washed in PBS and then lysed in methanol, scraped into Eppendorf tubes, and centrifuged at 12,000 rpm for 10 min at 4°C. Supernatant was used for analysis of bile acid content. Electrospray mass spectrometry was performed with a Perkin-Elmer Sciex API-III instrument (Perkin-Elmer, Alberta, Canada) modified with a nanoelectrospray source from Protana (Odense, Denmark). Palladium-coated borosilicate glass capillaries (Protana) were used for sample injection. The instrument was operated in the negative mode with Q1 IS voltage set to 600 V. The IN voltage was set to 100 V, and the ORI voltage set to 100 V. A curtain gas of ultrapure nitrogen was pumped into the interface at 0.6 l/min to aid evaporation of solvent droplets and to prevent particulate matter from entering the analyzer.

Chemical identity of peaks was confirmed by the fragmentation pattern of selected ions (Q3 mode) using argon gas, as well as by comparison with known standards.

Data analysis and statistics. Differences in responses among the three main classes of bile acids (epimers of DCA and CDCA, homologues of DCA, and taurine conjugates of DCA and CDCA) were examined by ANOVA. Differences between individual bile acids were then examined by the Newman-Keuls multiple range test. Differences of $P<0.05$ were considered significant.

RESULTS

Dose-response studies for bile acid-induced secretion in T84 cells. Figure 2 shows the time course of $I_{sc}$ responses to 0.5 mM DCA or 0.5 mM CDCA added bilaterally to monolayers of T84 cells mounted in Ussing chambers. Responses to both bile acids reached a maximum by 5 min and declined to near baseline values by 15 min. At this concentration, there was no effect of the bile acids on transepithelial resistance, implying no loss of monolayer integrity. Secretion was not induced by a concentration of 0.2 mM DCA, and transepithelial resistance rapidly decreased when the DCA concentration reached 1 mM (data not shown). Accordingly, a concentration of 0.5 mM was chosen for subsequent studies in the assessment of structure-activity relationships.

Effect of nuclear substituents on secretory responses. Table 1 summarizes the relationship between bile acid structure and effects on transepithelial secretory responses. DCA was the most efficacious secretagogue, inducing an $I_{sc}$ response of $15.7±12.5$ $\mu$A/cm$^2$ ($n=17$; $P<0.01$ compared with other bile acids). The 12β-hydroxy epimer of DCA (lagodeoxycholic acid) was $\sim50$% as effective as DCA in stimulating secretory responses, whereas its 3β-hydroxy epimer (isoDCA) was inactive.

CDCA, as noted, had about one-half the efficacy of DCA. Both its 3β-hydroxy (isoCDCA) and 7β-epimer (UDCA) were devoid of secretory activity. Cholic acid, a common trihydroxy natural primary bile acid, also did not induce secretion. Similarly, no responses were seen to lithocholic acid, a monohydroxy bile acid that is a major fecal bile acid in humans. UDCA, a bacterial metabolite of CDCA and an agent widely used in the treatment of cholestatic liver disease, was also devoid of secretory activity.

Effect of side-chain length on secretory responses. DCA possesses a side chain of five carbon atoms. Experiments were

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Table 1. Relationship between nuclear substituents and bile acid-induced secretion in T84 cell monolayers

