

Mast Cells and Histamine Contribute to Bile Acid-stimulated Secretion in the Mouse Colon

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Abstract

Certain dihydroxy bile acids cause secretory diarrhea when present in the colonic lumen at inappropriately high concentrations. However, the mechanism underlying the secretagogue activity has not been fully elucidated. Experiments were performed to test whether mast cells and one of their major mediators, histamine, might contribute to the secretory effect. Chenodeoxycholic acid, a secretory bile acid, and ursodeoxycholic acid, a nonsecretory, hydrophilic bile acid, were compared for their ability to induce chloride secretion across segments of mouse colon mounted in Ussing chambers. Chenodeoxycholic acid, but not ursodeoxycholic acid, induced dose-dependent, biphasic chloride secretion that was greater after serosal than mucosal addition and was greater in distal versus proximal colonic segments. The secretory effect of chenodeoxycholic acid was inhibited by H₁ histamine receptor antagonists and modified by the cyclooxygenase inhibitor indomethacin. However, it was unaffected by an H₂ histamine receptor antagonist or by atropine. Secretory effects of chenodeoxycholic acid were diminished in magnitude and delayed in colonic tissues from mice with a genetic deficiency of tissue mast cells. Concentrations of chenodeoxycholic acid inducing secretion also released histamine from tissue segments. These data indicate that mast cells and histamine-mediated processes contribute significantly to the secretory effects of dihydroxy bile acids in the murine colon. (*J. Clin. Invest.* 1995. 95:2831–2839.) **Key words:** mast cells • bile acids • secretory diarrhea • inflammation • chloride secretion

Introduction

Bile acids are water-soluble end products of cholesterol metabolism that participate in fat digestion in the gastrointestinal tract.

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A pool of bile acids is stored in the gallbladder and secreted with meals. In the small intestinal lumen, bile acids are present at millimolar concentrations during digestion. In health, the vast majority of the bile acid pool is efficiently absorbed via both active and passive mechanisms by the small intestine. Only a small fraction of the bile acid pool, therefore, escapes the enterohepatic circulation to enter the colon (1). The aqueous concentration of bile acids in the cecum is therefore likely to be in the micromolar range (2). However, in patients who have lost normal ileal absorptive function, bile acids are malabsorbed. A compensatory increase in bile acid biosynthesis in the liver results in a new steady state in which increased fecal loss is balanced by increased hepatic biosynthesis. Bile acids pass into the colon in greatly increased amounts, and in patients with diarrhea, markedly elevated concentrations of bile acids (in the millimolar range) in the aqueous phase of feces have been reported to be present (2–4).

The increased concentration of bile acids in colonic contents has been considered to play a contributory role in the pathogenesis of diarrhea, because the common dihydroxy bile acids induce a concentration-dependent secretion when infused into the colon (5–8) and because administration of cholestyramine, a polymeric bile acid sequestrant, lowers the concentration of bile acids in the aqueous phase of feces and ameliorates the diarrhea (9). Bile acid-induced diarrhea has also been induced by administration of chenodeoxycholic acid to patients with gallstones; the incidence and magnitude of diarrhea are dose dependent (10). Bile acid-induced diarrhea is thought to have several causes. Among these, active chloride secretion can be induced by bile acids in the colon (6–8). An additional contributory mechanism may be due to the loss of ileal function. This can result in decreased absorption, providing an increased load of fluid and electrolytes to the colon (11).

Only two natural bile acids have been found to induce colonic secretion in perfusion studies (7, 12). These are chenodeoxycholic acid (CDCA),¹ one of the two major primary bile acids in man, and deoxycholic acid (DCA), a secondary bile acid formed in the colon by bacterial 7-dehydroxylation of cholic acid and the predominant fecal bile acid in man (1). Both of these bile acids have two α -hydroxyl groups on the steroid nucleus, are surface active, and are cytotoxic (13). Both unconjugated and glycine- or taurine-conjugated bile acids induce secretion, but for a given bile acid, secretion is induced by the unconjugated bile acid at a lower concentration than its corresponding conjugated derivative (6, 14). Ursodeoxycholic acid (UDCA), the 7 β -epimer of CDCA (a bile acid that is used therapeutically for gallstone dissolution and cholestatic liver

1. *Abbreviations used in this paper:* CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; DPH, diphenhydramine; I_{sc} , short circuit current; PD, potential difference; TTX, tetrodotoxin; UDCA, ursodeoxycholic acid.

disease), does not induce colonic secretion (8, 15). UDCA differs from CDCA and DCA in being less surface active and noncytotoxic.

Multiple mechanisms have been postulated for the stimulatory effects of bile acids on intestinal electrolyte and water transport. These include direct mucosal injury (7, 12); enhancement of mucosal permeability (7, 16, 17); and stimulation of cAMP production (16, 18, 19), mucosal prostaglandin synthesis (20, 21), calcium influx and calcium release from intracellular stores in epithelial cells (22–24), and local neural enteric reflexes (25). A secretory effect of conjugated bile acids when applied mucosally is observed only in association with an increase in permeability (26, 27), suggesting that enhancement of mucosal permeability may be necessary to permit access for these large, charged molecules to the basolateral membrane and/or to the subepithelium, where they then can exert their secretory effect. Indeed, most studies have shown evidence for direct mucosal injury (7, 12) or enhancement of mucosal permeability (7, 16, 17) during experiments in which secretion was induced by elevated concentrations of bile acids in colonic perfusates. The final common path for the secretory effect of bile acids is considered to be increased chloride secretion by the enterocyte. However, a component of bile acid-induced secretion has also been proposed to be initiated by stimulation of local neural enteric reflexes (25) that in turn induces secretion by the enterocyte. Thus the precise mechanism by which dihydroxy bile acids exert their effects remains unresolved.

Elucidation of the mechanisms by which bile acids or other agents induce chloride secretion in colonic perfusion studies is complicated by the intrinsic complexity of this tissue, which is made up of many different but interacting cell types. The enterocytes themselves differ in function, as secretion is thought to be mediated predominantly by crypt enterocytes and absorption by villus (or surface) enterocytes (28). Electrolyte and water transport by epithelial cells is further modulated by mediators secreted from underlying nonepithelial cells, as well as by enterochromaffin cells and by intramural and extrinsic neural pathways (28).

Among the nonepithelial cells present in the small and large intestinal mucosa are mast cells, which are now widely recognized as effectors of the intestinal secretion induced by antigenic challenge of sensitized tissues (29). Their role in secretory responses to nonimmune agonists has been less well studied. However, previous studies from our laboratory have shown that bile acids are capable of activating mast cells both *in vitro* and *in vivo* (13). We have therefore performed experiments using mouse colonic segments examined *in vitro* to test whether mast cells are involved in bile acid-induced secretion.

Methods

Experimental design. Colon segments from normal or mast cell-deficient mice were mounted in Ussing chambers, and electrogenic chloride secretion was assessed by quantifying changes in short circuit current (I_{sc}). After the addition of tetrodotoxin (TTX) to abolish local neural reflexes, the response to bile acids applied to the serosal or mucosal compartment was measured without and with the prior addition of putative antagonists. Histamine release from tissues induced by bile acids, as well as unreleased tissue histamine, was measured by immunoassay.

Chemicals. CDCA (3 α ,7 α -dihydroxy-5 β -cholan-24-oic acid) and UDCA (3 α ,7 β -dihydroxy-5 β -cholan-24-oic acid) of 99% purity (by HPLC [30]) were supplied by the Pharmazell Division of Diamalt (Raubling, FRG; courtesy of Dr. Thilo Messerschmidt). TTX, diphen-

hydramine (DPH), pyrilamine, cimetidine, atropine, and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of at least analytical grade and were obtained commercially.

Animals. C57BL/6J mice were obtained from Simonsen Laboratories (Gilroy, CA). Mast cell-deficient mice (WB/ReJ-W/+ \times C57BL/6J-W^{+/+})F1-(W/W^y) and their congenic normal littermates (+/+) were obtained from The Jackson Laboratories (Bar Harbor, ME). All mice were housed in separate quarters under filter hoods and were allowed free access to food and water. Experiments were conducted when the mice were 12–18 wk of age.

Transport studies in isolated mouse colon. Mice were killed by cervical dislocation. The colon was removed and opened along the mesenteric border, and full-thickness segments were mounted in Ussing chambers. The chambers used in these studies had an aperture of 0.28 cm². Both sides of the tissue segments were bathed with 10 ml of Ringer's solution (140 mM Na⁺, 5.4 mM K⁺, 1.2 mM Ca²⁺, 1.2 mM Mg²⁺, 119.8 mM Cl⁻, 25 mM HCO₃⁻, 0.4 mM H₂PO₄⁻, 2.4 mM HPO₄²⁻, 10 mM glucose). The Ringer's solution was maintained at 37°C, pH 7.4 and was gassed with 95% O₂/5% CO₂. In some studies, chloride-free solutions were made by replacing chloride with isethionate. The transmural potential difference (PD) was monitored using calomel electrodes connected to the bathing solutions via 3 M KCl-filled agar bridges. Current was passed across the tissues via Ag/AgCl electrodes connected to the chamber with short, Ringer's-filled agar bridges. The current necessary to nullify the PD was supplied by an automatic voltage clamp (Physiologic Instruments, San Diego, CA) and recorded continuously as I_{sc} via an interface to a personal computer fitted with custom software (Acquire and Analyze; Physiologic Instruments). The tissue conductance was calculated from the change in I_{sc} observed upon imposing a bipolar 5-mV step in PD, using Ohm's law.

For experiments with antagonists, only the distal half of the colon was used. The studies were performed in a paired fashion so that antagonist-treated tissues could be compared with control tissues from the same animal. Tissues were allowed to equilibrate under short circuited conditions for at least 15 min, at which time TTX (3×10^{-7} M) was added to the serosal side of all tissues to eliminate neuronal activity (see Results). Antagonists were added to either the serosal, the mucosal, or both bathing solutions after TTX treatment and at least 15 min before addition of CDCA from the serosal or mucosal side.

Histamine assay. Histamine release from buffered 0.9% saline- or bile acid-treated colonic tissue was measured by a commercially available ELISA assay (AMAC, Westbrook, ME). Briefly, tissues were incubated with the indicated solutions for 30 min at 37°C, at which time supernatants were harvested and frozen at -20°C until analysis. Residual histamine was released into a fresh aliquot of saline solution by boiling for 10 min, and the saline containing released histamine was again separated from the tissue segment and frozen until required.

Statistical analyses. Data are presented as mean \pm SEM for a given number of experiments. In all figures in which the time course of responses is depicted, the x axis represents the time elapsing after short circuiting the tissue in the Ussing chamber. Statistical significance was determined by Student's t test for paired and unpaired samples, as appropriate. $P < 0.05$ was considered to represent a significant effect.

Results

Secretory response induced by CDCA: features of the response in the colon as a whole. In pilot experiments, conducted without TTX, tissues from C57BL/6J mice exhibited very unstable and oscillatory baseline I_{sc} values. Because of this, consistent responses to bile acid addition could not be obtained. Pretreatment of tissues with TTX eliminated the spontaneous oscillatory I_{sc} , attributable to neuronal activity presumably induced by mounting the colon segments, and abruptly reduced I_{sc} values to a uniform and stable baseline in all tissues (Fig. 1).