<table>
<thead>
<tr>
<th>Trivial Name</th>
<th>A/B RJ, A Ring Substituent</th>
<th>B Ring Substituent</th>
<th>C Ring Substituent</th>
<th>$\Delta I_{sc}$, $\mu$A/cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxycholic</td>
<td>5β,3α-OH</td>
<td></td>
<td></td>
<td>12α-OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.7±12.5 ($n=17$)</td>
</tr>
<tr>
<td>Isodeoxycholic</td>
<td>5β,3β-OH</td>
<td></td>
<td></td>
<td>12α-OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5±0.8 ($n=4$)</td>
</tr>
<tr>
<td>Lagodeoxycholic</td>
<td>5α,3α-OH</td>
<td></td>
<td></td>
<td>12β-OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.0±1.7 ($n=4$)</td>
</tr>
<tr>
<td>Chenedoxycholic</td>
<td>5β,3α-OH</td>
<td></td>
<td></td>
<td>7α-OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.2±5.5 ($n=7$)</td>
</tr>
<tr>
<td>Isocchenodeoxycholic</td>
<td>5β,3β-OH</td>
<td></td>
<td></td>
<td>7α-OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.3±0.7 ($n=5$)</td>
</tr>
<tr>
<td>Ursodeoxycholic</td>
<td>5β,3α-OH</td>
<td></td>
<td></td>
<td>7β-OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0±0.0 ($n=5$)</td>
</tr>
<tr>
<td>Lithocholic</td>
<td>5β,3α-OH</td>
<td></td>
<td></td>
<td>Monohydroxy bile acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.2±0.7 ($n=8$)</td>
</tr>
<tr>
<td>Cholic</td>
<td>5β,3α-OH</td>
<td></td>
<td></td>
<td>Trihydroxy bile acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7α-OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0±0.0 ($n=3$)</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n$ = no. of experiments. A/B RJ, A/B ring juncture; $\Delta I_{sc}$, change in short-circuit current. Bile acids were added to both apical and basolateral compartments at a concentration of 0.5 mM. The secretory effect of DCA was greater than that of no addition, as well as greater than that of all other bile acids ($P<0.01$). The secretory effect of cholesteryl and chenodeoxycholic acid was also greater than that of no addition ($P<0.01$).
designed to examine the effects of shorting or lengthening the side chain on the secretory actions of this bile acid. These data are shown in Table 2, which compares the secretory effects of dinorDCA (3 carbon atoms in the side chain) with that of norDCA (4 carbon atoms in the side chain), DCA (5 carbons atoms in the side chain), and homodoxycholate (6 carbon atoms in the side chain). Elongation of the side chain by one carbon atom decreased the secretory activity of DCA by ~50%, whereas decreasing the length of the side chain by either one or two carbon atoms completely abolished secretory activity (P < 0.01, compared with DCA) Thus DCA, possessing the natural C-5 side chain of mammalian bile acids, was the most efficacious homologue.

Effect of conjugation of DCA and CDCA with taurine on secretory responses. Secretory responses induced by the taurine conjugates of DCA and CDCA were examined (Table 3) because taurodeoxycholate has been previously reported to induce secretion in T84 cells (7), yet taurine-conjugated bile acids are considered to be membrane impermeable because of their charge and size. Responses to bilateral addition of taurodeoxycholate (ΔIsuc = 14.3 ± 6.3 μA/cm²) were not different from those elicited when taurodeoxycholate was present solely in the basolateral compartment (ΔIsuc = 12.3 ± 7.5 μA/cm²). When added to the apical compartment alone (at 0.5 mM), taurodeoxycholate was without effect. Similar observations were made for taurochenodeoxycholate. The secretory activity of taurodeoxycholate was about twice as great as that of taurochenodeoxycholate. Of interest, we found that secretory responses to the unconjugated bile acid DCA were also greater when the bile acid was added to the basolateral side.

Bile acid uptake: effect of site of addition. Lack of a secretory effect of a bile acid could be caused by either the absence of uptake or absence of a pharmacodynamic effect (or both). Electrospray mass spectrometry was used to measure uptake from basolateral vs. apical compartments for DCA, taurodeoxycholate, and UDCA. Mass spectra are shown in Fig. 3 for a basolateral concentration of 0.5 mM and apical concentrations of 0.5, 1.0, and 3.0 mM taurodeoxycholate. No uptake of apical taurodeoxycholate occurred until its concentration reached 3.0 mM. At this concentration, there was a marked drop in transepithelial resistance, indicating damage to tight junctions that, in turn, is likely to permit taurodeoxycholate to reach the basolateral membrane.

Figure 4 compares the uptake of DCA, taurodeoxycholate, and UDCA from the basolateral and apical compartments. For these experiments, taurodeoxycholate and UDCA were present at 0.5 mM, whereas DCA was present at 0.2 mM. The uptake rate of DCA was multiplied by 2.5 to permit an approximate comparison with taurodeoxycholate and UDCA. Uptake from the basolateral compartment for all bile acids was found to be greater than from the apical compartment. Relative rates of uptake (basolateral/apical) were ~2 for DCA, 6 for UDCA, and 16 for taurodeoxycholate. These values likely underestimate the differences between basolateral and apical uptake, given the greater diffusion barriers that persist to basolateral uptake. Uptake of taurodeoxycholate from the apical compartment was extremely low and was not significantly different from zero, consistent with the known membrane impermeability of taurine-conjugated bile acids. No bile acid biotransformation occurred based on mass spectrometry. In separate experiments, we found that lithocholic acid (0.2 mM) was also readily taken up into T84 cells (amount of uptake = 0.764 arbitrary units; n = 2), indicating that, similar to UDCA, its lack of secretory activity is not due to lack of uptake.