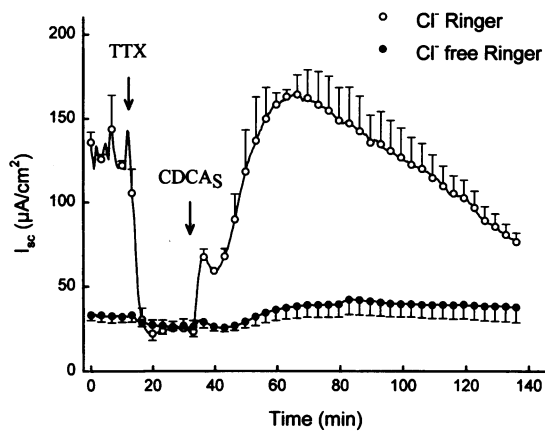


Figure 1. Effect of CDCA (1 mM) on the time course of changes in I_{sc} across segments of mouse colonic tissue mounted in Ussing chambers. All tissues were studied in the presence of TTX ($0.3 \mu\text{M}$) added serosally at the time indicated; CDCA was also added to the serosal side as indicated by the arrow. Tissues were studied in regular, chloride-containing Ringer's solution, or in chloride-free Ringer's solution. Values are means \pm SEM for four experiments.

Previous experiments with epithelial cell monolayers have shown that bile acids induce secretion mainly by acting on the basolateral side of the cell. We therefore first examined the response to CDCA added to the serosal side of the colonic tissue. As shown in Fig. 1, 1 mM CDCA induced a biphasic secretory response. The secretion started within a few minutes after addition of CDCA. The first I_{sc} peak was observed within 10 min, followed by a prolonged second secretory phase, maximal at ~ 40 – 50 min, that was sustained for at least 2 h (Fig. 1). Addition of 1 mM CDCA to the serosal compartment had no effect on transepithelial conductance other than that consistent with the extent of active secretion, indicating that serosal CDCA at 1 mM does not alter the integrity of the tight junctions (Fig. 2 B). To examine the dependence of I_{sc} changes induced by CDCA on active chloride transport, the chloride in the Ring-

er's bathing solution was substituted by isethionate. Under these conditions, the I_{sc} response to serosal CDCA was completely abolished (Fig. 1).

Addition of CDCA (1 mM) to the mucosal side of colonic tissues also resulted in a biphasic increase in I_{sc} . However, this change was accompanied by the gradual appearance of a marked increase in tissue conductance. The findings suggest that mucosal CDCA initiated a secretory response in colonic tissue, but that this response was eventually abolished by damage to the epithelium. Thus, to test viability of tissues after treatment with CDCA, 1 mM theophylline was added to both sides of the tissue at the end of the experiment (~ 2 h). Exposure of colonic tissue to 1 mM serosal CDCA did not affect its subsequent ability to display a secretory response to theophylline (1 mM). However, tissues treated with mucosal CDCA did not respond to theophylline, consistent with the view that their elevated conductance indicated cell injury (data not shown).

Comparison of distal and proximal tissues. Proximal and distal colon segments differed in their response to CDCA. When CDCA (1 mM) was added to the serosal bathing solution, the maximal change in I_{sc} (observed during the second peak of the response) was markedly greater in the distal two thirds of the colon than in the proximal one third of the colon (Fig. 2 A; Fig. 3, left panel) ($114.7 \pm 16.8 \mu\text{A}/\text{cm}^2$ versus $42.1 \pm 15.9 \mu\text{A}/\text{cm}^2$, respectively, $P < 0.05$, $n = 4$).

Addition of 1 mM CDCA to the mucosal bathing solution also induced a biphasic secretory response in distal colon segments. The first peak was similar to that observed with serosal addition, but the second peak was abbreviated and diminished, probably owing to delayed cytotoxic effects as indicated by the rise in conductance during this second phase of the response (Fig. 2, C and D). For mucosal addition, there was no significant difference in the magnitude of the response between proximal and distal tissues. However, the first peak was significantly later, and the response was mainly monophasic in the proximal colon compared with the biphasic response in the distal colon (Fig. 3, right panel). In aggregate, because of the differences between responses of proximal and distal colon, all subsequent comparisons of ion transport were made between tissues from distal colon segments.

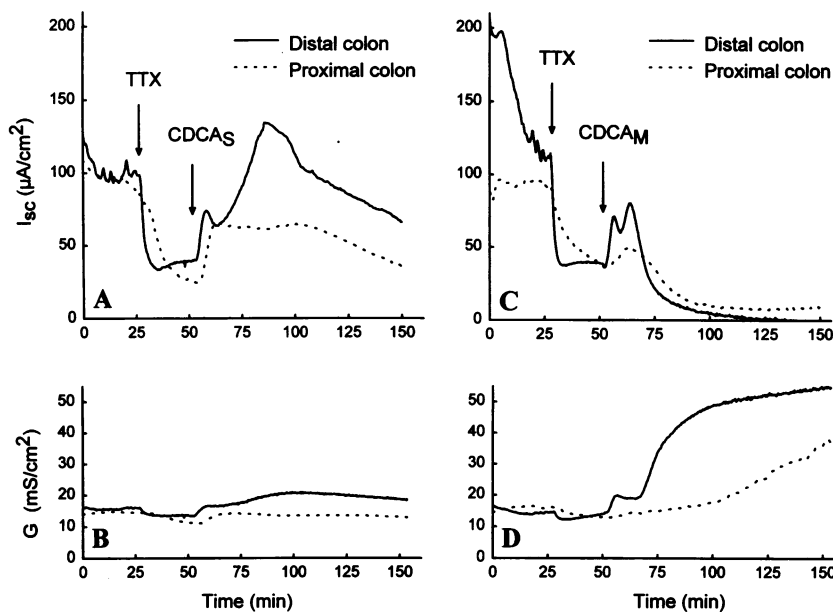


Figure 2. Comparison of the effects of CDCA (1 mM) on the time courses of ion transport (A and C) and tissue conductance (abbreviated as G; shown in B and D) of mouse colon after addition to either the serosal (A and B) or mucosal (C and D) aspect of the tissue. Results obtained with distal and proximal colonic tissue segments are shown. CDCA was added at the time indicated. Each trace depicts the data from a single experiment, representative of at least four similar experiments.

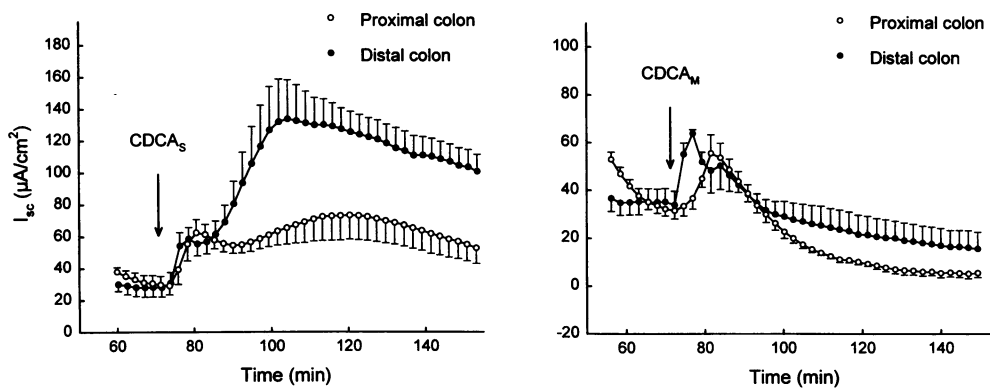


Figure 3. Summary of the effects of CDCA (1 mM) on the time course of I_{sc} responses across mouse proximal and distal colon after either serosal (left panel) or mucosal (right panel) addition. Values are means \pm SEM for four experiments in each case.

Dose dependency of CDCA-induced secretion and cell injury. Addition of 2 mM CDCA to the serosal side of distal colon caused a prompt rise in I_{sc} , with a peak increase of $\sim 200 \mu\text{A}/\text{cm}^2$ (Fig. 4 A). However, with this concentration of serosal CDCA, the second phase of the response was associated with a marked increase in conductance, suggesting an effect on tight junction integrity (Fig. 4 B). 2 mM CDCA added to the mucosal bathing solution caused an even faster increase in conductance that began even before the resolution of the first peak of secretion (Fig. 4 B).

Addition of 0.5 mM CDCA to the serosal side of distal colon induced a biphasic secretory response, with a first peak of similar magnitude to that evoked by 1 mM CDCA, but with a smaller and later second phase. The changes in I_{sc} induced by mucosal addition of 0.5 mM CDCA were very similar to those observed with 1 mM CDCA, but without an associated significant rise in conductance (Fig. 4, C and D).

Effect of UDCA on transport responses. Serosal addition of UDCA at concentrations of ≤ 2 mM had no effect on I_{sc} or conductance of distal colon. The prior addition of UDCA to the serosal compartment also did not influence the secretory response to subsequently added CDCA (data not shown). Mucosal addition of 1 or 2 mM UDCA did induce a small monophasic

effect on I_{sc} across distal colon (maximal increase of $11.7 \pm 2.9 \mu\text{A}/\text{cm}^2$ and $34.0 \pm 10.6 \mu\text{A}/\text{cm}^2$, respectively) (Fig. 5), but there was no effect of mucosal UDCA on the conductance of the tissue (Fig. 5) or its responsiveness to subsequently added theophylline (data not shown). However, when CDCA was added to the mucosal side after the secretory response to 1 mM mucosal UDCA, the conductance rose rapidly without an increase in I_{sc} , suggesting that toxic effects of CDCA were potentiated in the face of prior exposure to UDCA (Fig. 5).

Effect of pharmacological antagonists. To examine the mechanisms responsible for bile acid-induced secretion, we tested the effect of antagonists of candidate mediators. In previous studies, we showed that bile acids are able to activate mast cells (13). One likely mast cell-derived secretagogue is histamine, which is known to activate epithelial chloride secretion primarily via its actions on epithelial H_1 histamine receptors (31). Colonic tissue was pretreated with the H_1 histamine receptor antagonists pyrilamine or DPH before stimulation with CDCA. Both antagonists markedly inhibited the secretory response induced by serosal addition of 1 mM CDCA. In tissues pretreated with pyrilamine (200 μM), CDCA increased I_{sc} by $40.6 \pm 7.2 \mu\text{A}/\text{cm}^2$ compared with $133.0 \pm 12.9 \mu\text{A}/\text{cm}^2$ in control tissues (Fig. 6 and see Fig. 8). This inhibitory effect of