Effect of atropine on DCA-induced secretory responses. Studies in other systems have indicated that bile acids may induce biological effects through interacting with muscarinic cholinergic receptors. To test whether DCA-induced secretion in T84 cells is mediated by such an interaction, responses to DCA were studied in the presence and absence of the muscarinic antagonist atropine (100 nM). We found that atropine had no significant effect on DCA-induced secretion. Responses to DCA (0.5 mM) in the presence of atropine were 93.5% of those in cells treated with DCA alone (n = 5; not significant). In contrast, atropine virtually abolished responses to the cholinergic agonist carbachol. Responses to carbachol (100 μM) in atropine-treated cells were only 3.6 ± 3.1% of those in cells treated with carbachol alone (n = 3).

Table 2. Relationship between side-chain length and bile acid-induced secretion in T84 cell monolayers

<table>
<thead>
<tr>
<th>Trivial Name</th>
<th>Length of Side Chain</th>
<th>ΔIsuc, μA/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinordeoxycholate</td>
<td>3 carbons</td>
<td>0.0±0.0 (n=4)</td>
</tr>
<tr>
<td>Nordeoxycholate</td>
<td>4 carbons</td>
<td>0.5±0.8 (n=4)</td>
</tr>
<tr>
<td>Deoxycholate</td>
<td>5 carbons</td>
<td>15.7±12.5 (n=17)</td>
</tr>
<tr>
<td>Homodeoxycholate</td>
<td>6 carbons</td>
<td>7.8±4.8 (n=6)</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of experiments. Bile acids were added to both apical and basolateral compartments to a concentration of 0.5 mM. The secretory effect of deoxycholate (DCA) was greater than that of dinordeoxycholic acid (ΔIsuc = 14.3 ± 6.3 μA/cm²) and also greater than that of homodeoxycholate acid (P = 0.04).

Table 3. Sidedness of effects of deoxycholate, taurodeoxycholate, or taurochenodeoxycholate on bile acid-induced secretion in T84 cell monolayers

<table>
<thead>
<tr>
<th>Bile Acids</th>
<th>Site of Addition</th>
<th>ΔIsuc, μA/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurodeoxycholate</td>
<td>Apical and basolateral</td>
<td>14.3±6.3 (n=5)</td>
</tr>
<tr>
<td>Taurochenodeoxycholate</td>
<td>Apical</td>
<td>1.7±0.7 (n=5)</td>
</tr>
<tr>
<td>Taurocholic acid</td>
<td>Basolateral</td>
<td>12.3±7.5 (n=14)</td>
</tr>
<tr>
<td>Taurochenodeoxycholate</td>
<td>Apical</td>
<td>2.0±1.7 (n=6)</td>
</tr>
<tr>
<td>Taurodeoxycholate</td>
<td>Basolateral</td>
<td>8.8±4.8 (n=6)</td>
</tr>
<tr>
<td>Deoxycholate</td>
<td>Apical and basolateral</td>
<td>24.5±8.3 (n=3)</td>
</tr>
<tr>
<td>Deoxycholate</td>
<td>Apical</td>
<td>5.5±3.3 (n=3)</td>
</tr>
<tr>
<td>Deoxycholate</td>
<td>Basolateral</td>
<td>32.8±7.0 (n=3)</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of experiments. Bile acids were present at a concentration of 0.5 mM. Responses to taurodeoxycholate, taurocheno- doxycholate, and deoxycholate were all greater when the bile acids were present on the basolateral than on the apical side (P<0.05 in each case).