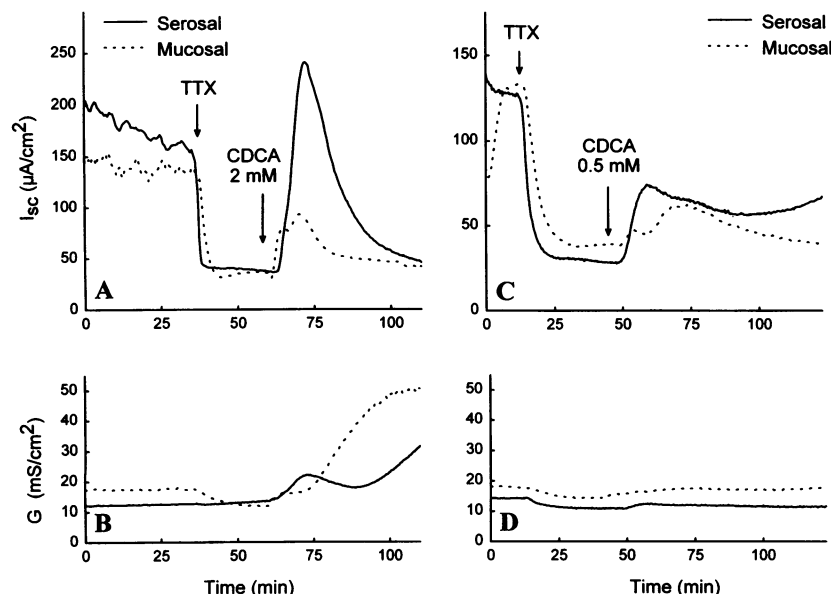


Figure 4. Effect of either 2 mM (A and B) or 0.5 mM (C and D) CDCA on the time courses of ion transport responses (A and C) and tissue conductance (B and D) across mouse colonic tissue mounted in Ussing chambers. CDCA was added to either the serosal or mucosal side of distal colonic tissues. Each trace depicts data from a single experiment, representative of at least four such experiments.

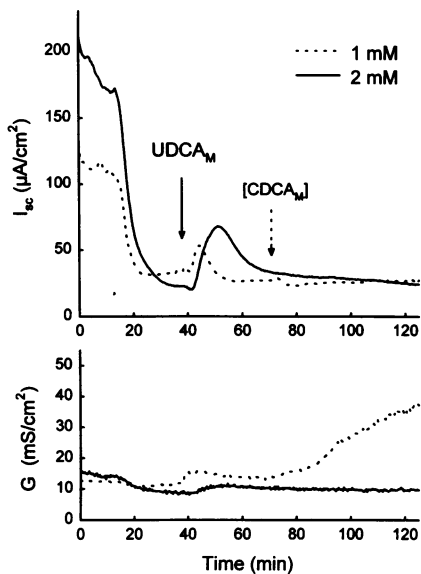


Figure 5. Effect of mucosal addition of UDCA at either 1 or 2 mM on the time course of ion transport responses (*upper panel*) or conductance (*lower panel*) of mouse distal colon. For the tissue treated with 1 mM UDCA only, CDCA (1 mM) was added subsequently at the time indicated by the arrow and appeared to result in an increase in conductance. Each trace depicts data from a single experiment, representative of at least three similar experiments.

pyrilamine was reproduced by pretreatment of tissues with DPH (200 μ M) (see Fig. 8).

DPH or pyrilamine also inhibited secretion induced by mucosal addition of 1 mM CDCA. The response to mucosal bile acid was either absent or reduced to a small monophasic response in the presence of the antagonists compared with the biphasic response observed in paired control tissues (data not shown). In some settings, histamine H_1 antagonists may display anticholinergic effects, particularly at high doses (32, 33). To determine whether the ability of DPH and pyrilamine to inhibit CDCA-induced chloride secretion is likely due to their antihistaminic or anticholinergic actions, we tested the effect of atropine. However, atropine was without effect on CDCA-induced chloride secretion (see Fig. 8), indicating that cholinergic mechanisms were likely not involved. In addition, the histamine H_1 antagonists, at the concentration used in our studies, had no effect on the secretory response to theophylline (see Fig. 9). This finding makes it unlikely that histamine H_1 antagonists exert nonspecific inhibitory effects on the chloride secretory response. In contrast to the inhibition induced by the H_1 histamine receptor antagonists, treatment of colonic tissue with cimetidine, an H_2 receptor antagonist, before stimulation with CDCA was without effect on changes in I_{sc} (see Fig. 8).

Prostaglandins are also known to be potent agonists of intestinal chloride secretion (28). Indomethacin, an inhibitor of prostaglandin synthesis, altered the pattern of the I_{sc} response to either serosal or mucosal CDCA in that the first (smaller) peak of the secretory response was abolished by this agent. However, the maximal increase in I_{sc} induced by serosal CDCA and the overall duration of the secretory response were unaffected by indomethacin (Figs. 7 and 8). In the presence of indomethacin, ongoing secretion could be markedly inhibited by subsequent

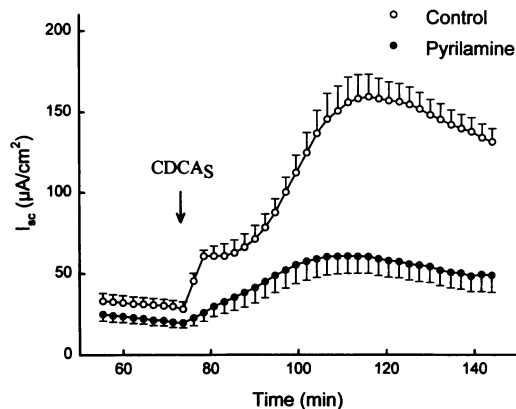


Figure 6. Effect of serosal addition of CDCA (1 mM) on the time course of I_{sc} across mouse distal colon in the absence (*Control*) or presence of pyrilamine (200 μ M, added to the serosal side of the tissue 15–20 min before CDCA). Values are means \pm SEM for five experiments.

addition of 100 μ M DPH and could be completely reversed by 200 μ M DPH (Fig. 9).

Experiments with mast cell-deficient W/W^v mice. Mast cells are thought to be the major cellular repository of histamine outside of the stomach and the central nervous system of rodents. Experiments with mast cell-deficient mice (W/W^v) and their mast cell-replete littermate mice ($+/+$) were performed to confirm the likely involvement of mast cells in CDCA-induced colonic chloride secretion. As before, tissues were pretreated with TTX. Although not apparent from the single time course shown in Fig. 10, the addition of TTX had, on average, a similar inhibitory effect on basal I_{sc} in tissues derived from either W/W^v or $+/+$ mice ($n = 7$; data not shown). In colonic tissue derived from W/W^v mice, the I_{sc} response induced by serosal addition of CDCA (1 mM) had a time course and amplitude different from those of the secretory response in $+/+$ mice (Fig. 10). The second peak of secretion was significantly delayed in W/W^v compared with $+/+$ mice ($P < 0.01$), and its area under the curve was smaller. In addition, the first peak of the I_{sc} response was significantly lower in W/W^v mice compared with $+/+$ mice ($P < 0.01$), as was its area under the curve (Fig. 10). The I_{sc} responses in the second phase of CDCA-induced secretion were also less in mast cell-deficient mice than in $+/+$ controls, although this did not reach statistical significance, probably owing to the limited number of animals that could be studied (Fig. 10).