Discussion

Previous studies in humans and animal models have provided important information regarding the structural properties required for bile acids to elicit secretory responses in the colon. These studies have suggested that two α-hydroxy groups are essential for secretory activity because both DCA and CDCA (4, 9, 10, 12, 20, 22, 23, 32) and their taurine conjugates (6–8, 25, 34) induced secretory responses, whereas the trihydroxy bile acid cholic acid (4, 10, 23) and its taurine conjugate (6, 7, 23, 34) did not. Furthermore, UDCA, the 7β-hydroxy epimer...
of CDCA, was also demonstrated to lack prosecretory activity (4, 9). The present study, carried out on isolated colonic epithelial cells grown as polarized monolayers, sheds further light on the structure-activity relationships for bile acids in promoting colonic epithelial secretion. The data presented here confirm the prosecretory activity of DCA and CDCA and their conjugates but indicate that colonic epithelial secretion can also be induced by lagodeoxycholic acid, the 12β-epimer of DCA. Furthermore, similar to UDCA, the 3β-epimer of CDCA (isoCDCA) is also devoid of secretory activity. Our experiments also show that lithocholic acid, a major natural fecal bile acid in humans, is devoid of secretory activity. Previous colonic perfusion studies have shown that replacing any hydroxy group of DCA or CDCA by an oxo group abolishes their secretory activity (4). Thus, if one considers all of the possible epimers and oxo derivatives of CDCA and DCA, there are 18 possible disubstituted bile acids and, of these, 8 have now been tested. On the basis of the data presented here and elsewhere (4, 10), it seems highly likely that only DCA, lagodeoxycholic acid, and CDCA possess prosecretory activity.

Our studies using side-chain homologues of DCA demonstrate that the natural isopentanoic acid side chain that is present in bile acids of virtually all mammals is associated with the greatest secretory activity. Lengthening the side chain by one carbon atom diminishes secretory activity, whereas shortening the side chain by a single carbon abolishes secretory activity. Together, our data thus demonstrate a strict structural specificity for bile acids in their ability to induce secretory responses across colonic epithelial cells.

In the present study, we used a relatively high bile acid concentration of 0.5 mM to induce epithelial secretory responses. At this concentration, none of the bile acids tested...
altered transepithelial resistance over the duration of the experimental period, whereas lower concentrations (≤0.2 mM) were without secretory activity. Thus our present findings mirror findings from previous studies carried out in perfused human intestine, which demonstrated that prosecretory effects of bile acids occur only at relatively high (mM) concentrations (23). However, it is interesting to note that, in colonic epithelial preparations from some animal models, bile acid-induced secretion has been found to occur at lower concentrations (e.g., 10–100 μM of DCA or its conjugates) (22, 26, 34). The precise reasons for, and the physiological relevance of, such species differences in the secretory activities of bile acids remains unclear. However, it is also important to note that the full extent of epithelial secretory responses to bile acids in vivo is likely to reflect not only direct effects on the enterocyte but also the influences of numerous other factors, including mucosal blood flow, the presence of an unstirred mucous layer, and the recruitment of paracrine and/or neurocrine pathways.

Bile acids have long been believed to be rapidly deconjugated after entering the cecum and therefore are believed to exist primarily in their unconjugated form in the colon (11). In unpublished studies, the use of mass spectrometry to analyze human cecal content has allowed us to confirm this belief. However, in the work reported here, in addition to performing structure-activity relationships with unconjugated bile acids, we also examined secretory responses to the taurine conjugates of DCA and CDCA because these are the dominant form of bile acids in the small intestine and conjugated bile acids have often been used in previous studies to examine colonic secretion. The lack of a secretory effect of the taurine conjugates of DCA and CDCA when applied apically to T84 cells confirms the well-accepted concept that conjugated bile acids are impermeable to cell membranes and are too large to pass via intact epithelial tight junctions. Our data further indicate that taurine-conjugated bile acids present in the apical compartment will not induce secretion unless the integrity of the paracellular junctions is compromised, permitting them to reach the basolateral domain. Dharmathaporn et al. (7) reached similar conclusions, finding that taurodeoxycholate was more potent when added basolaterally to colonic epithelial cells and that apical addition of the bile acid was only secretory at concentrations >1 mM, which were also associated with a marked fall in transepithelial resistance. In the present study, we did not examine secretory responses to glycine-conjugated bile acids because these are not present in appreciable concentrations in colonic content. This is likely because glycine-conjugated bile acids are cleaved more rapidly than taurine-conjugated bile acids by bacterial deconjugases (13). However, we predict that the secretory properties of glycine-conjugated bile acids would be similar, if not identical, to those of their corresponding taurine conjugates.