The second peak of CDCA-induced chloride secretion in both W/W^v and $+/+$ mice could be promptly and completely reversed by addition of pyrilamine (200 μ M) (Fig. 10). The results with the H_1 receptor antagonist suggest the presence of histamine in non-mast cell stores in W/W^v mice (see the following sections).

Histamine release induced from normal mouse colon by CDCA. The results with the H_1 histamine receptor antagonists in C57BL/6J mice suggested that histamine is a key mediator of bile acid-induced colonic chloride secretion. We therefore tested whether CDCA was able to release colonic tissue histamine stores, as demonstrated previously with isolated mast cells (13). Incubation of colonic tissue with 1 mM CDCA caused a significant release of histamine into the supernatant compared

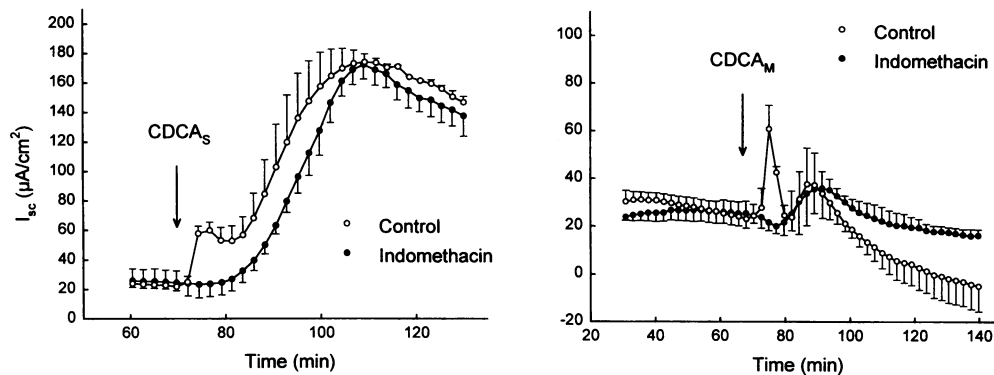


Figure 7. Effect of indomethacin ($50 \mu\text{M}$) on the time course of ion transport responses induced by either serosal (left panel) or mucosal (right panel) addition of CDCA (1 mM) to mouse distal colon. Results for tissues studied in the absence (Control) or presence of indomethacin are shown. Values are means \pm SEM for four experiments.

with tissue incubated in saline alone. Furthermore, the amount of histamine released from distal colon segments was considerably greater than that released from proximal colon segments. In the distal colon, CDCA (1 mM) released $328 \pm 60 \text{ pg}$ of histamine per mg of tissue into the supernatant, compared with the spontaneous release of histamine in saline of $153 \pm 24 \text{ pg/mg}$ ($P < 0.05$). In the proximal colon segments, CDCA released $164 \pm 23 \text{ pg/mg}$, compared with a spontaneous release of $82 \pm 13 \text{ pg/mg}$ ($P < 0.01$) (Fig. 11).

We also measured residual histamine in tissues exposed to CDCA, which would represent histamine that was still contained in intracellular stores after bile acid treatment. In control distal colonic tissue the histamine content was $750 \pm 146 \text{ pg/mg}$. In tissues treated with 1 mM CDCA, this was significantly reduced to $106 \pm 10 \text{ pg/mg}$ residual histamine. In the proximal colon, the corresponding values were $126 \pm 17 \text{ pg/mg}$ versus $59 \pm 7 \text{ pg/mg}$ (Fig. 11).

From these data, it is apparent that total tissue histamine (i.e., that released into the supernatant plus residual) is markedly lower in CDCA-treated distal colon samples compared with equivalent controls, whereas total histamine in proximal samples was similar for controls and tissues treated with the

bile acids (Fig. 11). The most likely explanation for the histamine deficit in the distal colon is degradation of released histamine to a nonimmunoreactive metabolite.

Histamine release from colonic tissue of mast cell-deficient mice. We measured histamine in the supernatant of W/W^v colonic tissue and the residual histamine in the tissue after incubation in 1 mM CDCA or saline. The tissue was taken from the middle of the colon in order to obtain an average value for colonic histamine. CDCA (1 mM) released $18.6 \pm 0.3 \text{ pg}$ of histamine per mg of tissue into the supernatant compared with $7.5 \pm 0.5 \text{ pg/mg}$ for tissue exposed to saline ($P < 0.001$, $n = 3$). The residual histamine in the tissue treated with CDCA was $31.9 \pm 0.1 \text{ pg/mg}$ compared with $97 \pm 8 \text{ pg/mg}$ in control tissue ($P < 0.01$, $n = 3$). The residual histamine content of control tissue in W/W^v mice was comparable to the amount of residual histamine we measured in the proximal colon of normal C57BL/6J mice ($126 \pm 17 \text{ pg/mg}$). However, the histamine released into the supernatant by CDCA was markedly less in W/W^v mice than in C57BL/6J mice.