The mechanisms by which bile acids trigger epithelial secretion are not yet fully known. However, the potent secretory activity and more rapid uptake of bile acids, particularly conjugated bile acids, from the basolateral compartment imply that a bile acid transporter is likely to be important in initiating this response. A possible candidate for such a transporter is the heterodimer organic solute transporter OSTα/OSTβ, which has been shown to mediate the basolateral transport of conjugated bile acids out of the ileal enterocyte (5) and hepatocyte (3). However, studies in rabbit suggest that expression of OSTα/OSTβ is lower in the colon than in the ileum and that the multidrug resistance protein Mrp3 could also be a candidate basolateral transporter for bile acids (35). However, Mrp3 appears to mediate bile acid efflux, rather than influx, and appears only to have a minor role in this regard in rat intestine (28). Whether these or other conjugated bile acid transporters are present in T84 cells remains to be determined. Bile acids have also been found to have the ability to elicit cellular responses through interactions with G-protein-coupled receptors (16, 21), particularly the muscarinic M3 receptor, in some experimental systems (27). However, our data indicate that it is unlikely that the prosecretory effects of bile acids in colonic epithelia are mediated through such interactions. First, we found that the muscarinic antagonist atropine, which abolished secretory responses to the cholinergic agonist carbachol, had no effect on responses to DCA. Furthermore, the bile acid-activated G-protein-coupled receptors that have been described by Maruyama et al. (21) and Kawamata et al. (16) are activated most potently by lithocholic acid, a bile acid that had no secretory activity in our cells. These putative bile acid-activated G-protein-coupled receptors have also been shown to be positively linked to G proteins and elevations in intracellular cAMP, whereas the prosecretory effects of bile acids in epithelial cells are thought to be mediated by intracellular calcium (7).

The more rapid uptake of unconjugated bile acids, such as DCA and UDCA, from the basolateral compartment does not appear to have been noted previously, although Huang et al. (12) noted that secretory responses occurred at lower concentrations when DCA was added to the basolateral compartment compared with those when the bile acid was added to the apical compartment. Dihydroxy bile acids are considered to be absorbed rapidly by passive diffusion of the protonated molecule across the lipid domains of membranes (1, 18). However, carrier-mediated uptake of unconjugated bile acids in isolated hepatocytes (2) and LLC-PK1 cells transfected with ntcp (a sodium-dependent bile acid transporter) (24) has been reported. It is possible that similar transporters may have contributed to unconjugated bile acid uptake in our studies. We also found that, similar to UDCA, lithocholic acid is rapidly taken up into T84 cells, indicating that, for both bile acids, their lack of secretory activity is not attributable to a lack of uptake. Our experiments were not designed to elucidate the mechanisms by which bile acids activate the transport machinery of colonic epithelial cells to induce secretion. However, it is likely that these responses are mediated via the CFTR chloride channel that is thought to be the predominant exit pathway for chloride in colonic epithelia. On the basis of previous studies, the signaling cascade activated by bile acids is likely to involve phosphatidylinositol 4,5-bisphosphate hydrolysis to liberate inositol 1,4,5-trisphosphate, release of calcium from intracellular stores, and activation of basolateral potassium channels (19, 25). Efflux of potassium would then hyperpolarize the cell and provide the electrical driving force for chloride secretion across the apical membrane.

Further studies are required before we can understand the full implications of bacterial modification of bile acids in regulating colonic fluid transport. For example, the 7-dehydroxylation of cholic acid converts a nonsecretory trihydroxy...
bile acid (cholic) to DCA, a bile acid with potent secretory activity. In contrast, 7-dehydroxylation of CDCA, a secretory bile acid, converts it to lithocholic acid, a bile acid that is devoid of secretory activity. Thus bacterial 7-dehydroxylation per se might not be expected to significantly alter the secretory activity of luminal bile acids. On the other hand, bacterial epimerization of DCA at C-3 or CDCA at C-3 or C-7 decreases the secretory activity of luminal bile acids and might therefore contribute to constipation if bile acid-induced secretion is an important modulator of colonic hydration. Evidence supporting the view that bile acids are indeed important modulators of colonic water movement comes from observations that administration of bile acid sequestrants, such as cholestyramine, is frequently associated with constipation (17).

In conclusion, our studies demonstrate that bile acid-induced secretion across colonic epithelial cells displays remarkable structural specificity, suggesting the presence of an intracellular “receptor” for secretory bile acids. Our data also suggest that bacterial modification of colonic bile acids is likely to play a significant role in modulating their secretory properties. A greater understanding of the role that bacteria play in the metabolism of colonic bile acids will lead to a greater understanding of the factors that regulate colonic epithelial transport in health and disease.

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