Discussion

The goal of this study was to examine mechanisms mediated by subepithelial cell types that might contribute to the secretory

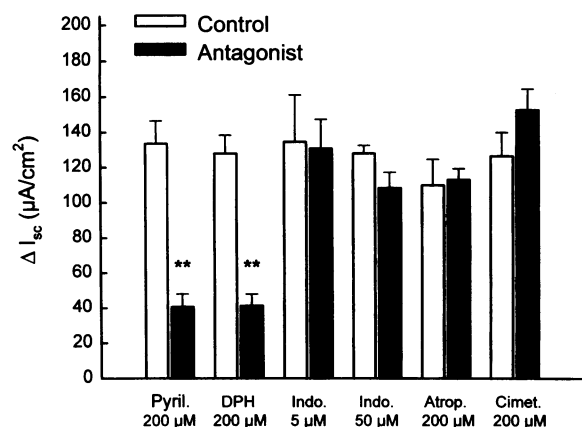


Figure 8. Maximal changes in I_{sc} induced by serosal addition of CDCA (1 mM) across mouse distal colon in the presence of various antagonists (closed bars), as noted on the x axis. DPH and pyrilamine were added to the serosal side of the tissue; the other named inhibitors were added to both sides. All antagonists were added to the tissues 15–20 min before CDCA. Parallel control data obtained in the absence of antagonist treatment are depicted by the open bars. Data are means \pm SEM for at least three experiments for each case.

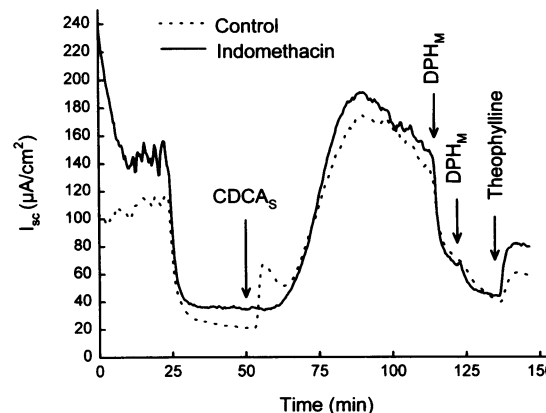


Figure 9. Effect of the sequential addition of indomethacin ($50 \mu\text{M}$) and DPH (100 and $200 \mu\text{M}$) on the time course of the secretory response evoked by 1 mM CDCA added to the serosal aspect of mouse colon. Theophylline (1 mM) was added at the conclusion of the experiment as indicated, to confirm tissue integrity. The data are from a single experiment, representative of at least three similar experiments.

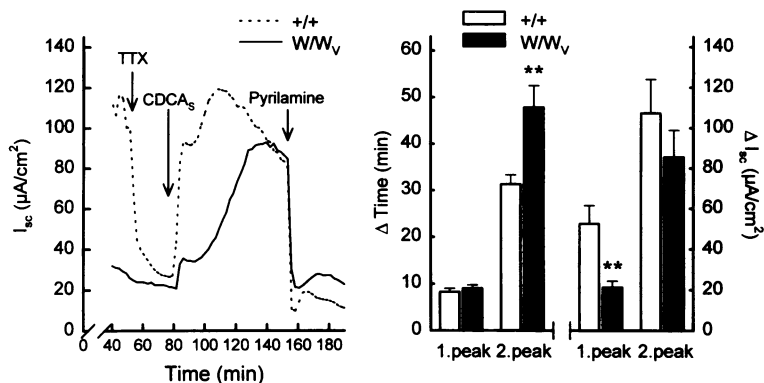


Figure 10. (Left panel) Time course of the effect of serosal addition of CDCA (1 mM) on ion transport response of colonic tissues obtained from either *W/W v* or *+/+* mice. Pyrilamine (200 μ M) was added at the end of the experiment, as indicated. The data are from a single experiment, representative of seven such experiments. (Right panel) Time elapsed after CDCA addition until the maximums of the biphasic I_{sc} response (Δ Time) and magnitude of the two phases of the response (ΔI_{sc}) in *+/+* and *W/W v* mice. Values are means \pm SEM for seven experiments. Asterisks denote responses in *W/W v* mice that are significantly different from those in *+/+* mice (** $P < 0.01$, Student's t test).

effect of bile acids in the mammalian colon. The starting point for the study was twofold: our previous observations that bile acids are capable of causing mast cell activation both in vivo and in vitro, and the growing appreciation of the role played by immune and inflammatory cell types, including mast cells, in regulating intestinal secretion. We hypothesized, based on these prior findings, that mast cells and their mediators might contribute to the secretory response evoked by colonic exposure to pathophysiological concentrations of bile acids. The findings reported in this paper are in support of such a hypothesis. These experiments show that CDCA, a common biliary bile acid in man, induces electrogenic chloride secretion in the mouse colon and that a major component of this secretion appears to be attributable to histamine. The source of this histamine is likely to be predominantly mast cells. Other cell sources are also possible, but unlikely, except in the case of the mast cell-deficient animals. Chloride secretion was induced at concentrations of CDCA (0.5 and 1 mM) that are well within those reported to be present in the aqueous phase of colonic content in patients with bile acid malabsorption and diarrhea. They are

also in the range that we have reported to activate cultured mast cells in vitro (13). CDCA also released histamine when incubated with colonic segments, although the current data do not allow us to conclude definitely that this ability to release histamine is directly responsible for the observed effects of CDCA on transport. The work extends studies from a number of laboratories in showing that both enterocytes and nonepithelial cells are involved in bile acid-induced secretion. UDCA, in agreement with studies in other species including humans, did not have secretory or toxic effects.

Mice were chosen for the present study because of the availability of strains that display genetically determined deficiencies in tissue mast cells. The secretory effect of CDCA in *W/W v* (mast cell-deficient) mice was attenuated and delayed compared with that in normal littermate controls, suggesting that mast cells are involved in the response to CDCA in the normal animals. However, secretory responses to CDCA were not absent in the mutant mice, and the colonic tissue of such animals were shown to contain histamine at an undefined site that was released by incubation with CDCA.

The chloride secretory response induced by either serosal or mucosal application of CDCA was biphasic, suggesting that the total response was caused by more than one secondary mediator and/or by more than one cell type capable of producing secretagogues. Studies using pharmacological antagonists indicated that not only histamine (acting through H_1 receptors), but also cyclooxygenase products contribute to the secretory effects of CDCA in the mouse colon. Treatment with histamine H_1 antagonists (but not with the H_2 antagonist cimetidine or with atropine) markedly inhibited both phases of secretory responses or abolished them once initiated. A role for histamine in mediating the secretory effect of CDCA was further substantiated by the ability of CDCA to release histamine from mouse colonic tissues and the finding that CDCA-induced secretion and histamine release were both greater in distal than in proximal colon segments. Indomethacin, a cyclooxygenase inhibitor, reduced the first, transient phase of the secretory response to CDCA, but had little effect on the magnitude of the later component. These data suggest that prostaglandins are likely to be involved in only the early phase of responses and that histamine is the quantitatively more important mediator in the model system examined in these studies.

Of note, the studies reported here were all conducted in the presence of a neurotoxin, TTX. This treatment provided a stable baseline of ion transport and abolished the frequently high levels of spontaneously oscillatory ion transport that occurred upon

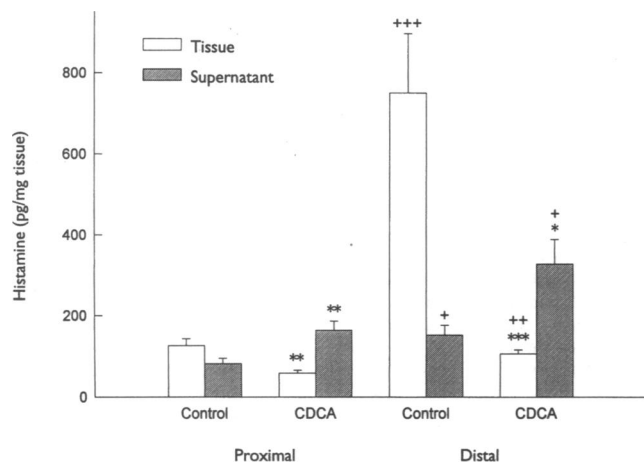


Figure 11. Histamine release (Supernatant) and residual tissue histamine (Tissue) from mouse proximal and distal colon in the presence of either buffer alone (Control) or 1 mM CDCA. Values are means \pm SEM for at least seven experiments. Values designated by asterisks are significantly different from the corresponding control values (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's t test). Values designated by plus signs are significantly different from the corresponding values in proximal tissue (+ $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$, Student's t test).

mounting the mouse colonic tissue in the Ussing chamber. In one respect, this experimental strategy is useful because it allows the isolation of effects that are independent of the enteric nervous system. On the other hand, it excludes examination of the interrelationship between mast cell activation and neural control of ion transport in this model. The use of TTX may have led to an underestimation of the contribution of mast cells, since it is known, from a number of models of immune-related chloride secretion, that the effects of mast cell activation and released mediators on chloride secretion can be amplified by effects on the enteric nervous system (29).

The serosal addition of CDCA had a greater effect on chloride secretion than did mucosal addition for both proximal and distal tissues. CDCA molecules will flip-flop rapidly across the lipid domains of all membranes (34), suggesting that the biochemical mechanism of CDCA-induced secretion is initiated by interaction with signal transduction mechanisms present on the basolateral membrane. When CDCA is added to the mucosal side, it can reach only the basolateral membrane by passing transcellularly, and such transcellular transport is likely to involve binding to intracellular proteins, if transport through colonic enterocytes is similar to that through small intestinal enterocytes (35). Were such binding to occur, the basolateral membrane might be exposed to a much lower intracellular concentration than that present when CDCA was added to the serosal compartment. The entry of CDCA into the colonic enterocyte is likely to cause acidification of the cell (36), as well as calcium mobilization (22–24), and both of these might contribute to an increase in paracellular permeability. If this occurs, CDCA would also enter the mucosa via the paracellular pathway and interact directly with the basolateral membrane of the enterocytes, as well as with mast cells. In conditions of intestinal inflammation, in which both intestinal permeability and the number of mucosal mast cells might be expected to be elevated (37–40), the tissue might be expected to respond to much lower concentrations of CDCA present in the colonic contents.

In the person without bile acid malabsorption, the aqueous concentration of CDCA in the cecum is extremely low because of its intrinsic insolubility at the acidic pH of cecal content as well as its rapid conversion by bacterial 7-dehydroxylation to lithocholic acid, which is also extremely insoluble (2, 41). DCA, the other secretory bile acid, also has an extremely low aqueous concentration in the cecum because of its insolubility (2). Thus the effects described here are unlikely to occur in the healthy individual with neither bile acid malabsorption, nor changes in colonic permeability and/or mast cell numbers, unless CDCA is ingested orally in large amounts. The effects might also be induced by the continuous ingestion of cathartic agents.

In summary, we present evidence in this paper that mast cells and, in particular, one of their major mediators, histamine, contribute to the ability of bile acids such as CDCA to induce colonic secretion when present at that site at inappropriately high concentrations. We certainly do not mean to suggest that release of mediators from mast cells is the only mechanism underlying bile acid-induced chloride secretion. However, the clinical relevance of these findings is reinforced by the preliminary observation that histamine apparently also contributes to the secretory effects of CDCA in surgical specimens of human colon mounted in Ussing chambers (Gelbmann, C. M., et al., unpublished observations). Thus, a consideration of the mechanisms discussed here might have therapeutic implications for

the management of patients with secretory diarrhea caused by bile acid malabsorption. This would be particularly true in the setting of a concurrent increase in colonic bile acid concentrations, mucosal permeability, and expansion of the mucosal mast cell population, such as might be expected to occur in inflammatory bowel disease (37–40, 42).

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